GMO DETECTION WITH NANOBIOSENSING SYSTEM INTEGRATION OF ARTIFICIAL INTELLIGENCE

YAPAY ZEKÂNIN ENTEGRE EDİLDİĞİ NANOBİYOSENSÖR SİSTEMİYLE GDO TAYİNİ

YEŞİM TAŞKIN

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ABSTRACT

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Genetically modified organisms (GMOs) and their products have been in the food and feed sectors for decades. There are almost 32 crops approved in 44 countries. Every country has different legislation regarding the threshold values and allowed GM events. In the case of Turkey, there are not any GMOs that have been given approval for food use except for three microbial food enzymes. In order to determine GMO levels in the specified limits, rapid and sensitive GMO detection is required.

The aim of this study is to propose a new method that makes critical decisions regarding GMO and to develop an amplification-free, DNA-based nanobiosensor that will allow rapid, qualitative determination of the Cry1Ac gene in soybean event MON87701. The new methodology with gold nanoparticles (AuNPs) offers an excellent platform based on the genomic DNA (gDNA) sequence of interest's hybridization with a complementary sequence. gDNA isolated from the Certified Reference Materials (CRM) was prepared with high purity. The probes used were the exact complementary of the gene of interest. Optimal conditions for the GMO nanobiosensor have been determined and with three batches of citrate reduction synthesized AuNPs.

Firstly, heat treatment is applied to unamplified gDNA and the complementary probe to allow hybridization, later addition of AuNPs. Detection was accomplished through aggregation of AuNPs which was associated with color changes of the reaction after addition of NaCl. The aggregation levels were evaluated using UV–vis absorption or by visual observation immediately.

A correlation was obtained between the GMO level according to the Cry1Ac gene with a colorimetric change of red to purple. The detection limit is as low as nanomolar, and the detection time estimated as 10 min. The method proposed here has the potential to be an alternative method available in food.

Finally, a successful prediction analytics model has been developed from the obtained data set with the machine learning algorithm, support vector machine, that will automatically classify and predict GM levels from different batches of AuNPs. Further models are integrated into a user-friendly website.

Keywords: gold nanoparticules (AuNP), genetically modified organism (GMO), nanobiosensors, MON87701, Cry1Ac, support vector machine (SVM)

ÖZET

YAPAY ZEKÂNIN ENTEGRE EDİLDİĞİ NANOBİYOSENSÖR SİSTEMİYLE GDO TAYİNİ

Yeşim TAŞKIN

Yüksek Lisans, Gıda Mühendisliği Bölümü

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Mayıs 2022, 140 sayfa

Genetik modifiye organizmalar (GDO) ve ürünleri uzun yıllardır gıda ve yem sektöründe yer almaktadır. Şu anda 44 ülkede onaylanmış yaklaşık 32 ürün mevcuttur. Her ülkenin izin verilen eşik değerler ve izin verilen GD çeşitleri ile ilgili farklı bir mevzuatı vardır. Türkiye'de, üç mikrobiyal gıda enzimi dışında hiçbir GDO'ya gıda kullanımı için onay verilmemiştir. GDO düzeylerinin belirlenen limitler içerisinde olduğunun tespiti için hızlı ve hassas GDO tespiti şarttır.

Bu çalışmanın amacı, GDO ile ilgili kritik kararlar veren yeni bir yöntem önermek ve soya fasulyesi çeşidi MON87701'de Cry1Ac geninin hızlı, kalitatif tespitine olanak sağlayacak, amplifikasyon gerektirmeyen, DNA tabanlı bir nanobiyosensör geliştirmektir. Altın nanopartikül (AuNP) metodolojileri, ilgilenilen genomik DNA (gDNA) dizisinin tamamlayıcı dizi ile hibridizasyon prensibine dayalı işleyen bir platform sunar. Bu amaçla öncelikle Sertifikalı Referans Materyalden (CRM) izole edilen gDNA'lar yüksek saflıkta elde edilmiştir. Sentezlenen problar, ilgilenilen genin tam eşleniğidir. GDO nanobiyosensörü için en uygun koşullar belirlenmiş ve üç grup sitrat indirgeme ile AuNP'ler sentezlenmiştir.

İlk olarak, amplifiye edilmemiş gDNA'ya ve hibridizasyona izin vermek için tamamlayıcı proba uygulanan ısıl işlem, daha sonra AuNP'lerin eklenmesini izlemektedir. Tespit, NaCl ilavesinden sonra reaksiyonun renk değişiklikleri ile ilişkili olan AuNP'lerin toplanması/kümeleşmesi yoluyla gerçekleşmektedir. Kümeleşme seviyeleri, UV-Vis absorpsiyon spektroskopisi kullanılarak veya anında görsel gözlem ile değerlendirilmiştir.

Kırmızıdan, mor renge kolorimetrik değişim ile Cry1Ac gen düzeylerine göre GDO seviyesi arasında korelasyon elde edilmiştir. Tespit sınırı nanomolar düzeyi kadar düşüktür ve tespit süresi 10 dakika olarak bulunmuştur. Burada önerilen metot, gıdalarda GDO tespitinde mevcut alternatif yöntem olma potansiyeline sahiptir.

Son olarak, elde edilen veri setinden makine öğrenme algoritması ile başarılı bir tahmin analitik modeli geliştirilmiştir, destek vektör makinesi algoritması ile farklı AuNP partilerinden GM seviyelerini otomatik olarak sınıflandırılmış ve yüksek doğrulukta tahmin edilmiştir. Daha sonra modeller, kullanıcı dostu bir web sitesine entegre edilmiştir.

Anahtar kelimeler: altın nanopartikül (AuNP), genetik modifiye organizma (GMO), nanobiyosensör, MON87701, Cry1Ac, destek vektör makinesi (DVM)

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ABBREVIATIONS AND SYMBOLS

Symbols

g	gram
ml	milliliter
µl	microliter
ng	nanogram
pM	picomolar
nm	nanometer

Abbreviations

AOCS	American Oil Chemists' Society
AuNP	Gold nanoparticle
bp	base pair
CRM	Certified Reference Material
DNA	Deoxyribonucleic Acid
ds	double stranded
EC-JRC-IRMM	European Commission Joint Research Center – Institute for Reference Materials and Measurements
EFSA	European Food Safety Authority
ELISA	Enzyme-linked Immunosorbent Assay
ERM	European Reference Material
EU	European Union
FDA	Food and Drug Administration
gDNA	Genomic Deoxyribonucleic Acid
GM	Genetically Modified

GMO	Genetically Modified Organism
ISO	International Organization for Standardization
kb	kilo base
Le1	Lectin
LOD	Limit of detection
LSPR	Localized surface plasmon resonance
PCR	Polymerase Chain Reaction
Q-PCR	Quantitative Polymerase Chain Reaction
rpm	Revolution per Minute
RT-PCR	Real Time Polymerase Chain Reaction
RSD_r	Relative Repeatability Standard Deviation
SVM	Support Vector Machine
TEM	Transmission Electron Microscopy
UV-Vis	Ultraviolet–Visible
WHO	World Health Organization

1. INTRODUCTION

Humans have been growing crops for years to feed ourselves and the animals. Over time crops became domesticated and farther developed by conventional breeding. Further, improvements in the area of biotechnology allowed for the development of modern agriculture and “genetic engineering” [1]. Genetic engineering provides a direct method to introduce one or more useful genes. Finally, the end product is called Genetically Modified Organism (GMO). Quantitative-Real-Time polymerase chain reaction is considered the gold standard and the reference method for GMO testing, according to its sensitivity, high specificity.

On the other hand, novel approaches using the biosensor devices are becoming a promising strategy day by day due to the easy monitoring. There has been a big interest in the use of nanoparticles for detection. Nanoparticles can have a number of advantages over their bulk form. Especially gold nanoparticles (AuNPs) have been widely employed in sensors based on their unique properties [2].

Aim of this study is to develop a simple, rapid and selective DNA based gold nanobiosensors for GMO detection. The interaction between gold nanoparticles with single stranded and double stranded nucleic acids has facilitated this detection. We have optimized and shown that our method primarily works with synthetic oligonucleotide probes. Further exploite with genomic DNA (gDNA) for sequence-specific detection that differs in aggregation with respects to soybean event MON87701 levels according to Cry1Ac gene. However according to AuNP batches that are differentiate in size and concentration and different gDNA batches, can lead to different levels of aggregation. The results presented herein lead us to propose a range for each GMO levels.

Under the optimum conditions, a correlation was obtained between the GMO level with colorimetric change of red to purple. It provides visual Cry1Ac gene detection without the need of cycles of amplification. This gold nanobiosensor based detection can give qualitative information for the GMO level in the sample prior to routine PCR analysis.

Using the method described herein, we successfully developed an algorithm for ML with support vector machine. Through training support vector machine's predictive analysis data-classification, test data achieved a good percent of success rate. Further models are integrated into a user-friendly website.

This thesis study positioned at the intersection of biotechnology, nanotechnology and computer science.

This application provides decition tool prior to PCR analysis.

This solution is rapid, specific, cost effective diagnostic and an accessiable technology.

This thesis study has three main headings,

i) Oligonucleotide probe nanobiosensor for GMO detection
ii) Genomic DNA probe nanobiosensor for GMO detection through different size and concentration of AuNPs
iii) Development of machine learning algorithm and a website

2. GENERAL INFORMATION

2.1. Genetically Modified Organism

There are different definitions for genetically modified organisms (GMO) in different sources. “The International Service for the Acquisition of Agri-biotech Applications” (ISAAA) define GMO as “a GM or transgenic crop is a plant that has a novel combination of genetic material obtained through the use of modern biotechnology” [3].

World Health Organization (WHO) define GMO as “Genetically modified organisms (GMOs) can be defined as organisms (i.e. plants, animals or microorganisms) in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating and/or natural recombination. The technology is often called modern biotechnology recombinant DNA technology or genetic engineering” [4].

The Food and Drug Administration (FDA) define genetic modification as “A GMO is a plant, animal, or microorganism that has had its genetic material (DNA) changed using technology that generally, involves the specific modification of DNA, including the transfer of specific DNA from one organism to another” [5]. The United States Department of Agriculture (USDA) summarized it as “An organism produced through genetic modification” [6]. In the US, there are currently four crops that are genetically engineered to produce soybeans, cotton, rapeseed, and maize. Another 20 + species such as papaya and sugar beets are the popular varieties.

Through genetic engineering, a desirable trait can be inserted to plant/crop in a short time. Goal is to make it more effective at fighting against pests and diseases or to give a wanted characteristics [7]. GMOs provide a number of benefits, including a significant increase in agricultural productivity, quality and better nutritional properties of plants [8]. Advances have also been made with crops that can endure metals like boron or resistance to salt, drought, frost, other environmental stressors [9].

GMOs, have been planted and sold for almost two decades. Commercial GMOs are grown in more than twenty-nine countries and in total, 190.4 million hectares of engineered crops grew in 2019 [10]. With the planet population set to reach 9 billion by 2050, the food has to be of course available and accessible. GM crops could help to reduce hunger and malnutrition [11, 12]. However, for this to be done, new policies, larger investment

in agricultural research is needed. This accomplished by finding and extracting genes of interest from organisms and incorporating the wanted DNA sequence into the crops genome [13].

If we generalize it, genetic engineering is a procedure that entails;

1- identify the gene that has desired trait
2- copy the gene from the organism
3- incorporate the information into the new organism
4- grow the new organism
5- conform the existence and expression of the added gene or desired characteristic
6- testing

A general gene construct in a GMO is formed of, the promoter, the selected gene, the terminator [14].

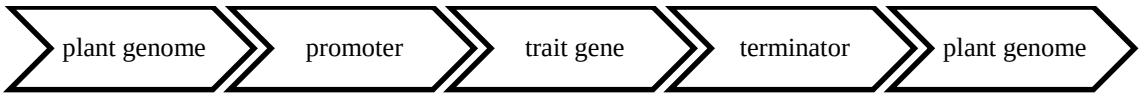


Figure 2.1. General gene construct of a GMO

2.1.1. Methods Used to Transfer DNA

The first part includes, DNA transfer to plant cell. Genetic modification, a number of genetic engineering approaches are characterized as a sort of genetic alteration that has a wanted change in a crop genome to achieve certain end product.

Microbial Vectors

Agrobacterium tumefaciens cause an appearance of soilcrown gall disease on plants. It is a special pathogen because, it can deliver a part of its own DNA into the new cell. Then the new cell reads and expresses the transferred DNA. In line with this situation studies allow for a new strains of *Agrobacterium* that deliver new DNA into the cells [15].

Microprojectile Bombardment

Klein et all [16] found that naked DNA can be transferred through cells via shooting. For most of the cereals like corn and rice microprojectile bombardment is an amazing tool. Many examples of this method is commercial produced.

Electroporation

In *electroporation,* simply crops protoplasts absorbs DNA from the medium. Further electrical pulse is applied this causes destabilization in the cell membrane and desired gene enters the cell. Further new trait owning cells, grow to big, transgenic plants. Electroporation can be limited.

Microinjection

DNA is injected into cells directly. Some of the cells continue to survive and have the introduced DNA. However, this method can ben expensive, not as efficient.

Nontransgenic Molecular Methods of Manipulation

Plants can have gene traits introduced to them but not have them into the receiving genome. Target DNA can be incorporated to the cell, and further expressing a novel protein [15].

2.1.2. CRISPR

CRISPR (clustered regularly interspaced short palindromic repeats/Cas9) gene-editing technology has been boosted vastly by researchers. CRISPR is a very popular technology because of its precise addition and change in DNA with targeted specificity as well as comfort. CRISPR already showed important improvements recently for plants production [17-19].

CRISPR vs. GMO

CRISPR gene-editing differs from the GM technics especially, CRISPR might ve utilized to make modification to the DNA, whereas GMOs can be foreign DNA (transgenic) [20, 21]. Also difference in regulation side, GM plants are many times subjected to strict biosafety assessments, but some countries have not regulated CRISPR plant/food due to not having transgenic DNA. Ishii and Ishii 2021, showed that, developers can prove that a CRISPR is not a GMO. GMO versus CRISPR is summarized in Table 2.1 [17, 22].

Table 2.1. CRISPR vs. GMO

	GMO	CRISPR
DNA origin and technique	A change is inserted at a location in the genome, using genes from another species or synthetic genes.	A change is made at a precise spot within a genome.
Detection	The transferred gene cannot be generated by breeding and makes the plant distinguishable from native plants.	Indistinguishable from traditional selective breeds.
Timing	+++++	++

2.1.3. Classification of Genetically Modified Organisms

Classification can be done three different ways, classification based on the origin of new DNA, level of available knowledge about the gene structure, classification by approval status.

Classification via the line of new insert DNA

1st generation/single trait transgenes were developed using enzymatic cut and paste method. The genetic elements further inserted in a circular cloning vectors (also contains marker gene) [23]. As 2nd generation GMOs are mostly hybrid crossbreed of 1st generation or re-transformed 1st generation GMOs. "Stacked hybrid GMOs are difficult to discriminate from their parental generation" [24, 25] The 3rd generation/near intragenic are big region of insert is host originated. In as a 4th generation of GMOs/intragenics and cisgenics, the incorporated element is obtained from the genes available. That's why monitoring the insert only is not a direct means of GM [26].

Classification based on the level of knowledge

Either same desired trait is incorporated or created with novel constructions, the number of GMOs carrying that target might rise over time. GMOs that may now be detected in the food system can be divided into the following groups based on the current state of knowledge about their genetic structure.

GMOs fully characterized/knowledge level 1 conclude, when whole insert sequences are recognized. This category has all GMOs authorized for commercial use in the European Union. Level 2, "all GMOs where the fusion of elements within the construct is defined." Level 3 includes, "some trait genes, in particular from the 1st generation of GMOs, have also been used quite frequently." GMOs transformed with only new genes/knowledge level 4 class is undetectable except a generic vector test, and will suggest that the GMO is an unknown [25].

Classification of GMOs by Approval Status

Legal classification GMOs are either authorized/approved, or un-authorized. "Presence of some GMOs for which authorization is pending or not renewed, is or has been tolerated for a limited period within the EU, provided that the quantity is below a specified threshold and that the presence is adventitious and technically unavoidable" [27].

GMOs are authorized/approved GMOs are GMOs that are legally classified to be safe for food use and environmental health. They should describe risk assessment and analytical methods for detection and quantification. All the approved GMOs shorted by plant or country can be found in ISAAA's page of GMO Approval Database [28].

GMOs are unauthorized/ unapproved GMOs (UGM) and derived materials can be seen on the European shelfs. There is now 0% tolerance for UGM. The detection of an UGM on the market can be the result of a contamination [29].

2.1.4. Genetically Modified Plants in the World

The USDA authorized the Flavr Savr tomato to be the first GM food for commercial production. From the start of genetically modified crop was commercialized in 1996, GM crops continue to hold the promise of improving agriculture and millions farmers continue to plant them [30]. The most recent revision is on October 2021, USA approved maize event DBN9858.for food and feed.

The acceptance and commercialization of biotech crops in 2008 are discussed. The impact of biotech plants was described, including their contribution to global food and feed impact, more sustainable agriculture, and poverty and hunger reduction in underdeveloped nations [31].

Objectives for developing GM crops are;

Herbicide tolerance eliminate the nearby weeds, but allow the main crop untouched. Monsanto company firstly presented glyphosate-resistant soybean and further presented maize frequently referred as Roundup Ready [32]. Insect resistance plants were first brought up to market with maize and potato via the entomocidal δ-endotoxin (Bt) also referred as Cry proteins [33]. Resistance to viruses, fungi, and bacteria, pathogens and diseases are a serious constraint on agricultural production across the planet. While plants are inherently resistant to the great majority of them, those that do manage to develop skills can be exceedingly troublesome and costly [34]. Drought tolerance is needed because of climate change causes drop in terms of yield and required for specific stress-related genes [35]. Salinity resistance is important trait that needs to be improved when soil salinity in high i.e.ion exclusion, osmotic tolerance [36]. Enhanced nutritional value: Malnutrition is one of the planet's most serious health issue. Plant-based meals include a wide range of elements that are important. Biofortification is the main term used when the nutritional quality of food is enhanced. Biofortified main crops such as rice and wheat aids essential micronutrients to help under development as are novel crop types that can fight chronic illness. GM Golden Rice enhanced to have more β-carotene concentrations [37, 38].

Worldwide events by GM trait

A global knowledge sharing network ISAAA database of events by GM trait is listed as follows: herbicide tolerance, anti allergenic, drought stress tolerance, enhanced photosynthesis/yield/vitamin, fertility, lower; gossypol, modified; α-amylase, amino/fatty/oil acid, flower/fruit color, starch/carbohydrate, reduction; nicotine black spot , non-browning, nopaline synthesis, visual marker and wood increase etc. [39].

2.1.4.1. Soybean

Soybean (*Glycine max* L. Merr.) is an Asiatic plant grown in many parts of the planet for its oil and proteins content and relatively low in carbohydrates. It is widely utilized in the production of animal and human food. Soybean is one of the crucial plant after wheat, maize, rice and potato [40]. Soybeans, like other legumes, are packed with vitamins and minerals. As calorie content soybeans have 36% of protein and in very high quality scored

by FDA and WHO with 1/highest score. This signify that soy protein is as quality as meat proteins. Soybean has 40% of the calories from fat, other legumes (except nuts) contain between 2-14% fat. Fiber, when whole, single serving of soy has 8 g dietary fiber [41].

Soybean in the World

Global soybean cultivation has increased quickly over the past years. Most of the world's soybean comes from mainly United States and Brazil [42].

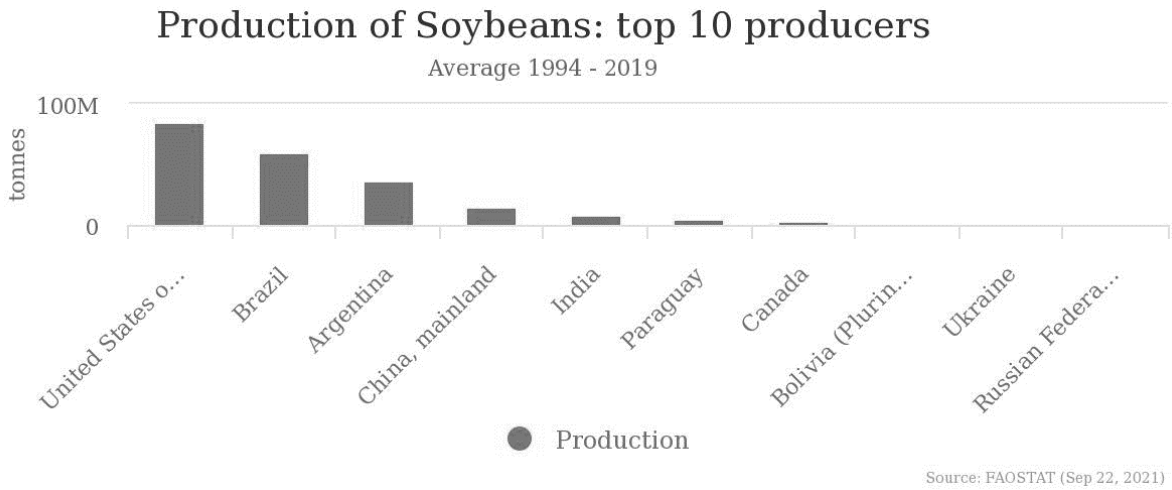


Figure 2.2. Top 10 producer of soybean [42].

Processed soybeans are the mainly animal feed and the 2nd largest source of vegetable oil. The US is the world's leading soybean grower and make up 90% oil-seed production [43]. As can be seen in Figure 2.3. the world's soy is mainly used for animal feed. All of the soybean (*Glycine max* L.) GM events till today is listed in Table 2.2.

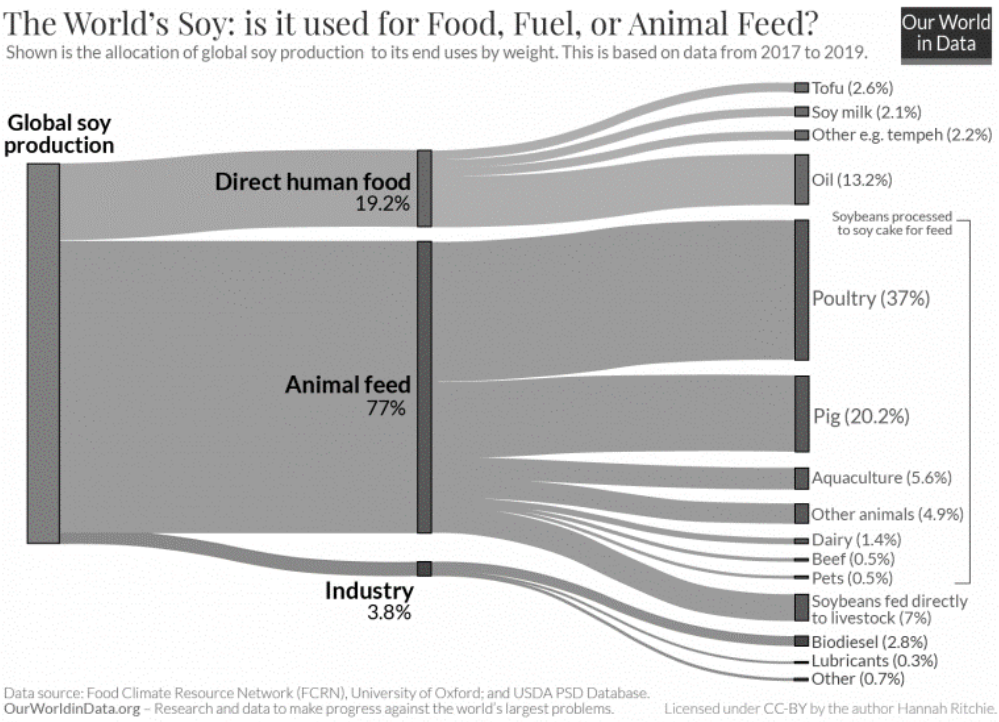


Figure 2.3. Global soy production use [44].

Table 2.2. Soybean GM Events [45].

Event Name	Gene Introduced	Gene Source	Product
MON87701	Cry1Ac	*Bacillus thuringiensis* subsp. Kurstaki strain HD73	Cry1Ac delta-endotoxin
MON87701xMON89788	Cry1Ac	*Bacillus thuringiensis* subsp. Kurstaki strain HD73	Cry1Ac delta-endotoxin
	cp4 epsps	*Agrobacterium tumefaciens* strain CP4	herbicide tolerant form of EPSPS enzyme
356043	gm-hra	*Glycine max*	modified ALS enzyme
	gat4601	*Bacillus licheniformis*	glyphosate N-acetyltransferase enzyme
A5547-127	pat	*Streptomyces viridochromogenes*	PAT enzyme
MON87708	dmo	*Stenotrophomonas maltophilia* strain DI-6	dicamba mono-oxygenase enzyme
	cp4 epsps *	*Agrobacterium tumefaciens* strain CP4	herbicide tolerant form of EPSPS enzyme
BPS-CV127-9	csr1&2	*Arabidopsis thaliana*	modified-acetohydroxyacid synthase
MON87705	fatb1-A	*Glycine max*	no functional enzyme is produced
	fad2-1A	*Glycine max*	no functional enzyme is produced
	cp4 epsps	*Agrobacterium tumefaciens* strain CP4	herbicide tolerant form of EPSPS enzyme
305423	gm-hra *	Glycine max	ALS enzyme
	gm-fad2-1	Glycine max	no functional enzyme is produced
FG72	2mepsps	*Zea mays*	EPSP synthase
	hppdPF W336	*Pseudomonas fluorescens* strain A32	modified p-hydroxyphenylpyruvate dioxygenase enzyme
A2704-12	pat	*Streptomyces viridochromogenes*	PAT enzyme
MON40-3-2	cp4 epsps	*Agrobacterium tumefaciens* strain CP4	herbicide tolerant form of EPSPS enzyme
MON89788	cp4 epsps	*Agrobacterium tumefaciens* strain CP4	herbicide tolerant form of EPSPS enzyme
DAS-44406-6	aad-12	*Delftia acidovorans*	AAD-12 protein
	2mepsps	*Zea mays*	EPSP synthase
	pat	*Streptomyces viridochromogenes*	PAT enzyme

*Selection marker

Soybean in Turkey

Soybean production in Turkey dates back to 1930s in the Black Sea Region, and recently, it is still grown as a second crop. It is being processed as food, feed and industrial raw material. Soybean production in Turkey is not much and only a pew percent of the consumption is met by local production. Because of its favorable climatic circumstances, Turkey has a large potential for soybean cultivation. However, compatibility of soybean is low concerning alternative products due to high input costs [46].

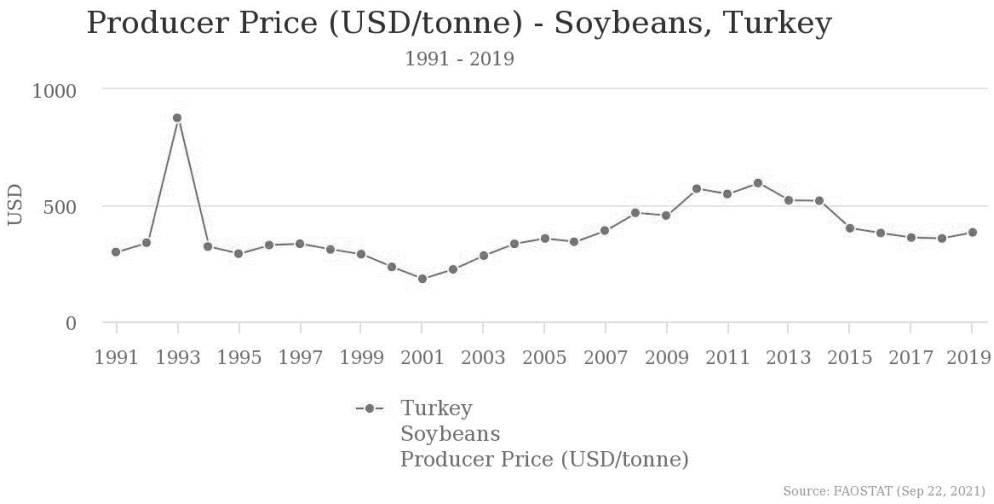


Figure 2.4. Soybean cost to producers in 1991-2019.

Cry1Ac Gene

Cry1Ac provides protection from feeding damage by target lepidopteran pest via expression of the *Bacillus thuringiensis* subsp. *kurstaki* strain HD73 insecticidal protein Cry1Ac. It is a delta-endotoxin, conferring resistance to lepidopteran insects. In the Table 2.3. the list of Cry1Ac genes present in different plants is given.

Table 2.3. Events with Cry1Ac.

Event	Trade Name
Soybean - *Glycine max L.*	
DAS81419	NA
DAS81419 x DAS44406	Conkesta Enlist E3™ Soybean
MON87701	NA
MON87701 x MON89788	Intacta™ Roundup Ready™ 2 Pro
MON87751 x MON87701 x MON87708 x MON89788	NA
Cotton - *Gossypium hirsutum L*	
281-24-236 x 3006-210-23 (MXB-13)	WideStrike™ Cotton
281-24-236 x 3006-210-23 x COT102	NA
281-24-236 x 3006-210-23 x COT102 x 81910	NA
3006-210-23	NA
3006-210-23 x 281-24-236 x MON1445	WideStrike™ Roundup Ready™ Cotton
3006-210-23 x 281-24-236 x MON8891	Widestrike™ Roundup Ready Flex™ Cotton
3006-210-23 x 281-24-236 x MON88913 x COT102	Widestrike™ x Roundup Ready Flex™ x VIPCOT™ Cotton
3006-210-23 x 281-24-236 x MON88913 x COT102 x 81910	NA
31707	BXN™ Plus Bollgard™ Cotton
31803	BXN™ Plus Bollgard™ Cotton
31807 x 31808	NA
31807	BXN™ Plus Bollgard™ Cotton
31808	BXN™ Plus Bollgard™ Cotton
42317	BXN™ Plus Bollgard™ Cotton
BNLA-601	NA
COT102 x MON15985	Bollgard® III
COT102 x MON15985 x MON8891	Bollgard® III x Roundup Ready™ Flex™
COT102 x MON15985 x MON88913 x MON88701	n/a
Event 1	NA
Event 1	JK 1
GHB614 x LLCotton25 x MON15985	NA
GHB614 x MON15985	NA
LLCotton25 x MON15985	Fibermax™ Liberty Link™ Bollgard II™
MON1076	Bollgard™ Cotton
MON15985	Bollgard II™ Cotton
MON15985 x MON1445	Roundup Ready™ Bollgard II™ Cotton
MON531	Bollgard™ Cotton, Ingard™

MON531 x MON1445	Roundup Ready™ Bollgard™ Cotton
MON757	Bollgard™ Cotton
MON88701 x MON88913 x MON15985	NA
MON88913 x MON15985	Roundup Ready™ Flex™ Bollgard II™ Cotton
Maize - *Zea mays L.*	
DBT418	Bt Xtra™ Maize
Rice - *Oryza sativa L.*	
GM Shanyou 63	BT Shanyou 63
Huahui-1/TT51-1	Huahui-1
Tomato - *Lycopersicon esculentum*	
5345	NA
Eggplant - *Solanum melongena*	
Event EE1	BARI Bt Begun-1, -2, -3 and -4
Sugarcane - *Saccharum sp*	
CTC91087-6	NA
CTC93209-4	NA
Poplar - *Populus sp*	
Bt poplar	NA
Hybrid poplar clone 741	NA

NA: not available

MON87701 event

In this study focused on MON 87701 event. An insect resistant soybean. MON 87701, which was developed by *Agrobacterium*-mediated transformation of soybean using the 2T-DNA plasmid vector PV- GMIR9, produces Cry1Ac (Figure 2.5) [47, 48].

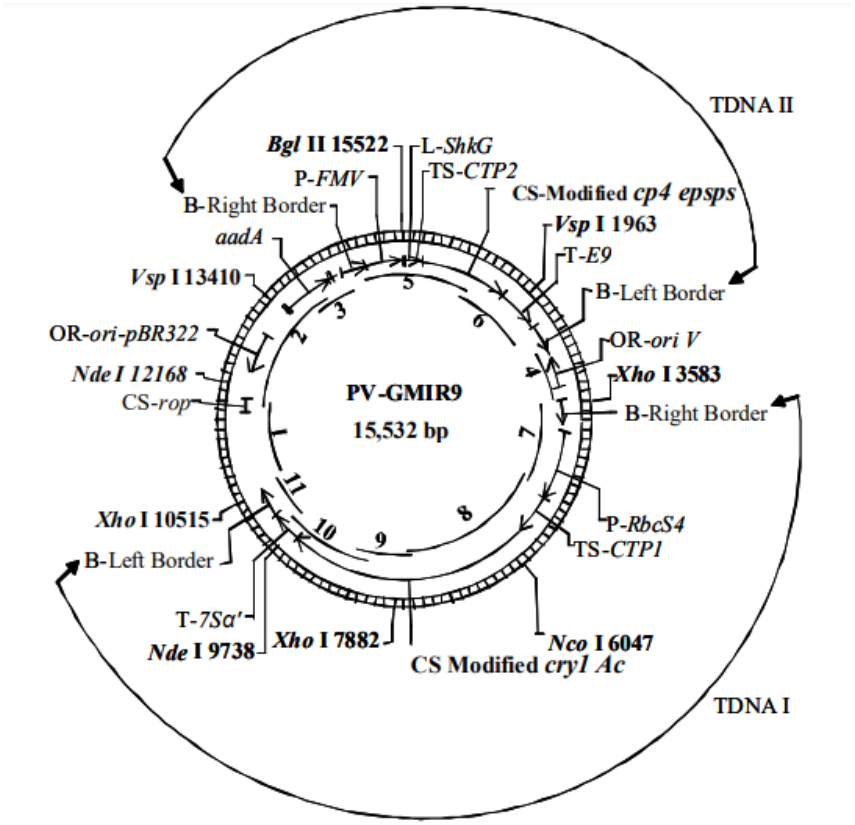


Figure 2.5. Plasmid map of PV-GMIR9 [49, 50]

2.1.5. Monitoring and Labeling of GMOs

GMO regulations in United States with 3 agencies work together, FDA, EPA and USDA. All foods, including GMOs, are regulated by the FDA. Foods and GMO ingredients must all fulfill the same safety requirements, according to the FDA. The EPA controls the safety of the chemicals that protect GMO plants and are used in certain GMO plants to make them pest and disease resistant. The USDA Animal and Plant Health Inspection Service, APHIS, establishes rules to ensure that GMO plants do not damage other plants. Food manufacturers, importers and retailers must identify foods that are bioengineered or include bioengineered components.

In Europe, the use of GM materials is ruled mainly for labeling and traceability. Traceability, bodies must provide an indication that the product/ingredient has GM material. GM Labelling is for the case of packaged GM materials; they must state that in the list of ingredients. These phrases must be prominently displayed for the product even if the product does not have packaging. These labeling rules do not apply to GM that

include no more than 0.9 percent of the total food/feed components, unless their presence is unintended or technically unavoidable [51, 52].

2.1.5.1. GMO regulations in Turkey

GMO regulations in Turkey have been in place since 1998, with the scope growing to encompass biosecurity, import, processing, export, control and further introduction of Biosafety Law in 2010 numbered 5977, which rules since September 26, 2010. Analyses of all GMOs can be performed via GMO and Biosafety Analysis Laboratories under TS EN ISO/IEC 17025 accreditation [53].

In Turkey GM variety crops has limited use as feed. A total of 39 GM varieties, including 22 corn and 14 soybeans and 4 genetically modified food enzyme have been approved. (Table 2.4) Although the products do not contain GMOs, residual DNA can be detected due to storage, transportation or contamination from the other product [54].

Table 2.4. Current GMO list (Turkey) [54].

No	GM Plant	Gene Introduced	Code
1.	Maize	Bt11	SYN-BTØ11-1
2.	Maize	DAS1507	DAS-Ø15Ø7-1
3.	Maize	DAS59122	DAS-59122-7
4.	Maize	NK603	MON-ØØ6Ø3-6
5.	Maize	GA21	MON-ØØØ21-9
6.	Maize	MON89034	MON-89Ø34-3
7.	Maize	4114	DP-ØØ4114-3
8.	Soybean	SYHT0H2	SYN-ØØØH2-5
9.	Maize	MON88017	MON-88Ø17-3
10.	Maize	MON810	MON-ØØ81Ø-6
11.	Maize	59122xNK603	DAS-59122-7xMON-ØØ6Ø3-6
12.	Maize	MIR604	SYN-IR6Ø4-5
13.	Maize	MON863	MON-ØØ863-5
14.	Maize	T25	ACS-ZMØØ3-2
15.	Soybean	MON87701	MON-877Ø1-2
16.	Soybean	MON87701xMON89788	MON-877Ø1-2xMON-89788-1
17.	Soybean	356043	DP-356Ø43-5
18.	Soybean	A5547-127	ACS-GMØØ6-4
19.	Maize	Bt11xMIR604	SYN-BTØ11-1 x SYN-IR6Ø4-5
20.	Maize	MIR162	SYN-IR162-4
21.	Maize	MIR604xGA21	SYN-IR6Ø4-5 x MON-ØØØ21-9
22.	Maize	MON863xMON810	MON-ØØ863-5 x MON-ØØ81Ø-6
23.	Maize	MON863xNK603	MON-ØØ863-5 x MON-ØØ6Ø3-6
24.	Maize	MON89034xMON88017	MON-89Ø34-3xMON-88Ø17-3
25.	Soybean	MON87708	MON-877Ø8-9
26.	Soybean	BPS-CV127-9	BPS-CV127-9
27.	Soybean	MON87705	MON-877Ø5-6
28.	Maize	MON87460	MON 8746Ø-4
29.	Soybean	305423	DP-3Ø5423-1
30.	Soybean	FG72	MST-FGØ72-2
31.	Maize	MON87427	MON-87427-7
32.	Maize	DAS40278-9	DAS-4Ø278-9
33.	Soybean	A2704-12	ACS-GMØØ5-3
34.	Soybean	MON40-3-2	MON-Ø4Ø32-6
35.	Soybean	MON89788	MON-89788-1
36.	Soybean	DAS-44406-6	DAS-444Ø6-6

Addition to this list, 4 genetically modified food enzyme have been approved.

2.2. GMO Detection

The analytical strategy for GMO detection and quantification begins with sampling and sample preparation. GMO detection methods mostly focuses on finding adapted DNAs [55]. Detection of GMOs via RNAs, proteins, metabolites, and phenotypes could be difficult [25]. In the case of processed foods RNAs and proteins can be easily denatured. DNA, on the other hand, is more stable and can be extracted largely and relatively cheaply. Detection procedure can be divided into two groups, based on nucleic acid or protein, furthermore as challenges, limitations aroused, novel and improved methodologies for GMO detection methodologies developed [14].

2.2.1. Protein Based GMO Detection

Immunological procedures are another term for protein-based approaches. The detection principle is conjugation of antigen+antibody. The conjugation follows 2nd combination in which a colorimetric response occurs. Specificity of antibodies varies. Widely used protein-based techniques are lateral flow strips (LFS) and enzyme linked immunosorbent assays (ELISA). These techniques are mostly applied for quick detection in the field however, not the best option for detection of GMO [56].

2.2.1.1. ELISA Method

Detects a specific protein quickly. The protein sample is introduced after the antibody has been immobilized on a solid substrate. An antibody-antigen conjugate is generated. The presence of the target protein may then be visualized using the second antibody, which has been labeled [57].

2.2.1.2. LFS Method

The sample travels through a material propelled and the secondary antibody is added. In the case of target presence, the following protein attach and move, until being absorbed by the main antibody, which is immobilized in a reading well [56].

2.2.2. Nucleic Acid-Based GMO Detection

Methods targeting DNA are more time intensive, but may provide all degrees of specificity and capacity to measure the target. There is popular unity for the DNA based GMO detection [25, 29].

2.2.2.1. Polymerase Chain Reaction

In terms of GMO detection, PCR-based approaches are still the gold standard [55]. PCR-based GMO detection approaches have markers specific to GM type. As a result, these PCR methods can be sort by element-specific, construct-specific, and event-specific [25].

The element-specific targets, promoter, a terminator. The construct-specific targets, combination of two elements. The event-specific targets, transgenic construct that is incorporated. The event-specific PCR approach is specialized for measurement of GMOs [58, 59]. EURL GMFF published a report providing a compass and guidance on how methods for GMO analysis should be evaluated.

2.2.3. Novel Methods for GMO Detections

The rapid development and expanding GM plants makes GMO testing difficult. That's why detection methodologies have evolved in the past decades. Today, there is more need for novel, accessible, automated, real-time, data-driven, more specific and sensitive approaches for a strong GMO detection system. The increase in GMO research and commercialization is resulting in a continuous increase in the studies. For example lab on the chip-based technology will soon enable a routine sensing of GMO [60, 61].

In 2021, development of a new LAMP-TaqMan method for detection of GMOs studied to detect NOS and also showed short time and with very low copies of sequences is possible [62]. Multiplex and regenerable surface plasmon resonance biosensor for GMO detection described [63].

A portable biosensing device was constructed based on a recombinase polymerase amplification-lateral flow strip (RPA-LFS) method for rapid detection of MON810 [64].

Through novel applications of nanotechnology, nanosensors/nanobiosensors are taking a lead role in detection over conventional methods. Use of different nanomaterials, such

as nanowires, nanotubes, layers (i.e., graphene) or colloids in portable devices that could be easily deployed across fields and locations have supported such approaches. The use of nanoparticle may not just leave the PCR technic out of game, it can act as a preliminary decision mechanism.

2.3. Nanoparticles

2.3.1. Gold Nanoparticles

There has been marvelous interest in the use of nanoparticles for detection for years. Nanoparticles have number of advantages over small molecule agents or in bulk form. Especially gold nanoparticles (AuNPs) have been widely used in nanobiotechnology, because of their unique characteristics and diverse surface capabilities [65, 66]. When reduced to nanoscale (less than a hundred nm), AuNPs have distinct physicochemical features (mechanical, electrical, optical), that set them apart from their bulk form [67, 68].

The simplicity with which AuNP may be functionalized makes it a flexible platform for nanobiological assemblies easy and controlled attachment, such as oligonucleotides, antibodies, and small molecules that enable broadened functionality and detection [69, 70]. AuNPs have been utilized for centuries because of their behaviour with visible light. Still these unique optoelectronic properties have been researched and used in operations such as sensors and many more. Advantages of AuNPs are; good biocompatibility, easy synthesis, monodispersity, less toxicity and easy functionalization and capability of visual detection using naked eyes [67, 71, 72].

The optical charcteristic of AuNPs are adjustable by changing the size, shape, surface chemistry, or aggregation condition. Diameter changes also produce colour changes from red to blue, aggregated AuNP color changes with respects characteristic move among the surface plasmon resonance [73-75].

2.3.1.1. Synthesis of Gold Nanoparticles

Among different synthesis methods, diverse physical and chemical approaches have been developed [76]. Apart from physical and chemical approaches, a growing shift toward adapting non toxic and eco friendly green synthesis methods is happening [77-80]. It is

critical to choose the right synthesis approach for AuNPs, to tune for GMO detection applications.

In chemical methods, reduction of gold is frequently used. Turkevich and friends in 1951, studied one of the most known approach; AuNPs are prepared from the reduction of Au ions by reducing element, citrate. Citrate ions also serve as capping agents to stabilize the AuNPs [81]. Frens in 1973, demonstrated that the size variation of the resulting particles by the citrate reduction can be done by simply changing the concentration of sodium citrate [82]. By varying preparation factors such as concentrations, pH, temperature, and reducing agents, e.g. the pH of the solution can influence the morphology of the resulting AuNPs. AuNPs are stable for a long time once synthesized. They are inexpensive, despite the fact that the material from which they are made is costly [83].

As physical synthesis technique, laser ablation in combination with the laser-induced size control provides a versatile full physical preparation method of size-selected gold nanoparticles [84].

2.3.1.2. Characterization of Gold Nanoparticles

It is important to characterize gold nanoparticles to assess the surface modification [85]. The most common techniques are Ultraviolet-Visible (UV-Vis) Spectroscopy, Microscopic Imaging, Dynamic Light Scattering (DLS), Gel Electrophoresis [86].

Transmission electron microscopy (TEM) is the most popular approach for acquiring reliable data regarding the average size and size distribution of AuNPs [87]. However, TEM measurements have several drawbacks when it comes to sizing regularly and in particular, does not allow for quick and real-time monitoring [88].

Scanning electron microscopy (SEM) was used to validate the synthesis of AuNPs and study the morphology. However, SEM has limited resolution of, makes it hard to see differences in the size and shape [89].

Dynamic light scattering (DLS) is used commonly used to measure the hydrodynamic size of AuNPs and colloids in a liquid. AuNPs are extraordinary light scatterers at or near their SPR wavelength [90].

Gel Electrophoresis can be used to separate gold nanoparticles based on size, shape, and charge, separate also mixture of gold spheres and long rods. Its best performance

necessitates a relatively large amount of AuNP in the loading cell for visible detection of bands [91].

The different colors of AuNP come from an optical feature called as localized surface plasmon resonance (LSPR). The collective oscillation of electrons in the conduction band of AuNPs in resonance with a specific wavelength of light. LSPR of AuNPs results in a distinct absorbance band in the visible region, which can be quantified by UV-Vis spectroscopy. The UV range extends from 100–400 nm, and the visible spectrum ranges from 400–700 nm. LSPR Peaks occurs around 515-520 wavelength, refer to measure the concentration of gold nanoparticles in solution, refer to Lambert- Beer Law [92]. When the size changes, the maximum extinction of the surface plasmon band shifts in the visible region. Well plasmonic materials have optical absorption spectra with a maximum at the LSPR frequency wavelength, which for AuNPs it is centered near 520 nm. AuNP resonate at frequencies within the visible spectrum of light. Smaller nano gold particles look red. Larger nano gold particles look blue. The LSPR spectrum is dependented on the morphology of AuNPs. The LSPR spectrum redshifts by a few nm when ligands attach to the AuNPs and this is the basis of LSPR biosensing. Aggregation states may also be used to detect by a shift in colour of the solution from red to blue/purple. Various AuNPs have been studied for targeting ie. DNA and many more biological specimens [93, 94]. The great part of the studies targets specific sequences through hybridization of a probe to the target analyte. This studies are mainly simple and quick and use surface functionalization as well as do not require sophisticated instrument [95, 96]. Many examples can be shown to the advantages of combining AuNPs within the sensor systems to enhance the selectivity and sensitivity [97-99].

2.3.1.3. Mechanism and principles of AuNPs

“Complexes between DNA and negatively charged AuNP have been studied for many years and many creative schemes have exploited AuNP covalently functionalized with DNA sequences to bind specific target DNA sequences” [100, 101]. It has been widely assumed that the negative phosphate backbone of DNA repels negatively charged AuNP [102]. A handfull of interaction can be used to attach biological elements to nanoparticles, the most cases elements are simply adsorbed directly to the nanoparticle’s surface or to the capping agents [103]. Citrate capped AuNPs are dispersed when in buffer solution.

This is resulting DNAs to repuls each other. By simply addition of ions via salt, DNA adsorption is boosted. Salt has 3 overall effects. 1- reduces the attraction between DNA and AuNPs, thus causing faster adsorption, 2- reduces the attraction between DNA strands, thus causing a higher loading of DNA, 3- reduces the attraction between the AuNPs, thus causing aggregation [104].

DNA nanobiosensor has bioreceptor is a singe-stranded oligonucleotide (ssDNA); probe that is immobilized. The analyte, a complementary target DNA, is recruited to the surface via base-pairing interactions with the probe. The DNA nanobiosensors are based on the immobilization of a single-stranded on a transducer surface that recognizes its complementary DNA sequence via the hybridization reaction [105, 106]. Furthermore, due to the weak immobilization, some of the oligonucleotides may detach during reaction [107].

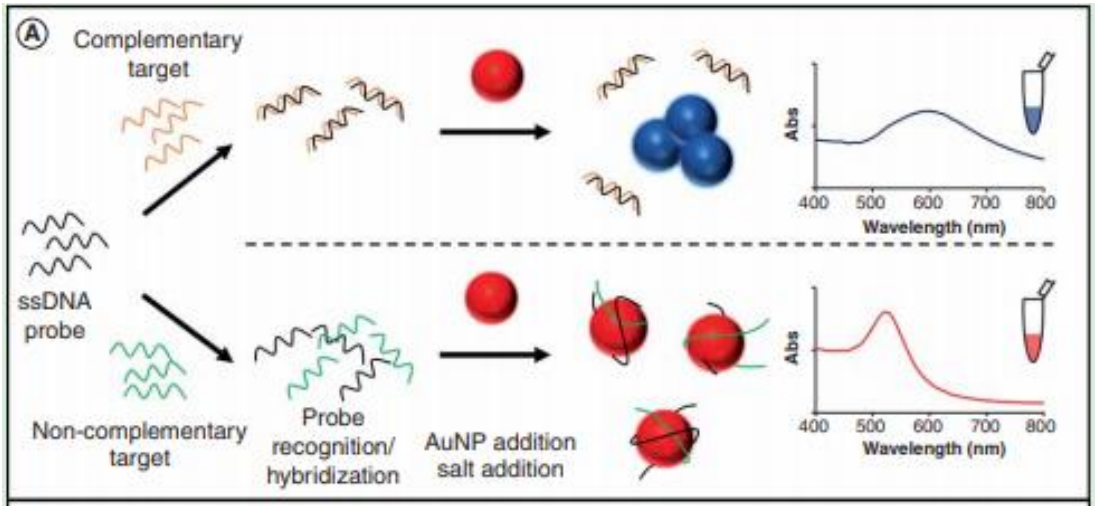


Figure 2.6. Mechanism of gold nanoparticle hybridization assay.

As can be seen in Figure 2.6. anneal of target sequence to the complementary sequence results in dsDNA, causing relation with AuNPs is lessen and further aggregation with inducer occurs (blue). When there are non-complementary sequences allow to remain single stranded DNA in mixture, causing absorption on the AuNP and stabilizes against aggregation (red) [108].

DNA can adsorb on AuNPs surface via nucleobase-gold interactions. Naturally, ssDNA adsorbs on AuNP surface stronger and faster than dsDNA. The surface of AuNPs is able

to differentiate the state of probe/oligonucleotide. The difference can be visualized after addition of ions to induce aggregation to AuNP (Figure 2.7) [109].

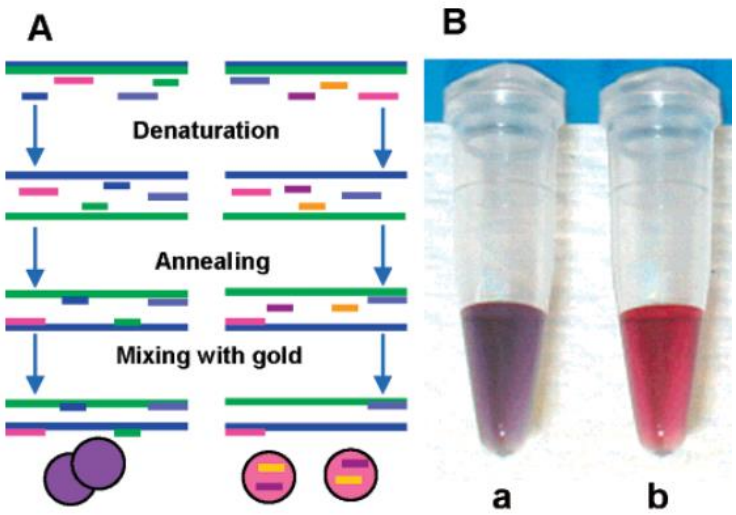


Figure 2.7. Principle of gDNA detection assay.

Li and Rothberg in 2004 (Figure 2.6) are very clearly explaining the principle of gDNA assay. (A) The mixture of DNA and probes is denatured and annealed with complementary oligonucleotide and added AuNP. The long lines represent the gDNA, and medium bars represent complementary oligonucleotide that bind, resulting in aggregation. The shorter bars are non-complementary oligonucleotide that do not bind and adsorb to the AuNP's surface, inhibit aggregation and causing mixture remain red. (B) a) complementary probe and (b) non-complementary probe [110].

Often PCR amplified target product used with this colorimetric sensing strategy. Another example from Zou group in 2018, developed target DNA sequence based AuNP's assemblies induced PCR product. "In the presence of target DNA, the two DNA-functionalized AuNP probes selectively hybridized, resulted in the aggregation of AuNPs" [111].

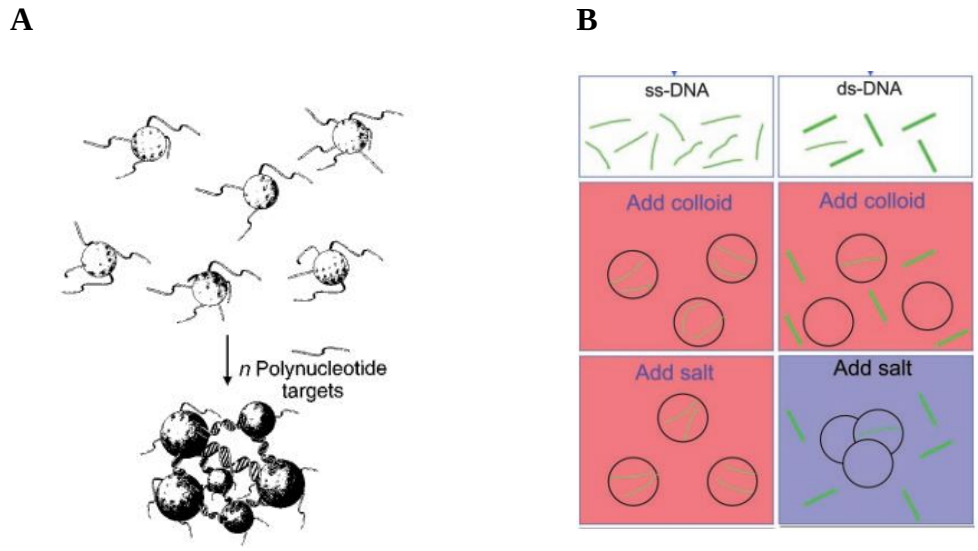


Figure 2.8. Principle of synthetic DNA detection assay.

(A) Colorimetric polynucleotide detection method based on oligonucleotide-modified AuNP probes. By hybridization through freeze and thaw process single stranded target oligonucleotide into a solution containing the appropriate probes resulted in the formation of a network of nanoparticles with a red to purple color change [112].

(B) Colorimetric method for separating single and double stranded probes.

2.4. Nanobiosensors

Biosensors are known as analytical tools that convert a biological event into a detectable signal [113, 114]. The combining of nano sized particles with biosensors makes them called nanobiosensor, a term introduced by Malik at al. [115].

Nanobiosensors are widely used in broad fields of technology such as tissue engineering, biomaterials, biomarker, water quality, contaminant detection in food, quality evaluation of foods; in agricultural applications such as nanofertilizers, nanopesticides, or as environmental remediation agents; medical technology, preventing, clinical testing, treating, monitoring of human diseases, drug delivery, diagnosing mostly cancer today still new nanobiosensors being developed [116-120].

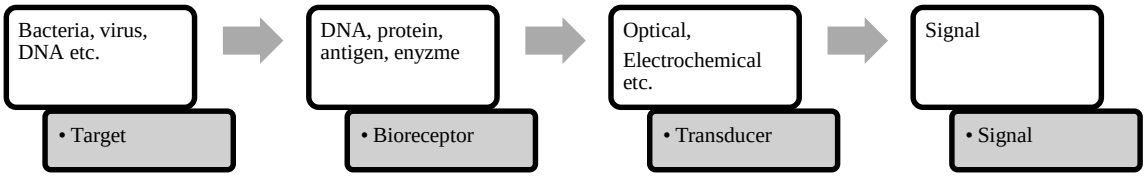


Figure 2.9. Elements that made uf nanobiosensor [121].

Nanobiosensors can be thought of as a fusion of biological function and nanofabrication technology. They can be classified by samples, such as food, biological elements, enzymes or aptamers. Transducer can be classified such as electrochemical, optical, colorimetric, mechanical, or magnetic, signal and amplification processing methods [122]. For instance, if any antigen or enzyme is monitored by biosensors, these devices are named antigen biosensors or enzyme biosensors [123]. Likewise, in the case of biosensors classification with respect to their sensing mechanism, the main types are electrochemical, and optical (Figure 2.9) [124]. AuNP-based biosensors can be categorized mainly into optical biosensors, electrochemical, and piezoelectric biosensors [125] [115].

In the case of electrochemical nanobiosensors, they analyze the reactions via electrical change. For that reason, AuNPs have the effectiveness of nanobiosensor [114].

Piezoelectric biosensors are working on a principle of affinity interaction, a sensor part working on the principle of oscillations change due to a mass bound on the piezoelectric crystal surface [126].

2.4.1. Applications of Gold Nanobiosensor for GMO detection

For more than 25 years, modern biotechnological approaches in food and agriculture shows an excellent approach to cover the global food security issue.

Some industrialized nations, such as the US and Canada, are making progress in the creation and exports of biotechnological goods, but other countries, such as Turkey, have had major timetable delays in recent biotechnology endeavors. Notably, regulation diversity of the countries is the main reason for complication in the international trade [127]. For all that, post market environmental monitoring has been still conducting for GMOs according to the monitoring plan as contained in GM notifications and regulations in Europe and Eurasian countries [128, 129]. Today, Quantitative-Real-Time PCR is seen

as the validated method for GMO detection, on the other hand, novel approaches using the biosensor devices are becoming a promising strategy day by day due to the easy monitoring and automated portable options. To establish a monitoring policy requires simple, quick and portable analytical approach for identification and quantification [130, 131]. The excite in DNA nanobiosensors for GMO testing has been rising becasuse to their feasibility simplicity, being able to portable, such as visual/optical or electrochemical apparatus. To do the cooccurring detection of multiple events, applications of DNA nanobiosensors have rised for high throughput GMO detection [132, 133].

Kalogianni et al., developed a nanobiosensor for visual testing of GMO sequences of 35S promoter and NOS terminator. Their work, the dipstick test allows oligonucleotide-conjugated AuNP's couple with the target DNA. Causing a characteristic color due to the pilling of the nanoparticles on test strip [132].

An assay with oligonucleotide modified AuNPs, provides an approach without the need of PCR equipment for quantitative detection of targets sequence. Zhu et al., reported a electrochemiluminescence emission measurements, based barcode DNA and hybridization using AuNPs by paramagnetic beads for GMO detection from raw materials [134].

In Plácido's work in 2018, magnetic nanoparticles were used to immobilizate Roundup Ready soya. In their work, covalent immobilization of probe onto the gold shell; magnetogenoassays exhibited low detection limits around nM [135].

Recently, SPR technique was improved with the addition of AuNPs. To detect GM tobacco, DNA isolated from different parts of the plant and hybridized with the biotinylated probe that was immobilized on a sensor. Functionalized AuNPs coated more biotinylated probe were let the detection methods, sensitivity improved. This SPR sensor can detect non-amplified gDNA at pM concentration [136].

Biospecific interaction method was worked using SPR and detect GM RR soybean event gene sequences. Biotinylated oligonucleotides containing soybean lectin and RR gene sequences immobilized on a sensor chip and suitable probes used to detect GM RR soybean gene efficiently [137].

For DNA sensing applications, a direct binding of thiol oligonucleotide probes to gold sensor surfaces has been utilized. SPR transduction relaying method and resulted in low limit of detection [138].

Further SPR device biosensor that has been applied to detect GMOs through sequence of 35S promoter and NOS terminator. The method has been optimized using oligonucleotides, later utilized to real food analysis such as soya flour. They used two different methods to obtain single stranded DNA from double stranded, thermal denaturation and magnetic particle separation. In conclusion the amplified DNA was detectable by the immobilized probe only when target sequence separated from its complementary strand using magnetic particle separation. When thermal denaturation was performed, no meaningful analytical signal was produced [139].

Change in LSPR signal of DNA probe-AuNP–modified cuvette attached to sensor chip which surface was functionalized with a capture DNA has been used for designing portable sensors. The analysis shows a SPR sensor chip coated with AuNPs that detects changes in refractive index. The target DNA from the GM plant is added into the cuvette, after hybridization, chip recognized target DNA with nM detection limit [140].

To find Cry1Ab gene, AuNPs used as signal amplification nano-probes for colorimetric and chemiluminescent detection. The results gives well correlativity when compared to commercial ELISA kit [131].

In 1997, colorimetric detection method based on modified AuNPs probes was published. Addition of a target oligonucleotide into a mixture with the specific probes. Followed with hybridization by freeze and thaw process of the mixture, allowed change of a range of imperfect targets [112].

In another study, electrochemical nanobiosensor detects NPT-II, in GMOs [141], an electrochemical DNA biosensor was fabricated by self-assembling probe ssDNA with a nano gold decorated on an electrode. When method has been optimized, voltametric response of electrochemical indicator methylene blue correlated with presence of target ssDNA gene sequence. Same responses seen with immobilizations differential pulse voltammetry and cyclic voltammetry and electrochemical impedance spectroscopy. A new label-free DNA electrochemical biosensor for detection of the PAT transgene in GM plants. AuNP was modified to the glassy carbon electrode and probe was attached by the

interaction of AuNP with DNA. The response grew following the immobilization of DNA probes hybridization with the complementary ssDNA [142].

Detection with SERS and separation by magnetism was achieved to detect the 35S gene carrying Bt-176 maize. The principle relies on, 35S DNA specific oligonucleotide probe attached onto gold coated magnetic nanospheres. Further target oligonucleotide hybridized with probes on the nanoparticles. The target concentration and the SERS response has the linear relationship [143].

CP4 protein widely present in the GM crops. A study in 2019 presented, an electrochemical immunosensor for CP4-EPSPS has been designed by combining a portable bioanalytical device with a screen-printed carbon electrode of GM crops. Consequently, nanoparticle improved immunosensor could detect CP4-EPSPS at very low quantity [144].

International standard for GMO detection has been achieved 10 times lower by an electrochemical biosensor. The quick detection succeeds with applying electroactive redox tags with the enzyme EXO III and AuNP based probe attachment. The electroactive redox tags were capable for immediate detection of different DNA components of GMO. Further adoption of enzymatic signal with AuNP based signal amplification has improved the sensitivity [145].

An electrochemical DNA nanobiosensor was developed for GMO event MON89788 soybean sequence specific. Detection with rolling circle amplification (RCA) and gold nanoparticle cube (AuNPC)-labeled multiple probes. The mass amount of DNA strands extended infinitely on AuNPC, meaning high detection signal. Results of the chronocoulometry signal gave a good linear the target specific gene sequence [146].

Lastly, a sandwich-type genosensor successfully detected the target CaMV35S in GM tomato. Using gold-silver core shell loaded iron oxide nanocomposite as label of signal DNA probe [147].

2.5. Artificial Intelligence & Machine Learning

Machine learning (ML) is a subdivision of Artificial Intelligence (AI), which develops learning algorithms to make foretell prediction among data and build a model. The

development of ML algorithms in biotechnology has been aided by recent advances in computing [148]

Machine learning tasks are typically classified into broad categories, supervised and semi-supervised, un-supervised and reinforcement learning. A dataset must be provided as inputs and outputs for supervised learning. The most prevalent sort of learning in biotechnology is supervised learning. Another categorization of machine learning tasks is when considering the desired outputs, in classification, the outputs are divided into classes. Then further the model provides assigns inputs for not yet seen. There are a many ML algorithms, examples for statistical learning are linear, logistic regression, k-nearest neighbor, random forest and support vector machine (SVM) (Figure 2.10) [149].

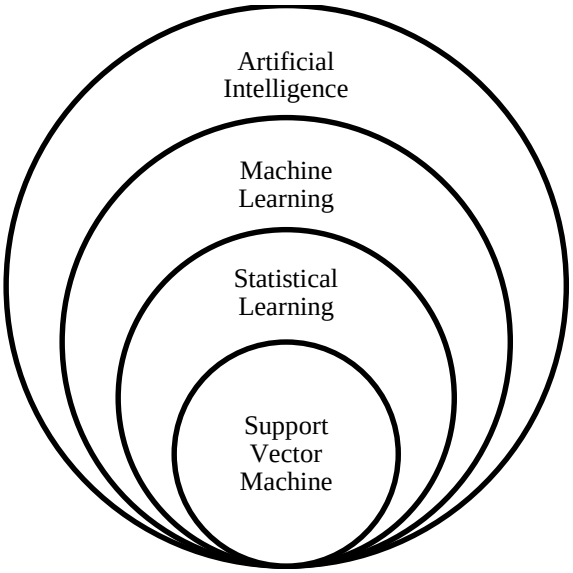


Figure 2.10. Machine learning is a type of artificial intelligence; support vector machine is a statistical learning approach.

At present, there is a growing number of solutions that provide AI and ML based systems. AI which is tested multiple times on health care and has been successful in some cases however clinical diagnostic often requires more context [150]. Although AI has many advantages, many challenges lie ahead. Because ML based approaches rely largely on huge volumes of high-quality training data, it's crucial to collect data them.

2.5.1. Support Vector Machine

A support vector machine (SVM) is a computer algorithm that learns by assign labels to data. SVMs have also been successfully adapted to a variety of applications and widely

used in classification data, i.e. microarray gene expression pattern recognition. The idea behind the SVM algorithm, one needs only to grasp four basic concepts: the separating hyperplane, the maximum-margin hyperplane, the margin (Figure 2.10) [151].

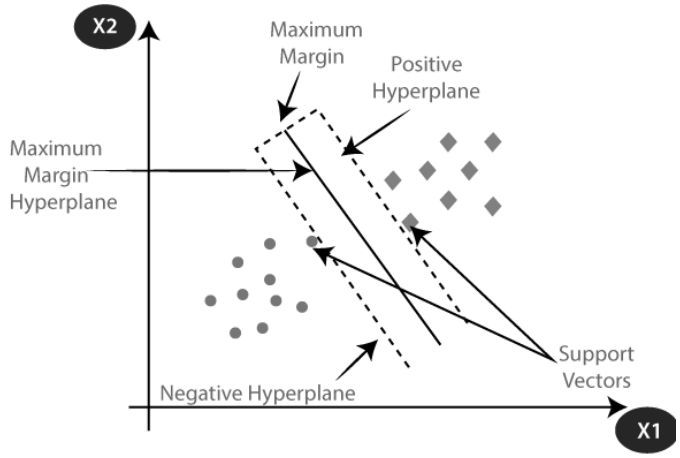


Figure 2.11. General classification hyperplane representation of SVM algorithm.

SVMs categorize data by locating a hyperplane (decision boundaries) that distinguishes between groups. To separate classes, there are many possible hyperplanes, SVM finds the best plane. The plane that has the maximum distance between data. "If there are just two input characteristics, the hyperplane is merely a line. The hyperplane becomes a two-dimensional plane when the number of input characteristics reaches three." The advantages of SVM are; effective in high dimensional spaces, uses support vectors, also memory efficient and versatile. SVM allow us to work with relatively smaller data set. Further SVM can also leads to rejection of cases with missing or false response values [152].

2.5.2. Artificial intelligence Based Biosensors

The integration of sensors and AI technologies benefits many areas. Biosensors are widely used to collect parameters, where they are used as a source of data that guides the decision-making process.

For the future, the detection and classification can be completely on-line for the food industry with fully automated biosensors that incorporate AI for processing and modeling [150]. There are plenty of examples of how data science and the prediction power of machine learning can be used to revolutionize [153]. Like, automated image analysis using machine learning technologies offering to replace laborious culture reading

(PhenoMATRIX and APAS Independence). Using machine learning to predict laboratory test results [154] or ML applied to diagnosis of human diseases [155]. For instance, Chen's 2020 paper aims to provide AI-assisted drying of fruits and vegetables to provide energy for the drying process [156]. Amperometric glucosemeter and lateral flow pregnancy tests are the most commercialized biosensors. Gold-labeled lateral-flow immunoassay for HIV testing has very high sensitivity and specificity; 100%/99.8% is achieved with commercially available AuNP-based points of care tests (Uni-Gold Recombigen™ HIV-1).

What's new, due to the pandemic of the COVID-19 around the planet, the need of quick, easy, inexpensive detection methods gains importance and nanotechnology in diagnostics, can provide monitoring [157].

2.6. Aim of The Study

Gold nanoparticles are well recognized tools for visual DNA detection. Most studies however, been restricted to synthetic sequences or PCR amplification. Hereafter we describe a colorimetric genomic DNA detection nanobiosensor that can detect unamplified genomic DNA sequences specifically, using bare AuNPs. The oligonucleotide/probe, in the presence of target MON88701 genomic DNA, cause the AuNPs to aggregate, giving a red to purple colorimetric change. Further we would like to highlight the implementation of this new emerging technology to GMO detection with support vector machine design.

To make a brief conclusion, the importance of rapid detection of GMOs is growing. It is believed that the GMO detection must be simple, rapid and in-field. Since DNA hybridization-based methods skip the time-consuming amplification step, these kinds of methods would also show their good performance together with ML. Thus, our gold nanobiosensor based detection, together with ML algorithm and a website can give qualitative information for the GMO level in the sample prior to routine PCR analysis.

3. MATERIALS AND METHOD

3.1. Materials

3.1.1. Certified Reference Materials

Certified reference material (CRM), GM and non-GM soybean, maize and cotton flours provided from American Oil Chemists' Society (AOCS) and Joint Research Centre (JRC), for genomic DNA-based detection with AuNP system. In this research, CRM containing varieties was used and their certificate of analysis reports and product descriptions are as follows.

Table 3.1. Certificate of analysis and product descriptions of AOCS 0809-A2, MON 87701 soybean [158].

"Certificate of Analysis AOCS 0809-A2, MON 87701 Soybean"			
Certified Presence	**Certified Value**	**Measurement Uncertainty**	**Test Method**
MON87701 Soybean present	≥ 984 g/kg	8 g/kg	event-specific realtime PCR
"This is the MON 87701 soybean CRM prepared by AOCS for Monsanto Company. It was produced in October 2019. The certified value is based on a sample purity of 99.2% with 95% confidence. This certificate is valid through: July 2022. AOCS 0809-A2 is arrived in 27mL glass headspace vials."			

Table 3.2. Certificate of analysis and product descriptions of AOCS 0906-A2, non-modified soybean [159].

"Certificate of Analysis **AOCS 0906-A2, non-modified soybean"**			
Certified Presence	**Certified Value**	**Measurement Uncertainty**	**Test Method**
MON 89788, MON 87701, MON 87705, MON 87708, MON 87751 and MON 87769 absent	< 0.8 g/kg	0	event-specific realtime PCR
"This is the non-modified soybean CRM prepared by AOCS for Monsanto Company. It was produced in April 2021. The certified value is based on a sample impurity of 0% with 95% confidence. This certificate is valid through: July 2022. AOCS 0906-A2 has been prepared by AOCS from non-modified soybean seed. AOCS 0906-A2 is arrived in 27mL glass headspace vials. Users are informed that this reference material has been produced from non-modified soybean seed."			

Table 3.3. Certificate of analysis and product descriptions of ERM-BF410ak Roundup Ready® Soya (blank) [160].

"Certificate of Analysis **ERM-BF410ak** **Roundup Ready® Soya (blank)"**			
Certified Presence	**Certified Value**	**Measurement Uncertainty***	**Test Method**
GTS 40-3-2 soya absent	< 0.7 g/kg	-	event-specific realtime PCR
"This is the Roundup Ready® Soya (blank) CRM prepared by Monsanto Company. The powders arrived in 10mL amber glass vials. **The certified uncertainty is the expanded uncertainty (U), corresponding to a level of confidence of about 95%. This certificate is valid through: July 2022"			

Table 3.4. Certificate of analysis and product descriptions of AOCS 0804-D2, MON 15985 cotton [161].

"Certificate of Analysis **AOCS 0804-D2, MON 15985 cotton MON-15985-7"**			
Certified Presence	**Certified Value**	**Measurement Uncertainty**	**Test Method**
MON 15985 cotton present	≥ 996 g/kg	2 g/kg	event-specific real-time PCR
"This is the MON 15985 cotton CRM prepared by AOCS for Monsanto Company. It was produced in July 2020. The certified value is based on a sample purity of 100 with 95% confidence, the true value is ≥ 996 g/kg. AOCS 0804-D2 is available in 27mL glass headspace vials."			

These materials are then stored in the dark at +4°C before further use in DNA extraction step. Sample ID was given to all replicates during DNA isolation. Soybean blanks received the codes MON0-X, MON87701 samples received the codes MON100-X, Roundup Ready samples received the codes RR-X and cotton samples received the codes CT-X. X indicating sequence numbers they received in isolation order.

3.1.2. Equipments

The equipment used in the study are as follows.

1) Heated Block (Major Science MD-01N)
2) Microcentrifuge (Nüve NF 800)
3) Spectrophotometer (Nanodrop 2000c and SPECTROstar Nano)
4) Refrigerator -20°C and +4°C
5) Incubator (Nüve EN400)
6) Vortex (Biosan V1+)
7) Plate Centrifuge (MiuLab MINIP2500 Micro-Plate Centrifuge)
8) Thermal Cycler (Nanobiz CubeCycler®)
9) Transmission Electron Microscope FEI Teknai F30

3.1.3. Chemicals

The chemicals used for DNA extraction in our study are present in the "GeneMATRIX Food-Extract DNA Purification Kit". Buffer FE, Res FE, Lyse FE, PR, Sol FE, Wash FEX, Elution, DNA and Proteinase K (20 mg/ml). In our assay RNA presence was unwanted, what's why Rnaz A enzyme has been prepared.

The chemicals used for AuNP analysis in our study are; hydrochloric acid 65% (HCl) nitric acid 35% (HNO_3) (Sigma-Aldrich), Gold(III) chloride trihydrate ($HAuCl_4.3H_2O$) (Sigma-Aldrich), trisodium citrate tribasic dihydrate ($C_6H_5Na_3O_7.2H_2O$) (Sigma-Aldrich) as well as ultra pure water.)

3.1.4. Kits

GeneMATRIX Food-Extract DNA Purification Kit (EURx Molecular Biology Products).

3.1.5. Probes

Oligonucleotide probes for nucleic acid detection are generated using the EU approved reference methods for GMO Analysis with RealTime-PCR. (“PCR method for detection of soybean event MON87701”, event-specific, Cry1Ac). The sequences are 22 base pairs in length. The oligonucleotide probes are unique to the target, showing few or no complementarities with other organism genes [162, 163].

Table 3.5. Oligonucleotide probe sequences.

Oligonucleotide Name	**5’-3’**	**Base**	**MW**	**Tm (˚C)**	**OD (260nm)**
Target oligonucleotide	TTT AAA CTG AAG GCG GGA AAC G	22	6832 g/mol	58	35
Complementary oligonucleotide	C GTT TCC CGC CTT CAG TTT AAA	22	6636 g/mol	58	41

3.2. Method

3.2.1. Synthesis of Citrate Gold Nanoparticles (AuNPs)

Prior to sythesis all glassware used was cleaned in a bath of freshly prepared aqua regia solution. Aqua regia is a highly corrosive mixture of hydrochloric acid (HCl) and nitric acid (HNO_3) HCl/HNO_3, 3:1. Glasswares soaked in the solution, then rinsed thoroughly with pure water and oven dried for 24 hours before use.

Citrate AuNPs were synthesized via applying the citrate reduction approach according to procedures described by Turkevich [81]. In this approach, citrate serves as reducing agent and stabilizer (Figure 3.1).

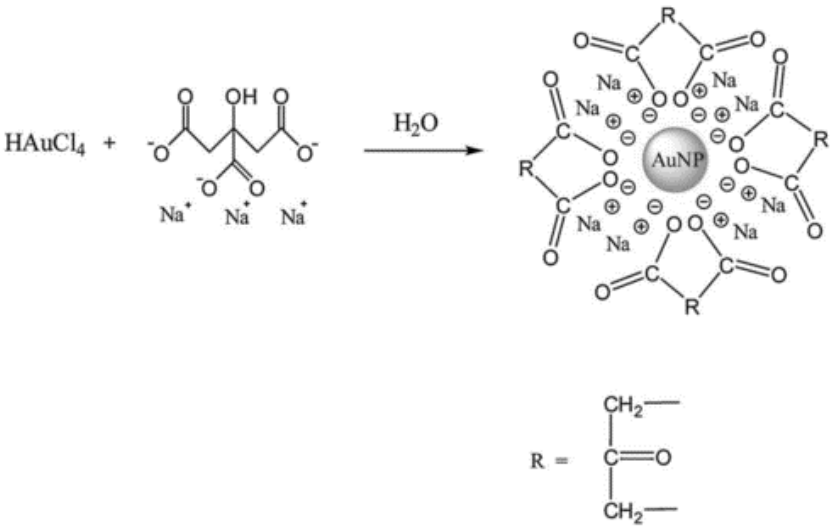


Figure 3.1. Citrate reduced AuNPs synthesis reaction [164].

The synthesis was repeated multiple times for this study, changing the parameters in order to achieve nanoparticles with morphology. AuNP batches named S1, Y5, Y6, Y7, Y8 indicating synthesis in different day. Synthesis of batch number S1 prepared at Day Lab (Michigan, USA) in 2019. Batch numbers Y5 and Y6 prepared at FoodOmics Lab (Ankara, TR) in April, 2021. Batch numbers Y7 and Y8 prepared at FoodOmics Lab (Ankara, TR) in October, 2021. Table 3.2. summarizes these features.

Table 3.6. AuNP batches prepared with different concentration and volumes.

Batch Number	**Gold(III) chloride trihydrate ($HAuCl_4.3H_2O$)**		**Trisodium citrate tribasic dihydrate ($C_6H_5Na_3O_7.2H_2O$)**	
	Concentration(mM)**	Volume (ml)	Concentration(mM)	Volume (ml)
S1*	1.11	90	38.80	10
Y5	1.11	45	38.76	5
Y6	1.0	50	38.80	5
Y7	1.25	40	38.80	4
Y8	1.0	100	38.80	10

* Synthesis Timeline, S1 is kindly provided by Prof Day and Dr. Saroopa, synthesis has been conducted in Day lab at Michigan State University, 2019.

**For accurate precision weighing, the Gold(III) chloride trihydrate was first prepared to be 5 mM stok. Then further diluted.

Citrate Gold Nanoparticles Synthesis Procedure

1. 50 mL of 0.01 M Gold(III) chloride trihydrate ($HAuCl_4.3H_2O$) was boiled at with vigorous magnetic stirring for until boils and temperature riches 100 ˚C.
2. 5 mL of trisodium citrate tribasic dihydrate ($C_6H_5Na_3O_7.2H_2O$) (dissolved in ultra pure water) was then added quickly.
3. Kept stirring and boiling for additional 10 min.
4. The color of the mixture changed from colorless to black, finally deep red, and a reduction completed.
5. Solution was removed from heat and then lowered to room temperature later stored in +4°C refrigerator, out of light.)

In the literature, it had been seen that 10:1 is always used as the volume ratio of Gold (III) chloride trihydrate/citrate tribasic dihydrate. We complied with this ratio as well. We prepared the citrate fresh each time and preheat before reaction. After quick addition of the citrate, we turned off the heating setting as soon as we saw the red color formation. Further cooling the, now gold nanoparticle solution stored in +4°C refrigerator, in dark before proceeding to characterization [81].

3.2.1.1. UV-Vis Spectral Analysis

The UV-Vis absorption spectra were recorded on SPECTROstar Nano spectrophotometer. All the data were acquired using 96 well sterile plates. Measurement type: Absorbance spectrum No. of flashes per well is 22. Wavelength range is 400-700 nm. Wavelength step width is 10 nm. Settling time is 0,2 s. Absorbance values are displayed as OD.

The absorption at 520 nm, 620 was picked to monitor the DNA and AuNP chelation/binding (bonding of ions/chemical to a substrate). UV-Vis studies presents adsorption at 520 nm decreased while 600-700 nm increased. This blue shift means of aggregation. The absorbance intensity at 520 nm changed as the GMO level changed from 0 to 100%. These alterations finally resulted in a simple foundation for visualization and colorimetric sensing. As a result, a noticeable colour shift from red to purple was noted.

3.2.1.2. TEM Analysis

Transmission electron microscopy (TEM) FEI Teknai F30, used to determine the size and distribution. For this purpose, one drop of (3 µl) the AuNP solution was dropped on a 400 mesh copper grid and allowed to dry under infrared lamp (Beurer IL 21). TEM pictures were taken filament at an accelerating voltage of 300 kV and 1000-75000 X magnifications. Particle size determination were performed using ImageJ Software. In images scale is presented. For each image new scale has been set to determine diameters and exported into Excel, where histograms and plots are prepared [165].

3.2.2. DNA Extraction

Scope of the DNA isolation is to extract genomic DNA from raw or processed food/plant, and further be used as the target DNA from GMO via GeneMATRIX Food-Extract DNA Purification Kit [166]. CRM soybean flours that are blank has marked as MON0 and soybean flours that contains 98.4% GM marked as MON100.

The present method starts with grinded seed and remaining structures are solubilized by lysis. Further, Proteinase K enzyme breaks proteins, RNAz applied to obtain RNA-free DNA. Solution was added to precipitate enzyme inhibitors. Later DNA binds to spin-columns and then eluted in buffer.

DNA Extraction Kit Procedure

The kit procedure steps were as follows;

1. “Add 30 µl of buffer to spin-filter to activate.
2. Homogenization of sample (even less than 100 mg of sample material in 2 ml Eppendorf tube). Addition of 750 µl Res buffer. Suspend the sample thoroughly.
3. Addition 60 µl Lyse buffer and 10 µl Proteinase K and 10 µl of RNase A.
4. Mix by fold every 2 minute, inverting the tube and incubate the mixture for 45 min at 65°C
5. Centrifuge the lysate in a microcentrifuge for 7 min at maximum speed (18.000 x g).
6. Transfer 400 µl of the supernatant to a new 2 ml microcentrifuge tube.
7. Add 400 µl PR buffer. Vortex for 15 secs and incubate on ice for 7 min.

8. Centrifuge for 3 min at maximum speed.

9. Transfer 600 µl of the supernatant to a new tube.

10. Add 600 µl Sol buffer.

11. Add 600 µl of 96% ethanol and mix several times inverting the tube.

12. Centrifuge briefly.

13. Transfer 600 µl of the supernatant to the spin-column placed in the collection tube.

14. Centrifuge for 3 min at 12 000 x g.

15. Take out spin-column, discard flow-through and place back spin-column in the collection tube.

16. Repeat 13–15 steps.

17. Transfer the remaining supernatant to the spin-column placed in the collection tube. Centrifuge for 3 min at 18 000 x g to filtrate the remains of the lysate through the resin.

18. Take out spin-column, discard flow-through.

19. Add 500 µl Wash buffer to the spin-column and centrifuge for 3 min at 12 000 x g. Take out spin-column, discard flow-through and place back spin-column in the collection tube.

20. Add 500 µl Wash buffer to the spin-column and centrifuge for 3 min at 12 000 x g. Remove spin-column, pour off supernatant, replace spin-column.

21. Spin down at 12 000 x g for 3 min to remove traces of buffer.

22. Place the spin-column in a new collection tube and add 100 µl of Elution buffer that is heated to 70°C to elute bound DNA.

23. Incubate the spin-column/collection tube for 10 min at 25°C.

24. Centrifuge the spin-column for 3 min at 12 000 x g.

25. Cap the collection tube. Store DNA" [166].

3.2.2.1. DNA Characterization

The DNA extract characterization process provided, DNA quantity and quality informations, that was essential for further steps. By measuring the absorbance at wavelengths 260 and 280 nm and further calculation of Abs (260/280) ratio provides measurement of protein contamination in a DNA sample. While nucleic acids, including DNA have a strong absorbance at 260 nm. The absorbance at 230 nm was an indication

of possible polysaccharide or other contaminants. NanoDrop have enabled this rapid quantifications of DNAs (Thermo Fisher Scientific) [167].

3.2.3. Oligonucleotide Probe Assay/Oligonucleotide Detection with Nanobiosensor

Firstly, nanobiosensors feasibility is studied with synthetic DNA. Three different but critical parameters were studied that influences the color change and absorbance, salt, oligonucleotide/probe concentration and reaction completion time. PCR strips were used as a reaction wells.

Order of addition:

1. Preparation of GMO level mix (target and complementary oligonucleotide) in PCR reaction wells.

2. Gold Nanoparticles

3. NaCl in PBS

Table 3.7. AuNP-probe assay's parameters

Oligo Name (10µl each)	**0% non-GM**	**25% MON87701**	**50% MON87701**	**75% MON87701**	**100% MON87701**
Target	-	12,5 µM	25 µM	37,5 µM	50 µM
Complementary	50 µM	50 µM	50 µM	50 µM	50 µM

To obtain different GMO levels synthetically, different oligonucleotide concentrations were used. In example, solution containing the half-complementary oligonucleotide was classified as 50% GMO. The complementary oligonucleotide and target oligonucleotide mixture was heated to 51°C for ten minutes for annealing, then 30°C for one minute to cool, according to the concentration and volumes in Table 3.7. After annealing, AuNP were added to the mixture and followed by pipetting them to the plate and addition of salt to induce color change. Characterized by UV-Vis spectrophotometer, absorbance values to demonstrate the shift of the solution was noted immediately (in 1 minute), in five minutes and in ten minutes. Wells were also photographed.

Salt Concentration

The ionic strength is a parameter that we can optimize and manage the electrostatic interactions between AuNPs and probe. If the ionic force was too low, the AuNPs present no aggregation, if much, the opposite effect [168]. As a result, concentration of NaCl was a key element tough it was first to optimized.

Oligonucleotide/Probe Concentrations

Having a good relation between probe surface coverage, particle stability, and hybridization efficiency was critical in order to obtain best result for oligonucleotide assay. The concentration be required to high enough to stabilize the AuNPs yet low enough that target sequences to be hybridized.

3.2.4. Genomic DNA Probe Assays/GMO Detection with Nanobiosensor

After synthetic DNA studies, studies were started with gDNA isolated from real life sample CRM. Four critical parameters were studied that influences the color change and absorbance, probe and salt concentration, gDNA purity and reaction completion time. PCR strips were used as a reaction wells.

Genomic DNA purity

Our approach relied on the nanobiosensors ability to detect dsDNA-probe hybridization. Any potential disturbance that is presence in the gDNA, contamination and such will stabilize the AuNP in solution giving the undesired false negatives.

Probe Concentrations

Our main approach is to develop nanobiosensor for gDNA-probe assay and probe concentration plays an important role. Explanations for the changes observed, leads to a change in interparticle distance (probes are single stranded oligonucleotides and as assays principle, they hinder AuNP getting closer). Or in a way increased in the number of

oligonucleotide suppresses aggregation. To ensure the optimize aggregation according to GMO levels, the concentration of probes was needed to be optimized.

Salt Concentration

Increasing ionic concentration could block out the electrostatic forces between the AuNPs and initiate aggregation. We conducted the assay over a range of salt concentrations in order to determine its robustness. This was to make sure that non-target samples show little to no colour change when NaCl added. Thus, we carry out the different concentrated and sized AuNP batches with varying concentrations of NaCl in PBS [169].

Reaction Time

It has been observed that absorbance values continue to change even after 10 minutes. As demonstrated in this work we have to performed detection and complete the absorbance read the in 10 min. Which was crucial for to be in line with subsequent trials.

According to EURL GMFF report "definition of minimum performance requirements for analytical methods of GMO testing"; this part refers to nanobiosensing system based approaches for the detection of GMOs, together with DNA extraction. This part was addressed to any undertaking method development and optimization. Outcomes were given the results and conclusion parts under the following, "minimum performance requirements for GMO detection with nanobiosensing system" section. Method acceptance criteria were; DNA quality and purity, practicability, selectivity, limit of detection, robustness and precision - relative repeatability standard deviation (RSDr) The calculations for the limits of detection (LOD) were based on the standard deviation of y-intercepts of the regression lines (σ) and the slope (S) [170].

$$LOD = 3.3\ \sigma/S$$

3.2.5. Support Vector Machine Algorithm

Supervised algorithms used 2 data sets: a training set, to learn about the classes; and a test set, to determine the accuracy of the algorithm and give prediction for the data analysis.

In the training set we had x number of classes. The aim of the SVM were to build an x-dimensional hyperplane that gives the maximum separation. After we build the SVM, data were classified as with respect to the hyperplane [171]. To understand the SVM, we determine kernel, C and gamma [172].

Data split; in the circumstance of ML, the split of our absorbance spectrum value regarding different GMO levels dataset was the first steps that we undertook. The datasplit and having different AuNP batches for training and testing, aided us evaluate model performance. We utilized the training set to train our model, and then used the test set as a collection of data points to assess if the model can adapt well, to unknown data.

Unknown sample read; The specificity of the detection method verified experimentally. Gene-specific nanobiosensor exclusively detects the targeted GM event Cry1Ac, meaning SVM algorithm also need to be tested with unknown samples. Further analyzing the algorithm's prediction percentage. The unknown sample prediction of accuracy is then compared to their actual GM levels. As unknown sample read; A non-complementary sequence corresponding to a specific sequence of the Roundup Ready blank. For complementary sequence, MON15895 99.6% GM Cotton events were chosen.

Regularization (C); The C value shows penalty for a wrong classification.

Gamma; it described the impact of train dataset, for low gamma, datas that are far from the hyperplane will also be taken into regard [172].

Kernel; several kernels exist in SVM including Gaussian, polynomial, linear, hyperbolic tangent, laplacian and radial base function (RBF) kernels [173].

Kernel Trick; can be used to look at classification from another angle to improve classification by addition of new dimensions. SVM kernel trick allows to deal with non-linearly separable datasets. More conceptually, the kernel trick illustrated our case in different ways and showed how machine learning algorithm "saw" different data representation.

The aims of the present research;

- use SVM, to model the GMO levels from absorbance spectrum data;
- to show the SVM performance of prediction.

3.2.6. Website Development

We have designed and established a website that will facilitate the use of the GMO nanobiosensor in the field and further showcase our data. By having a website that looks good and clearly delivers information to your users.

Our goal for the website is to explain what GMO detection with a nanobiosensor system is and how this website works. Also, demonstraid, how to upload your data and how to read the results? Our target audience was, customs in Turkey and all countries where GMOs are regulated. It was aimed to use this website as a decision mechanism prior to PCR.

Server was responsible for sending, processing, and receiving data requests. They are the intermediary between the database and the browser. The server was obtained from Hacettepe University Computer Center, in order to run the SVM algorithm and serve the website. As an operating system, Ubuntu 20.04.4 LTS installed on the server. Python (v3.6.15) programming language and Django (v2.1.5) were the Web Framework that were used to develop the website. For the database, we have used SQLite (v3.36.0). Moreover, Gunicorn and nginx was installed for deployment of the website.

Bootstrap (v5.1.3) has been used for design and customize responsive website with JavaScript. Steps were including, create an HTML page, load Bootstrap from the local storage, include jQuery, load Bootstrap JavaScript, put it all together. Further we have designed our page, added navigation bar, include custom CSS, add an overlay, include a page title and body text, images, created a call-to-action button. Finally got our website live. The website has been designed in Turkish and English languages for the convenience of users.

4. RESULTS AND DISCUSSION

In this study, we demonstrated that detection of sequences using AuNPs is possible with unamplified target genomic DNA with complementary probes resulted in change the interactions with AuNPs, which aggregates after ionic solution addition by colorimetric swich of red to blue in AuNP solution with presence of Cry1Ac target gene.

Overall, citrate stabilized AuNPs synthesized by reduction of gold ions by reducing agents citrate. Further MON87701 soybean CRMs (non-modified and ≥98.4% GM) were used for the experiments since they contain Cry1Ac gene sequence. Roundup Ready® Soya (blank), MON 15985 cotton (≥ 99.6% GM) was also tested for selectivity. Genomic DNAs were isolated from these samples.

Firstly, oligonucleotide/probe assay, later gDNA/GMO detection with nanobiosensor assay was optimized. Using the optimized conditions, sensitivity and selectivity tests were completed. A color change and aggregation indicating different GMO levels were expected. The selectivity tests for non-target sample was expected to be remain its red color and show no aggregation, though for target sample was expected to aggregate and show deep-red and purplish color.

SVM is a ML algorithm based on statistical learning, mainly used for data classification analysis. Taking the raw absorbance spectrum data OD's as an example, the SVM was used to train and predict the label data of GM levels, and the relative error of predicted vs. actual sample value was analyzed to verify the SVM data prediction.

Lastly we have designed and established a website that will facilitate the use of the GMO nanobiosensor in the field and further showcase our data. Results data headers can be followed in Figure 4.1.

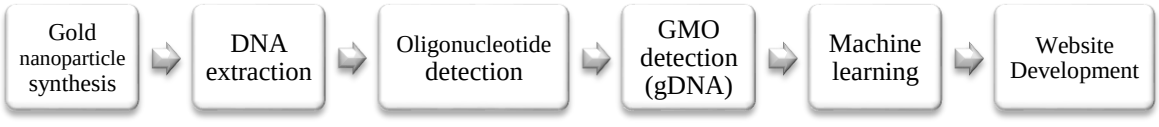


Figure 4.1. Flow chart of result data heaters.

4.1. Synthesis of Citrate Gold Nanoparticles (AuNPs)

The most popular protocol for the synthesis of AuNP based on the reduction of Au in solution by citrate, that has been developed by Turkevitch [81]. Later Frens suggested that, simply changing the ratio of gold to citrate, it was possible to get different diameter nanoparticle. Following in this study, gold solution's concentration and citrate solution's concentration varied in different batches Table 4.1 [82].

4.1.1. UV-Vis Spectral Analysis

AuNP solutions exhibited a characteristic UV−Vis spectrum due to the presence of a localized surface plasmon resonance (LSPR) in the visible proportion of the spectrum. The size of AuNP and the molar concentration may be derived directly from the UV-Vis spectra, according to the literature. The concentration of each batch was obtained by measuring based on the calculated molar absorptivity with the UV–vis spectrophotometer. The value of the maximum band of SPR absorption was connected to the concentration of the AuNPs via Lambert–Beer law [174].

$$A = \varepsilon * c * b$$

Where, absorbance equals to the solutions, molar concentration (c), molar absorptivity (ε), and the path length (b).

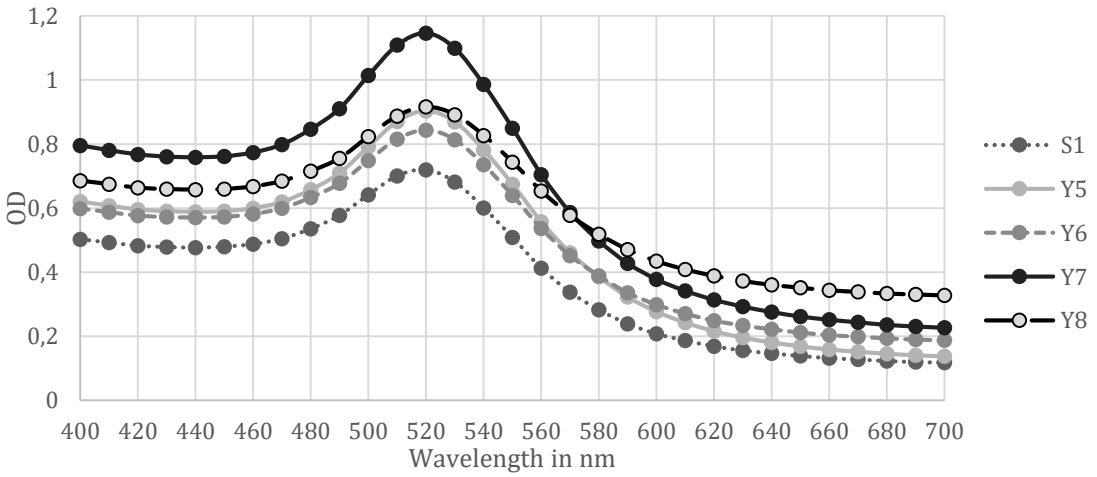


Figure 4.2. Synthesized UV-Vis spectrum of AuNPs maximum absorbance at 522nm.

It was observed that the Y7 batch, which gave the highest OD value at 520 nm, was actually due to fact that it contained more $HAuCl_4.3H_2O$ from the synthesis stage than all the batches. Although Y6 and Y8 contained synthesis material at the same concentration, the reason for showing different maximum OD values and bandwidth can be shown as the prolongation of the reaction time with the increase in volume. Y5 and Y8 showed the same maximum OD value, while Y8 has larger in bandwidth. This will later be seen by TEM analysis, that Y5 batch with narrower peak resulted in smaller particle. In addition, Y5 batch had more concentrated $HAuCl_4.3H_2O$ solution.

Table 4.1. Concentration of all AuNP batches prepared with different concentration and volumes.

Batch Number	**($HAuCl_4.3H_2O$)**		**($C_6H_5Na_3O_7.2H_2O$)**		**Concentration of AuNP solution (nM)**	**OD (520nm)**
	Concentration (mM)	**Volume (ml)**	**Concentration (mM)**	**Volume (ml)**		
S1	1.11	90	38.80	10	3.75	0,72
Y5	1.11	45	38.76	5	4.70	0,904
Y6	1.0	50	38.80	5	4,40	0,844
Y7	1.25	40	38.80	4	5,96	1,146
Y8	1.0	100	38.80	10	4,77	0,916

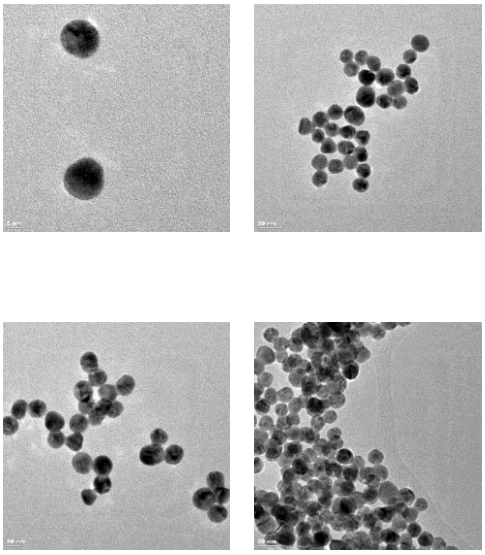
Using UV-Vis spectrum results and Beer Lambert's law together, Table 4.1. summarizes concentration data of the calculated AuNP batches are listed. The increase in the maximum peak in OD 520 nm indicates the difference in concentration despite the same volume of solution in the spectrum cells. The OD of each nanoparticle batch was determined and spectrum was given in Figure 4.2. All batches were added to the microplate in the same volume and reads were performed simultaneously. The results were graphed as the average of two parallel readings. The maximum absorbance values in the table differ due to the synthesis at different days and the differences indicated in Table 3.6. under methods section.

4.1.2. TEM Analysis

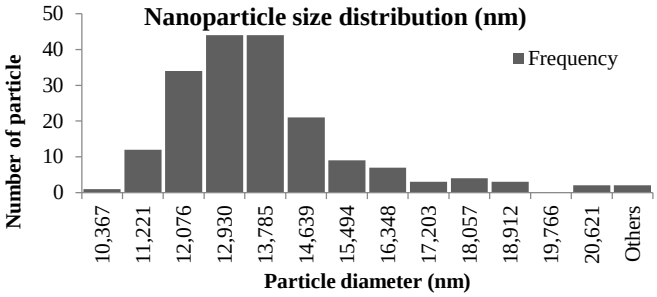
Transmission electron microscopy (TEM) is a very popular method used to determine the size distribution of the sample. TEM analyses of samples were performed at the Bilkent University UNAM.

Typically, TEM pictures of each sample were taken at various different magnifications in order to obtain information about the sample in general far view, also as close as possible visualization of the particles. When the representative group of pictures was collected, the next step was to count as many particles as possible, thus satisfactory data to be seen. AuNP in the desired morphology (diameter, size and shape) was obtained with our procedure. Gold nanoparticle sample's images, size distribution graphs and characterization result tables are given in Figure 4.3-4.7.

A



B



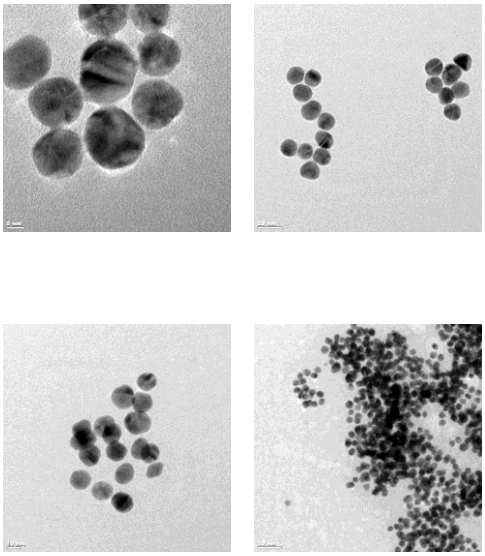
C

Batch Number	Gold(III) chloride trihydrate concentration (mM)	Trisodium citrate tribasic concentration (mM)	AuNP solution diameter (nm)	AuNP solution concentration (nM)
S1	1.11	38.80	~ 13.8	3.75

Figure 4.3. **(A)** TEM images of S1 with increasing magnification. **(B)** Nanoparticle size distribution bars of S1. **(C)** Characterization results obtained from batch S1.

The S1 batch, was for the most part, containing particles with a diameter of 12.9-13.8 nm, also 3.75 nM concentration and reduction endup with uniform, homogeneous, wineish-red colored AuNP solution.

A



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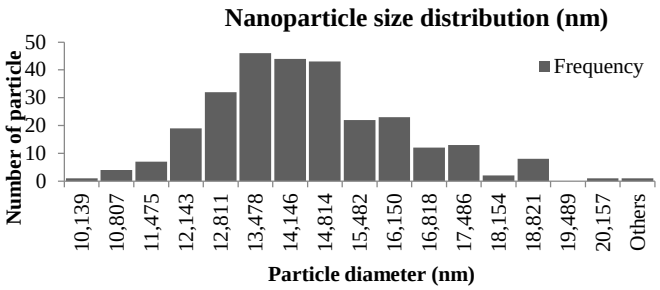


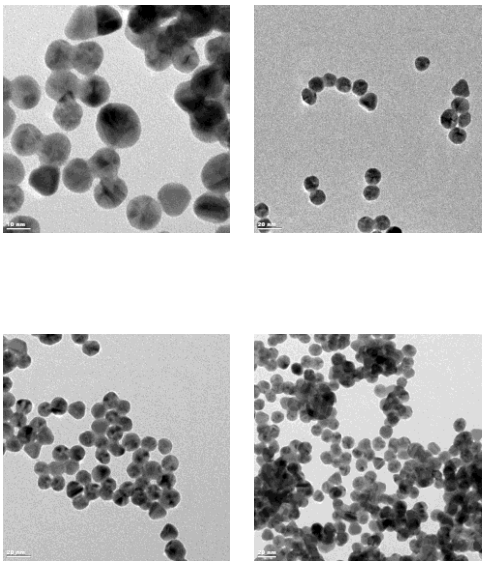
C

Batch Number	Gold(III) chloride trihydrate concentration (mM)	Trisodium citrate tribasic concentration (mM)	AuNP solution diameter (nm)	AuNP solution concentration (nM)
Y5	1.11	38.76	~ 13.4	4.70

Figure 4.4. **(A)** TEM images of Y5 with increasing magnification. **(B)** Nanoparticle size distribution bars of Y5. **(C)** Characterization results obtained from batch Y5.

The Y5 batch, was for the most part, containing particles with a diameter of 13.47-14.81 nm, also 4.70 nM concentration and reduction endup with uniform, homogeneous, wineish-red colored AuNP solution.

A



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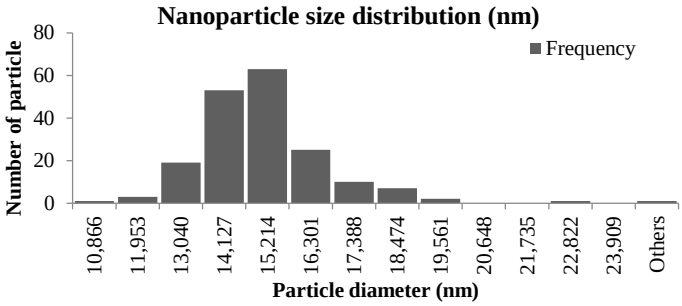


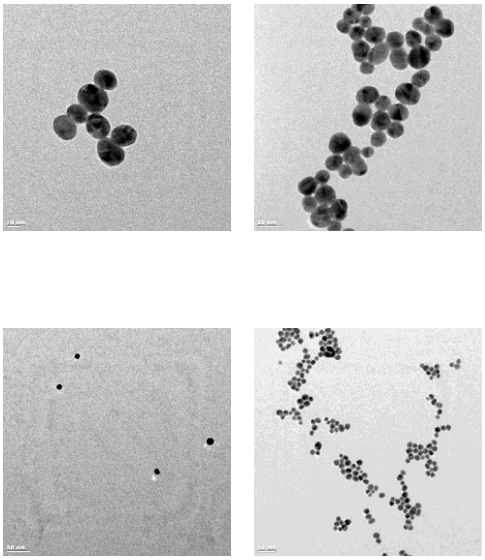
C

Batch Number	Gold(III) chloride trihydrate concentration (mM)	Trisodium citrate tribasic concentration (mM)	AuNP solution diameter (nm)	AuNP solution concentration (nM)
Y6	1.0	38.8	~ 15.2	4,40

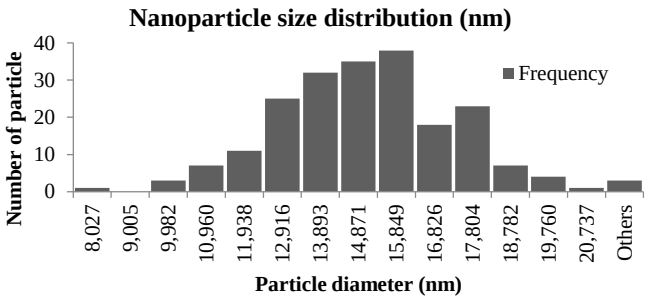
Figure 4.5. **(A)** TEM images of Y6 with increasing magnification. **(B)** Nanoparticle size distribution bars of Y6. **(C)** Characterization results obtained from batch Y6.

The Y6 batch, was for the most part, containing particles with a diameter of 14.12-15.21 nm, also 4.40 nM concentration and reduction endup with uniform, homogeneous, wineish-red colored AuNP solution.

A



B



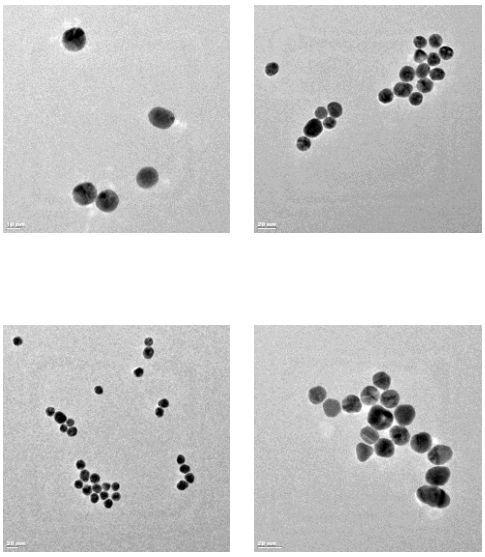
C

Batch Number	Gold(III) chloride trihydrate concentration (mM)	Trisodium citrate tribasic concentration (mM)	AuNP solution diameter (nm)	AuNP solution concentration (nM)
Y7	1.25	38.8	~ 15.8	5.96

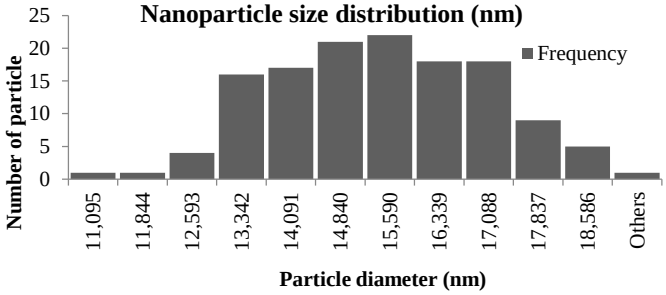
Figure 4.6. (A) TEM images of Y7 with increasing magnification. (B) Nanoparticle size distribution bars of Y7. (C) Characterization results obtained from batch Y7.

The Y7 batch, was for the most part, containing particles with a diameter of 14.87-15.84 nm, also 5.96 nM concentration and reduction endup with uniform, homogeneous, wineish-red colored AuNP solution.

A



B



C

Batch Number	Gold(III) chloride trihydrate concentration (mM)	Trisodium citrate tribasic concentration (mM)	AuNP solution diameter (nm)	AuNP solution concentration (nM)
Y8	1.0	38.8	~ 15.6	4.77

Figure 4.7. (A) TEM images of Y8 with increasing magnification. **(B)** Nanoparticle size distribution bars of Y8. **(C)** Characterization results obtained from batch Y8.

The Y8 batch, was for the most part, containing particles with a diameter of 14.80-15.60 nm, also 4.77 nM concentration and reduction endup with uniform, homogeneous, wineish-red colored AuNP solution.

The growth of gold nanoparticles reduction by citrate had been explored. It was found that AuNPs can be produced in 13-16 nm diameter range of sizes by following the work of Turkevich and Frens.

4.1.3. Gold Nanoparticle Optimizations

In order to develop optimum condition for gold nanoparticle based biosensor, investigation of the main particles limitations, behavior in different colloidal condition has been performed.

4.1.4. Robustness

The robustness is the methods ability to stay unaffected by minor but purposeful alterations from experimental circumstances. For a nanobiosensor, the following factors could be tested: salt concentration, sensitivity of the AuNP against different concentration of oligonucleotide probe, sensitivity of the AuNP against commercial soybean extracted genomic DNA.

Salt concentration

As shown in Figure. 4.8. when the concentration of salt was increased to 0.10 M, the color of the AuNPs solution did not change and its UV–vis absorption spectra remain same, AuNPs were. However, the color shifted to blue when salt is increased. Therefore, higher than 0.15 M sodium chloride was choosed to work with bare gold nanoparticle.

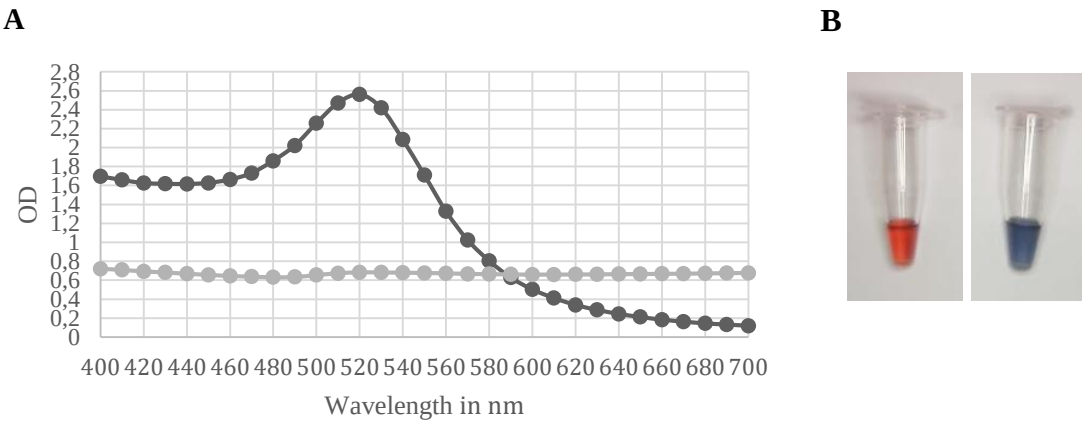


Figure 4.8. (A) UV-Vis spectra of disperse and aggregated **(B)** Visual readout of left, dispersed AuNP, right, NaCl induced heavily agglomerated AuNP.

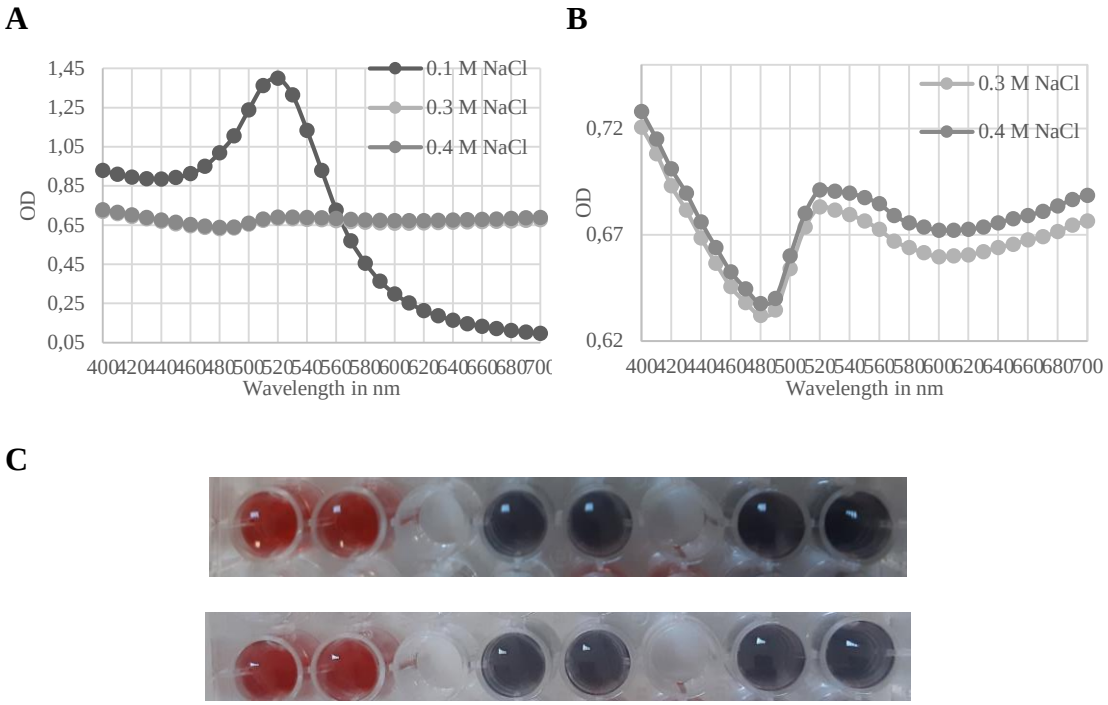
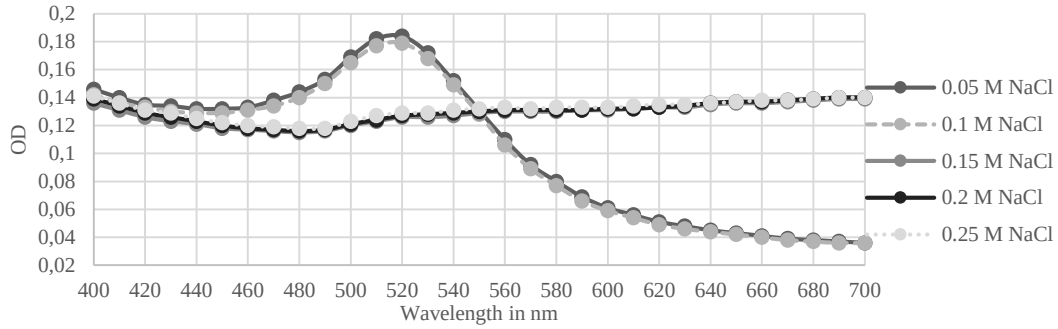


Figure 4.9. (A) Absorbance spectrum of AuNP with diluted S1 batch and different levels of salt. **(B)** Absorbance spectrum of AuNP with 0.3 and 0.4 M NaCl **(C)** Visual readout NaCl induced aggregation of 0.1, 0.3 and 0.4 M salt from left to right.

It is seen in Figure 4.9. salt optimization and AuNP experiments performed at low concentrations, diluted S1 batch, 0.1 M NaCl did not aggregate (A), while 0.3-0.4 M NaCl (B) changed the spectrum completely. That means at low AuNP concentrations since the effect of salt is more intense, the NaCl ratio in the solutions should be reduced. In the presence of excessive aggregation, two different salts concentrations, it is difficult to distinguish between them by naked eye. Hence difference can be followed with absorbance spectrum (Figure 4.9 and Table 4.2).

A



B

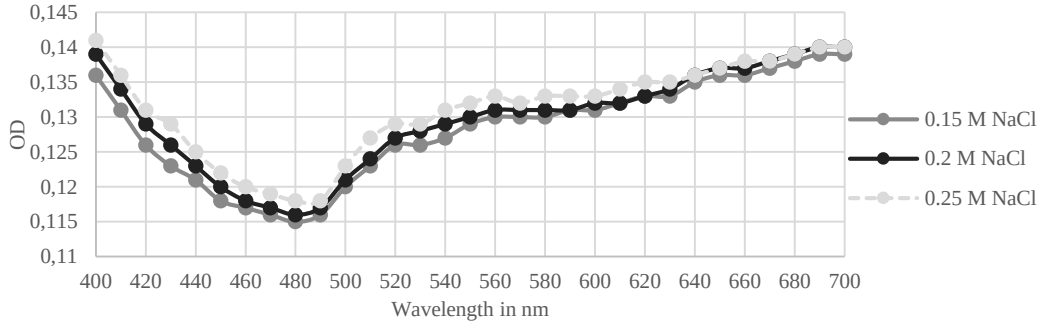


Figure 4.10. AuNP's (1.5 nM) behavior with addition of different salt concentration (0.05-025 M NaCl containing PBS).

It was observed that the AuNP concentrations decreased to as low as 1.5 nM, the salt concentration, which initiates the aggregated been as low as to 0.15 M. That means at low AuNP concentrations since the effect of salt is more intense, the NaCl ratio in the solutions should be reduced (Figure 4.10).

Sensitivity of the AuNP against different concentration of oligonucleotide probe

Sensitivity defines, protection of the AuNPs (1.5 nM) against pure oligonucleotide in micromolar, nanomolar and picomolar concentration.

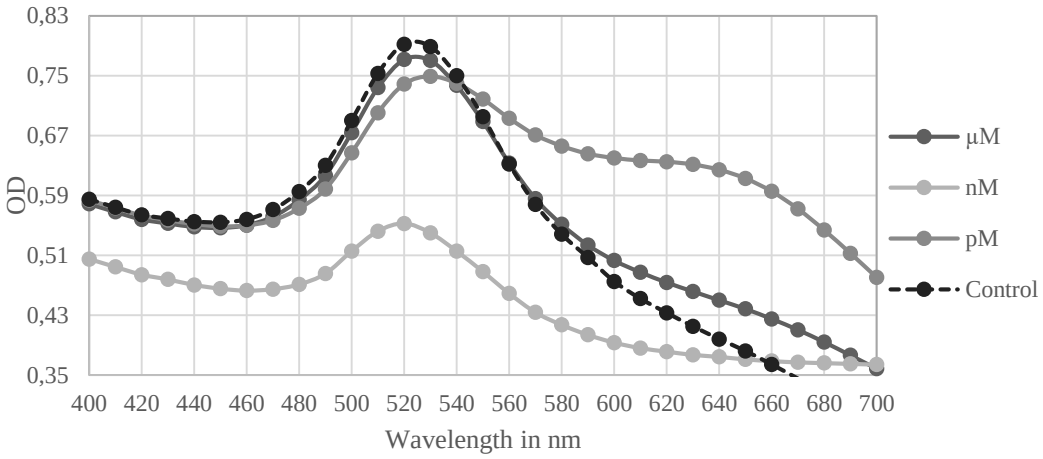


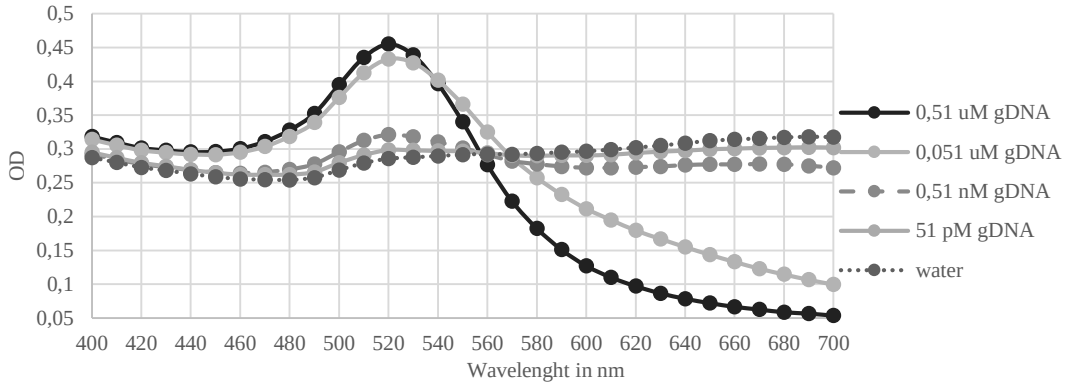
Figure 4.11. AuNP's behavior with different oligonucleotide's concentration.

As shown in Figure.4.11. we observed a heavy aggregation in picomolar concentration probe, on the contrary, when oligonucleotides are in micromolar concentration AuNP's are more protected against salt induced aggregation seen in 600-700 nm wavelength region. Due to pM being aggregated and µM being too protecting, the interaction of probe and AuNP at nanomolar concentration is choose to be optimum, in order to observe aggregation and allow color transformation at different probe levels compared to control.

Sensitivity of the AuNP against commercial soybean extracted genomic DNA

To define the limits of the AuNP assay for the detection of soybean, we investigated the protection of the AuNPs (1.5 nM) against gDNA from the commercial soybean ranging from 0,56 µM to 56 pM.

A



B

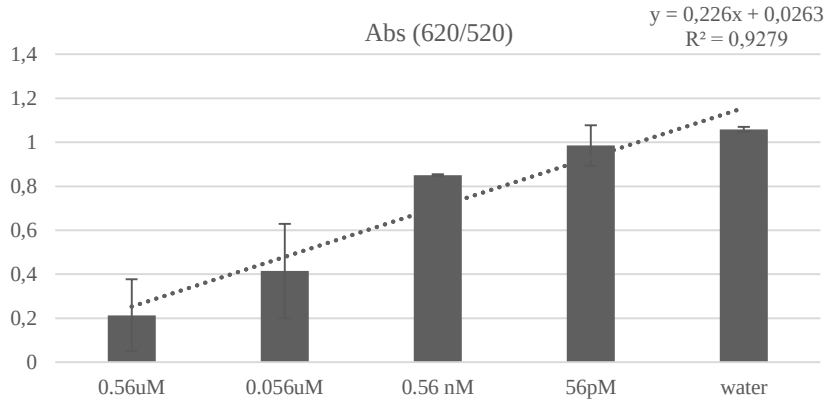


Figure 4.12. (A) Absorbance spectrum of AuNP with gDNA after salt addition **(B)** Abs (620/520) bar graph of sensitivity of the AuNP with commercial soybean extracted gDNA.

Bars represent ± standard error of the mean of 3 parallel. After the AuNP was mixed to the extracted gDNAs, salt addition is followed. Here, it has been seen how dsDNA actually protects against salt and prevents aggregation by entering between the AuNP in solution. It was observed, with the same amount of salt added, up to 0.56 nM gDNA level aggregation was prevented, dsDNAs have protection ability.

As shown in Figure. 4.12., we observed an apparent aggregation in AuNP from from 0,56 µM to 56 pM, as referred to control reactions (i.e., only water). As decrease in AuNP

aggregation seen as increase in DNA concentration backs up the hypothesis of AuNP-DNA interaction is a colorimetric change.

4.2. DNA Extraction

4.2.1. DNA Quality and Purity & Applicability

The DNA concentrations were found to be appropriate for the subsequent analyses. The yields of the DNA were done with reference to mass of DNA (ng/µL). The kit has been optimized by the company for DNA isolation from a variety of food samples/plant, the quality and purity of the genomic DNA obtained with the kit has been found to be suitable for nanobiosensor. Determined by Nanodrop 2000c. The results obtained are shown and discussed below Table (4.2-4.11).

Table 4.2. DNA concentration (ng/µL) of samples extracted 1st batch.

GMO Level	Sample ID	Nucleic Acid	A260	A280	260/280
AOCS 0906-A2, non-modified soybean < 0.08% GM soybean	MON01	35,7	0,715	0,39	1,83
	MON02	38,2	0,764	0,428	1,78
	MON03	53,7	1,073	0,576	1,86
	MON04	47,1	0,942	0,493	1,91
	MON05	41,6	0,833	0,445	1,87
	MON06	41,1	0,823	0,423	1,95

The average of the first batch was 42,90 ng/L, the standard deviation was 6,53 ng/L, and the coefficient of variation resulted in 15.22%.

Table 4.3. DNA concentration (ng/µL) of samples extracted 2nd batch.

GMO Level	Sample ID	Nucleic Acid	A260	A280	260/280
AOCS 0906-A2, non-modified soybean < 0.08%	MON07	60,3	1,207	0,734	1,64
	MON08	87,8	1,756	0,986	1,78
	MON09	66,5	1,33	0,651	2,04
	MON010	67,7	1,354	0,759	1,78
	MON011	71	1,419	0,746	1,9
	MON012	64,2	1,285	0,704	1,82
	MON013	65,5	1,309	0,704	1,86
	MON014	78,2	1,565	0,88	1,78

The second batch's average was 70,15 ng/L, the standard deviation was 8,15 ng/L, and the coefficient of variation was 12,64%.

Table 4.4. DNA concentration (ng/µL) of samples extracted 3rd batch.

GMO Level	Sample ID	Nucleic Acid	A260	A280	260/280
AOCS 0906-A2, non-modified soybean < 0.08%	MON015	120,3	2,406	1,1381	1,84
	MON016	112,1	2,243	1,231	1,82
	MON017	125,5	2,509	1,809	1,39
	MON018	107,1	2,143	1,116	1,92
	MON019	114,9	2,299	1,525	1,51
	MON020	146,2	2,924	1,71	1,81
	MON021	258	5,16	2,922	1,77
	MON022	476,2	9,524	5,068	1,88
	MON023	127,5	2,55	1,709	1,49

The third batch's average was 176,42 ng/L, the standard deviation was 121,60 ng/L, and the coefficient of variation was 68,93%.

Table 4.5. DNA concentration (ng/µL) of samples extracted 4th batch.

GMO Level	Sample ID	Nucleic Acid	A260	A280	260/280
AOCS 0809-A2 ≥ 98.4% GM MON87701 soybean	MON1001	30,6	0,611	0,326	1,87
	MON1002	28,4	0,569	298	1,91
	MON1003	46	ND	ND	1,72
	MON1004	20,9	0,417	0,221	1,89
	MON1005	30,1	0,601	0,323	1,86
	MON1006	22,7	0,454	0,249	1,82
	MON1007	43	0,86	0,412	2,09

The fourth batch's average was 29,78 ng/L, the standard deviation was 8,89 ng/µL, and the coefficient of variation was 29,83%.

Table 4.6. DNA concentration (ng/µL) of samples extracted 5th batch.

GMO Level	Sample ID	Nucleic Acid	A260	A280	260/280
AOCS 0809-A2 ≥ 98.4% GM MON87701 soybean	MON1008	56,5	1,131	0,637	1,78
	MON1009	51,1	ND	ND	1,72
	MON10010	51,8	1,036	0,585	1,77
	MON10011	58,5	1,17	0,599	1,95
	MON10012	80,5	1,61	0,993	1,82
	MON10013	62,6	1,252	0,651	1,92
	MON10014	48,4	0,976	0,498	1,96

The fifth batch's average was 58,49 ng/L, the standard deviation was 10,85 ng/µL, and the coefficient of variation was 18,55%.

Table 4.7. DNA concentration (ng/µL) of samples extracted 6th batch.

GMO Level	Sample ID	Nucleic Acid	A260	A280	260/280
AOCS 0809-A2 ≥ 98.4% GM MON87701 soybean	MON10015	120,3	2,406	1,1381	1,84
	MON10016	112,1	2,243	1,231	1,82
	MON10017	125,5	2,509	1,809	1,39
	MON10018	107,1	2,143	1,116	1,92
	MON10019	114,9	2,299	1,525	1,51
	MON10020	146,2	2,924	1,71	1,81
	MON10021	258	5,16	2,922	1,77
	MON10022	476,2	9,524	5,068	1,88
	MON10023	127,5	2,55	1,709	1,49

The sixth batch's average was 125,84 ng/L, the standard deviation was 41,12 ng/µL, and the coefficient of variation was 32,67%.

Table 4.8. DNA concentration (ng/µL) of samples extracted 7th batch.

GMO Level	Sample ID	Nucleic Acid	A260	A280	260/280
ERM-BF410ak <0.07% GM Roundup Ready® soya	RR1	71,3	ND	ND	1,77
	RR2	32,8	ND	ND	1,82
	RR3	49,2	0,984	0,595	1,82

The seventh batch's average was 51,10 ng/L, the standard deviation was 19,32 ng/µL, and the coefficient of variation was 37,81%.

Table 4.9. DNA concentration (ng/µL) of samples extracted 8th batch.

GMO Level	Sample ID	Nucleic Acid	A260	A280	260/280
AOCS 0804-D2 MON 15895 99.6% GM cotton	CT1	51,5	1,03	0,517	1,99
	CT2	40,5	0,809	0,414	1,95
	CT3	120,8	2,416	1,333	1,81

The eighth batch's average was 70,93 ng/L, the standard deviation was 43,53 ng/µL, and the coefficient of variation was 61,37%.

Table 4.10. DNA concentration (ng/µL) of commercial soybean samples extracted 9th batch

Sample	Nucleic Acid	A260 (Abs)	A280 (Abs)	260/280
HU-1	665,4	13,30	6,67	2,0
HU-2	638,8	12,77	6,37	2,01
HU-3	564,6	11,29	6,25	1,81
HU-4	693	13,859	7,22	1,92
HU-5	459	9,18	4,86	1,89
HU-6	553,8	11,07	5,56	1,99

The ninth batch's average was 595,76 ng/L, the standard deviation was 80,70 ng/µL, and the coefficient of variation was 14,55%.

The most used way to determine DNA yield and purity is measurement of absorbance. Absorbance readings were performed at 260nm where DNA absorbs light most strongly, and further concentration estimated. To evaluate DNA purity, absorbance measured at 280 nm to detect other possible contaminants (carry outs from plant, organic materials, extraction chemicals). The most common purity calculation is the ratio of the absorbance at 260nm divided by the reading at 280nm. Good-quality/pure DNA was achivied that has Abs (260/280) ratio of 1.8–2.0. When compared with the "Report on the Validation of a DNA Extraction Method for Soybean Seeds" report, it has been observed that our method concluded with comparable results in all DNA extraction batches.

It has been observed that DNA yield from commercial soybean was better than CRMs. It is known that this situation is related to the particle diameter and the grinding state of the grains, which affect the DNA extraction yield. Coefficients of variation results were similar to literature and JRC reports [170].

4.3. Oligonucleotide Probe Assay/Oligonucleotide Detection with Nanobiosensor

Oligonucleotide detection carried out with synthetic DNAs. The test run in three technical replicates. The data was then averaged and normalized in the bar graphs, by dividing the absorbance ratio by the minimum value, in order to reduce data redundancy and improve integrity.

Oligonucleotide detection carried out with synthetic DNAs. Sequences that specific to Cry1Ac gene region, given in the Table 3.5.

Salt concentration optimization 0.1 M NaCl in PBS

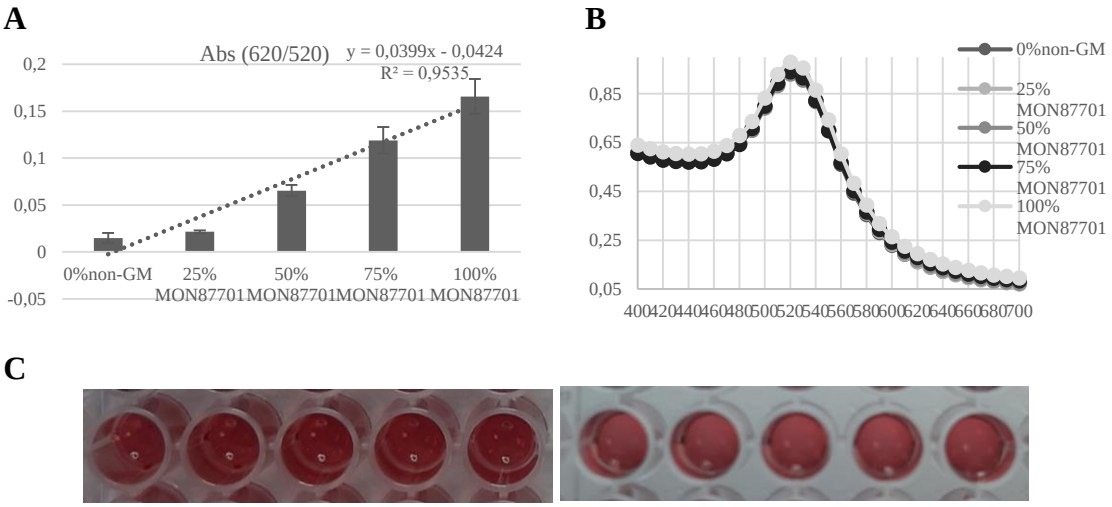


Figure 4.13. (A) Abs (620/520) bar graph for different levels of Cry1Ac synthetic DNAs **(B)** UV-Vis absorption spectroscopy of AuNPs with different levels of Cry1Ac synthetic DNAs **(C)** Visual readout.

It should be emphasized that although the color remains red, a change in absorbance is actually observed and also be followed with absorbance 620/520 nm. In the mixtures above (Figure 3.13.) salt concentration is 0.1 M and volume added was 20 μM, these numbers were sufficient to observe a significant change in absorbance, according to increasing Cry1Ac gene presence.

Salt concentration optimization;0.137 M NaCl in PBS

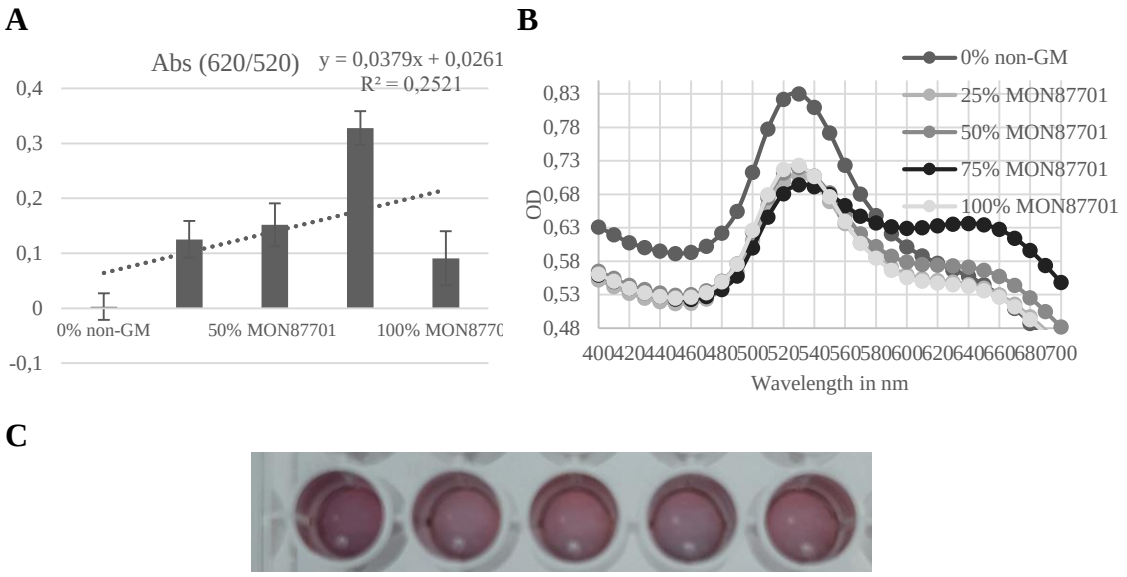


Figure 4.14. (A) Abs (620/520) bar graph for different levels of Cry1Ac synthetic DNAs **(B)** Absorbance spectrum of AuNPs with different levels of Cry1Ac synthetic DNAs **(C)** Visual readout increasing GMO levels from left to right.

As seen when excessive amount of salt added to mixture, in all MON87701 levels, color turns purple (a slight brighter color at level 0%). A change in absorbance spectrum could be captured and also be followed with absorbance 620/520 nm. In the mixtures above (Figure 4.14.) salt concentration is 0.137 M and volumes is 20 µM, these numbers are sufficient to observe a significant change in absorbance, according to increasing Cry1Ac gene presence.

The color of AuNP is very susceptible to level of accumulation happening suspension can be easily induced with electrolytes (NaCl). We choose two oligonucleotides/probes that has complementary sequences to each other. Mixture of these two target and complementary probe has been prepared for 50 µM each and dilutions of them are prepared with ratios of 25, 50, 75, 100% along with non-complementary so called 0% (Note that, 0% MON87701 has only has sequences that does not hybridizes together, and 100% MON87701 has the same volume and concentration of perfectly matching

sequences). Prior to addition of AuNP to mixture, probe mixture annealed at 51 °C temperature for 5 minute and cooled to 30 °C. At the same time, the unconsumed probes also remain in the solution. When gold nanoparticle was exposed to this mixture, later the salt added, solution occurred with immediate aggregation. When the no hybridization occurs at the 0% due to ssDNA affinity and adsorption to AuNP, aggregation prevented.

If a target sequence exists, they will form dsDNA covering the Cry1Ac gene, that are designed to be perfectly matched. dsDNA cannot prevent salt induced aggregation.

Moderate amounts of salt cause aggregation of the AuNP if 22 mers oligonucleotide probe and its exact complementary probe is added to the solution. In addition, also found that increasing temperature of hybridization also results in too much aggregation that does not allow for the gradual change with Cry1Ac level. Increasing salt concentration allow the electrostatic compel between the AuNPs and mediate aggregation. In all cases, there are strong Van der Waals attraction and dipolar interactions between the oligonucleotide/probe and the gold nanoparticle.

Although volume and concentration fluctuations were attempted, the expected red to purple color change could not be observed. This is because the oligonucleotide had maximum purity that does not allow a smooth transition in color. It does not have the impurities that come with gDNA, so either complete aggregation or stabilization was seen. The solution's absorbance spectra and colour are preserved in these conditions. The reason for the stabilization that is the oligonucleotides/probe causes negative charges to the AuNPs to further enhance their repulsion [110].

4.4. Genomic DNA Probe Assays/GMO Detection with Nanobiosensor

It has been also studied that genomic DNA could be detected sequence specifically by AuNPs [175, 176], and designed a series of test to find the best method. The gDNA left together with complementary probes that bind within the Cry1Ac gene sequence. After the heat threatment and addition of AuNP colloids, we were able to see visual colorimetric change from red to purple as well as with absorbance spectrum shift. The positives, targets sequences resulted darker red/purple and the negatives and non-target soybean genomic sequences remained red, as should be expected.

Different but critical parameters were studied that influences the color change and absorbance, salt concentration, gDNA purity, volume of the reagents and reaction completion time.

4.4.1. Precision - Relative Repeatability Standard Deviation (RSDr)

Repeatability, where "test results are obtained with the same method, on identical test items, in the same laboratory, by the same operator, using the same equipment". Repeatability is a measure of the ability of the method to generate similar results (or to give a range) for multiple replicates of the same sample. Repeatability carried out by Yeşim Taşkın, same analyst, same instrument and laboratory and varies in the AuNP batches that are synthesized in in different days and genomic DNA's that were isolated in different days but from same sample.

Table 4.11. AuNP gDNA-probe probe assay's parameters for Y6 and Y8

Analyte	**Volume (µl)**	**Concentration**
gDNA	10	30 ng/µl final concentration (2.5ng/µl)
Oligonucleotide probe	10	1pM final concentration (0.08pM)
AuNP	90	4,40 and 4,77 nM
PBS containing salt	10	0.15 M

Table 4.12. AuNP gDNA-probe probe assay's parameters for Y5

Analyte	**Volume (µl)**	**Concentration**
gDNA	10	30 ng/µl final concentration (2.5ng/µl)
Oligonucleotide probe	10	1pM final concentration (0.08pM)
AuNP	90	4,70 nM
PBS containing salt	10	0.05 M

Assay was carried out on five GMO levels for event MON87701 soybean (<0.08% non-modified soybean, 25%, 50%, 75% and ≥ 98.4% GM MON87701), 10 test replicates repeated within 2 days, resulting total of 50 data set for each AuNPs. Obtain absorbance spectrum from 400-700 nm wavelength for 1, 5 and 10-minute raw data has been given in APPENDIX 1.

Protocol;

The complementary oligonucleotide-probe specific to the MON87701 event and extracted gDNAs are diluted. The gDNA and probe mix was heat treated at 95°C for ten minutes, then immediately followed by incubation at 55°C for five minutes and 30°C for one minute to cool the mix, according to the concentration and volumes in Table 4.11-12 (only difference was NaCl's concentration, due to AuNP batch having smaller diameter, the aggregation accomplished with less salt)

After hybridization, AuNP were added to the mixture and followed by pipetting them to the plate and addition of salt to induce color change. Characterized by UV-Vis spectrophotometer, absorbance values to demonstrate the SPR position change of the solution was noted immediately (in 1 minute), in five minutes and in ten minutes. Wells were also photographed.

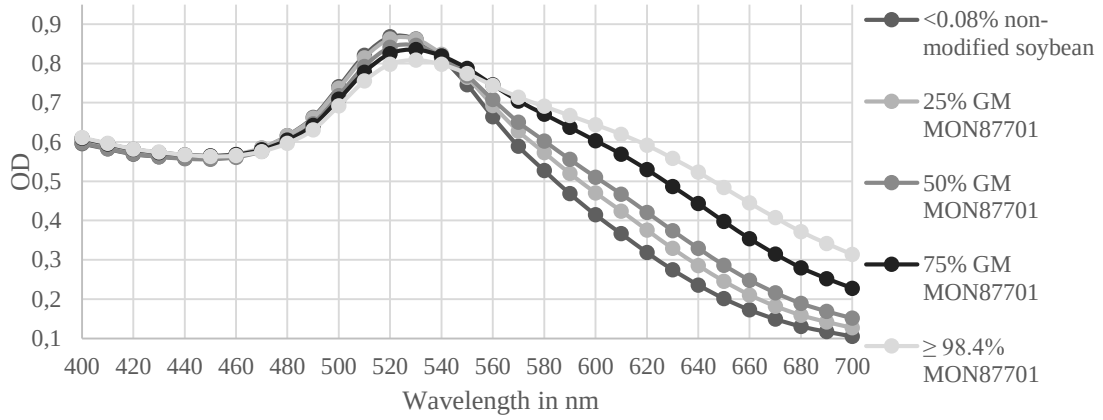
UV-Visible Spectral Analysis

Data normalized in the bar graphs, by dividing the absorbance ratio by the minimum value, in order to reduce data redundancy.

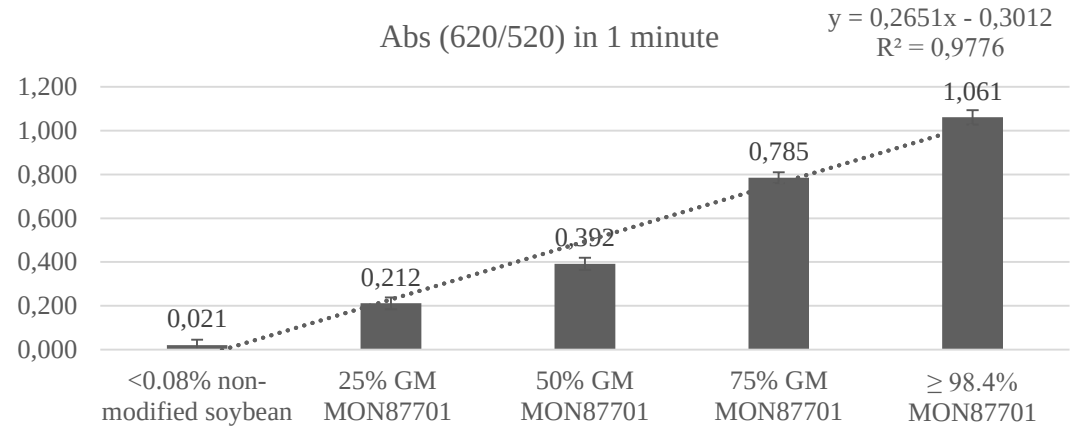
Batch Number Y5

Results of initial read in 1 minute

A



B



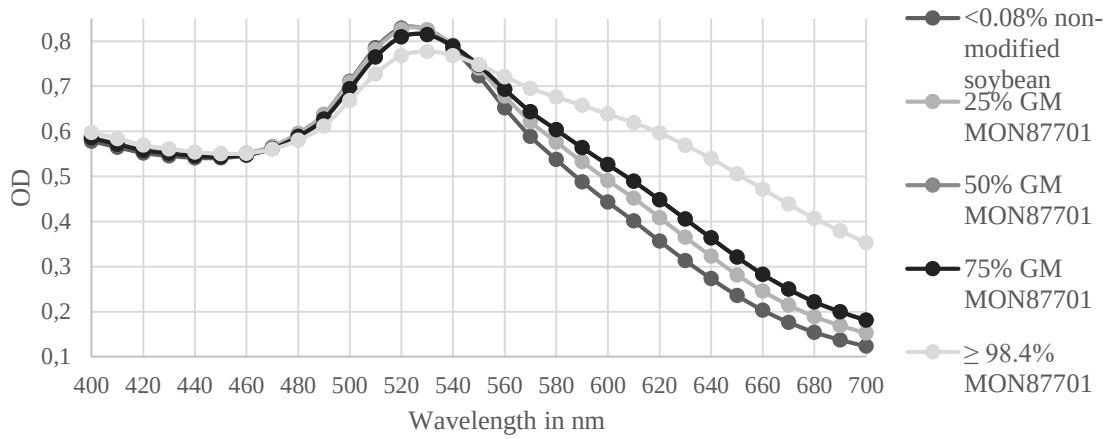
C

1'	Abs (620/520)	Standard deviation	Coefficient of variation
<0.08% non-modified soybean	0,021	0,025	6,711
25% GM MON87701	0,212	0,027	6,203
50% GM MON87701	0,392	0,028	5,552
75% GM MON87701	0,785	0,025	3,901
≥ 98.4% MON87701	1,061	0,033	4,431

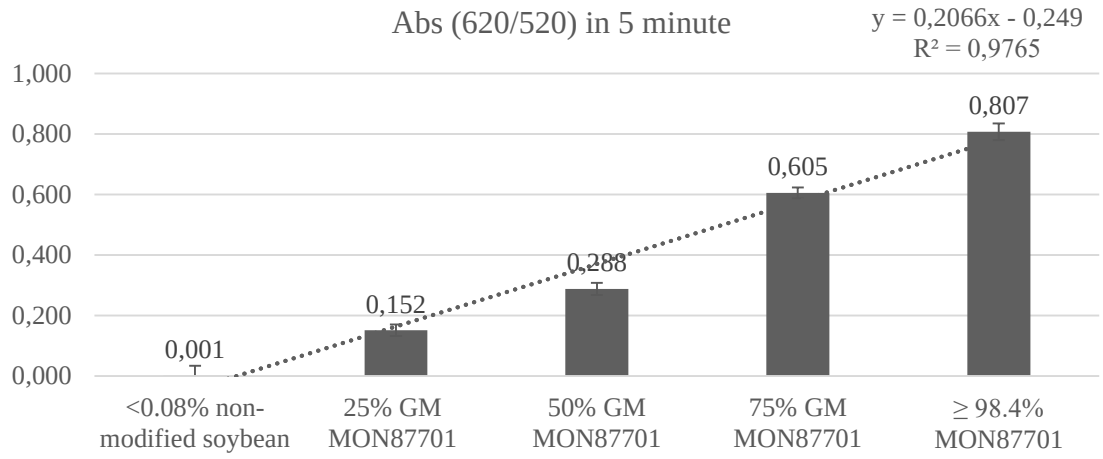
Figure 4.15. Batch Y5 **(A)** Absorbance spectrum with different levels of GMO in 1 minute **(B)** Abs (620/520) bar graphs with different levels of GMO in 1 minute **(C)** Normalized results of Abs (620/520), standard deviation and coefficient of variation with in 1 minute.

Reads in 5 minute

A



B



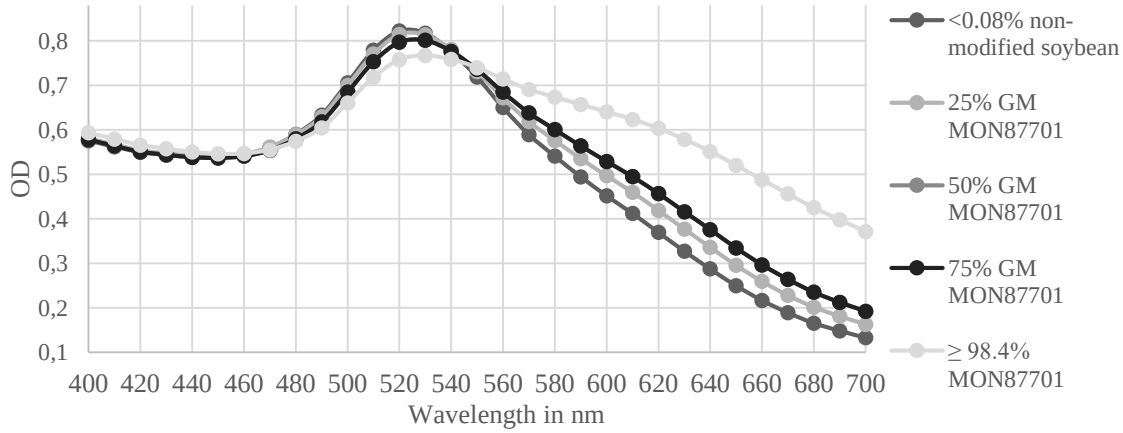
C

5'	Abs (620/520)	Standard deviation	Coefficient of variation
<0.08% non-modified soybean	0,001	0,033	7,562
25% GM MON87701	0,152	0,019	3,768
50% GM MON87701	0,288	0,020	3,559
75% GM MON87701	0,605	0,018	2,645
≥ 98.4% MON87701	1,061	0,033	4,431

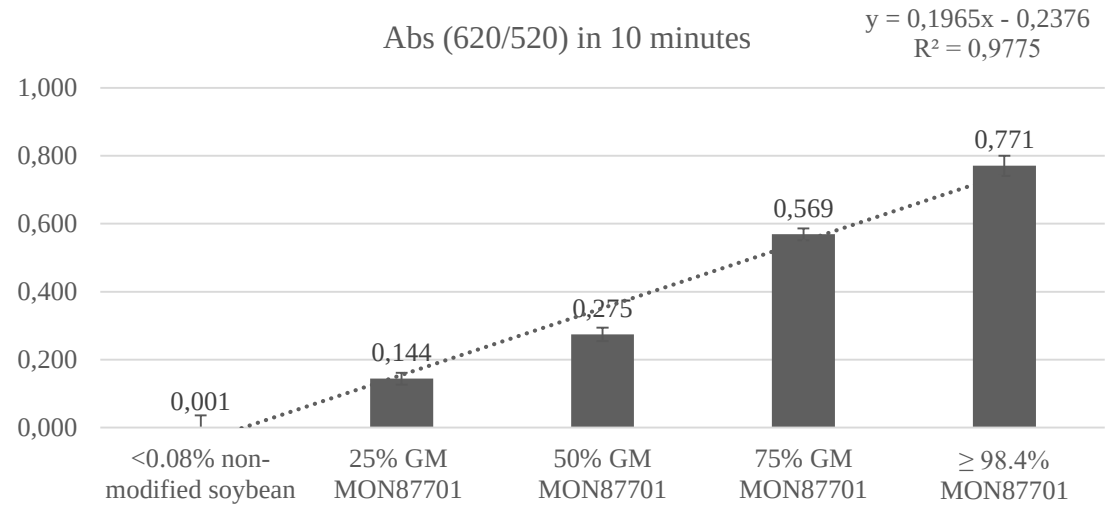
Figure 4.16. Batch Y5 **(A)** Absorbance spectrum with different levels of GMO in 5 minute **(B)** Abs (620/520) bar graphs with different levels of GMO in 5 minute **(C)** Normalized results of Abs (620/520), standard deviation and coefficient of variation with in 5 minute.

Reads in 10 minute

A



B



C

10'	Abs (620/520)	Standard deviation	Coefficient of variation
<0.08% non-modified soybean	0,001	0,036	7,977
25% GM MON87701	0,144	0,018	3,401
50% GM MON87701	0,275	0,020	3,465
75% GM MON87701	0,569	0,017	2,461
≥ 98.4% MON87701	0,771	0,030	3,729

D

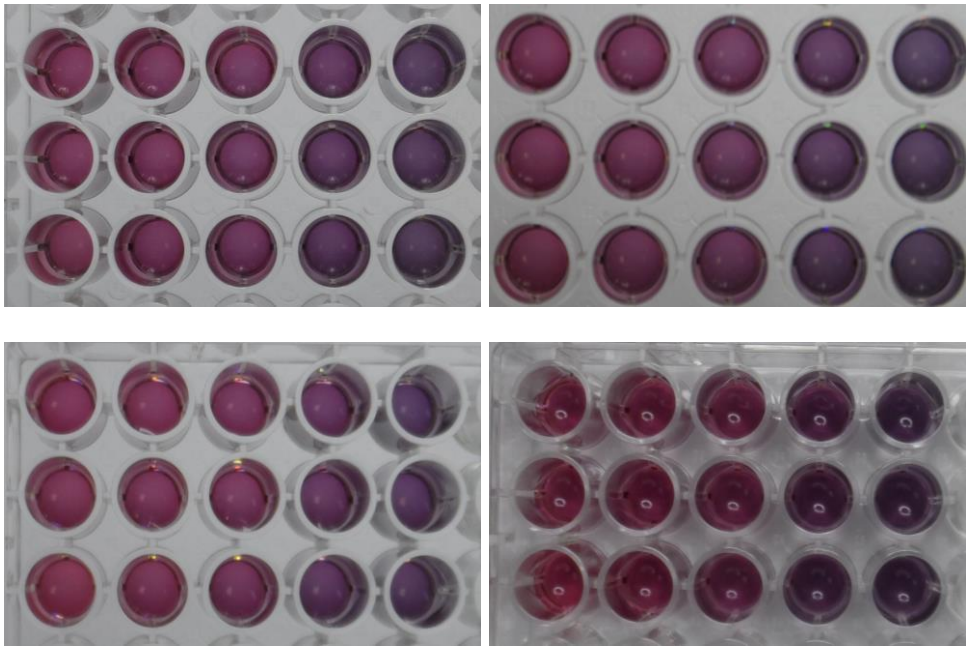


Figure 4.17. Batch Y5 **(A)** Absorbance spectrum with different levels of GMO in 10 minute **(B)** Abs (620/520) bar graphs with different levels of GMO in 10 minute **(C)** Normalized results of Abs (620/520), standard deviation and coefficient of variation with in 10 minute. **(D)** Visual readout, increasing GMO levels from left to right.

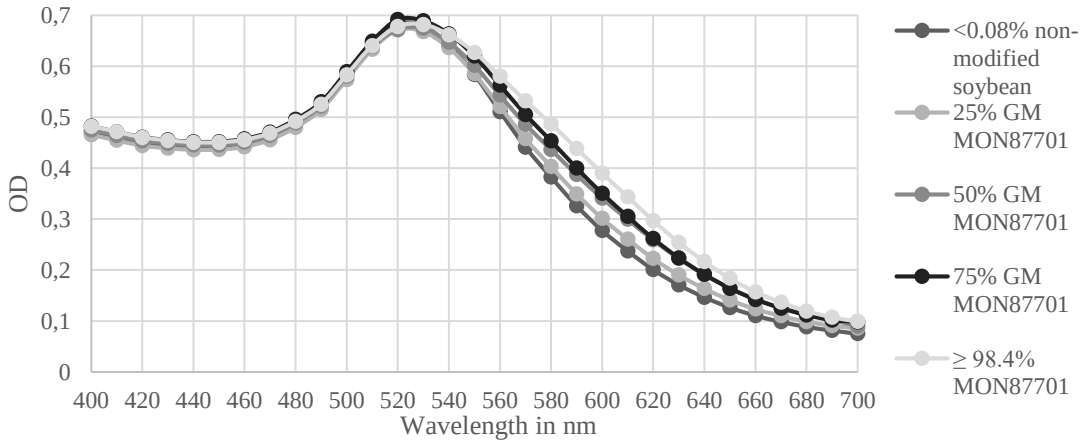
Spectrum data received for batch Y5, arranged bar graphs and color transformations in wells were given, Figure 4.15-17. Graphs were obtained with the mean and standard deviations of 10 replicates of this batch. Batch number Y5 had smallest diameter (~ 13.4 nm) and 4.7 nM concentration. It was expected that the hybridization between the probe strand and gDNA thermally show different level of aggregation upon AuNP and salt aggregation. The more the target gDNA presence, more probes will hybridize and less protection for the AuNP, meaning more aggregation. Wells having target gDNA (≥ 98.4% GM) present in solution that was hybridized with the complementary probe, dsDNA could not protect the AuNP, and they aggregate after salt addition with consequent colour change from red to purple (Figure 4.17 D). Contrarily, with the non-complementary sample (<0.08% non-modified) double stranded, gDNA prevents aggregation after salt addition, stabilization and electrostatic repel occurred in the reaction mixture and remains its reddish colour. As can be seen, the absorbance values continued to change within 10 minutes. The absorption at 520 nm, 620 was chosen to observe the relations of DNA and

AuNP. UV-Vis spectrum distinctly presented the decrease of adsorption at 520 nm, meanwhile increased in the 600-700 nm area. That blue-shift occurred, showed development of aggregates increase, with respects to GMO level increase. It was evidently observed with the bar graph that the increase is linear. It was important that the $R^2 = 0,97$ values of each graphs indicated consistency.

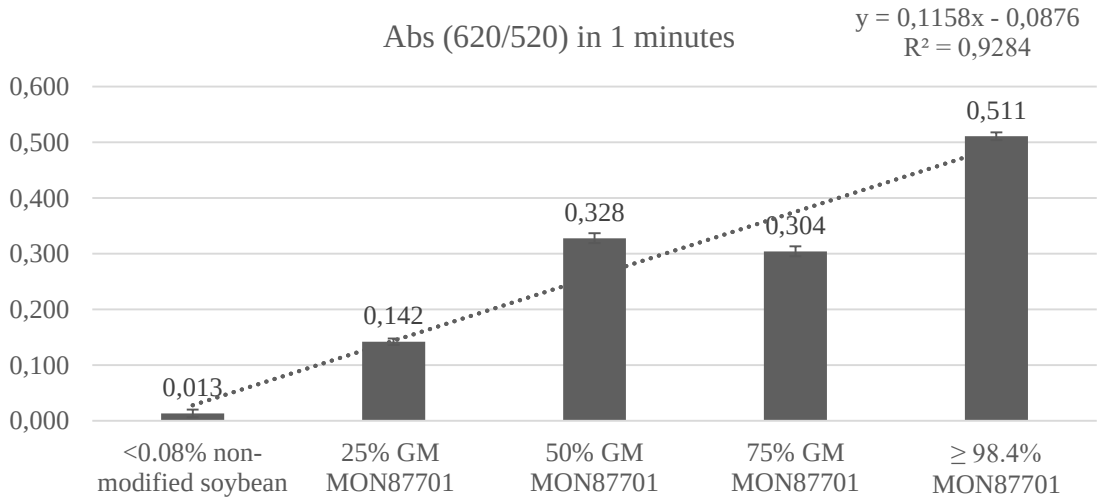
Batch Number Y6

Results of initial read in 1 minute

A



B



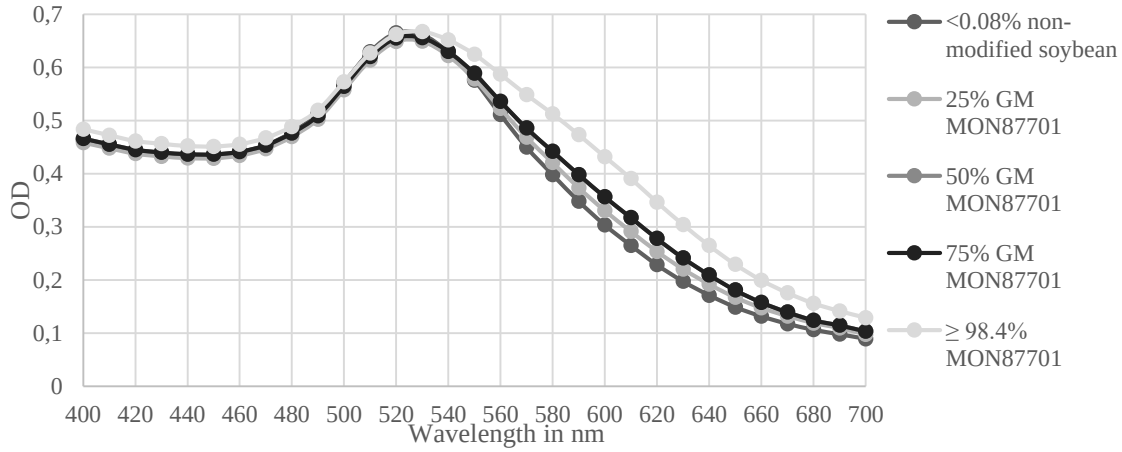
C

1’	Abs (620/520)	Standard deviation	Coefficient of variation
<0.08% non-modified soybean	0,013	0,007	2,333
25% GM MON87701	0,142	0,006	1,760
50% GM MON87701	0,328	0,009	2,338
75% GM MON87701	0,304	0,009	2,330
≥ 98.4% MON87701	0,511	0,007	1,560

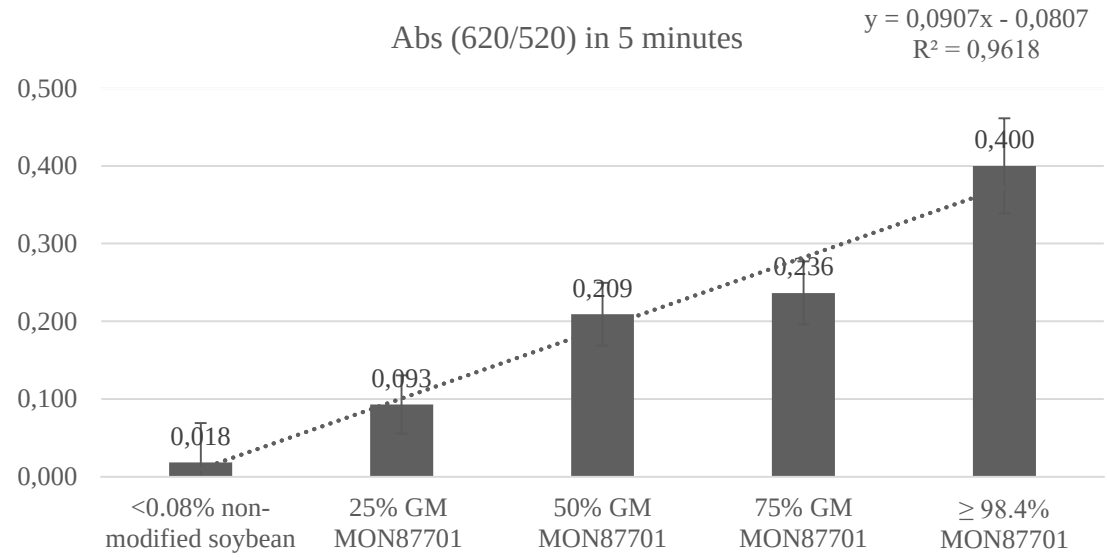
Figure 4.18. Batch Y6 **(A)** Absorbance spectrum with different levels of GMO in 1 minute **(B)** Abs (620/520) bar graphs with different levels of GMO in 1 minute **(C)** Normalized results of Abs (620/520), standard deviation and coefficient of variation with in 1 minute.

Reads in 5 minute

A



B



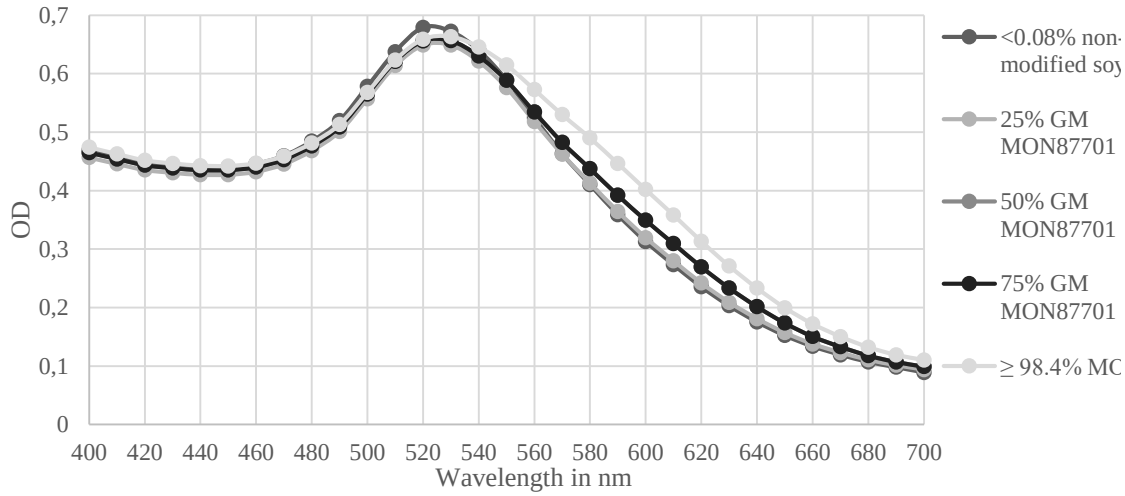
C

5’	Abs (620/520)	Standard deviation	Coefficient of variation
<0.08% non-modified soybean	0,018	0,051	14,625
25% GM MON87701	0,093	0,037	10,048
50% GM MON87701	0,209	0,040	9,816
75% GM MON87701	0,236	0,041	9,652
≥ 98.4% MON87701	0,400	0,061	12,863

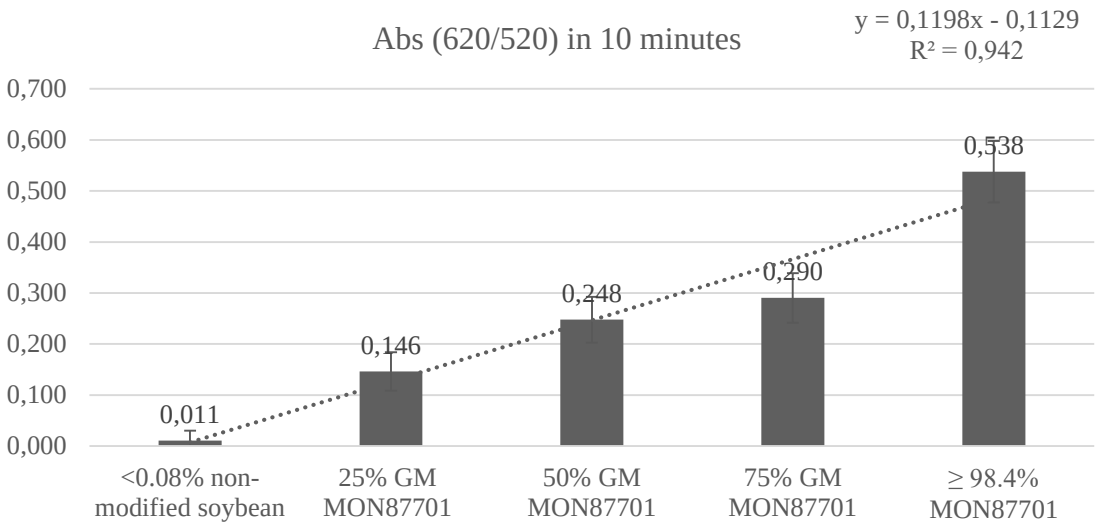
Figure 4.19. Batch Y6 **(A)** Absorbance spectrum with different levels of GMO in 5 minute **(B)** Abs (620/520) bar graphs with different levels of GMO in 5 minute **(C)** Normalized results of Abs (620/520), standard deviation and coefficient of variation with in 5 minute.

Reads in 10 minute

A



B



C

10’	Abs (620/520)	Standard deviation	Coefficient of variation
<0.08% non-modified soybean	0,011	0,020	5,703
25% GM MON87701	0,146	0,038	9,692
50% GM MON87701	0,248	0,045	10,590
75% GM MON87701	0,290	0,049	11,063
≥ 98.4% MON87701	0,538	0,060	11,499

D

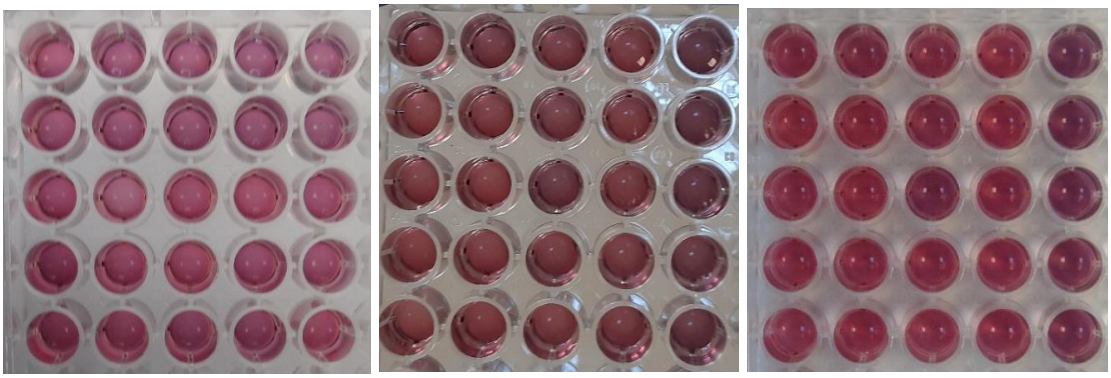


Figure 4.20. Batch Y6 **(A)** Absorbance spectrum with different levels of GMO in 10 minute **(B)** Abs (620/520) bar graphs with different levels of GMO in 10 minute **(C)** Normalized results of Abs (620/520), standard deviation and coefficient of variation with in 10 minute. **(D)** Visual readout, increasing GMO levels from left to right.

Spectrum data received for batch Y6, arranged bar graphs and color transformations in wells were given, Figure 4.18-20. Graphs were obtained with the mean and standard deviations of 10 replicates of this batch. Batch number Y6 had diameter (~ 15.2 nm) and 4.4 nM concentration. It was expected that the hybridization between the probe strand and gDNA thermally show different level of aggregation upon AuNP and salt aggregation. The more the target gDNA presence, more probes hybridized and less protection for the AuNP, meaning more aggregation. Wells having target gDNA (≥ 98.4% GM) present in solution that was hybridized with the complementary probe, showed a clear change, aggregation, purplish color observed (Figure 4.17 D). Conversely, the presence of non-complementary sample (<0.08% non-modified) maintained its reddish color. As can be seen, the absorbance values continued to change within 10 minutes. The absorption at 520 nm, 620 was chosen to observe the relations of DNA and AuNP. UV-

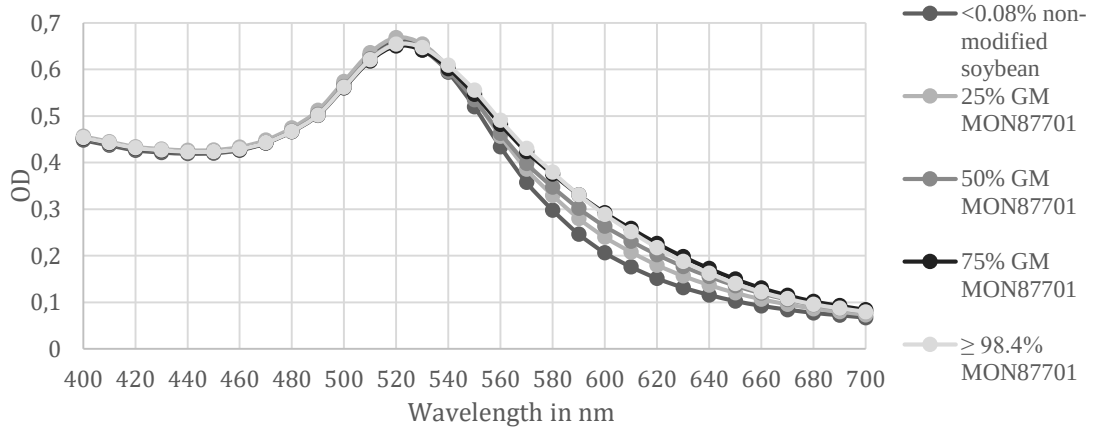
Vis spectrum distinctly presented the decrease of adsorption at 520 nm, meanwhile increased in the 600-700 nm area. Level ≥ 98.4% of GM sample, monitored by the spectrum, where the shift becomes clearer as time passes and was clearly separated from the smaller GM levels. It was evidently observed with the bar graph that the increase is linear. It was important that the R^2 = 0,92-0,96 values of each graphs indicated consistency.

Batch Number Y8

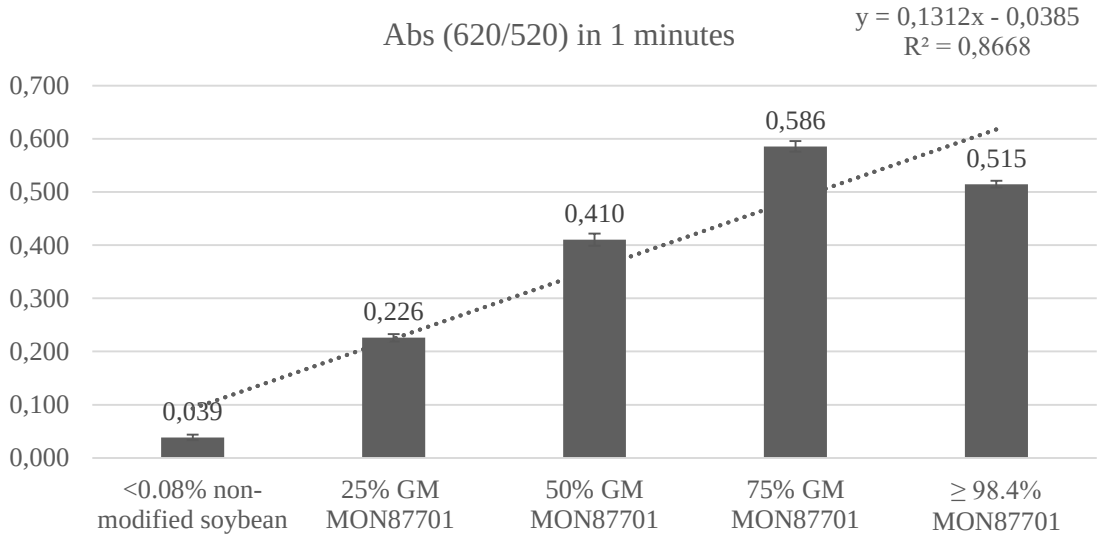
First 3 replicate that had been carried out with Y8, color change between non modified and ≥ 75% GM soybean-AuNP well is distinct and easily detectable with naked eye. On the other hand, at the level of ≥ 98.4% GM soybean well although there was a color transformation to purple, aggregation was not enough due to impurities may present in the DNA extract. This batch has also been optimized. Under the APPENDIX, gDNA purities effectiveness on aggregation level has been discussed in more details.

Results of initial read in 1 minute

A



B



C

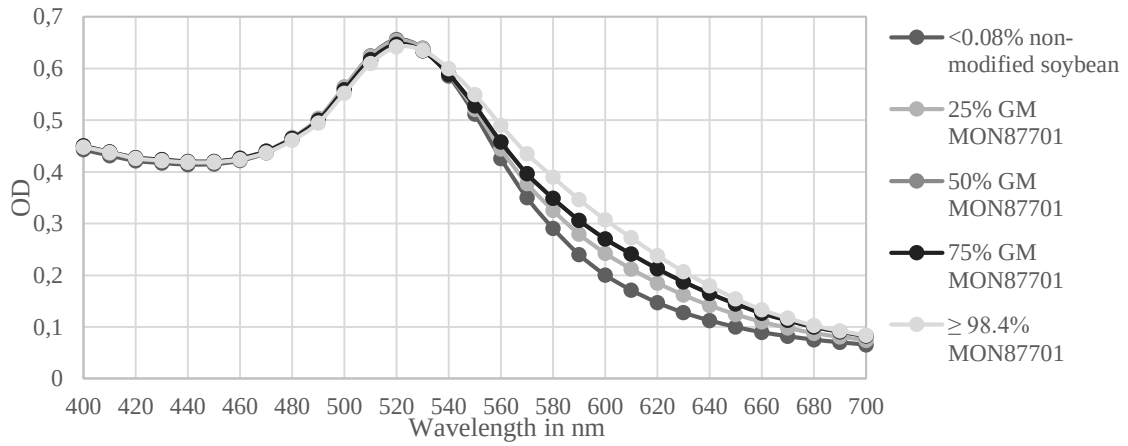
1’	Abs (620/520)	Standard deviation	Coefficient of variation
<0.08% non-modified soybean	0,039	0,005	2,239
25% GM MON87701	0,226	0,007	2,627
50% GM MON87701	0,410	0,012	3,737
75% GM MON87701	0,586	0,010	2,839
≥ 98.4% MON87701	0,515	0,006	1,946

Figure 4.21. Batch Y8 **(A)** Absorbance spectrum with different levels of GMO in 1 minute **(B)** Abs (620/520) bar graphs with different levels of GMO in 1 minute **(C)**

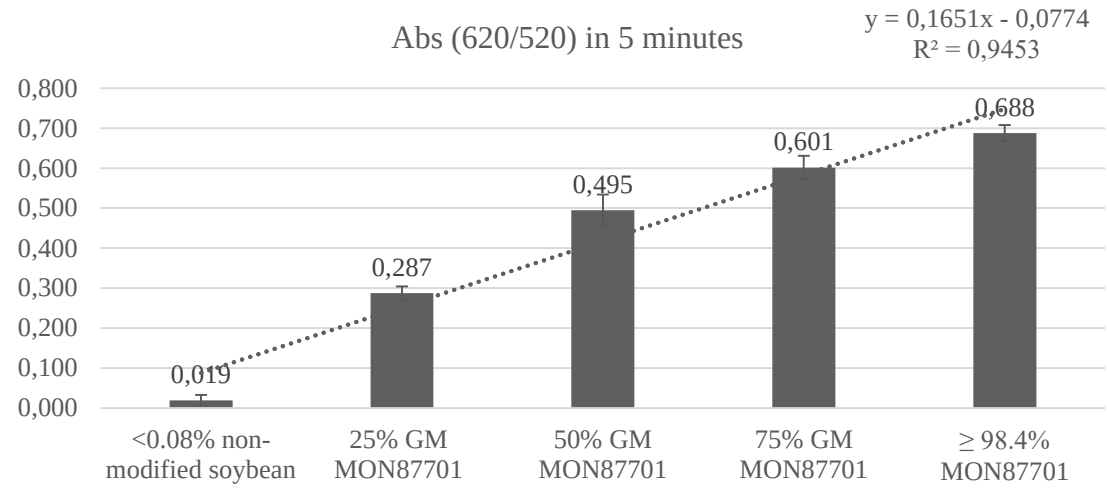
Normalized results of Abs (620/520), standard deviation and coefficient of variation with in 1 minute.

Reads in 5 minute

A



B



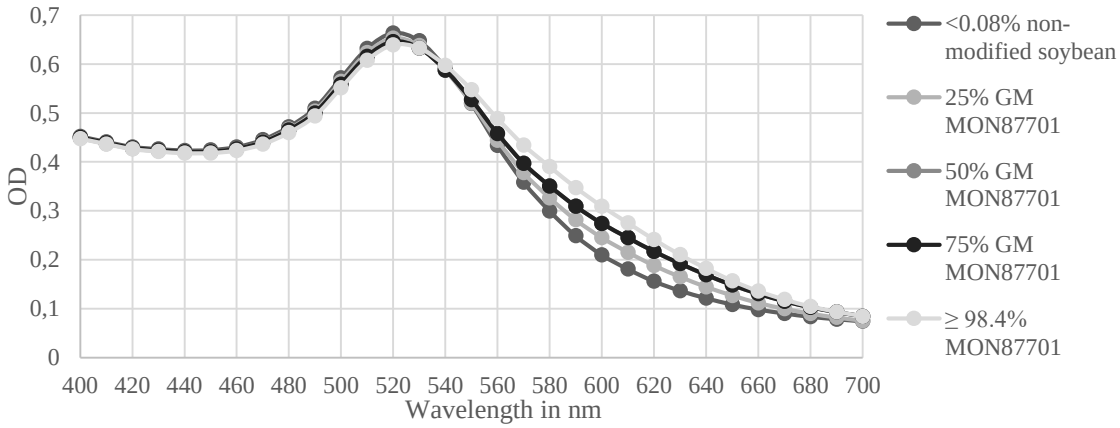
C

5’	Abs (620/520)	Standard deviation	Coefficient of variation
<0.08% non-modified soybean	0,019	0,014	6,136
25% GM MON87701	0,287	0,017	6,072
50% GM MON87701	0,495	0,039	11,938
75% GM MON87701	0,601	0,029	8,315
≥ 98.4% MON87701	0,688	0,020	5,372

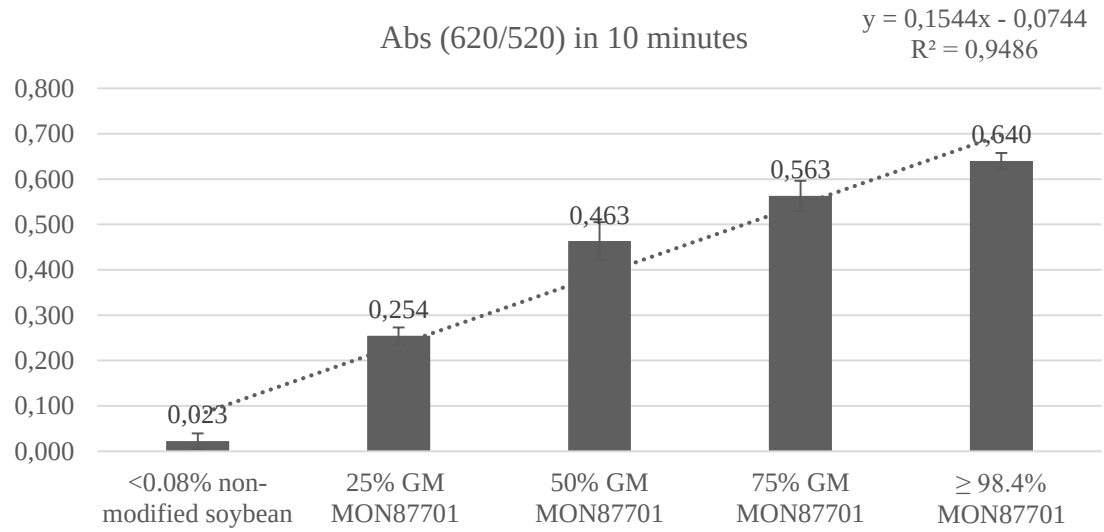
Figure 4.22. Batch Y8 **(A)** Absorbance spectrum with different levels of GMO in 5 minute **(B)** Abs (620/520) bar graphs with different levels of GMO in 5 minute **(C)** Normalized results of Abs (620/520), standard deviation and coefficient of variation with in 5 minute.

Reads in 10 minute

A



B



C

10’	Abs (620/520)	Standard deviation	Coefficient of variation
<0.08% non-modified soybean	0,023	0,017	7,158
25% GM MON87701	0,254	0,019	6,424
50% GM MON87701	0,463	0,042	12,491
75% GM MON87701	0,563	0,034	9,368
≥ 98.4% MON87701	0,640	0,017	4,549

D

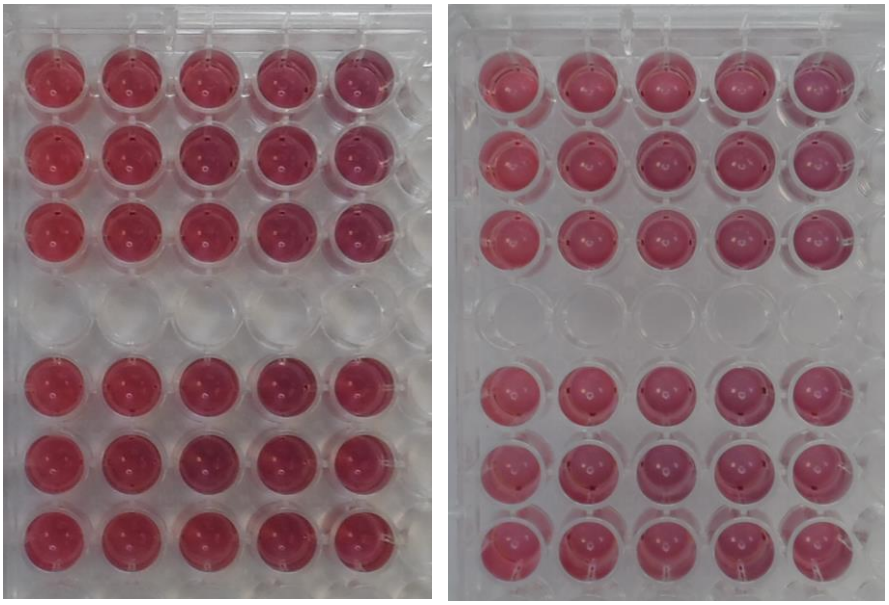


Figure 4.23. Batch Y8 **(A)** Absorbance spectrum with different levels of GMO in 10 minute **(B)** Abs (620/520) bar graphs with different levels of GMO in 10 minute **(C)** Normalized results of Abs (620/520), standard deviation and coefficient of variation with in 10 minute. **(D)** Visual readout, increasing GMO levels from left to right.

Spectrum data received for batch Y8, arranged bar graphs and color transformations in wells were given, Figure 4.21-23. Graphs were obtained with the mean and standard deviations of 10 replicates of this batch. Batch number Y8 had biggest diameter (~ 15.6 nm) and 4.7 nM concentration. It has been observed that level ≥ 98.4% GM takes time to complete its aggregation and separate from level 75% GM in this batch replicates. It is thought that this may be due to the fact that salt additions done from the level <0.08% to increasing levels. The color change owing to blue shift were evident even though it was difficult to see with naked eye. On the other hand, in between GM levels (25, 50 and 75%) although there was a color transformation, it is difficult to distinguish with the pictures as well. This might be due to length of isolated gDNA or any contaminant that may present while isolation or AuNP batch having the bigger nanoparticle. R^2 was found as 0,86-0,94.

The distinction ability of AuNPs comes from the complicated relation between DNA. This relation has been shown with findings by Li and Rothberg, who studied a colorimetric approach for sequence detection using AuNPs. Their technique is based on

the fact that bare AuNPs can distinguish ssDNA and dsDNA. ssDNA being able to bind to AuNP and protects them against salt formed the principle of our nanobiosensor. In this hybridization assay, the colorimetric result is achieved by a progressive aggregation of AuNP, with respect to hybridization with different level of target sequence presence in the mixture. This aggregation mainly take place when level change in complementary/target sequence presence and an increase in ionic force provided by salt.

It is expected that the hybridization of the probe strand and gDNA thermally will show different level of aggregation upon AuNP and salt aggregation due to different level of hybridization. The more the target gDNA presence, more probes will hybridize and less protection for the AuNP, meaning more aggregation.

When a target gDNA (≥ 98.4% GM) present in solution that was hybridized with the complementary probe, dsDNA could not protect the AuNP, and they aggregate after salt addition with consequent colour change from red to purple (Figure 4.17 D). Contrarily, with the non-complementary sample (<0.08% non-modified) double stranded, gDNA prevents aggregation after salt addition, stabilization and electrostatic repel occurred in the reaction mixture and remains its reddish colour.

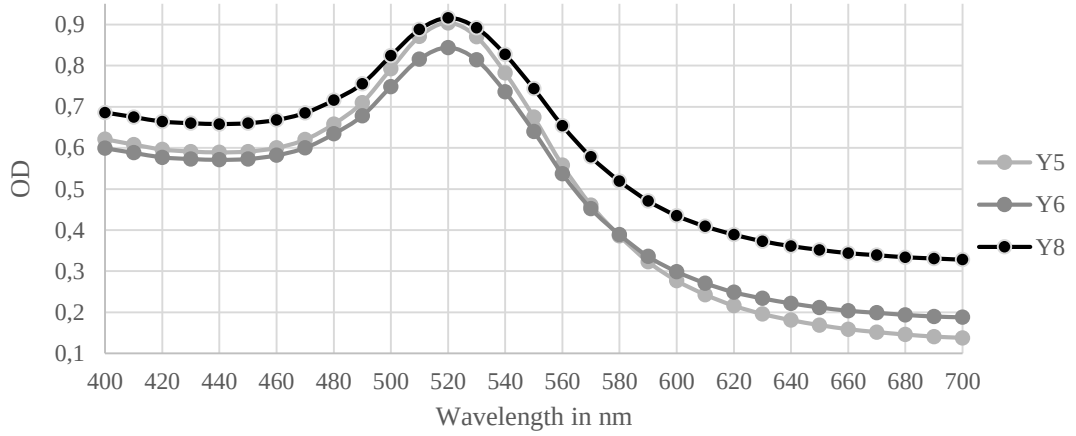
Kalidasan et all in 2013 showed similar visual detection results with respect to *Salmonella* genomic DNA using bare gold nanoparticles at room temperature. As a result, when the target sequence of *Salmonella* is present in the reaction this caused AuNPs to aggregate, however when non-complementary human genomic sequence is present, mixture remained red [169].

Simplifying the detection method, one of the major challenge in our nanobiosensors, was the complex process of preparing the sample and protocol. The efforts to develop simple colorimetric nanobiosensors in open environments continues to be restricted by temperature change, the presence of other chemicals. It was difficult to obtain reproducible results because of when the diluted gDNA batch, as well as AuNP batches change, the color drastically changes.

Repeatability Results Overlook

Repeatability, was tested where “test results are obtained with the same method, in the same laboratory, by the same operator, using the same equipment”, further in this section we compared the results of different AuNP batches results together.

A



B

Batch Number	Nanoparticle Diameter (nm)	Concentration (nM)	OD at 520 nm
Y5	~ 13.4	4.70	0,904
Y6	~ 15.2	4,40	0,844
Y8	~ 15.6	4,77	0,916

Figure 4.24. (A) Absorbance spectrum of three naked AuNPs **(B)** Three batch of AuNP's characterization results.

While batch number Y6 and Y8 has similar in nanoparticle diameter, Y5 and Y8 shows similarity and with respects to concentration. Therefore, comparing them in pairs will give more meaningful results and understanding. Results in the Figure 4.24. are given at the end of reaction at 10 minute.

Table 4.13. GMO levels mean Abs (620/520) in batch Y5, Y6 and Y8

A

Average of Batch Y5	Abs (620/520)	Normalized
<0.08% non-modified soybean	0,450	0,001
25% GM MON87701	0,515	0,144
50% GM MON87701	0,574	0,275
75% GM MON87701	0,706	0,569
≥ 98.4% MON87701	0,797	0,771

B

Average of Batch Y6	Abs (620/520)	Normalized
<0.08% non-modified soybean	0,344	0,011
25% GM MON87701	0,390	0,146
50% GM MON87701	0,424	0,248
75% GM MON87701	0,439	0,290
≥ 98.4% MON87701	0,523	0,538

C

Average of Batch Y8	Abs (620/520)	Normalized
<0.08% non-modified soybean	0,235	0,023
25% GM MON87701	0,289	0,254
50% GM MON87701	0,337	0,463
75% GM MON87701	0,359	0,563
≥ 98.4% MON87701	0,377	0,640

It can be seen in the Table 4.13. that AuNP batches with three different diameters and concentrations, resulted in different absorbance values for the same GMO levels.

A

Y6 & Y8	Abs (620/520)	±	Normalized	±
<0.08% non-modified soybean	0,289	0,054	0,016	0,005
25% GM MON87701	0,339	0,050	0,200	0,054
50% GM MON87701	0,380	0,043	0,355	0,107
75% GM MON87701	0,399	0,039	0,426	0,136
≥ 98.4% MON87701	0,450	0,072	0,589	0,051

B

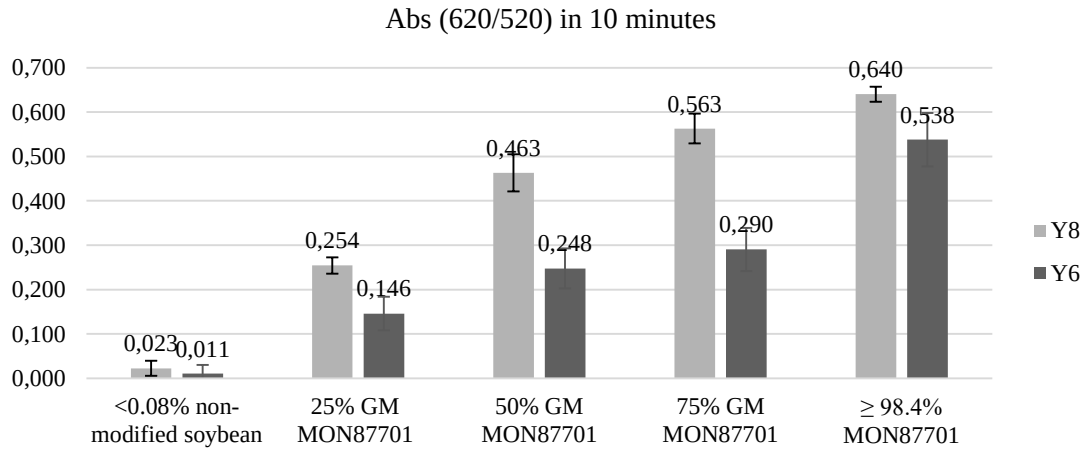


Figure 4.25. (A) Abs (620/520) means of batch Y6 and Y8 **(B)** Abs (620/520) bar graph batch Y6 and Y8.

Aggregation levels that can be monitored in the GMO nanobiosensor driven with AuNP of the same diameter but different concentration. The peak absorbance value difference seen in the naked fresh AuNPs (due to different concentration of gold in solution), also reflected in the aggregation results of nanobiosensor results. Y8 batch, which has a higher intensity in its bare form, also resulted in higher intensity after nanobiosensor assay. This shows the reason for the absorbance ratio difference results to be seen in different batches.

A

Y5 & Y8	Abs (620/520)	±	Normalized	±
<0.08% non-modified soybean	0,343	0,108	0,012	0,011
25% GM MON87701	0,402	0,113	0,199	0,055
50% GM MON87701	0,455	0,119	0,369	0,094
75% GM MON87701	0,533	0,173	0,566	0,003
≥ 98.4% MON87701	0,587	0,210	0,705	0,065

B

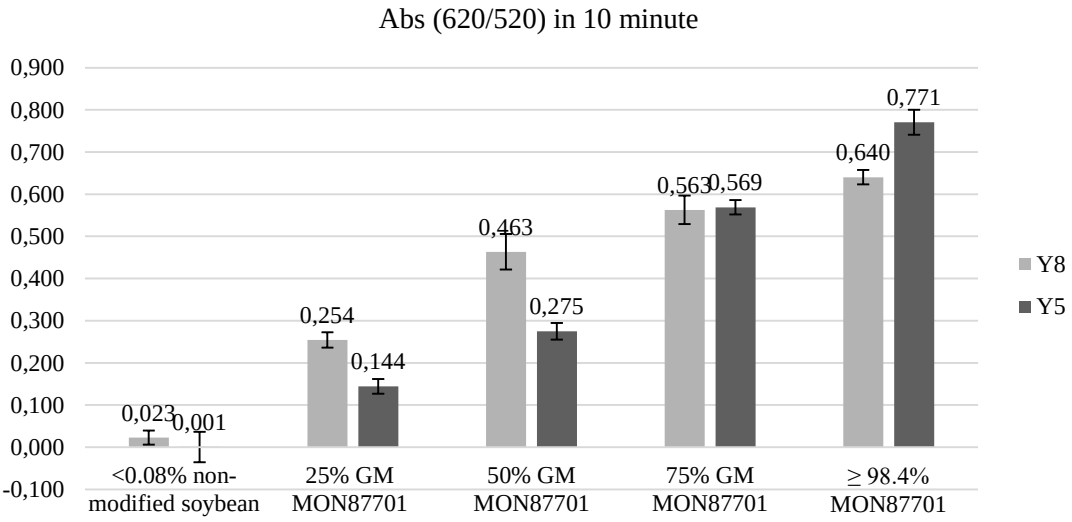


Figure 4.26. (A) Abs (620/520) means of batch Y5 and Y8 **(B)** Abs (620/520) bar graph batch Y5 and Y8.

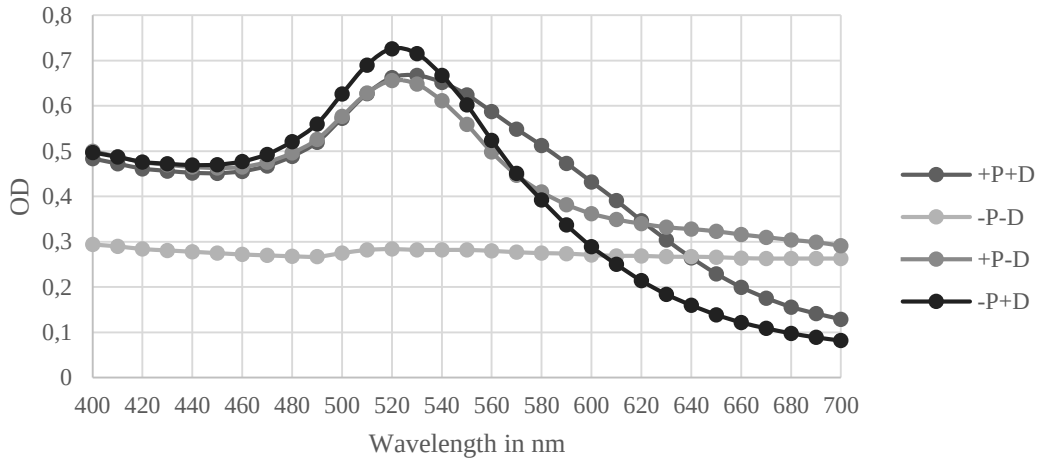
Batch number Y5 has smaller in diameter (~ 13.4 nm) than Y8 batch (~ 15.6) but both batches has the same concentration. At all GMO levels, aggregation was observed more clearly in the solution containing small diameter nanoparticle, visually. This was also observed more distinct with the absorbance spectrum. This is because in two different solutions at the same concentration, the solution containing smaller diameter of AuNP, contains more surface area that can be in a contact with gDNA and salt. This though to be another reason for the absorbance ratio difference results to be seen in different batches.

This concludes that, colorimetric nanobiosensors show some limitations such as signal reproducibility, standardization, and the need for confirmation with conventional methods.

Zhang et all in 2013 commented regarding the particle diameter difference; while the method works well for 13 nm, it usually does to work for bigger in diameter. Zhang also pointed that concentration and extinction coefficient plays a big role. They showed that when particle gets larger less DNA was able to attached. This means most of the DNA was discarded when added to large AuNPs. Also in the literature, a similar DNA concentration was used regardless of AuNP size, showed different aggregation level [104].

Controls of the Nanobiosensor

A



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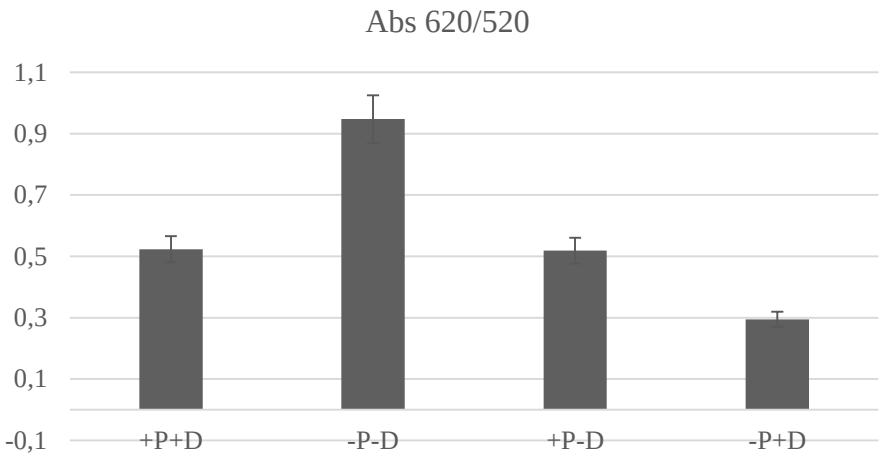

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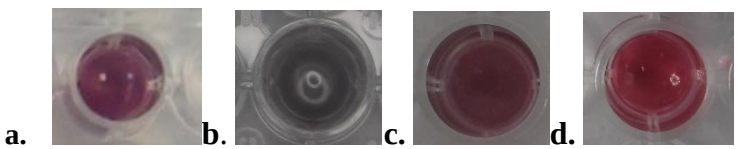


Figure 4.27. (A) Absorbance spectrum of the AuNPs and its corresponding plot. Positive probe + Positive DNA (+P+D), Negative probe + Negative DNA (-P-D), Positive probe + Negative DNA (+P-D), Negative probe + Positive DNA (+P-D). **(B)** Abs (620/520)

bar graphs with its corresponding controls of Abs (620/520) **(C)** Visual readout, a. (+P+D), b. (-P-D), c. (+P-D), d. (-P+D).

Controls are for experiments first step. The same amount salt was not sufficient to destabilize AuNP when only isolated gDNA presented, aggregation does not happen and the solution remains its red color. This approach governs limitations, since gDNAs can be longer than probes and cannot always give purple for aggregation. Colors of control wells should not be compared with GMO level wells. A different feature that influences controls are probe and gDNA density at the AuNP surface.

4.4.2. Practicability-Cost-effectiveness Analysis

The simplicity and effectiveness of method, the costs per sample of the practice. Comparison of novel methods requires such as, not generally available or expensive equipment, required to perform the analysis in considerably higher time, cost etc. Cost-effectiveness analysis (CEA) is a method that brought together information on an intervention's cost with an estimate of the outcomes. In our project CEAs will provide information to compare both detection methods of Real Time-PCR and GMO nanobiosensor. Guide us to make strategic decision and priority setting. Cost Effectiveness Ratio (CER) =cost/outcome. Method with lower CEA are considered to be more cost-effective (i.e., the cost is less for the outcome expected). Common expenses are excluded (pipette, pipette tip, electricity expenses, DNA isolation step, etc.) Ranking from 0-10. 10 being the highest.

$$CER = \frac{equivalent\ total\ cost}{total\ of\ effectiveness\ measure}$$

Table 4.14. Cost comparison of standart method and novel method

Standart Method	**Cost (€)**	**Novel Method**	**Cost (€)**
RT-PCR Instrument	15.500	Microplate Reader	9.500
PCR kit	590	AuNP synthesis chemicals	230
Total	16.090	**Total**	9.730

Table 4.15. CER calculation of standart method and novel method

Method	Cost (€/Year)	Required Pre-education	Speed of Result	False Result Rate	Total Ranking	CER (€/Ranking)
Standart Method	7080	8	6	1	14	505,714
Novel Method	1560	5	2	4	7	222,857

Addition to this in Ankara Food Control Laboratory Directorate, MON87701 Soybean type identification analysis is ~80 €/per sample, MON87701 Soybean quantification analysis is ~225 €/per sample. (1€=16₺).

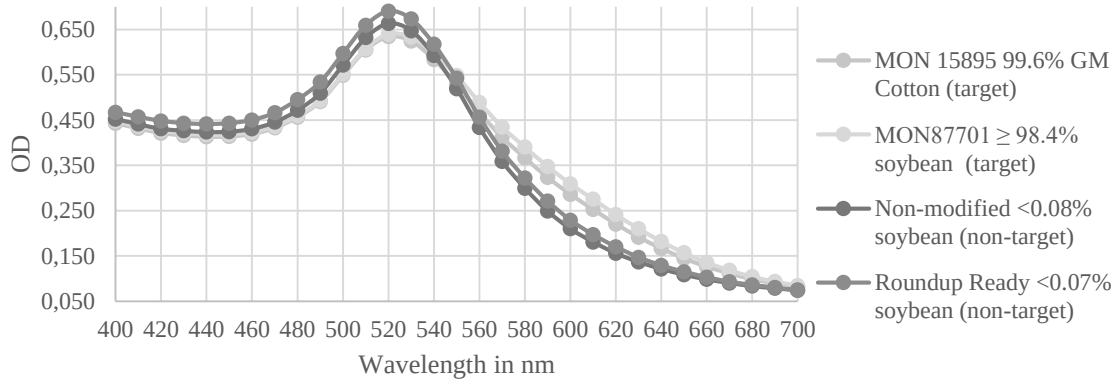
GMO nanobiosensor cost, when considering all expenses are present; 1 gram of gold(III) chloride trihydrate 137.00 €. It is possible to synthesize 40 ml gold nanoparticles 500 times with 1 gram. Considering that 100 µl of AuNP was used in 1 analysis. It means that 20.000 analyzes can be performed where maximum efficiency is achieved. Which means the cost is less than 1 €/per sample.

Considering the ranking, pricing and CER obtained, it can be concluded that the nanobionsensor is more cost effective; while keeping in mind that this method is relatively new and has limitations that has not been took in account [177, 178].

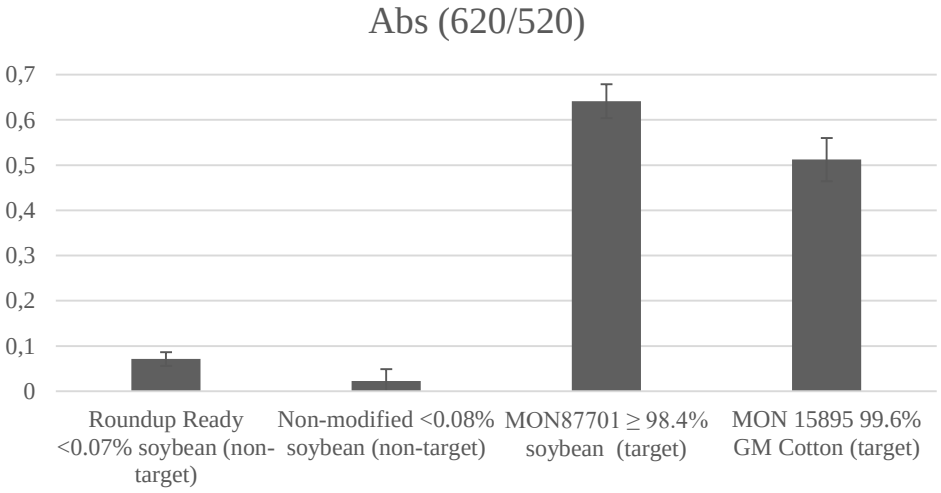
4.4.3. Specificity/Selectivity

The nanobiosensor responds exclusively to the target. This demonstrated by similarity by test with non-target and target sample. Our gene-specific method exclusively detected the targeted GM event of Cry1Ac. The specificity of the detection method verified by testing the lack of the target sequence. A non-complementary sequence corresponding the <0.07% Roundup Ready soya (blank); and a target MON15895 99.6% GM Cotton events were chosen to study the selectivity.

A



B



C

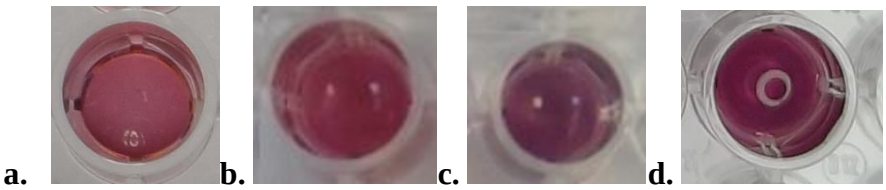


Figure 4.28. (A) Absorbance spectrum of AuNP with target and non-target gDNA. **(B)** Abs (620/520) bar graphs with target and non-target gDNA **(C)** Visual readout, a. <0.07% Roundup Ready soybean (non-target), b. <0.08% non-modified soybean, c. ≥ 98.4% GM soybean, d. 99.6% GM MON15895 Cotton.

It was observed that, none of the non-target samples (a, b) significantly affected the absorbance spectrum of AuNPs. On the other hand, addition of Cry1Ac gene resulted in a purple color formation of the AuNP solution, indicating aggregation.

As expected, the intensity ratio of MON15895 99.6% GM Cotton and ≥ 98.4% MON87701 GM soybean was significantly higher than that of <0.07% Roundup Ready soybean (non-target) and <0.08% non-modified soybean (non-target) presence. <0.07% Roundup Ready soybean (non-target), showed the current intensity close to the blank sample (similar to <0.08% non-modified soybean), even more protection against aggregation, conforming the specifity of nanobiosensor. For target, MON15895 99.6% GM Cotton, due to Cry1Ac gene level, showed the current intensity close to the target sample (similar to ≥ 98.4% MON87701 GM soybean), Purple color meaning aggregation, confirmed the specifity of nanobiosensor.

4.4.4. Limit of Detection (LOD)

The minimum amount of sample that can be detected is LOD. Addition of 10 µl volume of 30 ng/µl gDNA to detection solution containing 120 µl in final reaction volume, results with 2.5 ng/µl final reaction concentration.

LOD also estimated with signal to noise ratio from 10 minute graphs of three batches of AuNP. LOD is calculated as three times of the standart deviation divided by slope of the plot. It was found to be 0.317, 1.061 and 0.501 ng/µl lowest concentration of target DNA, GM sample which gives rise to Y5, Y6 and Y8 respectively.

According to ENGL, published average 1C (unreplicated haploid soybean genom) value for the soybean genome is 1,13 pg. If 1 genom is 1,13 pg, then 1 ng of soybean DNA is 1000 pg/1,13 = 884,95 copy soybean genom. Furthermore 30 ng gDNA means 30x884,95 = 26548,5 genome copies.

26548,5 genome copies in 120 µl of total reaction volume 26548,5/120 = 221,2 genome copies in 1 µl which also equals to 250 pg genome copies.

GMO content 25% means; 25*/100*26548,5= 6637,125 target GM genome copies. GMO content 50% means; 50*/100*26548,5= 13274,25 target GM genome copies. GMO content 75% means; 75*/100*26548,5= 19911,325 target GM genome copies. GMO content 100% means; 100*/100*26548,5= 26548,5 target GM genome copies [179-181].

4.5. Support Vector Machine Algorithm

In this thesis, success rate on the data sets were analyzed by using the GMO levels and absorbance data in the SVM algorithm C software language. Even though the SVM algorithm was written in the C programming language, it was important that it is suitable to run in many languages such as Python, Java, R and etc.

First of all, the results related to the classification performance of the SVM were obtained with the full spectrum data of all three batches together. As well as with kernel trick, Abs (620/520). While developing SVM, many kernel function options (linear, radial, polynomial, etc.) have been tried and the classification performance of radial base function (RBF)-based SVM resulted in the best classification performance. The RBF has proven its flexibility enough that many examples applied in a variety of engineering applications [182, 183].

Once labeling has done, the SVM algorithm ran. We have a space that is divided into 5, meaning there were 5 different classifications available (0, 25, 50, 75 and 100 % GMO level labels). Giving the three bathes of AuNP raw data (absorbance at each wavelength from 400-700 nm with step width of 10 nm. Prediction resulted in 58% success rate. This is mainly due to different bathes resulted in different absorbance with respects to same GMO level. With the so called “heat map” visualization given with all raw data, it is clear to say that SVM algorithm was unable to see difference in batches (Figure 4.29).

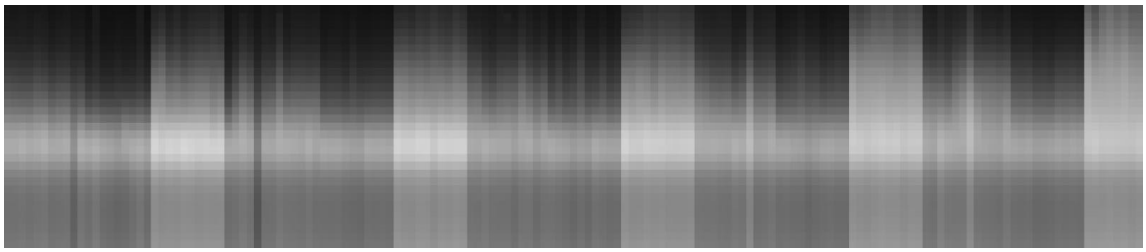


Figure 4.29. Heat map of three batches of AuNP.

Therefore, it was decided to move on with a kernel trick, using the absorbance ratio of 620 nm to 520 nm data for all batches. We analyzed that the change will be seen when these are taken as the reference point. With this kernel trick, the SVM model was reanalyzed. (derivative kernel trick). By training and classifying three AuNP batches

separately (among themselves) Y5, Y6 and Y8 with SVM using full spectrum and kernel trick success rates are shown in Table 4.16.

Table 4.16. Success rate of different AuNP batches.

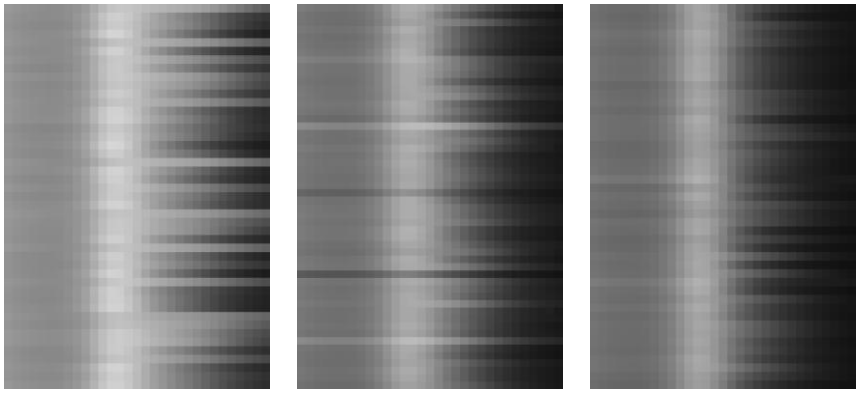
Batch Number	SVM Input	Classification Success Rate (%)
Y5	Full spectrum	95.55
	Kernel trick	93.33
Y8	Full spectrum	86.66
	Kernel trick	55.55
Y6	Full spectrum	63.46
	Kernel trick	50.00

AuNP batch Y5 having the smallest diameter of 13 nm and 4.7 nM concentration resulted in the best color darkening. With aggregation increasing significantly and linearly with the increase in the GMO level, and the data fed to the SVM algorithm was the best eligible. It has fewer false negatives and positives with a small amount of misplaced data, which is eliminated in the algorithm. Y5 batch containing 5 classes, 32 columns and 46 rows with total of 1472 data was classified with a 95.55% success rate.

AuNP batch Y8 having the diameter of 15 nm and 4.7 nM concentration resulted with slight darkening. This resulted in the aggregation increasing slightly and according to the increase in the GMO level. Due to the increase in the diameter of this batch, the transition between classes had become more intertwined and the classification of the algorithm has become more difficult, although the success rate was quite good. Y8 batch containing 5 classes, 32 columns and 46 rows with total of 1472 data has resulted in 86.66%.

Batch number Y6 gold nanoparticles has the diameter of 15 nm and 4.4 nM concentration resulted in which the color change was difficult to perceive with the eye, but a clear distinction can be seen with the wavelength reading. The 10 technical repeat show that false positives are more likely to occur in this batch. Y6 batch containing 5 classes, 32 columns and 53 rows with total of 1696 data has resulted in 63.46% success rate. The relevant outputs of the algorithm, kernel type, gamma, C values and heat maps for each type, are summarized in table 4.17.

Table 4.17. SVM algorithm model outputs.

AuNP batch	**Y5**	**Y6**	**Y8**
Kernel type	RBF	RBF	RBF
Gamma	5,06E+15	5,06E+15	5,06E+15
C	1,25E+17	3,13E+18	3,13E+18
Heat Maps			

C and Gamma are the parameters for SVM with a RBF kernel. They appear as the optimum results given by autolearn. Our SVM model seeked to find a margin that apart all classes. Nevertheless, if any samples are mislabeled, this might result in models that are not well-fitting. SVM model allowed some examples to be "ignored", that lead to a better overall fit. The parameter C which controled the influence of each individual support vector; this process involveed trading error penalty for stability. A small C gave us higher bias and lower variance. A small gamma implied the class of this support vector will have influence on deciding the class of the vector even if the distance between them was large. So a small gamma gave us low bias and high variance. The larger C value meant greater penalty of the error, with our colorimetric change as well as absorbence results supports the Y5 nanoparticle batch having smaller C, was the best interms of SVM model.

Unknown read; The specificity of the detection method verified experimentally. Gene-specific nanobiosensor exclusively detects the targeted GM event Cry1Ac, meaning SVM algrithm also need to be tested with unknown samples. The unknown sample prediction of accuracy is then compared to their actual GM levels. As unknown sample read; A non-complementary sequence of the blank <0.07% Roundup Ready soyben CRM with 2

parallels. For complementary sequence MON15895 99.6% GM Cotton CRM with 2 paralels were chosen.

Table 4.18. Unknown read results of Roundup Ready soybean and MON15895 cotton.

Sample	Actual	Predicted Result (% GMO)
<0.07% Roundup Ready soya1	0	0
<0.07% Roundup Ready soya1	0	0
MON15895 99.6% GM cotton1	100	100
MON15895 99.6% GM cotton2	100	75

As a result, four unknown samples, data that the algorithm had never seen before, were classified with 75% success rate (Table 4.18). As kernel RBF and parameters gamma is 5,06E+15 and C value as 3,13E+18. Misclassification of 1 out of 4 samples gave 75% success rate, the success rate will increase as the number of data increases. The fact that the GMO level in cotton is perceived one class less is because the algorithm can reach 86.66% as the maximum success.

4.6. Website Development

Our website had the hostname of http://gmonanobiosensor.hacettepe.edu.tr Operating system was Ubuntu 20.04.4 LTS. Database type was Sqlite3 (v3.36.0) [184].

We designed our website to be “one page”. On the navigation bar, sections involve;

- What is GMO detection with nanobiosensor system web tool? Provides brief information on GMO’s and our gold nanobiosensor based detection kit.
- How does it work? Provides steps that the person who has the “GMO detection with nanobiosensor kit” should follow as well as the principle.
- How to upload data? Provides users, in what order and in what format the user's data will be uploaded to the website
- How to read results? Provides users how the user will interpret the results.

- Upload&Classify section. Browsing the file, and mandatory section selection. Result window.
- About us section.

Thus, individuals who do not have any programming skills and customs employees will be able to obtain information of the sample with this website. Person who has the GMO nanobiosensor kit, can easily following the steps in the website and have an idea of the sample prior to the PCR analysis.

CONCLUSION

Nanomaterials including AuNPs, have a diverse range of characteristics that are valuable in a variety of applications such as point-of-care testing. In fact, test systems that are incorporating particularly AuNPs, have been changing the game. Noteworthy advances in properties will guide production and sensitive diagnostic.

In this study, we demonstrated that detection of sequences using AuNPs is possible with unamplified target genomic DNA with complementary probes. A correlation was obtained between the GMO level according to the Cry1Ac gene with a colorimetric change of red to purple. The detection limit is as low as nanomolar, and the detection time very short compared to rutine analysis. Finally, a successful prediction analytics model has been developed from the obtained data set with the machine learning algorithm, support vector machine, that will automatically classify and predict GM levels from different batches of AuNPs. It was then integrated into a user friendly website.

According to EURL GMFF report "definition of minimum performance requirements for analytical methods of GMO testing" which we now called as "minimum performance requirements for GMO detection with nanobiosensing system" [170]. Method acceptance criterias were; DNA quality and purity, Practicability, Specificity/Selectivity, LOD, Robustness and Precision-RSDr. According to the results obtained within the scope of the report; we take DNA quality and purity & applicability criteria, good quality-pure DNA with the absence of inhibitors has been obtained. Practicability wise, we conducted cost-effectiveness analysis for feasibility and efficiency of implementation, considering the ranking, pricing it can be concluded that the nanobionsensor is more cost effective; while keeping in mind that this method is relatively new and has limititations that has not been took in account. According to definition of Specificity/Selectivity the method should only respond to the target sample, meaning the nanobiosensor should only gives signal for the target sequence. It has been seen that for non modified (non-target) Roundup Ready blank, showed the current intensity close to the blank sample (similar to <0.08% non-modified soybean), even more protection against aggregation and also target, MON15895 99.6% GM Cotton, due to Cry1Ac gene presence, the showed the current intensity close to the target sample (similar to ≥ 98.4% MON87701 GM soybean), purple color meaning aggregation, conforming the specifity of nanobiosensor. In our case of

LOD, detection solution containing 120 µl in final reaction volume, results with 2.5 ng/µl final reaction concentration, 26548,5 genome copies in total reaction volume 221,2 genome copies in 1 µl, 250 pg genome copies has been reached with ensured level of confidence. Robustness tested for nanobiosensors various parameters and seen that concentrations of salt, morphology of AuNP, heat threatment, all plays a crucial role but with our optimum experimental conditions described, robustness state is provided. Lastly Precision-RSDr conditions where tested and acceptance criterion of ≤25% was obtained (0, 25, 50, 75 and 100 % GMO replicates standart deviations are 8.95, 9.16, 10.44, 15.27 and, 17.57 respectively). This study showed that limited quantification is possible with GMO nanobiosensor, and further detailed studies are required. Since novel detection methods perform better in some cases, validation is needed. The performance management of gold nanobiosenors may be very sensitive to environmental factors such as temperature and light, necessitating the use of particular ranges based on the circumstances [185]. On the other hand, international standardization and regulation are required for nanomaterial use [186].

Progress in development of nanobiosensor with real samples is not far away in fact is some versions are already on the market. The impact of these novel nanobiosensor will greatly contribute to the improvement of detection assays. Portable technologies including mostly nanobiosensing are widely used for the smarter food safety.

In conclusion, the most significant advantage of the proposed DNA nanobiosensor is its simplicity. It is rapid and inexpensive. It provides visual Cry1Ac gene sequences within a few minutes without the need of cycles and cycles of PCR. The nanobiosensor-based detection gives qualitative information for the GMO level in the sample can be obtained prior to routine PCR analysis. The GMO detection nanobiosensor system integration with machine learning has been shown to have a potential to be leading tool in the food industry.

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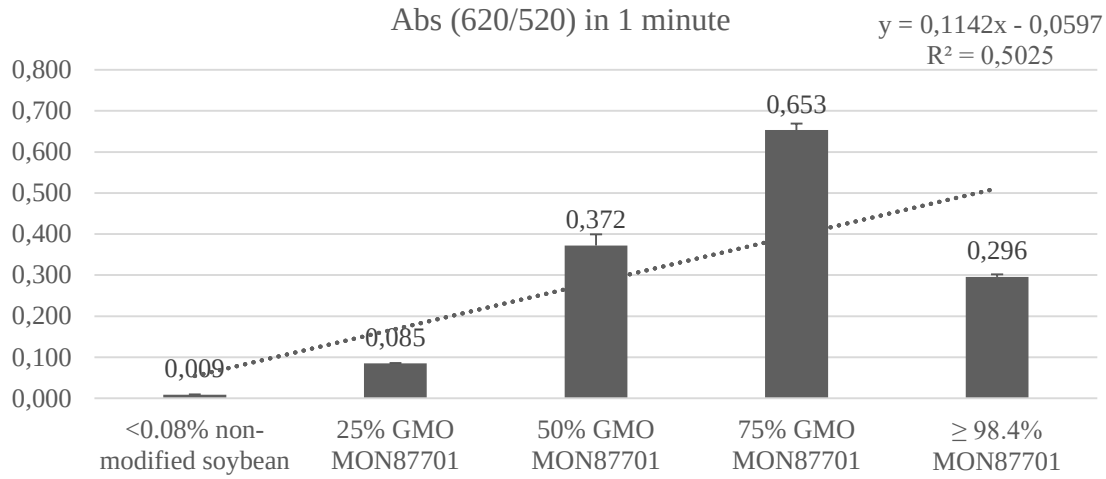
APPENDIX

Batch Number Y8 Trial 1

Error bars refer to standard deviations from 3 sets of experiments.

Reads in 1 minute

A



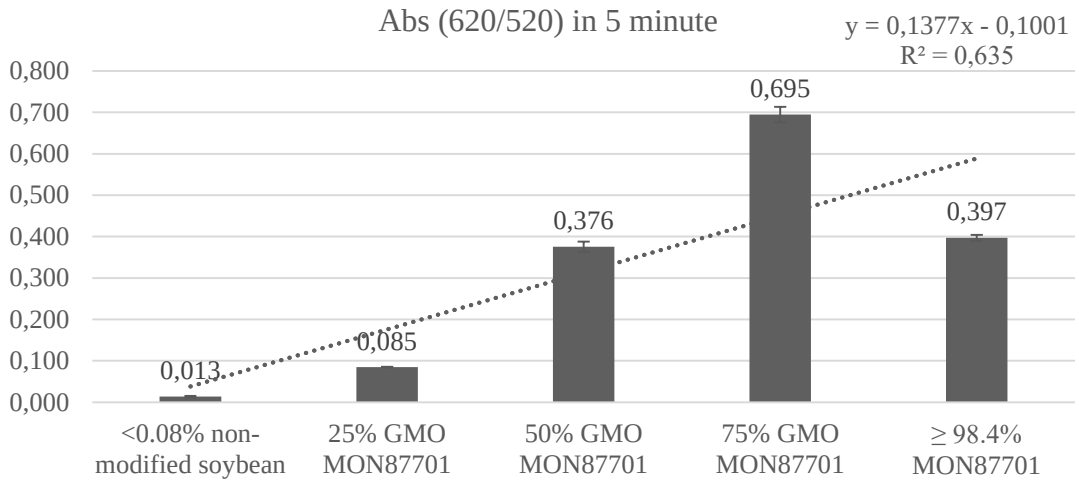
B

1'	Abs (620/520)	Standard deviation	Coefficient of variation
<0.08% non-modified soybean	0,009	0,001	0,421
25% GM MON87701	0,085	0,001	0,972
50% GM MON87701	0,372	0,027	18,107
75% GM MON87701	0,653	0,016	9,482
≥ 98.4% MON87701	0,296	0,006	4,569

Figure A.1. Batch Y8 **(A)** Abs (620/520) bar graphs with different levels of GMO in 1 minute **(B)** Normalized results of Abs (620/520), standard deviation and coefficient of variation with in 1 minute

Reads in 5 minute

A



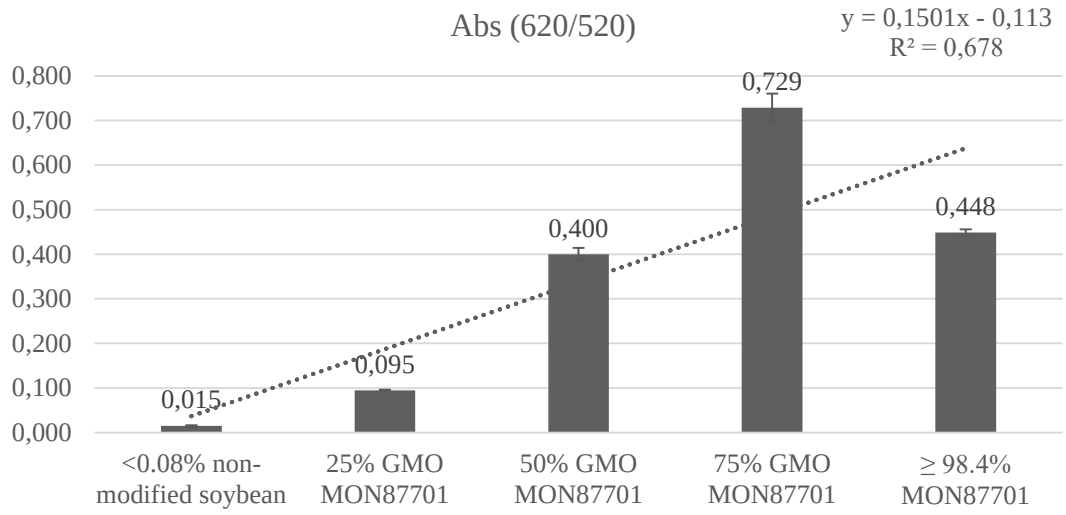
B

5'	Abs (620/520)	Standard deviation	Coefficient of variation
<0.08% non-modified soybean	0,013	0,002	1,375
25% GM MON87701	0,085	0,001	0,432
50% GM MON87701	0,376	0,012	8,515
75% GM MON87701	0,695	0,019	10,987
≥ 98.4% MON87701	0,397	0,008	5,660

Figure A.2. Batch Y8 **(A)** Abs (620/520) bar graphs with different levels of GMO in 5 minute **(B)** Normalized results of Abs (620/520), standard deviation and coefficient of variation with in 5 minute

Reads in 10 minute

A



B

10'	Abs (620/520)	Standard deviation	Coefficient of variation
<0.08% non-modified soybean	0,015	0,002	1,460
25% GM MON87701	0,095	0,001	0,687
50% GM MON87701	0,400	0,015	9,881
75% GM MON87701	0,729	0,031	17,432
≥ 98.4% MON87701	0,448	0,008	5,470

C

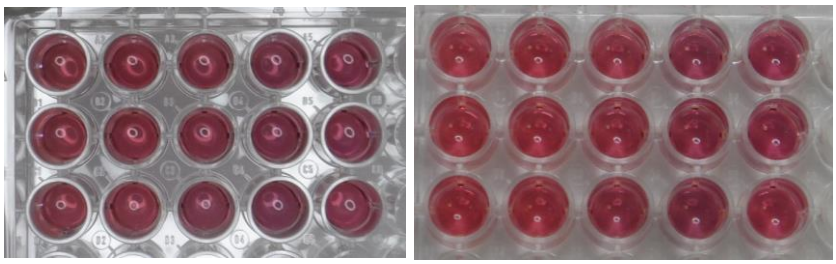


Figure A.3. Batch Y8 **(A)** Abs (620/520) bar graphs with different levels of GMO in 10 minute **(B)** Normalized results of Abs (620/520), standard deviation and coefficient of variation with in 10 minute **(C)** Visual readout, increasing GMO levels from left to right.

In this first 3 replicate, color change between non modified and ≥ 75% GM soybean-AuNP well is distinct and easily detectable with naked eye. On the other hand, at the level

of ≥ 98.4% GM soybean-AuNP well although there is a color transformation to purple and aggregation it was not enough due to impurities may present in the extract.

In the reaction well of ≥ 98.4% GM soybean, there is only 10 µl of extract which cause only that well to inhibit AuNP's aggregation. In the dilution of the other GMO levels wells the mixtures were able to dilute impurities. Even though the yield is the same as other extract, DNA contaminants the ratio of A260/A230 is a sensitive indicator of contaminants (e.g. ethanol) that absorb at 230 nm. These contaminants are significantly more numerous than those absorbing at 280 nm, and include chaotropic salts, EDTA, non-ionic, proteins, and phenol. Substances like polysaccharides, protein and fats (e.g., plants materials) absorb at this wavelength. Protein contamination affects the A260/A230. (Soybeans is a plant that rich in protein). With more concentrated DNA samples, the impact of protein contamination tends to be underestimate but in this case it cannot be due to 30ng/µl addition of gDNA to mixture occurs. To a certain extent, ethanol, commonly used in wash buffers presence may also influence aggregation. Therefore, any sample with contamination may lead to reduced A260/A230 ratios, slightly increased A260/A280 ratios, and greater variability in the measurements. Other reason could be the length of isolated gDNA or breakage in the Cry1Ac region from chemical or enzymatic reagents. Also most of the sensor work did not consider potential interactions between the contaminants and AuNP surface.

APPENDIX 1 – Absorbance Spectrums

Table A.1. Batch Y6 in 1 min

Wavelength [nm]	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640	650	660	670	680	690	700
<0.08% non-modified soybean	0,482	0,47	0,46	0,454	0,451	0,452	0,458	0,472	0,5	0,537	0,599	0,663	0,702	0,7	0,665	0,608	0,536	0,465	0,404	0,345	0,293	0,25	0,211	0,178	0,151	0,13	0,112	0,099	0,088	0,08	0,074
	0,489	0,477	0,467	0,461	0,458	0,459	0,465	0,48	0,507	0,544	0,607	0,67	0,709	0,706	0,669	0,611	0,537	0,466	0,404	0,345	0,293	0,25	0,211	0,179	0,153	0,132	0,115	0,102	0,091	0,083	0,077
	0,47	0,46	0,45	0,445	0,442	0,443	0,449	0,463	0,489	0,526	0,587	0,649	0,686	0,684	0,647	0,589	0,515	0,445	0,386	0,33	0,282	0,242	0,207	0,178	0,153	0,133	0,117	0,105	0,095	0,088	0,082
	0,469	0,458	0,448	0,442	0,439	0,44	0,446	0,461	0,487	0,523	0,584	0,646	0,683	0,679	0,641	0,583	0,511	0,441	0,382	0,326	0,277	0,237	0,2	0,17	0,146	0,126	0,11	0,098	0,088	0,081	0,075
	0,464	0,453	0,443	0,438	0,434	0,435	0,44	0,455	0,48	0,515	0,576	0,636	0,674	0,672	0,637	0,58	0,508	0,439	0,379	0,323	0,275	0,235	0,199	0,17	0,146	0,126	0,111	0,099	0,089	0,081	0,076
	0,484	0,472	0,463	0,457	0,454	0,455	0,461	0,475	0,503	0,541	0,604	0,668	0,708	0,708	0,67	0,611	0,535	0,462	0,4	0,341	0,29	0,247	0,208	0,177	0,152	0,131	0,115	0,102	0,091	0,084	0,077
	0,458	0,448	0,436	0,432	0,429	0,430	0,436	0,450	0,476	0,512	0,572	0,632	0,667	0,659	0,618	0,558	0,485	0,415	0,358	0,304	0,257	0,219	0,184	0,156	0,134	0,116	0,101	0,090	0,081	0,074	0,068
	0,464	0,453	0,442	0,437	0,435	0,435	0,441	0,456	0,482	0,518	0,578	0,639	0,675	0,67	0,632	0,575	0,503	0,433	0,373	0,317	0,269	0,229	0,192	0,163	0,14	0,12	0,105	0,093	0,084	0,078	0,071
	0,484	0,473	0,463	0,458	0,455	0,455	0,462	0,476	0,503	0,54	0,602	0,665	0,704	0,704	0,67	0,615	0,544	0,475	0,416	0,357	0,306	0,263	0,223	0,19	0,163	0,14	0,122	0,109	0,098	0,09	0,083
	0,413	0,403	0,393	0,389	0,386	0,386	0,391	0,404	0,426	0,457	0,511	0,563	0,594	0,588	0,556	0,507	0,447	0,389	0,34	0,292	0,251	0,216	0,184	0,157	0,136	0,117	0,103	0,092	0,083	0,077	0,071
	0,485	0,474	0,463	0,458	0,455	0,456	0,463	0,48	0,508	0,547	0,612	0,676	0,712	0,7	0,65	0,582	0,5	0,423	0,363	0,307	0,26	0,222	0,188	0,16	0,138	0,119	0,105	0,093	0,084	0,078	0,071
Mean	0,469273	0,458273	0,448	0,442818	0,439818	0,440545	0,446545	0,461091	0,487364	0,523636	0,584727	0,646091	0,683091	0,679091	0,641364	0,583545	0,511	0,441182	0,382273	0,326091	0,277545	0,237273	0,200636	0,170727	0,146545	0,126364	0,110545	0,098364	0,088364	0,081273	0,075
25% GM MON87701	0,504	0,492	0,481	0,475	0,471	0,471	0,476	0,49	0,516	0,552	0,614	0,678	0,72	0,725	0,701	0,655	0,595	0,534	0,478	0,422	0,369	0,322	0,276	0,236	0,202	0,172	0,148	0,13	0,114	0,103	0,094
	0,493	0,481	0,471	0,465	0,462	0,463	0,469	0,483	0,509	0,546	0,608	0,67	0,708	0,708	0,678	0,628	0,565	0,502	0,446	0,389	0,336	0,29	0,246	0,208	0,177	0,151	0,13	0,114	0,101	0,092	0,084
	0,333	0,324	0,317	0,313	0,311	0,312	0,316	0,326	0,344	0,368	0,41	0,451	0,475	0,47	0,443	0,404	0,356	0,312	0,273	0,236	0,203	0,175	0,15	0,129	0,112	0,097	0,086	0,078	0,071	0,066	0,061
	0,453	0,443	0,432	0,427	0,424	0,424	0,43	0,443	0,467	0,501	0,558	0,617	0,652	0,651	0,619	0,568	0,502	0,439	0,384	0,331	0,286	0,247	0,212	0,182	0,158	0,138	0,123	0,111	0,101	0,093	0,087
	0,482	0,471	0,46	0,455	0,451	0,452	0,457	0,471	0,495	0,53	0,588	0,646	0,7	0,676	0,641	0,587	0,519	0,453	0,397	0,343	0,296	0,256	0,222	0,192	0,169	0,15	0,134	0,123	0,113	0,106	0,087
	0,488	0,476	0,466	0,46	0,457	0,456	0,461	0,475	0,5	0,536	0,596	0,658	0,697	0,702	0,675	0,629	0,568	0,507	0,454	0,4	0,352	0,308	0,266	0,229	0,197	0,169	0,147	0,13	0,115	0,105	0,096
	0,469	0,458	0,447	0,443	0,44	0,44	0,446	0,461	0,486	0,522	0,583	0,643	0,68	0,677	0,643	0,589	0,521	0,455	0,398	0,342	0,292	0,25	0,211	0,179	0,152	0,131	0,113	0,101	0,089	0,082	0,089
	0,475	0,464	0,453	0,449	0,446	0,447	0,453	0,468	0,495	0,533	0,597	0,660	0,697	0,689	0,646	0,582	0,504	0,431	0,369	0,311	0,261	0,221	0,185	0,156	0,134	0,115	0,101	0,089	0,080	0,074	0,088
	0,466	0,456	0,445	0,44	0,437	0,437	0,442	0,455	0,48	0,514	0,572	0,631	0,668	0,669	0,64	0,593	0,531	0,47	0,417	0,363	0,315	0,274	0,234	0,2	0,171	0,147	0,129	0,114	0,103	0,094	0,087
	0,493	0,482	0,47	0,465	0,462	0,462	0,469	0,484	0,51	0,548	0,612	0,676	0,714	0,712	0,675	0,619	0,547	0,476	0,416	0,358	0,307	0,264	0,225	0,192	0,166	0,143	0,125	0,112	0,101	0,093	0,086
Mean	0,4656	0,4547	0,4442	0,4392	0,4361	0,4364	0,4419	0,4556	0,4802	0,515	0,5738	0,633	0,6711	0,6679	0,6361	0,5854	0,5208	0,4579	0,4032	0,3495	0,3017	0,2607	0,2227	0,1903	0,1638	0,1413	0,1236	0,1102	0,0988	0,0908	0,0859
50% GM MON87701	0,487	0,476	0,465	0,459	0,455	0,455	0,46	0,474	0,499	0,534	0,593	0,653	0,692	0,693	0,667	0,623	0,566	0,509	0,46	0,41	0,363	0,32	0,278	0,239	0,205	0,175	0,149	0,129	0,113	0,1	0,091
	0,489	0,477	0,467	0,461	0,458	0,458	0,464	0,478	0,503	0,537	0,597	0,656	0,693	0,694	0,669	0,627	0,574	0,52	0,473	0,424	0,376	0,332	0,287	0,246	0,21	0,178	0,153	0,133	0,116	0,104	0,094
	0,481	0,471	0,461	0,455	0,452	0,452	0,458	0,472	0,497	0,532	0,591	0,651	0,687	0,683	0,647	0,592	0,523	0,459	0,404	0,35	0,304	0,264	0,228	0,197	0,171	0,15	0,133	0,12	0,11	0,102	0,096
	0,454	0,444	0,434	0,429	0,426	0,427	0,433	0,447	0,471	0,506	0,566	0,624	0,659	0,654	0,618	0,564	0,495	0,43	0,376	0,324	0,278	0,238	0,203	0,173	0,148	0,128	0,112	0,1	0,089	0,082	0,076
	0,448	0,436	0,426	0,421	0,417	0,418	0,423	0,436	0,458	0,49	0,546	0,602	0,636	0,636	0,609	0,566	0,508	0,452	0,404	0,356	0,311	0,272	0,234	0,201	0,173	0,148	0,129	0,114	0,101	0,092	0,084
	0,523	0,512	0,502	0,497	0,494	0,494	0,500	0,514	0,539	0,573	0,632	0,692	0,699	0,728	0,699	0,652	0,592	0,533	0,482	0,432	0,386	0,344	0,304	0,268	0,237	0,210	0,189	0,172	0,158	0,149	0,098
	0,45	0,44	0,429	0,424	0,421	0,421	0,426	0,439	0,462	0,494	0,549	0,604	0,639	0,64	0,616	0,576	0,523	0,47	0,424	0,377	0,332	0,291	0,251	0,214	0,183	0,155	0,134	0,117	0,103	0,094	0,085
	0,479	0,468	0,456	0,451	0,447	0,446	0,45	0,462	0,485	0,518	0,575	0,632	0,67	0,675	0,657	0,623	0,575	0,527	0,484	0,439	0,394	0,35	0,305	0,263	0,225	0,191	0,163	0,141	0,123	0,111	0,099
	0,483	0,472	0,462	0,458	0,454	0,454	0,46	0,473	0,498	0,532	0,592	0,653	0,691	0,693	0,666	0,621	0,563	0,505	0,455	0,404	0,356	0,312	0,269	0,23	0,196	0,167	0,144	0,126	0,112	0,102	0,093
	0,458	0,448	0,436	0,432	0,428	0,429	0,435	0,449	0,473	0,507	0,564	0,621	0,656	0,652	0,621	0,573	0,513	0,456	0,406	0,357	0,313	0,272	0,233	0,199	0,169	0,144	0,124	0,11	0,097	0,088	0,081
Mean	0,4752	0,4644	0,4538	0,4487	0,4452	0,4454	0,4509	0,4644	0,4885	0,5223	0,5805	0,6388	0,6722	0,6748	0,6469	0,6017	0,5432	0,4861	0,4368	0,3873	0,3413	0,2995	0,2592	0,223	0,1917	0,1646	0,143	0,1262	0,1122	0,1024	0,0897
75% GM MON87701	0,505	0,493	0,482	0,476	0,472	0,471	0,476	0,489	0,512	0,546	0,606	0,666	0,707	0,715	0,7	0,668	0,622	0,573	0,526	0,476	0,425	0,375	0,325	0,278	0,237	0,201	0,172	0,149	0,131	0,117	0,106
	0,503	0,492	0,481	0,475	0,471	0,471	0,476	0,489	0,514	0,549	0,609	0,669	0,71	0,715	0,695	0,659	0,609	0,557	0,508	0,455	0,402	0,352	0,302	0,258	0,219	0,186	0,159	0,138	0,121	0,109	0,099
	0,452	0,441	0,432	0,427	0,424	0,425	0,431	0,445	0,47	0,504	0,562	0,62	0,7	0,653	0,619	0,566	0,5	0,436	0,38	0,326	0,278	0,237	0,201	0,171	0,146	0,126	0,111	0,099	0,089	0,082	0,095
	0,468	0,457	0,447	0,442	0,438	0,437	0,443	0,455	0,478	0,511	0,568	0,625	0,663	0,668	0,646	0,606	0,551	0,496	0,446	0,395	0,347	0,304	0,263	0,227	0,196	0,17	0,149	0,133	0,12	0,111	0,103
	0,474	0,463	0,452	0,446	0,442	0,442	0,447	0,46	0,483	0,515	0,572	0,63	0,668	0,673	0,652	0,613	0,56	0,505	0,456	0,405	0,356	0,312	0,27	0,232	0,2	0,173	0,151	0,134	0,12	0,11	0,102
	0,481	0,469	0,460	0,454	0,451	0,452	0,458	0,472	0,499	0,536	0,597	0,659	0,696	0,694	0,658	0,603	0,534	0,467	0,408	0,351	0,300	0,257	0,216	0,183	0,156	0,134	0,117	0,104	0,093	0,085	0,089
	0,483	0,471	0,461	0,456	0,453	0,453	0,459	0,474	0,5	0,536	0,598	0,66	0,697	0,695	0,663	0,612	0,547	0,483	0,427	0,371	0,32	0,275	0,233	0,197	0,168	0,144	0,124	0,109	0,097	0,089	0,098
	0,5	0,489	0,477	0,472	0,468	0,469	0,474	0,488	0,514	0,55	0,611	0,672	0,711	0,713	0,687	0,645	0,589	0,531	0,48	0,426	0,374	0,326	0,279	0,238	0,202	0,172	0,148	0,129	0,114	0,103	0,094
	0,474	0,463	0,452	0,447	0,443	0,444	0,449	0,462	0,486	0,519	0,577	0,636	0,674	0,676	0,653	0,612	0,559	0,504	0,454	0,402	0,353	0,309	0,265	0,227	0,194	0,166	0,144	0,127	0,113	0,103	0,095
	0,484	0,473	0,462	0,457	0,454	0,454	0,46	0,474	0,499	0,533	0,593	0,653	0,691	0,691	0,663	0,618	0,559	0,501	0,449	0,397	0,348	0,304	0,262	0,225	0,194	0,167	0,145	0,129	0,116	0,106	0,098
Mean	0,4824	0,4711	0,4606	0,4552	0,4516	0,4518	0,4573	0,4708	0,4955	0,5299	0,5893	0,649	0,6917	0,6893	0,6636	0,6202	0,563	0,5053	0,4534	0,4004	0,3503	0,3051	0,2616	0,2236	0,1912	0,1639	0,142	0,1251	0,1114	0,1015	0,0979
≥ 98.4% MON87701	0,499	0,488	0,477	0,47	0,466	0,467	0,471	0,485	0,509	0,543	0,602	0,661	0,701	0,707	0,688	0,654	0,609	0,56	0,514	0,464	0,413	0,364	0,314	0,269	0,229	0,194	0,166	0,145	0,127	0,113	0,103
	0,486	0,475	0,464	0,459	0,455	0,454	0,46	0,472	0,497	0,53	0,588	0,646	0,683	0,686	0,666	0,631	0,583	0,534	0,488	0,437	0,387	0,339	0,29	0,247	0,21	0,178	0,152	0,132	0,116	0,105	0,095
	0,468	0,457	0,447	0,441	0,438	0,438	0,444	0,458	0,482	0,516	0,573	0,632	0,678	0,668	0,641	0,598	0,542	0,486	0,436	0,385	0,337	0,294	0,252	0,215	0,184	0,158	0,136	0,12	0,106	0,097	0,088
	0,462	0,452	0,441	0,435	0,432	0,432	0,437	0,45	0,473	0,505	0,562	0,619	0,655	0,658	0,634	0,595	0,541	0,487	0,439	0,388	0,341	0,298	0,257	0,221	0,19	0,164	0,143	0,127	0,114	0,104	0,097
	0,467	0,456	0,444	0,44	0,436	0,437	0,443	0,457	0,482	0,516	0,577	0,635	0,688	0,67	0,639	0,591	0,529	0,468	0,413	0,359	0,308	0,264	0,224	0,19	0,162	0,139	0,12	0,107	0,095	0,087	0,098
	0,486	0,475	0,464	0,458	0,454	0,454	0,458	0,47	0,493	0,526	0,582	0,639	0,68	0,69	0,68	0,654	0,614	0,569	0,523	0,472	0,418	0,367	0,314	0,267	0,227	0,192	0,164	0,143	0,125	0,113	0,102
	0,501	0,49	0,479	0,474	0,47	0,47	0,474	0,487	0,511	0,545	0,604	0,664	0,705	0,713	0,697	0,665	0,619	0,568	0,519	0,465	0,412	0,361	0,311	0,267	0,229	0,195	0,169	0,147	0,129	0,117	0,106
	0,49	0,478	0,466	0,46	0,456	0,456	0,46	0,474	0,497	0,531	0,589	0,647	0,66	0,696	0,681	0,651	0,607	0,557	0,509	0,454	0,399	0,347	0,296	0,251	0,212	0,179	0,153	0,133	0,116	0,104	0,094
	0,488	0,477	0,466	0,461	0,457	0,456	0,461	0,474	0,497	0,531	0,587	0,644	0,68	0,682	0,662	0,63	0,589	0,549	0,513	0,474	0,431	0,388	0,34	0,293	0,25	0,21	0,177	0,151	0,13	0,115	0,103
	0,481	0,47	0,459	0,454	0,45	0,449	0,454	0,467	0,49	0,522	0,578	0,634	0,669	0,671	0,652	0,62	0,58	0,54	0,504	0,465	0,424	0,382	0,336	0,29	0,248	0,209	0,176	0,151	0,13	0,114	0,102
	0,474	0,463	0,452	0,447	0,443	0,442	0,447	0,46	0,482	0,514	0,569	0,624	0,658	0,658	0,639	0,609	0,571	0,532	0,498	0,461	0,42	0,378	0,332	0,286	0,243	0,204	0,172	0,147	0,126	0,112	0,099
Mean	0,482	0,471	0,459909	0,454455	0,450636	0,450455	0,455364	0,468545	0,492091	0,525364	0,582818	0,640455	0,677909	0,681727	0,661727	0,627091	0,580364	0,531818	0,486909	0,438545	0,39	0,343818	0,296909	0,254182	0,216727	0,183818	0,157091	0,136636	0,119455	0,107364	0,098818

Table A.2. Batch Y6 in 5 min

Wavelength [nm]	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640	650	660	670	680	690	700
<0.08% non-modified soybean	0,467	0,456	0,445	0,44	0,437	0,437	0,443	0,457	0,482	0,517	0,576	0,635	0,672	0,671	0,639	0,588	0,523	0,461	0,407	0,354	0,307	0,266	0,228	0,195	0,167	0,144	0,126	0,113	0,1	0,091	0,085
	0,469	0,458	0,447	0,442	0,439	0,439	0,445	0,459	0,484	0,519	0,578	0,637	0,673	0,67	0,637	0,586	0,521	0,458	0,404	0,351	0,304	0,264	0,226	0,194	0,167	0,144	0,126	0,113	0,101	0,092	0,085
	0,469	0,459	0,449	0,444	0,442	0,442	0,448	0,462	0,488	0,524	0,584	0,645	0,682	0,681	0,644	0,588	0,516	0,45	0,393	0,34	0,294	0,255	0,219	0,189	0,165	0,144	0,128	0,115	0,105	0,096	0,09
	0,53	0,519	0,507	0,501	0,497	0,497	0,503	0,517	0,544	0,582	0,646	0,713	0,757	0,761	0,732	0,68	0,612	0,543	0,485	0,427	0,376	0,332	0,289	0,253	0,222	0,196	0,175	0,159	0,145	0,135	0,126
	0,466	0,455	0,445	0,44	0,436	0,436	0,442	0,456	0,481	0,515	0,575	0,635	0,671	0,67	0,638	0,585	0,517	0,451	0,395	0,341	0,295	0,255	0,219	0,188	0,163	0,142	0,125	0,112	0,101	0,093	0,087
	0,484	0,472	0,462	0,457	0,454	0,454	0,46	0,475	0,501	0,538	0,601	0,664	0,704	0,704	0,669	0,613	0,541	0,472	0,412	0,355	0,305	0,263	0,224	0,192	0,165	0,143	0,125	0,111	0,1	0,092	0,084
	0,469	0,457	0,447	0,441	0,437	0,437	0,441	0,453	0,474	0,505	0,558	0,611	0,699	0,650	0,634	0,607	0,572	0,536	0,503	0,466	0,425	0,384	0,340	0,295	0,253	0,215	0,183	0,158	0,136	0,121	0,090
	0,465	0,455	0,443	0,439	0,436	0,437	0,442	0,456	0,482	0,517	0,577	0,636	0,672	0,668	0,633	0,58	0,512	0,448	0,393	0,339	0,292	0,251	0,214	0,182	0,157	0,135	0,118	0,105	0,094	0,086	0,079
	0,469	0,458	0,447	0,443	0,439	0,44	0,446	0,459	0,485	0,521	0,579	0,64	0,678	0,677	0,644	0,592	0,526	0,463	0,408	0,354	0,307	0,267	0,229	0,197	0,17	0,148	0,13	0,116	0,105	0,096	0,09
	0,403	0,393	0,384	0,38	0,377	0,377	0,382	0,394	0,414	0,444	0,494	0,543	0,573	0,568	0,538	0,493	0,437	0,384	0,34	0,297	0,259	0,226	0,195	0,169	0,146	0,128	0,113	0,102	0,092	0,086	0,08
	0,477	0,466	0,456	0,451	0,448	0,449	0,455	0,47	0,497	0,534	0,595	0,656	0,69	0,679	0,635	0,571	0,497	0,429	0,374	0,323	0,28	0,243	0,209	0,18	0,156	0,136	0,121	0,108	0,098	0,09	0,084
Mean	0,469818	0,458909	0,448364	0,443455	0,440182	0,440455	0,446091	0,459818	0,484727	0,519636	0,578455	0,637727	0,679182	0,672636	0,640273	0,589364	0,524909	0,463182	0,410364	0,358818	0,313091	0,273273	0,235636	0,203091	0,175545	0,152273	0,133636	0,119273	0,107	0,098	0,089091
25% GM MON87701	0,484	0,472	0,461	0,455	0,451	0,451	0,455	0,468	0,491	0,525	0,582	0,641	0,68	0,686	0,666	0,628	0,576	0,524	0,477	0,428	0,381	0,337	0,293	0,253	0,219	0,188	0,163	0,144	0,127	0,115	0,105
	0,472	0,461	0,449	0,445	0,441	0,441	0,446	0,46	0,483	0,516	0,574	0,631	0,666	0,667	0,641	0,599	0,545	0,493	0,446	0,398	0,351	0,309	0,267	0,229	0,197	0,169	0,146	0,128	0,114	0,103	0,094
	0,34	0,331	0,324	0,32	0,318	0,318	0,322	0,332	0,35	0,374	0,415	0,457	0,48	0,475	0,449	0,411	0,364	0,321	0,284	0,248	0,216	0,189	0,163	0,142	0,124	0,109	0,097	0,088	0,08	0,074	0,07
	0,471	0,46	0,449	0,445	0,441	0,441	0,447	0,46	0,482	0,515	0,572	0,629	0,665	0,665	0,635	0,587	0,525	0,465	0,413	0,363	0,319	0,28	0,244	0,214	0,189	0,167	0,15	0,138	0,126	0,118	0,111
	0,448	0,438	0,427	0,423	0,419	0,42	0,425	0,439	0,463	0,497	0,554	0,612	0,646	0,643	0,61	0,558	0,493	0,431	0,379	0,327	0,282	0,243	0,208	0,178	0,153	0,133	0,117	0,105	0,095	0,087	0,081
	0,497	0,485	0,474	0,468	0,464	0,464	0,469	0,481	0,506	0,54	0,599	0,66	0,7	0,705	0,681	0,639	0,583	0,527	0,476	0,426	0,379	0,336	0,294	0,256	0,223	0,194	0,17	0,151	0,135	0,124	0,114
	0,468	0,457	0,447	0,442	0,439	0,44	0,446	0,46	0,485	0,52	0,58	0,64	0,676	0,673	0,64	0,589	0,524	0,462	0,407	0,353	0,305	0,263	0,224	0,191	0,163	0,14	0,122	0,108	0,096	0,088	0,081
	0,457	0,446	0,435	0,431	0,427	0,427	0,432	0,445	0,468	0,501	0,557	0,614	0,649	0,649	0,621	0,576	0,518	0,462	0,413	0,364	0,320	0,280	0,242	0,209	0,181	0,157	0,138	0,123	0,111	0,101	0,094
	0,454	0,443	0,433	0,428	0,425	0,424	0,429	0,442	0,465	0,498	0,553	0,609	0,644	0,646	0,62	0,577	0,522	0,468	0,42	0,37	0,326	0,286	0,247	0,213	0,184	0,16	0,14	0,125	0,113	0,103	0,096
	0,475	0,464	0,454	0,449	0,446	0,446	0,451	0,465	0,49	0,526	0,586	0,646	0,684	0,682	0,65	0,598	0,533	0,471	0,417	0,365	0,318	0,277	0,238	0,205	0,177	0,154	0,135	0,121	0,109	0,1	0,093
Mean	0,456556	0,445667	0,435333	0,430556	0,427111	0,427222	0,432222	0,445222	0,468333	0,501222	0,557222	0,613889	0,649	0,649111	0,621333	0,576222	0,518333	0,462444	0,413222	0,364222	0,319667	0,28	0,242	0,209	0,181	0,157111	0,137778	0,123111	0,110556	0,101333	0,093889
50% GM MON87701	0,471	0,46	0,449	0,443	0,439	0,439	0,444	0,457	0,48	0,513	0,568	0,625	0,66	0,662	0,639	0,601	0,55	0,502	0,46	0,416	0,374	0,334	0,294	0,256	0,222	0,191	0,164	0,143	0,125	0,111	0,101
	0,472	0,461	0,449	0,444	0,44	0,44	0,445	0,458	0,481	0,513	0,569	0,625	0,659	0,66	0,638	0,603	0,556	0,511	0,472	0,43	0,388	0,348	0,305	0,265	0,228	0,195	0,167	0,145	0,126	0,112	0,101
	0,484	0,472	0,463	0,458	0,455	0,455	0,461	0,474	0,499	0,534	0,592	0,65	0,686	0,683	0,65	0,597	0,533	0,472	0,42	0,369	0,324	0,284	0,247	0,215	0,188	0,165	0,146	0,133	0,121	0,112	0,105
	0,473	0,462	0,452	0,448	0,444	0,445	0,45	0,464	0,488	0,521	0,58	0,638	0,673	0,669	0,635	0,583	0,518	0,457	0,405	0,355	0,311	0,272	0,235	0,204	0,177	0,156	0,138	0,125	0,113	0,105	0,099
	0,448	0,437	0,427	0,422	0,419	0,419	0,424	0,437	0,459	0,491	0,545	0,601	0,635	0,635	0,61	0,567	0,512	0,459	0,414	0,368	0,326	0,288	0,25	0,216	0,187	0,162	0,141	0,125	0,112	0,102	0,094
	0,465	0,454	0,443	0,438	0,435	0,435	0,440	0,453	0,476	0,509	0,565	0,621	0,656	0,656	0,630	0,588	0,534	0,482	0,437	0,392	0,349	0,310	0,269	0,233	0,202	0,173	0,150	0,133	0,118	0,107	0,098
	0,448	0,438	0,427	0,422	0,419	0,419	0,424	0,436	0,458	0,489	0,544	0,598	0,632	0,634	0,611	0,573	0,524	0,475	0,432	0,388	0,346	0,306	0,266	0,229	0,196	0,167	0,144	0,126	0,111	0,101	0,092
	0,479	0,468	0,456	0,451	0,447	0,446	0,45	0,462	0,485	0,517	0,573	0,63	0,667	0,672	0,653	0,62	0,573	0,526	0,485	0,442	0,399	0,357	0,314	0,272	0,234	0,201	0,173	0,151	0,132	0,12	0,108
	0,47	0,46	0,45	0,445	0,441	0,442	0,446	0,459	0,483	0,517	0,573	0,631	0,668	0,669	0,643	0,601	0,546	0,494	0,447	0,4	0,356	0,314	0,272	0,235	0,202	0,172	0,149	0,132	0,117	0,106	0,097
	0,442	0,431	0,421	0,416	0,413	0,413	0,418	0,432	0,454	0,486	0,541	0,594	0,626	0,624	0,595	0,553	0,5	0,449	0,406	0,362	0,322	0,283	0,245	0,211	0,181	0,155	0,134	0,118	0,105	0,095	0,097
Mean	0,4652	0,4543	0,4437	0,4387	0,4352	0,4353	0,4402	0,4532	0,4763	0,509	0,565	0,6213	0,6562	0,6564	0,6304	0,5886	0,5346	0,4827	0,4378	0,3922	0,3495	0,3096	0,2697	0,2336	0,2017	0,1737	0,1506	0,1331	0,118	0,1071	0,0992
75% GM MON87701	0,488	0,476	0,465	0,459	0,455	0,454	0,459	0,471	0,493	0,525	0,581	0,638	0,676	0,684	0,668	0,638	0,594	0,55	0,508	0,463	0,418	0,373	0,326	0,283	0,243	0,208	0,18	0,158	0,14	0,126	0,115
	0,481	0,47	0,458	0,453	0,449	0,449	0,454	0,467	0,489	0,522	0,578	0,635	0,672	0,676	0,657	0,624	0,578	0,531	0,488	0,441	0,394	0,349	0,303	0,261	0,224	0,192	0,166	0,145	0,129	0,116	0,106
	0,451	0,441	0,431	0,427	0,423	0,424	0,429	0,443	0,468	0,501	0,557	0,615	0,65	0,649	0,618	0,569	0,506	0,447	0,395	0,343	0,297	0,256	0,218	0,186	0,16	0,138	0,121	0,109	0,098	0,09	0,084
	0,468	0,458	0,447	0,442	0,439	0,439	0,443	0,456	0,479	0,511	0,566	0,624	0,662	0,667	0,645	0,605	0,552	0,498	0,45	0,4	0,354	0,312	0,271	0,236	0,205	0,179	0,158	0,142	0,129	0,119	0,111
	0,473	0,462	0,451	0,446	0,442	0,441	0,446	0,459	0,481	0,512	0,568	0,625	0,662	0,667	0,647	0,61	0,56	0,508	0,462	0,414	0,368	0,325	0,284	0,247	0,215	0,187	0,165	0,148	0,134	0,123	0,115
	0,481	0,470	0,459	0,453	0,450	0,450	0,455	0,468	0,491	0,524	0,580	0,637	0,674	0,677	0,654	0,615	0,563	0,511	0,464	0,416	0,370	0,326	0,284	0,246	0,212	0,184	0,161	0,143	0,129	0,118	0,110
	0,501	0,49	0,479	0,474	0,47	0,471	0,476	0,49	0,516	0,551	0,611	0,671	0,709	0,708	0,678	0,632	0,571	0,512	0,46	0,407	0,358	0,314	0,272	0,235	0,204	0,177	0,156	0,139	0,125	0,115	0,107
	0,548	0,535	0,523	0,516	0,511	0,51	0,514	0,527	0,551	0,583	0,642	0,702	0,739	0,742	0,718	0,68	0,629	0,578	0,53	0,48	0,431	0,384	0,337	0,294	0,256	0,223	0,197	0,176	0,159	0,147	0,137
	0,456	0,444	0,434	0,429	0,426	0,426	0,431	0,444	0,466	0,498	0,552	0,607	0,643	0,646	0,624	0,587	0,538	0,488	0,444	0,397	0,353	0,311	0,27	0,233	0,201	0,173	0,151	0,134	0,12	0,11	0,101
	0,465	0,454	0,443	0,439	0,435	0,435	0,44	0,453	0,476	0,509	0,565	0,62	0,656	0,657	0,633	0,593	0,541	0,49	0,445	0,398	0,354	0,313	0,273	0,237	0,206	0,179	0,158	0,14	0,126	0,116	0,107
Mean	0,4812	0,47	0,459	0,4538	0,45	0,4499	0,4547	0,4678	0,491	0,5236	0,58	0,6374	0,6743	0,6773	0,6542	0,6153	0,5632	0,5113	0,4646	0,4159	0,3697	0,3263	0,2838	0,2458	0,2126	0,184	0,1613	0,1434	0,1289	0,118	0,1093
≥ 98.4% MON87701	0,48	0,468	0,457	0,452	0,448	0,448	0,453	0,465	0,487	0,52	0,575	0,632	0,668	0,673	0,655	0,623	0,58	0,535	0,494	0,449	0,403	0,358	0,313	0,27	0,233	0,199	0,172	0,151	0,134	0,121	0,111
	0,469	0,458	0,446	0,441	0,437	0,437	0,441	0,454	0,476	0,508	0,563	0,618	0,652	0,655	0,636	0,604	0,561	0,517	0,476	0,432	0,386	0,342	0,297	0,256	0,219	0,187	0,162	0,142	0,126	0,113	0,103
	0,48	0,469	0,458	0,453	0,45	0,45	0,455	0,468	0,492	0,525	0,581	0,639	0,674	0,676	0,651	0,61	0,556	0,503	0,456	0,407	0,361	0,318	0,276	0,239	0,207	0,18	0,158	0,141	0,126	0,116	0,107
	0,465	0,454	0,444	0,439	0,435	0,435	0,439	0,452	0,475	0,507	0,563	0,619	0,655	0,658	0,635	0,596	0,545	0,493	0,446	0,398	0,353	0,311	0,27	0,235	0,204	0,178	0,156	0,14	0,126	0,117	0,108
	0,464	0,453	0,442	0,437	0,434	0,434	0,44	0,453	0,477	0,511	0,568	0,626	0,662	0,661	0,633	0,588	0,53	0,473	0,422	0,37	0,321	0,278	0,237	0,202	0,173	0,149	0,13	0,116	0,104	0,095	0,1
	0,487	0,476	0,464	0,459	0,455	0,453	0,458	0,469	0,49	0,521	0,575	0,63	0,669	0,681	0,673	0,653	0,621	0,584	0,546	0,501	0,453	0,404	0,353	0,304	0,261	0,223	0,192	0,168	0,148	0,133	0,121
	0,494	0,482	0,471	0,465	0,461	0,46	0,465	0,477	0,499	0,531	0,587	0,644	0,683	0,693	0,683	0,659	0,622	0,581	0,539	0,491	0,442	0,393	0,343	0,297	0,256	0,22	0,19	0,166	0,146	0,131	0,119
	0,484	0,472	0,46	0,455	0,451	0,45	0,454	0,466	0,488	0,519	0,575	0,63	0,668	0,678	0,668	0,645	0,609	0,567	0,527	0,479	0,429	0,38	0,33	0,283	0,242	0,207	0,178	0,156	0,137	0,123	0,112
	0,472	0,461	0,451	0,445	0,441	0,441	0,445	0,457	0,479	0,51	0,564	0,617	0,652	0,654	0,636	0,606	0,569	0,534	0,502	0,467	0,429	0,389	0,345	0,301	0,259	0,219	0,186	0,159	0,137	0,121	0,108
	0,464	0,452	0,442	0,436	0,433	0,432	0,436	0,448	0,469	0,5	0,552	0,604	0,638	0,64	0,623	0,595	0,56	0,527	0,497	0,464	0,428	0,39	0,347	0,304	0,262	0,223	0,19	0,162	0,14	0,123	0,122
	0,456	0,445	0,435	0,43	0,426	0,425	0,429	0,441	0,462	0,492	0,544	0,596	0,628	0,629	0,611	0,584	0,549	0,515	0,486	0,453	0,417	0,379	0,336	0,292	0,25	0,212	0,179	0,153	0,132	0,117	0,104
Mean	0,474091	0,462727	0,451818	0,446545	0,442818	0,442273	0,446818	0,459091	0,481273	0,513091	0,567909	0,623182	0,659	0,663455	0,645818	0,614818	0,572909	0,529909	0,490091	0,446455	0,402	0,358364	0,313364	0,271182	0,233273	0,199727	0,172091	0,150364	0,132364	0,119091	0,110455

Table A.3. Batch Y6 in 10 min

Wavelength [nm]	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640	650	660	670	680	690	700
<0.08% non-modified soybean	0,466	0,455	0,445	0,44	0,437	0,437	0,443	0,456	0,481	0,516	0,574	0,632	0,668	0,668	0,637	0,587	0,525	0,466	0,414	0,364	0,319	0,279	0,24	0,207	0,179	0,155	0,137	0,122	0,11	0,101	0,093
	0,467	0,456	0,445	0,441	0,438	0,438	0,443	0,457	0,481	0,516	0,574	0,633	0,668	0,666	0,634	0,583	0,52	0,459	0,407	0,357	0,311	0,272	0,234	0,202	0,175	0,152	0,134	0,119	0,108	0,099	0,091
	0,474	0,463	0,453	0,448	0,445	0,446	0,451	0,466	0,491	0,527	0,587	0,647	0,685	0,682	0,647	0,591	0,522	0,456	0,4	0,348	0,303	0,264	0,229	0,198	0,174	0,153	0,137	0,123	0,112	0,104	0,097
	0,462	0,452	0,442	0,437	0,434	0,434	0,440	0,453	0,477	0,512	0,571	0,629	0,664	0,661	0,628	0,575	0,511	0,449	0,398	0,348	0,303	0,264	0,228	0,197	0,171	0,149	0,131	0,117	0,106	0,097	0,090
	0,476	0,465	0,454	0,449	0,445	0,445	0,45	0,463	0,487	0,522	0,58	0,639	0,676	0,676	0,647	0,599	0,538	0,479	0,429	0,379	0,333	0,293	0,255	0,221	0,192	0,167	0,147	0,131	0,118	0,108	0,09
	0,482	0,471	0,46	0,455	0,452	0,452	0,458	0,472	0,497	0,534	0,594	0,656	0,696	0,696	0,664	0,611	0,544	0,479	0,424	0,371	0,323	0,282	0,243	0,209	0,181	0,158	0,139	0,124	0,112	0,103	0,094
	0,456	0,446	0,434	0,43	0,427	0,427	0,433	0,448	0,472	0,506	0,566	0,624	0,657	0,651	0,613	0,559	0,493	0,431	0,38	0,331	0,286	0,248	0,212	0,182	0,157	0,135	0,118	0,105	0,094	0,087	0,079
	0,462	0,451	0,44	0,435	0,432	0,433	0,439	0,452	0,476	0,511	0,569	0,627	0,662	0,659	0,626	0,576	0,514	0,454	0,403	0,352	0,308	0,268	0,231	0,199	0,171	0,148	0,13	0,116	0,104	0,096	0,087
	0,467	0,455	0,446	0,441	0,438	0,438	0,444	0,458	0,483	0,518	0,577	0,637	0,673	0,673	0,641	0,59	0,525	0,463	0,41	0,358	0,312	0,272	0,234	0,203	0,176	0,153	0,136	0,122	0,11	0,101	0,094
	0,402	0,392	0,382	0,379	0,376	0,376	0,38	0,392	0,413	0,443	0,493	0,542	0,572	0,566	0,536	0,491	0,435	0,383	0,34	0,298	0,261	0,229	0,198	0,172	0,15	0,131	0,117	0,105	0,095	0,088	0,082
	0,477	0,466	0,456	0,451	0,448	0,449	0,455	0,47	0,497	0,534	0,596	0,657	0,691	0,681	0,635	0,571	0,495	0,427	0,373	0,322	0,278	0,242	0,208	0,179	0,156	0,136	0,12	0,108	0,098	0,091	0,084
Mean	0,462818	0,452	0,441545	0,436909	0,433818	0,434091	0,439636	0,453364	0,477727	0,512636	0,571	0,629364	0,664727	0,661727	0,628	0,575727	0,511091	0,449636	0,398	0,348	0,303364	0,264818	0,228364	0,197182	0,171091	0,148818	0,131455	0,117455	0,106091	0,097727	0,089182
25% GM MON87701	0,488	0,477	0,466	0,46	0,456	0,456	0,46	0,472	0,495	0,529	0,586	0,645	0,683	0,69	0,668	0,63	0,579	0,527	0,48	0,432	0,387	0,345	0,302	0,263	0,229	0,198	0,173	0,154	0,137	0,125	0,1
	0,468	0,457	0,447	0,442	0,439	0,438	0,444	0,457	0,48	0,514	0,57	0,626	0,662	0,662	0,635	0,594	0,541	0,49	0,444	0,397	0,353	0,311	0,27	0,234	0,202	0,173	0,151	0,133	0,119	0,108	0,099
	0,355	0,346	0,338	0,334	0,331	0,331	0,335	0,345	0,363	0,387	0,428	0,469	0,493	0,489	0,463	0,426	0,38	0,338	0,302	0,267	0,235	0,207	0,181	0,157	0,139	0,122	0,11	0,1	0,091	0,085	0,079
	0,459	0,448	0,438	0,433	0,429	0,429	0,435	0,447	0,471	0,504	0,56	0,617	0,653	0,653	0,624	0,576	0,515	0,456	0,404	0,355	0,311	0,273	0,237	0,207	0,181	0,159	0,142	0,129	0,118	0,109	0,102
	0,46	0,45	0,439	0,434	0,43	0,43	0,435	0,448	0,472	0,505	0,562	0,618	0,653	0,65	0,621	0,575	0,517	0,46	0,411	0,363	0,318	0,279	0,241	0,208	0,18	0,157	0,138	0,124	0,111	0,102	0,094
	0,485	0,474	0,462	0,457	0,453	0,452	0,456	0,469	0,491	0,524	0,581	0,639	0,677	0,683	0,663	0,627	0,577	0,528	0,484	0,438	0,395	0,355	0,315	0,277	0,244	0,214	0,189	0,169	0,152	0,14	0,11
	0,467	0,457	0,446	0,442	0,439	0,439	0,444	0,459	0,483	0,518	0,577	0,636	0,672	0,67	0,637	0,588	0,525	0,465	0,413	0,361	0,315	0,273	0,234	0,201	0,173	0,149	0,13	0,116	0,103	0,095	0,086
	0,466	0,455	0,445	0,440	0,436	0,436	0,442	0,455	0,478	0,512	0,568	0,625	0,661	0,662	0,634	0,588	0,531	0,474	0,425	0,376	0,332	0,292	0,254	0,221	0,192	0,167	0,148	0,133	0,120	0,110	0,102
	0,46	0,449	0,439	0,434	0,431	0,431	0,436	0,448	0,471	0,505	0,559	0,615	0,651	0,652	0,625	0,58	0,523	0,469	0,42	0,372	0,329	0,29	0,251	0,219	0,191	0,167	0,147	0,132	0,12	0,111	0,103
	0,475	0,464	0,454	0,449	0,446	0,446	0,452	0,465	0,49	0,525	0,584	0,643	0,68	0,679	0,648	0,598	0,536	0,475	0,423	0,372	0,327	0,287	0,248	0,215	0,186	0,163	0,143	0,128	0,116	0,107	0,099
Mean	0,4583	0,4477	0,4374	0,4325	0,429	0,4288	0,4339	0,4465	0,4694	0,5023	0,5575	0,6133	0,6485	0,649008	0,6218	0,5782	0,5224	0,4682	0,4206	0,3733	0,330214	0,2912	0,2533	0,2202	0,1917	0,1669	0,1471	0,1318	0,1187	0,1092	0,0974
50% GM MON87701	0,474	0,463	0,452	0,447	0,443	0,443	0,448	0,46	0,483	0,516	0,571	0,628	0,663	0,665	0,642	0,604	0,555	0,507	0,465	0,422	0,382	0,343	0,302	0,265	0,231	0,199	0,172	0,151	0,133	0,119	0,108
	0,472	0,461	0,45	0,445	0,441	0,441	0,447	0,459	0,481	0,514	0,57	0,625	0,659	0,661	0,638	0,601	0,554	0,509	0,469	0,428	0,387	0,348	0,306	0,266	0,23	0,197	0,17	0,148	0,131	0,117	0,106
	0,484	0,473	0,463	0,457	0,454	0,454	0,459	0,472	0,497	0,531	0,588	0,647	0,683	0,68	0,647	0,596	0,533	0,474	0,423	0,374	0,329	0,289	0,252	0,218	0,19	0,166	0,147	0,132	0,119	0,111	0,103
	0,466	0,455	0,446	0,441	0,438	0,438	0,443	0,456	0,481	0,515	0,572	0,63	0,664	0,662	0,628	0,576	0,512	0,452	0,402	0,353	0,309	0,27	0,234	0,202	0,176	0,153	0,136	0,122	0,111	0,102	0,096
	0,466	0,455	0,445	0,44	0,437	0,436	0,442	0,453	0,476	0,508	0,563	0,619	0,654	0,654	0,628	0,586	0,531	0,479	0,433	0,389	0,346	0,308	0,27	0,235	0,205	0,179	0,158	0,141	0,127	0,117	0,108
	0,466	0,455	0,445	0,440	0,437	0,436	0,441	0,453	0,476	0,509	0,564	0,620	0,655	0,656	0,630	0,589	0,536	0,485	0,442	0,398	0,356	0,317	0,278	0,241	0,209	0,180	0,158	0,140	0,124	0,130	0,103
	0,445	0,435	0,424	0,419	0,416	0,416	0,42	0,433	0,454	0,485	0,538	0,591	0,626	0,626	0,604	0,567	0,52	0,473	0,433	0,392	0,351	0,313	0,273	0,237	0,204	0,175	0,151	0,133	0,118	0,107	0,097
	0,474	0,464	0,452	0,447	0,443	0,442	0,446	0,457	0,479	0,51	0,564	0,62	0,656	0,66	0,643	0,612	0,571	0,528	0,491	0,453	0,412	0,373	0,331	0,29	0,252	0,218	0,189	0,165	0,146	0,132	0,119
	0,472	0,46	0,452	0,447	0,444	0,443	0,448	0,461	0,484	0,517	0,574	0,631	0,668	0,67	0,645	0,604	0,55	0,5	0,455	0,409	0,366	0,325	0,283	0,245	0,212	0,182	0,158	0,139	0,124	0,112	0,103
	0,442	0,431	0,421	0,416	0,413	0,413	0,418	0,43	0,453	0,485	0,539	0,592	0,624	0,622	0,594	0,551	0,498	0,449	0,407	0,364	0,324	0,287	0,249	0,215	0,185	0,159	0,138	0,122	0,108	0,098	0,09
Mean	0,4661	0,4552	0,445	0,4399	0,4366	0,4362	0,4412	0,4534	0,4764	0,509	0,5643	0,6203	0,6552	0,6556	0,6299	0,5886	0,536	0,4856	0,442	0,3982	0,3562	0,3173	0,2778	0,2414	0,2094	0,1808	0,1577	0,1393	0,1241	0,1145	0,1033
75% GM MON87701	0,483	0,472	0,461	0,455	0,451	0,45	0,454	0,466	0,488	0,52	0,574	0,63	0,668	0,675	0,659	0,629	0,589	0,546	0,506	0,462	0,419	0,376	0,331	0,289	0,251	0,216	0,189	0,166	0,148	0,134	0,123
	0,477	0,466	0,455	0,45	0,446	0,446	0,451	0,463	0,485	0,517	0,572	0,628	0,664	0,668	0,65	0,617	0,574	0,53	0,489	0,445	0,4	0,357	0,312	0,271	0,234	0,202	0,176	0,155	0,138	0,125	0,115
	0,480	0,468	0,458	0,452	0,449	0,449	0,454	0,466	0,489	0,522	0,578	0,635	0,672	0,673	0,650	0,611	0,560	0,508	0,463	0,416	0,371	0,329	0,288	0,251	0,218	0,189	0,167	0,149	0,134	0,123	0,113
	0,471	0,46	0,45	0,444	0,44	0,441	0,445	0,457	0,48	0,512	0,567	0,624	0,661	0,666	0,645	0,607	0,555	0,503	0,456	0,409	0,364	0,323	0,283	0,247	0,216	0,189	0,168	0,152	0,138	0,128	0,119
	0,468	0,456	0,446	0,441	0,437	0,436	0,441	0,453	0,475	0,507	0,562	0,618	0,655	0,659	0,64	0,605	0,556	0,506	0,461	0,415	0,37	0,328	0,288	0,251	0,219	0,191	0,168	0,151	0,136	0,126	0,116
	0,483	0,471	0,46	0,456	0,452	0,453	0,458	0,472	0,498	0,533	0,593	0,653	0,69	0,687	0,654	0,604	0,541	0,479	0,426	0,373	0,325	0,282	0,241	0,207	0,178	0,154	0,135	0,121	0,109	0,1	0,092
	0,472	0,461	0,45	0,446	0,442	0,443	0,449	0,462	0,486	0,52	0,578	0,637	0,672	0,672	0,646	0,602	0,547	0,493	0,445	0,396	0,35	0,307	0,265	0,228	0,196	0,168	0,146	0,128	0,114	0,103	0,094
	0,546	0,534	0,521	0,515	0,511	0,509	0,514	0,527	0,549	0,582	0,639	0,697	0,734	0,736	0,717	0,686	0,644	0,601	0,561	0,519	0,474	0,429	0,382	0,337	0,295	0,258	0,227	0,203	0,183	0,169	0,155
	0,455	0,444	0,434	0,429	0,426	0,426	0,431	0,443	0,465	0,497	0,551	0,606	0,642	0,644	0,623	0,586	0,537	0,489	0,446	0,401	0,357	0,316	0,275	0,239	0,207	0,179	0,157	0,139	0,125	0,115	0,106
	0,461	0,45	0,441	0,435	0,432	0,432	0,437	0,449	0,472	0,504	0,559	0,614	0,649	0,651	0,627	0,589	0,539	0,49	0,447	0,402	0,359	0,32	0,281	0,246	0,215	0,187	0,165	0,147	0,133	0,121	0,112
Mean	0,4796	0,4682	0,4576	0,4523	0,4486	0,4485	0,4534	0,4658	0,4887	0,5214	0,5773	0,6342	0,6707	0,6731	0,6511	0,613629	0,5642	0,5145	0,47	0,4238	0,3789	0,3367	0,2946	0,2566	0,2229	0,1933	0,1698	0,1511	0,1358	0,1244	0,1145
≥ 98.4% MON87701	0,474	0,463	0,452	0,447	0,443	0,442	0,447	0,459	0,481	0,513	0,567	0,622	0,658	0,662	0,645	0,614	0,573	0,531	0,492	0,449	0,406	0,363	0,319	0,278	0,24	0,207	0,181	0,159	0,142	0,129	0,118
	0,465	0,454	0,443	0,438	0,434	0,433	0,438	0,45	0,471	0,503	0,556	0,609	0,643	0,646	0,627	0,597	0,556	0,515	0,477	0,434	0,391	0,349	0,304	0,264	0,228	0,196	0,17	0,149	0,133	0,121	0,111
	0,514	0,502	0,492	0,487	0,483	0,483	0,487	0,5	0,524	0,556	0,612	0,669	0,705	0,706	0,681	0,641	0,59	0,539	0,492	0,445	0,399	0,357	0,315	0,278	0,245	0,217	0,194	0,175	0,161	0,149	0,14
	0,485	0,473	0,463	0,458	0,453	0,453	0,458	0,47	0,494	0,526	0,582	0,638	0,675	0,678	0,654	0,615	0,562	0,509	0,462	0,414	0,368	0,326	0,286	0,25	0,219	0,192	0,171	0,155	0,141	0,131	0,122
	0,483	0,472	0,461	0,456	0,452	0,451	0,455	0,467	0,488	0,519	0,573	0,627	0,662	0,667	0,651	0,624	0,587	0,548	0,512	0,473	0,431	0,391	0,346	0,304	0,264	0,229	0,199	0,176	0,156	0,141	0,129
	0,484	0,473	0,461	0,456	0,451	0,45	0,454	0,465	0,485	0,515	0,567	0,621	0,659	0,67	0,664	0,647	0,62	0,587	0,554	0,515	0,472	0,427	0,378	0,331	0,287	0,248	0,215	0,189	0,166	0,15	0,136
	0,552	0,54	0,528	0,523	0,518	0,517	0,52	0,531	0,551	0,581	0,635	0,689	0,728	0,739	0,736	0,721	0,694	0,661	0,629	0,591	0,548	0,504	0,457	0,41	0,365	0,324	0,288	0,259	0,234	0,216	0,2
	0,477	0,466	0,453	0,448	0,444	0,442	0,446	0,458	0,478	0,507	0,56	0,614	0,651	0,66	0,653	0,635	0,605	0,57	0,535	0,495	0,45	0,406	0,358	0,312	0,271	0,235	0,203	0,179	0,159	0,144	0,13
	0,467	0,456	0,446	0,44	0,437	0,436	0,44	0,452	0,473	0,504	0,556	0,609	0,642	0,645	0,628	0,6	0,565	0,532	0,502	0,47	0,435	0,398	0,354	0,311	0,268	0,228	0,194	0,166	0,143	0,126	0,112
	0,461	0,45	0,44	0,435	0,431	0,43	0,434	0,445	0,466	0,497	0,548	0,6	0,633	0,636	0,619	0,592	0,558	0,525	0,496	0,464	0,43	0,394	0,352	0,31	0,268	0,229	0,195	0,167	0,144	0,127	0,113
	0,456	0,445	0,434	0,429	0,425	0,424	0,429	0,44	0,461	0,491	0,543	0,594	0,626	0,627	0,61	0,582	0,547	0,514	0,485	0,454	0,419	0,382	0,339	0,296	0,255	0,216	0,183	0,157	0,135	0,12	0,107
Mean	0,483455	0,472182	0,461182	0,456091	0,451909	0,451	0,455273	0,467	0,488364	0,519273	0,572636	0,626545	0,662	0,666909	0,651636	0,624364	0,587	0,548273	0,512364	0,473091	0,431727	0,390636	0,3462	0,304	0,264545	0,229182	0,199364	0,175545	0,155818	0,141273	0,128909

Table A.4. Batch Y8 in 1 min

Wavelength [nm]	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640	650	660	670	680	690	700
	0,457	0,446	0,435	0,431	0,428	0,43	0,436	0,452	0,48	0,52	0,584	0,648	0,679	0,661	0,603	0,525	0,437	0,359	0,299	0,247	0,206	0,176	0,151	0,131	0,115	0,101	0,091	0,083	0,076	0,071	0,066
	0,45	0,44	0,429	0,424	0,422	0,422	0,429	0,445	0,472	0,511	0,574	0,637	0,668	0,649	0,592	0,514	0,425	0,347	0,286	0,234	0,194	0,164	0,141	0,122	0,108	0,096	0,087	0,08	0,073	0,069	0,064
	0,449	0,438	0,427	0,423	0,42	0,421	0,428	0,444	0,471	0,509	0,573	0,635	0,667	0,649	0,593	0,517	0,429	0,351	0,291	0,239	0,198	0,168	0,144	0,125	0,11	0,097	0,087	0,08	0,073	0,068	0,063
	0,448	0,437	0,427	0,422	0,42	0,421	0,428	0,443	0,47	0,509	0,572	0,633	0,664	0,646	0,59	0,516	0,429	0,353	0,294	0,243	0,204	0,174	0,149	0,13	0,114	0,102	0,091	0,084	0,077	0,072	0,067
<0.08% non-modified soybean	0,429	0,418	0,408	0,403	0,4	0,401	0,407	0,421	0,447	0,483	0,541	0,599	0,627	0,611	0,56	0,492	0,414	0,345	0,292	0,246	0,209	0,181	0,156	0,136	0,119	0,105	0,094	0,085	0,078	0,072	0,067
	0,441	0,429	0,419	0,414	0,411	0,412	0,417	0,432	0,457	0,494	0,556	0,621	0,662	0,659	0,615	0,544	0,455	0,372	0,305	0,248	0,205	0,173	0,147	0,128	0,112	0,1	0,09	0,083	0,076	0,071	0,067
	0,439	0,428	0,418	0,413	0,411	0,412	0,418	0,433	0,46	0,497	0,557	0,617	0,647	0,632	0,58	0,51	0,429	0,357	0,301	0,252	0,213	0,184	0,159	0,139	0,123	0,109	0,098	0,089	0,082	0,076	0,071
	0,454	0,443	0,432	0,427	0,424	0,425	0,432	0,447	0,474	0,512	0,575	0,636	0,667	0,651	0,597	0,525	0,441	0,366	0,308	0,257	0,217	0,186	0,16	0,139	0,123	0,109	0,098	0,088	0,081	0,076	0,07
	0,467	0,455	0,444	0,44	0,437	0,438	0,445	0,46	0,488	0,528	0,594	0,657	0,69	0,672	0,614	0,537	0,448	0,369	0,307	0,254	0,212	0,181	0,156	0,136	0,12	0,107	0,097	0,088	0,081	0,076	0,07
Mean	0,448222	0,437111	0,426556	0,421889	0,419222	0,420222	0,426667	0,441889	0,468778	0,507	0,569556	0,631444	0,663444	0,647778	0,593778	0,52	0,434111	0,357667	0,298111	0,246667	0,206444	0,176333	0,151444	0,131778	0,116	0,102889	0,092556	0,084444	0,077444	0,072333	0,067222
	0,459	0,448	0,437	0,432	0,429	0,43	0,437	0,452	0,479	0,518	0,582	0,644	0,676	0,66	0,607	0,535	0,453	0,382	0,326	0,277	0,238	0,206	0,177	0,154	0,133	0,116	0,102	0,091	0,082	0,075	0,069
	0,453	0,442	0,431	0,427	0,425	0,425	0,432	0,447	0,473	0,51	0,573	0,634	0,665	0,649	0,597	0,529	0,451	0,382	0,328	0,281	0,243	0,212	0,184	0,16	0,14	0,122	0,107	0,096	0,086	0,079	0,072
	0,456	0,444	0,433	0,428	0,425	0,426	0,433	0,448	0,474	0,512	0,575	0,636	0,667	0,651	0,6	0,53	0,451	0,382	0,327	0,28	0,241	0,21	0,183	0,159	0,139	0,121	0,107	0,095	0,086	0,079	0,071
	0,447	0,435	0,426	0,421	0,418	0,419	0,425	0,44	0,467	0,504	0,565	0,625	0,655	0,64	0,589	0,519	0,439	0,368	0,312	0,264	0,224	0,193	0,166	0,144	0,126	0,111	0,099	0,089	0,082	0,075	0,07
25% GM MON87701	0,442	0,43	0,419	0,415	0,412	0,412	0,418	0,432	0,456	0,491	0,549	0,606	0,636	0,622	0,577	0,516	0,445	0,383	0,335	0,292	0,256	0,226	0,199	0,175	0,154	0,135	0,119	0,106	0,095	0,087	0,079
	0,436	0,425	0,415	0,411	0,407	0,408	0,414	0,429	0,454	0,489	0,548	0,606	0,635	0,621	0,573	0,509	0,434	0,368	0,317	0,271	0,234	0,204	0,177	0,154	0,135	0,119	0,105	0,094	0,085	0,079	0,073
	0,457	0,446	0,434	0,429	0,426	0,427	0,433	0,448	0,475	0,514	0,576	0,639	0,672	0,659	0,607	0,536	0,452	0,377	0,317	0,264	0,221	0,189	0,161	0,14	0,123	0,109	0,098	0,089	0,081	0,076	0,071
	0,476	0,465	0,453	0,448	0,445	0,446	0,452	0,467	0,494	0,533	0,599	0,667	0,71	0,706	0,66	0,588	0,497	0,411	0,342	0,28	0,233	0,197	0,168	0,145	0,128	0,113	0,102	0,093	0,085	0,08	0,074
	0,481	0,469	0,457	0,452	0,449	0,449	0,456	0,471	0,498	0,537	0,602	0,665	0,699	0,686	0,636	0,569	0,49	0,419	0,363	0,312	0,27	0,236	0,205	0,179	0,157	0,138	0,122	0,108	0,097	0,089	0,081
Mean	0,456333	0,444889	0,433889	0,429222	0,426222	0,426889	0,433333	0,448222	0,474444	0,512	0,574333	0,635778	0,668333	0,654889	0,605111	0,536778	0,456889	0,385778	0,329667	0,280111	0,24	0,208111	0,18	0,156667	0,137222	0,120444	0,106778	0,095667	0,086556	0,079889	0,073333
	0,472	0,46	0,449	0,444	0,441	0,441	0,448	0,462	0,489	0,527	0,589	0,651	0,683	0,668	0,617	0,549	0,47	0,4	0,345	0,296	0,255	0,222	0,193	0,167	0,146	0,128	0,113	0,102	0,092	0,085	0,078
	0,455	0,444	0,433	0,428	0,425	0,426	0,431	0,446	0,47	0,506	0,566	0,625	0,656	0,644	0,6	0,541	0,473	0,413	0,366	0,325	0,288	0,257	0,227	0,199	0,175	0,151	0,132	0,115	0,102	0,092	0,082
	0,449	0,438	0,427	0,422	0,419	0,42	0,425	0,44	0,465	0,502	0,563	0,623	0,653	0,639	0,591	0,526	0,451	0,385	0,334	0,289	0,251	0,22	0,193	0,169	0,148	0,129	0,113	0,1	0,089	0,081	0,073
	0,436	0,424	0,415	0,409	0,406	0,407	0,412	0,426	0,45	0,484	0,541	0,598	0,627	0,616	0,574	0,516	0,45	0,39	0,344	0,302	0,267	0,237	0,208	0,183	0,161	0,14	0,123	0,109	0,097	0,088	0,08
50% GM MON87701	0,447	0,436	0,426	0,421	0,418	0,418	0,423	0,436	0,46	0,493	0,55	0,606	0,635	0,625	0,587	0,534	0,472	0,417	0,375	0,337	0,305	0,277	0,248	0,222	0,197	0,172	0,151	0,133	0,117	0,104	0,094
	0,436	0,425	0,415	0,412	0,408	0,408	0,414	0,428	0,453	0,487	0,546	0,603	0,633	0,62	0,575	0,514	0,443	0,379	0,329	0,284	0,247	0,217	0,189	0,165	0,145	0,127	0,112	0,101	0,09	0,083	0,076
	0,455	0,443	0,432	0,427	0,424	0,424	0,43	0,445	0,471	0,508	0,568	0,629	0,662	0,651	0,605	0,542	0,467	0,399	0,344	0,293	0,252	0,218	0,188	0,164	0,143	0,126	0,112	0,1	0,09	0,084	0,077
	0,459	0,448	0,436	0,431	0,428	0,429	0,434	0,449	0,475	0,511	0,573	0,634	0,667	0,656	0,611	0,55	0,477	0,41	0,356	0,306	0,263	0,228	0,197	0,171	0,149	0,13	0,114	0,102	0,092	0,085	0,077
	0,457	0,446	0,435	0,43	0,427	0,427	0,433	0,448	0,474	0,511	0,574	0,635	0,667	0,654	0,606	0,54	0,462	0,392	0,335	0,283	0,242	0,208	0,179	0,155	0,136	0,12	0,106	0,095	0,086	0,08	0,074
Mean	0,451778	0,440444	0,429778	0,424889	0,421778	0,422222	0,427778	0,442222	0,467444	0,503222	0,563333	0,622667	0,653667	0,641444	0,596222	0,534667	0,462778	0,398333	0,347556	0,301667	0,263333	0,231556	0,202444	0,177222	0,155556	0,135889	0,119556	0,106333	0,095	0,086889	0,079
	0,452	0,441	0,43	0,425	0,421	0,422	0,427	0,442	0,468	0,504	0,565	0,625	0,657	0,645	0,6	0,539	0,467	0,402	0,352	0,305	0,265	0,232	0,201	0,174	0,151	0,131	0,114	0,101	0,089	0,082	0,073
	0,46	0,449	0,438	0,433	0,43	0,431	0,437	0,452	0,477	0,514	0,575	0,635	0,667	0,656	0,611	0,55	0,479	0,416	0,365	0,32	0,281	0,247	0,216	0,189	0,165	0,143	0,124	0,109	0,097	0,088	0,079
	0,451	0,44	0,428	0,424	0,42	0,42	0,426	0,44	0,464	0,499	0,558	0,616	0,647	0,636	0,596	0,54	0,475	0,417	0,371	0,328	0,291	0,26	0,23	0,202	0,177	0,154	0,133	0,117	0,102	0,092	0,082
	0,457	0,446	0,436	0,431	0,427	0,428	0,433	0,447	0,471	0,506	0,564	0,622	0,653	0,646	0,608	0,555	0,493	0,438	0,393	0,353	0,317	0,285	0,254	0,225	0,198	0,172	0,15	0,131	0,115	0,103	0,093
75% GM MON87701	0,432	0,421	0,412	0,407	0,403	0,404	0,408	0,421	0,444	0,477	0,532	0,587	0,616	0,608	0,572	0,521	0,463	0,409	0,366	0,326	0,29	0,258	0,227	0,199	0,173	0,15	0,131	0,115	0,102	0,092	0,084
	0,445	0,433	0,422	0,417	0,414	0,415	0,42	0,434	0,457	0,491	0,549	0,605	0,636	0,626	0,589	0,536	0,474	0,417	0,371	0,329	0,291	0,258	0,227	0,198	0,172	0,149	0,13	0,115	0,102	0,093	0,084
	0,472	0,46	0,449	0,444	0,44	0,441	0,446	0,46	0,485	0,522	0,583	0,643	0,678	0,671	0,633	0,578	0,512	0,451	0,399	0,35	0,307	0,27	0,235	0,204	0,177	0,154	0,134	0,118	0,105	0,096	0,088
	0,452	0,442	0,43	0,425	0,423	0,422	0,428	0,441	0,466	0,5	0,56	0,618	0,651	0,643	0,604	0,551	0,486	0,426	0,375	0,327	0,285	0,249	0,215	0,187	0,163	0,141	0,124	0,11	0,098	0,091	0,083
	0,453	0,442	0,431	0,425	0,422	0,422	0,428	0,441	0,465	0,499	0,557	0,615	0,647	0,64	0,604	0,553	0,492	0,435	0,388	0,342	0,302	0,268	0,235	0,206	0,181	0,158	0,138	0,122	0,109	0,099	0,09
Mean	0,452667	0,441556	0,430667	0,425667	0,422222	0,422778	0,428111	0,442	0,466333	0,501333	0,560333	0,618444	0,650222	0,641222	0,601889	0,547	0,482333	0,423444	0,375556	0,331111	0,292111	0,258556	0,226667	0,198222	0,173	0,150222	0,130889	0,115333	0,102111	0,092889	0,084
	0,462	0,45	0,439	0,434	0,43	0,43	0,435	0,449	0,473	0,509	0,568	0,627	0,66	0,655	0,62	0,571	0,511	0,455	0,408	0,362	0,321	0,283	0,247	0,213	0,184	0,157	0,135	0,118	0,103	0,093	0,083
	0,449	0,438	0,427	0,423	0,419	0,419	0,425	0,438	0,461	0,495	0,553	0,611	0,642	0,636	0,601	0,552	0,493	0,438	0,393	0,349	0,309	0,274	0,239	0,207	0,179	0,154	0,132	0,116	0,101	0,091	0,082
	0,463	0,451	0,439	0,434	0,43	0,43	0,436	0,45	0,474	0,509	0,569	0,628	0,662	0,655	0,619	0,569	0,508	0,451	0,405	0,359	0,318	0,281	0,246	0,214	0,185	0,159	0,136	0,118	0,103	0,093	0,083
	0,444	0,433	0,423	0,418	0,414	0,415	0,42	0,434	0,457	0,491	0,549	0,606	0,639	0,632	0,596	0,545	0,483	0,425	0,376	0,329	0,287	0,25	0,215	0,185	0,16	0,138	0,12	0,106	0,095	0,086	0,079
≥ 98.4% MON87701	0,443	0,433	0,423	0,419	0,415	0,415	0,42	0,433	0,457	0,491	0,549	0,606	0,637	0,63	0,594	0,542	0,481	0,424	0,377	0,332	0,292	0,257	0,223	0,193	0,167	0,144	0,125	0,11	0,098	0,089	0,082
	0,449	0,438	0,428	0,423	0,42	0,42	0,426	0,439	0,464	0,498	0,557	0,615	0,647	0,638	0,601	0,546	0,482	0,422	0,373	0,326	0,285	0,25	0,216	0,187	0,161	0,14	0,122	0,108	0,097	0,088	0,081
	0,453	0,442	0,43	0,425	0,422	0,422	0,427	0,441	0,466	0,502	0,562	0,623	0,658	0,652	0,614	0,559	0,49	0,425	0,369	0,315	0,269	0,23	0,196	0,168	0,146	0,126	0,111	0,099	0,089	0,082	0,075
	0,466	0,455	0,444	0,438	0,435	0,435	0,441	0,455	0,481	0,519	0,581	0,644	0,679	0,672	0,629	0,57	0,496	0,427	0,369	0,314	0,268	0,23	0,196	0,168	0,146	0,127	0,113	0,101	0,091	0,084	0,077
	0,454	0,444	0,432	0,427	0,424	0,425	0,431	0,445	0,471	0,507	0,569	0,63	0,664	0,654	0,61	0,548	0,474	0,405	0,347	0,293	0,249	0,213	0,182	0,157	0,137	0,12	0,107	0,096	0,087	0,081	0,075
Mean	0,453667	0,442667	0,431667	0,426778	0,423222	0,423444	0,429	0,442667	0,467111	0,502333	0,561889	0,621111	0,654222	0,647111	0,609333	0,555778	0,490889	0,430222	0,379667	0,331	0,288667	0,252	0,217778	0,188	0,162778	0,140556	0,122333	0,108	0,096	0,087444	0,079667

Table A.5. Batch Y8 in 5 min

Wavelength [nm]	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640	650	660	670	680	690	700
	0,456	0,444	0,434	0,43	0,427	0,428	0,435	0,451	0,479	0,518	0,582	0,645	0,676	0,657	0,6	0,523	0,434	0,358	0,298	0,247	0,207	0,177	0,152	0,132	0,116	0,103	0,092	0,084	0,077	0,072	0,067
	0,445	0,434	0,424	0,42	0,417	0,418	0,425	0,44	0,467	0,505	0,569	0,63	0,661	0,642	0,585	0,508	0,421	0,343	0,283	0,232	0,192	0,163	0,14	0,121	0,107	0,096	0,086	0,079	0,073	0,069	0,064
	0,446	0,434	0,424	0,42	0,417	0,418	0,425	0,44	0,467	0,505	0,569	0,63	0,661	0,643	0,588	0,512	0,425	0,349	0,289	0,237	0,198	0,168	0,144	0,125	0,11	0,097	0,087	0,08	0,073	0,068	0,063
	0,436	0,426	0,416	0,411	0,409	0,409	0,416	0,431	0,457	0,495	0,555	0,615	0,644	0,627	0,573	0,5	0,416	0,343	0,286	0,237	0,199	0,17	0,146	0,127	0,112	0,099	0,089	0,081	0,075	0,069	0,065
<0.08% non-modified soybean	0,427	0,416	0,406	0,401	0,398	0,399	0,405	0,419	0,443	0,479	0,537	0,594	0,621	0,604	0,554	0,487	0,411	0,344	0,292	0,247	0,211	0,184	0,16	0,14	0,123	0,11	0,098	0,089	0,081	0,075	0,07
	0,431	0,419	0,408	0,404	0,4	0,401	0,406	0,42	0,445	0,481	0,542	0,606	0,646	0,642	0,6	0,532	0,446	0,364	0,3	0,244	0,201	0,17	0,145	0,126	0,111	0,099	0,089	0,082	0,075	0,07	0,066
	0,456	0,444	0,434	0,43	0,427	0,428	0,435	0,451	0,479	0,518	0,582	0,645	0,676	0,657	0,6	0,523	0,434	0,358	0,298	0,247	0,207	0,177	0,152	0,132	0,116	0,103	0,092	0,084	0,077	0,072	0,067
	0,445	0,434	0,424	0,42	0,417	0,418	0,425	0,44	0,467	0,505	0,569	0,63	0,661	0,642	0,585	0,508	0,421	0,343	0,283	0,232	0,192	0,163	0,14	0,121	0,107	0,096	0,086	0,079	0,073	0,069	0,064
	0,446	0,434	0,424	0,42	0,417	0,418	0,425	0,44	0,467	0,505	0,569	0,63	0,661	0,643	0,588	0,512	0,425	0,349	0,289	0,237	0,198	0,168	0,144	0,125	0,11	0,097	0,087	0,08	0,073	0,068	0,063
Mean	0,443111	0,431667	0,421556	0,417333	0,414333	0,415222	0,421889	0,436889	0,463444	0,501222	0,563778	0,625	0,656333	0,639667	0,585889	0,511667	0,425889	0,350111	0,290889	0,24	0,200556	0,171111	0,147	0,127667	0,112444	0,1	0,089556	0,082	0,075222	0,070222	0,065444
	0,462	0,45	0,44	0,435	0,433	0,433	0,439	0,454	0,482	0,52	0,583	0,645	0,676	0,66	0,607	0,536	0,455	0,386	0,331	0,283	0,245	0,213	0,185	0,161	0,141	0,123	0,109	0,098	0,088	0,081	0,074
	0,452	0,44	0,429	0,426	0,422	0,423	0,429	0,444	0,47	0,507	0,569	0,629	0,659	0,644	0,593	0,526	0,449	0,381	0,33	0,284	0,247	0,216	0,189	0,165	0,144	0,126	0,111	0,099	0,088	0,081	0,074
	0,452	0,44	0,43	0,425	0,422	0,423	0,43	0,444	0,47	0,508	0,57	0,63	0,661	0,645	0,594	0,525	0,447	0,38	0,327	0,281	0,243	0,213	0,186	0,163	0,142	0,125	0,11	0,098	0,087	0,08	0,073
	0,439	0,427	0,416	0,412	0,409	0,41	0,416	0,431	0,456	0,493	0,553	0,612	0,641	0,625	0,575	0,507	0,429	0,36	0,307	0,26	0,222	0,192	0,166	0,144	0,126	0,111	0,099	0,089	0,081	0,075	0,07
25% GM MON87701	0,437	0,427	0,416	0,411	0,408	0,409	0,415	0,428	0,452	0,487	0,544	0,6	0,629	0,615	0,57	0,51	0,441	0,381	0,334	0,292	0,258	0,23	0,203	0,179	0,158	0,14	0,123	0,11	0,098	0,089	0,082
	0,432	0,421	0,409	0,406	0,403	0,403	0,409	0,424	0,447	0,483	0,542	0,598	0,626	0,611	0,564	0,5	0,427	0,362	0,312	0,268	0,232	0,203	0,177	0,155	0,137	0,12	0,106	0,095	0,086	0,079	0,073
	0,462	0,45	0,44	0,435	0,433	0,433	0,439	0,454	0,482	0,52	0,583	0,645	0,676	0,66	0,607	0,536	0,455	0,386	0,331	0,283	0,245	0,213	0,185	0,161	0,141	0,123	0,109	0,098	0,088	0,081	0,074
	0,452	0,44	0,429	0,426	0,422	0,423	0,429	0,444	0,47	0,507	0,569	0,629	0,659	0,644	0,593	0,526	0,449	0,381	0,33	0,284	0,247	0,216	0,189	0,165	0,144	0,126	0,111	0,099	0,088	0,081	0,074
	0,452	0,44	0,43	0,425	0,422	0,423	0,43	0,444	0,47	0,508	0,57	0,63	0,661	0,645	0,594	0,525	0,447	0,38	0,327	0,281	0,243	0,213	0,186	0,163	0,142	0,125	0,11	0,098	0,087	0,08	0,073
Mean	0,448889	0,437222	0,426556	0,422333	0,419333	0,42	0,426222	0,440778	0,466556	0,503667	0,564778	0,624222	0,654222	0,638778	0,588556	0,521222	0,444333	0,377444	0,325444	0,279556	0,242444	0,212111	0,185111	0,161778	0,141667	0,124333	0,109778	0,098222	0,087889	0,080778	0,074111
	0,467	0,455	0,444	0,44	0,436	0,436	0,443	0,457	0,483	0,52	0,581	0,642	0,673	0,659	0,609	0,542	0,465	0,398	0,345	0,298	0,259	0,227	0,197	0,172	0,151	0,132	0,116	0,105	0,094	0,087	0,08
	0,456	0,444	0,433	0,429	0,426	0,426	0,432	0,445	0,47	0,505	0,565	0,623	0,653	0,641	0,598	0,54	0,473	0,415	0,371	0,33	0,295	0,266	0,236	0,209	0,184	0,16	0,14	0,123	0,108	0,097	0,087
	0,447	0,435	0,425	0,421	0,417	0,417	0,423	0,438	0,463	0,499	0,559	0,618	0,648	0,634	0,586	0,522	0,449	0,384	0,334	0,29	0,254	0,224	0,197	0,173	0,152	0,133	0,116	0,103	0,091	0,083	0,075
	0,431	0,42	0,409	0,405	0,402	0,402	0,408	0,421	0,445	0,479	0,535	0,591	0,619	0,607	0,565	0,507	0,441	0,383	0,337	0,297	0,262	0,234	0,206	0,182	0,16	0,141	0,123	0,11	0,098	0,089	0,081
50% GM MON87701	0,45	0,438	0,427	0,422	0,419	0,419	0,424	0,437	0,46	0,493	0,549	0,604	0,632	0,622	0,583	0,531	0,471	0,418	0,377	0,341	0,31	0,283	0,256	0,23	0,205	0,181	0,159	0,141	0,124	0,112	0,1
	0,432	0,421	0,411	0,407	0,404	0,403	0,409	0,423	0,447	0,482	0,54	0,596	0,625	0,612	0,567	0,507	0,437	0,375	0,326	0,283	0,247	0,218	0,191	0,168	0,147	0,13	0,114	0,102	0,092	0,084	0,077
	0,467	0,455	0,444	0,44	0,436	0,436	0,443	0,457	0,483	0,52	0,581	0,642	0,673	0,659	0,609	0,542	0,465	0,398	0,345	0,298	0,259	0,227	0,197	0,172	0,151	0,132	0,116	0,105	0,094	0,087	0,08
	0,456	0,444	0,433	0,429	0,426	0,426	0,432	0,445	0,47	0,505	0,565	0,623	0,653	0,641	0,598	0,54	0,473	0,415	0,371	0,33	0,295	0,266	0,236	0,209	0,184	0,16	0,14	0,123	0,108	0,097	0,087
	0,447	0,435	0,425	0,421	0,417	0,417	0,423	0,438	0,463	0,499	0,559	0,618	0,648	0,634	0,586	0,522	0,449	0,384	0,334	0,29	0,254	0,224	0,197	0,173	0,152	0,133	0,116	0,103	0,091	0,083	0,075
Mean	0,450333	0,438556	0,427889	0,423778	0,420333	0,420222	0,426333	0,440111	0,464889	0,500222	0,559333	0,617444	0,647111	0,634333	0,589	0,528111	0,458111	0,396667	0,348889	0,306333	0,270556	0,241	0,212556	0,187556	0,165111	0,144667	0,126667	0,112778	0,1	0,091	0,082444
	0,452	0,44	0,43	0,425	0,422	0,422	0,428	0,442	0,467	0,503	0,564	0,624	0,655	0,643	0,598	0,537	0,466	0,402	0,352	0,307	0,268	0,235	0,205	0,178	0,155	0,134	0,117	0,103	0,091	0,083	0,075
	0,459	0,447	0,436	0,432	0,429	0,429	0,435	0,449	0,475	0,511	0,571	0,631	0,662	0,65	0,606	0,546	0,478	0,416	0,367	0,323	0,286	0,254	0,223	0,196	0,171	0,149	0,13	0,115	0,101	0,092	0,083
	0,449	0,437	0,426	0,422	0,418	0,419	0,424	0,438	0,461	0,495	0,554	0,611	0,641	0,631	0,59	0,536	0,473	0,415	0,371	0,33	0,295	0,265	0,235	0,208	0,183	0,159	0,139	0,121	0,106	0,095	0,086
	0,444	0,432	0,422	0,418	0,415	0,414	0,42	0,433	0,456	0,49	0,547	0,603	0,633	0,624	0,587	0,536	0,478	0,424	0,383	0,345	0,311	0,282	0,253	0,225	0,198	0,173	0,151	0,132	0,116	0,103	0,093
75% GM MON87701	0,425	0,414	0,404	0,4	0,396	0,396	0,401	0,414	0,436	0,468	0,523	0,576	0,605	0,596	0,56	0,511	0,453	0,401	0,36	0,322	0,288	0,257	0,227	0,199	0,174	0,151	0,132	0,116	0,102	0,092	0,084
	0,441	0,43	0,419	0,414	0,411	0,411	0,417	0,43	0,453	0,487	0,544	0,599	0,628	0,619	0,581	0,529	0,467	0,412	0,368	0,327	0,29	0,259	0,228	0,2	0,175	0,152	0,133	0,117	0,104	0,094	0,086
	0,452	0,44	0,43	0,425	0,422	0,422	0,428	0,442	0,467	0,503	0,564	0,624	0,655	0,643	0,598	0,537	0,466	0,402	0,352	0,307	0,268	0,235	0,205	0,178	0,155	0,134	0,117	0,103	0,091	0,083	0,075
	0,459	0,447	0,436	0,432	0,429	0,429	0,435	0,449	0,475	0,511	0,571	0,631	0,662	0,65	0,606	0,546	0,478	0,416	0,367	0,323	0,286	0,254	0,223	0,196	0,171	0,149	0,13	0,115	0,101	0,092	0,083
	0,449	0,437	0,426	0,422	0,418	0,419	0,424	0,438	0,461	0,495	0,554	0,611	0,641	0,631	0,59	0,536	0,473	0,415	0,371	0,33	0,295	0,265	0,235	0,208	0,183	0,159	0,139	0,121	0,106	0,095	0,086
Mean	0,447778	0,436	0,425444	0,421111	0,417778	0,417889	0,423556	0,437222	0,461222	0,495889	0,554667	0,612222	0,642444	0,631889	0,590667	0,534889	0,470222	0,411444	0,365667	0,323778	0,287444	0,256222	0,226	0,198667	0,173889	0,151111	0,132	0,115889	0,102	0,092111	0,083444
	0,457	0,446	0,435	0,43	0,427	0,427	0,432	0,445	0,47	0,504	0,562	0,62	0,654	0,648	0,614	0,564	0,506	0,451	0,406	0,362	0,322	0,286	0,25	0,218	0,188	0,161	0,139	0,121	0,106	0,095	0,085
	0,448	0,438	0,426	0,422	0,418	0,419	0,424	0,437	0,46	0,493	0,55	0,607	0,638	0,632	0,597	0,549	0,491	0,437	0,393	0,352	0,314	0,28	0,246	0,214	0,186	0,16	0,138	0,121	0,106	0,095	0,086
	0,459	0,448	0,436	0,431	0,428	0,428	0,433	0,446	0,47	0,505	0,564	0,622	0,655	0,648	0,613	0,563	0,503	0,449	0,404	0,36	0,322	0,286	0,252	0,22	0,191	0,164	0,141	0,123	0,107	0,096	0,086
	0,433	0,422	0,412	0,407	0,404	0,404	0,41	0,423	0,446	0,479	0,535	0,591	0,622	0,615	0,58	0,53	0,47	0,413	0,367	0,321	0,281	0,246	0,212	0,183	0,158	0,137	0,119	0,106	0,094	0,086	0,079
≥ 98.4% MON87701	0,434	0,423	0,413	0,409	0,406	0,406	0,411	0,424	0,447	0,481	0,537	0,593	0,624	0,617	0,58	0,53	0,47	0,415	0,37	0,327	0,288	0,255	0,222	0,193	0,167	0,144	0,125	0,11	0,098	0,089	0,081
	0,441	0,431	0,419	0,416	0,413	0,413	0,419	0,432	0,456	0,489	0,548	0,605	0,636	0,627	0,589	0,536	0,473	0,415	0,367	0,323	0,283	0,249	0,216	0,187	0,163	0,141	0,122	0,108	0,097	0,088	0,08
	0,457	0,446	0,435	0,43	0,427	0,427	0,432	0,445	0,47	0,504	0,562	0,62	0,654	0,648	0,614	0,564	0,506	0,451	0,406	0,362	0,322	0,286	0,25	0,218	0,188	0,161	0,139	0,121	0,106	0,095	0,085
	0,448	0,438	0,426	0,422	0,418	0,419	0,424	0,437	0,46	0,493	0,55	0,607	0,638	0,632	0,597	0,549	0,491	0,437	0,393	0,352	0,314	0,28	0,246	0,214	0,186	0,16	0,138	0,121	0,106	0,095	0,086
	0,459	0,448	0,436	0,431	0,428	0,428	0,433	0,446	0,47	0,505	0,564	0,622	0,655	0,648	0,613	0,563	0,503	0,449	0,404	0,36	0,322	0,286	0,252	0,22	0,191	0,164	0,141	0,123	0,107	0,096	0,086
Mean	0,448444	0,437778	0,426444	0,422	0,418778	0,419	0,424222	0,437222	0,461	0,494778	0,552444	0,609667	0,641778	0,635	0,599667	0,549778	0,490333	0,435222	0,39	0,346556	0,307556	0,272667	0,238444	0,207444	0,179778	0,154667	0,133556	0,117111	0,103	0,092778	0,083778

Table A.6 Batch Y8 in 10 min

Wavelength [nm]	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640	650	660	670	680	690	700
	0,456	0,444	0,434	0,43	0,427	0,429	0,435	0,451	0,478	0,518	0,582	0,644	0,675	0,657	0,599	0,522	0,434	0,358	0,299	0,248	0,209	0,179	0,153	0,133	0,117	0,104	0,093	0,085	0,078	0,073	0,068
	0,485	0,474	0,463	0,459	0,456	0,456	0,462	0,477	0,503	0,541	0,603	0,663	0,693	0,675	0,618	0,543	0,456	0,38	0,32	0,269	0,23	0,201	0,177	0,159	0,144	0,132	0,122	0,115	0,108	0,104	0,1
	0,446	0,434	0,424	0,42	0,417	0,418	0,425	0,44	0,467	0,505	0,568	0,629	0,66	0,643	0,587	0,512	0,425	0,349	0,289	0,238	0,198	0,169	0,144	0,125	0,11	0,097	0,087	0,079	0,073	0,068	0,064
	0,436	0,425	0,416	0,41	0,408	0,409	0,415	0,43	0,457	0,495	0,556	0,615	0,645	0,627	0,571	0,498	0,414	0,341	0,285	0,236	0,198	0,17	0,146	0,127	0,112	0,099	0,089	0,081	0,074	0,069	0,065
<0.08% non-modified soybean	0,426	0,416	0,405	0,401	0,398	0,398	0,404	0,418	0,443	0,478	0,536	0,592	0,619	0,603	0,552	0,486	0,409	0,343	0,292	0,247	0,212	0,185	0,161	0,141	0,125	0,11	0,099	0,089	0,081	0,075	0,07
	0,434	0,424	0,413	0,408	0,405	0,405	0,411	0,424	0,448	0,484	0,544	0,607	0,648	0,645	0,603	0,536	0,449	0,369	0,304	0,248	0,206	0,175	0,149	0,13	0,115	0,103	0,093	0,086	0,079	0,074	0,07
	0,456	0,444	0,434	0,43	0,427	0,429	0,435	0,451	0,478	0,518	0,582	0,644	0,675	0,657	0,599	0,522	0,434	0,358	0,299	0,248	0,209	0,179	0,153	0,133	0,117	0,104	0,093	0,085	0,078	0,073	0,068
	0,485	0,474	0,463	0,459	0,456	0,456	0,462	0,477	0,503	0,541	0,603	0,663	0,693	0,675	0,618	0,543	0,456	0,38	0,32	0,269	0,23	0,201	0,177	0,159	0,144	0,132	0,122	0,115	0,108	0,104	0,1
	0,446	0,434	0,424	0,42	0,417	0,418	0,425	0,44	0,467	0,505	0,568	0,629	0,66	0,643	0,587	0,512	0,425	0,349	0,289	0,238	0,198	0,169	0,144	0,125	0,11	0,097	0,087	0,079	0,073	0,068	0,064
Mean	0,452222	0,441	0,430667	0,426333	0,423444	0,424222	0,430444	0,445333	0,471556	0,509444	0,571333	0,631778	0,663111	0,647222	0,592667	0,519333	0,433556	0,358556	0,299667	0,249	0,21	0,180889	0,156	0,136889	0,121556	0,108667	0,098333	0,090444	0,083556	0,078667	0,074333
	0,456	0,445	0,434	0,429	0,427	0,428	0,434	0,449	0,476	0,514	0,577	0,638	0,668	0,653	0,6	0,529	0,449	0,381	0,327	0,28	0,242	0,211	0,183	0,16	0,139	0,121	0,106	0,095	0,085	0,077	0,071
	0,453	0,441	0,431	0,427	0,424	0,425	0,431	0,446	0,471	0,509	0,57	0,63	0,66	0,644	0,594	0,526	0,45	0,383	0,332	0,287	0,25	0,22	0,192	0,168	0,147	0,129	0,113	0,1	0,09	0,082	0,075
	0,456	0,444	0,434	0,429	0,426	0,426	0,433	0,448	0,474	0,511	0,573	0,633	0,663	0,647	0,596	0,528	0,45	0,383	0,331	0,285	0,249	0,219	0,192	0,169	0,148	0,131	0,115	0,103	0,093	0,085	0,078
	0,44	0,429	0,42	0,415	0,412	0,413	0,419	0,433	0,459	0,496	0,555	0,613	0,643	0,627	0,577	0,509	0,432	0,364	0,312	0,266	0,229	0,199	0,172	0,151	0,132	0,117	0,104	0,094	0,086	0,079	0,074
25% GM MON87701	0,437	0,426	0,416	0,411	0,408	0,409	0,414	0,428	0,452	0,486	0,544	0,599	0,627	0,613	0,568	0,509	0,441	0,381	0,335	0,295	0,262	0,234	0,208	0,185	0,163	0,144	0,127	0,113	0,101	0,091	0,084
	0,43	0,419	0,409	0,404	0,402	0,403	0,409	0,422	0,447	0,482	0,54	0,596	0,624	0,609	0,562	0,498	0,425	0,361	0,312	0,269	0,234	0,205	0,18	0,157	0,138	0,121	0,107	0,096	0,086	0,08	0,073
	0,456	0,445	0,434	0,429	0,427	0,428	0,434	0,449	0,476	0,514	0,577	0,638	0,668	0,653	0,6	0,529	0,449	0,381	0,327	0,28	0,242	0,211	0,183	0,16	0,139	0,121	0,106	0,095	0,085	0,077	0,071
	0,453	0,441	0,431	0,427	0,424	0,425	0,431	0,446	0,471	0,509	0,57	0,63	0,66	0,644	0,594	0,526	0,45	0,383	0,332	0,287	0,25	0,22	0,192	0,168	0,147	0,129	0,113	0,1	0,09	0,082	0,075
	0,456	0,444	0,434	0,429	0,426	0,426	0,433	0,448	0,474	0,511	0,573	0,633	0,663	0,647	0,596	0,528	0,45	0,383	0,331	0,285	0,249	0,219	0,192	0,169	0,148	0,131	0,115	0,103	0,093	0,085	0,078
Mean	0,448556	0,437111	0,427	0,422222	0,419556	0,420333	0,426444	0,441	0,466667	0,503556	0,564333	0,623333	0,652889	0,637444	0,587444	0,520222	0,444	0,377778	0,326556	0,281556	0,245222	0,215333	0,188222	0,165222	0,144556	0,127111	0,111778	0,099889	0,089889	0,082	0,075444
	0,466	0,455	0,445	0,439	0,436	0,436	0,443	0,457	0,483	0,52	0,581	0,64	0,671	0,657	0,608	0,541	0,465	0,399	0,347	0,3	0,262	0,23	0,201	0,175	0,153	0,134	0,119	0,106	0,096	0,088	0,081
	0,456	0,445	0,434	0,43	0,427	0,427	0,432	0,446	0,471	0,506	0,565	0,622	0,652	0,64	0,597	0,539	0,474	0,416	0,372	0,333	0,299	0,27	0,24	0,214	0,188	0,164	0,143	0,125	0,111	0,099	0,089
	0,444	0,433	0,423	0,418	0,415	0,415	0,421	0,435	0,46	0,496	0,556	0,614	0,644	0,629	0,582	0,518	0,445	0,381	0,332	0,289	0,253	0,224	0,197	0,173	0,152	0,133	0,116	0,103	0,091	0,083	0,075
	0,43	0,419	0,409	0,404	0,401	0,401	0,407	0,419	0,443	0,477	0,533	0,587	0,616	0,604	0,562	0,506	0,441	0,384	0,341	0,302	0,269	0,241	0,214	0,19	0,167	0,147	0,129	0,114	0,102	0,092	0,083
50% GM MON87701	0,454	0,443	0,432	0,427	0,424	0,424	0,429	0,442	0,465	0,498	0,553	0,607	0,636	0,625	0,586	0,535	0,475	0,423	0,384	0,349	0,319	0,293	0,267	0,242	0,217	0,193	0,171	0,151	0,134	0,12	0,109
	0,432	0,421	0,411	0,407	0,404	0,404	0,41	0,423	0,447	0,481	0,539	0,595	0,623	0,61	0,566	0,506	0,437	0,375	0,329	0,287	0,252	0,223	0,196	0,173	0,152	0,133	0,118	0,105	0,094	0,086	0,079
	0,466	0,455	0,445	0,439	0,436	0,436	0,443	0,457	0,483	0,52	0,581	0,64	0,671	0,657	0,608	0,541	0,465	0,399	0,347	0,3	0,262	0,23	0,201	0,175	0,153	0,134	0,119	0,106	0,096	0,088	0,081
	0,456	0,445	0,434	0,43	0,427	0,427	0,432	0,446	0,471	0,506	0,565	0,622	0,652	0,64	0,597	0,539	0,474	0,416	0,372	0,333	0,299	0,27	0,24	0,214	0,188	0,164	0,143	0,125	0,111	0,099	0,089
	0,444	0,433	0,423	0,418	0,415	0,415	0,421	0,435	0,46	0,496	0,556	0,614	0,644	0,629	0,582	0,518	0,445	0,381	0,332	0,289	0,253	0,224	0,197	0,173	0,152	0,133	0,116	0,103	0,091	0,083	0,075
Mean	0,449778	0,438778	0,428444	0,423556	0,420556	0,420556	0,426444	0,44	0,464778	0,5	0,558778	0,615667	0,645444	0,632333	0,587556	0,527	0,457889	0,397111	0,350667	0,309111	0,274222	0,245	0,217	0,192111	0,169111	0,148333	0,130444	0,115333	0,102889	0,093111	0,084556
	0,449	0,438	0,427	0,422	0,419	0,419	0,425	0,439	0,464	0,5	0,56	0,619	0,65	0,638	0,594	0,533	0,463	0,401	0,352	0,307	0,27	0,237	0,207	0,18	0,156	0,136	0,118	0,104	0,092	0,083	0,075
	0,454	0,442	0,432	0,427	0,424	0,425	0,43	0,445	0,47	0,506	0,566	0,625	0,656	0,644	0,6	0,541	0,472	0,412	0,364	0,322	0,284	0,253	0,222	0,195	0,17	0,148	0,129	0,113	0,1	0,09	0,082
	0,446	0,435	0,425	0,42	0,417	0,417	0,423	0,436	0,46	0,494	0,552	0,609	0,639	0,628	0,589	0,533	0,47	0,414	0,37	0,331	0,296	0,267	0,237	0,21	0,185	0,161	0,141	0,123	0,108	0,097	0,087
	0,445	0,434	0,424	0,419	0,416	0,417	0,422	0,434	0,457	0,49	0,545	0,599	0,63	0,622	0,586	0,537	0,48	0,43	0,39	0,354	0,322	0,294	0,265	0,238	0,211	0,185	0,162	0,143	0,125	0,112	0,101
75% GM MON87701	0,426	0,416	0,406	0,402	0,399	0,398	0,403	0,415	0,438	0,47	0,524	0,577	0,606	0,597	0,561	0,511	0,454	0,403	0,362	0,325	0,292	0,262	0,232	0,204	0,179	0,156	0,136	0,119	0,105	0,094	0,086
	0,439	0,428	0,418	0,413	0,41	0,41	0,415	0,428	0,451	0,484	0,54	0,595	0,625	0,615	0,578	0,527	0,467	0,413	0,371	0,331	0,297	0,266	0,235	0,207	0,181	0,157	0,138	0,121	0,107	0,097	0,088
	0,449	0,438	0,427	0,422	0,419	0,419	0,425	0,439	0,464	0,5	0,56	0,619	0,65	0,638	0,594	0,533	0,463	0,401	0,352	0,307	0,27	0,237	0,207	0,18	0,156	0,136	0,118	0,104	0,092	0,083	0,075
	0,454	0,442	0,432	0,427	0,424	0,425	0,43	0,445	0,47	0,506	0,566	0,625	0,656	0,644	0,6	0,541	0,472	0,412	0,364	0,322	0,284	0,253	0,222	0,195	0,17	0,148	0,129	0,113	0,1	0,09	0,082
	0,446	0,435	0,425	0,42	0,417	0,417	0,423	0,436	0,46	0,494	0,552	0,609	0,639	0,628	0,589	0,533	0,47	0,414	0,37	0,331	0,296	0,267	0,237	0,21	0,185	0,161	0,141	0,123	0,108	0,097	0,087
Mean	0,445333	0,434222	0,424	0,419111	0,416111	0,416333	0,421778	0,435222	0,459333	0,493778	0,551667	0,608556	0,639	0,628222	0,587889	0,532111	0,467889	0,411111	0,366111	0,325556	0,290111	0,259556	0,229333	0,202111	0,177	0,154222	0,134667	0,118111	0,104111	0,093667	0,084778
	0,455	0,444	0,434	0,428	0,425	0,425	0,43	0,443	0,467	0,502	0,56	0,618	0,65	0,645	0,61	0,561	0,502	0,449	0,404	0,361	0,322	0,287	0,251	0,219	0,189	0,162	0,139	0,121	0,106	0,095	0,085
	0,45	0,439	0,429	0,424	0,42	0,42	0,425	0,438	0,461	0,495	0,552	0,608	0,639	0,633	0,598	0,549	0,491	0,438	0,395	0,353	0,316	0,283	0,249	0,218	0,189	0,163	0,141	0,123	0,108	0,097	0,087
	0,456	0,444	0,433	0,428	0,424	0,425	0,43	0,443	0,467	0,501	0,56	0,617	0,65	0,642	0,608	0,558	0,5	0,446	0,402	0,36	0,321	0,287	0,253	0,221	0,192	0,165	0,142	0,124	0,108	0,096	0,086
	0,431	0,421	0,411	0,406	0,404	0,404	0,409	0,421	0,445	0,478	0,533	0,588	0,619	0,613	0,579	0,529	0,471	0,416	0,371	0,327	0,288	0,253	0,22	0,19	0,164	0,142	0,123	0,109	0,097	0,088	0,081
≥ 98.4% MON87701	0,435	0,424	0,414	0,409	0,406	0,406	0,411	0,424	0,448	0,481	0,537	0,592	0,623	0,615	0,579	0,529	0,47	0,415	0,371	0,329	0,292	0,259	0,226	0,197	0,171	0,148	0,129	0,113	0,1	0,09	0,082
	0,441	0,43	0,419	0,415	0,412	0,413	0,418	0,431	0,455	0,489	0,547	0,603	0,634	0,625	0,587	0,535	0,472	0,414	0,368	0,324	0,286	0,252	0,22	0,19	0,165	0,143	0,125	0,11	0,098	0,089	0,081
	0,455	0,444	0,434	0,428	0,425	0,425	0,43	0,443	0,467	0,502	0,56	0,618	0,65	0,645	0,61	0,561	0,502	0,449	0,404	0,361	0,322	0,287	0,251	0,219	0,189	0,162	0,139	0,121	0,106	0,095	0,085
	0,45	0,439	0,429	0,424	0,42	0,42	0,425	0,438	0,461	0,495	0,552	0,608	0,639	0,633	0,598	0,549	0,491	0,438	0,395	0,353	0,316	0,283	0,249	0,218	0,189	0,163	0,141	0,123	0,108	0,097	0,087
	0,456	0,444	0,433	0,428	0,424	0,425	0,43	0,443	0,467	0,501	0,56	0,617	0,65	0,642	0,608	0,558	0,5	0,446	0,402	0,36	0,321	0,287	0,253	0,221	0,192	0,165	0,142	0,124	0,108	0,096	0,086
Mean	0,447667	0,436556	0,426222	0,421111	0,417778	0,418111	0,423111	0,436	0,459778	0,493778	0,551222	0,607667	0,639333	0,632556	0,597444	0,547667	0,488778	0,434556	0,390222	0,347556	0,309333	0,275333	0,241333	0,210333	0,182222	0,157	0,135667	0,118667	0,104333	0,093667	0,084444

Table A.7. Batch Y5 in 1 min

Wavelength [nm]	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640	650	660	670	680	690	700
	0,596	0,582	0,569	0,563	0,559	0,559	0,566	0,583	0,617	0,665	0,743	0,824	0,872	0,869	0,823	0,755	0,674	0,599	0,537	0,476	0,42	0,37	0,319	0,273	0,231	0,195	0,165	0,141	0,122	0,108	0,096
	0,593	0,579	0,566	0,559	0,555	0,555	0,562	0,58	0,612	0,658	0,736	0,815	0,862	0,856	0,808	0,742	0,662	0,59	0,532	0,476	0,423	0,376	0,328	0,283	0,242	0,205	0,174	0,149	0,128	0,114	0,101
	0,596	0,582	0,569	0,562	0,559	0,559	0,567	0,585	0,619	0,668	0,749	0,831	0,877	0,869	0,816	0,741	0,651	0,568	0,5	0,435	0,377	0,327	0,278	0,235	0,2	0,169	0,143	0,123	0,108	0,097	0,086
	0,589	0,575	0,562	0,555	0,551	0,551	0,557	0,574	0,606	0,651	0,727	0,805	0,854	0,852	0,812	0,749	0,673	0,601	0,541	0,483	0,43	0,381	0,332	0,286	0,246	0,21	0,18	0,155	0,136	0,121	0,109
<0.08% non-modified soybean	0,597	0,583	0,569	0,562	0,558	0,557	0,564	0,581	0,613	0,659	0,737	0,817	0,864	0,861	0,817	0,752	0,672	0,597	0,535	0,475	0,42	0,37	0,321	0,276	0,236	0,201	0,172	0,149	0,13	0,117	0,104
	0,59	0,576	0,563	0,557	0,552	0,553	0,56	0,578	0,611	0,658	0,737	0,815	0,857	0,845	0,792	0,718	0,633	0,557	0,498	0,443	0,394	0,352	0,31	0,27	0,235	0,203	0,175	0,152	0,133	0,119	0,106
	0,595	0,582	0,569	0,562	0,558	0,558	0,565	0,582	0,614	0,66	0,736	0,815	0,863	0,86	0,816	0,752	0,675	0,604	0,545	0,487	0,436	0,388	0,34	0,295	0,255	0,218	0,188	0,163	0,143	0,128	0,117
	0,599	0,586	0,573	0,568	0,564	0,564	0,572	0,59	0,625	0,673	0,752	0,835	0,882	0,874	0,821	0,747	0,659	0,58	0,515	0,454	0,399	0,35	0,303	0,26	0,222	0,189	0,162	0,14	0,122	0,11	0,099
	0,61	0,598	0,584	0,578	0,573	0,573	0,581	0,598	0,63	0,676	0,753	0,832	0,879	0,873	0,826	0,759	0,679	0,606	0,545	0,487	0,434	0,387	0,34	0,297	0,258	0,224	0,195	0,173	0,154	0,141	0,129
Mean	0,596111	0,582556	0,569333	0,562889	0,558778	0,558778	0,566	0,583444	0,616333	0,663111	0,741111	0,821	0,867778	0,862111	0,814556	0,746111	0,664222	0,589111	0,527556	0,468444	0,414778	0,366778	0,319	0,275	0,236111	0,201556	0,172667	0,149444	0,130667	0,117222	0,105222
	0,612	0,598	0,585	0,578	0,574	0,574	0,581	0,598	0,631	0,679	0,758	0,84	0,889	0,887	0,843	0,776	0,698	0,627	0,567	0,509	0,456	0,406	0,354	0,305	0,261	0,221	0,187	0,159	0,137	0,12	0,107
	0,6	0,586	0,573	0,566	0,562	0,562	0,568	0,585	0,617	0,663	0,74	0,819	0,866	0,863	0,82	0,757	0,683	0,617	0,563	0,51	0,461	0,414	0,366	0,318	0,274	0,233	0,197	0,168	0,144	0,127	0,112
	0,597	0,583	0,569	0,563	0,558	0,558	0,565	0,582	0,614	0,66	0,738	0,816	0,862	0,858	0,814	0,75	0,674	0,605	0,549	0,495	0,444	0,397	0,348	0,301	0,259	0,219	0,185	0,158	0,136	0,12	0,105
	0,599	0,585	0,571	0,565	0,559	0,558	0,564	0,58	0,61	0,654	0,729	0,806	0,854	0,855	0,821	0,766	0,698	0,634	0,582	0,529	0,48	0,434	0,386	0,339	0,295	0,255	0,219	0,19	0,166	0,148	0,132
25% GM MON87701	0,602	0,587	0,574	0,567	0,562	0,562	0,568	0,584	0,615	0,66	0,737	0,815	0,863	0,863	0,824	0,764	0,692	0,623	0,567	0,51	0,459	0,412	0,363	0,317	0,275	0,236	0,203	0,176	0,155	0,139	0,125
	0,607	0,592	0,579	0,571	0,567	0,566	0,571	0,588	0,617	0,661	0,736	0,813	0,86	0,86	0,824	0,769	0,701	0,637	0,585	0,532	0,484	0,439	0,392	0,345	0,302	0,262	0,227	0,198	0,174	0,156	0,141
	0,606	0,592	0,58	0,573	0,568	0,567	0,573	0,589	0,62	0,664	0,738	0,816	0,865	0,867	0,832	0,777	0,709	0,646	0,594	0,542	0,494	0,448	0,399	0,352	0,307	0,265	0,229	0,199	0,175	0,156	0,141
	0,609	0,595	0,582	0,575	0,571	0,57	0,576	0,593	0,624	0,669	0,745	0,825	0,874	0,874	0,836	0,776	0,703	0,636	0,58	0,526	0,476	0,429	0,381	0,333	0,29	0,25	0,216	0,188	0,165	0,148	0,134
	0,586	0,573	0,559	0,552	0,548	0,547	0,552	0,567	0,596	0,638	0,71	0,783	0,83	0,83	0,797	0,746	0,681	0,622	0,573	0,527	0,482	0,44	0,396	0,352	0,309	0,27	0,234	0,205	0,181	0,163	0,147
Mean	0,602	0,587889	0,574667	0,567778	0,563222	0,562667	0,568667	0,585111	0,616	0,660889	0,736778	0,814778	0,862556	0,861889	0,823444	0,764556	0,693222	0,627444	0,573333	0,52	0,470667	0,424333	0,376111	0,329111	0,285778	0,245667	0,210778	0,182333	0,159222	0,141889	0,127111
	0,622	0,607	0,594	0,586	0,581	0,58	0,586	0,603	0,634	0,681	0,757	0,838	0,889	0,891	0,855	0,799	0,729	0,663	0,61	0,557	0,506	0,458	0,406	0,355	0,308	0,262	0,223	0,191	0,164	0,144	0,128
	0,591	0,577	0,564	0,557	0,552	0,552	0,557	0,573	0,602	0,645	0,718	0,792	0,838	0,84	0,806	0,755	0,692	0,635	0,589	0,543	0,498	0,455	0,408	0,36	0,314	0,27	0,231	0,198	0,17	0,15	0,132
	0,602	0,588	0,574	0,567	0,562	0,56	0,566	0,581	0,61	0,654	0,727	0,803	0,851	0,855	0,824	0,774	0,712	0,654	0,607	0,558	0,512	0,466	0,416	0,366	0,318	0,272	0,231	0,197	0,169	0,148	0,13
	0,586	0,572	0,559	0,552	0,546	0,544	0,549	0,563	0,59	0,63	0,699	0,77	0,817	0,823	0,798	0,754	0,699	0,646	0,603	0,56	0,519	0,479	0,435	0,39	0,346	0,303	0,264	0,231	0,202	0,181	0,162
50% GM MON87701	0,589	0,575	0,561	0,554	0,549	0,548	0,553	0,567	0,594	0,635	0,705	0,777	0,825	0,829	0,803	0,758	0,701	0,646	0,601	0,556	0,513	0,472	0,428	0,383	0,339	0,297	0,26	0,228	0,201	0,181	0,163
	0,59	0,576	0,562	0,556	0,55	0,549	0,553	0,568	0,596	0,637	0,709	0,782	0,83	0,836	0,81	0,765	0,706	0,649	0,602	0,553	0,507	0,462	0,414	0,367	0,322	0,279	0,241	0,21	0,184	0,165	0,147
	0,601	0,586	0,573	0,566	0,561	0,559	0,564	0,578	0,606	0,647	0,717	0,791	0,84	0,847	0,822	0,778	0,721	0,666	0,62	0,576	0,533	0,492	0,447	0,4	0,355	0,312	0,273	0,24	0,212	0,19	0,173
	0,592	0,579	0,566	0,559	0,554	0,553	0,558	0,573	0,601	0,643	0,715	0,789	0,838	0,842	0,811	0,758	0,691	0,628	0,575	0,524	0,478	0,436	0,393	0,351	0,312	0,275	0,242	0,214	0,19	0,171	0,156
	0,602	0,588	0,574	0,567	0,561	0,56	0,565	0,58	0,607	0,648	0,719	0,793	0,842	0,849	0,824	0,779	0,722	0,666	0,619	0,574	0,531	0,488	0,442	0,397	0,351	0,308	0,27	0,237	0,21	0,189	0,172
Mean	0,597222	0,583111	0,569667	0,562667	0,557333	0,556111	0,561222	0,576222	0,604444	0,646667	0,718444	0,792778	0,841111	0,845778	0,817	0,768889	0,708111	0,650333	0,602889	0,555667	0,510778	0,467556	0,421	0,374333	0,329444	0,286444	0,248333	0,216222	0,189111	0,168778	0,151444
	0,617	0,603	0,589	0,582	0,575	0,573	0,576	0,59	0,616	0,656	0,726	0,798	0,848	0,86	0,842	0,807	0,761	0,717	0,679	0,641	0,603	0,564	0,518	0,468	0,418	0,367	0,319	0,276	0,239	0,209	0,184
	0,612	0,598	0,583	0,576	0,57	0,568	0,572	0,586	0,613	0,653	0,722	0,794	0,841	0,848	0,826	0,788	0,74	0,695	0,66	0,626	0,591	0,556	0,514	0,469	0,421	0,372	0,324	0,282	0,244	0,215	0,19
	0,604	0,591	0,577	0,569	0,563	0,561	0,565	0,577	0,601	0,638	0,704	0,773	0,819	0,829	0,813	0,781	0,739	0,698	0,665	0,632	0,598	0,564	0,525	0,481	0,436	0,388	0,342	0,3	0,264	0,235	0,21
	0,607	0,592	0,578	0,57	0,564	0,562	0,565	0,578	0,603	0,642	0,709	0,778	0,825	0,835	0,818	0,784	0,74	0,695	0,66	0,623	0,586	0,55	0,51	0,466	0,423	0,379	0,337	0,3	0,267	0,242	0,219
75% GM MON87701	0,599	0,586	0,572	0,564	0,558	0,555	0,558	0,569	0,593	0,629	0,694	0,761	0,808	0,818	0,805	0,776	0,737	0,699	0,666	0,633	0,599	0,565	0,525	0,483	0,44	0,396	0,354	0,317	0,284	0,258	0,234
	0,616	0,601	0,586	0,578	0,571	0,569	0,571	0,583	0,607	0,644	0,71	0,779	0,827	0,838	0,825	0,796	0,756	0,715	0,682	0,648	0,614	0,58	0,541	0,5	0,456	0,411	0,369	0,33	0,296	0,27	0,245
	0,606	0,592	0,579	0,571	0,564	0,561	0,564	0,575	0,599	0,634	0,698	0,765	0,811	0,822	0,81	0,784	0,747	0,711	0,682	0,652	0,622	0,591	0,556	0,516	0,476	0,433	0,391	0,352	0,317	0,287	0,261
	0,609	0,595	0,582	0,573	0,568	0,565	0,568	0,58	0,604	0,641	0,707	0,775	0,823	0,834	0,819	0,788	0,748	0,709	0,675	0,641	0,608	0,574	0,536	0,494	0,451	0,406	0,363	0,326	0,292	0,264	0,241
	0,615	0,601	0,586	0,579	0,573	0,57	0,573	0,585	0,609	0,645	0,711	0,78	0,825	0,834	0,817	0,786	0,743	0,703	0,671	0,64	0,609	0,58	0,545	0,507	0,468	0,426	0,386	0,35	0,317	0,291	0,268
Mean	0,609444	0,595444	0,581333	0,573556	0,567333	0,564889	0,568	0,580333	0,605	0,642444	0,709	0,778111	0,825222	0,835333	0,819444	0,787778	0,745667	0,704667	0,671111	0,637333	0,603333	0,569333	0,53	0,487111	0,443222	0,397556	0,353889	0,314778	0,28	0,252333	0,228
	0,624	0,609	0,594	0,586	0,578	0,575	0,577	0,588	0,61	0,647	0,71	0,777	0,822	0,836	0,827	0,804	0,774	0,745	0,723	0,699	0,675	0,648	0,617	0,578	0,536	0,489	0,44	0,393	0,348	0,31	0,275
	0,619	0,605	0,591	0,583	0,577	0,574	0,577	0,59	0,614	0,652	0,718	0,788	0,835	0,844	0,829	0,799	0,76	0,724	0,695	0,665	0,635	0,604	0,565	0,522	0,476	0,426	0,378	0,333	0,292	0,258	0,229
	0,612	0,597	0,583	0,574	0,567	0,563	0,565	0,576	0,597	0,632	0,694	0,759	0,802	0,815	0,805	0,781	0,75	0,718	0,694	0,667	0,641	0,614	0,584	0,548	0,511	0,47	0,43	0,392	0,355	0,324	0,296
	0,597	0,583	0,569	0,56	0,553	0,55	0,551	0,561	0,582	0,616	0,675	0,736	0,778	0,788	0,779	0,757	0,728	0,699	0,676	0,652	0,628	0,604	0,576	0,542	0,507	0,468	0,43	0,394	0,359	0,331	0,303
≥ 98.4% MON87701	0,604	0,589	0,575	0,567	0,56	0,556	0,558	0,569	0,589	0,623	0,684	0,747	0,79	0,799	0,788	0,765	0,734	0,703	0,68	0,656	0,632	0,608	0,58	0,547	0,512	0,473	0,435	0,397	0,363	0,334	0,306
	0,602	0,587	0,572	0,565	0,558	0,554	0,556	0,566	0,586	0,619	0,679	0,739	0,778	0,783	0,767	0,74	0,706	0,675	0,652	0,631	0,611	0,592	0,57	0,544	0,517	0,485	0,454	0,424	0,395	0,371	0,347
	0,62	0,606	0,592	0,585	0,577	0,573	0,575	0,585	0,605	0,638	0,697	0,76	0,802	0,813	0,804	0,784	0,755	0,728	0,706	0,685	0,664	0,642	0,617	0,587	0,555	0,52	0,484	0,449	0,415	0,386	0,359
	0,608	0,594	0,58	0,573	0,565	0,561	0,563	0,573	0,594	0,627	0,687	0,75	0,793	0,803	0,793	0,771	0,741	0,711	0,688	0,665	0,642	0,619	0,592	0,56	0,526	0,49	0,453	0,418	0,385	0,357	0,331
	0,615	0,601	0,586	0,578	0,571	0,566	0,567	0,576	0,595	0,626	0,684	0,743	0,784	0,795	0,788	0,772	0,748	0,724	0,705	0,687	0,668	0,648	0,626	0,599	0,569	0,535	0,5	0,467	0,434	0,405	0,378
Mean	0,611222	0,596778	0,582444	0,574556	0,567333	0,563556	0,565444	0,576	0,596889	0,631111	0,692	0,755444	0,798222	0,808444	0,797778	0,774778	0,744	0,714111	0,691	0,667444	0,644	0,619889	0,591889	0,558556	0,523222	0,484	0,444889	0,407444	0,371778	0,341778	0,313778

Table A.8. Batch Y5 in 5 min

Wavelength [nm]	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640	650	660	670	680	690	700
	0,585	0,572	0,559	0,552	0,548	0,548	0,553	0,569	0,6	0,643	0,716	0,791	0,836	0,834	0,796	0,741	0,676	0,617	0,57	0,524	0,48	0,438	0,391	0,344	0,299	0,256	0,218	0,187	0,161	0,141	0,125
	0,569	0,556	0,543	0,537	0,532	0,531	0,536	0,551	0,58	0,622	0,692	0,763	0,804	0,8	0,762	0,709	0,648	0,593	0,552	0,512	0,474	0,437	0,396	0,353	0,311	0,27	0,232	0,199	0,172	0,151	0,134
	0,581	0,567	0,554	0,548	0,543	0,544	0,55	0,568	0,599	0,644	0,721	0,796	0,841	0,833	0,787	0,722	0,646	0,578	0,524	0,471	0,422	0,378	0,331	0,287	0,247	0,21	0,179	0,154	0,134	0,119	0,105
	0,574	0,56	0,548	0,542	0,538	0,538	0,544	0,559	0,59	0,633	0,705	0,779	0,823	0,821	0,781	0,723	0,653	0,59	0,538	0,489	0,444	0,402	0,358	0,316	0,277	0,24	0,209	0,184	0,163	0,146	0,133
<0.08% non-modified soybean	0,578	0,565	0,552	0,547	0,542	0,542	0,549	0,567	0,598	0,643	0,719	0,795	0,839	0,832	0,786	0,722	0,646	0,578	0,523	0,47	0,422	0,378	0,332	0,289	0,249	0,213	0,182	0,157	0,136	0,121	0,109
	0,581	0,568	0,556	0,549	0,546	0,545	0,552	0,568	0,599	0,642	0,716	0,791	0,835	0,83	0,787	0,726	0,654	0,589	0,535	0,484	0,437	0,394	0,349	0,306	0,267	0,232	0,201	0,176	0,156	0,141	0,128
	0,573	0,559	0,547	0,54	0,536	0,535	0,541	0,556	0,586	0,629	0,701	0,775	0,821	0,82	0,783	0,727	0,659	0,597	0,544	0,494	0,448	0,404	0,359	0,314	0,274	0,236	0,204	0,177	0,156	0,139	0,126
	0,583	0,569	0,556	0,549	0,544	0,543	0,549	0,565	0,595	0,639	0,713	0,788	0,833	0,831	0,792	0,733	0,663	0,598	0,545	0,493	0,445	0,401	0,355	0,311	0,27	0,232	0,2	0,174	0,153	0,137	0,123
	0,581	0,567	0,553	0,547	0,543	0,543	0,549	0,566	0,597	0,642	0,717	0,79	0,83	0,819	0,77	0,702	0,626	0,559	0,506	0,458	0,416	0,378	0,34	0,302	0,267	0,234	0,205	0,18	0,16	0,143	0,129
Mean	0,578333	0,564778	0,552	0,545667	0,541333	0,541	0,547	0,563222	0,593778	0,637444	0,711111	0,785333	0,829111	0,824444	0,782667	0,722778	0,652333	0,588778	0,537444	0,488333	0,443111	0,401111	0,356778	0,313556	0,273444	0,235889	0,203333	0,176444	0,154556	0,137556	0,123556
	0,596	0,582	0,568	0,562	0,557	0,556	0,562	0,578	0,608	0,653	0,727	0,802	0,847	0,846	0,808	0,753	0,687	0,628	0,581	0,536	0,492	0,45	0,404	0,358	0,313	0,269	0,231	0,198	0,172	0,151	0,134
	0,582	0,569	0,556	0,549	0,544	0,543	0,548	0,563	0,591	0,633	0,704	0,775	0,817	0,816	0,781	0,732	0,675	0,623	0,585	0,547	0,51	0,474	0,432	0,388	0,344	0,299	0,258	0,222	0,192	0,169	0,149
	0,591	0,579	0,564	0,558	0,552	0,552	0,557	0,573	0,603	0,645	0,718	0,79	0,834	0,83	0,792	0,738	0,675	0,619	0,576	0,533	0,493	0,454	0,411	0,366	0,323	0,279	0,241	0,208	0,181	0,16	0,142
	0,592	0,579	0,566	0,559	0,555	0,554	0,559	0,574	0,603	0,645	0,717	0,791	0,837	0,839	0,806	0,754	0,691	0,633	0,585	0,54	0,497	0,456	0,412	0,368	0,327	0,286	0,251	0,221	0,196	0,177	0,161
25% GM MON87701	0,59	0,577	0,564	0,557	0,552	0,552	0,557	0,573	0,601	0,643	0,714	0,789	0,834	0,833	0,8	0,747	0,684	0,626	0,579	0,534	0,492	0,452	0,409	0,365	0,324	0,284	0,249	0,22	0,196	0,177	0,161
	0,568	0,554	0,542	0,536	0,531	0,53	0,534	0,548	0,576	0,615	0,683	0,752	0,795	0,796	0,765	0,717	0,66	0,607	0,564	0,524	0,485	0,449	0,41	0,369	0,33	0,291	0,258	0,229	0,204	0,185	0,169
	0,578	0,563	0,55	0,543	0,539	0,537	0,542	0,556	0,584	0,626	0,695	0,766	0,811	0,812	0,782	0,733	0,674	0,619	0,575	0,531	0,489	0,45	0,407	0,364	0,322	0,281	0,245	0,214	0,189	0,169	0,152
	0,586	0,572	0,559	0,552	0,547	0,546	0,552	0,567	0,596	0,638	0,711	0,784	0,829	0,829	0,794	0,74	0,675	0,615	0,565	0,517	0,473	0,431	0,387	0,344	0,302	0,264	0,23	0,202	0,179	0,161	0,146
	0,593	0,577	0,564	0,557	0,552	0,551	0,556	0,571	0,599	0,641	0,712	0,785	0,829	0,83	0,796	0,744	0,681	0,623	0,577	0,532	0,489	0,45	0,407	0,363	0,322	0,282	0,247	0,218	0,194	0,175	0,159
Mean	0,586222	0,572444	0,559222	0,552556	0,547667	0,546778	0,551889	0,567	0,595667	0,637667	0,709	0,781556	0,825889	0,825667	0,791556	0,739778	0,678	0,621444	0,576333	0,532667	0,491111	0,451778	0,408778	0,365	0,323	0,281667	0,245556	0,214667	0,189222	0,169333	0,152556
	0,596	0,583	0,57	0,563	0,557	0,556	0,561	0,576	0,605	0,646	0,717	0,791	0,837	0,841	0,81	0,763	0,705	0,653	0,611	0,57	0,53	0,491	0,446	0,4	0,353	0,306	0,265	0,228	0,198	0,175	0,155
	0,572	0,559	0,546	0,539	0,534	0,532	0,537	0,551	0,579	0,618	0,686	0,755	0,797	0,799	0,771	0,727	0,675	0,627	0,59	0,554	0,518	0,482	0,442	0,399	0,355	0,311	0,27	0,235	0,205	0,181	0,161
	0,583	0,57	0,557	0,55	0,544	0,542	0,547	0,56	0,588	0,627	0,696	0,766	0,812	0,815	0,788	0,746	0,693	0,645	0,606	0,568	0,531	0,493	0,45	0,405	0,359	0,313	0,271	0,234	0,203	0,179	0,159
	0,584	0,57	0,557	0,551	0,545	0,543	0,547	0,561	0,587	0,625	0,692	0,761	0,806	0,814	0,791	0,751	0,7	0,652	0,612	0,574	0,538	0,503	0,463	0,421	0,381	0,34	0,302	0,27	0,241	0,22	0,201
50% GM MON87701	0,583	0,57	0,556	0,55	0,544	0,543	0,548	0,562	0,588	0,628	0,696	0,768	0,813	0,818	0,79	0,744	0,685	0,629	0,583	0,538	0,497	0,459	0,419	0,379	0,342	0,305	0,272	0,244	0,219	0,199	0,182
	0,582	0,569	0,555	0,549	0,543	0,541	0,546	0,559	0,585	0,622	0,69	0,759	0,804	0,81	0,788	0,748	0,697	0,648	0,608	0,57	0,533	0,497	0,458	0,416	0,375	0,334	0,297	0,265	0,237	0,216	0,198
	0,578	0,564	0,55	0,543	0,538	0,535	0,539	0,552	0,578	0,616	0,681	0,75	0,793	0,8	0,777	0,738	0,688	0,641	0,604	0,567	0,531	0,496	0,457	0,416	0,375	0,334	0,295	0,262	0,233	0,21	0,19
	0,578	0,564	0,549	0,542	0,537	0,535	0,539	0,553	0,579	0,618	0,684	0,753	0,798	0,803	0,778	0,737	0,683	0,634	0,594	0,554	0,516	0,48	0,44	0,399	0,358	0,317	0,281	0,25	0,224	0,203	0,185
	0,609	0,594	0,58	0,573	0,567	0,565	0,568	0,582	0,608	0,646	0,712	0,782	0,826	0,831	0,808	0,766	0,714	0,664	0,623	0,581	0,541	0,502	0,459	0,416	0,373	0,332	0,295	0,263	0,237	0,217	0,199
Mean	0,585	0,571444	0,557778	0,551111	0,545444	0,543556	0,548	0,561778	0,588556	0,627333	0,694889	0,765	0,809556	0,814556	0,789	0,746667	0,693333	0,643667	0,603444	0,564	0,526111	0,489222	0,448222	0,405667	0,363444	0,321333	0,283111	0,250111	0,221889	0,2	0,181111
	0,604	0,59	0,576	0,569	0,561	0,558	0,561	0,572	0,597	0,633	0,697	0,764	0,81	0,821	0,807	0,78	0,744	0,709	0,682	0,655	0,626	0,597	0,561	0,521	0,477	0,429	0,383	0,339	0,3	0,267	0,239
	0,591	0,578	0,564	0,557	0,55	0,548	0,551	0,563	0,587	0,623	0,687	0,752	0,795	0,802	0,785	0,754	0,716	0,68	0,654	0,628	0,603	0,577	0,544	0,508	0,467	0,423	0,378	0,337	0,299	0,268	0,241
	0,58	0,566	0,553	0,545	0,539	0,536	0,538	0,549	0,571	0,605	0,666	0,729	0,772	0,781	0,768	0,744	0,709	0,677	0,652	0,628	0,603	0,578	0,547	0,512	0,472	0,429	0,386	0,346	0,309	0,278	0,251
	0,589	0,576	0,561	0,554	0,547	0,544	0,546	0,556	0,578	0,611	0,67	0,733	0,775	0,786	0,776	0,751	0,719	0,688	0,663	0,638	0,614	0,588	0,559	0,526	0,492	0,455	0,419	0,385	0,352	0,324	0,299
75% GM MON87701	0,597	0,584	0,571	0,564	0,557	0,554	0,556	0,568	0,591	0,626	0,688	0,754	0,797	0,807	0,793	0,765	0,728	0,691	0,662	0,633	0,604	0,576	0,542	0,505	0,467	0,426	0,388	0,354	0,322	0,296	0,273
	0,596	0,583	0,569	0,561	0,554	0,551	0,554	0,565	0,587	0,621	0,682	0,747	0,788	0,796	0,781	0,753	0,716	0,68	0,652	0,625	0,6	0,575	0,546	0,514	0,481	0,445	0,41	0,378	0,348	0,324	0,302
	0,597	0,582	0,569	0,561	0,555	0,552	0,555	0,567	0,59	0,627	0,69	0,756	0,801	0,811	0,796	0,765	0,724	0,686	0,655	0,624	0,592	0,561	0,525	0,487	0,447	0,406	0,366	0,331	0,299	0,274	0,251
	0,608	0,593	0,579	0,572	0,565	0,562	0,564	0,575	0,597	0,631	0,692	0,756	0,8	0,809	0,797	0,771	0,736	0,702	0,674	0,645	0,616	0,586	0,552	0,515	0,476	0,436	0,397	0,362	0,331	0,305	0,282
	0,599	0,586	0,57	0,562	0,555	0,552	0,554	0,565	0,587	0,622	0,684	0,747	0,792	0,803	0,791	0,765	0,729	0,694	0,665	0,637	0,607	0,579	0,545	0,509	0,471	0,432	0,394	0,359	0,328	0,302	0,279
Mean	0,595667	0,582	0,568	0,560556	0,553667	0,550778	0,553222	0,564444	0,587222	0,622111	0,684	0,748667	0,792222	0,801778	0,788222	0,760889	0,724556	0,689667	0,662111	0,634778	0,607222	0,579667	0,546778	0,510778	0,472222	0,431222	0,391222	0,354556	0,320889	0,293111	0,268556
	0,61	0,596	0,581	0,573	0,566	0,562	0,563	0,573	0,594	0,627	0,686	0,748	0,791	0,803	0,796	0,777	0,752	0,728	0,71	0,693	0,674	0,655	0,63	0,6	0,565	0,525	0,482	0,44	0,398	0,361	0,328
	0,602	0,589	0,575	0,567	0,56	0,557	0,559	0,57	0,593	0,628	0,689	0,753	0,796	0,806	0,793	0,768	0,736	0,707	0,684	0,662	0,639	0,614	0,584	0,548	0,509	0,465	0,422	0,38	0,34	0,307	0,278
	0,598	0,584	0,569	0,561	0,553	0,549	0,55	0,559	0,579	0,61	0,667	0,726	0,767	0,777	0,768	0,75	0,725	0,698	0,679	0,659	0,639	0,619	0,596	0,569	0,538	0,506	0,471	0,439	0,408	0,382	0,355
	0,601	0,588	0,574	0,566	0,559	0,554	0,555	0,564	0,583	0,613	0,668	0,725	0,764	0,774	0,767	0,748	0,724	0,7	0,683	0,665	0,649	0,633	0,612	0,588	0,563	0,533	0,503	0,473	0,444	0,419	0,395
≥ 98.4% MON87701	0,596	0,583	0,569	0,561	0,554	0,55	0,551	0,56	0,58	0,611	0,668	0,727	0,767	0,776	0,767	0,746	0,718	0,691	0,67	0,651	0,631	0,612	0,589	0,562	0,534	0,502	0,47	0,44	0,411	0,386	0,362
	0,598	0,585	0,57	0,562	0,554	0,55	0,55	0,558	0,575	0,603	0,657	0,713	0,751	0,761	0,756	0,742	0,72	0,7	0,684	0,669	0,653	0,638	0,62	0,597	0,573	0,544	0,515	0,486	0,457	0,432	0,408
	0,585	0,571	0,557	0,549	0,542	0,538	0,538	0,547	0,566	0,597	0,652	0,71	0,75	0,761	0,753	0,733	0,708	0,684	0,665	0,646	0,627	0,607	0,584	0,556	0,526	0,492	0,458	0,425	0,394	0,366	0,34
	0,594	0,58	0,566	0,558	0,55	0,547	0,547	0,557	0,577	0,609	0,667	0,726	0,767	0,776	0,766	0,744	0,716	0,689	0,667	0,648	0,626	0,606	0,582	0,553	0,523	0,489	0,455	0,422	0,39	0,364	0,339
	0,593	0,578	0,564	0,556	0,55	0,545	0,546	0,555	0,574	0,606	0,662	0,72	0,757	0,761	0,747	0,722	0,691	0,663	0,642	0,624	0,606	0,59	0,572	0,549	0,526	0,498	0,471	0,444	0,419	0,397	0,376
Mean	0,597444	0,583778	0,569444	0,561444	0,554222	0,550222	0,551	0,560333	0,580111	0,611556	0,668444	0,727556	0,767778	0,777222	0,768111	0,747778	0,721111	0,695556	0,676	0,657444	0,638222	0,619333	0,596556	0,569111	0,539667	0,506	0,471889	0,438778	0,406778	0,379333	0,353444

Table A.9. Batch Y5 in 10 min

Wavelength [nm]	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640	650	660	670	680	690	700
	0,584	0,571	0,558	0,552	0,548	0,548	0,553	0,569	0,599	0,643	0,716	0,79	0,834	0,832	0,795	0,739	0,675	0,618	0,573	0,529	0,486	0,445	0,399	0,352	0,306	0,263	0,224	0,192	0,166	0,146	0,129
	0,571	0,558	0,545	0,539	0,534	0,534	0,539	0,553	0,581	0,623	0,692	0,762	0,803	0,799	0,762	0,712	0,653	0,602	0,564	0,527	0,491	0,457	0,418	0,375	0,332	0,289	0,25	0,216	0,187	0,165	0,145
	0,574	0,561	0,548	0,543	0,539	0,539	0,545	0,562	0,593	0,637	0,711	0,785	0,828	0,821	0,777	0,714	0,642	0,578	0,527	0,478	0,433	0,391	0,346	0,302	0,262	0,225	0,192	0,166	0,144	0,129	0,114
	0,57	0,556	0,545	0,538	0,534	0,534	0,54	0,555	0,585	0,628	0,699	0,772	0,816	0,813	0,773	0,716	0,648	0,586	0,536	0,489	0,446	0,406	0,363	0,322	0,283	0,248	0,217	0,191	0,169	0,153	0,139
<0.08% non-modified soybean	0,58	0,566	0,555	0,549	0,545	0,545	0,552	0,569	0,601	0,645	0,721	0,798	0,842	0,835	0,788	0,723	0,646	0,578	0,522	0,47	0,422	0,378	0,333	0,291	0,252	0,217	0,187	0,163	0,142	0,127	0,114
	0,579	0,566	0,554	0,548	0,543	0,543	0,549	0,565	0,595	0,638	0,712	0,786	0,829	0,825	0,782	0,723	0,653	0,59	0,538	0,489	0,444	0,402	0,359	0,317	0,278	0,241	0,21	0,184	0,163	0,147	0,133
	0,572	0,558	0,546	0,539	0,534	0,533	0,538	0,553	0,582	0,625	0,695	0,768	0,813	0,811	0,776	0,722	0,656	0,597	0,549	0,502	0,459	0,419	0,376	0,333	0,293	0,255	0,223	0,195	0,172	0,155	0,139
	0,578	0,565	0,552	0,545	0,54	0,54	0,545	0,56	0,59	0,632	0,704	0,776	0,821	0,818	0,78	0,725	0,658	0,598	0,549	0,501	0,458	0,417	0,374	0,331	0,29	0,252	0,22	0,192	0,169	0,152	0,137
	0,576	0,561	0,548	0,541	0,537	0,537	0,543	0,559	0,589	0,632	0,705	0,776	0,815	0,803	0,757	0,694	0,622	0,559	0,512	0,469	0,431	0,397	0,362	0,327	0,294	0,261	0,231	0,206	0,183	0,165	0,149
Mean	0,576	0,562444	0,550111	0,543778	0,539333	0,539222	0,544889	0,560556	0,590556	0,633667	0,706111	0,779222	0,822333	0,817444	0,776667	0,718667	0,650333	0,589556	0,541111	0,494889	0,452222	0,412444	0,37	0,327778	0,287778	0,250111	0,217111	0,189444	0,166111	0,148778	0,133222
	0,589	0,575	0,562	0,555	0,551	0,55	0,555	0,57	0,598	0,641	0,712	0,783	0,828	0,827	0,794	0,744	0,686	0,635	0,596	0,556	0,518	0,481	0,436	0,391	0,345	0,299	0,258	0,223	0,193	0,17	0,15
	0,576	0,563	0,55	0,543	0,539	0,538	0,543	0,558	0,585	0,626	0,696	0,766	0,808	0,807	0,773	0,724	0,667	0,618	0,58	0,544	0,508	0,473	0,432	0,389	0,345	0,301	0,261	0,226	0,196	0,173	0,153
	0,573	0,558	0,546	0,54	0,535	0,535	0,54	0,555	0,583	0,625	0,696	0,766	0,809	0,806	0,769	0,718	0,657	0,605	0,564	0,525	0,487	0,452	0,41	0,368	0,325	0,282	0,244	0,21	0,182	0,161	0,142
	0,587	0,573	0,561	0,554	0,549	0,548	0,553	0,568	0,597	0,637	0,708	0,78	0,825	0,827	0,795	0,744	0,684	0,629	0,584	0,542	0,502	0,463	0,422	0,38	0,339	0,3	0,265	0,235	0,209	0,189	0,172
25% GM MON87701	0,586	0,572	0,56	0,553	0,548	0,547	0,553	0,568	0,597	0,638	0,71	0,783	0,828	0,827	0,793	0,74	0,677	0,619	0,573	0,528	0,486	0,447	0,406	0,364	0,324	0,286	0,253	0,224	0,2	0,181	0,165
	0,565	0,551	0,538	0,532	0,526	0,525	0,53	0,544	0,57	0,608	0,675	0,743	0,784	0,785	0,755	0,71	0,654	0,605	0,565	0,527	0,492	0,458	0,421	0,383	0,346	0,309	0,275	0,246	0,221	0,201	0,183
	0,587	0,573	0,559	0,552	0,546	0,545	0,55	0,565	0,593	0,634	0,704	0,775	0,82	0,821	0,789	0,74	0,68	0,625	0,58	0,536	0,496	0,458	0,416	0,374	0,333	0,293	0,258	0,227	0,202	0,182	0,164
	0,579	0,564	0,551	0,544	0,539	0,538	0,543	0,558	0,586	0,628	0,698	0,769	0,813	0,813	0,779	0,728	0,666	0,609	0,564	0,52	0,48	0,441	0,4	0,358	0,319	0,28	0,246	0,217	0,193	0,174	0,158
	0,591	0,576	0,563	0,555	0,55	0,549	0,553	0,567	0,594	0,634	0,704	0,773	0,816	0,815	0,783	0,735	0,677	0,625	0,584	0,543	0,506	0,47	0,431	0,391	0,352	0,313	0,278	0,247	0,222	0,201	0,182
Mean	0,581444	0,567222	0,554444	0,547556	0,542556	0,541667	0,546667	0,561444	0,589222	0,630111	0,700333	0,770889	0,814556	0,814222	0,781111	0,731444	0,672	0,618889	0,576667	0,535667	0,497222	0,460333	0,419333	0,377556	0,336444	0,295889	0,259778	0,228333	0,202	0,181333	0,163222
	0,591	0,577	0,564	0,557	0,552	0,551	0,556	0,569	0,597	0,638	0,708	0,779	0,824	0,827	0,799	0,754	0,699	0,652	0,614	0,576	0,54	0,503	0,462	0,416	0,371	0,324	0,282	0,245	0,213	0,188	0,167
	0,572	0,558	0,544	0,538	0,533	0,532	0,536	0,55	0,576	0,615	0,682	0,75	0,792	0,793	0,766	0,724	0,673	0,629	0,595	0,561	0,527	0,495	0,457	0,414	0,372	0,327	0,287	0,251	0,22	0,195	0,174
	0,575	0,561	0,548	0,541	0,536	0,534	0,538	0,55	0,576	0,614	0,68	0,747	0,79	0,794	0,771	0,733	0,687	0,646	0,615	0,582	0,551	0,519	0,48	0,437	0,393	0,347	0,303	0,264	0,231	0,204	0,18
	0,585	0,57	0,558	0,551	0,545	0,544	0,548	0,561	0,587	0,625	0,692	0,761	0,806	0,812	0,789	0,749	0,698	0,65	0,611	0,574	0,538	0,504	0,466	0,426	0,387	0,347	0,311	0,28	0,252	0,23	0,211
50% GM MON87701	0,578	0,564	0,551	0,545	0,539	0,537	0,541	0,555	0,581	0,619	0,687	0,756	0,8	0,805	0,778	0,733	0,677	0,625	0,582	0,541	0,503	0,467	0,431	0,394	0,358	0,322	0,289	0,261	0,235	0,214	0,196
	0,583	0,57	0,556	0,55	0,544	0,542	0,546	0,559	0,584	0,621	0,686	0,754	0,798	0,804	0,782	0,744	0,695	0,65	0,613	0,578	0,544	0,51	0,472	0,432	0,391	0,35	0,312	0,28	0,251	0,229	0,209
	0,574	0,56	0,547	0,54	0,534	0,532	0,535	0,548	0,574	0,612	0,676	0,743	0,787	0,792	0,769	0,728	0,678	0,632	0,594	0,557	0,523	0,49	0,454	0,416	0,378	0,339	0,303	0,272	0,244	0,222	0,203
	0,573	0,558	0,545	0,538	0,533	0,531	0,535	0,548	0,573	0,61	0,675	0,743	0,786	0,79	0,766	0,727	0,677	0,629	0,592	0,555	0,521	0,488	0,452	0,414	0,376	0,337	0,302	0,272	0,245	0,225	0,205
	0,573	0,559	0,546	0,539	0,533	0,532	0,535	0,548	0,574	0,612	0,679	0,746	0,791	0,796	0,772	0,732	0,681	0,633	0,593	0,554	0,517	0,48	0,441	0,399	0,359	0,32	0,284	0,253	0,227	0,206	0,188
Mean	0,578222	0,564111	0,551	0,544333	0,538778	0,537222	0,541111	0,554222	0,580222	0,618444	0,685	0,753222	0,797111	0,801444	0,776889	0,736	0,685	0,638444	0,601	0,564222	0,529333	0,495111	0,457222	0,416444	0,376111	0,334778	0,297	0,264222	0,235333	0,212556	0,192556
	0,595	0,581	0,567	0,56	0,553	0,55	0,552	0,563	0,585	0,62	0,681	0,745	0,789	0,8	0,788	0,765	0,734	0,705	0,683	0,66	0,637	0,613	0,581	0,545	0,505	0,459	0,414	0,371	0,331	0,297	0,266
	0,584	0,571	0,557	0,549	0,543	0,541	0,543	0,555	0,579	0,614	0,676	0,741	0,783	0,79	0,772	0,742	0,705	0,671	0,646	0,623	0,598	0,574	0,543	0,508	0,469	0,425	0,383	0,343	0,306	0,275	0,248
	0,579	0,565	0,55	0,543	0,537	0,534	0,536	0,547	0,569	0,602	0,661	0,723	0,765	0,774	0,762	0,738	0,704	0,673	0,65	0,628	0,604	0,581	0,552	0,519	0,483	0,442	0,402	0,363	0,327	0,297	0,27
	0,589	0,575	0,563	0,555	0,547	0,545	0,546	0,556	0,579	0,612	0,672	0,735	0,777	0,788	0,776	0,751	0,717	0,683	0,657	0,632	0,606	0,58	0,552	0,521	0,487	0,452	0,418	0,386	0,356	0,329	0,304
75% GM MON87701	0,598	0,584	0,571	0,564	0,557	0,554	0,556	0,567	0,588	0,623	0,684	0,747	0,789	0,799	0,786	0,76	0,725	0,692	0,665	0,639	0,613	0,587	0,557	0,523	0,488	0,45	0,414	0,38	0,349	0,323	0,299
	0,59	0,576	0,563	0,556	0,549	0,545	0,548	0,559	0,58	0,614	0,675	0,738	0,779	0,787	0,771	0,743	0,706	0,671	0,644	0,619	0,594	0,571	0,544	0,514	0,483	0,449	0,415	0,385	0,356	0,333	0,311
	0,592	0,577	0,564	0,556	0,549	0,546	0,549	0,56	0,583	0,618	0,68	0,743	0,787	0,795	0,78	0,752	0,713	0,677	0,648	0,619	0,592	0,563	0,531	0,497	0,461	0,424	0,388	0,356	0,326	0,301	0,279
	0,578	0,563	0,55	0,542	0,536	0,533	0,535	0,545	0,566	0,599	0,658	0,719	0,76	0,77	0,758	0,734	0,701	0,669	0,643	0,617	0,591	0,565	0,534	0,5	0,466	0,429	0,394	0,361	0,331	0,307	0,283
	0,592	0,578	0,563	0,554	0,548	0,545	0,546	0,556	0,578	0,611	0,671	0,733	0,775	0,785	0,774	0,75	0,716	0,684	0,658	0,631	0,606	0,581	0,551	0,519	0,485	0,448	0,413	0,38	0,349	0,325	0,3
Mean	0,588556	0,574444	0,560889	0,553222	0,546556	0,543667	0,545667	0,556444	0,578556	0,612556	0,673111	0,736	0,778222	0,787556	0,774111	0,748333	0,713444	0,680556	0,654889	0,629778	0,604556	0,579444	0,549444	0,516222	0,480778	0,442	0,404556	0,369444	0,336778	0,309667	0,284444
	0,598	0,583	0,57	0,561	0,554	0,55	0,559	0,578	0,608	0,665	0,722	0,764	0,777	0,772	0,758	0,737	0,718	0,705	0,692	0,678	0,663	0,644	0,619	0,589	0,553	0,515	0,475	0,435	0,399	0,364
	0,594	0,58	0,566	0,559	0,552	0,549	0,551	0,561	0,583	0,616	0,676	0,738	0,779	0,789	0,777	0,754	0,724	0,697	0,677	0,657	0,637	0,616	0,589	0,557	0,521	0,48	0,439	0,398	0,36	0,328	0,298
	0,596	0,581	0,567	0,558	0,551	0,547	0,547	0,555	0,574	0,604	0,66	0,717	0,756	0,766	0,759	0,742	0,717	0,693	0,676	0,658	0,64	0,622	0,602	0,577	0,55	0,52	0,489	0,46	0,43	0,405	0,38
	0,601	0,588	0,574	0,567	0,559	0,554	0,555	0,563	0,582	0,611	0,665	0,722	0,76	0,769	0,761	0,743	0,719	0,696	0,679	0,664	0,648	0,633	0,615	0,594	0,571	0,544	0,517	0,49	0,464	0,44	0,416
≥ 98.4% MON87701	0,591	0,576	0,564	0,556	0,548	0,544	0,545	0,553	0,572	0,602	0,657	0,715	0,754	0,765	0,757	0,739	0,715	0,691	0,673	0,657	0,64	0,623	0,603	0,578	0,552	0,521	0,489	0,458	0,427	0,401	0,375
	0,595	0,581	0,568	0,559	0,552	0,547	0,547	0,555	0,572	0,601	0,655	0,711	0,75	0,761	0,756	0,742	0,721	0,7	0,686	0,672	0,658	0,645	0,627	0,604	0,579	0,549	0,518	0,486	0,455	0,427	0,399
	0,582	0,569	0,555	0,547	0,54	0,535	0,536	0,544	0,563	0,594	0,648	0,706	0,745	0,755	0,748	0,73	0,705	0,682	0,665	0,648	0,63	0,612	0,591	0,565	0,536	0,504	0,471	0,439	0,408	0,381	0,354
	0,594	0,58	0,566	0,558	0,55	0,547	0,547	0,556	0,576	0,608	0,665	0,723	0,763	0,771	0,761	0,74	0,711	0,684	0,664	0,645	0,626	0,607	0,585	0,559	0,531	0,5	0,468	0,437	0,408	0,382	0,358
	0,591	0,577	0,563	0,555	0,548	0,543	0,544	0,552	0,57	0,6	0,655	0,711	0,747	0,751	0,737	0,714	0,683	0,657	0,638	0,621	0,606	0,592	0,576	0,556	0,536	0,512	0,488	0,464	0,44	0,42	0,4
Mean	0,593556	0,579444	0,565889	0,557778	0,550444	0,546222	0,546889	0,555333	0,574444	0,604889	0,660667	0,718333	0,757556	0,767111	0,758667	0,740222	0,714667	0,690889	0,673667	0,657111	0,640333	0,623667	0,603556	0,578778	0,551667	0,520333	0,488222	0,456333	0,425222	0,398111	0,371556

APPENDIX 2 – Code for SVM Algorithm

```
#include <iostream>
#include <sstream>
#include <fstream>
#include <opencv2/opencv.hpp>
#include <opencv2/core.hpp>
#include <opencv2/highgui.hpp>
#include <opencv2/ml.hpp>

using namespace cv;
using namespace std;
using namespace cv::ml;

#define AUTOLEARN 1


float app_ver = 1.4f;

/// printfv macro for printf depend on verbose variable
#define printfv(...) if(verbose == 1) printf(__VA_ARGS__)

float test_svm_file(string file_name, int kernel_type, bool* use_kernel_trick) {
	// File pointer
	fstream fin;
	if (!fin.is_open())
		fin.open(file_name, ios::in);

	if (!fin.is_open()) {
		printf("ERROR! %s file is not found in specified folder. Check the file ! \n ",
file_name);
		return -1;
	}

	// Get line count to get row count as dataset size
	std::ifstream inFile(file_name);
	int dataset_size = count(std::istreambuf_iterator<char>(inFile), std::istreambuf_iterator<char>(),
'\n');

	// Read first line to get colm_size and not use...
	string line;
	getline(fin, line, '\n');
	int colm_count = count(std::begin(line), std::end(line), ',');

	fin.clear();
	fin.seekg(0);		// get to start of file

	// Model selection with SVM dat files
	char model_name[50];
	if (colm_count == 1) {
		*use_kernel_trick = 1;
		sprintf_s(model_name, 50, "svm_spectrum_data_t%d_kt.xml", kernel_type);
	}
	else if (colm_count == 31) {
		*use_kernel_trick = 0;
		sprintf_s(model_name, 50, "svm_spectrum_data_t%d.xml", kernel_type);
	}
```

```
	else {
		printf("ERROR: Test data colm size must be either 2 or 31 depends on kernel trick or
not.\n"
			"Other type data sets is not valid.\n");
		return -1;
	}

	// Read given test dataset
	vector< int > ID;
	Mat data(dataset_size, colm_count, CV_32F);
	for (int i = 0; i < dataset_size; i++) {

		getline(fin, line, ',');
		ID.push_back(stod(line));  // fist colm is label

		for (int j = 0; j < colm_count - 1; j++) {
			getline(fin, line, ',');
			data.at<float>(i, j) = (stof(line));
		}

		getline(fin, line, '\n');
		data.at<float>(i, colm_count - 1) = (stof(line));
	}

	Ptr<cv::ml::SVM> svm = Algorithm::load<cv::ml::SVM>(model_name);

	// Self test
	Mat output(dataset_size, 1, CV_32F);
	svm->predict(data, output);

	// print test result for debug
	printf("  Data ID \t|\t Result (%% GMO) \n");
	printf("-----------------------------------------------\n");
	for (int i = 0; i < dataset_size; i++)
		printf(" %7d \t|\t %5d \n", ID[i], (int)output.at<float>(i, 0));

}

float svm_perf_eval(string file_name, int kernel_type, bool* use_kernel_trick) {
	// File pointer
	fstream fin;
	if (!fin.is_open())
		fin.open(file_name, ios::in);

	if (!fin.is_open()) {
		printf("ERROR! %s file is not found in specified folder. Check the file ! \n ",
file_name);
		return -1;
	}

	// Get line count to get row count as dataset size
	std::ifstream inFile(file_name);
	int dataset_size = count(std::istreambuf_iterator<char>(inFile), std::istreambuf_iterator<char>(),
'\n');

	// Read first line to get colm_size and not use...
	string line;
	getline(fin, line, '\n');
	int colm_count = count(std::begin(line), std::end(line), ',');
```

```
	fin.clear();
	fin.seekg(0);		// get to start of file

	// Model selection with SVM dat files
	char model_name[50];
	if (colm_count == 1) {
		*use_kernel_trick = 1;
		sprintf_s(model_name, 50, "svm_spectrum_data_t%d_kt.xml", kernel_type);
	}
	else if (colm_count == 31) {
		*use_kernel_trick = 0;
		sprintf_s(model_name, 50, "svm_spectrum_data_t%d.xml", kernel_type);
	}
	else {
		printf("ERROR: Test data colm size must be either 2 or 31 depends on kernel trick or
not.\n"
			"Other type data sets is not valid.\n");
		return -1;
	}

	// Read given test dataset
	vector< int > labels;
	Mat data(dataset_size, colm_count, CV_32F);
	for (int i = 0; i < dataset_size; i++) {

		getline(fin, line, ',');
		labels.push_back(stof(line));		// fist colm is label

		for (int j = 0; j < colm_count - 1; j++) {
			getline(fin, line, ',');
			data.at<float>(i, j) = (stof(line));
		}

		getline(fin, line, '\n');
		data.at<float>(i, colm_count - 1) = (stof(line));
	}

	Ptr<cv::ml::SVM> svm = Algorithm::load<cv::ml::SVM>(model_name);

	// Self test
	Mat output(dataset_size, 1, CV_32F);
	svm->predict(data, output);

	// print test result for debug
	printf("  Data  \t|\t Label \t|\t Prediction (%% GMO)  \n");
	printf("--------------------------------------------------------------------------------\n");
	for (int i = 0; i < dataset_size; i++)
		printf(" %5.4f \t|\t %5d \t|\t %5d \n", data.at<float>(i, 0), labels[i],
(int)output.at<float>(i, 0));

	// Compare
	int  error_cnt = 0;
	for (int i = 0; i < dataset_size; i++) {
		float tmp1 = output.at<float>(i, 0);
		float tmp2 = (float)labels[i];

		if (tmp1 != tmp2)
			error_cnt++;
	}
```

```
	float succes_rate = ((1 - ((float)error_cnt / dataset_size)) * 100);
	return succes_rate;

}


float create_svm_file(string file_name, bool use_kernel_trick) {

	char file_dir[255];
	sprintf_s(file_dir, 255, "Dataset/%s.csv", file_name.c_str());

	// File pointer
	fstream fin;
	if (!fin.is_open())
		fin.open(file_dir, ios::in);

	if (!fin.is_open()) {
		printf("ERROR! %s file is not found in specified folder. Check the file ! \n", file_dir);
		return -1;
	}

	// Get line count to get row count as dataset size
	std::ifstream inFile(file_dir);
	int dataset_size = count(std::istreambuf_iterator<char>(inFile), std::istreambuf_iterator<char>(),
'\n') - 1;

	// Read first line to get colm_size and not use...
	string line;
	getline(fin, line, '\n');
	int colm_count = count(std::begin(line), std::end(line), ',');

	// Read and Splitting dataset
	int train_dimension;
	if (use_kernel_trick)
		train_dimension = 1;
	else
		train_dimension = colm_count - 1;

	Mat train_data(dataset_size, train_dimension, CV_32F);

	// Use whole dataset on training
	vector< int > train_labels;
	for (int i = 0; i < dataset_size; i++) {

		getline(fin, line, ',');
		train_labels.push_back(stof(line));  // fist colm is label

		for (int j = 0; j < colm_count - 1; j++) {
			getline(fin, line, ',');
			if (!use_kernel_trick)
				train_data.at<float>(i, j) = (stof(line));
		}

		getline(fin, line, '\n');
		if (use_kernel_trick)
			train_data.at<float>(i, 0) = (stof(line));
	}

	// configure SVM
	Ptr<SVM> svm = SVM::create();
```

```
	if (AUTOLEARN)
		svm->trainAuto(train_data, ROW_SAMPLE, train_labels);
	else {
		svm->setKernel(SVM::LINEAR);
		svm->setType(SVM::C_SVC);
		svm->setC(15);
		svm->setGamma(1);
		svm->train(train_data, ROW_SAMPLE, train_labels);
	}

	char svm_name[255];
	if (use_kernel_trick)
		sprintf_s(svm_name, 255, "svm_%s_kt.xml", file_name.c_str());
	else
		sprintf_s(svm_name, 255, "svm_%s.xml", file_name.c_str());
	svm->save(svm_name);

	// self test
	Mat output(dataset_size, 1, CV_32F);
	svm->predict(train_data, output);

	char image_name[255];
	sprintf_s(image_name, 255, "Dataset/%s_%d.png", file_name.c_str(), use_kernel_trick);
	Mat img;
	img = train_data.clone();
	resize(train_data, img, Size(), 100, 100, 0);
	imwrite(image_name, img*255);

	//Compare
	vector < int >  errors;
	for (int i = 0; i < dataset_size; i++) {
		float tmp1 = output.at<float>(i, 0);
		float tmp2 = (float)train_labels[i];

		if (tmp1 != tmp2)
			errors.push_back(i);
	}

	int error_cnt = errors.size();

	printf(" %d of %d data has been clasifed succesfully \n", dataset_size, error_cnt);
	float succes_rate = ((1 - ((float)error_cnt / dataset_size)) * 100);
	return succes_rate;
}


/** @brief This only prints error message when a number is missing on user input.
 */
int print_error() {
	printf("ERROR: User input value is missing! \n");
	exit(EXIT_FAILURE);
}

int main(int argc, char* argv[])
{

	bool verbose = 0;
	int diameter = 0;
	float concentration = 0;
```

```
	bool devel = 0;
	bool prediction = 0;
	char file_name_[255];

	// User input handler loop
	for (int i = 1; i < argc; i++) {
		if (strcmp(argv[i], "--help") == 0) {
			printf("GMO NanoBioSensor demo app \n");
			printf("App version: v%6.3f \n", app_ver);
			printf("Example usage: gdodt.exe -f data.csv -d 13 -c 4.7  \n");
			printf("Options:  \n");
			printf("\t -v or --verbose 	--> For print debug outputs, <bool>, Default:0
\n");
			printf("\t -f or --file_name 	--> Test data set file formatted as csv. <string>
\n");
			printf("\t -d or --diameter 	--> Gold NanoParticle diameter. [13 or 15]\n");
			printf("\t -c or --concentration 	--> Gold NanoParticle concentration. [4.4 or
4.7] \n");
			printf("\t -p or --prediction 	--> Prediction validation with known data set
\n");
			exit(EXIT_SUCCESS);
		}
		else if ((!strcmp(argv[i], "-v")) || (!strcmp(argv[i], "--verbose"))) {
			verbose = 1;
		}
		else if ((!strcmp(argv[i], "-f")) || (!strcmp(argv[i], "--file_name"))) {
			if ((i + 1 < argc) && (*argv[i + 1] != '-'))
				strcpy_s(file_name_, argv[++i]);
			else
				print_error();
		}
		else if ((!strcmp(argv[i], "-d")) || (!strcmp(argv[i], "--diameter"))) {
			if ((i + 1 < argc) && (*argv[i + 1] != '-'))
				diameter = strtol(argv[++i], NULL, 0);
			else
				print_error();
		}
		else if ((!strcmp(argv[i], "-c")) || (!strcmp(argv[i], "--concentration"))) {
			if ((i + 1 < argc) && (*argv[i + 1] != '-'))
				concentration = atof(argv[++i]);
			else
				print_error();
		}
		else if ((!strcmp(argv[i], "--developer_mode"))) {
			devel = 1;
		}
		else if ((!strcmp(argv[i], "-p")) || (!strcmp(argv[i], "--prediction"))) {
			prediction = 1;
		}
		else {
			printf("ERROR: Invalid input, please look --help for help \n");
			exit(EXIT_FAILURE);
		}
	}

	printfv("User selections: -v %d, -f %s, -d %d, -c %2.1f \n", verbose, file_name_, diameter,
concentration);

	float result;
	if (devel) { // SVM learning with knonw data and creation model.xml
```

```
		vector<string> file_name;
		file_name.push_back("spectrum_data_t1");
		file_name.push_back("spectrum_data_t2");
		file_name.push_back("spectrum_data_t3");

		for (int t = 0; t < file_name.size(); t++) {
			result = create_svm_file(file_name[t], 0);
			printf("Success rate of %s without KT is %f\n", file_name[t].c_str(), result);
			result = create_svm_file(file_name[t], 1);
			printf("Success rate of %s with    KT is %f\n", file_name[t].c_str(), result);
		}
	}
	else if (prediction) { // SVM Performans evaluation test with known data
		// Selection of dataset type
		int type = 1; // type 1 is default
		if (diameter == 13 && abs(concentration - 4.7f) < 0.001f)
			type = 1;
		else if (diameter == 15 && abs(concentration - 4.7f) < 0.001f)
			type = 2;
		else if (diameter == 15 && abs(concentration - 4.4f) < 0.001f)
			type = 3;
		else
			printfv("WARNING: Not define test data type.. diameter 13 and concentration
= 4.7 will use!\n");

		bool is_kernel_trick;
		result = svm_perf_eval(file_name_, type, &is_kernel_trick);

		if (result == -1)
			exit(EXIT_FAILURE);
		else
			printf("Success rate of %s is %f\n", file_name_, result);
	}
	else { // Real Test with unknown data
		// Selection of dataset type
		int type = 1; // type 1 is default
		if (diameter == 13 && abs(concentration - 4.7f) < 0.001f)
			type = 1;
		else if (diameter == 15 && abs(concentration - 4.7f) < 0.001f)
			type = 2;
		else if (diameter == 15 && abs(concentration - 4.4f) < 0.001f)
			type = 3;
		else
			printfv("WARNING: Not define test data type.. diameter 13 and concentration
= 4.7 will use!\n");

		bool is_kernel_trick;
		result = test_svm_file(file_name_, type, &is_kernel_trick);

		if (result == -1)
			exit(EXIT_FAILURE);


	}
}
```

APPENDIX 3 – Publications from M. Sc Thesis

Taskin, Y., Samaradivakara S., Day, B., Yilmaz, R., DNA-based Nanobiosensor for Visual Detection of a GMO Content in Soybean Event MON87701. 26. 2nd International /12th National Food Engineering Congress (2021, November) Ankara (Oral Presentation)

Taskin, Y., Samaradivakara S., Day, B., Yilmaz, R., The use of different size and concentration of gold nanoparticles for detection of Cry1Ac gene. 21st Biotechnology Congress with International Participant (2021, December) Ankara (Oral Presentation)

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