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Stool Samples DNA Extraction

Parkinson's Disease

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The subject of the study was DNA isolation from stool samples in persons who are at risk to get Parkinson's disease. The study was conducted at the University of Helsinki, Institute for Biotechnology at the DNA sequencing and genomics laboratory. This thesis is one part of the University of Helsinki Parkinson's disease study. The focus of this study was to isolate DNA from stool samples and to create a summary of the results of the data.

The University of Helsinki has collected 745 stool samples. The purpose was to randomize the stool samples first and then isolate the DNA. Isolated DNA samples were measured with a NanoDrop spectrophotometer apparatus to find out the DNA concentration of the samples. The theoretical part of the thesis includes information on Parkinson's disease and material handling. Based on an established protocol, DNA isolation was performed.

For the study, 745 pieces of stool samples were collected. The 745 stool samples were randomly divided into batches of 23 samples for DNA isolation. To each batch one blank for kit contamination control was added, for a total of 24 samples per isolation batch.

Based on the NanoDrop spectrophotometer result, the lowest result was 0.61 ng/μL and information will be the highest result was 26.6 μg/μL. Isolated DNA samples studies will continue by using PCR amplification, and will be samples sequenced, processed bioinformatically, and analyzed with statistical methods.

Keywords	Parkinson's Disease, DNA, Randomization, DNA Isolation, NanoDrop Spectrophotometer.

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Opinnäytetyön aiheena oli DNA:n eristäminen ulostenäytteistä henkilöiltä, joilla on riski sairastua Parkinsonin tautiin. Opinnäytetyö tehtiin Helsingin yliopiston biotekniikan instituutissa DNA-sekvensointi- ja genomiikkalaboratoriossa. Tämä opinnäytetyö on osana Helsingin yliopiston Parkinsonin taudin tutkimusta. Opinnäytetyön prioriteetti oli DNA:n eristäminen ulostenäytteistä ja yhteenvedon tekeminen datan tuloksista.

Helsingin yliopisto on kerännyt 745 ulostenäytettä. Tämän opinnäytetyön tarkoituksena oli satunnaistaa ulostenäytteet ensin ja sitten eristää DNA:ta. Eristetyt DNA:n näytteet mitattiin NanoDrop-spektrofotometrilaitteella, jotta saatiin selvitettyä näytteiden DNA-pitoisuutta. DNA:n eristys tehtiin tunnetun protokollan mukaan. Opinnäytetyön teoreettinen osa sisältää tietoa Parkinsonin taudista ja materiaalien käsittelystä.

Tutkimusta varten on kerätty 745 kappaletta ulostenäytteitä, joita näytteet jaettiin satunnaisesti 23 kappaleen eriin DNA:n eristämistä varten. Kullekin erille lisätiin yksi nollanäyte kontaminaatiokontrollia varten, yhteensä 24 näytettä per eristys erä.

NanoDrop-spektrofotometrin tuloksen perusteella alin tulos oli 0,61 ng/μL ja korkein tulos oli 26,6 μg/μL. Eristettyjen DNA-näytteiden tutkimukset jatkuvat käyttäen PCR-monistusta, ja näytteet sekvensoidaan, prosessoidaan bioinformatiikan avulla ja analysoidaan tilastollisin menetelmin.

Avainsanat	Parkinsonin tauti, DNA, satunnaistaminen, DNA eristäminen, NanoDrop spektrifotometri.

Contents

Abbreviations

αSYN	Alpha-Synuclein
CNS	Central Nervous System
EtOH	Ethanol
GI	Gastrointestinal
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
LPS	Lipopolysaccharide
NMS	Non-Motor Symptoms
PD	Parkinson’s Disease
SNCA	Synuclein Alpha

1 Introduction

The study was conducted at the DNA sequencing and genomics laboratory at Institute of the Biotechnology University of Helsinki. This work is based on previous studies which suggest that there is intestinal dysfunction present in Parkinson's disease which associates between certain gut bacteria and the Parkinson's disease [1]. The project of the Parkinson's disease has taken almost three years, which has included planning the project, raising fund for the project, collecting the stool samples from Germany, randomizing the samples, extracting the DNA from stool samples so that the DNA libraries will build by using PCR, sequenced, processing bioinformatically, and analysed with statistical methods.

Parkinson's disease is a neurodegenerative disorder and major cause of losing the dopaminergic neurons remains unknown. Symptoms will get worse over time [2]. There has been evidence that genetic and environmental factors are also connected with Parkinson's disease. The researchers [3] claim that there are different gene mutation in specific chromosomal regions. Gene mutations vary in different countries and main risks of developing Parkinson's disease in an individual are the presence of another affected family member and increasing of the person's age. The earlier the age of Parkinson's disease onset, the higher chance that the genetic factors play a significant role. Environmental influences play an important role in the cause of sporadic Parkinson's disease. Living in the countryside has been associated with the agricultural industry, which increases the risk of developing Parkinson's disease. It has been suggested that pesticides and herbicides may contribute to causing the increased risk of Parkinson's disease for those in rural areas [3; 4; 5].

The purpose of this study was to randomize the stool samples and extract the DNA. The samples were randomized to avoid the batch effects which can bias the result [6] and extract the DNA from stool samples. This project is part of the Parkinson's disease study to research the connection between of bacteria and Parkinson's disease.

2 Parkinson’s Disease (PD)

Parkinson’s disease is a slow chronic and progressive disorder [2] and typically diagnosed between ages 50 to 70 [7; 8]. Parkinson’s disease is degenerative disease and symptoms will continue and will get worse over time, and the major cause for losing the dopaminergic neurons remains unknown [2; 9]. When brain cells start to lose dopaminergic neurons in the substantia nigra, it will start to affect on mood and weaken slowly the control of the movements, posture, and balance [10; 11]. Parkinson’s disease has different symptoms and primary motor signs are: tremor (hands-, arms-, face- and legs shaking), bradykinesia (slowed movement), rigid muscles (muscle stiffness may limit and occur in any part of a body, that can cause pain) and postural instability (impaired balance, condition, loss of automatic movements, speech- and writing changes) [2; 11]. The Parkinson’s disease symptoms are described in Figure 1 below.

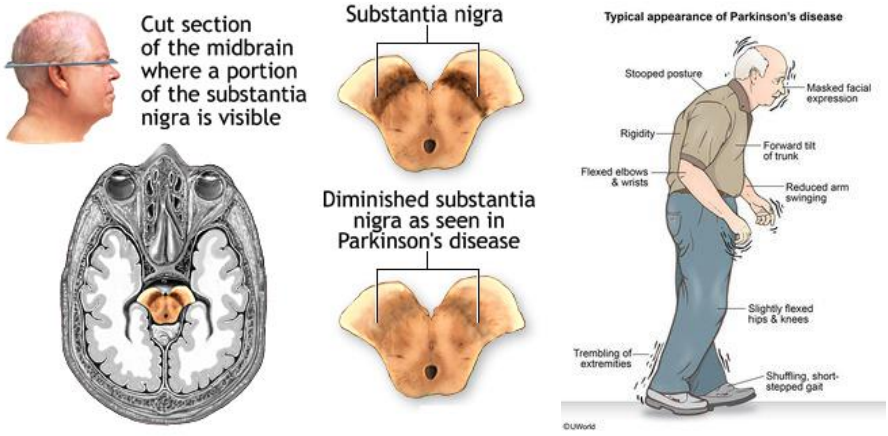


Figure 1. Figure on the right show person who has Parkinson's disease and the symptoms of Parkinson's disease; tremor, bradykinesia, rigid muscles and postural instability [12]. The figure on the left shows where a substantia nigra is located and differences in the brain structure between healthy person and person who has Parkinson's disease [13].

αSYN is naturally occurring protein found in human brain and all nerve cells of the body, and in red blood cells, heart, muscle and other cells [14; 15]. α-Synuclein is one of first genes that has been associated with Parkinson’s disease, and mutation in Synuclein alpha (SNCA) causes Parkinson’s disease autosomal dominant forms and is basis risk to developing the sporadic Parkinson’s disease [16].

The neuronal toxicities α-Synuclein cause is unknown in Parkinson's disease but it known that it plays a central role in the pathogenesis of Parkinson's disease [16]. Figure 2 shows the Lewy bodies in substantia nigra. Substantia nigra is located in the midbrain of Parkinson's disease patients, and can be seen in figure 1.

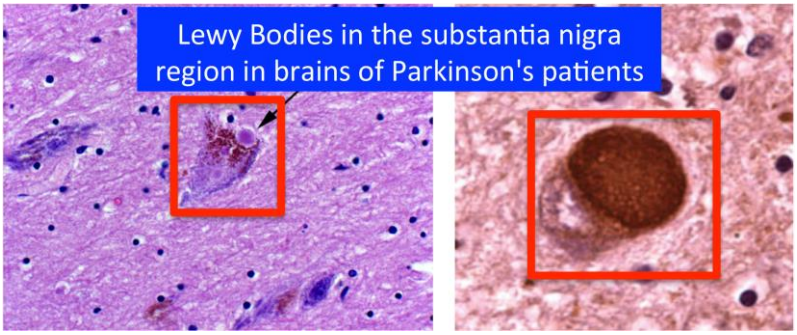


Figure 2. Lewy Bodies is located in the substantia nigra in midbrain of Parkinson's disease patients [17].

αSYN creates precipitates and forms a ball-shaped Lewy bodies in Parkinson's disease, and also develops Parkinson's disease [3; 5; 18].

2.1 Risk Factors of Parkinson's Disease

Nowadays researchers have managed to connect gut microbiota in PD, which have suggested intestinal environment can affect the activity of intestinal central nervous system (CNS). Evidence has also been suggesting that the vagus nerve might act as the direct canal through which substance from the intestine can pass to the brain. Changes in intestinal bacteria population have been associated with Parkinson's disease, autism, multiple sclerosis, schizophrenia, depression, anxiety and post-traumatic stress disorder [1].

The human intestine has more than 100 trillion bacteria along with abundant viruses and fungi, and the intestinal immune is constantly exposed to microbe antigens that cause stimuli that extends inflammatory reaction. Intestinal tissue damage that exposes to substances that irritate strong immune reactions, can increase the inflammation of the intestinal environment. Also, the introduction of aggressive pathogens, which can turn enteric inflammation can induce a numerous of effects that ultimately changes the CNS function. Chronic intestinal inflammation disorders may develop eventually Inflammatory Bowel

Disease (IBD) and Irritable Bowel Syndrome (IBS). Also, Lipopolysaccharide (LPS) increase intestinal permeability and are highly immunogenic and activate systemic inflammatory responses. Many of these diseases are related to advanced age and intestinal inflammation and forms of intestinal permeability increase with aging. Evidence has shown that Gastrointestinal (GI) and also Nonmotor Symptoms (NMS) are connected with the earliest stage of Parkinson's disease (PD) [1].

One of the early stage non-motor symptoms (NMS) of Parkinson's disease are constipation, rapid eye movement, sleeping behaviour disorder, hyposmia, anxiety, etc. Constipation is the most common and the second is NMS which is hyposmia in PD. Constipation is one of the pre-motor symptoms obvious years before CNS degenerations is apparent in diagnosis of PD, and also abnormal enteric αSYN is present before CNS neurodegeneration has advanced satisfactorily to produce motor symptoms [1].

2.2 Bacteria Connected to Parkinson's Disease

Researchers have discovered a connection between a few bacteria and Parkinson's disease (PD). The bacteria start their development in the guts and the pathology spreads to the brain later on. The pathology may be initiated by gut bacteria before diagnosing the Parkinson's disease, the patient suffers from early symptoms such as constipation. The bacteria that have been connected to Parkinson's disease are *Prevotellaceae, Lactobacillaceae, Verrucomicrobiaceae, Bradyrhizobiaceae and Clostridiales Incertae Sedis IV* [19].

The bacteria are located in the intestine and they're transmitted through metabolism to stool. The gut-brain incorporates bidirectional communication between the central nervous system and the enteric nervous system and endocrine systems. Regulation of immune responses is in the gut, as well as in the brain. The activity of intestine appears to be heavily influenced by microbes [1].

2.3 16s rRNA gene sequence

16s rRNA gene sequence is for bacterial identification. Each type of bacteria has different sequences, but they are similar to 16s rRNA sequences. The 16s rRNA gene is the most used marker gene for economical of large projects and the diversity reference data set for bacteria. 16s rRNA gene sequences are used for the study of bacterial taxonomy and phylogeny and 16s rRNA gene is generally selected for achieving high taxonomic resolution. 16s rRNA gene sequence informatics is to provide species and genus identification for taxa that are infrequently associated with human infectious disease and identification for isolations that do not fit slightly recognized biochemical profiles [20; 21].

3 Sample Management

It was stated in the previous chapter that bacteria are in a person's body and are found in stools. Researching the DNA of bacteria that are located in stools is more complex for the research to reach its desired result. This chapter briefs the theoretical part of the management of given samples, and then gives a practical explanation how the sample management was implemented for this study.

3.1 The Quantity of Samples Statistically

Important prerequisite is computational tools that are able to quickly and accurately compare large amounts of data produced from complex bacterial communities to identify the properties that distinguish them. The extracted DNA samples are sequenced and a metagenomic library is created, and the method of metagenomics can be used to search for new pathogens or microbes in the intestine [22].

Metagenomics purposes are to comprehend the function and structure of microbial populations exclusively through DNA analysis, and statistical method is for detecting the differentially large structures between microbial populations. Each sample is provided with count data that represent the relative abundance of specific features with each sample, for example, 16s rRNA clones a specific taxon. 16s rRNA were explained in previous chapter 2.3 [22].

The statistics are based on probability calculations that study the collection processing and statistical analysis of statistical data. The statistics can be used to measure observations and to deal with data generated by measurements. Statistic can be divided into theoretical and applied statistics and plays an important role in analyzing the results [22].

3.2 Sample Storage

Sampling, storing and processing of samples are one of the critical components of DNA-based microbial community analysis processes of environmental samples. DNA and RNA of a stool and the composition of its microbial community can change its form in warmer temperature. If stool samples stay at room temperature for 2 weeks, the DNA degeneration further increases and nearly all high-molecular weight fragments will disappear. In order to keep the stools highly molecular weight fragments the stool should be frozen -20°C as soon as a sample of it is taken, so the potential of the sample starting to ferment is as minimal as possible. The ideal temperature for sample storage is -80°C. The storage condition has a large influence on the taxonomic composition of the samples bacterial taxa [21; 23; 24].

3.3 Randomization of Samples

The randomizing of the sample right at the beginning prevents the variation of batch effect and the result. Randomization is to minimize the so-called batch effects. Batch effects causes are unknown technical variables in a study. One the reason that causes the batch effects are laboratory conditions, reagent lots and personal differences. This becomes a significant problem when batch effects are correlated with an outcome of interest and lead to incorrect conclusions [6].

3.4 Samples Contamination

It is needed to understand all sources that could possible contaminate the samples that affect PCR results, for avoiding contamination. Aseptic technique is routine that prevents samples, reagents, instruments and other solutions from being contaminated by unwanted micro-organisms. There is also related additional issue in the presentation of contaminating microbial DNA during sample preparation, and the reason might be DNA extraction kit, reagents, and molecular biology grade water. The low microbial biomass is easy to contaminate and can affect misleading DNA results [25; 26].

In today's laboratories, aseptic techniques are critical. The aseptic technique includes proper laboratory equipment such as laboratory coat, disposable protective gloves, sterile pipet tips, and tubes [25]. To prevent the sample contaminations, laminar has to be cleaned with 70% ethanol (EtOH), and also with DNA away reagent buffer. DNA away buffer destroys unwanted DNA and cleans better than 70% ethanol (EtOH). Ethanol (EtOH) cleans the laminar, but not the same way as DNA away buffer does. It's recommended that ethanol and DNA away buffer to be used before using the laminar and after using the laminar [25].

4 DNA Isolation

DNA extraction of bacteria from stool sample is one of the critical steps. Previous studies have demonstrated that many factors can affect the composition of the gut microbiota, including human genotype, diet etc. There are many different protocols to extract the DNA from bacteria, different sampling, and analytical methods can influence the decided microbiome composition. However, most of the protocol follows the same principle to extract the DNA: break the cell walls of bacteria and release the DNA, remove inhibitors and elute the DNA. Choosing the correct protocol is important to prevent the great impact on the purity and amount of DNA [27; 28; 29].

Stool sample cell lysis can be done in three different ways: enzymatically, chemically or mechanically, as described by protocol [27; 29; 30]. Enzymatically and chemically lysis methods are careful but also have limited access to all target organisms and selectivity for different cell type. Mechanical bead cell lysing, which is used for this study, is the most effective method since it not only breaks the cell wall of bacteria but also homogenizes the sample even more. The effect of beads, lysis, and homogenization gives the reagents in the next step an ideal environment for removing PCR inhibitors from the sample, although beads provide an effective way of revealing the DNA but lysing the sample too much, is considered to be too destructive for chromosomal DNA studies [29].

Stool samples contain many different inhibitors and one of them are complex polysaccharides and bile salts [31; 32; 33]. Bile salt is conjugated bile acid formed in the liver and is one of major bile acid found in humans approximately 0.7 % [34].
If Bile salt and complex polysaccharides are not removed properly it can inhibit the PCR results, depending on cofactors, in the PCR reaction. The PCR is an enzymatic reaction and therefore sensitive to inhibitors [35; 36].

The inhibitor removal is done with appropriate bile salt method [30; 37]. Bile salts can be inhibited by binding them to molecules such as magnesium hydroxide, cholestyramine, sucralfate, meciadanol or aluminium hydroxide [38]. The remaining inhibitors can be removed by a simple washing step since the biding eliminates most inhibitors from the samples. For example, silica membrane [37] DNA binding and washing steps reduced the amount of PCR inhibitors from 12.5% to 1.1%. The removal of inhibitor is commonly done by pelleting the inhibitors through centrifugations or binding the DNA molecules to another molecule and by washing surroundings [39].

Stool samples contain normal proteins as well as reagents from extraction kits that inhibit PCR reactions. Proteinase K is one of the most commonly used enzymes, which is a board-spectrum serine protease digest inhibits the proteins in DNA extraction [40, 41]. It has been described in the manual [41] that Proteinase K cleaves peptide bonds at the aromatic, carboxyl sides of aliphatic or hydrophobic amino acid. Proteinase K activates by urea and dodecylsulfate that is caused primarily by denaturation of the protein substrates. Combination of dodecylsulfate and Proteinase K achieves to protect RNA from degradation during isolation of polysomal RNA, in which ribonuclease can't prevent inhibition without the combination of dodecylsulfate and Proteinase K [41]. Figure 4 shows the Proteinase K activity aliphatic reaction.

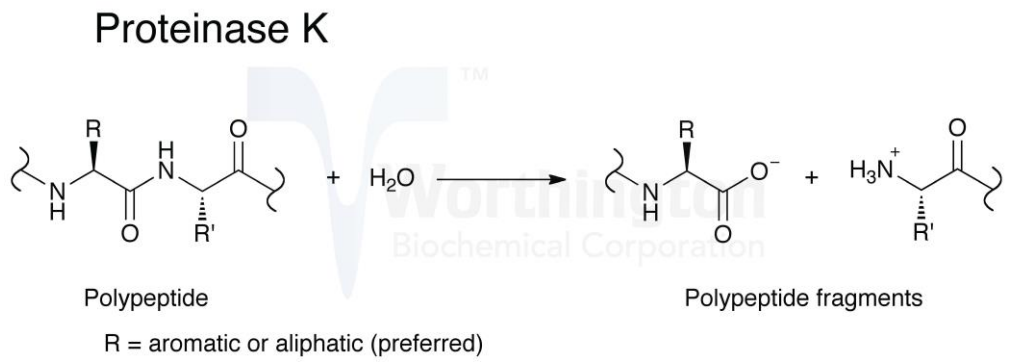


Figure 4. An example of Proteinase K chemical polypeptides activity reaction [42].

In the manual [43] it says that recommended working concentration of Proteinase K is 0.05–1 mg/mL, and that the activity of the enzyme is stimulated by 0.2-1% SDS or 1-4 M Urea [41].

The final DNA processing is the washing and elution step. Washing part purifies the DNA from most of the remaining PCR inhibitors and pollutant proteins. If a silica column filter is used, silicate filter is one of washing steps that can be performed and DNA binds to silicate in the presence of strong salts. This is potentially suitable for negative charge of both silica and DNA. The negatively charged ionized salt molecules set between the positive charges that form a hydrogen bond. This bond will not break as long as pH stays stable and amount of salt stays high. This allows washing further to remove the ethanol and salts from DNA. However, it has been established that DNA binds with silica based elution, which can be little as 21% of the original amount of DNA [32, 37].

Figure 5 show the silica spin filter, spin column based nuclein acid purification. Nucleic acid binds to the solid phase of silica under certain conditions [44].

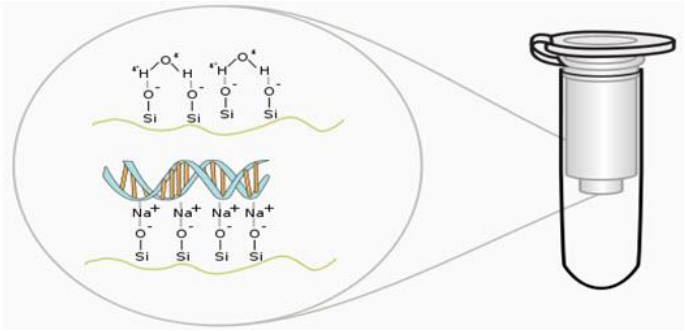


Figure 5. The principle of silica membrane is DNA binding. In the presence of chaotropic salt, DNA molecules bind into the hydrolyzed Na+ with hydrogen bond [44].

The elution buffer has been designed for storing the samples and the elution of DNA. The DNA, bound to the silicate filter, is eluted when the concentration of the binding salt is diluted enough. The DNA is eluted through the filter through a centrifuge [30].

For DNA, the 260/280 ratio is used to access purity and a ratio of ~ 2.0 and nucleic acid ~1.8 is generally accepted as pure. The 260/230 values of pure nucleic acid are often higher than the respective 260/280 values and expected values are commonly in the range of 2.0 – 2.2 [29, 30, 45].

4.1 DNA Extraction Kit

The PSP Spin Stool DNA Kit has compounded methods for collection, transportation, and storage of stool samples and consequent DNA purification. The PSP kit has been designed to be simple to isolate any microorganism's DNA or host DNA organism. The ideal of purified DNA is to trustworthy use in PCR and other any downstream analysis [27]. PSP Spin Stool DNA Plus Kit was chosen to extract DNA from the stool sample. Researchers in the article [37] had been testing different kind extraction kit, and SPS Spin Stool DNA Plus Kit was chosen based on the resulting stool sample collection, transportation, and storage [27].

4.2 Controls of DNA Extraction

In the DNA extraction two controls were used. One blank negative PSP kit control and one positive ZymoBIOMICS control. The blank control kit makes sure, that the kit reagents are not contaminating the samples, and the kit blank control also is sequenced and compared with the samples [30]. During the DNA extraction process, control is prepared at the same time. DNA extracted by protocol [Appendix 1]. In the DNA isolation, for example, positive control ZymoBIOMICS can be used. This helps to prevent misreading the analytical results, therefore standardization is critical for minimizing bias and quality control of entire microbiomics workflows. ZymoBIOMICS microbial community standard mocks the community of microbial containing of two fungal strains and eight bacterial. It includes two tough-to-lyse yeasts (e.g. Saccharomyces cerevisiae), three easy-to-lyse Gram-negative bacteria (e.g. Escherichia coli) and five though-to-lyse Gram-positive bacteria (e.g. Listeria monocytogenes). The microbial standard is particularly characterized and contains irrelevant impurities < 0.01%. ZymoBIOMICS can be used to expose error, artifacts, and bias in metagenomics or microbiomics workflows [46]. ZymoBIOMICS microbial community standard was prepared at the end of stool samples DNA extraction [Appendix 1]. Table 1 shows Microbial community standard mock the microbial that are contained in a standard solution.

Table 1. ZymoBIOMICS microbial community standard mocks the community of microbial containing of two fungal strains and eight bacterial [46].

Contains bacteria species
Pseudomonas aeruginosa
Escherichia coli
Salmonella enterica
Lactobacillus fermentum
Enterococcus faecalis
Staphylococcus aureus
Listeria monocytogenes
Bacillus subtilis
Saccharomyces cerevisiae
Cryptococcus neoformans

Blank PSP kit is negative control and ZymoBIOMICS is positive control, which helps to prevent misreading the analytical results [30; 46].

5 Materials and Methods

Stool samples were from Germany and the total number of samples was 745 pieces. They were collected from people who weren't diagnosed with Parkinson's disease before the day the stool was collected, but they were classified as a risk of Parkinson's disease group.

Stool sample microbiomes of 745 risk of developing Parkinson's disease patients and 34 kit blank kit controls were DNA extracted, to measure with NanoDrop and later will be studied by using PCR, sequenced, processed bioinformatically, and analyzed with statistical methods [19; 22]. Chapter 2.2 Bacteria connected to Parkinson's disease, explained the bacteria.

5.1 Collecting the Samples

Researchers require samples are first collected on container before transferring to sample tube. The reason why the sample is collected first on the container is to facilitate the transfer of stool samples easier to sample tube. There were three types of collector tubes from one patient, one for DNA, one for RNA and one for protein analysis. DNA and RNA tube were contained the stabilizer solution and protein did not contain in stabilizer solution [30].

Stool sample of this study was collected in the tube, which helps to collect, store and transport the sample. DNA Stabilizer solution prevents any degeneration of the DNA during the transportation, and the prelysis of bacteria fast and effective isolation of high-quality DNA from the stool sample. DNA stabilizer also preserves the microorganism titer [30].

5.2 Randomizations

When the stool samples were arrived at laboratory at department of the University of Helsinki, the samples have been stored at -80°C freezer, then taken to -20 to randomize and prepare batches, and put back to -80 again to minimize the degradation of bulk DNA by confining the activity of endogenous nuclease [30].

Samples were randomized by selecting randomly and marked to Excel. Sample boxes were marked with DNA extraction and the purification order number. One box contained 23 samples and one blank control kit. Table 2 shows the Excel table, where samples were read with laser barcode scanner. Barcode scanned samples' barcode and trend ID. The barcode was on tube's label. Information's helps in interpreting results, if there was something in the analysis results and also helps to choose the correct protocol of DNA isolations.

Table 2. Box number is extracting order number, barcode, and trend ID number is patient's personal number that has been given for DNA extraction and note.

Box Number	Barcode	Trend ID	Notes	Purification number	Trend ID	Tube Label
DNA_30	DA0237	7028	Tube is full of stool!	703	7028	DA0237
DNA_30	DA545	7272		704	7272	DA545
DNA_30	DA469	7211		705	7211	DA469
DNA_30	DA643	1227	Tube full of stool, may not have DNA stabilizer	706	1227	DA643
DNA_30	DA0133	7349		707	7349	DA0133
DNA_30	TYD836	1404	May have very little DNA stabilizer	708	1404	TYD836
DNA_30	DA0043	7065		709	7065	DA0043
DNA_30	DA0004	7148		710	7148	DA0004

5.3 DNA Isolation

DNA isolation was done by PSP Spin Stool DNA Plus Kit manual [30]. There were few samples that were a solid, or thick, or missing stabilizer in the tube. This kind of samples were pipetted by protocol [Appendix 1]. A deviant DNA extraction instruction was for the samples that were a missing stabilizer, or solid, or thick samples, the samples were weighed 200 mg into a 2,0 ml Safe-Lock-Tube and were added 1,2 ml nuclease-free H_2O, and vortexed for 1 minute [Appendix 1].

There were some stool samples that thawed quicker than the others, but generally, samples thaw equally. There were few samples in each box that thaw unequally from the norm. There is no significant addition of DNA damage to fragments or variation of bacterial species, unless the samples were kept melted for hours or days. The thawing of samples usually took about one hour [21; 23; 24].

ZymoBIOMICS standard were extracted by protocol [46], ZymoBIOMICS standard were pipetted 0.75µL into a 2,0 ml Safe-Lock-Tube and were added 1,325 mL DNA stabilizer, and continued the same way as protocol [Appendix 1].

5.4 Extracted DNA Measurement with NanoDrop.

Samples were measured with NanoDrop spectrophotometer and the result was saved in the folder and the result was printed out. The printed result was attached to the laboratory book. The DNA microtube samples were transferred to the storage box and exported to a -20°C freezer. The storage box was marked PD DNA Extraction and number order of extracted DNA. Figure 7 shows the extracted DNA microtube from the stool sample, and on the microtube the sample's ID label were attached.

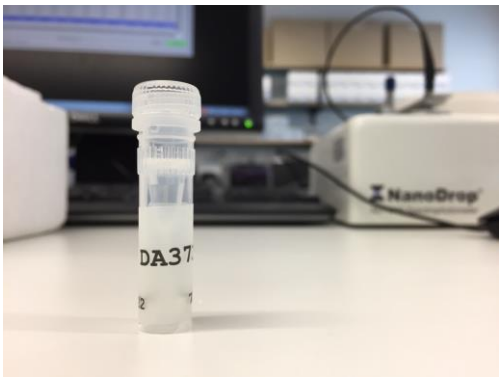


Figure 7. The collected DNA from stool samples was measured with NanoDrop spectrophotometer.

6 Results

745 amount of isolated DNA concentration results were between 0.61ng/μL and 26.6 μg/μL. The limit of result has been set below 20ng/μL because there are a few results that were low under 20 ng/μL and correlations were either normal, high or low result. The purity of extracted DNA values is commonly in the range of 1.8 – 2.0 [30].

Figure 8 shows the all extracted results that were isolated from 745 stool samples, 34 controls samples. Figure 8 also shows the highest result of 26.6 ng/μL, which is sample purification number 171 and the lowest result 0.61 ng/μL, which is sample purification number 4.

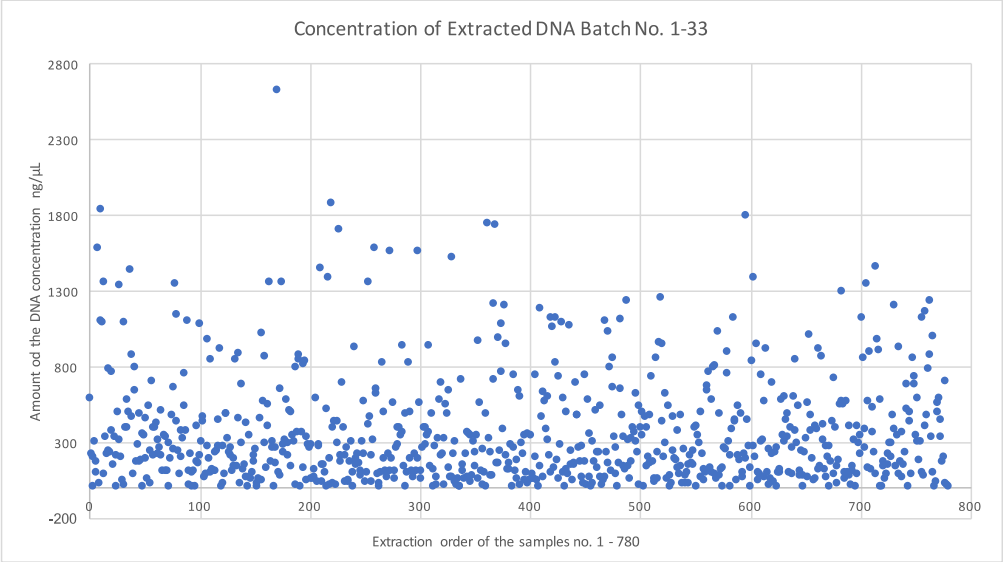


Figure 8. The result of total 780 samples, including 34 control samples.

All samples average concentration results were 377.80 ng/μL and blank control kits samples purity average were 0.11 ng/μL.

Figure 9 shows the low result that was lower 20 ng/µL of extracted DNA from stool. There were a few samples that were under 20 ng/µL, but the correlation was three different kind of: normal, low and high correlation.

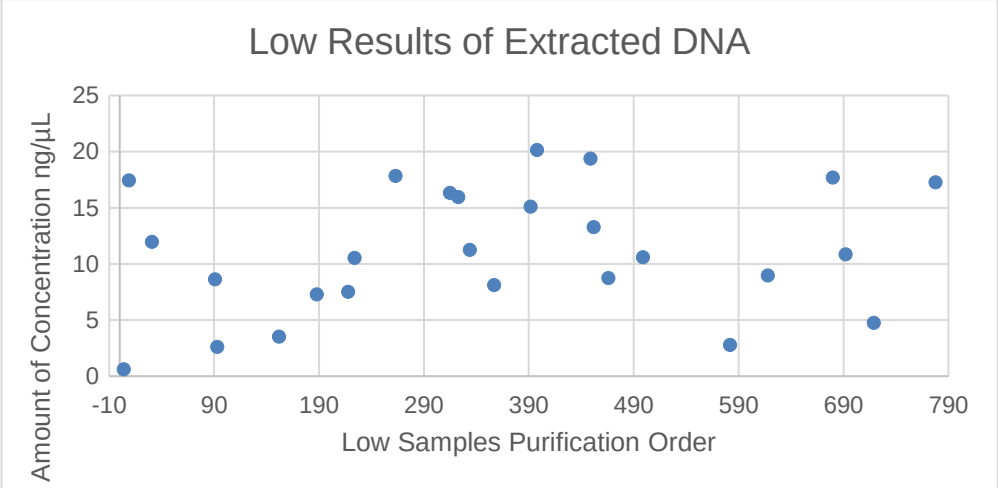


Figure 9. Low concentration result of extracted DNA from stool samples.

Since the results were low, it was wanted to see the purification in clustered column. In figure 10 the purification of low samples can be seen. The sample D-93 purification value was in 3.34, and sample D-4 and D-263 was the lowest purification. Sample D-4 purification value was -1.55, and sample D-263 purification value was 0.18. Most of the samples were in the wanted purification value ~1.8 – 2.0. Overall, the low results were in the limit value. Figure 10 shows the average of standard error results. Average of standard error means an average error in statistical science and can be used to measure a confidence interval of a certain probability.

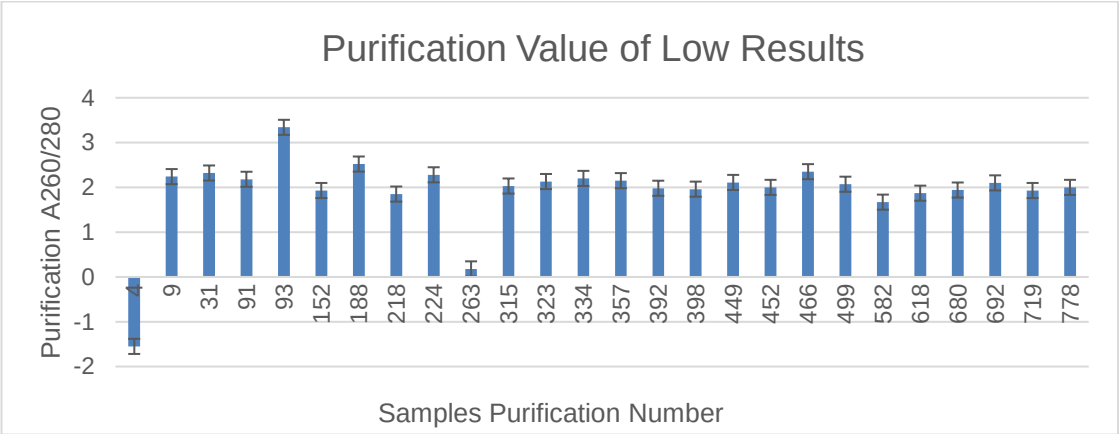


Figure 10. Purification of low results that were under 20 ng/µL and average of standard error.

Figure 11 has been shown the purities of the average and standard error of low samples in scatter.

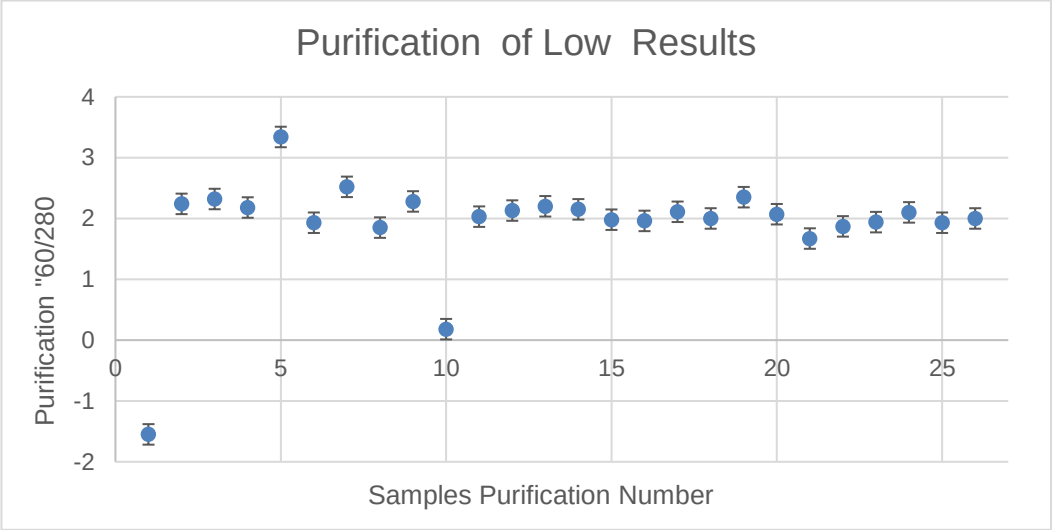


Figure 11. Purification of low results that were under 20 ng/µL.

The low sample concentration results might have affected the missing stabilizer, for example sample D-334 had a normal amount of stabilizer solution in the tube but had very little sample, and also sample D-522 tube had only half stabilizer solution, which might have been water or stabilizer solution. Anyway, this did not affect the concentration and purity result. D-522 concentration result was 198.89 ng/µL and purity result was 2.06. The sample tubes that had too much stool sample in the tube or missing the stabilizer, or very little stool sample in the tube, or too much stool sample and little stabilizer in the tube, didn't affect in the concentration and purity results. The results can be seen in Appendix 2.

The standard concentration result was 9.33 ng/µL and, spectrophotometer wavelength 260/280 purity was 2.17 nm, and the results process has been successful. By standard [47] manual, the concentration of ZymoBIOMICS. The standard is supposed to give a total of around 2 µg/µL total as output. The Microbial Community Standard contained bacterial mass, and bacterial mass and fungus were digested with PSP Spin Stool DNA Plus Kit from chromosomal DNA and the yield was 9.33 ng/µL. In manual [47] says good isolation the yield would be 2 µg in total. DNA mass calculated first by multiplying the final volume of DNA with DNA concentration result: 100 µL * 9.33 ng/µL = 0.933 µg. After

DNA mass calculation, the total loss of DNA is calculated by ideal DNA yield minus the results of DNA concentration yield: 2 µg – 0.933 µg = 1.065 µg. Total DNA loss was 1.065 µg. Percent yield is calculated by dividing the actual yield with theoretical yield, then multiplying by 100; (0.933 µg/µL / 2 µg) * 100 = 46.7%. The result of percent yield is 46.7%.

Blank control kit should be zero, so it does not count purity. Blank control kit result can be seen in appendix 2.

Low results will first drive by PCR (Polymerase Chain Reaction), in which it can be seen whether there is need to isolate the new DNA from the stool sample or not. PCR is a technique that is used in molecular biology laboratory to amplify a single or few copies of a section of DNA across several orders of magnitude, to produce thousands to millions of copies of a particular DNA sequence [47].

7 Conclusion

The purpose of the study was to understand the meaning of work, why stool samples were randomized and how the DNA extraction is done and what comes next. Chapter 2 explained the Parkinson's disease and the batch effects that cause the development of this disease.

The results were affected by many factors, for example, the amount of stool in DNA stabilizer tube, as some patient put too much stool sample in the tube, and some patients put too little stool sample in the DNA stabilizer tube. The result might also be effected during DNA extraction protocol, for example during centrifugation. Some of the samples required more than one centrifugation because of loosen sample or because sample was viscous and did not pass the spin filter. The sample results may have been affected by the quality of the stool, as some samples were diarrheal, sticky or loose/solid. It is also possible that the results are affected by how the food was digested in the intestines, as in some samples was possible to see with bare eyes that the sample food was not digested. It is possible that the patient had digestion problems. The thawing of samples began to predict if the sample was sticky or full of diarrhea sample, that was difficult to pipet to 2.0 mL safe-lock tube. Pipetting the difficult samples had to do by different protocol (Appendix 1). Predicting the samples did help to prevent the problem during filtration. To avoid the mistake during the DNA extraction, it was needed to work carefully and work by protocol [30].

Samples ratio of DNA and nuclein acid were measured with NanoDrop spectrophotometer. The approximate ratio of samples was around 2.0, which is accepted as pure, referring to the chapter 4.3 DNA concentration.

All problems that came during the DNA isolation from stool samples had to be written in laboratory book, to be able to go back to them and see what was the problem at that point, which will help to understand how these exact results were achieved, and it helps to understand why this kind results came. In the laboratory, the PSP spin stool DNA plus kit product number, batch- and expiration date number and also micro tube batch information had to be written in the book.

As standard concentration result was 9.33 ng/μL and spectrophotometer purity 260/280 was 2.17 nm, the result process has been successful since the standard expected yield is approximately 2 μg/μL DNA per preparation.

The stool samples randomizations lasted two weeks in February 2017 and DNA extraction started after randomization. The DNA of samples were extracted. It took 33 days to extract the DNA of samples, one sample box a day. The storage, randomization of samples, and stool samples were stored in PSP plus kit's stabilizer buffer as described in chapter 3.

The goal was to randomize the 745 pieces of stool samples and to extract the DNA from the stool sample. This study will be used to do the building of the DNA libraries by using the PCR, sequencing, processing bioinformatically, microbial investigation and analyzing with statistical methods. I did my DNA extraction part successfully.

References

1 Madelyn C. Houser1 and Malú G. Tansey. The gut-brain axis: is intestinal inflammation a silent driver of Parkinson's disease pathogenesis? NPJ Parkinson's Dis. 2017; 3: 3. DOI:10.1038/s41531-016-0002-0.

2 Parkinson's Disease Foundations. What is Parkinson's Disease [Internet]. Available from URL: http://www.pdf.org/about_pd [Cited: 15.5.2017].

3 Thomas T. Warner, Anthony H. V. Schapira. Genetic and environmental factors in the cause of Parkinson's disease. Ann Neurol. 2003;53 Suppl 3:S16-23; discussion S23-5. DOI: 10.1002/ana.10489.

4 J. R. Bedarf, F. Hildebrand, L. P. Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naïve Parkinson's disease patients. Genome Med. 2017 Apr 28;9(1):39. DOI 10.1186/s13073-017-0428-y.

5 Alison L. McCormack, Mona Thiruchelvam. Environmental risk factors and Parkinson's Disease: Selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat. Neurobiol Dis. 2002 Jul;10(2):119-27. DOI: 10.1006/nbdi.2002.0507.

6 Jeffrey T. Leek. Tackling the widespread and critical impact of batch effects in high-throughput data. Nat Rev Genet. 2010. Oct;11(10):733-9. DOI:10.1038/nrg2825.

7 Suomen Parkinson-liitto ry [Updated 22.9.2015]. Parkinsonin tauti [Internet]. Available from URL: https://www.parkinson.fi/parkinsonin-tauti [Cited: 15.5.2017].

8 Neurologian erikoislääkäri Sari Atula. Published 16.5.2016. Parkinsonin tauti [Internet]. Terveyskirjasto 2017 Duodecim Kustannus Oy. Artikkelin tunnus: dlk00055 (020.022). Available from URL: http://www.terveyskirjasto.fi/terveyskirjasto/tk.koti?p_artikkeli=dlk00055&p_hakusana=Parkinsonin%20tauti [Cited: 15.5.2017].

9 William J. Weiner M.D, Lisa M Shulman M.D, Anthony E. Lang M.D. F.R.CP. P. Parkinson's disease, Second Edition, A Complete guide for patients and families. ISBN:9781421410760. Publisher: John Hopkins University; 2nd Edition (November 13, 2006). Page 3

10 Bethany Brookshire. Published Online:17.1.2017. Explainer: What is dopamine [Internet]. Science News for Students. Available from URL: https://www.sciencenewsforstudents.org/article/explainer-what-dopamine [Cited: 19.5.2017].

11 Parkinsoninfo. Parkinsonin tauti johtuu hermosolujen tuhoutumisesta [Internet]. Available from URL: http://www.parkinsoninfo.fi/parkinsonin-tauti/parkinsonin-tauti-johtuu-hermosolujen-tuhoutumisesta [Cited: 19.5.2017].

12 Joseph V. Campellone, David Zieve, Isla Ogilvie. Revive date: 13.8.2015. Substantia nigra and Parkinson's Disease [Internet]. Medline Plus, Trusted Health Information for You. Available from URL: https://medlineplus.gov/ency /imagepages/19515.htm [Cited: 19.5.2017].

13 Disu Disability Support Uganda. Published Online 2.9.2016. Symptoms of Parkinson's Disease [Internet]. Available from URL: https://disu256.blogspot.fi/2016/09/parkinsons-disease-in-uganda.html?spref=pi [Cited: 19.5.2017].

14 Dennis J. Selkoe. Unravelling the mystery of alpha-Synuclein [Internet]. Parkinson's Disease Foundation. Available from URL: http://www.pdf.org/winter16_asynuclein [Cited: 24.5.2017].

15 Jiajie Diao, Jacqueline Burré. Native α-Synuclein induces clustering of synaptic-vesicle mimics via binding to phospholipids and synaptobrevin-2/VAMP2. Elife. 2013 Apr 30;2:e00592. DOI: 10.7554/eLife.00592.

16 Lingjia Xu, Jiali Pu. Alpha-Synuclein in Parkinson's disease: from Pathogenetic dysfunction to potential clinical application. Parkinsons Dis. 2016; 2016:1720621. DOI: 10.1155/2016/1720621.

17 Tina Alvarez. Published Online 23.5.2015. Figure of Alpha-Synuclein in Lewy Bodies [Internet]. Journey with Parkinson's. Available from URL: https://journeywithparkinsons.com/tag/medical-therapy/page/2/ [Cited: 24.5.2017].

18 Raimo Tuominen, Mart Saarman. Huhtikuu 2015. Suomen Parkinsonsäätiö sr [Internet]. Available from URL: http://www.parkinsonsaatio.fi/parkinsonin-tauti/ tutkimus/alfasynukleiini-parkinsonin-taudissa-ja-siihen-liittyva-kokeellinen [Cited: 24.5.2017].

19 Filip Scheperjans, Velma Aho, Pedro A. B. Pereira. Gut microbiota are related to Parkinson's disease and clinical phenotype. Mov Disord. 2015 Mar;30(3):350-8. DOI: 10.1002/mds.26069.

20 Pluses, Perils, and Pitfalls J. Michael Janda and Sharon L. Abbott. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory. J Clin Microbiol. 2007 Sepp; 45(9):2761-2764. DOI: 10.1128/JCM.01228-07.

21 Julia K. Goodrich, Sara C. Di Rienzi, Angela C. Poole, Omry Koren. Conducting a microbiome study. Cell. 2014 Jul 17;158(2):250-262. DOI: 10.1016/j.cell.2014.06.037.

22 James Robert White, Niranjan Nagarajan, Mihai Pop. Statistical methods for detecting differentially abundant features in clinical metagenomics samples. PLoS Comput Biol. 2009 Apr;5(4):e1000352. DOI: 10.1371/journal.pcbi.1000352.

23 Christian L. Lauber. Effect of storage conditions on the assessment of bacterial community structure in soil and human-associated samples. FEMS Microbiol Let. 2010 Jun;307(1):80-6. DOI:10.1111/j.1574-6968.2010.01965.x.

24 Silvia Cardona, Anat Eck, Montserrat Cassellas, Milagros Gallart, Carmen Alastrue, Fernando Azpiroz, Francisco Guarner. Storage conditions of intestinal microbiota matter in metagenomic analysis. BMC Microbiol. 2012 Jul 30; 12:158. DOI:10.1186/1471-2180-12-158.

25 Tomasz Bykowski and Brian Stevenson. Aseptic Technique APPENDIX 4D. Curr Protoc Microbiol. 2008 Nov;Appendix4:Appendix4D. DOI:10.1002/9780471729259.mca04ds11.

26 Susannah J Salter, Julian Parkhill, Alan W Walker. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol. 2014 Nov 12; 12:87. DOI: 10.1186/s12915-014-0087-z.

27 Gary D Wu, James D Lewis, Kyle Bittinger, Jennifer Hwang, Frederic D Bushman. Sampling and pyrosequencing methods for characterizing bacterial communities in the human gut using 16S sequence tags. BMC Microbiol. 2010 Jul 30;10:206. DOI: 10.1186/1471-2180-10-206.

28 Birgitte Smith, Nan Li, Anders Schou Andersen, Hans Christian Slotved, Karen Angeliki Krogfelt. Optimising bacterial DNA extraction from faecal samples: Comparison of three methods. Open Microbiol J. 2011;5:14-7. DOI: 10.2174/1874285801105010014.

29 Anne Salonen, Janne Nikkilä, Jonna Jalanka-Tuovinen, Outi Immonen, Airi Palva. Comparative analysis of fecal DNA extraction methods with phylogenetic microarray: Effective recovery of bacterial and archaeal DNA using mechanical cell lysis. J Microbiol Methods. 2010 May;81(2):127-34. DOI: 10.1016/j.mimet.2010.02.007.

30 PSP Spin Stool DNA Plus Kit. (REF 1038100x00) [Internet]. Stratec Molecular GmbH, D-13125 Berlin. Available from URL: http://www.stratec.com/share/molecular/Manuals/Single/Pathogens/PSPSpinStool_StoolPlusKit.pdf [Cited:11.8.2017].

31 Andrew Porterfield. Bitesize Bio. Sifting detritus – Extracting DNA and RNA samples from soil and feces [Internet]. Available from URL: http://bitesizebio.com/13530/sifting-detritus-extracting-dna-and-rna-samples-from-soil-and-feces/ [Cited: 31.8.2017].

32 Kelly A. Fitzpatrick, Gilbert J. Kersh, Robert F. Massung. Practical method for extraction of PCR-quality DNA from environmental soil samples. Appl Environ Microbiol. 2010 Jul;76(13):4571-3. DOI: 10.1128/AEM.02825-09. [Cited: 31.8.2017].

33 Joseph Bessetti. Promega Corporation. 03/2007. An introduction to PCR inhibitors [Internet]. Available from URL: https://fi.promega.com/-/media/files/resources/profiles-in-dna/1001/an-introduction-to-pcr-inhibitors.pdf?la=en [Cited: 31.8.2017].

34 Kim Barrett, Heddwen Brooks, Scott Boitano, Susan Barman. Ganing's Review of Medical Physiology, 23rd Edition. 2010. The McGraw-Hill Companies, Inc. ISBN: 978-0-07-160568-7, MHID: 0-07-160568-1. Page 438-440.

35 Pär-Gunnar Lantz, Mikael Matsson, Torkel Wadström, Peter Rådström. Removal of PCR inhibitors from human faecal samples through the use of an aqueous two-phase system for sample preparation prior to PCR. Journal of Microbiological Methods. Volume 28, Issue 3, 1.4.1997, Pages 159-167. DOI:10.1016/S0167-7012(97)00979-2.

36 Lurdes Monteiro, Dominique Bonnemaison, Jacques Bonnet, Francis Me'gra. Complex polysaccharides as PCR inhibitors in feces: Helicobacter pylori. J Clin Microbiol. 1997 Apr; 35(4): 995-998. PMCID: PMC229720.

37 C. Schrader, A. Schielke, L. Ellerbroek, R. Johne. PCR inhibitors - occurrence, properties and removal. J Appl Microbiol. 2012 Nov;113(5):1014-26. DOI: 10.1111/j.1365-2672.2012.05384.x.

38 Pamela Lipsett, Thomas R. Gadacz. Bile salt binding by maalox, sucralfate, and meciadanol: in vitro and clinical comparisonsly. J.Surg Res. 1989 Nov;47(5):403-6. DOI: 10.1016/0022-4804(89)90091-7.

39 Boris Böddinghaus, Thomas A. Wichelhaus, Volker Brade, Thomas Bitnner. Removal of PCR inhibitors by silica membranes: Evaluating the amplicor mycobacterium tuberculosis Kit. J Clin Microbiol. 2001 Oct;39(10):3750-2. DOI: 10.1128/JCM.39.103750-3752.2001.

40 Meredith Turnbough. What is the actual role of Proteinase K in human DNA extraction [Internet]. Available from URL: https://www.researchgate.net/post/What_is_the_actual_role_of_Proteinase_K_in_human_DNA_extraction [Cited: 6.9.2017].

41 Helmuth Hilz, Ulrich Wiegers, Peter Adamietz. Stimulation of Proteinase K action by denaturing agents: Application to the isolation of nucleic acids and the degradation of 'masked' proteins. Eur J Biochem. 1975 Aug 1;56(1):103-8. DOI: 10.1111/j.1432-1033.1975.tb02211.x PMID: 1236799.

42 Proteinase K [Internet]. Worthington Biochemical Corporation; 2017. Available from URL: http://www.worthington-biochem.com/PROK/images/reaction.jpg [Cited: 6.9.2017].

43 Proteinase K information: Proteinase K, recombinant, PCR Grade [Internet]. ThermoFisher Scientific Ltd. Available from URL: https://www.thermofisher.com/order/catalog/product/EO0491 [Cited: 6.9.2017].

44 Nirav Soni. Pharmatutor Pharmacy infopedia; 1.6.2016. Silica spin column [Internet]. Available from URL: http://www.pharmatutor.org/articles/extraction-and-purification-of-nucleic-acid-using-column-based-nucleic-acid-purification-pcia-technique [Cited: 8.9.2017].

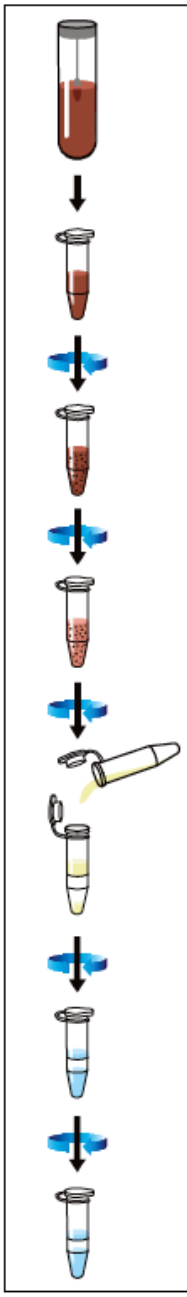
45 NanoDrop 2000/2000c Spectrophotometers [Internet]. ThermoFisher Scientific Ltd. Available from URL: https://www.thermofisher.com/order/catalog/product/ND2000CLAPTOP [Cited: 11.9.2017].

46 ZymoBIOMICS. Microbial community standard[Internet]. Catalog No. D6300. Available from URL: http://www.zymoresearch.com/downloads/dl /file /id /737 /d6300i.pdf [Cited: 10.9.2017].

47 PCR (Polymerase Chain Reaction) [Internet]. Genetic science learning center. Virtual Labs. Available from URL: http://learn.genetics.utah.edu/content/labs/pcr/ [Cited: 11.9.2017].

PSP Spin Stool DNA Plus Kit, DNA Extraction Protocol

Scheme of the PSP® Spin Stool DNA *Plus* Kit



Please read protocols prior the start of the preparation carefully

collect a spoon of the stool sample
transfer stool sample into the Stool Collection Tube, close the tube
mix for a short time by shaking

transfer 1.4 ml of the stabilized stool sample (Stool DNA Stabilizer with stool specimen) into a 2.0 ml Safe-Lock Tube

for enrichment of host DNA:
incubate 10 min at RT under shaking

for enrichment of bacterial DNA:
incubate 10 min at 95°C on a thermomixer under shaking,
add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min

spin down at 11.000 x g (11.000 rpm) for 1 min

transfer the supernatant to the **InviAdsorb**-Tube
mix it by vortexing for 15 sec.
incubate 1 min at RT
spin down for 3 min at full speed.

transfer the supernatant in a new 1.5 ml Receiver Tube
centrifuge the sample again at full speed for 3 min

add 25 µl **Proteinase K** in a new 2.0 ml Safe-Lock-Tube
transfer 800 µl of the sample supernatant to the same tube
mix shortly by vortexing
incubate for 10 min at 70 °C while continuously shaking on a thermomixer at 900 rpm

add 400 µl **Binding Buffer A** *(follow preparing instructions)* to the lysate
mix shortly by vortexing or pipetting up and down

transfer the whole mixture **in two steps** to the RTA Spin Filter
incubate for 1 min at RT
centrifuge at 11.000 x g (11.000 rpm) for 2 min
discard the filtrate and the RTA Receiver Tube

transfer the RTA Spin Filter in a new RTA Receiver Tube
pipet 500 µl **Wash Buffer I** onto the RTA-Spin Filter
centrifuge at 11.000 x g (11.000 rpm) for 1 min
discard the flow-through and the RTA Receiver Tube

put the RTA Spin Filter in a new RTA Receiver Tube
pipet 700 µl **Wash Buffer II** onto the RTA Spin Filter
centrifuge at 11.000 x g (11.000 rpm) for 1 min
discard the flow-through and reuse the RTA Receiver Tube

to eliminate any traces of ethanol, centrifuge again for 4 min at maximum speed,
discard the RTA Receiver Tube

transfer the RTA Spin Filter into a new 1.5 ml Receiver Tube
pipet 100-200 µl of **Elution Buffer** (preheated to 70°C) directly onto the center of the membrane of the RTA Spin Filter
incubate for 1 min at RT
centrifuge at 11.000 x g (11.000 rpm) for 1 min; discard the RTA-Spin Filter;
place the eluted total DNA immediately in a refrigerator or store it at –20°C

Protocol 2: Isolation of total DNA from 1.4 ml stabilized stool homogenate with and without enrichment of bacterial DNA

Please read protocols prior the start of the preparation and complete preparing steps!!

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer A*** *– see instruction page: 13*

Important Note: *Please note, that the extracted DNA from stool sample is by the majority from bacterial origin !*

Heat heating blocks (e.g. thermomixer) to 70°C and 95 °C

Preheat the ***Elution Buffer*** *to 70°C (e.g. transfer the needed volume into a tube and place it at the appropriate temperature into a thermomixer)*

1. Sample Homogenization and Prelysis

Transfer 1.4 ml of the collected and well homogenized stool sample (Stool DNA Stabilizer with stool specimen) after storage or directly after collection into the 2.0 ml Safe-Lock Tube.

~~Centrifuge the sample at 11.000 x g (11.000 rpm) for 1 min to pellet solid stool particles. This will lead to a reduced amount of extracted total DNA, but is not influencing the amount of human DNA~~

For an enrichment of bacterial DNA:

Incubate the sample for 10 min at 95°C in a thermomixer under continuously shaking at 900 rpm.
Add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min at RT.
Centrifuge the sample at 11.000 x g (11.000 rpm) for 1 min to pellet solid stool particles and beads.

Important Note: *The incubation step at 95°C will lead to maximize the amount of bacterial DNA, because of a very efficient destruction of the cell wall of e.g. gram+ bacteria.*

For an enrichment of host DNA, don't perform this high-temperature step

2. Removal of PCR Inhibitiors

ALL SUPERNATANT

Transfer the supernatant into an **InviAdsorb-Tube** and vortex vigorously for 15 sec.
Incubate the suspension for 1 min at room temperature. Centrifuge the sample at full speed for 3 min. FULL SPEED: 13.2 RPM

3. Second Sample Cleanup

ALL SUPERNATANT

Transfer the supernatant completely into a new 1.5 ml Receiver Tube. Discard the pellet.
Centrifuge the sample again at full speed for 3 min. FULL SPEED: 13.2 RPM

4. Proteinase K digestion

Transfer 25 µl **Proteinase K** into a new 2.0 ml Safe-Lock-Tube and pipet 800 µl of the supernatant from step 3 to the 1.5 ml Receiver Tube containing **Proteinase K**, mix shortly by vortexing and incubate the sample for 10 min at 70°C in a thermomixer under continuous shaking at 900 rpm.

NOT THE PROTEINASE K, ONLY BLANK
> DO TO STEP 5.

KIT CON. MOL/BLANK:
JUST PUT THE PROTEINASE K
IN THE END

PUT ON THIS MOMENT THE DNA ELUTION BUFFER IN TO + 70C INKUBATE

5. Binding of the DNA

Add 400 µl of **Binding Buffer A** to the lysate and mix shortly by vortexing or by pipetting up and down several times.
Transfer the mixture **in two steps** onto the membrane of the RTA Spin Filter. Incubate for 1 min at room temperature and centrifuge at 11.000 x g (11.000 rpm) for 2 min. Discard the filtrate and the RTA Receiver Tube.

6. Washing Steps

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 500 µl **Wash Buffer I** to the membrane of the RTA Spin Filter. Close the lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and the RTA Receiver Tube.

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 700 µl **Wash Buffer II** to the membrane of the RTA Spin Filter. Close the lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and put the RTA Spin Filter back to the same RTA Receiver Tube.

7. Ethanol Removal

To eliminate any traces of ethanol, centrifuge again for 4 min at maximum speed, discard the RTA Receiver Tube

MAX SPEED: 13.2 RPM

8. DNA Elution

INKUBATING FOR 5 MINUTE INSTEAD 1 MINUTE.

Place the RTA Spin Filter into a new 1.5 ml Receiver Tube and add 100 - 200 µl preheated (70°C) **Elution Buffer.** Incubate for ~~1~~ min. Centrifuge at 11.000 x g (11.000 rpm) for 1 min to elute the DNA. Finally discard the RTA Spin Filter.

DNA ELUTION WERE PUT + 70C INKUBATIN IN PROTOCOL 5. BINDING OF THE DNA.

Note: *The DNA can also be eluted with a lower volume of Elution Buffer (depends on the expected yield of genomic DNA). But pay attention that the minimum volume for the elution is **50 µl and that this volume can reduce the maximum yield.** If a quite large amount of DNA is expected, the volume of elution can be increased.*

Note: *For long-term storage, we recommend to keep the eluted DNA at –20°C.*

Instructions

The following notes are valid for all protocols:

Note: *The centrifugation steps were made with the **Centrifuge 5415 D from Eppendorf.** The indicated refers to this centrifuge.*

Protocol 1: Isolation of total DNA from up to 200 mg stool samples with and without enrichment of bacterial DNA

Please read protocols prior the start of the preparation and complete preparing steps!!

Attention: *Please be aware, that you have to prepare the **Binding Buffer A** – see instruction page: 13*

Important Note: *Please note, that the majority of extracted DNA from stool samples is of bacterial origin !*

Heat heating blocks (e.g. thermomixer) to 70°C and 95 °C

Preheat the Elution Buffer to 70°C (e.g. transfer the needed volume into a tube and place it at the appropriate temperature into a thermomixer)

1. Sample homogenization and prelysis

Weigh 200 mg of stool sample (fresh or frozen) into a 2.0 ml Safe-Lock-Tube and add 1.2 ml **Lysis Buffer P** to each stool sample .Vortex vigorously for 1 min. Even if you use less starting material, perform the protocol like described.

Important: *If the sample is liquid, pipet 200 µl into the 2.0 ml Safe-Lock-Tube. Cut-off the end of the pipet tip to make pipetting easier.*
If the sample is frozen, use a scalpel or spatula to scrape bits of stool into the provided 2.0 ml Safe-Lock-Tube on ice. Take care, that this samples do not thaw until Lysis Buffer P is added, otherwise the DNA in the sample may degrade. After addition of the buffer, the following steps can be performed at RT or like recommended.

Incubate the sample for 10 min at RT under continuous shaking at 900 rpm.
Centrifuge the sample at 11.000 x g (11.000 rpm) for 1 min to pellet solid stool particles.

For an enrichment of bacterial DNA:

Incubate the sample for 10 min at 95°C in a thermomixer under continuously shaking at 900 rpm.
Add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min at RT.
Centrifuge the sample at 11.000 x g (11.000 rpm) for 1 min to pellet solid stool particles and beads.

Important: *The incubation step at 95°C will maximize the yield of bacterial DNA, because of a very efficient disruption of the cell wall of e.g. gram positive bacteria.*

For an enrichment of host DNA, don't perform this high-temperature step

2. Removal of PCR inhibitiors

Transfer the supernatant into an **InviAdsorb-Tube** and vortex vigorously for 15 sec.
Incubate the suspension for 1 min at room temperature. Centrifuge the sample at full speed for 3 min.

Extracted DNA Results

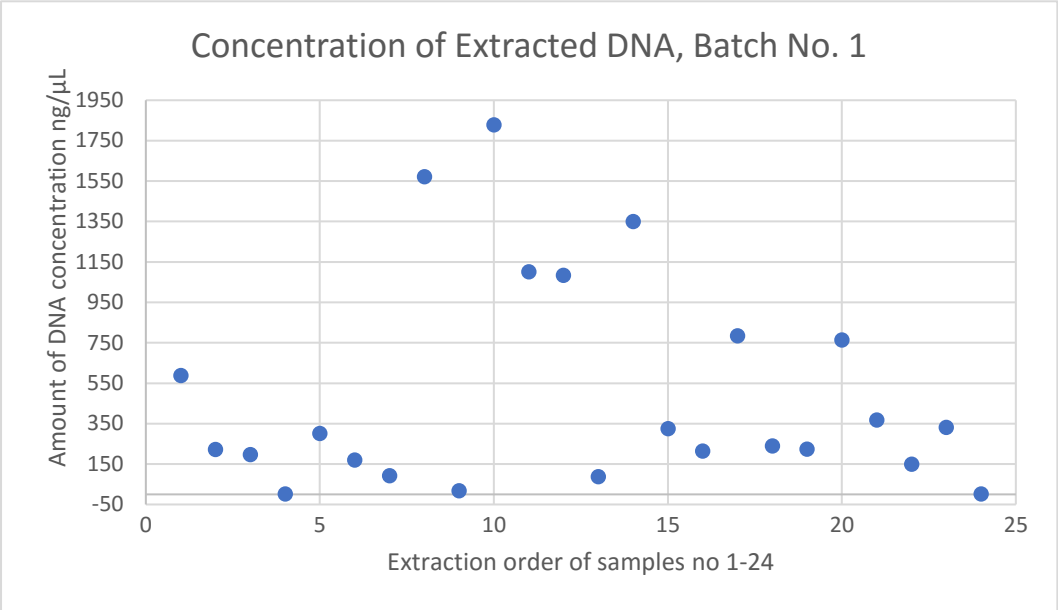


Figure 12. Extraction purification order no. 1-24. Batch no. 1.

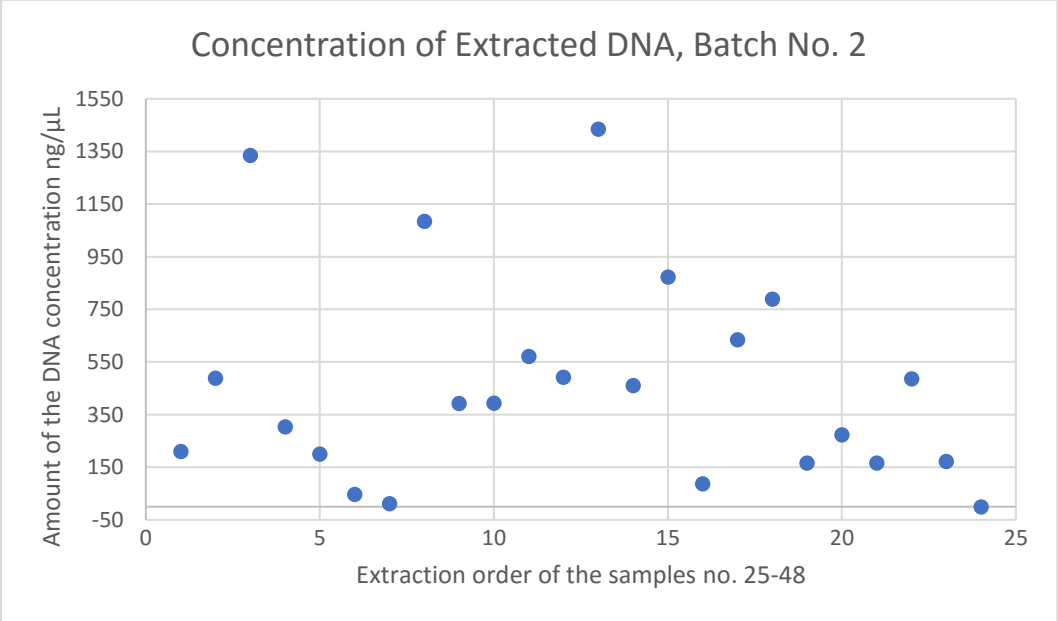


Figure 13. Extraction purification order no. 25-48. Batch no. 2.

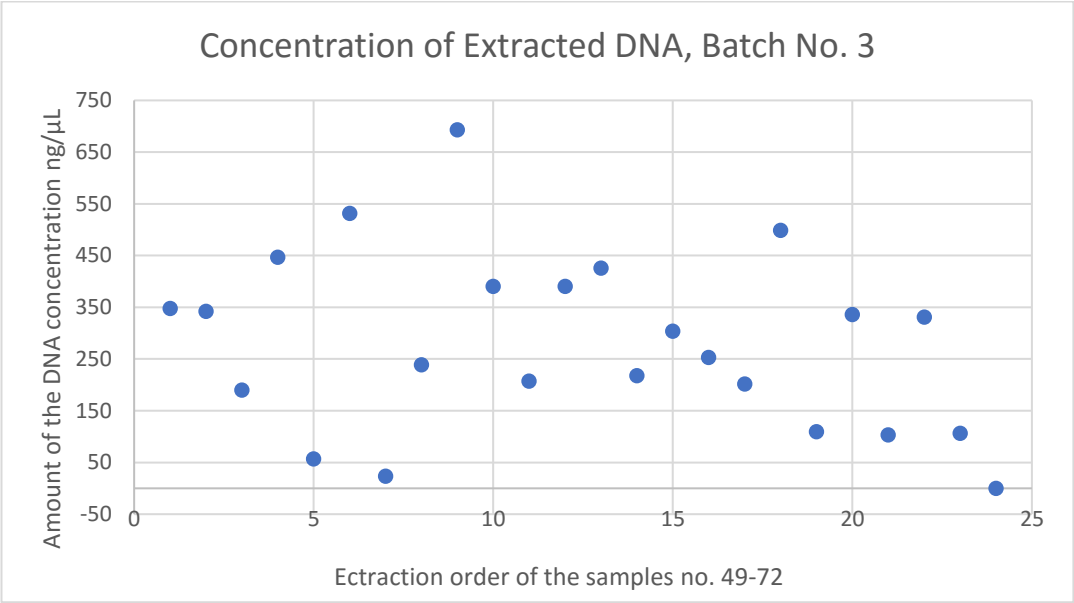


Figure 14. Extraction purification order no. 49-72. Batch no. 3.

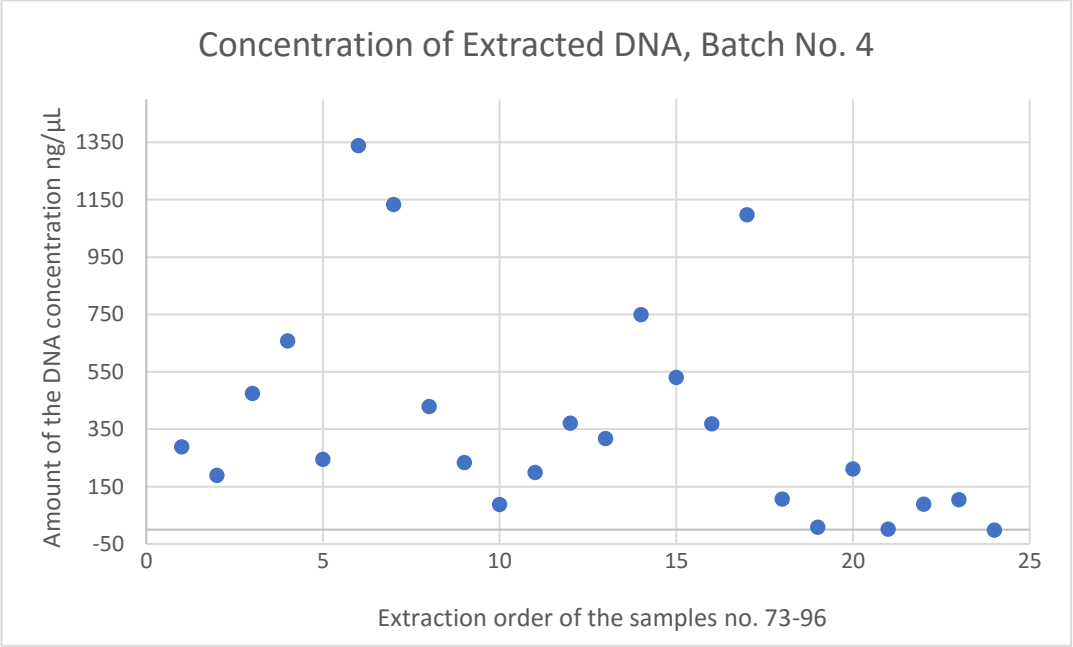


Figure 15. Extraction purification order no. 73-96. Batch no. 4

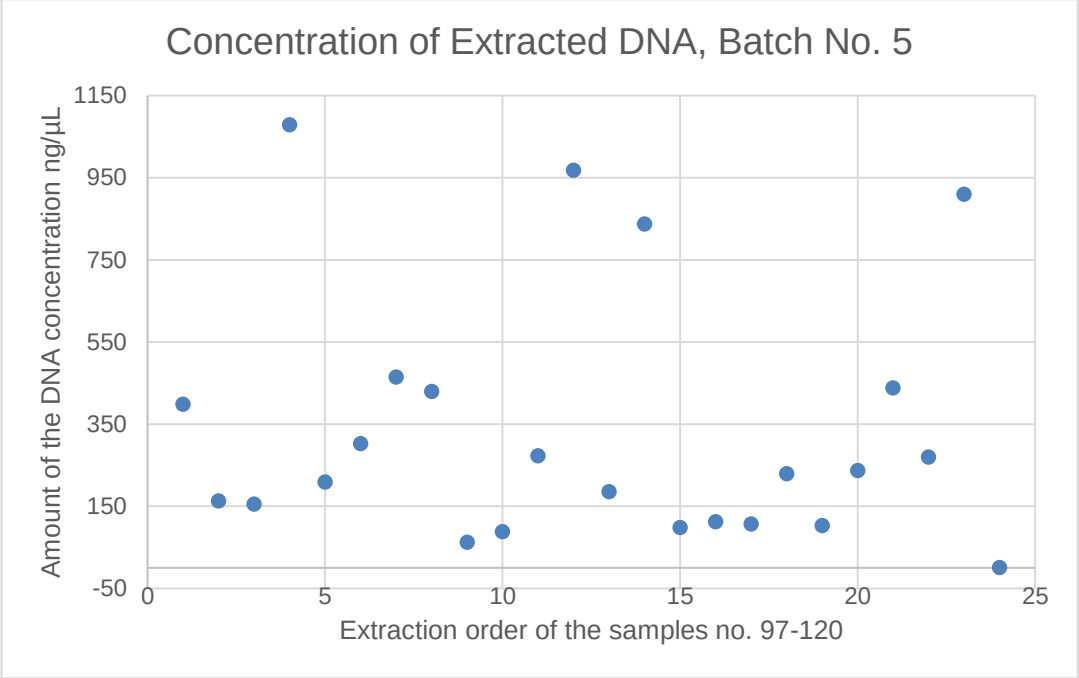


Figure 16. Extraction purification order no. 97-120. Batch no. 5.

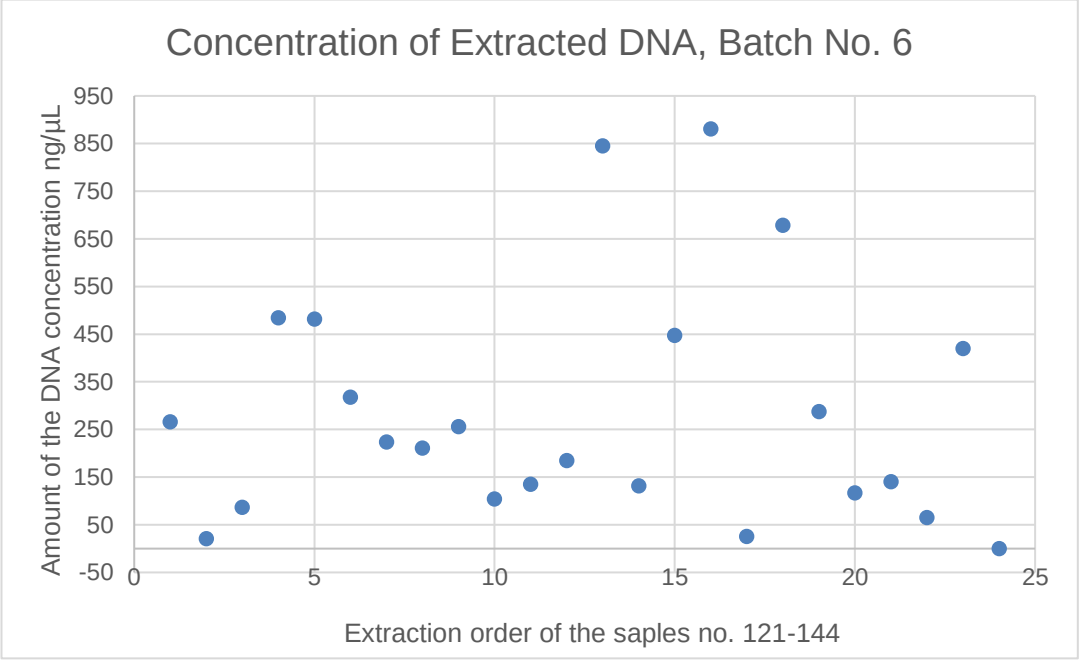


Figure 17. Extraction purification order no. 121-144. Batch no. 6.

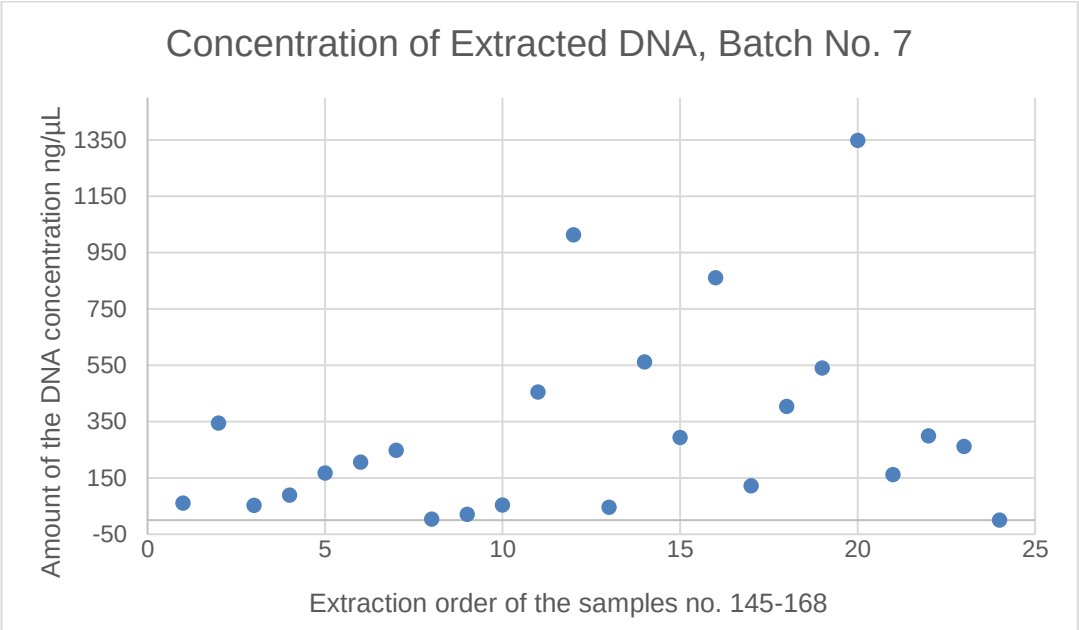


Figure 18. Extraction purification order no. 145-168. Batch no. 7.

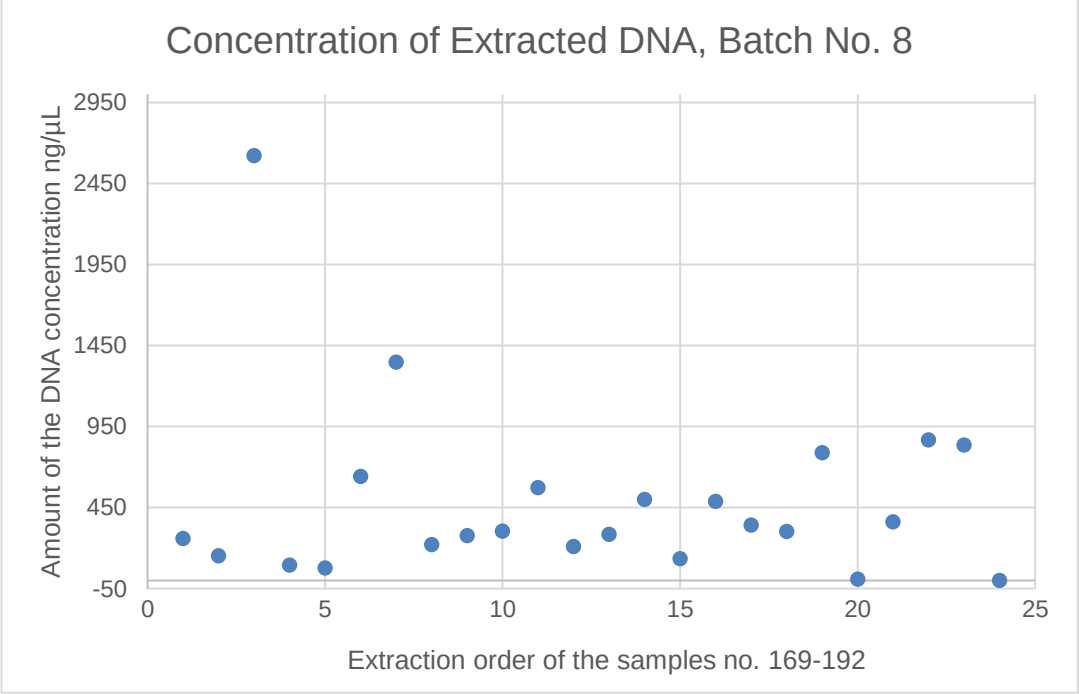


Figure 19. Extraction purification order no. 169-192. Batch no. 8.

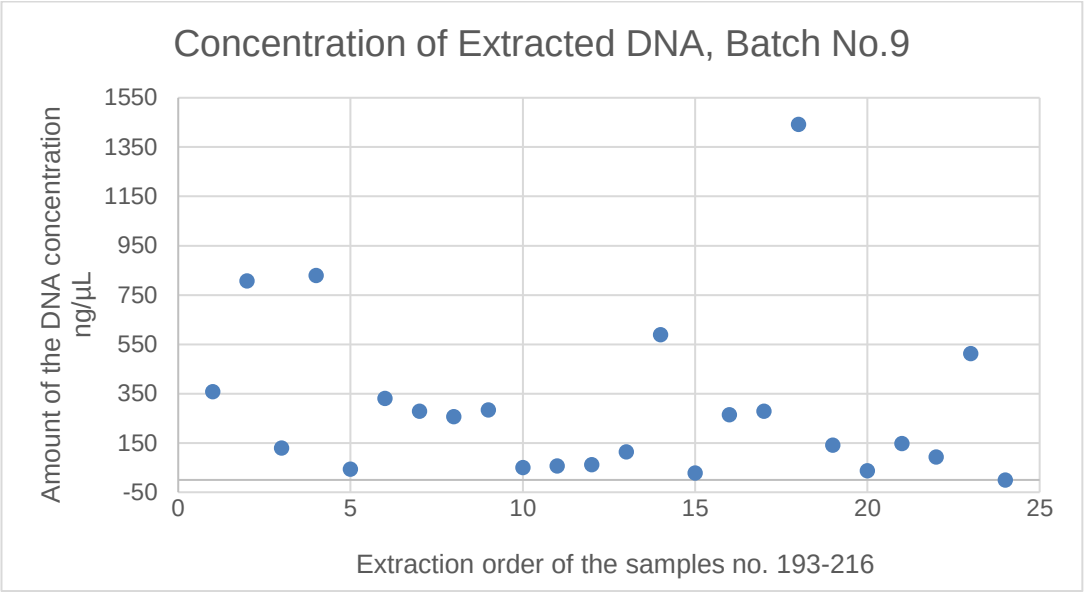


Figure 20. Extraction purification order no. 193-216. Batch no. 9.

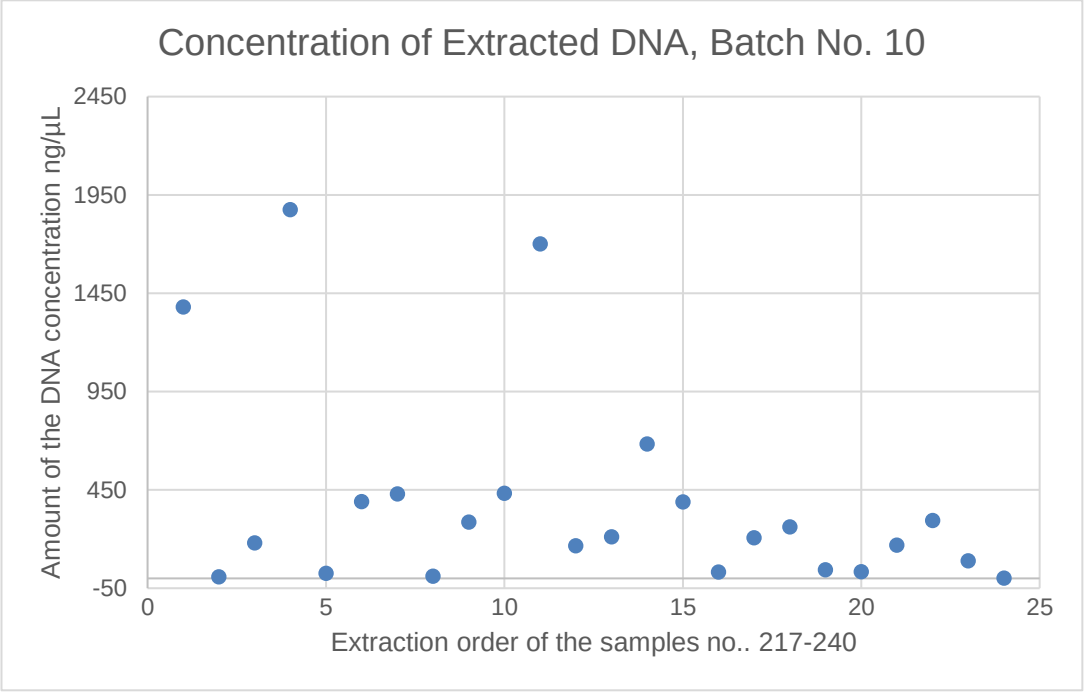


Figure 21. Extraction purification order no. 217-240. Batch no. 10.

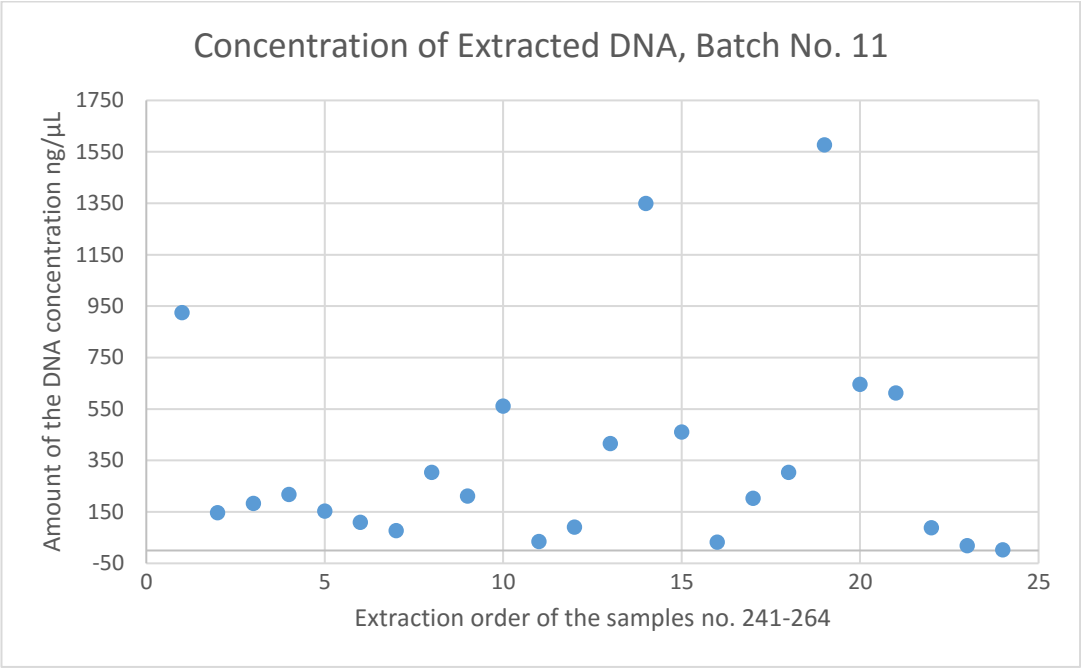


Figure 22. Extraction purification order no. 241-264. Batch no. 11.

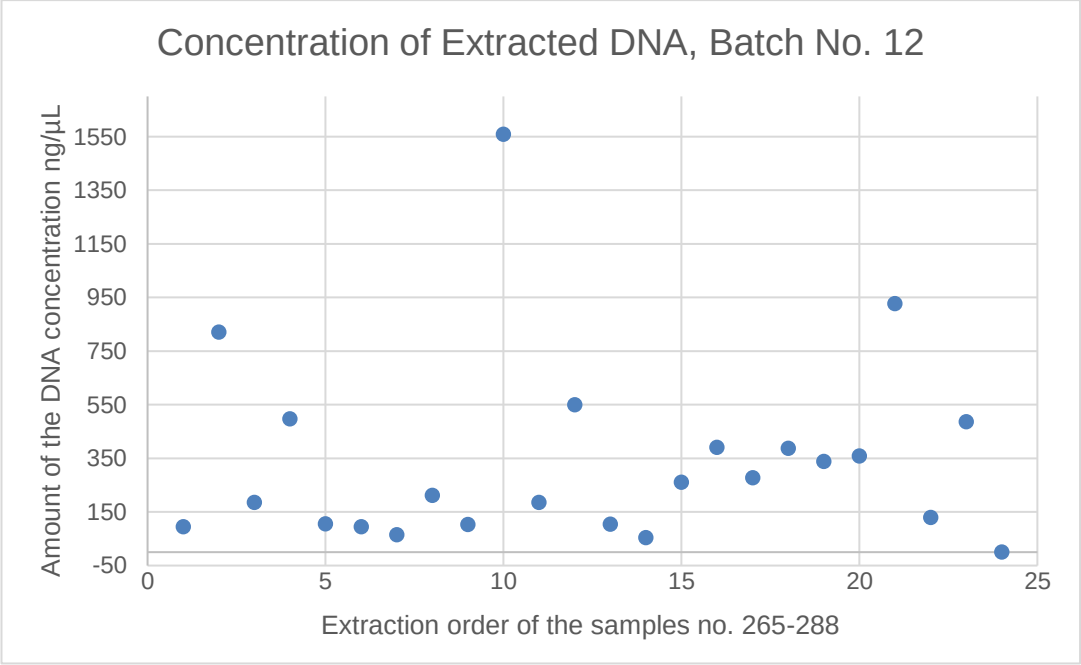


Figure 23. Extraction purification order no. 265-288. Batch no. 12.

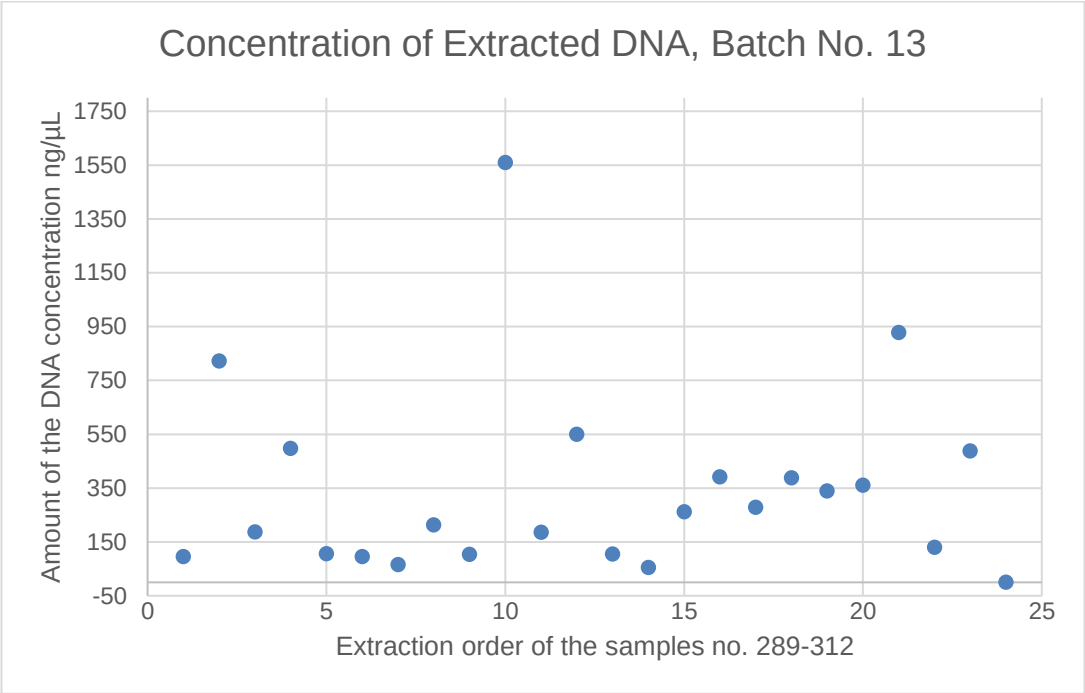


Figure 24. Extraction purification order no. 289-312. Batch no. 13.

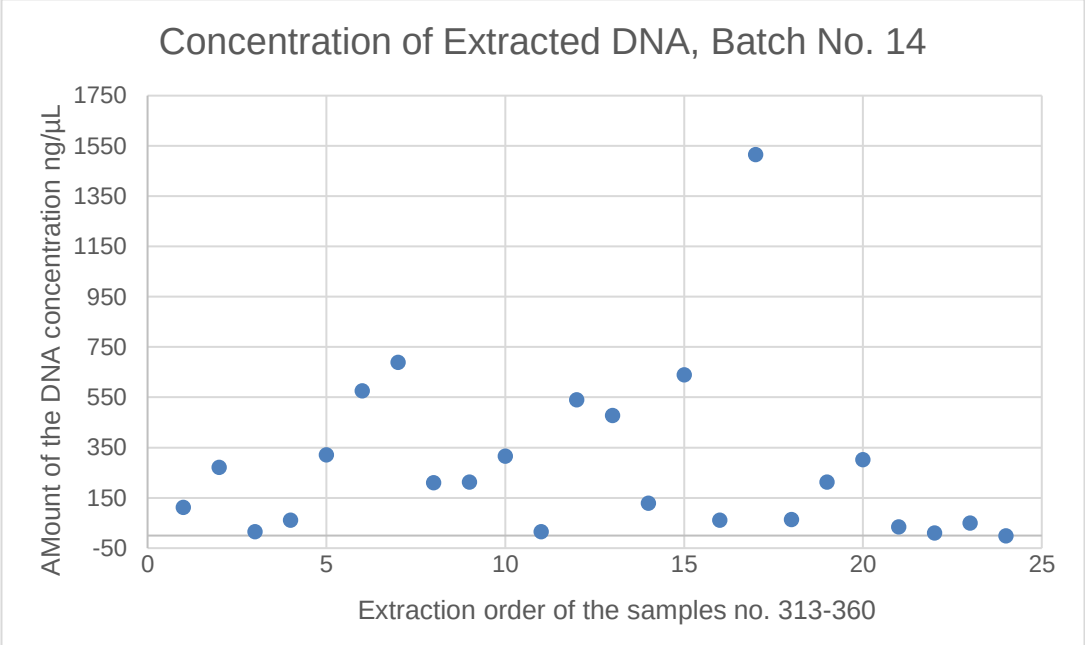


Figure 25. Extraction purification order no. 313-360. Batch no. 14.

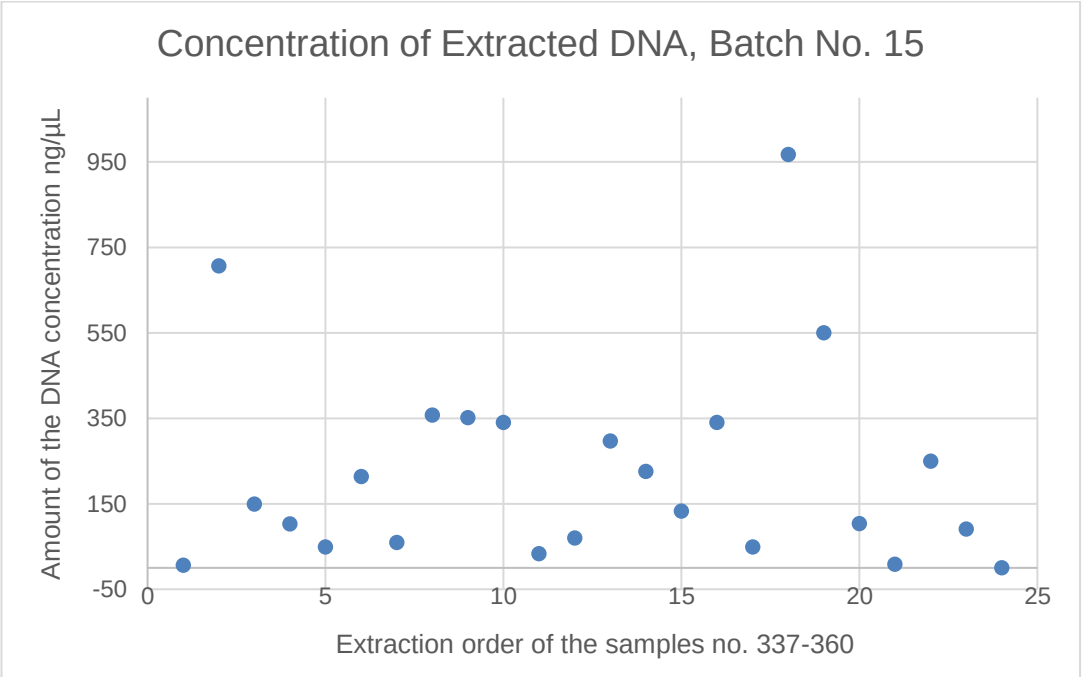


Figure 26. Extraction purification order no. 337-360. Batch no. 15.

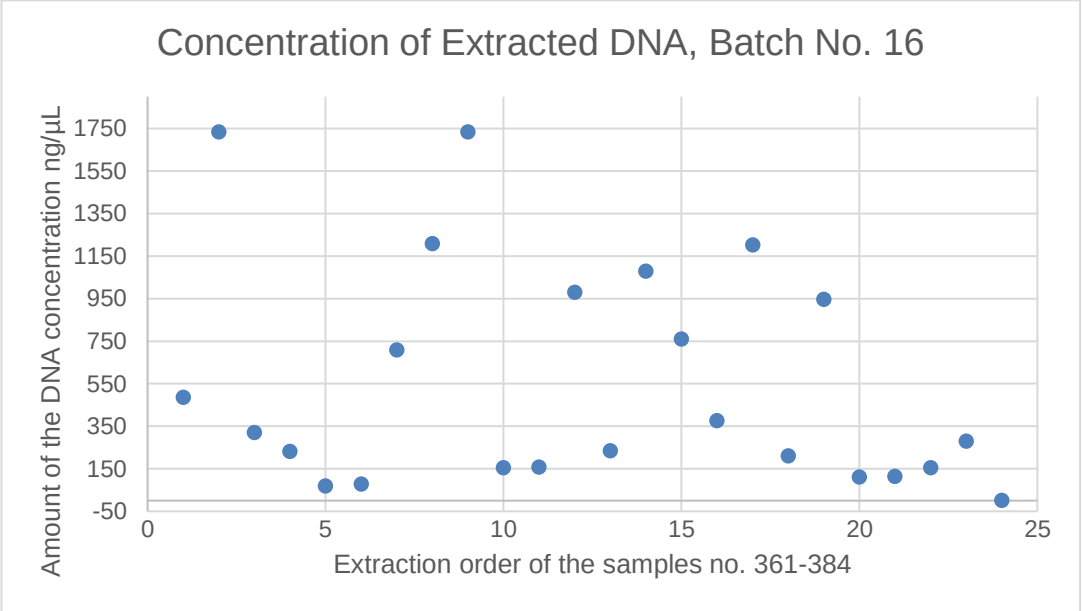


Figure 27. Extraction purification order no. 361-384. Batch no. 16.

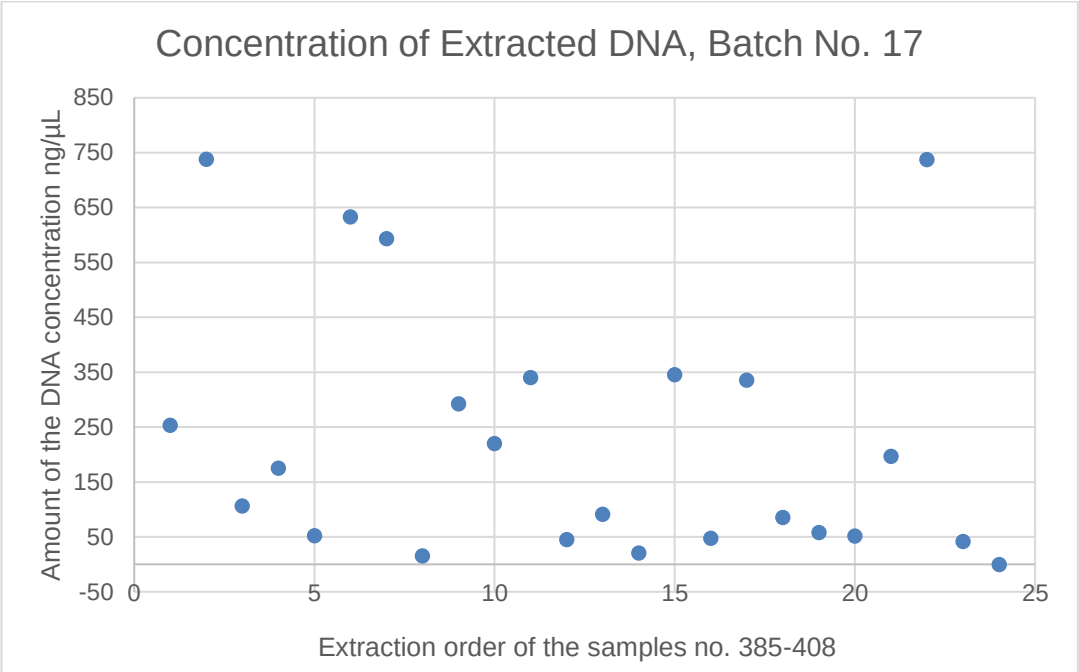


Figure 28. Extraction purification order no. 385-408. Batch no. 17.

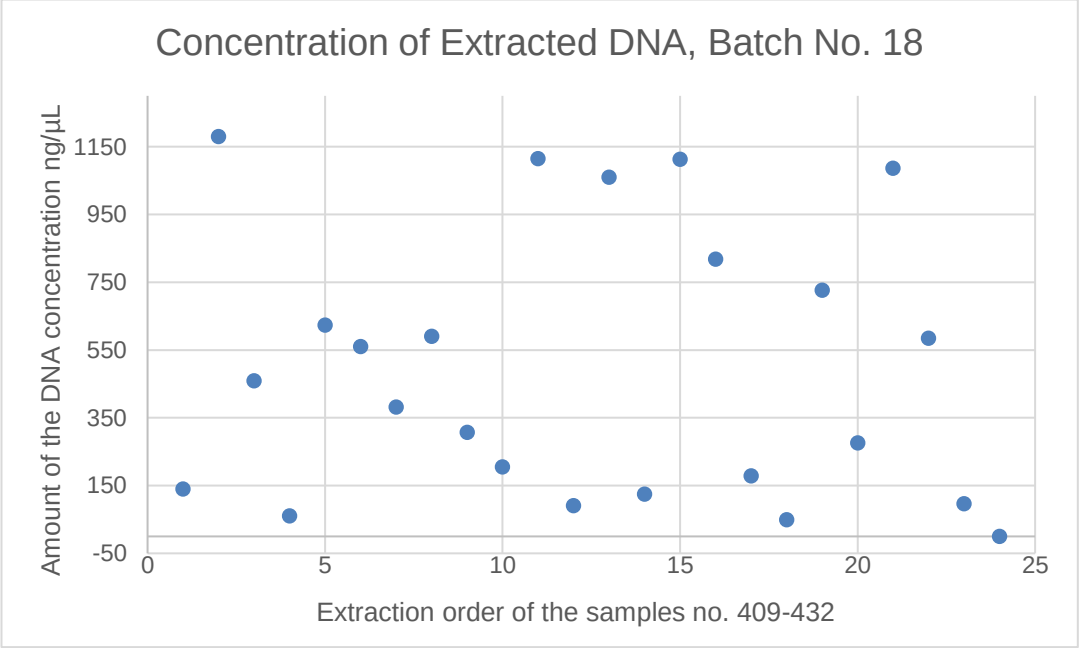


Figure 29. Extraction purification order no. 409-432. Batch no. 18.

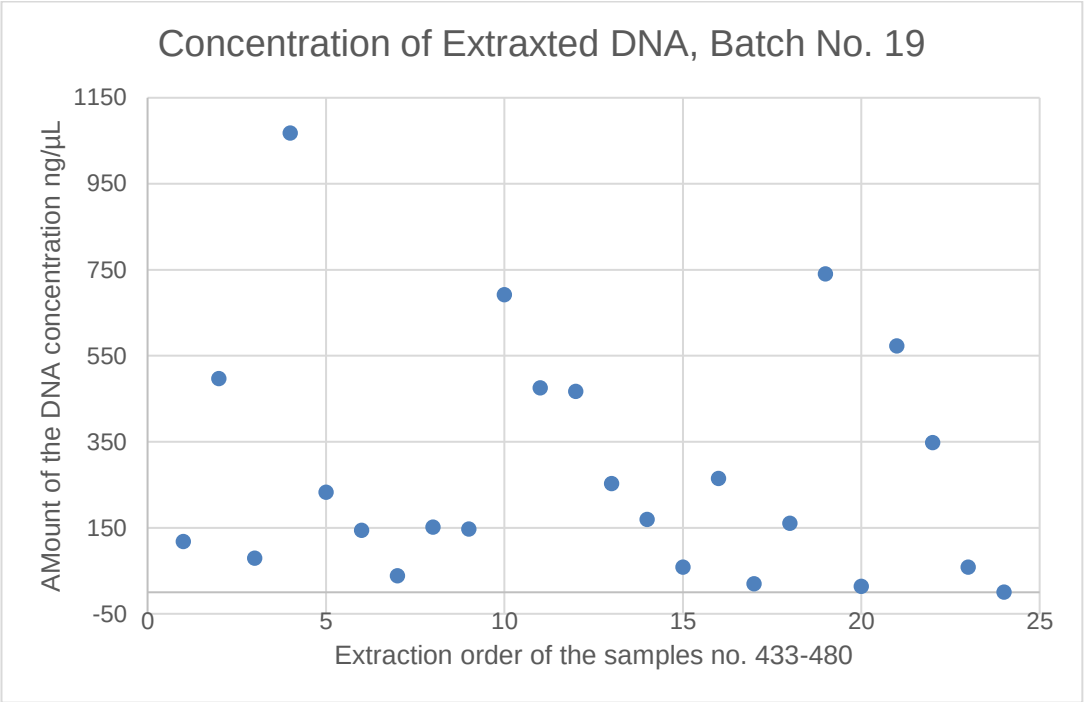


Figure 30. Extraction purification order no. 433-480. Batch no. 19.

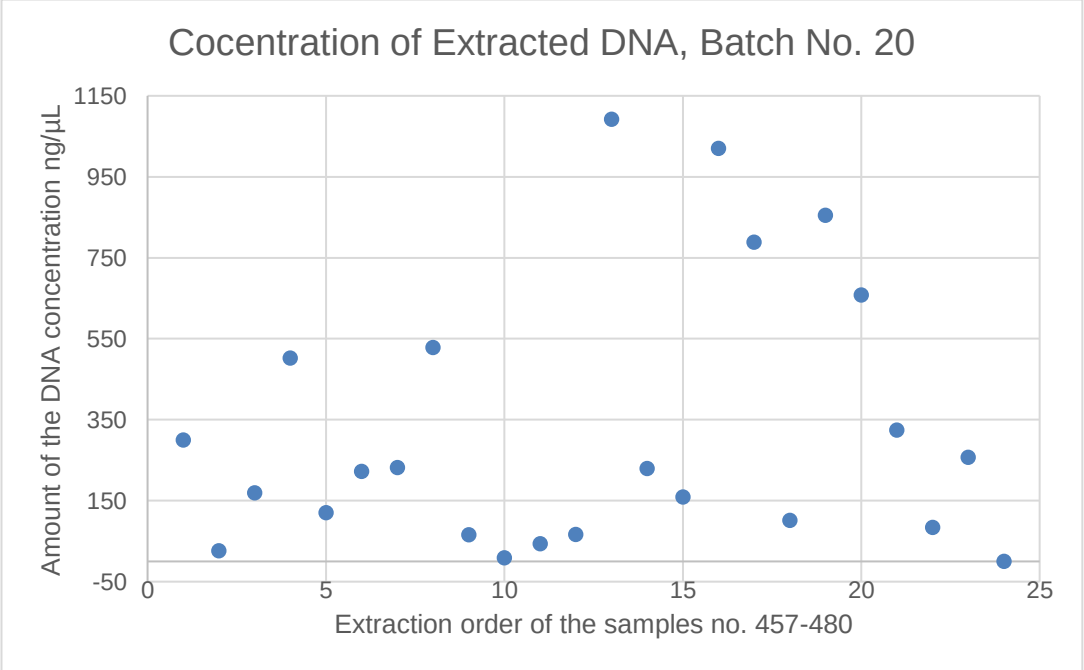


Figure 31. Extraction purification order no. 457-480. Batch no. 20.

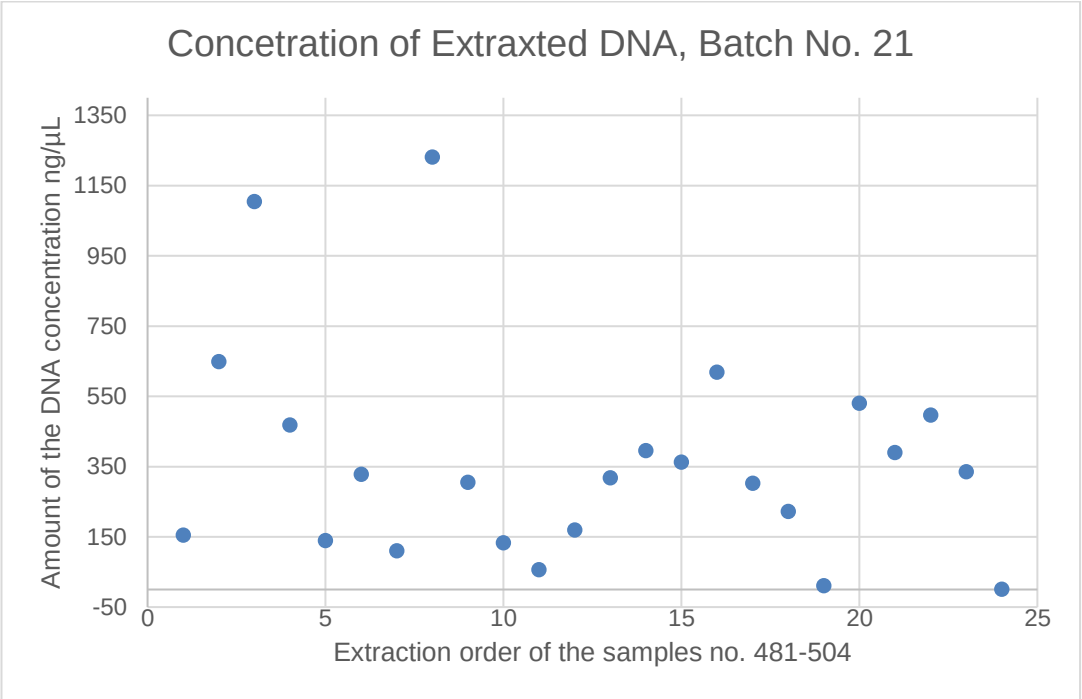


Figure 32. Extraction purification order no. 481-504. Batch no. 21.

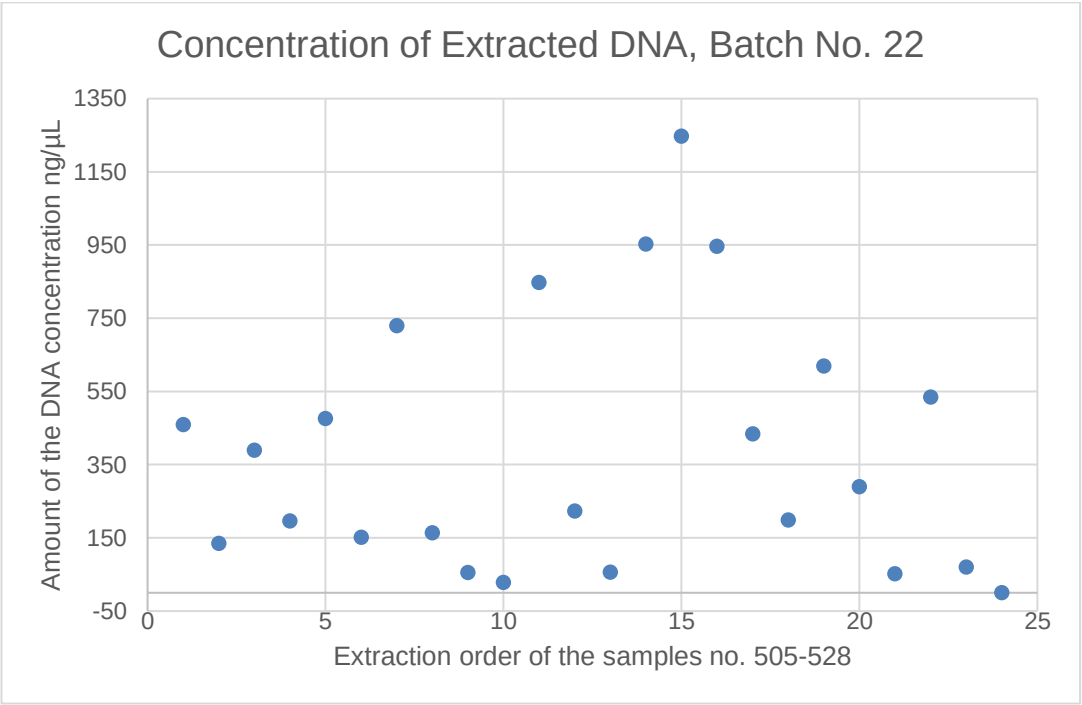


Figure 33. Extraction purification order no. 505-528. Batch no. 22.

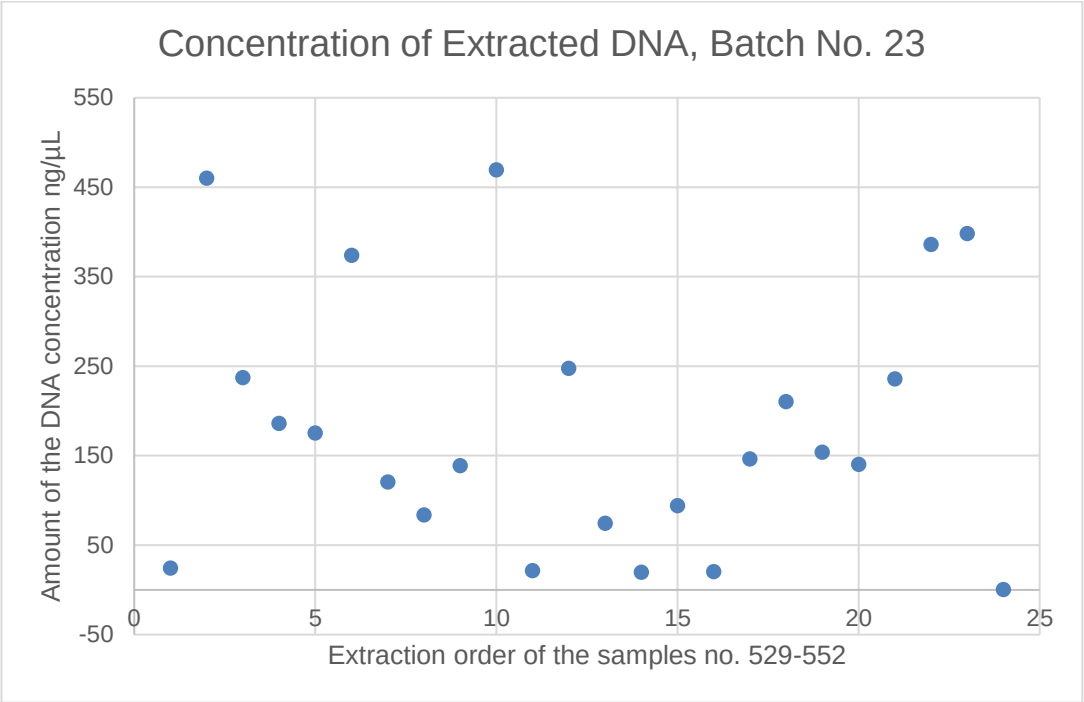


Figure 34. Extraction purification order no. 529-552. Batch no. 23.

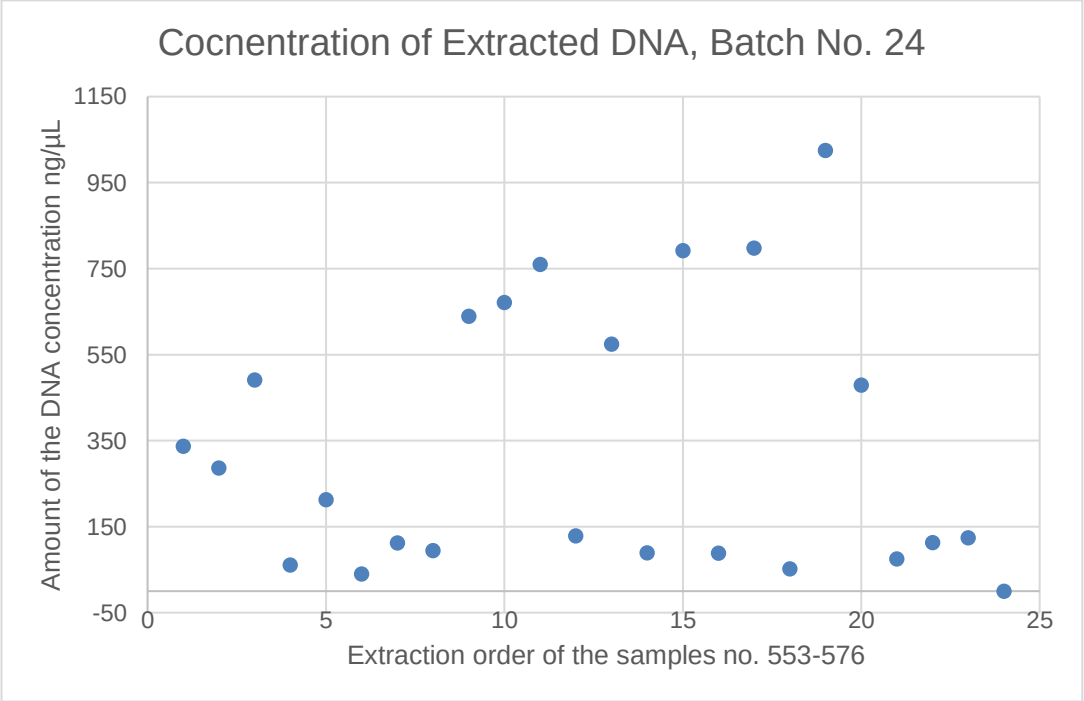


Figure 35. Extraction purification order no. 553-576. Batch no. 24.

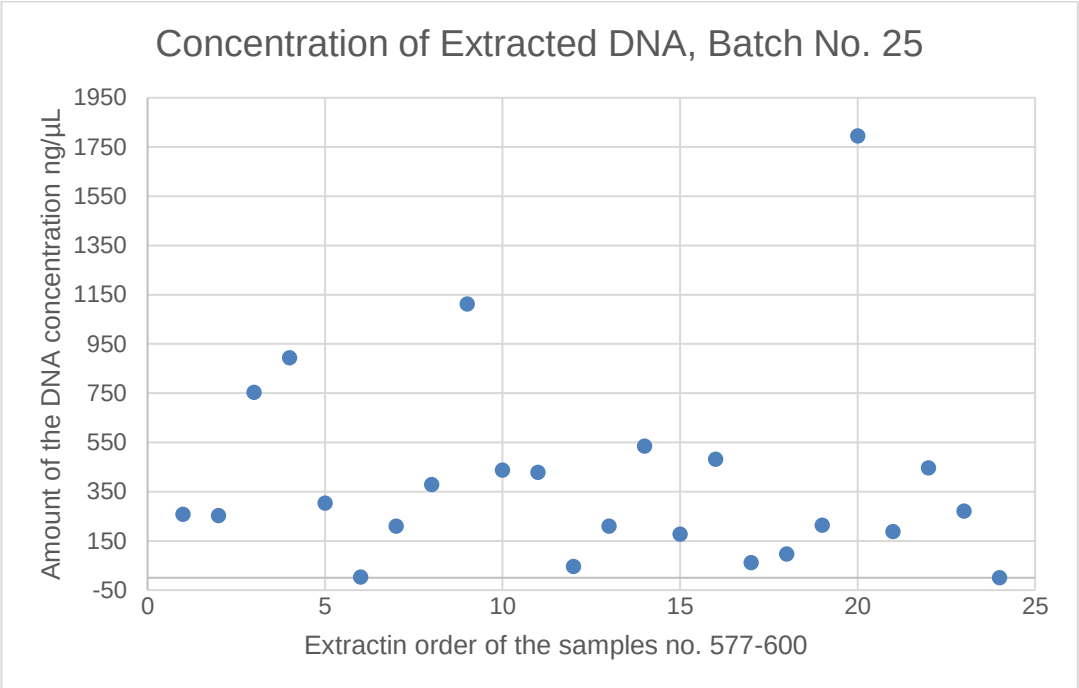


Figure 36. Extraction purification order no. 577-600. Batch no. 25.

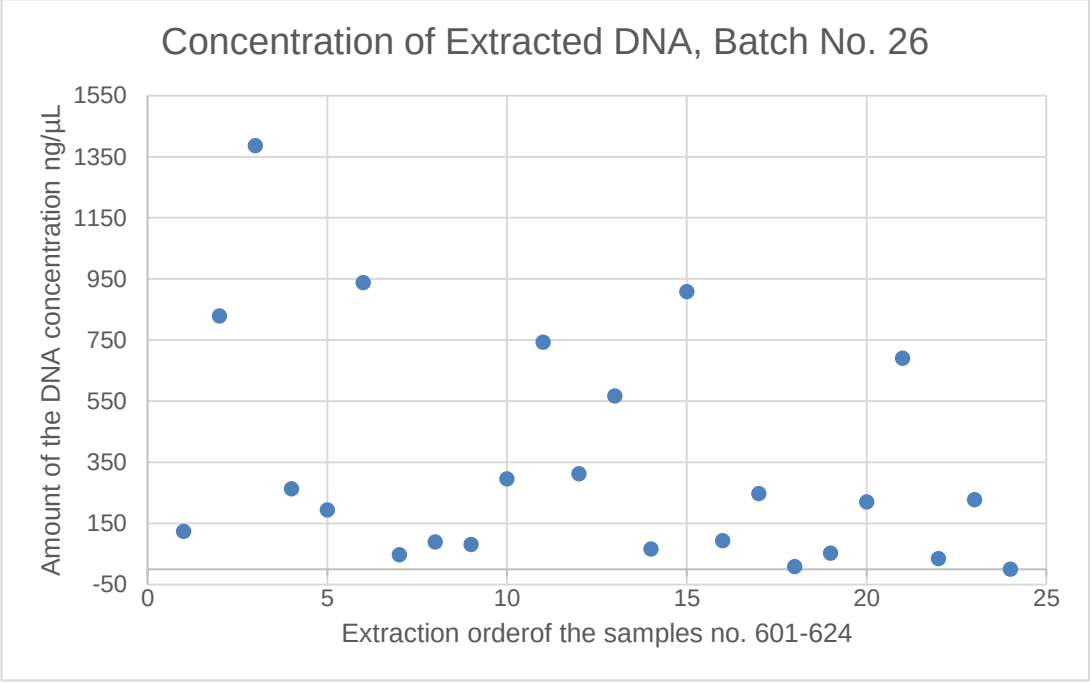


Figure 37. Extraction purification order no. 601-624. Batch no. 26.

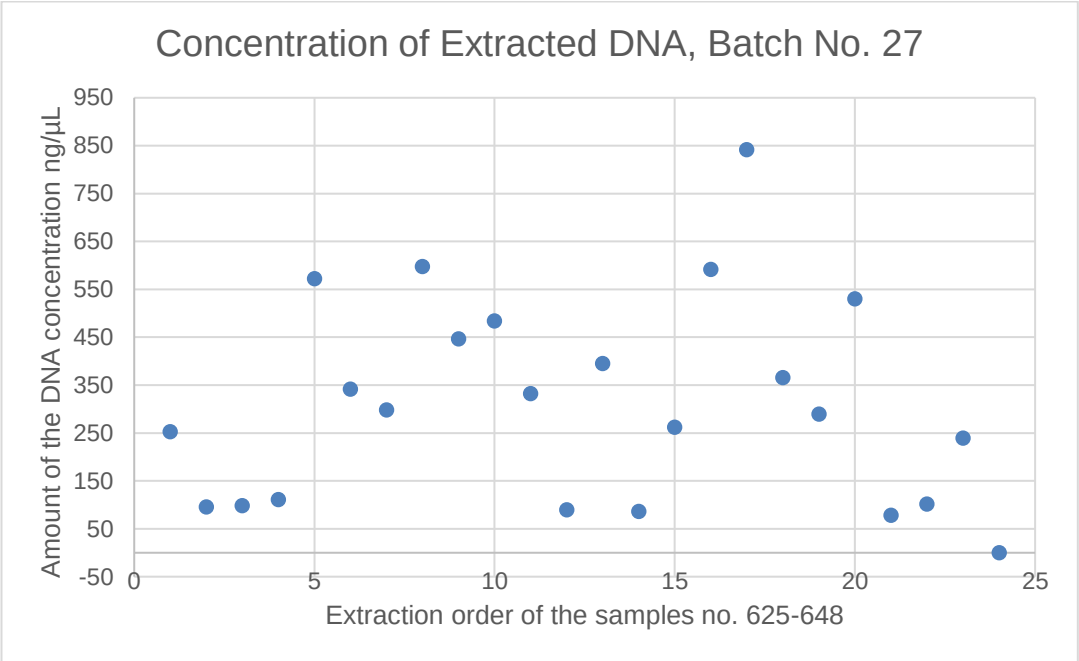


Figure 38. Extraction purification order no. 625-648. Batch no. 27.

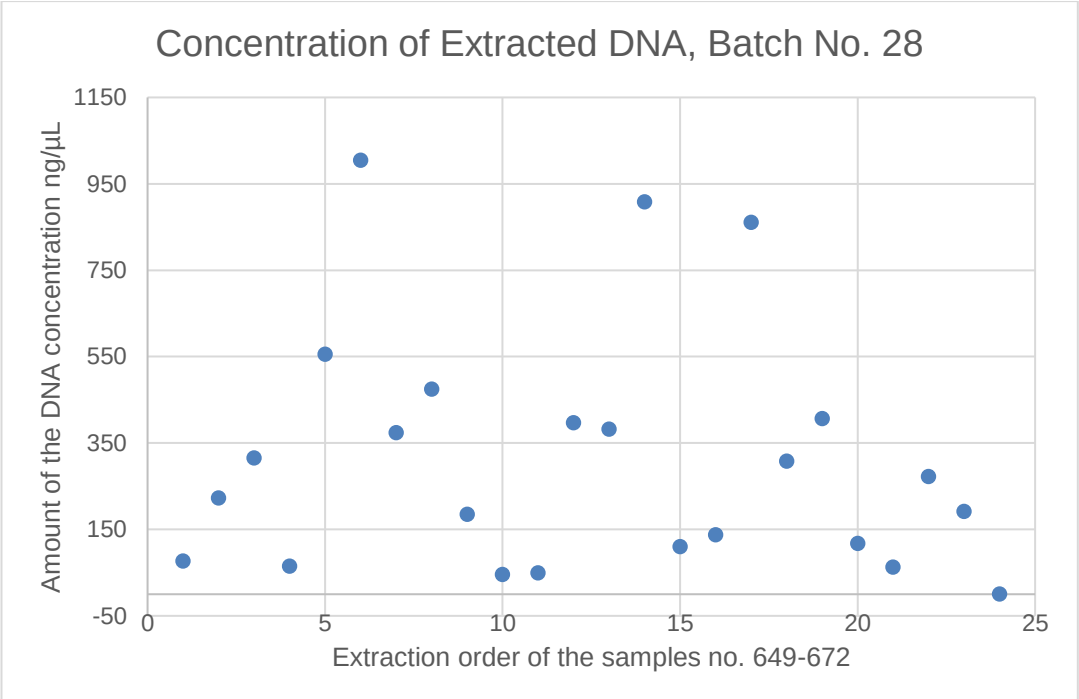


Figure 39. Extraction purification order no. 649-672. Batch no. 28.

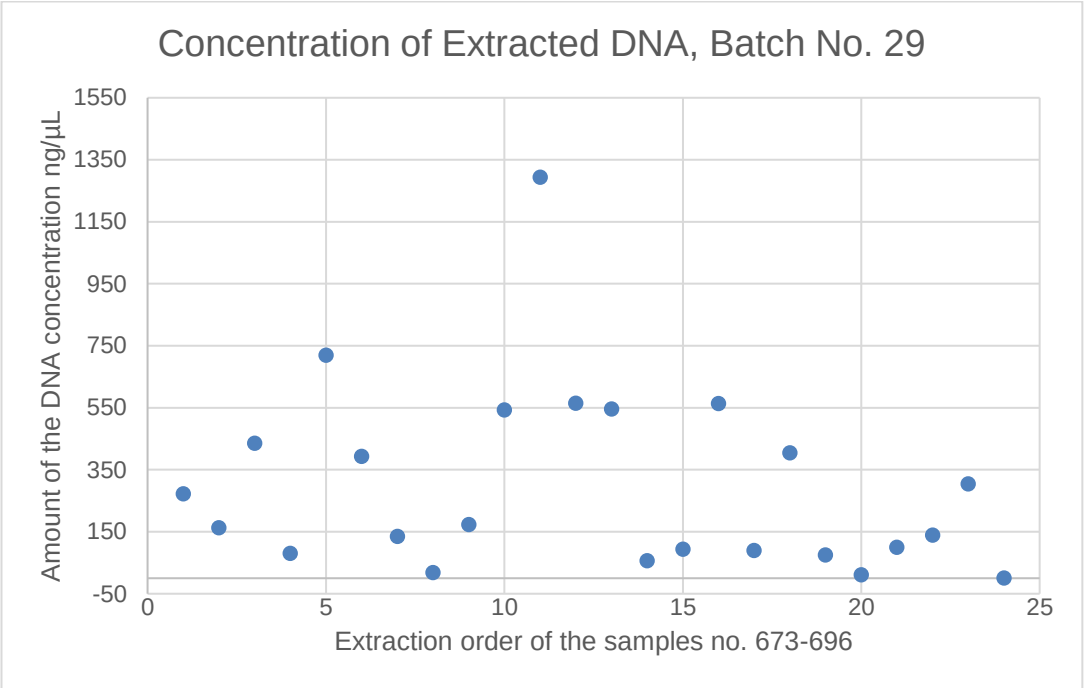


Figure 40. Extraction purification order no. 673-696. Batch no. 29.

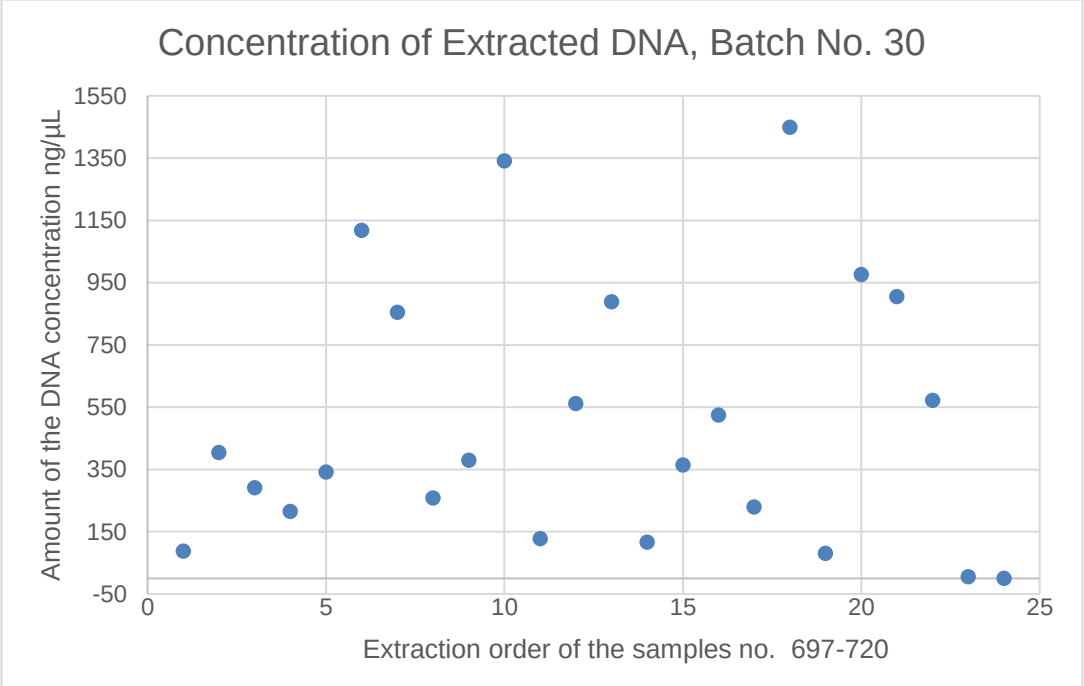


Figure 41. Extraction purification order no. 697-720. Batch no. 30.

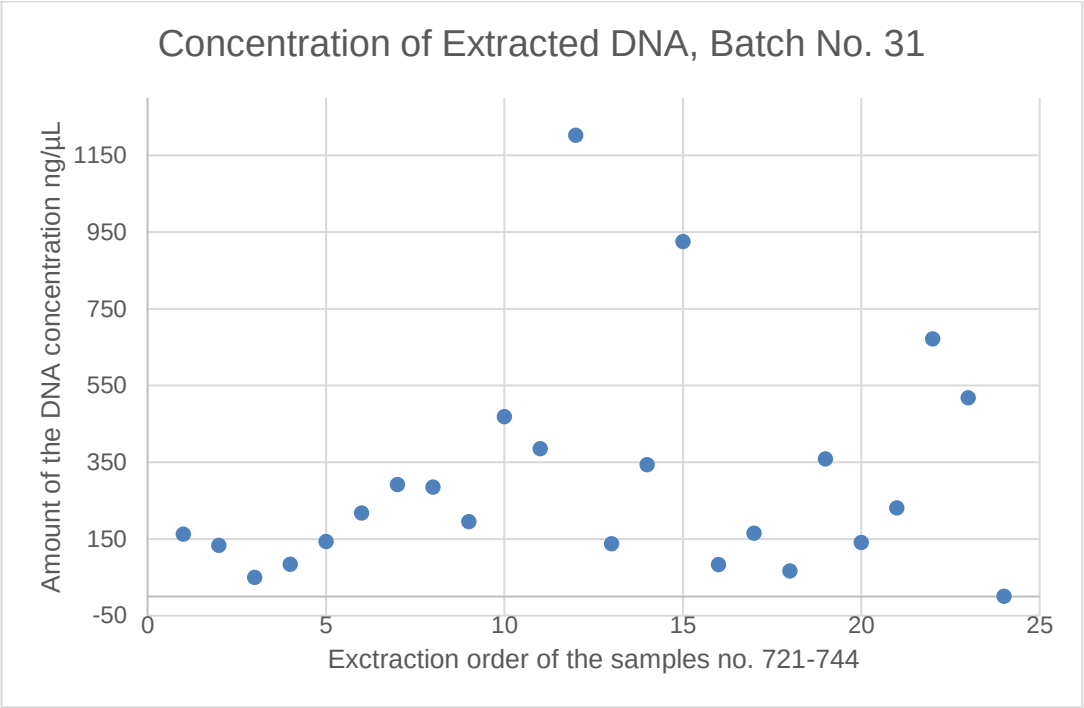


Figure 42. Extraction purification order no. 721-744. Batch no. 31.

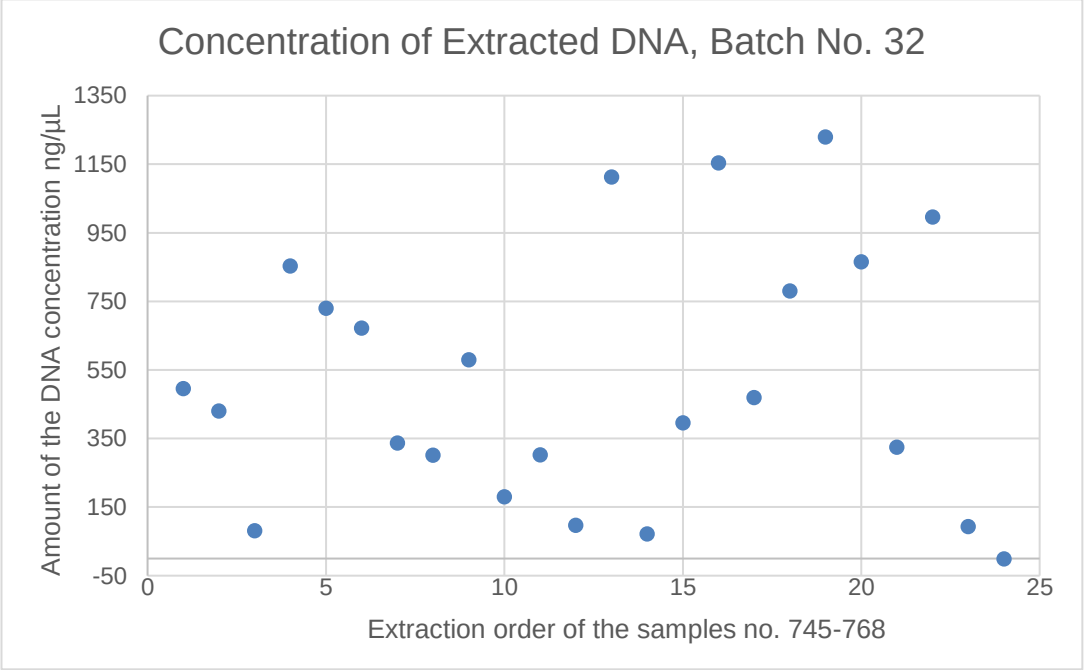


Figure 43. Extraction purification order no. 745-768. Batch no. 32.

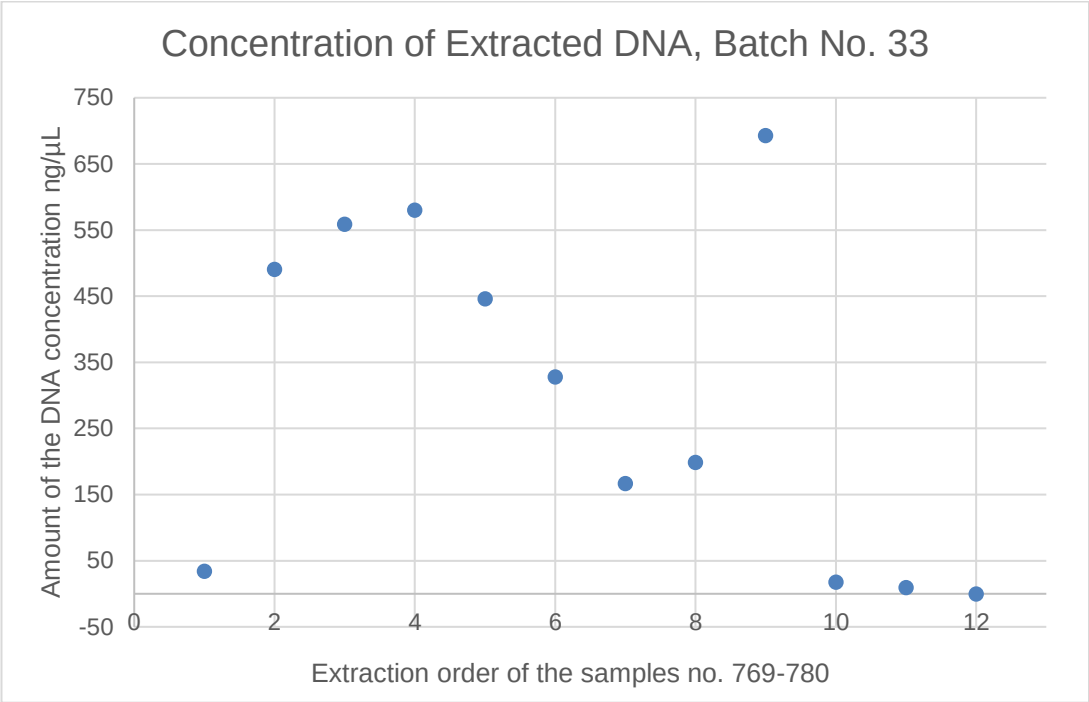


Figure 44. Extraction purification order no. 769-780. Batch no. 33.

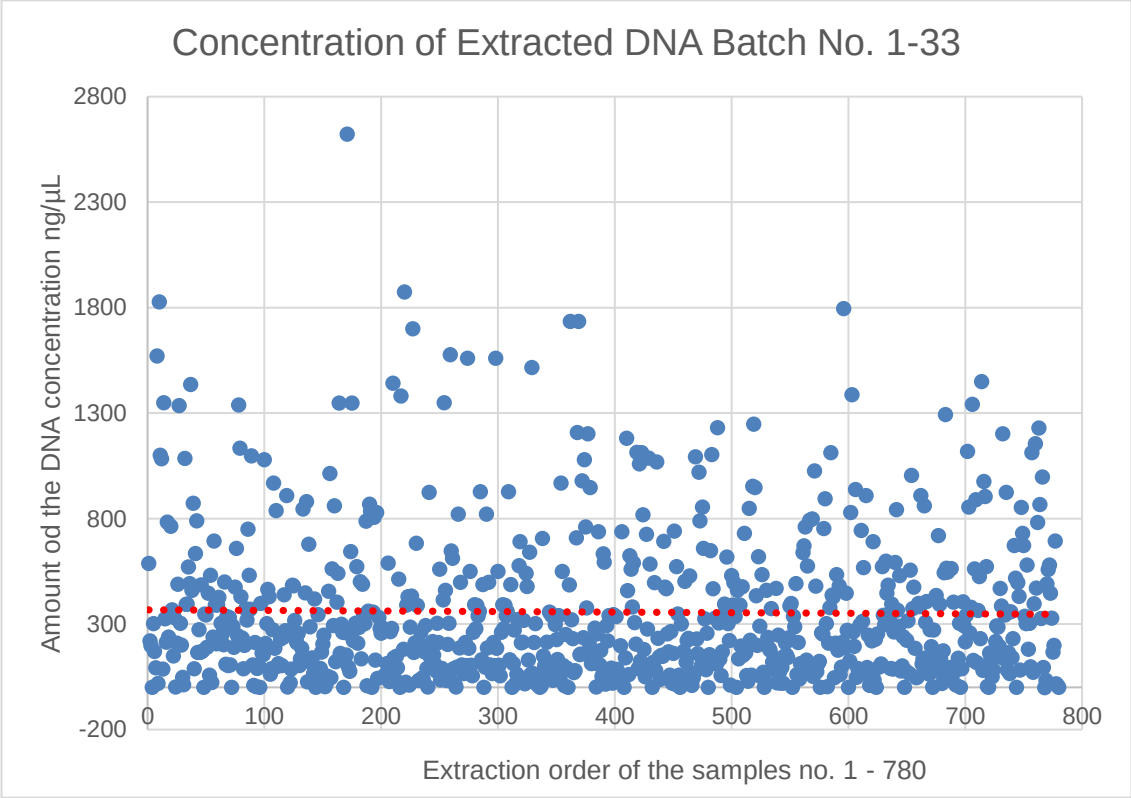


Figure 45. All samples purification number 1-782. Batch no. 1-33.

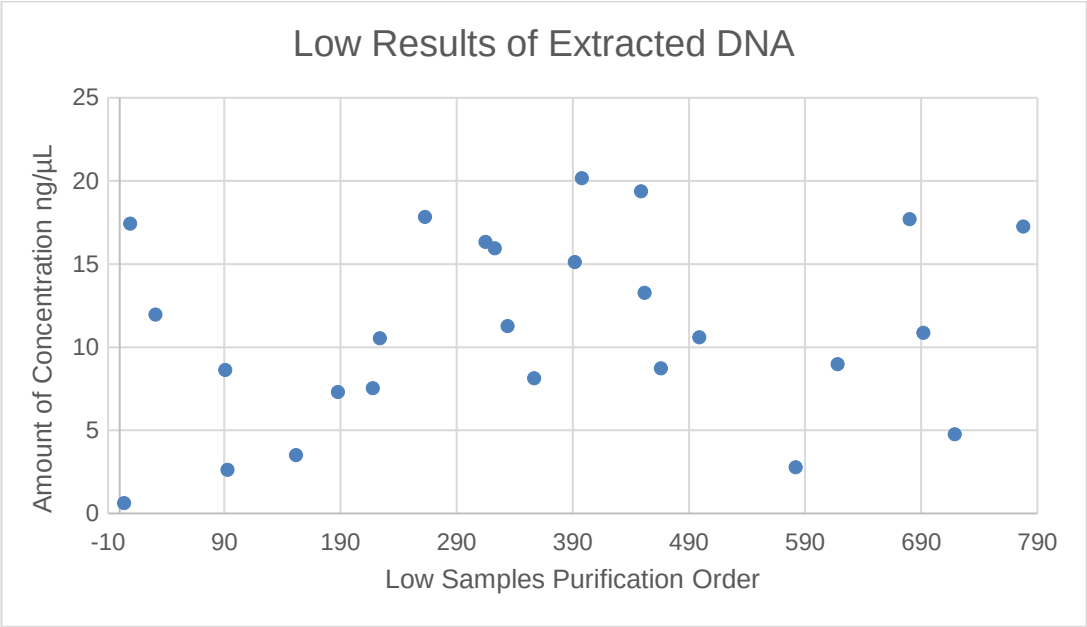


Figure 46. Low result under 20 ng/µL.

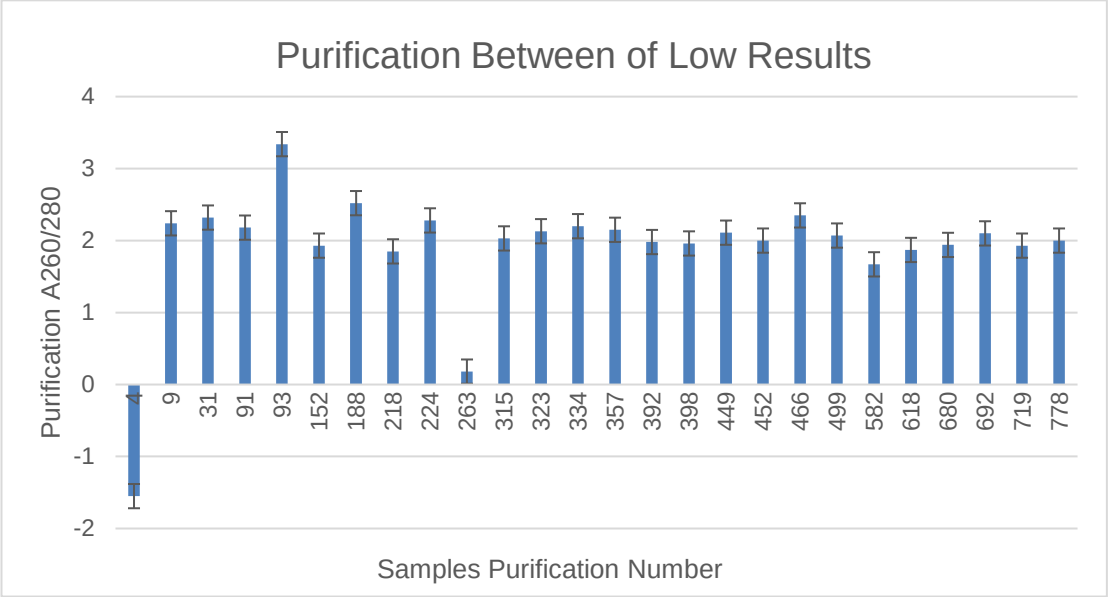


Figure 47. Low result average of standard error.

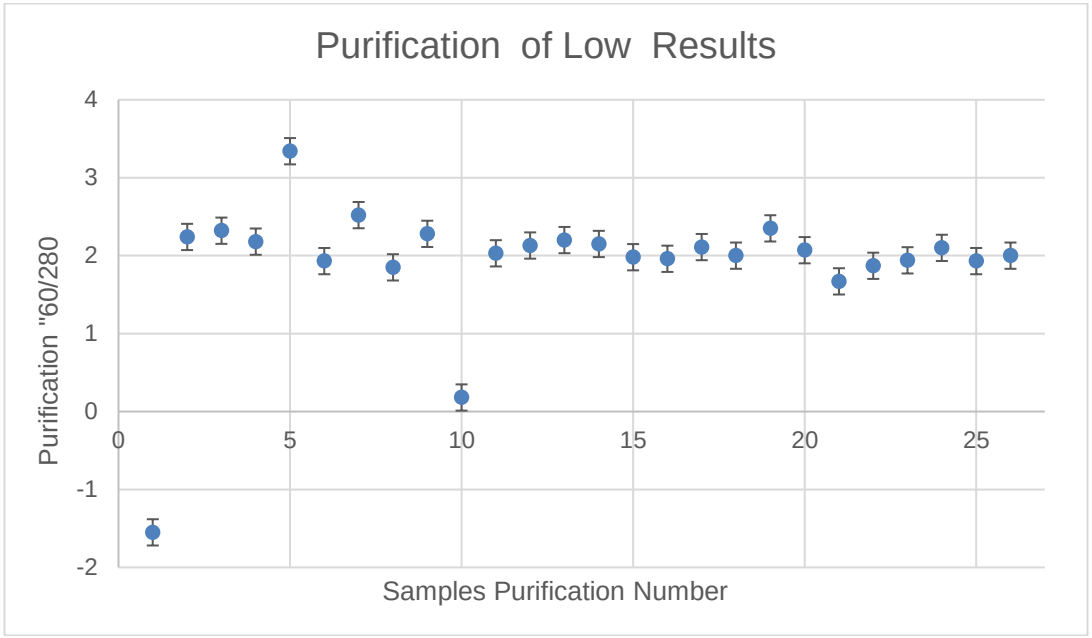


Figure 48. Low result average of standard error in scatter.

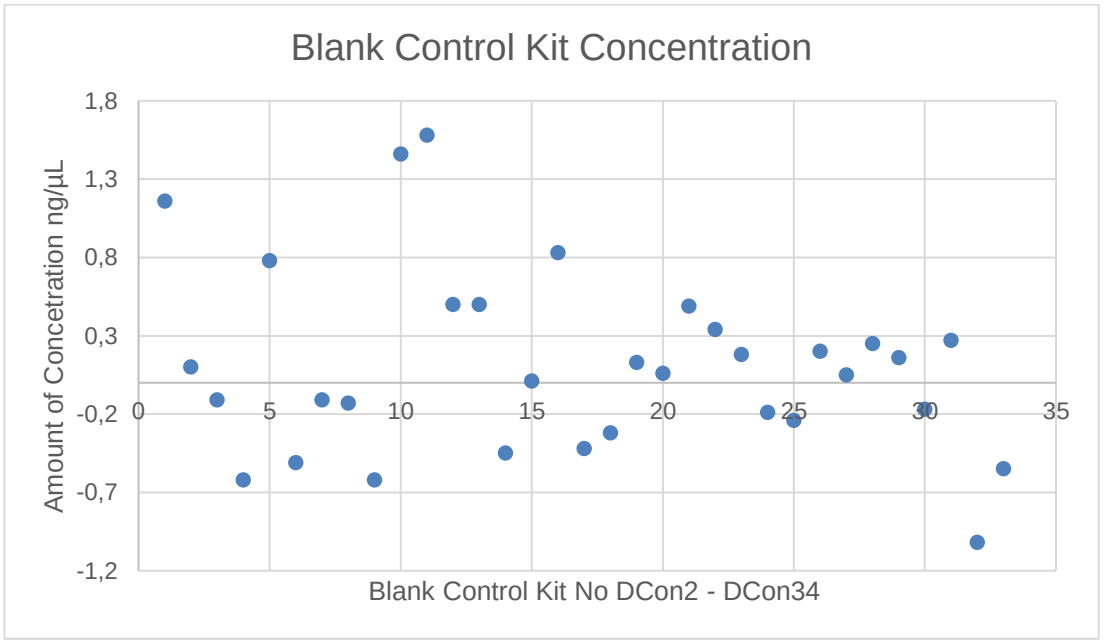


Figure 49. Blank control kit concentration.

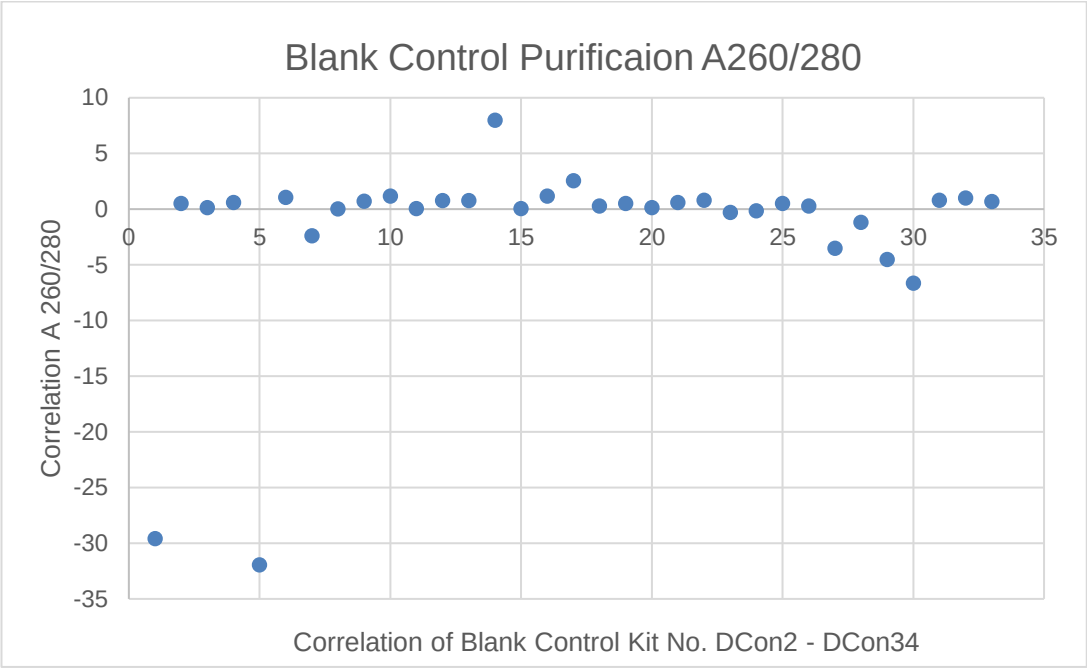


Figure 50. Blank control kit purification.

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Box Number	Barcode	Trend ID	Purification number	Sample ID	Day	ng/µL	A260	260 / 280	260 / 230	Note
DNA_01	DA267	7597	1	D-1	2-3.3.2017	586,7	11,734	1,97	2,27	
DNA_01	DA332	7348	2	D-2	2-3.3.2017	220,66	4,413	2,06	2,01	
DNA_01	DA0096	7156	3	D-3	2-3.3.2017	196,43	3,929	2,08	1,19	
DNA_01	DA0219	7195	4	D-4	2-3.3.2017	0,61	0,012	-1,55	0,02	Thick sample, Low correlation
DNA_01	DA0156	7544	5	D-5	2-3.3.2017	301,23	6,025	2,02	1,76	
DNA_01	DA0176	7337	6	D-6	2-3.3.2017	169,64	3,393	1,98	1,05	
DNA_01	TYD781	1652	7	D-7	2-3.3.2017	92,19	1,844	2,07	2,04	
DNA_01	DA535	1036	8	D-8	2-3.3.2017	1570,94	31,419	2,01	2,04	
DNA_01	DA593	7108	9	D-9	2-3.3.2017	17,42	0,348	2,24	1,3	Correlation ok
DNA_01	TYD811	1546	10	D-10	2-3.3.2017	1826,77	36,535	2,02	1,7	
DNA_01	TYD888	1450	11	D-11	2-3.3.2017	1100,61	22,012	2,07	2,24	
DNA_01	DA0061	7315	12	D-12	2-3.3.2017	1082,78	21,656	2,08	2,17	
DNA_01	DA522	1176	13	D-13	2-3.3.2017	86,19	1,724	2,06	2	
DNA_01	TYD797	7473	14	D-14	2-3.3.2017	1349	26,98	1,99	2,34	
DNA_01	DA647	1053	15	D-15	2-3.3.2017	324,74	6,495	2,09	2,2	
DNA_01	DA730	1321	16	D-16	2-3.3.2017	213,12	4,262	2,11	2,08	
DNA_01	DA670	1184	17	D-17	2-3.3.2017	783,57	15,671	2,05	2,04	
DNA_01	DA322	7414	18	D-18	2-3.3.2017	238,26	4,765	2,07	2,29	
DNA_01	DA380	7531	19	D-19	2-3.3.2017	223,77	4,475	2,03	1,97	
DNA_01	DA0054	7114	20	D-20	2-3.3.2017	762,48	15,25	1,99	2,17	
DNA_01	DA440	7653	21	D-21	2-3.3.2017	367,24	7,345	2,04	0,51	
DNA_01	DA262	7620	22	D-22	2-3.3.2017	149,04	2,981	1,99	2,15	
DNA_01	TYD986	7602	23	D-23	2-3.3.2017	331,19	6,624	2,13	2,2	
DNA_01	DCon2		24	D-24 Control	2-3.3.2017	1,16	0,023	-29,59	1,21	
DNA_02	DA644	1356	25	D-25	03/03/2017	210,01	4,2	2,03	1,28	
DNA_02	DA0171	7353	26	D-26	03/03/2017	488,91	9,778	1,97	1,5	
DNA_02	TYD961	1526	27	D-27	03/03/2017	1335,09	26,702	2	2,11	
DNA_02	DA0242	7525	28	D-28	03/03/2017	303,84	6,077	2,03	0,9	
DNA_02	DA0211	7013	29	D-29	03/03/2017	200,6	4,012	1,96	0,83	
DNA_02	DA351	7409	30	D-30	03/03/2017	46,75	0,935	1,96	0,39	
DNA_02	DA684	1254	31	D-31	03/03/2017	11,95	0,239	2,32	0,15	High correltaion
DNA_02	DA441	7500	32	D-32	03/03/2017	1084,73	21,695	2,03	1,07	
DNA_02	TYD902	1057	33	D-33	03/03/2017	392,3	7,846	2,05	1,1	
DNA_02	TYD853	1683	34	D-34	03/03/2017	393,79	7,876	1,98	1,21	
DNA_02	TYD834	9095	35	D-35	03/03/2017	570,78	11,416	1,95	1,45	
DNA_02	TYD882	9091	36	D-36	03/03/2017	491,99	9,84	1,97	1,71	
DNA_02	DA0073	7683	37	D-37	03/03/2017	1435,46	28,709	2,04	1,59	
DNA_02	TYD808	7467	38	D-38	03/03/2017	460,18	9,204	2,02	1,71	
DNA_02	DA0105	7524	39	D-39	03/03/2017	872,99	17,46	1,96	1,77	
DNA_02	DA397	7706	40	D-40	03/03/2017	87,47	1,749	2,04	1,34	
DNA_02	DA747	9087	41	D-41	03/03/2017	634,16	12,683	2,03	2,21	
DNA_02	DA265	7509	42	D-42	03/03/2017	788,39	15,768	2,07	2,42	
DNA_02	DA712	1229	43	D-43	03/03/2017	165,66	3,313	1,85	1,64	
DNA_02	TYD971	1544	44	D-44	03/03/2017	273,06	5,461	2,04	2,48	
DNA_02	TYD886	7420	45	D-45	03/03/2017	165,6	3,312	1,96	2,48	
DNA_02	DA0099	7648	46	D-46	03/03/2017	485,64	9,713	1,97	2,24	
DNA_02	DA0023	7378	47	D-47	03/03/2017	172,07	3,441	1,99	2,47	
DNA_02	DCon3		48	D-48 Control	03/03/2017	0,1	0,002	0,5	-0,1	
DNA_03	DA416	7472	49	D-49	07/03/2017	347,38	6,948	2,06	2,48	
DNA_03	DA630	1573	50	D-50	07/03/2017	342,11	6,842	1,95	1,76	
DNA_03	DA503	1597	51	D-51	07/03/2017	189,78	3,796	2,01	1,99	
DNA_03	DA517	1131	52	D-52	07/03/2017	446,76	8,935	1,94	1,61	
DNA_03	TYD822	1837	53	D-53	07/03/2017	56,85	1,137	1,8	1,12	Thick sample
DNA_03	DA681	1651	54	D-54	07/03/2017	531,24	10,625	1,96	2,09	
DNA_03	DA0249	7076	55	D-55	07/03/2017	23,17	0,463	1,98	0,69	Thick sample
DNA_03	TYD844	1435	56	D-56	07/03/2017	238,6	4,772	2,04	1,47	
DNA_03	DA0179	7036	57	D-57	07/03/2017	692,77	13,855	1,97	1,85	
DNA_03	DA589	1293	58	D-58	07/03/2017	390,13	7,803	2,07	2,07	
DNA_03	DA519	7507	59	D-59	07/03/2017	206,99	4,14	2,02	1,87	
DNA_03	DA289	7242	60	D-60	07/03/2017	390,18	7,804	1,96	2,12	
DNA_03	DA394	7226	61	D-61	25/04/2017	425,58	8,512	2,04	2,56	
DNA_03	DA626	1608	62	D-62	07/03/2017	217,77	4,355	1,98	1,98	
DNA_03	DA646	7491	63	D-63	07/03/2017	303,45	6,069	2,05	1,57	
DNA_03	DA534	1485	64	D-64	07/03/2017	252,79	5,056	2,03	1,51	
DNA_03	DA0114	7082	65	D-65	07/03/2017	201,66	4,033	1,93	1,35	
DNA_03	TYD805	1067	66	D-66	07/03/2017	498,62	9,972	1,97	1,55	
DNA_03	DA547	7515	67	D-67	07/03/2017	108,88	2,178	1,87	0,74	
DNA_03	DA444	7630	68	D-68	07/03/2017	335,72	6,714	1,99	1,99	
DNA_03	DA666	7550	69	D-69	07/03/2017	103,17	2,063	2,01	1,51	
DNA_03	TYD775	1761	70	D-70	07/03/2017	330,81	6,616	2,01	1,04	
DNA_03	DA742	1408	71	D-71	07/03/2017	106,25	2,125	2,1	1,71	
DNA_03	DCon4		72	D-72 Control	07/03/2017	-0,11	-0,002	0,12	0,09	

DNA_04	TYD754	1785	73	D-73	08/03/2017	289,35	5,787	2,04	1,85	
DNA_04	DA0009	7246	74	D-74	08/03/2017	189,41	3,788	1,99	2,55	
DNA_04	DA523	1225	75	D-75	08/03/2017	475,35	9,507	2,09	2,31	
DNA_04	DA516	1011	76	D-76	08/03/2017	657,86	13,157	2,05	2,39	
DNA_04	DA439	7425	77	D-77	08/03/2017	245,25	4,905	1,95	2,4	Thick sample
DNA_04	DA497	7530	78	D-78	08/03/2017	1338,93	26,779	1,98	2,17	
DNA_04	DA470	7419	79	D-79	08/03/2017	1133,7	22,674	1,99	2,28	Thick sample
DNA_04	DA327	9080	80	D-80	08/03/2017	429,09	8,582	1,93	2,16	
DNA_04	DA740	7483	81	D-81	08/03/2017	234,09	4,682	2	2,4	
DNA_04	TYD953	1461	82	D-82	08/03/2017	88,57	1,771	2,11	0,66	
DNA_04	DA0202	7144	83	D-83	08/03/2017	199,21	3,984	2,1	0,94	
DNA_04	DA0208	1465	84	D-84	08/03/2017	371,79	7,436	2,02	2,95	
DNA_04	DA381	7017	85	D-85	08/03/2017	318,16	6,363	2,11	2,68	
DNA_04	DA0122	7029	86	D-86	08/03/2017	749,84	14,997	2,01	2,12	Thick sample
DNA_04	DA310	7647	87	D-87	08/03/2017	530,66	10,613	1,97	2,19	
DNA_04	TYD820	1777	88	D-88	08/03/2017	369,52	7,39	2,07	0,96	
DNA_04	DA511	9014	89	D-89	08/03/2017	1097,67	21,953	1,99	2,03	
DNA_04	TYD960	1694	90	D-90	08/03/2017	106,81	2,136	2,07	6,32	
DNA_04	DA733	1282	91	D-91	08/03/2017	8,62	0,172	2,18	-0,69	Correlation ok
DNA_04	DA297	7154	92	D-92	08/03/2017	212,27	4,245	1,92	3,05	
DNA_04	DA721	1820	93	D-93	08/03/2017	2,6	0,052	3,34	-0,05	DNA isolation failed?
DNA_04	TYD767	1213	94	D-94	08/03/2017	89,28	1,786	2,01	6,32	
DNA_04	DA0193	7682	95	D-95	08/03/2017	104,58	2,092	2,07	2,81	
DNA_04	DCon5		96	D-96 Control	08/03/2017	-0,62	-0,012	0,58	0,38	
DNA_05	TYD765	9063	97	D-97	09/03/2017	397,97	7,959	2,01	2,07	
DNA_05	DA650	7213	98	D-98	09/03/2017	162,68	3,254	2,08	2,84	
DNA_05	TYD818	7601	99	D-99	09/03/2017	154,72	3,094	2,08	4,12	
DNA_05	DA0243	1237	100	D-100	09/03/2017	1078,29	21,566	1,98	2,45	Thick sample
DNA_05	TYD770	7468	101	D-101	09/03/2017	208,54	4,171	1,97	1,81	
DNA_05	DA251	7289	102	D-102	09/03/2017	301,78	6,036	2,03	0,5	Thick sample
DNA_05	TYD860	1454	103	D-103	09/03/2017	464,69	9,294	1,98	3,02	
DNA_05	DA530	1214	104	D-104	09/03/2017	429,55	8,591	1,98	2,37	
DNA_05	DA673	1206	105	D-105	09/03/2017	61,84	1,237	2,1	-5,68	
DNA_05	DA561	1633	106	D-106	09/03/2017	87,8	1,756	2,06	2,1	
DNA_05	TYD975	7296	107	D-107	09/03/2017	272,58	5,452	2,01	2,54	
DNA_05	DA527	1710	108	D-108	09/03/2017	968,07	19,361	2,02	2,24	
DNA_05	DA311	7418	109	D-109	09/03/2017	184,93	3,699	2,06	3,81	
DNA_05	DA307	7376	110	D-110	09/03/2017	836,9	16,738	2,02	2,19	
DNA_05	DA668	1487	111	D-111	09/03/2017	98,2	1,964	2,05	1,83	
DNA_05	TYD760	7314	112	D-112	09/03/2017	112,27	2,245	2,01	2,45	
DNA_05	DA0151	7137	113	D-113	09/03/2017	106,35	2,127	2,01	2,41	
DNA_05	DA0119	7239	114	D-114	09/03/2017	229,04	4,581	1,95	2,37	
DNA_05	DA590	1411	115	D-115	09/03/2017	102,56	2,051	2,03	31,87	
DNA_05	DA465	7411	116	D-116	09/03/2017	236,39	4,728	2,11	1,73	
DNA_05	DA540	9076	117	D-117	09/03/2017	437,76	8,755	2,02	2,88	
DNA_05	DA746	1654	118	D-118	09/03/2017	269,24	5,385	2,11	1,5	
DNA_05	DA0187	7303	119	D-119	09/03/2017	909,4	18,188	1,98	1,64	
DNA_05	DCon6		120	D-120_Control	09/03/2017	0,78	0,016	-31,97	0,14	
DNA_06	DA0158	7510	121	D-121	10/03/2017	265,45	5,309	1,95	2,85	
DNA_06	DA0092	1090	122	D-122	10/03/2017	20,52	0,41	2,25	1,45	
DNA_06	DA649	7628	123	D-123	10/03/2017	86,46	1,729	2,05	3,83	
DNA_06	DA0034	7016	124	D-124	10/03/2017	483,78	9,676	1,94	2,43	
DNA_06	DA663	1319	125	D-125	10/03/2017	481,22	9,624	1,92	1,8	
DNA_06	DA0065	7520	126	D-126	10/03/2017	317,39	6,348	1,97	2,61	
DNA_06	DA0100	7259	127	D-127	10/03/2017	223,35	4,467	1,99	2,3	
DNA_06	DA360	7564	128	D-128	10/03/2017	210,49	4,21	1,97	3,07	
DNA_06	DA571	1326	129	D-129	10/03/2017	255,87	5,117	2,07	3,3	
DNA_06	DA258	1403	130	D-130	10/03/2017	103,53	2,071	2,03	4,89	
DNA_06	DA315	7264	131	D-131	10/03/2017	135,01	2,7	2,01	2,68	
DNA_06	TYD869	1423	132	D-132	10/03/2017	184,69	3,694	2,01	2,89	
DNA_06	DA368	7281	133	D-133	10/03/2017	845,02	16,9	2	2,39	
DNA_06	DA0181	7427	134	D-134	10/03/2017	131,27	2,625	2,13	0,99	
DNA_06	DA537	1794	135	D-135	10/03/2017	447,13	8,943	2,01	2,92	
DNA_06	DA692	9092	136	D-136	10/03/2017	880,29	17,606	1,99	1,84	
DNA_06	DA0226	7553	137	D-137	10/03/2017	25,37	0,507	2,1	-1,74	
DNA_06	TYD845	1551	138	D-138	10/03/2017	678,47	13,569	2	2,45	
DNA_06	TYD792	7675	139	D-139	10/03/2017	287,45	5,749	2,1	3,19	
DNA_06	DA512	1406	140	D-140	10/03/2017	116,71	2,334	2	4,41	
DNA_06	TYD755	1456	141	D-141	10/03/2017	140,15	2,803	2,07	3,04	
DNA_06	TYD870	1434	142	D-142	10/03/2017	64,85	1,297	2,07	132,26	
DNA_06	TYD885	9055	143	D-143	10/03/2017	419,43	8,389	1,98	2,57	
DNA_06	DCon7		144	D-144 Control	10/03/2017	-0,51	-0,01	1,03	0,53	

DNA_07	DA483	7540	145	D-145	14/03/2017	60,04	1,201	2,06	0,79	
DNA_07	DA460	7394	146	D-146	14/03/2017	343,7	6,874	1,96	2,14	
DNA_07	DA313	7070	147	D-147	14/03/2017	51,58	1,032	1,96	0,59	
DNA_07	DA471	7341	148	D-148	14/03/2017	88,85	1,777	1,96	2,59	
DNA_07	TYD900	7695	149	D-149	14/03/2017	167,22	3,344	2,01	2,17	
DNA_07	TYD799	1457	150	D-150	14/03/2017	205,27	4,105	2,11	1,99	
DNA_07	DA606	1477	151	D-151	14/03/2017	247,97	4,959	2,08	2,02	
DNA_07	TYD980	1496	152	D-152	14/03/2017	3,5	0,07	1,93	-0,52	Correlation ok
DNA_07	DA354	7344	153	D-153	14/03/2017	20,34	0,407	2,03	1,67	
DNA_07	DA428	7439	154	D-154	14/03/2017	53,23	1,065	2,15	2,5	
DNA_07	DA0048	7236	155	D-155	14/03/2017	455,04	9,101	2,07	2,08	
DNA_07	DA504	1159	156	D-156	14/03/2017	1012,83	20,257	1,98	2,29	
DNA_07	TYD977	1625	157	D-157	14/03/2017	45,38	0,908	2,03	1,71	
DNA_07	DA363	7254	158	D-158	14/03/2017	561,42	11,228	2,04	2,31	
DNA_07	DA0200	7097	159	D-159	14/03/2017	293,05	5,861	1,96	2,25	
DNA_07	DA294	7586	160	D-160	14/03/2017	860,52	17,21	1,98	2,16	
DNA_07	DA587	1082	161	D-161	14/03/2017	121,62	2,432	1,99	1,62	
DNA_07	TYD995	1222	162	D-162	14/03/2017	402,83	8,057	2,02	2,24	
DNA_07	DA636	1337	163	D-163	14/03/2017	539,51	10,79	1,94	2,13	
DNA_07	TYD777	1427	164	D-164	14/03/2017	1348,09	26,962	1,99	2,13	
DNA_07	DA479	7624	165	D-165	14/03/2017	160,82	3,216	1,95	1,63	Solid stool sample
DNA_07	TYD801	1274	166	D-166	14/03/2017	299,31	5,986	2,01	2,39	
DNA_07	TYD921	1392	167	D-167	14/03/2017	261,24	5,225	1,96	2,41	
DNA_07	DCon8		168	D-168 Control	14/03/2017	-0,11	-0,002	-2,4	1,04	
DNA_08	DA434	7650	169	D-169	15/03/2017	259,07	5,181	2,09	2,32	
DNA_08	DA400	7038	170	D-170	15/03/2017	153,1	3,062	2	1,57	
DNA_08	DA411	7396	171	D-171	15/03/2017	2622,52	52,45	2,05	2,34	Thick sample
DNA_08	DA656	1283	172	D-172	15/03/2017	95,22	1,904	2,02	1,77	
DNA_08	DA0152	7302	173	D-173	15/03/2017	76,29	1,526	1,99	1,13	
DNA_08	DA377	7534	174	D-174	15/03/2017	643,02	12,86	1,99	2,28	
DNA_08	DA0050	1169	175	D-175	15/03/2017	1347,35	26,947	2,01	2,34	Thick sample
DNA_08	DA0180	9067	176	D-176	15/03/2017	221,68	4,434	2,08	2,04	
DNA_08	DA610	7678	177	D-177	15/03/2017	275,78	5,516	2,08	1,89	
DNA_08	DA329	7122	178	D-178	15/03/2017	304,14	6,083	2,03	2,2	
DNA_08	DA660	1555	179	D-179	15/03/2017	572,36	11,447	2,02	2,23	
DNA_08	TYD873	9056	180	D-180	15/03/2017	209,18	4,184	1,84	1,87	
DNA_08	DA538	7445	181	D-181	15/03/2017	284,43	5,689	2,03	2,22	
DNA_08	DA612	1368	182	D-182	15/03/2017	500,6	10,012	1,98	2,2	
DNA_08	TYD909	7158	183	D-183	15/03/2017	134,37	2,687	2,09	2,66	
DNA_08	DA316	1051	184	D-184	15/03/2017	488,91	9,778	2,1	2,22	
DNA_08	TYD859	1731	185	D-185	15/03/2017	341,78	6,836	2,13	2,26	
DNA_08	TYD779	1653	186	D-186	15/03/2017	301,99	6,04	2,04	2,34	
DNA_08	DA0116	7487	187	D-187	15/03/2017	787,82	15,756	1,99	2,28	Thick sample.
DNA_08	DA0117	7478	188	D-188	15/03/2017	7,28	0,146	2,52	-1,01	Correllation ok
DNA_08	DA576	1174	189	D-189	15/03/2017	361,47	7,229	2,08	1,95	
DNA_08	DA413	7263	190	D-190	15/03/2017	868,15	17,363	2,02	2,23	
DNA_08	DA493	1782	191	D-191	15/03/2017	834,93	16,699	1,99	1,34	
DNA_08	DCon9		192	D-192 Control	15/03/2017	-0,13	-0,003	-0,006	0,42	
DNA_09	DA0095	1096	193	D-193	16/03/2017	356,92	7,138	3,562	2,08	
DNA_09	TYD757	1384	194	D-194	16/03/2017	806,02	16,12	8,026	2,17	
DNA_09	DA0153	7471	195	D-195	16/03/2017	128,56	2,571	1,266	1,94	
DNA_09	DA588	1085	196	D-196	16/03/2017	828,2	16,564	8,217	2,12	
DNA_09	DA707	1489	197	D-197	16/03/2017	42,46	0,849	0,427	1,23	
DNA_09	DA550	1646	198	D-198	16/03/2017	329,96	6,599	3,424	2,09	
DNA_09	DA0185	7047	199	D-199	16/03/2017	278,58	5,572	2,879	2,21	
DNA_09	TYD783	1270	200	D-200	16/03/2017	256,44	5,129	2,484	1,97	
DNA_09	DA675	1445	201	D-201	16/03/2017	282,67	5,653	2,773	1,64	
DNA_09	DA566	1121	202	D-202	16/03/2017	48,95	0,979	0,505	1,85	
DNA_09	DA508	1693	203	D-203	16/03/2017	56,47	1,129	0,569	1,25	
DNA_09	DA388	7160	204	D-204	16/03/2017	61,55	1,231	0,613	2,42	
DNA_09	DA361	7251	205	D-205	16/03/2017	112,82	2,256	1,14	1,38	
DNA_09	DA352	7703	206	D-206	16/03/2017	588,82	11,776	5,71	2,24	
DNA_09	DA349	7145	207	D-207	16/03/2017	27,7	0,554	0,274	0,61	
DNA_09	DA0059	7162	208	D-208	16/03/2017	264,35	5,287	2,615	1,55	
DNA_09	TYD958	1765	209	D-209	16/03/2017	278,7	5,574	2,716	2,25	
DNA_09	TYD890	1366	210	D-210	16/03/2017	1441,5	28,83	14,048	2,12	Thick sample
DNA_09	DA484	7608	211	D-211	16/03/2017	139,79	2,796	1,425	1,79	
DNA_09	DA292	7288	212	D-212	16/03/2017	36,02	0,72	0,368	1,59	
DNA_09	DA276	7397	213	D-213	16/03/2017	146,68	2,934	1,481	2,23	
DNA_09	DA613	1175	214	D-214	16/03/2017	92,13	1,843	0,903	2,31	
DNA_09	DA0062	7062	215	D-215	16/03/2017	512,35	10,247	5,184	2,01	
DNA_09	DCon10		216	D-216 Control	16/03/2017	-0,62	-0,012	0,71	686,1	

DNA_10	DA0038	7088	217	D-217	17/03/2017	1380,32	27,606	2,03	2,2	
DNA_10	DA437	7385	218	D-218	17/03/2017	7,51	0,15	1,85	0,6	Low correlation
DNA_10	DA0238	7012	219	D-219	17/03/2017	180,82	3,616	2,05	1,08	
DNA_10	DA0084	7528	220	D-220	17/03/2017	1874,28	37,486	2,04	2,33	
DNA_10	TYD766	7248	221	D-221	17/03/2017	24,75	0,495	1,87	0,44	
DNA_10	DA667	7171	222	D-222	17/03/2017	390,06	7,801	2,06	1,82	
DNA_10	DA750	1786	223	D-223	17/03/2017	429,4	8,588	2,03	2,25	
DNA_10	DA641	7557	224	D-224	17/03/2017	10,52	0,21	2,28	-1,42	High correltaion
DNA_10	DA748	1120	225	D-225	17/03/2017	285,21	5,704	2,09	2,33	
DNA_10	DA722	7687	226	D-226	17/03/2017	431,79	8,636	1,99	2,06	
DNA_10	DA546	7673	227	D-227	17/03/2017	1699,9	33,998	2,04	2,02	Thick sample
DNA_10	DA282	9071	228	D-228	17/03/2017	164,75	3,295	1,99	1,7	
DNA_10	DAJ130	7389	229	D-229	17/03/2017	211,18	4,224	2,1	2,36	
DNA_10	DA723	1446	230	D-230	17/03/2017	682,44	13,649	1,97	2,04	
DNA_10	DA476	7636	231	D-231	17/03/2017	387,81	7,756	2,04	2,28	
DNA_10	DA0053	7117	232	D-232	17/03/2017	31,31	0,626	2,02	0,58	
DNA_10	DA599	1050	233	D-233	17/03/2017	205,84	4,117	2,1	2,6	
DNA_10	TYD936	1233	234	D-234	17/03/2017	260,91	5,218	2,02	2,78	
DNA_10	DA395	7423	235	D-235	17/03/2017	44,18	0,884	2,05	2,64	
DNA_10	DA0155	7050	236	D-236	17/03/2017	34,45	0,689	2,08	-422,04	
DNA_10	TYD968	1620	237	D-237	17/03/2017	168,77	3,375	1,91	2,13	No stabilizer in tube
DNA_10	DA398	7725	238	D-238	17/03/2017	293,53	5,871	2,04	1,38	Very little material
DNA_10	DA0217	7545	239	D-239	17/03/2017	89,49	1,79	1,96	0,39	
DNA_10	DCon11		240	D-240 Control	17/03/2017	1,46	0,029	1,15	-1,72	
DNA_11	DA0075	7207	241	D-241	20/03/2017	924,34	18,487	9,08	2,04	
DNA_11	DA0209	7405	242	D-242	20/03/2017	145,96	2,919	1,424	2,05	
DNA_11	DA558	7430	243	D-243	20/03/2017	183	3,66	1,826	2	
DNA_11	DA0040	7422	244	D-244	20/03/2017	217,25	4,345	2,211	1,97	
DNA_11	TYD877	7340	245	D-245	20/03/2017	153,04	3,061	1,543	1,98	
DNA_11	DA432	7146	246	D-246	20/03/2017	108,52	2,17	1,111	1,95	
DNA_11	TYD992	1032	247	D-247	20/03/2017	76,72	1,534	0,757	2,03	
DNA_11	DA367	7187	248	D-248	20/03/2017	303,19	6,064	2,963	2,05	
DNA_11	DA446	7613	249	D-249	20/03/2017	211,67	4,233	2,094	2,02	
DNA_11	DA0160	7669	250	D-250	20/03/2017	560,41	11,208	5,615	2	
DNA_11	DA706	9012	251	D-251	20/03/2017	34,17	0,683	0,346	1,97	
DNA_11	DA330	7380	252	D-252	20/03/2017	89,76	1,795	0,904	1,99	
DNA_11	TYD788	1596	253	D-253	20/03/2017	414,87	8,297	4,028	2,06	
DNA_11	DA734	7686	254	D-254	20/03/2017	1348,45	26,969	13,278	2,03	
DNA_11	DA582	9053	255	D-255	20/03/2017	459,44	9,189	4,566	2,01	
DNA_11	TYD874	1418	256	D-256	20/03/2017	31,26	0,625	0,321	1,95	
DNA_11	DA0020	7360	257	D-257	20/03/2017	202,87	4,057	1,994	2,03	
DNA_11	DA0028	7155	258	D-258	20/03/2017	303,75	6,075	3,024	2,01	
DNA_11	DA716	7607	259	D-259	20/03/2017	1576,76	31,535	15,872	1,99	
DNA_11	DA0111	7224	260	D-260	20/03/2017	646,12	12,922	6,45	2	
DNA_11	DA502	1442	261	D-261	20/03/2017	612,04	12,241	5,987	2,04	
DNA_11	DA0199	7274	262	D-262	20/03/2017	87,76	1,755	0,889	1,97	
DNA_11	DA281	7024	263	D-263	20/03/2017	17,82	0,356	0,18	1,98	Low correlation
DNA_11	DCon12		264	D-264 Control	20/03/2017	1,58	0,032	0,03	1,04	
DNA_12	DA353	7200	265	D-265	22/03/2017	94,81	1,896	2,06	1,8	
DNA_12	DA0064	7680	266	D-266	22/03/2017	821,38	16,428	2	2,34	
DNA_12	DA0045	7002	267	D-267	22/03/2017	186,27	3,725	1,92	1,68	
DNA_12	DA0227	7092	268	D-268	22/03/2017	497,5	9,95	1,99	2,52	Thick sample
DNA_12	DA0056	7173	269	D-269	22/03/2017	106,45	2,129	2,03	1,59	
DNA_12	DA696	1421	270	D-270	22/03/2017	94,96	1,899	1,99	5,33	
DNA_12	DA586	1044	271	D-271	22/03/2017	65,41	1,308	1,95	2,87	
DNA_12	DA0233	1624	272	D-272	22/03/2017	212,34	4,247	2,01	2,63	
DNA_12	DA569	1757	273	D-273	22/03/2017	103,49	2,07	2,06	5,37	
DNA_12	DA0033	7273	274	D-274	22/03/2017	1559,4	31,188	2,02	1,87	Thick sample
DNA_12	DA495	7476	275	D-275	22/03/2017	185,79	3,716	2,03	1,27	
DNA_12	DA559	9083	276	D-276	22/03/2017	549,89	10,998	2,02	2,33	
DNA_12	DA0184	7198	277	D-277	22/03/2017	104,97	2,099	2,08	3,87	
DNA_12	DA0235	7546	278	D-278	22/03/2017	54,26	1,085	1,97	11,42	
DNA_12	DA659	1417	279	D-279	22/03/2017	261,61	5,232	2,06	2,48	
DNA_12	DA425	7696	280	D-280	22/03/2017	390,94	7,819	2,07	2,36	
DNA_12	DA417	7724	281	D-281	22/03/2017	278,08	5,562	2,02	2,76	
DNA_12	TYD897	1428	282	D-282	22/03/2017	388,02	7,76	2,03	2,86	
DNA_12	DA382	9086	283	D-283	22/03/2017	338,59	6,772	1,95	2,35	
DNA_12	DA645	7599	284	D-284	22/03/2017	359,82	7,196	1,98	2,55	
DNA_12	DA568	1728	285	D-285	22/03/2017	927,29	18,546	2,01	2,12	
DNA_12	TYD871	7527	286	D-286	22/03/2017	129,75	2,595	2,05	2,15	
DNA_12	DA0008	7297	287	D-287	22/03/2017	487,19	9,744	2,01	0,73	
DNA_12	DCon13		288	D-288 Control	22/03/2017	0,5	0,01	0,77	-4,14	

DNA_13	DA500	7099	289	D-265	22/03/2017	94,81	1,896	2,06	1,8	
DNA_13	DA0191	7664	290	D-266	22/03/2017	821,38	16,428	2	2,34	
DNA_13	DA715	7382	291	D-267	22/03/2017	186,27	3,725	1,92	1,68	
DNA_13	TYD938	1718	292	D-268	22/03/2017	497,5	9,95	1,99	2,52	
DNA_13	TYD895	1388	293	D-269	22/03/2017	106,45	2,129	2,03	1,59	
DNA_13	DA0027	7379	294	D-270	22/03/2017	94,96	1,899	1,99	5,33	
DNA_13	DA573	1231	295	D-271	22/03/2017	65,41	1,308	1,95	2,87	
DNA_13	DA387	1690	296	D-272	22/03/2017	212,34	4,247	2,01	2,63	
DNA_13	DA0172	7142	297	D-273	22/03/2017	103,49	2,07	2,06	5,37	
DNA_13	DA374	7352	298	D-274	22/03/2017	1559,4	31,188	2,02	1,87	
DNA_13	DA744	1781	299	D-275	22/03/2017	185,79	3,716	2,03	1,27	
DNA_13	DA477	7707	300	D-276	22/03/2017	549,89	10,998	2,02	2,33	
DNA_13	DA435	1524	301	D-277	22/03/2017	104,97	2,099	2,08	3,87	
DNA_13	DA0046	7279	302	D-278	22/03/2017	54,26	1,085	1,97	11,42	
DNA_13	DA295	7284	303	D-279	22/03/2017	261,61	5,232	2,06	2,48	
DNA_13	DA738	7559	304	D-280	22/03/2017	390,94	7,819	2,07	2,36	
DNA_13	TYD966	9064	305	D-281	22/03/2017	278,08	5,562	2,02	2,76	
DNA_13	DA611	1142	306	D-282	22/03/2017	388,02	7,76	2,03	2,86	
DNA_13	DA0044	7113	307	D-283	22/03/2017	338,59	6,772	1,95	2,35	
DNA_13	DA438	7496	308	D-284	22/03/2017	359,82	7,196	1,98	2,55	
DNA_13	DA0246	7431	309	D-285	22/03/2017	927,29	18,546	2,01	2,12	
DNA_13	DA514	7596	310	D-286	22/03/2017	129,75	2,595	2,05	2,15	
DNA_13	DA584	1103	311	D-287	22/03/2017	487,19	9,744	2,01	0,73	
DNA_13	DCon14		312	D-288 Control	22/03/2017	0,5	0,01	0,77	-4,14	
DNA_14	TYD802	1677	313	D-313	24/03/2017	112,15	2,243	2,03	1,55	
DNA_14	DA536	1484	314	D-314	24/03/2017	271,92	5,438	1,89	1,49	
DNA_14	TYD791	7110	315	D-315	24/03/2017	16,32	0,326	2,03	0,18	Correlation ok
DNA_14	DA305	1058	316	D-316	24/03/2017	61,41	1,228	2,06	0,77	
DNA_14	DA414	1644	317	D-317	24/03/2017	321,37	6,427	2,05	2,48	
DNA_14	DA376	1614	318	D-318	24/03/2017	576,66	11,533	1,94	2,13	
DNA_14	DA462	7192	319	D-319	24/03/2017	689,7	13,794	1,99	2,24	
DNA_14	DA0144	1558	320	D-320	24/03/2017	210,24	4,205	2,03	2,01	
DNA_14	DA419	7280	321	D-321	24/03/2017	213,39	4,268	1,97	2,57	
DNA_14	DA371	7216	322	D-322	24/03/2017	316,64	6,333	2	1,81	
DNA_14	DA0207	7312	323	D-323	24/03/2017	15,93	0,319	2,13	1,02	Correlation ok
DNA_14	DA669	1660	324	D-324	24/03/2017	540,5	10,81	2,01	2,43	
DNA_14	TYD796	1696	325	D-325	24/03/2017	477,57	9,551	1,96	1,81	
DNA_14	DA0137	7334	326	D-326	24/03/2017	129,27	2,585	2	3,64	
DNA_14	TYD962	1637	327	D-327	24/03/2017	640,16	12,803	2,06	2,44	Thick sample
DNA_14	DA422	1059	328	D-328	24/03/2017	61,07	1,221	2,04	1,56	
DNA_14	DA726	1673	329	D-329	24/03/2017	1516,13	30,323	2,08	2,26	Thick sample
DNA_14	DA0076	9005	330	D-330	24/03/2017	64,54	1,291	1,95	5,93	
DNA_14	DA702	1536	331	D-331	24/03/2017	212,74	4,255	2,05	3,22	
DNA_14	TYD810	9088	332	D-332	24/03/2017	302,49	6,05	2,03	2,55	
DNA_14	DA466	7497	333	D-333	24/03/2017	34,32	0,686	1,96	0,84	
DNA_14	DA728	1519	334	D-334	24/03/2017	11,25	0,225	2,2	-0,6	Sabilizer ok, verry little sample, Correlation ok
DNA_14	DA0079	7176	335	D-335	24/03/2017	50,72	1,014	2,04	6,73	
DNA_14	DCon15		336	D-336 Control	24/03/2017	-0,45	-0,009	7,97	-0,8	
DNA_15	DA463	7582	337	D-337	27/03/2017	5,67	0,113	1,76	0,3	Low correlation
DNA_15	DA614	7715	338	D-338	27/03/2017	706,15	14,123	2,04	1,84	
DNA_15	DA0072	7555	339	D-339	27/03/2017	149,02	2,98	2,05	1,04	
DNA_15	DA0013	7056	340	D-340	27/03/2017	102,86	2,057	1,98	1	
DNA_15	DA0012	7554	341	D-341	27/03/2017	48,54	0,971	2,01	0,94	
DNA_15	TYD894	9062	342	D-342	27/03/2017	213,28	4,266	2,02	0,89	
DNA_15	DA0192	7215	343	D-343	27/03/2017	58,91	1,178	1,97	1,07	
DNA_15	TYD850	9058	344	D-344	27/03/2017	356,92	7,138	2,07	1,94	
DNA_15	DA0104	7228	345	D-345	27/03/2017	351,28	7,026	2,05	2,08	
DNA_15	TYD955	1253	346	D-346	27/03/2017	339,76	6,795	2,07	2,13	
DNA_15	DA541	1809	347	D-347	27/03/2017	33,25	0,665	1,99	0,41	
DNA_15	DA557	1144	348	D-348	27/03/2017	69,38	1,388	2,01	0,9	
DNA_15	DA456	7612	349	D-349	27/03/2017	296,95	5,939	1,99	1,78	
DNA_15	DA0166	1219	350	D-350	27/03/2017	225,36	4,507	2,02	2,02	
DNA_15	DA505	1407	351	D-351	27/03/2017	132,68	2,654	2,07	2,03	
DNA_15	DA0212	7151	352	D-352	27/03/2017	340,23	6,805	2,04	2,11	
DNA_15	DA442	7504	353	D-353	27/03/2017	49	0,98	2,04	1,87	
DNA_15	DA403	7111	354	D-354	27/03/2017	967,3	19,346	2,08	2,1	
DNA_15	TYD753	1494	355	D-355	27/03/2017	549,76	10,995	2,04	2,2	
DNA_15	DA564	7442	356	D-356	27/03/2017	103,11	2,062	1,94	1,74	
DNA_15	DA671	7305	357	D-357	27/03/2017	8,11	0,162	2,15	0,56	Correlation ok
DNA_15	DA482	7502	358	D-358	27/03/2017	249,54	4,991	2	1,9	
DNA_15	DA552	7655	359	D-359	27/03/2017	90,3	1,806	2,04	1,49	
DNA_15	DCon16		360	D-360 Control	27/03/2017	0,01	0	0,03	0,04	

DNA_16	TYD878	9097	361	D-361	28/03/2017	485,6	9,712	2,01	2,33	
DNA_16	DA0188	7166	362	D-362	28/03/2017	1734,46	34,689	2,01	2,26	
DNA_16	DA0149	7190	363	D-363	28/03/2017	320,43	6,409	2,09	1,73	
DNA_16	TYD889	1509	364	D-364	28/03/2017	231,29	4,626	2,04	2,2	
DNA_16	TYD830	1382	365	D-365	28/03/2017	69,26	1,385	2	1,53	
DNA_16	DA0123	7366	366	D-366	28/03/2017	78,16	1,563	1,93	4,33	
DNA_16	DA383	7223	367	D-367	28/03/2017	708,48	14,17	2	2,35	
DNA_16	DA283	1415	368	D-368	28/03/2017	1208,23	24,165	2,01	2,05	
DNA_16	DA0001	7041	369	D-369	28/03/2017	1733,67	34,673	2,02	2,39	Very little material
DNA_16	DA0174	7165	370	D-370	28/03/2017	154,79	3,096	2,06	2,86	
DNA_16	TYD826	1143	371	D-371	28/03/2017	157,53	3,151	2,07	4,38	
DNA_16	DA616	7438	372	D-372	28/03/2017	979,38	19,588	1,99	2,17	
DNA_16	DA0247	7665	373	D-373	28/03/2017	235,01	4,7	2,06	3,18	
DNA_16	DA314	1563	374	D-374	28/03/2017	1079,11	21,582	2,04	2,42	
DNA_16	DA736	1360	375	D-375	28/03/2017	760,56	15,211	2,04	2,54	
DNA_16	TYD787	1318	376	D-376	28/03/2017	376,12	7,522	2,01	2,52	
DNA_16	TYD861	1135	377	D-377	28/03/2017	1202,14	24,043	2,11	2,04	Thick sample, tube is totally full of stool
DNA_16	DA259	7719	378	D-378	28/03/2017	211,02	4,22	1,98	3,51	
DNA_16	DA264	7177	379	D-379	28/03/2017	946,02	18,92	1,98	2,01	
DNA_16	TYD881	1303	380	D-380	28/03/2017	111,6	2,232	2,08	6,64	
DNA_16	TYD998	7571	381	D-381	28/03/2017	114,64	2,293	2,11	9,17	
DNA_16	TYD800	7238	382	D-382	28/03/2017	155,39	3,108	1,99	2,57	
DNA_16	TYD933	1686	383	D-383	28/03/2017	280,04	5,601	2,09	3,33	
DNA_16	DCon17		384	D-384 Control	28/03/2017	0,83	0,017	1,16	0,29	
DNA_17	DA598	7286	385	D-385	29/03/2017	252,92	5,058	2,03	2,11	
DNA_17	DA489	1060	386	D-386	29/03/2017	737,78	14,756	2,06	2,03	
DNA_17	TYD868	9098	387	D-387	29/03/2017	105,99	2,12	1,98	2,4	
DNA_17	DA524	1055	388	D-388	29/03/2017	174,59	3,492	1,99	1,18	
DNA_17	DA0055	7053	389	D-389	29/03/2017	51,58	1,032	1,96	1,57	
DNA_17	TYD884	1517	390	D-390	29/03/2017	632,69	12,654	2,01	1,88	
DNA_17	DA0236	7015	391	D-391	29/03/2017	593,21	11,864	2	1,82	
DNA_17	DA531	1278	392	D-392	29/03/2017	15,1	0,302	1,98	0,83	Correlation ok
DNA_17	TYD934	1547	393	D-393	29/03/2017	292,26	5,845	1,95	1,42	
DNA_17	DA430	7547	394	D-394	29/03/2017	219,79	4,396	1,97	2,33	
DNA_17	TYD768	1767	395	D-395	29/03/2017	340,17	6,803	1,97	1,95	
DNA_17	DA601	1701	396	D-396	29/03/2017	44,88	0,898	2,01	1,15	
DNA_17	DA654	7649	397	D-397	29/03/2017	91,16	1,823	1,96	2,01	
DNA_17	DA0141	7031	398	D-398	29/03/2017	20,14	0,403	1,96	0,34	Correlation ok
DNA_17	DA679	7684	399	D-399	29/03/2017	345,09	6,902	1,99	2,26	
DNA_17	TYD989	9066	400	D-400	29/03/2017	46,93	0,939	2	1,76	
DNA_17	TYD817	1584	401	D-401	29/03/2017	335,23	6,705	1,98	2,26	
DNA_17	TYD941	1346	402	D-402	29/03/2017	85,27	1,705	2,02	2,42	
DNA_17	DA0113	7364	403	D-403	29/03/2017	57,6	1,152	1,99	3,25	
DNA_17	DA0110	7481	404	D-404	29/03/2017	51,44	1,029	2,03	0,48	
DNA_17	DA637	9013	405	D-405	29/03/2017	196,53	3,931	2,03	0,83	
DNA_17	DA0080	7298	406	D-406	29/03/2017	737,01	14,74	2,05	0,49	Tube is totally full of stool
DNA_17	DA510	1188	407	D-407	29/03/2017	41,35	0,827	2	0,77	
DNA_17	DCon18		408	D-408 Control	29/03/2017	-0,42	-0,008	2,53	4,21	
DNA_18	DA0037	7291	409	D-409	30/03/2017	139,18	2,784	2,04	1,44	
DNA_18	DA0129	7354	410	D-410	30/03/2017	1179,81	23,596	2,01	2,23	
DNA_18	DA424	7625	411	D-411	30/03/2017	458,48	9,17	2,06	2,4	
DNA_18	DA725	1262	412	D-412	30/03/2017	60	1,2	2,06	21,43	
DNA_18	DA415	7426	413	D-413	30/03/2017	623,49	12,47	2,05	2,3	
DNA_18	TYD840	1655	414	D-414	30/03/2017	559,8	11,196	1,99	2,39	
DNA_18	DA320	7408	415	D-415	30/03/2017	381,37	7,627	2,05	2,42	
DNA_18	DA0082	7701	416	D-416	30/03/2017	589,97	11,799	2,01	2,46	
DNA_18	TYD851	1331	417	D-417	30/03/2017	307,08	6,142	2,07	2,17	
DNA_18	DA513	1414	418	D-418	30/03/2017	204,35	4,087	2,08	3,5	
DNA_18	DA652	1185	419	D-419	30/03/2017	1114,41	22,288	2,06	2,34	Thick sample
DNA_18	DA334	7221	420	D-420	30/03/2017	90,14	1,803	2,03	2,3	
DNA_18	DA528	1304	421	D-421	30/03/2017	1059,37	21,187	2	2,16	
DNA_18	DA591	9060	422	D-422	25/04/2017	124,84	2,497	1,93	1,23	So thick sample
DNA_18	DA0197	7022	423	D-423	30/03/2017	1112,14	22,243	2,04	2,45	
DNA_18	DA0159	7310	424	D-424	30/03/2017	817,73	16,355	2,09	1,93	
DNA_18	DA0232	7301	425	D-425	30/03/2017	178,64	3,573	2,09	3,19	
DNA_18	DA0240	7183	426	D-426	30/03/2017	49,26	0,985	2,22	4,44	
DNA_18	TYD849	1431	427	D-427	30/03/2017	725,94	14,519	2,05	1,86	
DNA_18	DA427	7519	428	D-428	30/03/2017	275,74	5,515	2,09	1,99	
DNA_18	TYD857	1351	429	D-429	30/03/2017	1086,11	21,722	2,02	2,25	very little material
DNA_18	DA0057	7159	430	D-430	30/03/2017	584,72	11,694	1,97	2,17	
DNA_18	TYD867	7343	431	D-431	30/03/2017	96,26	1,925	2,06	5,97	
DNA_18	DCon19		432	D-432 Control	30/03/2017	-0,32	-0,006	0,28	0,74	

DNA_19	DA319	1611	433	D-433	31/03/2017	117,63	2,353	2,04	1,45	
DNA_19	DA0241	7311	434	D-434	31/03/2017	496,77	9,935	1,98	2,1	
DNA_19	DA0101	7232	435	D-435	31/03/2017	78,78	1,576	2,07	1,1	
DNA_19	DA0005	7209	436	D-436	31/03/2017	1067,49	21,35	2,02	1,79	
DNA_19	DA629	1329	437	D-437	31/03/2017	232,39	4,648	2,06	2,05	
DNA_19	DA0093	1166	438	D-438	31/03/2017	143,82	2,876	2,08	1,87	
DNA_19	DA0010	7697	439	D-439	31/03/2017	38,09	0,762	2,01	1,23	
DNA_19	DA323	7063	440	D-440	31/03/2017	151,28	3,026	1,98	2,22	
DNA_19	TYD887	1631	441	D-441	31/03/2017	146,5	2,93	2,05	1,88	
DNA_19	DA0115	9001	442	D-442	31/03/2017	691,7	13,834	2,03	2,13	
DNA_19	DA579	7575	443	D-443	31/03/2017	475,19	9,504	2,03	2,29	
DNA_19	DA0074	7663	444	D-444	31/03/2017	467,13	9,343	2,02	2,29	
DNA_19	DA705	7229	445	D-445	31/03/2017	252,79	5,056	2,05	2,34	
DNA_19	DA737	7499	446	D-446	31/03/2017	169,1	3,382	2,02	2,26	
DNA_19	DA300	7325	447	D-447	31/03/2017	58,23	1,165	2	1,71	
DNA_19	DA287	7429	448	D-448	31/03/2017	264,54	5,291	2,05	1,62	
DNA_19	DA621	7381	449	D-449	31/03/2017	19,35	0,387	2,11	-2,11	Correlation ok
DNA_19	DA359	7212	450	D-450	31/03/2017	160,44	3,209	2	2,07	
DNA_19	TYD948	1171	451	D-451	31/03/2017	740,18	14,804	2,01	2,33	
DNA_19	DA0206	1167	452	D-452	31/03/2017	13,25	0,265	2	-4,79	Correlation ok
DNA_19	DA0006	7189	453	D-453	31/03/2017	572,28	11,446	2,02	2,18	
DNA_19	TYD979	1508	454	D-454	31/03/2017	347,56	6,951	2,02	2,45	
DNA_19	DA0052	9003	455	D-455	31/03/2017	58,23	1,165	2,17	3,79	
DNA_19	DCon20		456	D-456 Control	31/03/2017	0,13	0,003	0,51	0,12	
DNA_20	DA263	7345	457	D-457	03/04/2017	299,77	5,995	2,04	2,44	
DNA_20	DA708	1056	458	D-458	03/04/2017	25,96	0,519	2,05	5,87	
DNA_20	DA402	7266	459	D-459	03/04/2017	169,18	3,384	1,96	3,21	
DNA_20	DA431	7167	460	D-460	03/04/2017	502,36	10,047	2,02	1,78	
DNA_20	TYD776	7042	461	D-461	03/04/2017	120,25	2,405	2,04	6,37	
DNA_20	DA0143	7178	462	D-462	03/04/2017	222	4,44	1,94	3,35	
DNA_20	DA0154	7456	463	D-463	03/04/2017	231,73	4,635	1,99	3	
DNA_20	DA553	1556	464	D-464	03/04/2017	528,53	10,571	1,99	2,52	
DNA_20	TYD892	7267	465	D-465	03/04/2017	65,7	1,314	2,06	27,11	
DNA_20	DA665	1676	466	D-466	03/04/2017	8,72	0,174	2,35	-0,35	High correltaion
DNA_20	DA0035	7217	467	D-467	03/04/2017	43,34	0,867	2,07	-2,52	
DNA_20	DA0007	7363	468	D-468	03/04/2017	66,6	1,332	1,97	1,93	
DNA_20	DA632	1065	469	D-469	03/04/2017	1091,97	21,839	2,04	2,45	
DNA_20	TYD794	1193	470	D-470	03/04/2017	229,03	4,581	2,03	3,41	
DNA_20	DA253	7051	471	D-471	03/04/2017	158,94	3,179	2	4,13	
DNA_20	DA301	7392	472	D-472	03/04/2017	1020,19	20,404	2,03	1,38	
DNA_20	DA580	1170	473	D-473	03/04/2017	788,64	15,773	2,03	1,53	
DNA_20	DA0108	1534	474	D-474	03/04/2017	101,31	2,026	2,13	0,82	
DNA_20	DA0022	7120	475	D-475	03/04/2017	854,65	17,093	2,01	1,84	Tube full of stool
DNA_20	DA683	1670	476	D-476	03/04/2017	657,86	13,157	2,09	2,12	
DNA_20	DA642	1719	477	D-477	03/04/2017	323,9	6,478	2,02	3,05	
DNA_20	TYD903	1422	478	D-478	03/04/2017	83,55	1,671	2,07	-78,72	
DNA_20	DA562	1192	479	D-479	03/04/2017	257,1	5,142	2,11	3,04	
DNA_20	DCon21		480	D-480 Control	03/04/2017	0,06	0,001	0,12	0,04	
DNA_21	DA0078	7003	481	D-481	04/04/2017	154,39	3,088	2,08	1,21	
DNA_21	DA704	1277	482	D-482	04/04/2017	648,11	12,962	2,04	1,38	
DNA_21	TYD795	1792	483	D-483	04/04/2017	1103,75	22,075	2,04	2,1	
DNA_21	DA0138	7428	484	D-484	04/04/2017	467,73	9,355	2,03	2,18	
DNA_21	TYD997	1543	485	D-485	04/04/2017	138,76	2,775	2,04	1,9	
DNA_21	DA0250	7440	486	D-486	04/04/2017	327,53	6,551	1,99	0,65	
DNA_21	DA585	7535	487	D-487	04/04/2017	110,2	2,204	1,99	1,76	
DNA_21	DA0218	7127	488	D-488	04/04/2017	1230,89	24,618	2,04	2,05	
DNA_21	TYD848	1338	489	D-489	04/04/2017	304,79	6,096	2,09	2,09	
DNA_21	DA622	7585	490	D-490	04/04/2017	133,05	2,661	2,08	1,66	
DNA_21	TYD985	1650	491	D-491	04/04/2017	55,9	1,118	2	1,27	
DNA_21	TYD862	1548	492	D-492	04/04/2017	168,75	3,375	1,98	2,17	
DNA_21	TYD832	7579	493	D-493	04/04/2017	318,08	6,362	2,06	2,4	
DNA_21	DA0175	1168	494	D-494	04/04/2017	394,85	7,897	2,11	2,34	
DNA_21	DA0125	7084	495	D-495	04/04/2017	362,02	7,24	1,99	2,28	
DNA_21	DA0169	7652	496	D-496	04/04/2017	618,18	12,364	2,02	2,09	
DNA_21	DA0230	7451	497	D-497	04/04/2017	302,48	6,05	1,95	2,03	
DNA_21	DA600	7720	498	D-498	04/04/2017	222,07	4,441	1,99	1,55	
DNA_21	DA312	7493	499	D-499	04/04/2017	10,57	0,211	2,07	1,21	Correlation ok
DNA_21	TYD959	1598	500	D-500	04/04/2017	529,91	10,598	1,99	2,15	
DNA_21	TYD807	7435	501	D-501	04/04/2017	390,1	7,802	2,09	2,33	
DNA_21	DA662	1165	502	D-502	04/04/2017	496,39	9,928	2,05	2,06	
DNA_21	DA718	1688	503	D-503	04/04/2017	334,73	6,695	2,02	2,19	
DNA_21	DCon22		504	D-504	04/04/2017	0,49	0,01	0,59	0,76	

DNA_22	DA631	1256	505	D-505	05/04/2017	459,41	9,188	2,07	2,02	
DNA_22	TYD855	1561	506	D-506	05/04/2017	135,36	2,707	2,04	1,74	
DNA_22	DA0225	7362	507	D-507	05/04/2017	390,08	7,802	1,99	2,03	
DNA_22	DA655	1018	508	D-508	05/04/2017	196,14	3,923	2	2,01	
DNA_22	DA337	7711	509	D-509	05/04/2017	476,34	9,527	1,94	0,53	Very little material
DNA_22	DA677	1148	510	D-510	05/04/2017	151,54	3,031	2,04	1,41	
DNA_22	TYD782	1612	511	D-511	05/04/2017	729,6	14,592	1,97	2,16	Tube is totally full of stool
DNA_22	DA0094	7157	512	D-512	05/04/2017	164,28	3,286	1,99	2,03	
DNA_22	DA699	7271	513	D-513	05/04/2017	56,03	1,121	1,92	1,82	
DNA_22	DA700	1448	514	D-514	05/04/2017	28,51	0,57	1,94	0,84	
DNA_22	TYD806	1771	515	D-515	05/04/2017	848,26	16,965	2,05	2,29	
DNA_22	TYD949	1635	516	D-516	05/04/2017	223,56	4,471	2,09	2,06	
DNA_22	DA651	1264	517	D-517	05/04/2017	56,85	1,137	1,95	2,07	
DNA_22	DA318	7505	518	D-518	05/04/2017	953,2	19,064	2,07	2	
DNA_22	DA0047	7046	519	D-519	05/04/2017	1247,49	24,95	2,06	2,29	
DNA_22	DA693	7275	520	D-520	05/04/2017	947,1	18,942	2,04	2,02	
DNA_22	DA521	1478	521	D-521	05/04/2017	434,03	8,681	2,03	2,4	
DNA_22	TYD804	1595	522	D-522	05/04/2017	198,89	3,978	2,06	1,91	Only half of stabilizer, could be water? The protein tube looks like it has either DNA stabilizer or water in it!
DNA_22	DA0234	7387	523	D-523	05/04/2017	619,48	12,39	2,1	2,25	
DNA_22	DA0015	7055	524	D-524	05/04/2017	290,28	5,806	2,01	2,22	
DNA_22	TYD786	7048	525	D-525	05/04/2017	52,03	1,041	1,96	1,6	
DNA_22	DA719	1177	526	D-526	05/04/2017	534,63	10,693	1,94	2,07	
DNA_22	TYD769	1538	527	D-527	05/04/2017	70,54	1,411	1,99	1,41	
DNA_22	DCon23		528	D-528 Control	05/04/2017	0,34	0,007	0,79	0,35	
DNA_23	DA542	1158	529	D-529	07/04/2017	24,38	0,488	2,09	2,21	
DNA_23	DA556	1470	530	D-530	07/04/2017	459,92	9,198	2,08	-5,23	
DNA_23	DA0086	7010	531	D-531	07/04/2017	236,95	4,739	2,05	-2,31	
DNA_23	DA399	7490	532	D-532	07/04/2017	185,78	3,716	2,09	4,64	
DNA_23	DA678	1749	533	D-533	07/04/2017	175,39	3,508	2,05	-3,14	
DNA_23	DA451	1602	534	D-534	07/04/2017	373,63	7,473	2,07	-1,3	Tube didn't have stabilizer
DNA_23	DA0085	7567	535	D-535	07/04/2017	120,53	2,411	2,14	-1,28	
DNA_23	DA0003	7147	536	D-536	07/04/2017	83,75	1,675	2,02	-2,07	
DNA_23	TYD864	1469	537	D-537	07/04/2017	138,58	2,772	2,04	2,2	
DNA_23	DA639	1704	538	D-538	07/04/2017	469,4	9,388	2,08	5,11	
DNA_23	DA711	1700	539	D-539	07/04/2017	21,46	0,429	2,2	-1,02	
DNA_23	DA687	1479	540	D-540	07/04/2017	247,51	4,95	2,12	5,11	
DNA_23	DA0170	7543	541	D-541	07/04/2017	74,48	1,49	2,11	-46,31	
DNA_23	DA695	7693	542	D-542	07/04/2017	19,63	0,393	1,96	0,81	
DNA_23	TYD763	1220	543	D-543	07/04/2017	94,05	1,881	2,1	2,43	
DNA_23	TYD883	1441	544	D-544	07/04/2017	20,31	0,406	2,21	1,22	
DNA_23	DA492	7027	545	D-545	07/04/2017	146,36	2,927	2,04	2,31	
DNA_23	DA333	7034	546	D-546	07/04/2017	210,21	4,204	2,08	11,26	
DNA_23	DA412	1153	547	D-547	07/04/2017	153,85	3,077	2,1	3,52	
DNA_23	DA0087	7538	548	D-548	07/04/2017	140,31	2,806	2,12	-2,7	
DNA_23	DA689	1788	549	D-549	07/04/2017	235,78	4,716	2,06	2,19	
DNA_23	DA565	1714	550	D-550	07/04/2017	385,93	7,719	2,07	-8,97	Thick sample
DNA_23	DA0030	7059	551	D-551	07/04/2017	398,12	7,962	2,04	2,78	
DNA_23	DCon24		552	D-552 Control	07/04/2017	0,18	0,004	-0,32	0	
DNA_24	DA0150	7252	553	D-553	10/04/2017	336,97	6,739	2,01	2,2	
DNA_24	DA325	7603	554	D-554	10/04/2017	286,19	5,724	2,1	2,61	
DNA_24	DA555	1393	555	D-555	10/04/2017	490,87	9,817	1,96	2,23	
DNA_24	DA0198	7479	556	D-556	10/04/2017	60,78	1,216	2,04	4,41	
DNA_24	DA0032	7175	557	D-557	10/04/2017	212,7	4,254	2,03	2,02	
DNA_24	DA408	7489	558	D-558	10/04/2017	40,1	0,802	2,07	-9,61	
DNA_24	DA634	7633	559	D-559	10/04/2017	111,72	2,234	1,95	2,89	
DNA_24	DA0031	7009	560	D-560	10/04/2017	94,23	1,885	2,03	2,98	
DNA_24	DA285	7398	561	D-561	10/04/2017	639,07	12,781	2,02	2,46	
DNA_24	DA0135	7149	562	D-562	10/04/2017	670,9	13,418	2,06	2,4	Thick sample
DNA_24	DA0189	7133	563	D-563	10/04/2017	759,94	15,199	2,04	2,28	
DNA_24	TYD842	1194	564	D-564	10/04/2017	128,46	2,569	2,04	3,04	
DNA_24	DA0183	7172	565	D-565	10/04/2017	574,5	11,49	1,98	1,68	
DNA_24	DA633	1491	566	D-566	10/04/2017	88,83	1,777	2,04	1,18	
DNA_24	DA373	7011	567	D-567	10/04/2017	791,6	15,832	2,04	2,04	
DNA_24	DA494	7705	568	D-568	10/04/2017	88,43	1,769	2,04	1,37	
DNA_24	DA348	7448	569	D-569	10/04/2017	797,8	15,956	2	1,92	
DNA_24	DA525	1412	570	D-570	10/04/2017	51,75	1,035	1,99	4,57	
DNA_24	DA491	7393	571	D-571	10/04/2017	1025,04	20,501	2,02	1,7	
DNA_24	DA0164	7589	572	D-572	10/04/2017	479,23	9,585	2,05	1,67	
DNA_24	TYD999	9093	573	D-573	10/04/2017	74,58	1,492	2	1,1	
DNA_24	TYD956	1754	574	D-574	10/04/2017	112,78	2,256	2,03	1,95	
DNA_24	DA0136	7595	575	D-575	10/04/2017	123,58	2,472	1,98	3,32	
DNA_24	DCon25		576	D-576 Control	10/04/2017	-0,19	-0,004	-0,16	-0,25	

DNA_25	DA640	1042	577	D-577	11/04/2017	257,27	5,145	2,12	2,19	
DNA_25	DA688	1115	578	D-578	11/04/2017	252,07	5,041	2,07	1,98	
DNA_25	DA0196	7020	579	D-579	11/04/2017	752,56	15,051	2,04	1,72	Thick sample
DNA_25	DA548	1317	580	D-580	11/04/2017	893,57	17,871	2,01	2,21	
DNA_25	DA529	1486	581	D-581	11/04/2017	302,53	6,051	1,98	1,86	
DNA_25	DA0167	7269	582	D-582	11/04/2017	2,76	0,055	1,67	0,19	Low correlation
DNA_25	DA0213	7581	583	D-583	11/04/2017	209,45	4,189	2,11	2,45	
DNA_25	TYD943	1399	584	D-584	11/04/2017	378,07	7,561	2,03	1,72	
DNA_25	DA532	1715	585	D-585	11/04/2017	1111,45	22,229	2,03	1,92	
DNA_25	DA338	7347	586	D-586	11/04/2017	437,25	8,745	1,98	2,07	
DNA_25	DA0157	7721	587	D-587	11/04/2017	427,85	8,557	2,01	2,06	
DNA_25	DA543	7452	588	D-588	11/04/2017	45,73	0,915	2,01	0,98	
DNA_25	DA273	7621	589	D-589	11/04/2017	209,51	4,19	1,87	0,44	Thick sample
DNA_25	TYD828	1365	590	D-590	11/04/2017	534,14	10,683	2,08	2,36	
DNA_25	DA653	1488	591	D-591	11/04/2017	176,66	3,533	1,92	1,8	
DNA_25	DA0142	7283	592	D-592	11/04/2017	480,93	9,619	2,05	2,21	
DNA_25	DA506	1200	593	D-593	11/04/2017	60,39	1,208	2,07	1,95	
DNA_25	DA0060	7103	594	D-594	11/04/2017	96,04	1,921	2,01	2,16	
DNA_25	DA366	1589	595	D-595	11/04/2017	213,3	4,266	2,04	1,99	
DNA_25	DA570	1440	596	D-596	11/04/2017	1794,74	35,895	2,06	2,14	
DNA_25	TYD825	1140	597	D-597	11/04/2017	187,26	3,745	2,01	1,99	
DNA_25	DA0223	7616	598	D-598	11/04/2017	445,82	8,916	2	2,3	
DNA_25	DA551	1069	599	D-599	11/04/2017	270,75	5,415	2,04	2,3	
DNA_25	DCon26		600	D-600 Control	11/04/2017	-0,24	-0,005	0,49	-0,52	
DNA_26	DA343	7516	601	D-601	12/04/2017	123,97	2,479	2	0,85	
DNA_26	DA713	7539	602	D-602	12/04/2017	828,89	16,578	2,01	2,07	
DNA_26	DA372	7390	603	D-603	12/04/2017	1386,7	27,734	2,03	2,1	
DNA_26	DA682	7453	604	D-604	12/04/2017	262,7	5,254	2,06	1,83	
DNA_26	DA0002	7134	605	D-605	12/04/2017	193,64	3,873	2,05	2	
DNA_26	DA0145	7075	606	D-606	12/04/2017	937,87	18,757	2,03	1,55	
DNA_26	DA685	1205	607	D-607	12/04/2017	47,32	0,946	2	0,77	
DNA_26	TYD901	1433	608	D-608	12/04/2017	89,79	1,796	2,05	1,55	
DNA_26	DA0239	7361	609	D-609	12/04/2017	80,79	1,616	1,97	2,03	
DNA_26	DA560	1647	610	D-610	12/04/2017	295,39	5,908	2,07	1,95	
DNA_26	DA549	1709	611	D-611	12/04/2017	742,7	14,854	2,07	2,21	
DNA_26	TYD821	1330	612	D-612	12/04/2017	312,19	6,244	1,97	1,98	
DNA_26	TYD957	1350	613	D-613	12/04/2017	567,18	11,344	1,93	2,16	
DNA_26	DA0244	7019	614	D-614	12/04/2017	66,69	1,334	2,04	2,43	
DNA_26	DA572	7140	615	D-615	12/04/2017	908,56	18,171	2,04	2,17	
DNA_26	DA275	7604	616	D-616	12/04/2017	93,95	1,879	2,02	2	
DNA_26	DA0081	7206	617	D-617	12/04/2017	247,03	4,941	2,05	1,88	
DNA_26	TYD772	7676	618	D-618	12/04/2017	8,96	0,179	1,87	0,25	Low correlation
DNA_26	TYD846	1687	619	D-619	12/04/2017	52,46	1,049	2,03	0,71	
DNA_26	TYD847	7666	620	D-620	12/04/2017	220,17	4,403	2,1	2,14	
DNA_26	DA581	1560	621	D-621	12/04/2017	690,48	13,81	2,05	2,17	
DNA_26	DA447	7080	622	D-622	12/04/2017	35,02	0,7	1,93	2,1	
DNA_26	DA396	7008	623	D-623	12/04/2017	227,78	4,556	2,05	2,22	
DNA_26	DCon27		624	D-624 Control	12/04/2017	0,2	0,004	0,28	0,36	
DNA_27	TYD815	7723	625	D-625	13/04/2017	252,7	5,054	2,04	2,36	
DNA_27	DA694	7691	626	D-626	13/04/2017	95,13	1,903	2,01	2,26	
DNA_27	DA480	7484	627	D-627	13/04/2017	98,27	1,965	2,12	2,04	
DNA_27	DA727	1265	628	D-628	13/04/2017	110,92	2,218	2,02	1,89	
DNA_27	DA277	7339	629	D-629	13/04/2017	571,96	11,439	1,97	2,22	
DNA_27	DA0146	7164	630	D-630	13/04/2017	341,55	6,831	2	2,18	
DNA_27	DA701	7704	631	D-631	13/04/2017	297,56	5,951	2	2,05	
DNA_27	TYD756	9061	632	D-632	13/04/2017	597,07	11,941	1,96	1,83	
DNA_27	DA0224	7119	633	D-633	13/04/2017	446,12	8,922	2,06	1,53	
DNA_27	DA509	1212	634	D-634	13/04/2017	483,54	9,671	2,03	2,07	
DNA_27	DA0011	7049	635	D-635	13/04/2017	332,28	6,646	2,07	1,97	
DNA_27	DA739	1066	636	D-636	13/04/2017	89,44	1,789	1,95	1,29	
DNA_27	TYD827	1438	637	D-637	13/04/2017	394,51	7,89	2,01	1,86	
DNA_27	DA0018	7330	638	D-638	13/04/2017	85,72	1,714	1,95	0,77	
DNA_27	DA0222	1766	639	D-639	13/04/2017	261,8	5,236	2,03	2,23	
DNA_27	DA554	1216	640	D-640	13/04/2017	591,07	11,821	2,01	2,02	
DNA_27	DA379	7128	641	D-641	13/04/2017	841,43	16,829	1,99	2,26	
DNA_27	TYD967	7651	642	D-642	13/04/2017	365,13	7,303	2,02	2,23	
DNA_27	DA252	7629	643	D-643	13/04/2017	289,04	5,781	2,12	2,17	
DNA_27	DA406	7203	644	D-644	13/04/2017	530,07	10,601	2,04	1,82	Tube is totally full of stool
DNA_27	DA478	7372	645	D-645	13/04/2017	77,94	1,559	1,95	1,87	
DNA_27	DA0067	7466	646	D-646	13/04/2017	101,35	2,027	2,02	2,27	
DNA_27	DA0126	7580	647	D-647	13/04/2017	239,36	4,787	1,99	2,11	
DNA_27	DCon28		648	D-648 Control	13/04/2017	0,05	0,001	-3,53	0,06	
DNA_28	DA355	9006	649	D-649	18/04/2017	76,8	1,536	1,98	1,44	
DNA_28	DA389	1580	650	D-650	18/04/2017	223,07	4,461	1,96	1,74	
DNA_28	DA0121	7634	651	D-651	18/04/2017	315,44	6,309	1,99	1,37	
DNA_28	TYD778	7313	652	D-652	18/04/2017	65,17	1,303	1,96	1,79	
DNA_28	DA0088	7482	653	D-653	18/04/2017	555,48	11,11	2	1,95	
DNA_28	DA270	7402	654	D-654	18/04/2017	1004,56	20,091	2,07	2,09	
DNA_28	-	9075	655	D-655	18/04/2017	373,85	7,477	2,01	2,04	Missing barcode
DNA_28	DA299	7244	656	D-656	18/04/2017	474,7	9,494	2	2,02	Tube is totally full of stool and leaking
DNA_28	DA594	1772	657	D-657	18/04/2017	184,64	3,693	2,04	1,99	
DNA_28	DA407	7615	658	D-658	18/04/2017	45,91	0,918	2,01	0,82	
DNA_28	DA533	7407	659	D-659	18/04/2017	49,21	0,984	1,97	2,74	
DNA_28	TYD880	1444	660	D-660	18/04/2017	396,79	7,936	2,05	2,15	
DNA_28	DA280	7327	661	D-661	18/04/2017	382,27	7,645	1,98	1,52	

DNA_28	TYD835	1610	662	D-662	18/04/2017	908,24	18,165	2	1,99	
DNA_28	TYD981	1663	663	D-663	18/04/2017	110,24	2,205	2,06	1,98	
DNA_28	DA0178	7090	664	D-664	18/04/2017	137,43	2,749	2,09	1,6	
DNA_28	DA0204	1083	665	D-665	18/04/2017	860,89	17,218	2,07	1,93	Tube is full of stool
DNA_28	DA515	1163	666	D-666	18/04/2017	307,87	6,157	2,08	2,14	
DNA_28	DA0098	9068	667	D-667	18/04/2017	406,45	8,129	1,95	1,23	
DNA_28	TYD990	7295	668	D-668	18/04/2017	117,93	2,359	2,05	2,16	
DNA_28	DA0147	7057	669	D-669	18/04/2017	62,54	1,251	2	1,63	
DNA_28	DA0173	7182	670	D-670	18/04/2017	272,52	5,45	1,96	1,5	
DNA_28	DA370	7040	671	D-671	18/04/2017	191,87	3,837	1,97	1,53	
DNA_28	DCon29		672	D-672 Control	18/04/2017	0,25	0,005	-1,19	0,22	
DNA_29	DA0124	7403	673	D-673	19/04/2017	272,22	5,444	2,05	1,97	
DNA_29	DA741	1703	674	D-674	19/04/2017	162,4	3,248	1,98	1,88	
DNA_29	DA288	7319	675	D-675	19/04/2017	434,91	8,698	2,03	1,93	
DNA_29	TYD965	1151	676	D-676	19/04/2017	79,51	1,59	2,06	2,98	
DNA_29	DA443	7132	677	D-677	19/04/2017	718,87	14,377	2,05	2,06	
DNA_29	DA472	7262	678	D-678	19/04/2017	392,45	7,849	2,09	2,07	
DNA_29	DA426	7404	679	D-679	19/04/2017	134,69	2,694	2,06	2,17	
DNA_29	DA520	7672	680	D-680	19/04/2017	17,68	0,354	1,94	16,36	Correlation ok
DNA_29	DA0066	7370	681	D-681	19/04/2017	173,03	3,461	1,9	1,69	Tube may not have stabilizer
DNA_29	DA279	7375	682	D-682	19/04/2017	542,59	10,852	2,06	2,26	
DNA_29	TYD964	1458	683	D-683	19/04/2017	1292,79	25,856	2,02	2,05	
DNA_29	DA0132	7413	684	D-684	19/04/2017	564,52	11,29	1,97	1,97	
DNA_29	DA0245	7230	685	D-685	19/04/2017	545,84	10,917	1,94	1,98	Tube is full of stool
DNA_29	DA0161	7102	686	D-686	19/04/2017	55,77	1,115	2,02	1	
DNA_29	DA0089	7218	687	D-687	19/04/2017	93,57	1,871	1,99	1,95	
DNA_29	TYD798	1778	688	D-688	19/04/2017	563,26	11,265	2,01	2,3	
DNA_29	TYD843	1768	689	D-689	19/04/2017	89,47	1,789	2,02	2,25	
DNA_29	DA518	1747	690	D-690	19/04/2017	403,77	8,075	2,09	2,25	
DNA_29	TYD891	1236	691	D-691	19/04/2017	74,6	1,492	1,98	3,39	
DNA_29	DA0139	7208	692	D-692	19/04/2017	10,84	0,217	2,1	1,12	Correlation ok
DNA_29	TYD969	7437	693	D-693	19/04/2017	99,3	1,986	2,05	2,07	
DNA_29	TYD940	1273	694	D-694	19/04/2017	138,54	2,771	2,08	1,73	
DNA_29	DA686	9084	695	D-695	19/04/2017	304,1	6,082	2,06	1,58	
DNA_29	DCon30		696	D-696 Control	19/04/2017	0,16	0,003	-4,53	0,12	
DNA_30	DA0039	7078	697	D-697	20/04/2017	87,25	1,745	1,89	5,02	
DNA_30	TYD951	7326	698	D-698	20/04/2017	404,31	8,086	2,07	1,75	
DNA_30	DA657	7406	699	D-699	20/04/2017	290,91	5,818	2,06	2,29	
DNA_30	DA268	7384	700	D-700	20/04/2017	215,32	4,306	2,02	3,94	
DNA_30	DA390	7614	701	D-701	20/04/2017	341,33	6,827	2,03	2,46	
DNA_30	DA0210	7045	702	D-702	20/04/2017	1117,76	22,355	2,05	2,3	
DNA_30	DA0237	7028	703	D-703	20/04/2017	854,96	17,099	1,98	2,43	Tube is full of stool
DNA_30	DA545	7272	704	D-704	20/04/2017	258,3	5,166	2,04	2,23	
DNA_30	DA469	7211	705	D-705	20/04/2017	379,19	7,584	2,08	2,57	
DNA_30	DA643	1227	706	D-706	20/04/2017	1341,18	26,824	2,04	1,76	Tube full of stool, may not have a stabilizer
DNA_30	DA0133	7349	707	D-707	20/04/2017	127,23	2,545	1,99	12,5	
DNA_30	TYD836	1404	708	D-708	20/04/2017	561,78	11,236	2,07	1,93	May have very little DNA stabilizer
DNA_30	DA0043	7065	709	D-709	20/04/2017	888,4	17,768	2,01	1,9	
DNA_30	DA0004	7148	710	D-710	20/04/2017	116,25	2,325	2,02	3,13	
DNA_30	TYD939	7643	711	D-711	20/04/2017	364,25	7,285	1,99	2,65	
DNA_30	DA269	7309	712	D-712	20/04/2017	524,58	10,492	2,01	1,84	
DNA_30	DA342	7369	713	D-713	20/04/2017	229,82	4,596	2,05	3,61	
DNA_30	TYD970	1342	714	D-714	20/04/2017	1449,06	28,981	2,05	2,26	
DNA_30	DA271	7587	715	D-715	20/04/2017	80,62	1,612	2	11,63	
DNA_30	DA296	7169	716	D-716	20/04/2017	976,08	19,522	1,97	2,17	
DNA_30	DA386	7071	717	D-717	20/04/2017	904,79	18,096	2,01	2,08	
DNA_30	DA278	7668	718	D-718	20/04/2017	572,06	11,441	1,92	1,65	
DNA_30	TYD793	1301	719	D-719	20/04/2017	4,74	0,095	1,93	1,04	Correlation ok
DNA_30	DCon31		720	D-720 Control	20/04/2017	-0,17	-0,003	-6,66	-0,34	

DNA_31	TYD922	1691	721	D-721	21/04/2017	162,32	3,246	1,97	2,34	
DNA_31	DA0026	1221	722	D-722	21/04/2017	133,07	2,661	1,99	1,27	
DNA_31	DA526	7061	723	D-723	21/04/2017	49,54	0,991	1,93	2,91	
DNA_31	DA331	7565	724	D-724	21/04/2017	83,93	1,679	2,07	3,05	
DNA_31	DA335	7126	725	D-725	21/04/2017	142,77	2,855	2,09	2,75	
DNA_31	TYD823	1681	726	D-726	21/04/2017	216,83	4,337	2,01	1,68	Second DNA tube with barcode TYD789, same trend ID. Two DNA tubes sent by mistake. DNA Box number 33.
DNA_31	TYD931	1460	727	D-727	21/04/2017	291,25	5,825	2,06	1,66	
DNA_31	TYD932	1376	728	D-728	21/04/2017	284,99	5,7	2,04	2,59	
DNA_31	DA0017	9073	729	D-729	21/04/2017	194,89	3,898	1,98	2,22	
DNA_31	DA698	7367	730	D-730	21/04/2017	468,35	9,367	2,02	2,42	
DNA_31	TYD833	9096	731	D-731	21/04/2017	384,86	7,697	1,99	2,21	
DNA_31	DA596	1246	732	D-732	21/04/2017	1202,1	24,042	2,03	2,15	
DNA_31	DA661	7323	733	D-733	21/04/2017	136,75	2,735	1,88	2,54	
DNA_31	DA293	7572	734	D-734	21/04/2017	342,9	6,858	2	2,06	
DNA_31	DA0103	7139	735	D-735	21/04/2017	924,54	18,491	2,09	2,15	
DNA_31	DA487	9079	736	D-736	21/04/2017	82,59	1,652	1,98	2,91	
DNA_31	DA461	7181	737	D-737	21/04/2017	164,42	3,288	1,94	2,14	
DNA_31	TYD982	1092	738	D-738	21/04/2017	65,85	1,317	1,98	1,4	
DNA_31	DA392	7626	739	D-739	21/04/2017	358	7,16	2,07	2,18	
DNA_31	DA0216	7199	740	D-740	21/04/2017	140,75	2,815	1,93	2,62	
DNA_31	TYD946	1518	741	D-741	21/04/2017	230,3	4,606	2,06	2,5	
DNA_31	DA0049	7332	742	D-742	21/04/2017	671,45	13,429	2,03	1,87	
DNA_31	DA254	7328	743	D-743	21/04/2017	517,62	10,352	2,06	2,4	
DNA_31	DCon32		744	D-744 Control	21/04/2017	0,27	0,005	0,79	0,38	
DNA_32	DA501	1084	745	D-745	25/04/2017	495,87	9,917	1,9	1,5	Tube is totally full of stool
DNA_32	DA0165	7365	746	D-746	25/04/2017	430,5	8,61	2,03	2,39	
DNA_32	DA676	7285	747	D-747	25/04/2017	81,41	1,628	2,04	1,27	
DNA_32	TYD983	7573	748	D-748	25/04/2017	853,07	17,061	2,06	2,3	
DNA_32	DA385	7121	749	D-749	25/04/2017	730,12	14,602	1,98	2,23	
DNA_32	DA266	7645	750	D-750	25/04/2017	672,59	13,452	1,95	2,06	
DNA_32	TYD829	1195	751	D-751	25/04/2017	336,77	6,735	2,01	1,99	
DNA_32	TYD935	7549	752	D-752	25/04/2017	301,51	6,03	2	2,33	
DNA_32	DA0201	7185	753	D-753	25/04/2017	580,17	11,603	1,9	1,75	
DNA_32	DA429	7503	754	D-754	25/04/2017	180,28	3,606	1,98	2,42	
DNA_32	DA321	7294	755	D-755	25/04/2017	302,79	6,056	2,01	2,35	
DNA_32	DA628	7374	756	D-756	25/04/2017	97,15	1,943	2,15	1,32	
DNA_32	DA583	1230	757	D-757	25/04/2017	1112,7	22,254	2	2,16	
DNA_32	DA445	1048	758	D-758	25/04/2017	71,74	1,435	2,14	6,47	
DNA_32	DA291	7646	759	D-759	25/04/2017	395,76	7,915	1,93	2,39	
DNA_32	DA0131	7307	760	D-760	25/04/2017	1154,21	23,084	2,06	2,35	
DNA_32	DA272	7590	761	D-761	25/04/2017	470,08	9,402	2,08	2,09	
DNA_32	DA0203	1806	762	D-762	25/04/2017	780,78	15,616	2,04	2,29	Tube has little stool and DNA stabilizer
DNA_32	TYD1000	1615	763	D-763	25/04/2017	1229,75	24,595	2,02	2,12	
DNA_32	DA393	7035	764	D-764	25/04/2017	865,62	17,312	2,03	1,69	Thick sample
DNA_32	DA274	7138	765	D-765	25/04/2017	325,49	6,51	2,04	2,39	
DNA_32	TYD898	1449	766	D-766	25/04/2017	996,55	19,931	2,02	2,19	
DNA_32	DA720	1675	767	D-767	25/04/2017	93,39	1,868	2,08	3,34	
DNA_32	DCon33		768	D-768_b_Contr	25/04/2017	-1,02	-0,02	1	0,36	
DNA_33	TYD954	1341	769	D-769	25/04/2017	33,77	0,675	2,12	3,47	
DNA_33	DA0097	7179	770	D-770	25/04/2017	490,04	9,801	2,02	2,53	
DNA_33	DA0107	7068	771	D-771	25/04/2017	558,46	11,169	2	2,35	
DNA_33	TYD924	1779	772	D-772	25/04/2017	579,94	11,599	1,94	2,09	
DNA_33	DA0021	7474	773	D-773	25/04/2017	445,55	8,911	1,91	2,05	
DNA_33	DA0214	7640	774	D-774	25/04/2017	327,68	6,554	1,99	2,36	
DNA_33	TYD947	1010	775	D-775	25/04/2017	166,24	3,325	1,98	2,52	
DNA_33	DA563	1634	776	D-776	25/04/2017	198,33	3,967	2	2,31	
DNA_33	DA302	7085	777	D-777	25/04/2017	692,8	13,856	2,01	2,21	
DNA_33	TYB_D_789	1681	778	D-778	25/04/2017	17,24	0,345	2	-3,51	Second DNA tube with barcode TYB_D_823, same trend ID. Two DNA tubes sent by mistake. DNA box 31. Correlation ok.
DNA_33	Z_Ext_Std	Standard	779	D-779_Ext_Std	25/04/2017	9,33	0,187	2,17	-3,89	
DNA_33	DCon34		780	D-780 Control	25/04/2017	-0,55	-0,011	0,66	1,63	

Table 4. Low sample concentration results.

Box Number	Barcode	Trend ID	Purification number	Sample ID	Day	ng/µL	A260	260 / 280	260 / 230	NOTE
DNA_01	DA0219	7195	4	D-4	2-3.3.2017	0,61	0,012	-1,55	0,02	Low correlation
DNA_01	DA593	7108	9	D-9	2-3.3.2017	17,42	0,348	2,24	1,3	
DNA_02	DA684	1254	31	D-31	03/03/2017	11,95	0,239	2,32	0,15	High correlation
DNA_04	DA733	1282	91	D-91	08/03/2017	8,62	0,172	2,18	-0,69	
DNA_04	DA721	1820	93	D-93	08/03/2017	2,6	0,052	3,34	-0,05	High correlation
DNA_07	TYD980	1496	152	D-152	14/03/2017	3,5	0,07	1,93	-0,52	
DNA_08	DA0117	7478	188	D-188	15/03/2017	7,28	0,146	2,52	-1,01	High correlation
DNA_10	DA437	7385	218	D-218	17/03/2017	7,51	0,15	1,85	0,6	
DNA_10	DA641	7557	224	D-224	17/03/2017	10,52	0,21	2,28	-1,42	High correlation
DNA_11	DA281	7024	263	D-263	20/03/2017	17,82	0,356	0,18	1,98	Low correlation
DNA_14	TYD791	7110	315	D-315	24/03/2017	16,32	0,326	2,03	0,18	
DNA_14	DA0207	7312	323	D-323	24/03/2017	15,93	0,319	2,13	1,02	
DNA_14	DA728	1519	334	D-334	24/03/2017	11,25	0,225	2,2	-0,6	
DNA_15	DA671	7305	357	D-357	27/03/2017	8,11	0,162	2,15	0,56	
DNA_17	DA531	1278	392	D-392	29/03/2017	15,1	0,302	1,98	0,83	
DNA_17	DA0141	7031	398	D-398	29/03/2017	20,14	0,403	1,96	0,34	
DNA_19	DA621	7381	449	D-449	31/03/2017	19,35	0,387	2,11	-2,11	
DNA_19	DA0206	1167	452	D-452	31/03/2017	13,25	0,265	2	-4,79	
DNA_20	DA665	1676	466	D-466	03/04/2017	8,72	0,174	2,35	-0,35	High correlation
DNA_21	DA312	7493	499	D-499	04/04/2017	10,57	0,211	2,07	1,21	
DNA_25	DA0167	7269	582	D-582	11/04/2017	2,76	0,055	1,67	0,19	Low correlation
DNA_26	TYD772	7676	618	D-618	12/04/2017	8,96	0,179	1,87	0,25	
DNA_29	DA520	7672	680	D-680	19/04/2017	17,68	0,354	1,94	16,36	
DNA_29	DA0139	7208	692	D-692	19/04/2017	10,84	0,217	2,1	1,12	
DNA_30	TYD793	1301	719	D-719	20/04/2017	4,74	0,095	1,93	1,04	
DNA_33	TYB_D_789	1681	778	D-778	25/04/2017	17,24	0,345	2	-3,51	

Table 5. Blank concentration kit result. Batches 1-33. DCon2 – Dcon34.

Box Number	Barcode	Trend ID	Purification number	Sample ID	Day	ng/µL	A260	260 / 280	260 / 230
DNA_01	DCon2	Con2	24	D-24 Control	2-3.3.2017	1,16	0,023	-29,59	1,21
DNA_02	DCon3	Con3	48	D-48 Control	03/03/2017	0,1	0,002	0,5	-0,1
DNA_03	DCon4	Con4	72	D-72 Control	07/03/2017	-0,11	-0,002	0,12	0,09
DNA_04	DCon5	Con5	96	D-96 Control	08/03/2017	-0,62	-0,012	0,58	0,38
DNA_05	DCon6	Con6	120	D-120_Control	09/03/2017	0,78	0,016	-31,97	0,14
DNA_06	DCon7	Con7	144	D-144 Control	10/03/2017	-0,51	-0,01	1,03	0,53
DNA_07	DCon8	Con8	168	D-168 Control	14/03/2017	-0,11	-0,002	-2,4	1,04
DNA_08	DCon9	Con9	192	D-192 Control	15/03/2017	-0,13	-0,003	-0,006	0,42
DNA_09	DCon10	Con10	216	D-216 Control	16/03/2017	-0,62	-0,012	0,71	686,1
DNA_10	DCon11	Con11	240	D-240 Control	17/03/2017	1,46	0,029	1,15	-1,72
DNA_11	DCon12	Con12	264	D-264 Control	20/03/2017	1,58	0,032	0,03	1,04
DNA_12	DCon13	Con13	288	D-288 Control	22/03/2017	0,5	0,01	0,77	-4,14
DNA_13	DCon14	Con14	312	D-288 Control	22/03/2017	0,5	0,01	0,77	-4,14
DNA_14	DCon15	Con15	336	D-336 Control	24/03/2017	-0,45	-0,009	7,97	-0,8
DNA_15	DCon16	Con16	360	D-360 Control	27/03/2017	0,01	0	0,03	0,04
DNA_16	DCon17	Con17	384	D-384 Control	28/03/2017	0,83	0,017	1,16	0,29
DNA_17	DCon18	Con18	408	D-408 Control	29/03/2017	-0,42	-0,008	2,53	4,21
DNA_18	DCon19	Con19	432	D-432 Control	30/03/2017	-0,32	-0,006	0,28	0,74
DNA_19	DCon20	Con20	456	D-456 Control	31/03/2017	0,13	0,003	0,51	0,12
DNA_20	DCon21	Con21	480	D-480 Control	03/04/2017	0,06	0,001	0,12	0,04
DNA_21	DCon22	Con22	504	D-504 Control	04/04/2017	0,49	0,01	0,59	0,76
DNA_22	DCon23	Con23	528	D-528 Control	05/04/2017	0,34	0,007	0,79	0,35
DNA_23	DCon24	Con24	552	D-552 Control	07/04/2017	0,18	0,004	-0,32	0
DNA_24	DCon25	Con25	576	D-576 Control	10/04/2017	-0,19	-0,004	-0,16	-0,25
DNA_25	DCon26	Con26	600	D-600 Control	11/04/2017	-0,24	-0,005	0,49	-0,52
DNA_26	DCon27	Con27	624	D-624 Control	12/04/2017	0,2	0,004	0,28	0,36
DNA_27	DCon28	Con28	648	D-648 Control	13/04/2017	0,05	0,001	-3,53	0,06
DNA_28	DCon29	Con29	672	D-672 Control	18/04/2017	0,25	0,005	-1,19	0,22
DNA_29	DCon30	Con30	696	D-696 Control	19/04/2017	0,16	0,003	-4,53	0,12
DNA_30	DCon31	Con31	720	D-720 Control	20/04/2017	-0,17	-0,003	-6,66	-0,34
DNA_31	DCon32	Con32	744	D-744 Control	21/04/2017	0,27	0,005	0,79	0,38
DNA_32	DCon33	Con33	768	D-768 _b_Control	25/04/2017	-1,02	-0,02	1	0,36
DNA_33	DCon34	Con34	780	D-780 Control	25/04/2017	-0,55	-0,011	0,66	1,63

Table 6. ZymoBIOMICS Standard result.

Box Number	Barcode	Trend ID	Purification number	Sample ID	Day	ng/µL	A260	260 / 280	260 / 230
DNA_33	Z_Ext_Std	Standard	779	D-779_Ext_Std	25/04/2017	9,33	0,187	2,17	-3,89