CRANBERRY-LINGONBERRY JUICE EFFECT ON GUT AND URINARY TRACT MICROBIOME

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Abstract

Cranberry, *Vaccinium oxycoccos*, and lingonberry, *Vaccinium vitis-idaea*, have long been known to provide many health benefits as a source of nutrition. Similar to other berries, these traditional herbs contain flavonoids and vitamins, but also have a special benefit to prevent urinary tract infection (UTI). Previous studies have shown that A-type proanthocyanins (PAC) of cranberries may influence the adhesion of bacteria causing urinary tract infection. UTI pathogens originate from the gut and earlier studies have shown that there is a connection between urinary tract and gut microbiome UTI causing pathogens. However, the cranberry mechanism of action on the gut and urinary tract microbiome is not yet elucidated.

This study aimed to investigate the Cranberry-Lingonberry juice (CLJ) effect on the gut and urinary tract bacterial communities. The hypothesis assumed that the metabolism of proanthocyanidins in the gut alters bacterial communities and reduces the amount of *E. coli* and possibly other proteobacteria in the urine. The research was done by examining urine and fecal samples from children with urinary tract infection for three (urine) to twelve (fecal samples) months. The samples were collected by Oulu University Hospital Child Health and Maternity Clinic from 77 patients who drank CLJ or flavonoid-free control juice in randomized trial. Total of 206 samples, including 40 urine and 166 fecal samples, were collected for study.

DNA was extracted from samples using two different DNA extraction protocols of QIAGEN, USA and quantified using Nanodrop spectrophotometer. The bacterial 16S rRNA was amplified by using Polymerase chain reaction (PCR), which also attached unique barcodes for each sample. Agarose gel electrophoresis was used to ensure amplification of PCR. All amplified PCR products were prepared for sequencing by Ion Torrent next generation sequencing.

The QIIME 2 next-generation microbiome bioinformatics platform was used to analyze the sequence data and metadata information. Greengenes 16S rRNA, Silva gene databases and Human oral microbiome database (HOMD) were used as alignment reference databases. Metadata information about sample material and collection time was used for grouping. The alpha - and beta diversity, as well as differential abundances between treatments, were analyzed using QIIME 2 platform and R-statistical program. Compliance data was used to limit the data to patients who used more than 80 % probability of CLJ or control juice in the second round of statistical analysis.

Altogether 183 samples were amplified, of which 150 was fecal and 40 urine, for downstream analysis. From the samples, 18 different phyla and 511 genera were identified, most of them even at species level.

Statistical analysis showed no significant differences in alpha- or beta diversity between CLJ and controls in any groups. Different abundances between treatment groups were found, but in the end none of them were statistically significant. By using HOMD-database, *E. coli* and other UTI-related species were identified from compliance 80 % limited data. Statistical analyses showed a significant decrease of these bacteria in the urinary tract and gut microbiomes of CLJ group patients. In the future, chemical studies about microbial metabolism products could be done from the samples to get a more specific view about CLJ treatment effect on gut and microbial communities, and cranberry juice polyphenols effect on the body.

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Abbreviations

AC-PAC	A-type cranberries proanthocyanidins
ASV	Amplicon sequence variant
BH-FDR	Benjamini-Hochberg false discovery rate
CLJ	Cranberry-Lingonberry juice
CLR	Centered log ratio
cp	chloroplast
cpDNA	chloroplast DNA
control	flavonoid-free control juice
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
fw	fresh weight
HOMD	Human Oral Microbiome Database
ISP	ion sphere particle
NGS	Next-Generation sequencing
OTU	Operational Taxonomic Unit
PAC	Proanthocyanidins
PC	Principal component
PCR	Polymerase chain reaction
PERMANOVA	Permutational multivariate analysis of variance
QIIME2	Quantitative Insights Into Microbial Ecology 2
rRNA	Ribosomal ribonucleic acid
St.	The DNA molecular weight standard
TBE	Tris base, boric acid and EDTA
UTI	Urinary tract infection

1. Literature review

1.1. Urinary tract infection

Urinary tract infection (UTI) is a common term for all infections in any part of the urinary tract. It's one of the most common and studied infections in human. UTI symptoms are painful urination, pelvic pain and traces blood in the urine. Typically, women have greater risk of developing UTI. The urinary tract can be divided into upper region, containing kidneys and ureters, and lower tract, containing bladder and urethra. Infection can be caused by various species of bacteria or fungi that colonize urinary tract, however, the gram-negative bacteria *Escherichia coli* is the most common pathogen (Imirzalioglu et al, 2008).

UTI has been quite thoroughly studied with consistent results. For a long time, it has been a common belief that healthy urinary tract is sterile from bacteria. Recently this old-time belief has turned upside-down as studies have found natural and beneficial colonization of the urinary tract. 16S sequencing technique from healthy women's urine showed that it contains a rich microbial flora. A study was done with 65 patients (41 suffer overactive bladder) urine samples contained members of the genera *Lactobacillus, Corynebacterium, Streptococcus*, A*ctinomyces* and *Staphylococcus* (Hilt et al., 2014).

The study has showed that the UTI caused by *E. coli* correlated with gender and was clearly more common in women (Behzadi et al., 2010). *E. coli* and *Klebsiella pneumoniae* are the most common UTI causing bacteria in children under 5 years. The study included 153 patients, of whom 67 were girls (Garout et al., 2015).

Overall only 5-25 % of UTI is caused by another organism than *E. coli* and *K. pneumoniae*, mainly gram-positive bacteria such as *Staphylococcus saprophyticus, Enterococcus faecalis,* and *Streptococcus agalactiae*. Typically, the infection is treated with antibiotics, but studies have also shown that antibiotic resistance develops against UTI-causing bacteria. This has pushed studies to find alternative medications (Imirzalioglu et al, 2008).

1.2. Cranberry and lingonberry for prevention of urinary tract infection

Cranberry has long been old folk medicine for urinary tract infection. There have been different theories about mechanism of action. Cranberry makes urine more acidic, and typically this is believed to be reason. Acidic conditions are less favorable for UTI causing pathogens like *E. coli*.

First study to show cranberries affecting urine acidity was made in 1914 by Blatherwick, but observations about cranberries benzoic acid affect to decrease urine pH was made by German physicians in 1880s. Cranberries benzoic acid is combined with glycine in the body and is excreted in urine as hippuric acid (Blatterwick, 1919). After that studies on cranberry effect on decreasing urine pH have been done regularly, but none has showed that cranberries benzoic acid could decrease urine acidity enough to make it bacteriostatic. Even four liter of cranberry juice per day is not enough to produce needed amount of hippuric acid (Raz et al., 2004).

Nowadays researchers have a different theory. Cranberry phytochemical compounds have been noticed to affect adhesion of pathogenic bacteria in urinary tract. One theory suggests that compounds make urinary cell walls more slippery for bacteria to stick (Gupta K et al., 2007; Lavigne et al., 2008) and other theory thinks that the compounds change bacterial mechanisms of attaching to urinary tract. *E. coli* has hairlike fimbria on their surface which bacteria use to adhere the receptors on uroepithelial cells (Liu Y et al., 2008). When adhering to receptors, bacterial fimbriae produce two adhesions, one mannose sensitive and one mannose resistant. (Raz et al., 2004).

Cranberries antiadherent properties were first studied by Sobota et al. in 1984. They found out that cranberry juice reduced *E. coli* from clinical isolates from patients with UTI (Sobota et al. 1984). After that more studies by different approaches have been done to confirm the hypothesis. In 1989 two phytochemical components of cranberries, fructose and proanthocyanidins (PAC), were showed to produce antiadherent properties against *E. coli* adhesion. Fructose has been shown to inhibit the mannose sensitive, type I fimbriae, adhesion - and PACs mannose-resistant, p-fimbriae, adhesions (Zafriri et al., 1989). From those, fructose can be found from many food sources and its role seems more minimal. PACs are more special and are found in significant numbers in genus *Vaccinium* species (Raz et al., 2004).

Many clinical studies have subsequently been conducted in patients with different background to confirm this hypothesis. Typically, in clinical trials, cranberry effect to prevent urinary tract symptoms is studied in adult women, elderly or pediatric patients. Studies suggest that PACs prevent UTI in two ways. Compounds strictly prevent *E. coli* adhesion to uroepithelial cells. PACs adhere to *E. coli* preventing them from adhering cellular receptors. This makes room for more beneficial bacteria to colonize urinary tract microbiome (Raz et al., 2004).

Cranberry is also shown to affect other UTI causing pathogens. Earlier *in vitro* studies have shown that cranberry juice extract could inhibit biofilm formation and enzymatic activities of *E. faecelis* strains isolated from urine (Wojnicz, 2016). Similar *in vitro* studies have also been done for other UTI causing pathogens with positive results. Case studies have also been reported for decades and there is strong evidence on the beneficial effects of cranberries in treating UTI. PACs have also shown to be effective in cases where UTI is caused by antibiotic resistant uropathogenic bacteria (Howell et al., 2002).

Mixed Cranberry-Lingonberry juice (CLJ) has been shown to beneficially affect patients suffering from UTI. The juice reduces *E. coli* biofilm formation and virulence. However, there is little evidence of the detailed mechanism or effect of the juice. The juice effect was studied in twenty healthy patients who drank juice for two weeks. Virulence gene expression levels between controls and patients were analyzed by qPCR, but no significant changes were found, indicating that there must be other mechanisms in place (Tapiainen et al., 2012).

A-type proanthocyanins (PAC) of cranberries can inhibit invasion of extra-intestinal pathogenic *E. coli* in gut epithelial cells *in-vitro*. The concentration of > 36 μg PAC/ml reduced *E. coli* invasion significantly by cross-linking surface virulence factors. The mechanism was identified by scanning electron microscopy. *E. coli* is typically connected to UTI *(*Polewski, 2016). Effects of probiotics and cross-effect were also studied in the same research, and results showed that synergy between bioactive PACs and probiotics produced the best results. PACs worked with *Lactobacillus* probiotics, meaning that PACs affect only *E. coli* adhesion in this case. These results suggest that combinatory medication would be also suitable to treat UTI (Polewski, 2016).

Other studies also suggest that the best results of UTI treatment would be reached with a combination of probiotics selected from common vaginal inhabitants and A-type PACs from cranberry juice. For example, *Lactobacillus* spp. dominate the vaginal cavity microbiome of healthy women and could colonize the free space gained from pathogenic *E. coli* communities due to PAC growth inhibition. A-type PACs also reduce fungal *Candida albicans* adhesion properties (Polewski, 2016; Rauf et al., 2019).

Studies have shown that gut microbiome might have a connection with UTI. Typically, UTI is caused by microbe originated from gut. Paalanne et al. (2018) showed that there were differences in gut microbiome at genus and family levels between healthy children and children with UTI. Less is still known about berry polyphenol mechanisms on gut - and urinary tract microbiomes. However, as is shown, every study offers a new piece in a puzzle, and maybe someday the puzzle will be completed.

1.3. Cranberry and lingonberry

Cranberry (*Vaccinium ocycoccos*) and lingonberry (*Vaccinium vitis-idaea*) are members of the Ericaceae family, which contains other familiar berries like blueberry, bilberry, and huckleberry. Most of them are well studied and contain bioactive compounds that are good for health. Studies about cranberry health effects are known for several decades in the past. Cranberry is not just one species; cranberries are a group of dwarf shrubs in the genus *Vaccinium*. Colloquially cranberry has been used to describe all group members. Lingonberry is also member of the same genus *Vaccinium* and provides mostly similar potential health benefits (Häkkinen et al., 2019). Overall all genera provide health effects, being full of vitamins and flavonoids, but still have some levels of differences (Baoru et al., 2015).

Cranberries have a wide phytochemical profile containing approximately 8000-10000 detected phytochemicals, of which many are a necessary part of the diet. For example, all cranberries are rich in ascorbic acid known as Vitamin C, which is also an antioxidant and an essential nutrient as part of the daily diet (Brown et al., 2011). Cranberry bioactive compounds have been studied in European cranberry, *Vaccinium oxycoccos*, and American cranberry, *V. macrocarpon.* Cranberries also contain phenolic acids, catechins and triterpenoids, of which some have biological effects, e.g. in relieving chronic diseases (Jurikova et al., 2018). For example, ursolic acid, a phenolic triterpenoid from *V. oxycoccos*, is protective against oxidative damage and lipid oxidation (Ramachandran et al., 2008). Anthocyanins, proanthocyanins and flavonols form the majority of cranberry flavonoids, and all are associated with human health benefits (Panche et al., 2016).

Lingonberry, *Vaccinium vitis-idaea,* contains also necessary parts of diet as vitamins, phenolics and omega-3-fatty acids. Mostly it contains same phenolics which are associated with human health benefits as cranberries, but those has concentration differences (Heinonen, 2017).
Both berries are acidic, because they contain citric and malic acid like many other berries. Lingonberry and cranberry contain also benzoic acid, which is untypical in other berries (Viljakainen, 2003). These compounds are components of tannins and make berry juice taste sour.

1.4. Flavonoids

The most important group of bioactive compounds in genus *Vaccinium* is flavonoids. They are a group of secondary plant metabolites that help the plant to survive, grow and reproduce. All flavonoids in plants are biosynthesized via the phenylpropanoid metabolic pathway. The pathway can use the amino acid phenylalanine or tyrosine as input substrate and produce a wide range of different

compounds after many steps that divide pathway to "subset pathways" often named after end products with specifying a name. When flavonoids are produced, phenylalanine is pathways input substrate (Ververidis et al., 2007).

Every compound has a specific role in the plant and can act e.g. as antioxidants, antimicrobials, and photoreceptors or insect repellant. Bioactive compounds ratio is not stable during flowering and fruit ripening in cranberries. Berry maturation has been shown to affect anthocyanin - and proanthocyanin (PACs) concentrations in *V. macrocarpon*. PAC levels are high at the beginning of flowering but decrease until late fruit maturation when levels start to increase. Anthocyanin level increases during maturation and only flavonol levels stay seemingly constant all the time (Vvedenskaya & Vorsa, 2004). Flavonoid concentration between *V. oxycoccos* and *V. macrocarpon* also differs. *V. oxycoccos* has the highest concentration of anthocyanins, and PACs are most abundant in *V. macrocarpon* (Povilaityte et al., 1998). Also, both species differ by phenolic compound concentrations in individual plants. For example, environmental factors have a significant influence on the phytochemical profile of the plant. Climate, cultivating style and area is just a couple of factors that have shown to affect individual berry phytochemical profile (Jurikova et al., 2018).

Flavonoids contain a variety of different compounds with different effects but have a similar general structure; the 15-carbon skeleton contains two phenyl rings and one heterocyclic ring. There are three main classes; bioflavonoids, isoflavonoids and neoflavonoids, or they can be divided into several subgroups according to chemical structure. From the human perspective, the most significant groups are anthocyanidins, anthoxantheins (flavones and flavonol), flavanones, flavanols and isoflavones (Panche et al., 2016).

For humans, flavonoids are part of the polyphenol class of phytonutrients containing over 6000 identified biochemical compounds. Many flavonoids can act as antiviral, -allergenic and -inflammatory biocompounds. Flavonoids may also function as antioxidants and prevent damage to cells caused by free radicals and even the formation of cancer (Panche et al., 2016).

The flavonoid profile of cranberries has been studied and compared with other berries. A profile consists of anthocyanidins, flavan-3-ols and flavonol aglycons. Flavan-3-ols are derivatives of flavans. The group consist, members that have a common structural skeleton, 2-phenyl-3,4-dihydro-2*H*-chromen-3-ol, e.g. catechin and proanthocyanin include it (Pappas et al., 2009).

Cranberries and lingonberry, *V. vitis-idae*, flavonols, quercetin, kaempferol and myricetin have antioxidative and other healthy properties, and are typical for other berries also. A study done by Ehala et al. (2005) shows that quercetin, which also has antihistamine properties, was the most common flavonoid in studied berries. Highest level of 1.2 mg/100 g fresh weight (fw) was found in bilberry, *V. myrtillus*. European cranberry, *V. oxycoccos*, has over half less (0.52 mg/100 mg fw).

Another study done for 25 berries by Häkkinen et al. (1998) has shown also that quercetin is the most common flavonol, but concentration levels were much higher. According to this study, lingonberry, *V. vitis-idae* get second place with content 7.4-14.6mg/100g fw and *V. oxycoccos* gets the third place with a level range between 8.3-12.1 mg/100 g fw. The same study showed that myricetin, also a potent antioxidant, was common in all berries, with concentrations ranging from 1.4 to 14.2 mg/100 g fw. Kaempferol wasn't detected from cranberry and lingonberry. Differences between studies might be related to processing procedures. For example, drying conditions have been showed to influence flavonoid content in fruits of European cranberry (Adamczak et al., 2009).

Anthocyanins are especially known for their health benefits. Those have anti-tumour, anti-ulcer, antioxidant and anti-inflammatory properties. Due to anthocyanin-related nutritional value, anthocyanin content has been studied from berries. Brown et al. (2011) studied it in cranberry juice produced from European and American cranberries. The content varied a lot between juice samples, and in general, juice produced from American cranberry, *V. macrocarpon,* seemed to contain more anthocyanins. Still, none of the cranberry juice samples was even close in anthocyanin content compared to bilberry juice. Bilberry is commonly known for its high anthocyanin levels (Brown, 2011; Forney et al., 2014). Brown et al. (2011) also showed that cranberry anthocyanin levels do not correlate with antioxidative properties. Therefore, other flavonoids, or their cross-effects, seem to be more important for antioxidative effect in cranberries.

Even though both cranberry and lingonberry contain much less anthocyanins than bilberry, *Vaccinium myrtillus,* studies have shown that they consist a variety of other phytochemicals that have health potential (Kylli et al., 2011; Jurikova et al., 2018). Anthocyanins are also predominant for cranberry, but for lingonberry, *V. Vitis-idea*, flavonols and procyanidins predominate. Comparing to bilberry, extract of lingonberry fruits contains higher total amount of phenolic compounds and flavonoids (Dróżdż et al., 2017). Both cranberry and lingonberry contain relatively huge amounts of proanthocyanidins which comprise 63-71 % of berry total phenolic compounds (Kylli et al., 2011).

1.5. Proanthocyanidins

Most interesting polyphenol class, according to urinary tract infection (UTI) related studies, is proanthocyanidins (PAC) that contains three groups: dimers and trimers, oligomers, and polymers. Cranberries and lingonberry contain all of these. The anti-inflammatory, antibacterial and antiviral properties of the berries are a result of these compounds (Česonienė et al., 2015).

The polyphenol structure of PACs consists of repeating units of catechin or epicatechin monomer, which is used for grouping. PACs are also called as condensed tannins, and chemically oligomeric

flavonoids, meaning that they contain few repeating units. The dimers contain two repeating units, trimers three and tetramers four. Tannins are a name for a group of polyphenolic biomolecules that bind and precipitate various organic compounds, mostly proteins. The chemical structure of tannins contains polymeric building blocks. In principle, polymers can contain an infinite number of repeating units in "loosely form", so they are considerably lighter in molecular weight compared to PACs (Česonienė et al., 2015).

Shorter PACs, built from catechin and epicatechin, are non-hydrolyzable tannins, meaning that they are formed by the condensation of flavans and they do not contain sugar residues. Hydrolyzable tannins have carbohydrate at the centre of the molecule — those form gallic or ellagic acids when heated. Hydrolyzable tannins have shown to have antibacterial properties against *Helicobacter pylori*. Polyphenols found from red wine and green tea are shown to inhibit the VacA toxin, which is a major virulence factor of *Helicobacter pylori*. Tannin effects against cancer have been studied in the 2000's (Funatogawa, 2004). Tannins may somehow improve protection against colon cancer by decreasing carcinogen-induced aberrant crypt formation, colonic cell proliferation and oxidative DNA damage. Both tannin groups are typically found in tea, coffee and wine (Beecher, 2004).

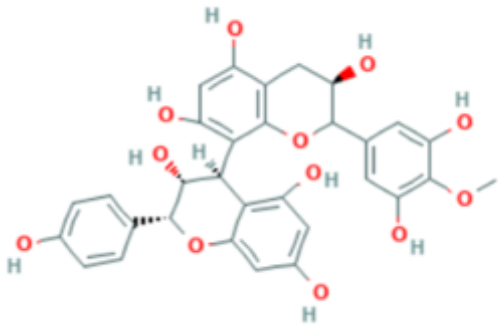

Figure 1 Proanthocyanin (National Center for Biotechnology Information. PubChem Database. Proanthocyanidin, CID=108065, https://pubchem.ncbi.nlm.nih.gov/compound/Proanthocyanidin (accessed on Oct. 29, 2019)

Among short PACs, A- and B-type proanthocyanidins that differ by structure, have a different effect on human microbiome. The A-type dimers and trimers are typical in European cranberry, *V. oxycoccos,* whose proanthocyanins levels are relatively high up to 63-71 % (1.5-2.0 mg/100g) of total amount of phenolic compounds (Määttä-Riihinen et al., 2005). A-type cranberries PACs (AC-PACs) are shown to affect urinary tract microbiomes pathogenic type bacterias adhesion to tissue walls (Kline & Lewis, 2016). *In vitro* studies has shown that polymeric PACs extracts of lingonberry and cranberry were antimicrobial against *Staphylococcus aureus*. Polymeric and oligomeric PACs of cranberry and oligomeric PACs of lingonberry affect *E. coli* adhesion (Kylli et al., 2011).

1.6. Cranberry and lingonberry health effects

In the past, cranberry has been used for many different health problems, including fever, blood disorder, stomach ailments and liver problems. Cranberry was typical natural medicine for Native Americans (Raz et al., 2004).

Studies done with spontaneously hypersensitive rats have shown that lingonberry lowers elevated blood pressure (Kivimäki et al., 2019). Lingonberry has also been shown to balance an otherwise unhealthy diet. In a study done with mice who had an unhealthy fatty diet comparable to humans, lingonberry prevented diet-induced obesity and low-grade inflammation. A reason for such results seems to be lingonberry effects on gut microbiome. Abundance of genera *Akkermansia* and *Faecalibacterium*, which are associated with healthy gut mucosa and anti-inflammation, increased by lingonberry consumption (Heyman-Lindén et al., 2016).

Antioxidant compounds of genus *Vaccinium* inhibit oxidation of low-density lipoproteins and reduce oxidative and inflammatory damage to the vascular endothelium. Studies done with mice have shown that cranberry polyphenols improve glucose and lipid homeostasis during high-fat and high-sucrose diet (Rauf et al., 2019; Jurikova, 2018).

PAC effect on colonic health has been studied with pig models that have a similar gastrointestinal tract as humans. In one study, six pigs were given 1% (w/w) of MegaNatural® Gold grape seed extract (GSE) once a day for six days. DNA was extracted from fecal samples and sequencing was done by using 16S rRNA barcode markers. Results showed that the major microbial metabolism products were 4-hydrophenylvaleric and 3-hydroxybenzoic acid, as their content in samples increased during treatments. The diet also caused an ecological shift in the pig gut microbiome, as Clostridiales, Lachnospiraceae, Lactobacillus and Ruminococcacceae abundance increased (Choy, Y. et al., 2014).

There is also some evidence about efficacy of cranberry PACs, quercetin and ursolic acid, against tumor development by inhibiting proliferation and colony formation, inducing apoptosis, and limiting tumor ability to invade. PACs also relax vessels and inhibit low-density lipoprotein oxidation, which can benefit health in case of cardiovascular disease (Cardano, 2013). However, more animal and *in vivo* studies are needed.

Although most PACs are not absorbed into bloodstream but are extracted into urine, as has been shown they can produce a health effect. Oligomeric and polymeric units have antioxidant and anti-inflammatory properties. PACs, formed of several monomers, can bind to excess activated enzymes, which are typical for the inflammatory condition. They have also a structure to attach to and increase barrier integrity, and large PACs can regulate cell signaling pathways by interacting with cell membrane proteins (Choy et al., 2014).

1.7. PAC metabolism in human

PACs, like other ingested polyphenols, are treated like xenobiotics in the body. This is because they are not a natural ingredient of the human body. The intestinal absorption of PACs is poor due to their structure and high molecular weight. Their decomposition is highly dependent on stomach pH value. They might be acid-catalyzed to monomeric flavan-3-ol units in the proper gastric environment (Spencer et al., 2000). Minor PAC monomers and dimers can be absorbed in the small intestine by Caco-2 epithelial cells, and studies have shown low plasma concentrations in patients after ingestion. Before absorption to bloodstream, compounds are first circulated in the liver where they are methylated, sulfated or glucuronidated by transferase enzymes. More studies have been done with B-type PACs (B1-B2), but studies in rats show that AC-PACs (A1-A2) are better absorbed in the small intestine than B2 PACs (Choy et al., 2014).

More complicated PACs, polymers and oligomers, are not absorbed and continue their pathway to the colon where microbial metabolism breaks down the compounds into smaller units. The body can absorb those to the bloodstream, but less is known on how cells can utilize their beneficial antioxidant properties. Overall, over 90 % of ingested polyphenols continue their way to the colon, where microbial metabolism seems to play a major role (Cassidy & Minihane, 2016).

Absorption of antioxidant compounds in intestine is typically limited. According Professor Lars Porskjær Christensen, from The University of Southern Denmark, antioxidant concentration in blood is very low, because most antioxidants have difficulties in passing through the cell membranes. Compounds of relatively polar and structural complexity must first be degraded and made less polar. He also criticizes typical belief on how antioxidants work in the body in the same way as *in vitro* studies, because many compounds are environmentally dependent. For example, vitamin C can work both as antioxidant or pro-oxidant. Christensen highlights that antioxidants are formed in the liver and for the most parts rapidly excreted in the urine, and how the remaining portion works and is absorbed to cells is still a mystery in many ways (Christensen et al., 2018).

1.8. Role of colonic microbiome in polyphenol metabolism

Diet effect on the gut microbiome has been studied a lot in recent years. For example, the diet has been shown to cause ecological migration in the gut microbiome. The number of known microbes that can utilize and catabolize biochemical compounds in the diet is increasing. In this way, human body is able to utilize many compounds that were initially too big to absorb. For microbes, these are

typically only by-products of metabolism that are utilized to buy space in a symbiotic microbial community (Chen et al., 2014).

In 2013, only a few bacterial species were identified to catabolize polyphenols (Cardona et al., 2013). These included *Escherichia coli*, *Bifidobacterium* sp., *Lactobacillus* sp., *Bacteroides* sp. and *Eubacterium* sp.. Only couple of years later more species were identified more accurately being involved in metabolism of polyphenols. *Adlercreutzia equolifacients* can dehydroxylate flavan-3ols, *Eggerthella* sp. SDG-2*, Eggerthella lenta, Slackia equolifaciens, Adlercreutzia equolifaciens* and *Lactobacillus plantarum* are shown to cleave C-ring from catechin and epicachin (Cardona et al., 2013; Braune & Blaut, 2016).

PACs are catabolized to chain fission products by intestinal bacteria. For example, 3-hydroxyphenyl acetic acid, 3,4-dihydroxyphenylacetic acid, 3-(3-hydroxyphenyl) propionic acid, and 5-(3′-hydroxyphenyl)-γ-valerolactone compounds are metabolites of PAC cleaving microbes among human microbiome. In these forms, they may be absorbed by the body, but their exact effects are still not well known (Cardona et al., 2013).

The effect of cranberry AC-PACs on the intestinal microbiome *in-vitro* has been studied in a project to develop nutrition strategies in the military environment (Laurel et al., 2018). Bacteria were extracted and grown from feces of three individuals in a nutrient-rich anaerobic medium supplemented with purified cranberry PAC at low and high doses. Results show that PAC might have a prebiotic effect on gut microbiome. Laurel et al. (2018) found out that abundance of *Ruminococcus* spp., which are associated with resistant starch degradation in the colon, was dose-dependently increased ($p<0.05$). Results also showed that butyrate production is PAC-dependent. PAC-dependent bacterial growth was revealed to be domain-dependent in studies that stimulate environmental conditions in different intestinal regions/domains (Laurel et al., 2018).

1.9. Polyphenols affecting microbiome composition

The exact information on the effect of polyphenols on gut microbiome is still low. Estimated different microbial species number ranges between 500-1000 in the gut microbiome, and their interactions with polyphenols are still a mystery. Some evidence has shown that dietary polyphenols affect microbial population composition and microbial activity. Most studies have focused on single polyphenol compounds and their effect on selected bacterial populations (Cardona et al., 2013).

Polyphenols have a positive effect on gut microbial content, and their abundance may alter Bacteroidetes/Firmicutes ratio (Stoupi at al., 2010). The effect of polyphenols was tested by a batch-culture model reflective of the distal region of the human large intestine, and the study showed that

flavan-3-ol monomers enhanced the growth of *E. coli* and bacteria in the *Clostridium coccoides-Eubacterium retale* group and inhibited the growth of *Clostridium histolyticum* (Tzounis et al., 2008). There are also more *in vitro* and *in vivo* evidence that flavan-3-ols may inhibit *Clostridium* spp. and favor *Lactobacillus* spp. (Cardona et al., 2013).

Phenolic extract of eight berries inhibits the growth of food-poisoning bacteria, that are pathogenic to humans, in a laboratory environment. Results showed that pathogenic strains were selectively inhibited. Cloudberry and raspberry extracts were the best inhibitors of bacterial growth, where the most effective compounds were ellagitannins. The extracts worked best against *Staphylococcus* but had no effect on probiotic bacteria *Lactobacillus rhamnosus,* indicating that the antibacterial effect is selective on pathogens. Only cranberry extract inhibits *Listeria* strain (Puupponen-Pimiä et al., 2005).

Another study showed that a 20-day treatment with polyphenol-rich red wine increased the relative abundances of the phyla Proteobacteria*,* Actinobacteria*,* Fusobacteria*,* Firmicutes*,* and Bacteroidetes found in fecal samples during treatment. Interestingly, non-alcoholic red wine only increased Fusobacteria and Bacteroidetes abundances (Queipo-Ortuño et al., 2012).

Many questions remain open, such as the role of microbial metabolites in humans and the mechanism leading to individual differences in the gut and urinary tract microbiomes. Relationship between the diet and microbiome has been shown, but the exact mechanisms are unclear. Microbiome and microbial metabolites seemingly modulate host health, but this crosstalk is still an ancient language that is under radical investigation (Wang et al., 2019).

2. Aims of the study

Cranberry has long been known to contain many health benefits as a source of nutrition, and especially in preventing urinary tract infection. However, the key to how this is happening is still mostly a mystery. Earlier studies have shown that there might be a connection between gut microbiome and urinary tract infection (Paalanne et al., 2018).

In this study, children with urinary tract infection were randomized to Cranberry-Lingonberry juice (CLJ) and control (flavonoid-free juice) consumption groups. Hypothesis was that the metabolism of proanthocyanidins in the gut alters bacterial composition and reduces the amount of *E. coli* and possibly other proteobacteria in the urine. The specific aims were

1) To compare microbiomes of the CLJ and control groups
2) Identify key microbes/groups

3. Materials and Methods

3.1. Samples and juices

Feces and urine samples were collected by the Oulu University Hospital Children's Clinic. Total of 77 children participated in the study. There was one real treatment, so the two groups were formed from patients, and one group drank Cranberry-Lingonberry juice (CLJ), and the other a control juice without cranberries. Research time was one year, and feces samples were collected after three -, six and twelve months. Urine samples were collected after three months. The assumption was that the changes would be identified in the urine sample after three months of CLJ use and to defined longtime effect in gut microbiota longer research time is needed.

Groups were coded by numbers. Number 1 was used to control group, and number 2 for CLJ group. Group 1 consisted of 116 samples from 42 patients and group 2 consists of 89 samples from 35 patients. Also, two samples were without tags. The total sample count was 207, including feces and urine.

Cranberry-Lingonberry juice (CLJ) used in this research, containing 2.0 g cranberry concentrate, 1.8 g of lingonberry concentrate with flavors, and 10.0 g sugar per one deciliter of juice. Placebo juice that control group used contained 10.2 g of added sugar, 5.5 g of citric acid, 5 g of natural cranberry aroma and 1 g of red anthocyanin color in one deciliter. It tastes, smells, and has the same color as CLJ but does not contain berry extracts. Juice products were provided by Eckes-Granini Finland.

3.2. DNA extraction

The DNA extraction was performed using two commercial extraction kits. For fecal samples, commercial QIAamp DNA stool kit was used. Extraction was performed manually according to the QIAamp handbook manual. In one extraction process, four to twelve samples were processed.

After fecal sample DNA extraction, the concentration of DNA quantity (ng/ul) and quality was measured by taking an A260/A280 and A260/A230 ratios, using Nanodrop spectrophotometer, (Thermo Fisher, USA). If yielded material was less than 20 ng/µl and quality seemingly bad, the sample was processed again. After verifying the success of the extraction, all extracted DNA sample materials were stored to -20 °C.

The extraction procedure can be divided to main points. First, Buffer ASL were used to lysis stool samples. Sample-buffer-mix were homogenized by vortex and stool lysate were add. Heat were used

to increase bacterial cells lysis. Heated mix were centrifuged to produce supernatant for later use. In the next steps, PCR inhibitors and DNA-damaging substances were absorbed from supernatant.

Last, DNA in the supernatant is purified. Procedure involves a couple of steps. First, proteins were digested, then DNA was bound to QIAamp silica membrane. The membrane was washed a couple of times to remove impurities before pure DNA is eluted to buffer that can be later used for PCR.

Urine samples were also extracted manually according to the extraction kit manual with some modification. For the extraction process, supernatant and pellet were made of urine samples. Pellet was used in later extraction process steps, which were mainly similar than with stool samples. Due to the different composition of sample material, the reactants, heating - and centrifugation times were mostly different for different sample materials. Urine volume varied clearly between samples. Quantity and quality of isolated DNA were also defined by Nanodrop. All extracted DNA samples were stored – 20 °C for later processing.

3.3. PCR

Before PCR, extracted microbial DNA samples were diluted to concentration 5 ng/ µl in a total volume of 50 µl and stored at -20 °C. For dilution calculations, data from Nanodrop was used. Samples that had lower yields were used without dilution. Later, the diluted samples were pipetted on a 96-well sample plate. Using a 96-well sample plate proved to be a better option than 1.5 ml Eppendorf tubes for PCR plate preparation.

For PCR, the master mix was made according to Table II recipe. Mastermix total volume depended on the number of samples to be amplified. Also, negative control samples and pipetting error were taken to account when planning master mix volume for the PCR process. All other reagents than barcoded reverse-primers and the sample DNA were mixed in 1.5 ml Eppendorf tube. The samples were spun down in a centrifuge to ensure mixing. dNTP mix of 10 mM were mixed and diluted from 100 mM nucleoside triphosphate solutions using Hypure Molecular biology grade water (Table I). The same water was used for master mix and diluting forward primer from stock. Reverse primers were already diluted to 10 mM or 5 mM.

Master mix was loaded to the 96-well plate by pipet or a multipipet. PCR-reaction was done in triplicate. Negative controls were used to test purity of reagents so that possible source of contamination could be found and eliminated. In negative controls, DNA was replaced by sterile water. One to three negative samples were added per plate depending on sample count. One 96-well plate could handle 29-30 samples and two to three controls. First, the mixed reagents were added to plate, followed by the unique barcode reverse-primer and finally DNA. Information about used

barcode reverse-primers and DNA samples were recorded for later purpose. Barcode primers were later used to target DNA sequences to the correct samples.

Table I dNTP mix

dNTP mix

reagent (concentration)	volume	final concentration	manufacturer
dH20	60 µl		
dATP (100 mM)	10 µl	10 mM	Thermo Fisher
dTTP (100 mM)	10 µl	10 mM	Thermo Fisher
dGTP (100 mM)	10 µl	10 mM	Thermo Fisher
dCTP (100 mM)	10 µl	10 mM	Thermo Fisher
Total	100 µl		

Table II PCR Master mix

PCR Master mix

reagent (concentration)	volume	final concentration	manufacturer
dH20	8. 9 µl or 7.9 µl		
PCR buffer Phusion GC (5X)	4 µl	1 X	Thermo Fisher
DMSO	0.5 µl	2.5 %	Thermo Fisher
dNTP mix (10 mM)	0.4 µl	200 µM	Thermo Fisher
forward primer F519 10 mM or 5 mM	1.0 µl or 2.0 µl	500 µM	Thermo Fisher
reverse primer R926 10 mM	1.0 µl	500 µM	Thermo Fisher
Phusion Polymerase 5 U/µl	0.2 µl	1 U	Thermo Fisher
DNA (5 ng/µl)	4 µl	20 µg	
Total	20 µl		

Table III PCR reaction protocol

	Temperature	Time
Initial denaturation:	98 °C	3 min
35 Cycles:	98 °C	10 s
	64 °C	15 s
	72 °C	30 s
Linked to:	72 °C	7 min
Hold:	4 °C	∞

Reagents, samples and plates were kept on ice throughout the preparation of the PCR. After 96-well plate was loaded, it was covered with a sealing tape. PCR-reaction was processed with Applied Biosystems Veriti 96 Well Thermal Cycler (Thermo Fischer) using the PCR protocol showed in Table III. After PCR-reaction was done, three identical reactions were combined into one, comprising a total volume of 60 µl. From that, 15 µl were analyzed by agarose gel electrophoresis. The rest of the PCR-product (45 µl) was stored at -20 °C.

3.4. Electrophoresis

A 1.4 % TBE (Tris base, boric acid and EDTA)- agarose gel was prepared for electrophoresis. 3 µl Ethidium bromide (10 mg/ml) was added to 50 ml liquid agarose gel, and mix was poured into the plate and left to cooldown. The ratio was same for every gel. Ethidium bromide was used to visualize the DNA in agarose gel after gel electrophoresis. When it intercalates between the nitrogenous bases of DNA, the DNA can be visualized under UV light.

The 15 µl of PCR samples were mixed with 3 µl DNA Loading dye (5X) produced by Thermo Fisher. In addition to the PCR samples, a DNA size standard for 16S by Thermo Fisher was loaded into the gel in total volumes of 18 µl. BioRad Power Pac 200 was used as the power supply and electrophoresis was performed for 1 hour at 100 V. If the PCR-reaction was successful, 16S bands could be visualized on the gel under UV light.

16S rRNA genes are widely used in phylogenetic studies, because it contains hypervariable species-specific sequences that can be used for identifying. In this case 16S clone products can be amplified from agarose gel according to typical size of the product. No products in negative controls ensured that the reactions were free from contamination and only 16S part of bacterial DNA was cloned (Yarza et al., 2014).

The electrophoresis gel images are shown in the Appendix 1. At its best, gel included 24 triplicate samples and three triplicate negative controls. Loading order and sample/barcode numbers are marked in the images. For clarity, a ladder was always added to the first well of each row.

3.5. Next-generation sequencing

Sequencing is used to transform the extracted 16S rRNA gene sequence information from samples to sequence library. In this study, 16S ribosomal RNA (rRNA) gene sequencing technique and reverse primer set F515-R926 were used. The 16S ribosomal RNA gene contains regions that are species-specific. F515-R926 primer set contains the regions V4-V5 of nine multivariable regions of bacterial 16S ribosomal RNA gene. In PCR-reaction, the primers were used to build up several copies of

species-specific 16S strands, and by using sample-specific barcode primers, it's possible to pool all samples together into one library dataset (Chakravorty et al. 2007; Joe et al., 2016).

Ion Torrent next-generation sequencing (NGS) platform (Thermo Fisher) was used to sequence 16S rRNA gene from the PCR-products. For that, the PCR-product samples were collected on a new 96-well plate by diluting them to a total volume of 50 µl. After all samples were transferred to new plates, they were sequenced at Biocenter Oulu Sequencing Center.

Ion Torrent NGS process starts with fragmenting a PCR-product DNA into millions of fragments. Those fragments are then labelled and cleaned from unwanted salts, enzymes and smaller fragments. After purification, each DNA fragment is bound to an ion sphere particle (ISP) and the binding is repeated until the ISP is covered with multiple copies of the same DNA fragment. The fragments are linked at one end of the ISP and biotinylated at the other end. That side will bind to magnetic beads so that empty ISPs are washed away. Enriched ISPs are then put to chips and sequencing can start (Rusk, N. 2010; Merriman, B. et al., 2012).

Ion Torrents semiconductor chips are full loaded with wells that capture chemical information from DNA sequencing and translate it to a digital form. Every time nucleotide is incorporated into a single strand of DNA, a hydrogen ion is released. This will lead to a change in pH in wells, and that change can be recorded and translated to a digital format (Merriman, B. et al., 2012; Rusk, N. 2010). Semiconductor chips are covered with liquid of one of four DNA nucleotides at a time. Nucleotide liquid is chanced every 15 seconds. A polymerase is used for connecting those nucleotides to a single strand of DNA. Every time when nucleotide incorporates into strand of DNA, pH change is measured and chanced to voltage. If there are two or more same complementary nucleotide in a strand of DNA, voltage is higher, and count can be recognized. The same process happens simultaneously in millions of wells. In the end, every attached nucleotide is recorded and translated to human-readable digital information (Rusk, N. 2010; Merriman et al., 2012).

3.6. Data processing

3.6.1. Metadata

For analyzing data build up with Ion Torrent NGS, metadata is formed. Metadata contains sample tags by rows and columns contain associated information. For QIIME 2 platform, first column has to include sample identification number (#SampleID). Barcode sequences and forward primer sequences must be inserted in the table. Other columns may include valuable information about samples e.g. treatment, sample material, age, sex etc. that can be used in statistical analysis.

In this study, information about treatment - and research groups, sample materials and collection time were used. The metadata was collected and are provided by the University Hospital of Oulu. An open-source Google Sheets add-on Keemei were used to validate QIIME 2 compatibility (Bolyen et al. 2018).

3.6.2. QIIME2

QIIME 2 is plugin compatible microbiome analysis software. The software produces and uses QIIME 2 artifacts (.qza). By using this file format, the software can track the type, format and provenance of data for researchers. QIIME 2 works via Python. QIIME 2 artifacts can also be used to build Visualization from analysis results. Visualization can be viewed using the qiime2view interface.

Fastq-file produced by Ion Torrent was imported to QIIME 2 environment where the software was used to study and edit sequence information. Pooled sequences were demultiplexed, and metadata were used to connect right samples and sequences. Sequences were trimmed to 300 bp and sampling depth of 32200 was selected for downstream analysis.

For taxonomic analysis, Silva 132, Greengenes and Human Oral Microbiome Database (HOMD) were used as 16S sequence reference data. There weren't major differences between the identified taxa in Silva 132- and Greengenes database, but taxonomy table produced with Silva worked better in the downstream analysis done by R.

3.7. Statistical analysis

For the microbiome analysis, QIIME 2 artifacts were changed to a format that can be handled by R programming language. QIIME2R is an R package for importing QIIME 2 artifacts into an R session. In this study, table.qza, rooted-tree.qza and taxonomy.qza artifacts and metadata.tsv file were used to build up phyloseq object. Phyloseq object stores all produced and OTU (operational taxonomic unit) clustered phylogenetic data into one R object. QIIME2 use DADA2 plugin to produce OTU artifact and strictly speaking those are ASVs (amplicon sequence variant) which are higher resolutions and -quality sequences. Although ASVs are a hash (a coded string of numbers and letters that represents unique sequences) and can be used to compare datasets denoised with exact same parameters.

The phyloseq package was used to analyze and graphically display sequence data. It leverages many other R tools for phylogenetic analysis and use ggplot2 graphic system. In this study, ALDEx2,

cowplot, metacoder, microbiome, phyloseq, tidyverse, vegan and ggplot2 packages were used to produce graphics of complex phylogenetic data (McMurdie & Holmes, 2013).

3.7.1. Relative abundance

Samples were divided by sample material, collection time and treatment into eight groups. This enabled comparison of long-term changes in gut microbiome between treatments.
After subgrouping, low abundance and unidentified phyla were filtered. Prevalence threshold of five percent was selected to remove ASVs which were present only one sample.

3.7.2. Alpha diversity

Alpha diversity is a local measure and describes diversity in a particular ecosystem. It can tell about the number of species in an environment under certain conditions. In this study, we were interested about Alpha diversity in gut and urinary tract microbiome after different treatment conditions. Alpha diversity was measured from microbiome of feces and urine samples in control and CLJ groups.
Observed species richness is one way to show Alpha diversity, but many different diversity indexes diversity indexes that can provide more information about community composition. Different indices have different assumptions and weights. The best index most often depends on the study design, and various indices give more perspective (Tuomisto, 2010).

In this study, various indices were used to show community richness in different conditions. Any observed rare ASVs were not trimmed before analysis because it can seriously alter the results. Some estimators give weight also for rare species.

The Shannon index assumes that all species are represented in a sample and are randomly sampled. It is a statistical information index and it shows how difficult it is to predict the identity of the randomly chosen individual. The Simpson index is a dominance index, meaning that it gives more weight to common or dominant species. It answers the question on the probability if two randomly selected individuals are of the same species. Chao1 is an abundance-based coverage estimator of species richness (Lee and Chao, 1994). Chao1 estimator takes into account the estimated number of unobserved species. Inverse Simpson estimator is inverse of the probability that two bacteria picked at random in the community belong to different OTU (Tuomisto, 2010).

Shapiro-Wilk normality test was used for observed and Chao1 estimator data in every group, to analyze distribution. Skewness plots were also built for studying the skewness of data (Figure 4). If data was not distributed normally, the Wilcoxon rank-sum test was used to test Alpha diversity

differences statistically. Wilcoxon rank-sum test is non-parametric test of the null-hypothesis that randomly selected ASV abundance from one sample is lower or greater than a random selection from another group. T-test was used for analysis of data with normal distribution.

3.7.3. Beta diversity

As the Alpha diversity describes how many different species there are and how balanced those are in a sample, beta diversity shows how different the microbial composition in one sample is compared to another. There are different metrics for beta diversity than alpha diversity.

Bray-Curtis and weighted UniFrac metrics take abundance into account, Jaccard and unweighted UniFrac only calculate beta diversity based on presence-absence data of an OTU/ASV. OTU-based metrics ignore taxonomy. Unifrac metrics incorporate phylogenetic information by using phylogenetic tree information about sequence distances. Unweighted branch is based on sequence distances, and weighted branch lengths are weighted with relative abundances (Tuomisto et al., 2010).

3.7.4. Differential abundance

Differential abundance was studied by metacoder and phyloseq R-package. The target was to identify significantly differentially abundant taxa for the treatment groups in the four different sample types. ANOVA-like differential expression (ALDEx2) method was used to test differentially abundances in ASVs between the treatment groups. This is a version of the Wilcoxon test developed for compositional NGS data (Gloor, 2015).

Metacoder-package for R was used to build differential heat trees from CLR-transformed data. Data were cleaned from unidentified and low abundance taxa before plotting. The tree branches represent identified taxa up to the species-level. The width of the branches was used to illustrate the amount of OTUs (ASV), and color panel was used to indicate which treatment has greater abundance using CLR-transformed data (Foster et al., 2015).

Differential abundance was also studied by QIIME 2 using phylum- and genus-level identification results.

3.7.5. Compliance

Clinical compliance data was used to trim the sample data of the time point of three months. Compliance shows the probability that patients had really used medication. The cut-off value was set at 80 %. After taking clinical compliance into account, there was a total of 54 samples left for

downstream analyses. CLJ group contained 29, 18 fecal and 11 urine, and control group 29 samples, 22 fecal and 7 urine.

After trimming, three-month samples were statistically analyzed by using the above-mentioned methods. Krona plots were used to visualize differential abundance between two treatments in feces and urine in the three-month samples.

4. Results

4.1. DNA extraction and PCR

DNA was extracted from 202 samples. Samples 100, 112, 204, 32, 33, 42, 69, 74, 86, 96 and 97 were re-processed, because low concentration results and there was sufficient amount of sample material to re-run. Three fecal samples contained an insufficient amount of sample material for DNA extraction. Two urine samples from hospital list were missing and one fecal sample falcon was empty. There were also three samples that were missing from the list. The average DNA yield from urine samples was much lower than that in fecal samples, as could be expected. Of all extracted samples, 47 had < 20 ng/ul concentration and were used without dilution in PCR.

PCR results are shown in Figures in the Attachment (Attach 1), and one example is shown in Figure 2. After NGS sequencing, there were a total of 115 samples in control group (1), of which 96 were feces and 19 urine samples. CLJ group (2) had 71 feces and 17 urine samples, 88 in total. After removing duplicates, a total of 197 samples were processed for downstream analysis.

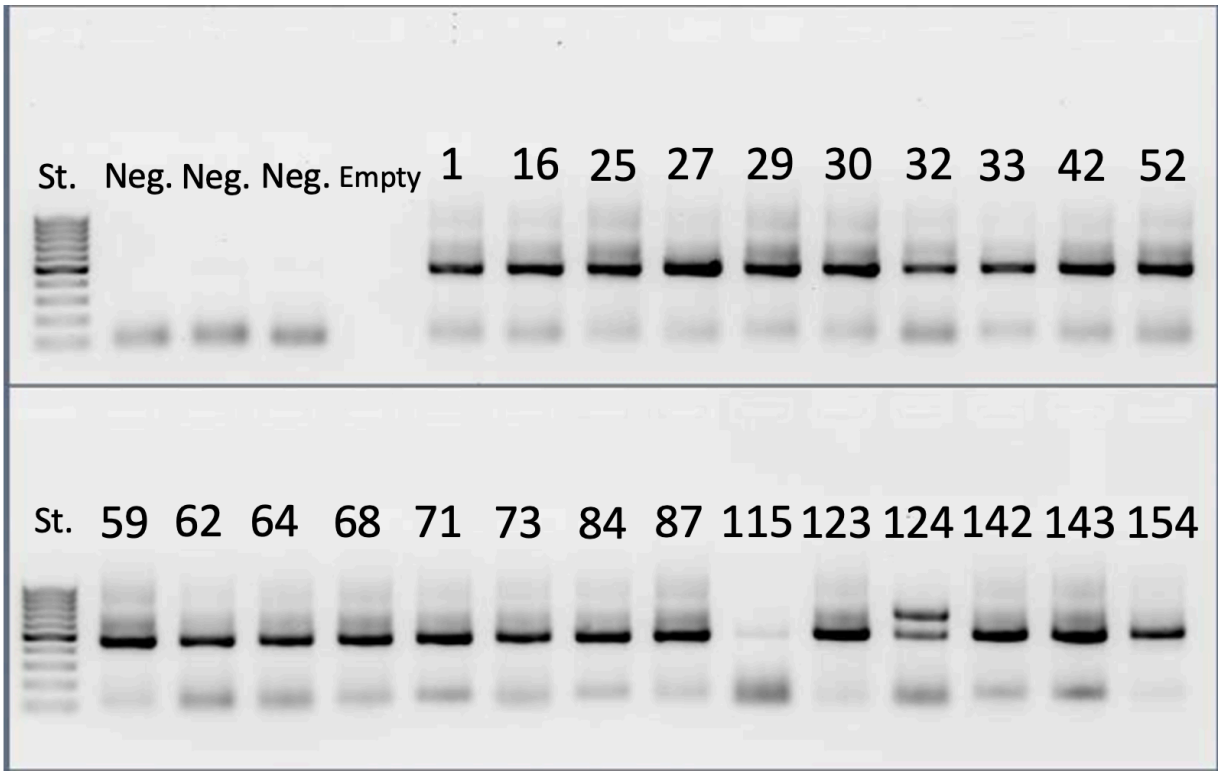

Figure 2 Agarose gel electrophoresis of PCR products amplified from bacterial 16S rRNA genes. The 1.4% agarose gel was made in TBE buffer, which was used in the electrophoresis at 100V for one hour. The samples are numbered above each well. The DNA molecular weight standard (st.) was GeneRuler DNA Ladder Mix (Thermo Fisher).

4.2. Relative abundance

Prevalence filtering caused a drop of 13 samples in the total count (184). In the Figure 3 groups of different treatments, but the same sample material and collection time, are presented side by side to allow easy analysis of any differences between treatments phylum-level relative abundances. Every analysis was produced according to the same grouping.

Phylum-level relative abundances were pretty similar between treatments. Bacteroidetes and Firmicutes play a dominant role in the majority of the samples. However, there were differences in the Bacteroidetes/Firmicutes ratio that could not be explained by treatment. Also, some samples contained Cyanobacteria that were not typically found in other samples. Actinobacteria were typically found almost in every sample. An interesting difference can be seen in urine sample groups. CLJgroup samples had less Proteobacteria members, and more of those belonging to Firmicutes.

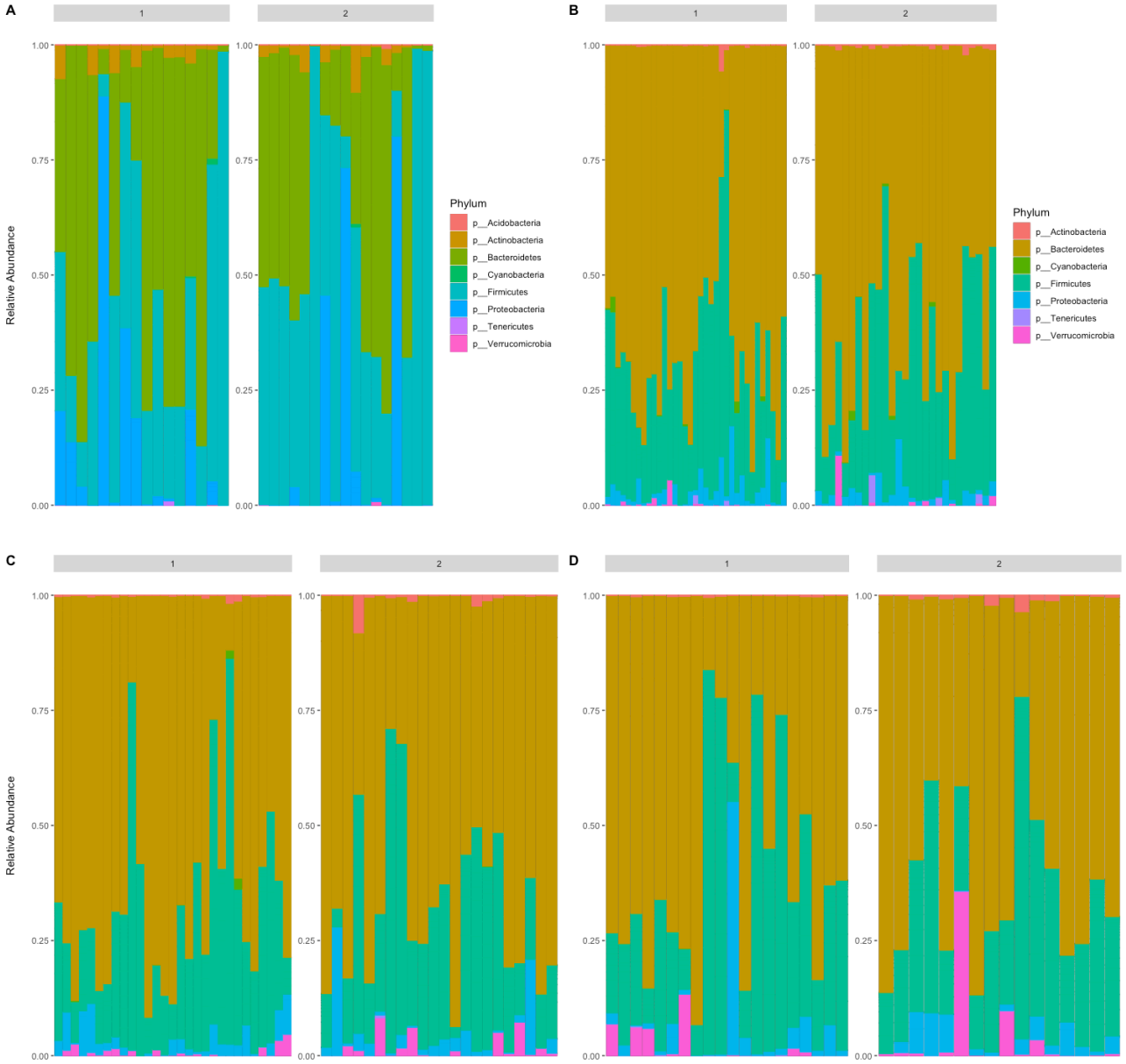

Figure 3 Phylum-level relative abundances in sample type groups. A=Urine 3 months samples, B: Feces 3 months, C= Feces 6 months, D=Feces 12 months. All feces groups have same legends, and urine owns. Plots are facet by treatments 1=control & 2=CLJ.

4.3. Alpha diversity

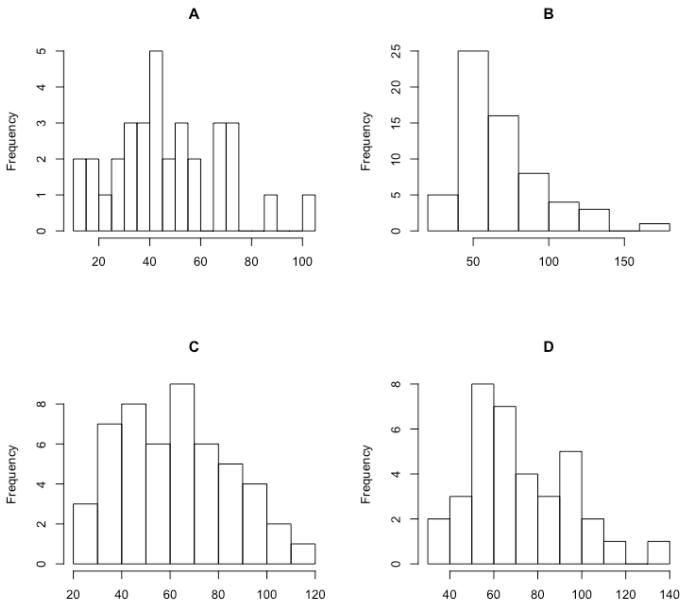

Figure 4 Plots of skewness of different sample groups. Urine 3 - and feces 6 and - 12 months samples are distributed normally, and feces 3 months are not distributed normally according to these plots and Shapiro-Wilk normality test. A=Urine 3 months, B= Feces 3 months, C= Feces 6 months, D=Feces 12 months.

Skewness of sample groups data are showed in Figure 4. Observed, Shannon, Simpson, Inverse Simpson and Chao1 diversities are presented in Figure 5 boxplots. ANOVA and Wilcoxon rank-sum test results are shown in Table IV. Feces 3 months sample data was not distributed normally, but the data in other three groups was normally distributed. There was no significant difference between treatments. As can be seen, most differences are found between sample types.

Table IV Alpha diversity indices with significance tested by t-test or Wilcoxon test

Group	Observed	Chao1	Shannon	Simpson	InvSimpson
	Wilcoxon rank sum test, p-value				
Feces 3 months	0.84	0.84	0.92	0.82	0.82
	t-test, p-value				
Feces 6 months	0.8746	0.9416	0.7988	0.4765	0.3587
Feces 12 months	0.6039	0.8238	0.6277	0.592	0.9618
Urine 3 months	0.4525	0.6814	0.8017	0.8319	0.6901

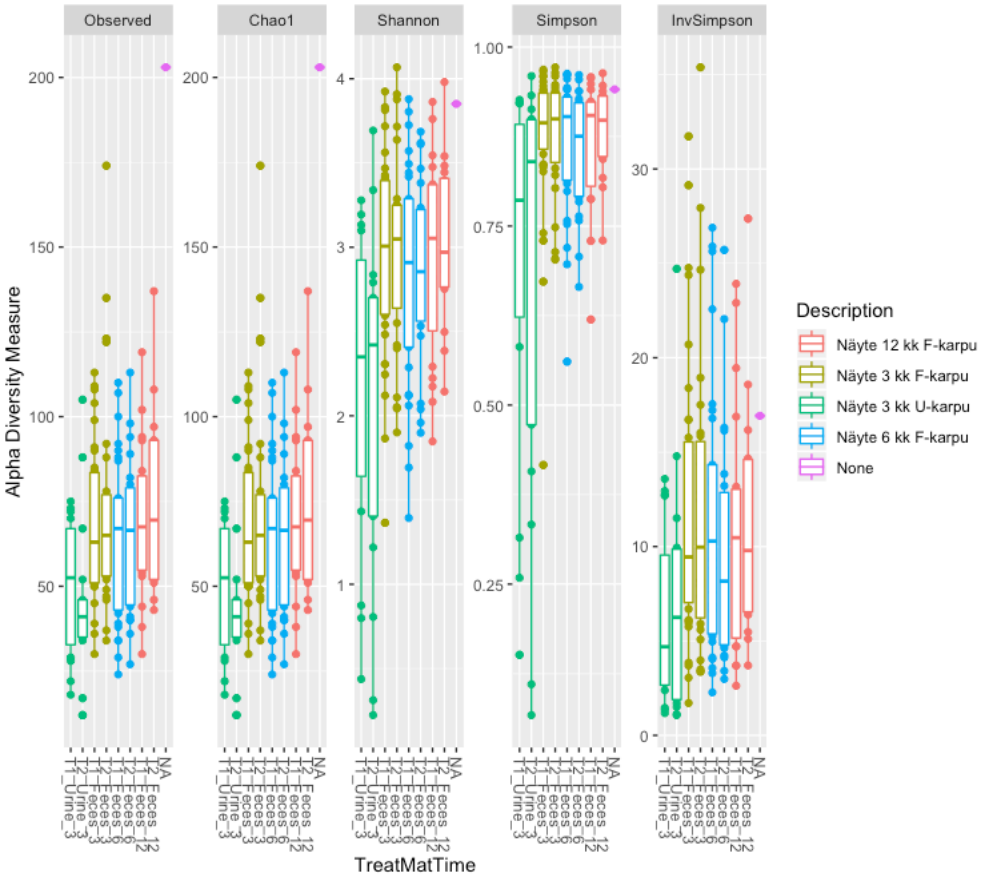

Figure 5 Alpha diversity metrics. Each measure is listed above each plot, and x-axel contains groups. Groups are divided by treatment, sample material and collection time in months. Last two are shown also as a color code: Green indicates urine samples, dark green indicates 3 months feces samples, blue indicates six months feces samples and red indicates twelve months feces samples.Y-axel contains abundance metrics values.

4.4. Beta-diversity

In this study, The Bray-Curtis dissimilarity metrics were used to build dendrogram from samples relative abundance data (Figure 7). The Bray-Curtis dissimilarity gets the value zero when the composition between samples is exactly the same, and one, when no taxa are shared. The treatment groups were color coded, control treatment (1) in red and CLJ treatment (2) in blue.

Beta diversity ordination in different sample types was generated using centered log-ratio transformed (CLR) counts. Transformation was performed using Microbiome R-package. Principal component analysis (PCA) histogram shows how much of variation are explained by first two principal components compared to other PCs (Figure 6). Those two PCs were mapped to two-dimensional space. Every group had some differences between treatments, and overlapping is typical for the same environment samples. Therefore, the beta-diversity analysis was continued further. Permutational multivariate analysis of variance (PERMANOVA) was used to test if there is more

clustering than expected by sampling variability. Homogeneity of multivariate dispersions were tested by ADONIS test, and for results, a permutation-based test was performed. Results are showed in Table V and Figure 8.

There were no significant differences in beta diversity indices between the treatment groups. The dendrogram shows that there is no partitioning between the treatment groups.

Ordination centroids and dispersion labeled method shows that there are no remarkable differences between ordination centroids and dispersion. The greatest differences can be seen in the six-months sample group.

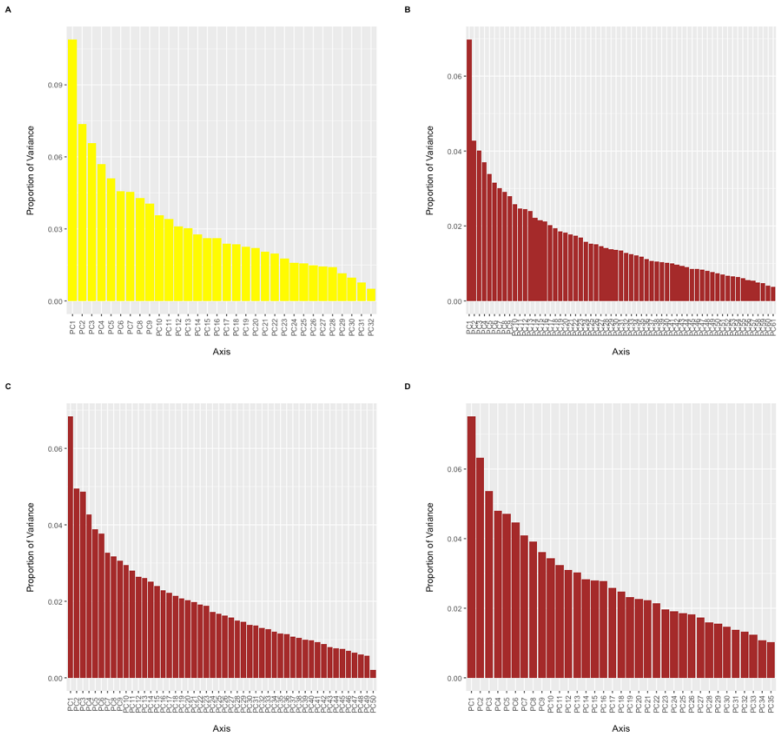

Figure 6 X-axel contains PCs formed from data, y-axel shows proportion of variance. Typically, first component includes most variance and after drop that continues gradient degree. In this study, drop isn't particularly huge. A= Urine 3 months, B= Feces 3 months, C= Feces 6 months, D=Feces 12 months)

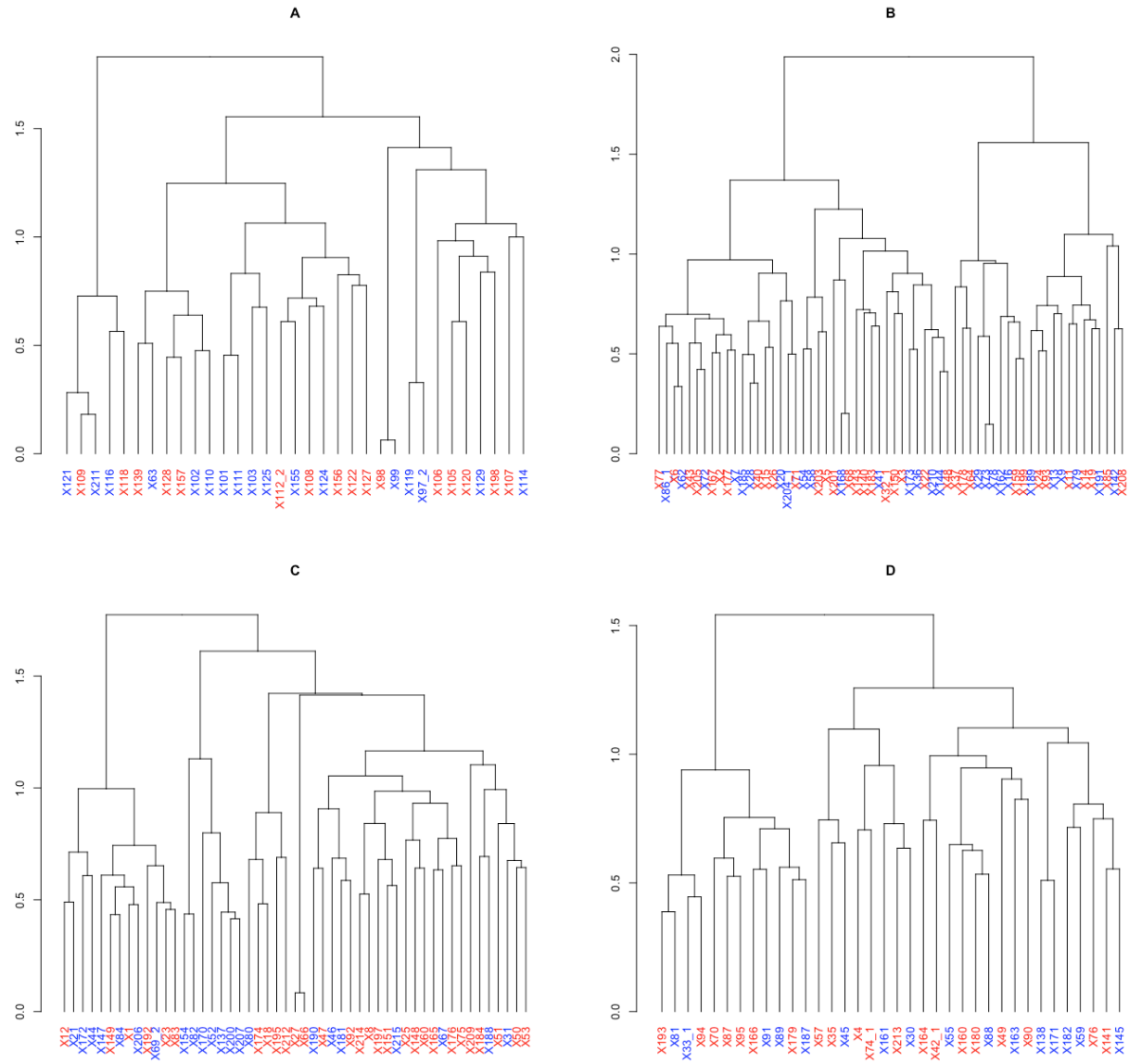

Figure 7 Bray-Curtis dissimilarity shows how different the samples in treatment groups are to each other. Samples under control treatment (1) are marked in red, samples under CLJ-treatment (2) are marked in blue. The longer the distance is, the more different is beta diversity between the treatments. A=Urine 3 months samples B: Feces 3 months, C= Feces 6 months, D=Feces 12 months

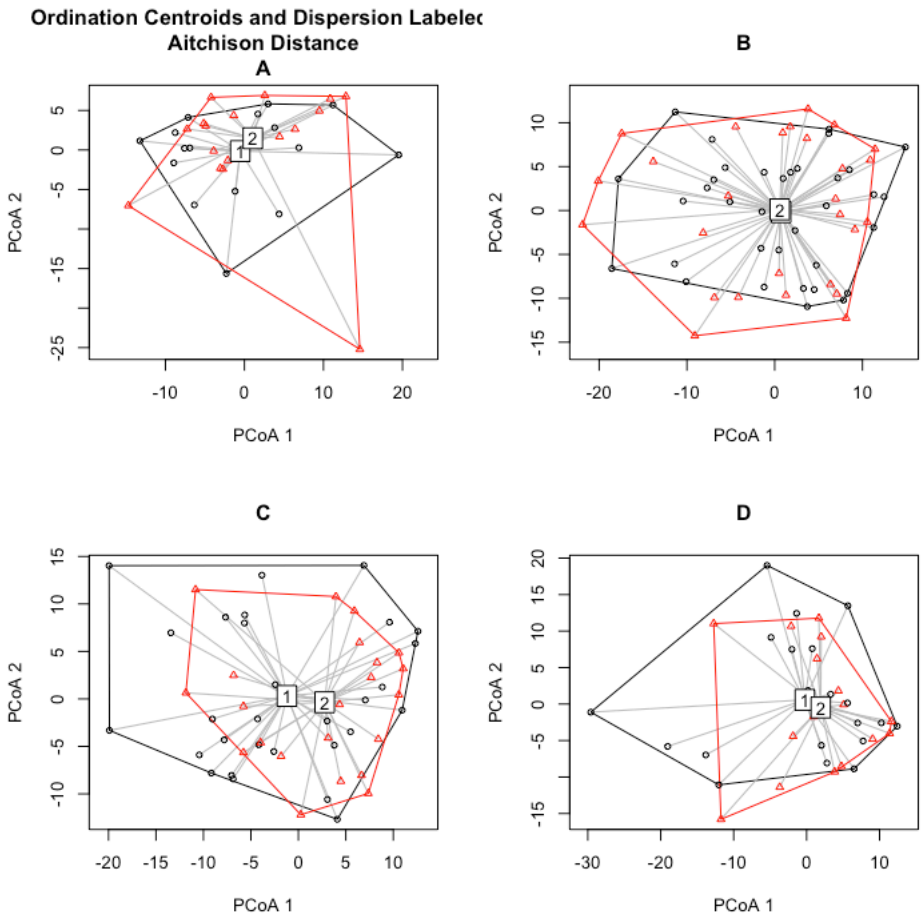

Figure 8 Beta diversity. Ordination centroids and dispersion labeled Aitchinson distance were used to illustrate beta diversity of samples in different treatments using two PCoA. Grey indicates control-treatment (1) and red indicates CLJ-treatment (2) plots. A=Urine 3 months samples B: Feces 3 months, C= Feces 6 months, D=Feces 12 months

Table V Beta diversity metrics

	Urine 3 months	Feces 3 months	Feces 6 months	Feces 12 months
ADONIS, p-value	0.997	0.698	0.283	0.756
Dispersion test / T1 & T2	24.16 & 23.69	33.23 & 33.26	31.40 & 31.08	32.03 & 32.09
Permutation test, p-value	0.753	0.984	0.784	0.973

4.5. Differential abundance

ALDEx2 test results are presented as effect size plots (Figure 9) that show no significant differences in abundance between treatment groups according to microbiome data. For a difference in taxa abundance to occur, it should exceed the dispersion. Benjamini-Hochberg false discovery rate (BH-FDR) corrected p-values should be present as red plot for taxa if those fall below the significance threshold (<0.05).

The effect size plots show the median log2 fold difference by the median log2 dispersion in every sample material time point. Differences between treatment groups can be read from y-axel. Data points plotted towards the bottom of the plot are more abundant in CLJ samples, and data points plotted towards the top of the plot are more abundant in control group. In every subgroup, there is a cluster in the middle of y-axel indicating that the abundances in the treatment groups are mostly similar by dispersion. The division on both sides of the y-axis origo shows different concentrations between the treatment groups, although not statistically significant. This is especially visible in urine plot. Dispersion in every treatment group is, overall, very similar.

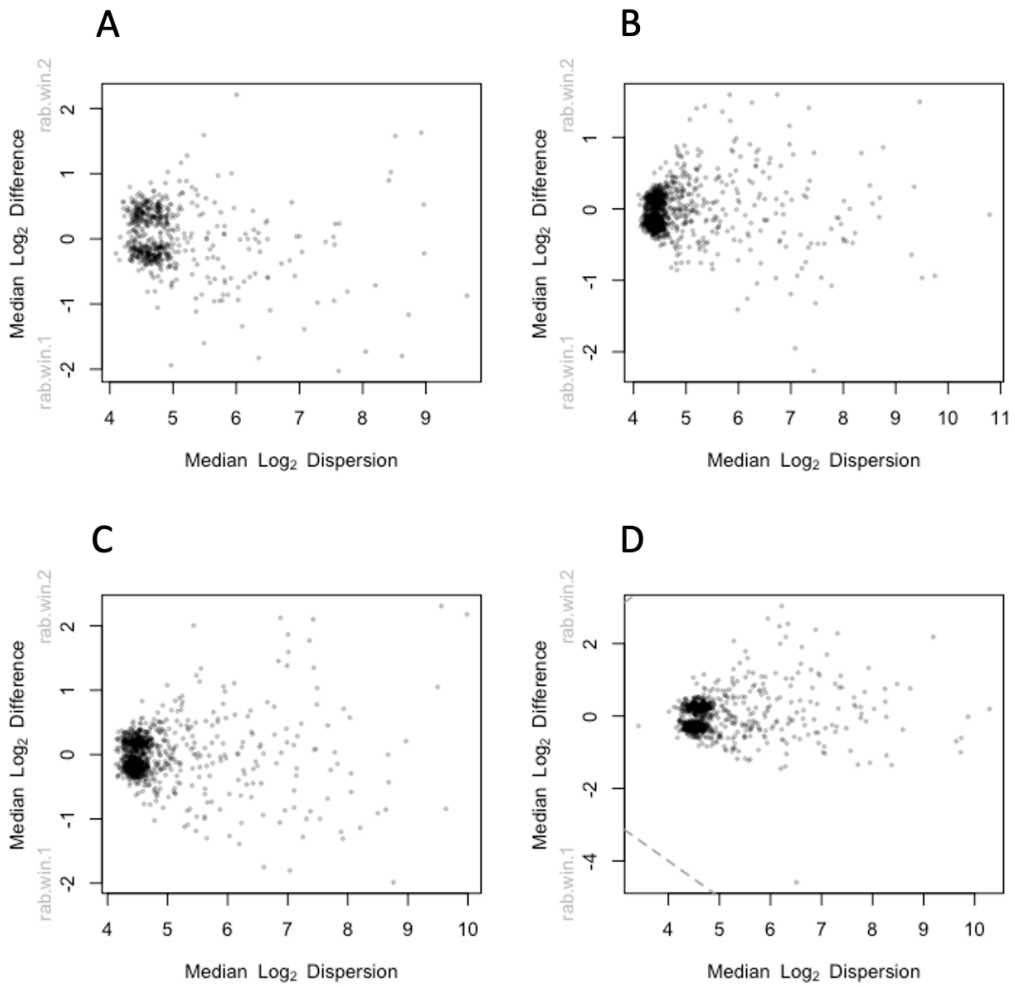

Figure 9 The effect size plots. Significantly different ASV should be plotted with red and wider diameter. A=Urine 3 months samples B: Feces 3 months, C= Feces 6 months, D=Feces 12 months

Species-level differences are shown in Figure 10 with heat tree plots containing CLR-transformed abundances for every treatment group. Log2 transformation was done to modify data divided by treatment to symmetric around zero. Species that were more abundant in control group (1), got

positive values and were colored turquoise. Species that were more abundant under CLJ treratment (2), got negative values and were colored tan. Differential abundance can also be seen from lower taxonomic nodes by the same rules. If there was no difference between treatments, the value became zero. Due to natural variation, exactly the same value is rare and small differences are always found.

Differential abundance for ASV in the different treatment groups were tested by Wilcoxon rank sum-test. There were no significant differences after false discovery rate (FDR) correction. Heat trees, containing log 2 ratios of median proportions and OTU/ASV counts were used to visualize species richness in sample types (Figure 10).

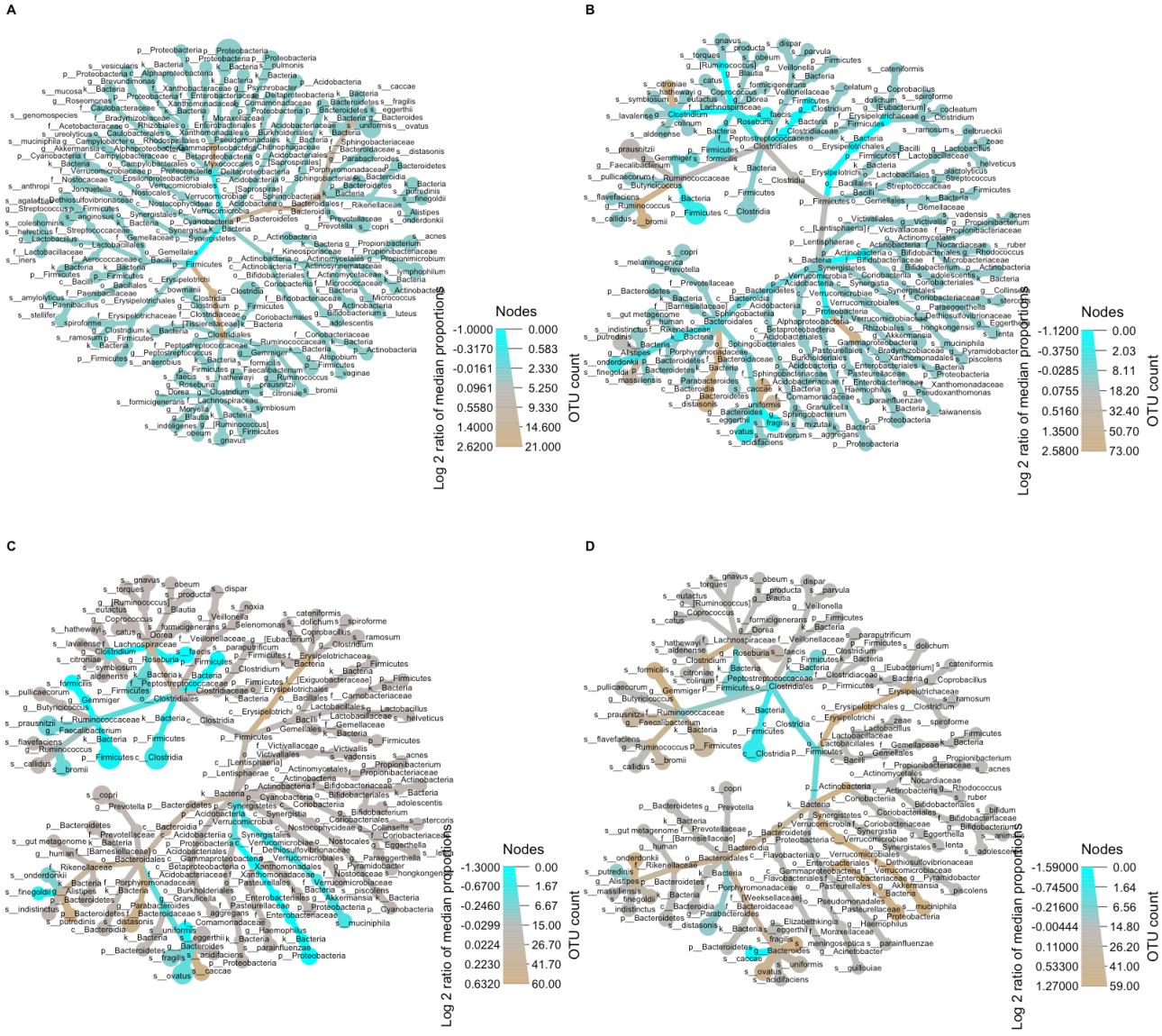

Figure 10 Heat tree maps containing centered CLR-transformed differential abundances between treatment groups. Value zero means no differences in abundance between treatments, distance from that shows difference level and direction. Turquoise-colored nodes indicate more abundance under treatment 1(control) and tan nodes under treatment 2 (CLJ). Node width shows OTU-count (ASV). A= Urine samples, three months, B= Feces samples, three months, C=Feces samples, six months, D= Feces samples, twelve months.

Kruskal-Wallis - and Mann-Whitney test results from phylum and genus-level data showed no significant differences between treatment groups in any subgroup. Some p-values reached the level of significance at genus-level in every subgroup, but after BH-FDR correction, the levels of significance were lost.

4.6.Compliance

Alpha – and Beta diversity were not significantly different between CLJ- and control treatment groups even when a compliance limit of 80 % was taken into account (data not shown). Differential abundance was also tested by Aldex2 -method, which showed no significant differences between ASVs. Besides the dispersion in urine samples being wider with compliance limit, there was no change in results (data not shown).

Krona plots (Figures 11-12) show relative abundances for data with compliance limit of 80 % in use. According to the Krona plots of urine samples, there are differences in abundance of some phyla and families between treatments. Proteobacteria were less abundant and Firmicutes were more abundant in the CLJ group. The CLJ group also had 5 % less members in the family Enterobacteriaceae (taxonomic family level under Proteobacteria phylum) than control group.

The krona plots (Figure 11-12) of the feces samples at three months were mainly similar between CLJ and control group after 80% compliance limit correction. However, some differences can be seen, e.g. the CLJ group has lower abundance of Proteobacteria compared to the control group.

QIIME2 environment was used to do Mann-Whitney and Kruskal-Wallis test for phylum and genera level taxa in both sample material groups. Mann-Whitney test for urine samples in genera level showed that genus *Finegoldia* had significantly different abundance between treatment groups. There were also some other statistically significant findings where the raw p-value was under 0.05, but these were lost after BH-FDR. Mann-Whitney test for feces samples in phylum level showed that the phylum Cyanobacteria had significantly different abundance between CLJ – and control group. Similarly, some findings at genera level had raw p-values under 0.05. All results are shown in table VII.

Table VII QIIME2 tests results

Urine 3 months

MannWhitney- Urine			
Genus	**Reject**	**Statistic**	**raw pvalue**
Finegoldia	**True**	38.5	0.0080
KruskalWallis- Urine			
Phylum	**Reject**	**Statistic**	**raw pvalue**
Proteobacteria	False	4.1957	0.0420
MannWhitney- Urine			
Genus	**Reject**	**Statistic**	**raw pvalue**
Fastidiosipila	False	38.5	0.0230
Negativicoccus	False	38.5	0.0200

Feces 3 months

MannWhitney			
Phylum	**Reject**	**Statistic**	**raw pvalue**
Cyanobacteria	**True**	197.5	0.0100
KruskalWallis-Feces			
Phylum	**Reject**	**Statistic**	**raw pvalue**
Actinobacteria	False	4.2193	0.0490
Genus	**Reject**	**Statistic**	**raw pvalue**
Prevotella	False	4.5167	0.0370
[Eubacterium] xylanophilum group	False	5.2780	0.0390
Erysipelotrichaceae UCG-003	False	4.5916	0.0270
MannWhitney- Feces			
Genus	**Reject**	**Statistic**	**raw pvalue**
Barnesiella	False	196.0	0.0450
Flavonifractor	False	195.5	0.0500
Veillonella	False	197.5	0.0130
Escherichia-Shigella	False	196.5	0.0370

To identify *E. coli* within the results, new reference database was used as a classifier. By using HOMD (Human oral microbiome database) newest version, new classifier was built and an earlier family-level group Enterobacteriacea was identified to be *E. coli.* However, the weakness of this

reference database was a much lower level of identification. Only 209 taxa were identified. Same tests were done as earlier in the QIIME2- environment, but some differences earlier identified were lost, and phylum level statistics was different by poorer identification levels.

ASV-based differential abundance test was done in R-platform using Wilcoxon rank sum test which showed some differences, but most of them were not classified to taxa and none reached level of significance after FDR correction. Table VIII consist results for differential abundance test classified with SILVA reference database which identified taxas more specific. Table VIII Urine n. 2 was classified to be genus *Megasphaera* member with HOMD reference database.

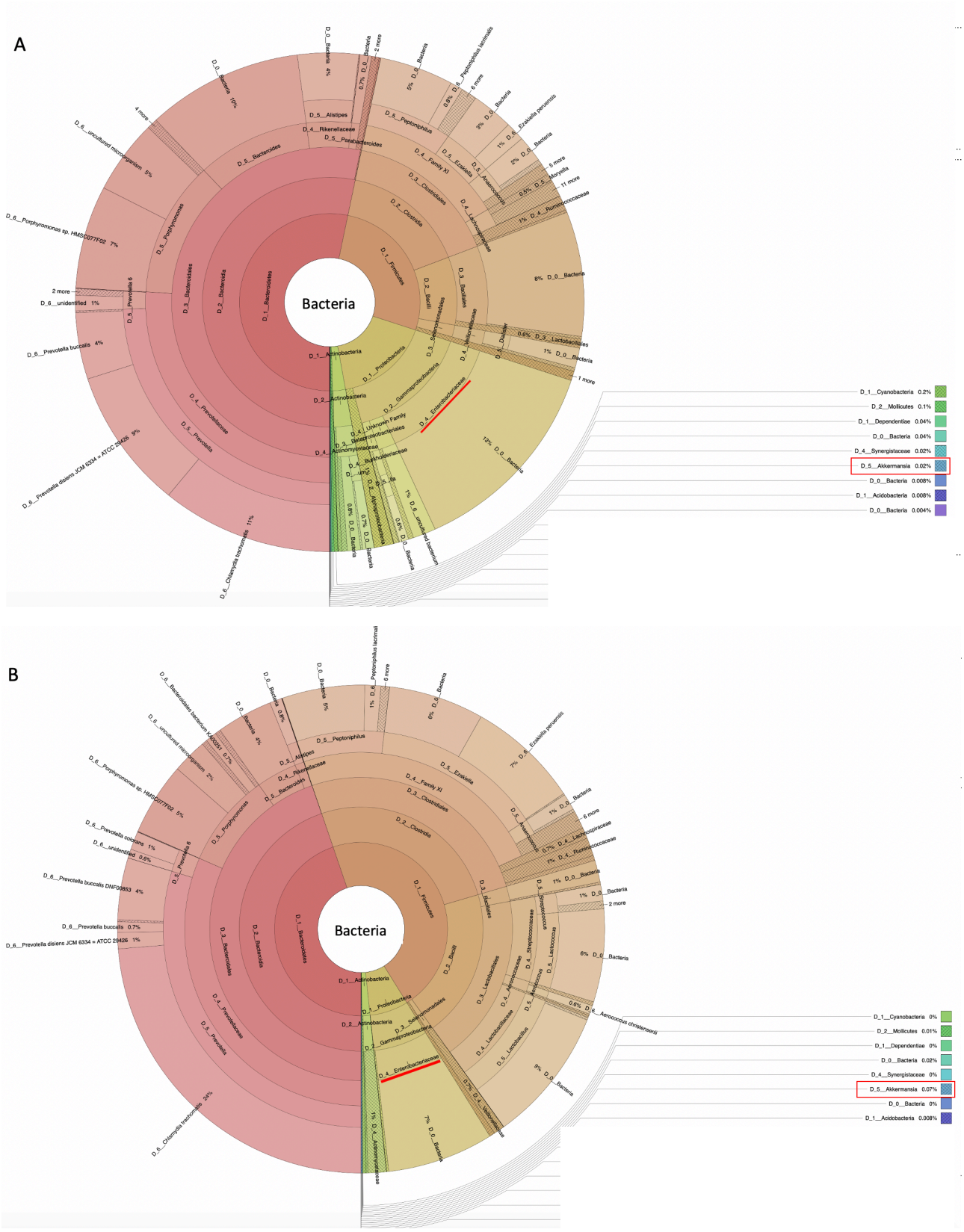

Figure 11 The relative abundance of taxa in urine samples after deployment of 80 % compliance limit. Used reference data Silva. Krona plots shows relative abundances at different taxonomic levels, from the center ring outward, lowest to highest identified taxonomic level is listed. When the highest identified taxonomic level is reached, outer ring contains lower taxonomic levels. Plots: A=Control, B= CLJ.

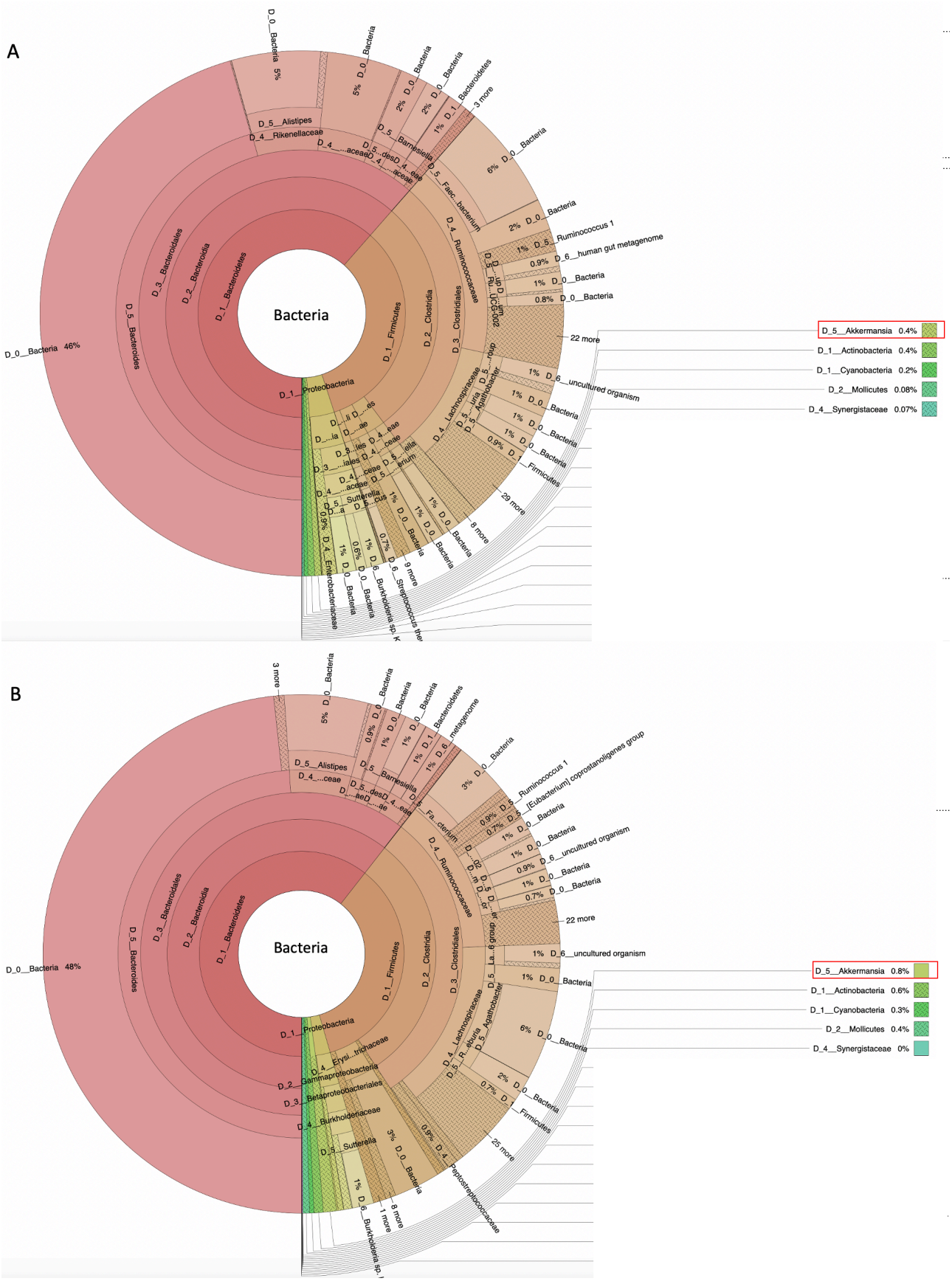

Figure 12 The relative abundance of taxa in feces samples after deployment of 80 % compliance limit. Used reference data Silva. Krona plots shows relative abundances at different taxonomic levels, from the center ring outward, lowest to highest identified taxonomic level is listed. When the highest identified taxonomic level is reached, outer ring contains lower taxonomic levels. Plots: A=Control, B= CLJ.

Table VIII ASV-based differential abundance test results

SILVA- Urine

	ASV	p_value	BH_FDR	Kingdom	Phylum	Class	Order	Family	Genus	Species
1	X8192e85fe5597e456b80adf23c6f855a	0.0204	0.3111	<NA>	<NA>	<NA>	<NA>	<NA>	<NA>	<NA>
2	fd40c2bafd3d2210e7eacb0ed6c6f9a0	0.0204	0.3111	k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	k__Bacteria	p__Firmicutes	c__Clostridia
3	X6321b1f518746de382217f61a70874cb	0.0204	0.3111	<NA>	<NA>	<NA>	<NA>	<NA>	<NA>	<NA>
4	X174a44be84eb24b6fd6aade8d1d7ad7a	0.0346	0.3111	<NA>	<NA>	<NA>	<NA>	<NA>	<NA>	<NA>
5	X77034556117fd9ae9546fdabcaa458b4	0.0441	0.3111	<NA>	<NA>	<NA>	<NA>	<NA>	<NA>	<NA>

SILVA- Feces

	ASV	p_value	BH_FDR	Kingdom	Phylum	Class	Order	Family	Genus	Species
1	cba56d7b61d990145e0a6f42c072eec6	0.0127	0.9677	k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Ruminococcaceae	g__Oscillospira	s__
2	X75851d70210b9cfcaa48f6f69f33e1d6	0.0162	0.9677	<NA>	<NA>	<NA>	<NA>	<NA>	<NA>	<NA>
3	X62f9258d944807cf94b3c9bfc19071d1	0.0237	0.9677	<NA>	<NA>	<NA>	<NA>	<NA>	<NA>	<NA>
4	X45730ee0adc0dc5f26f0a605ac7bc14a	0.0255	0.9677	<NA>	<NA>	<NA>	<NA>	<NA>	<NA>	<NA>
5	X4b29c8321281c949ef15f8072fcac607	0.0365	0.9677	<NA>	<NA>	<NA>	<NA>	<NA>	<NA>	<NA>

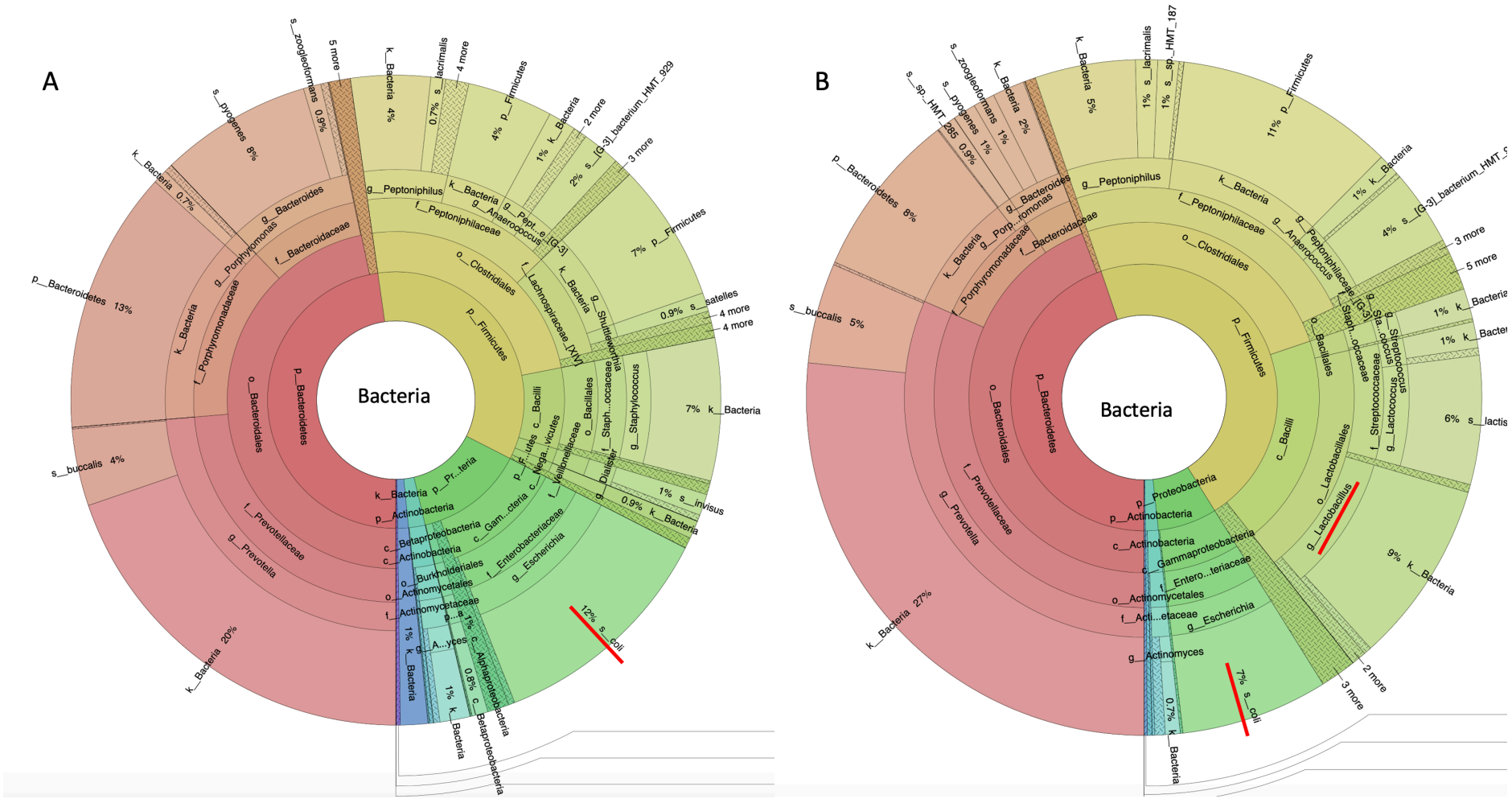

Figure 13 The relative abundance of taxa in urine samples after deployment of 80 % compliance limit. Used reference data HOMD. Krona plots shows relative abundances at different taxonomic levels, from the center ring outward, lowest to highest identified taxonomic level is listed. When the highest identified taxonomic level is reached, outer ring contains lower taxonomic levels. Plots: A=Control, B= CLJ.

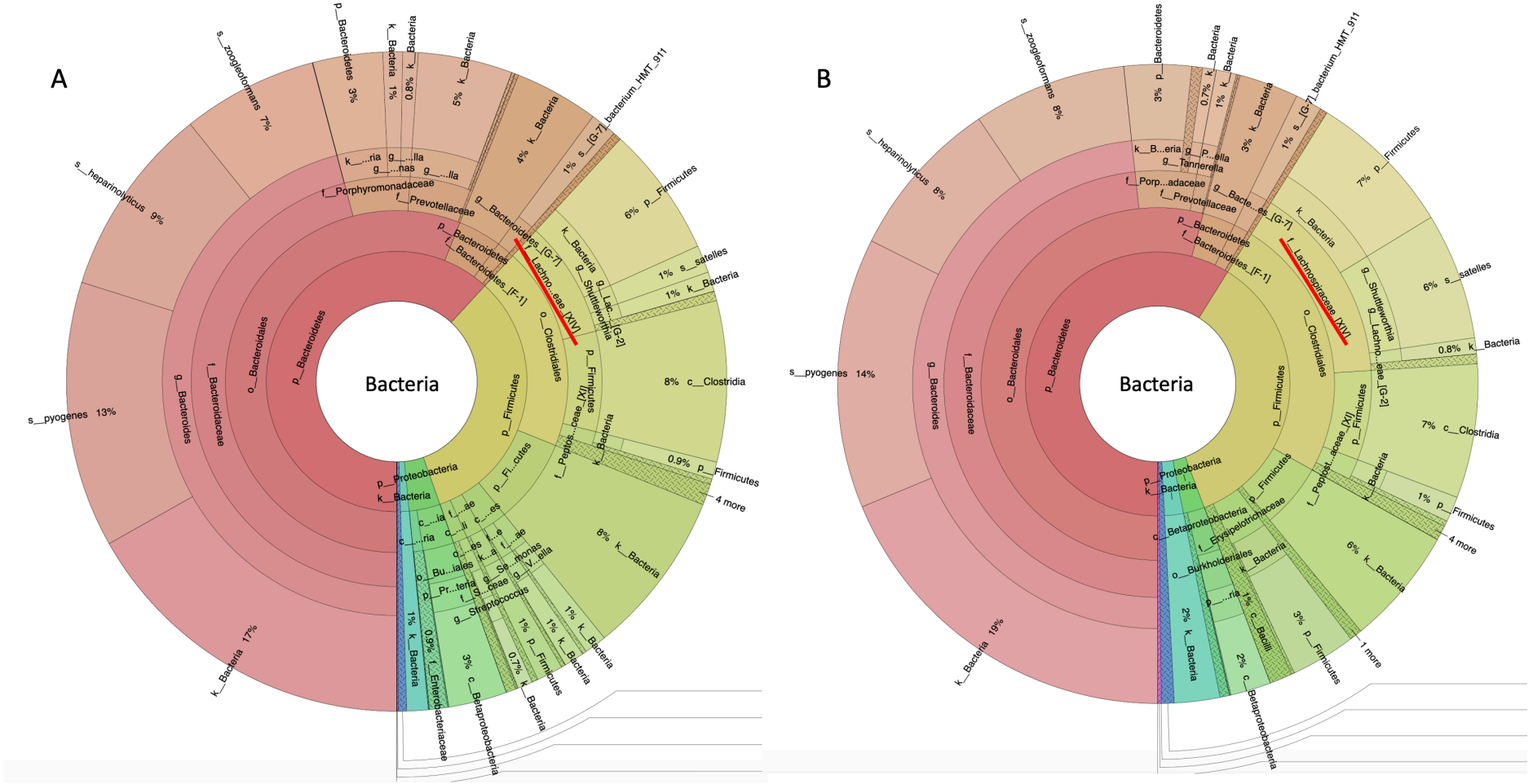

Figure 14 The relative abundance of taxa in feces samples after deployment of 80 % compliance limit. Used reference data HOMD. Krona plots shows relative abundances at different taxonomic levels, from the center ring outward, lowest to highest identified taxonomic level is listed. When the highest identified taxonomic level is reached, outer ring contains lower taxonomic levels. Plots: A=Control, B= CLJ.

4.7. Abundance of specific urinary tract causing bacteria

The heat tree analysis (Figure 10) and Krona plots (Figures 11-12) had already indicated differences in the Proteobacteria phylum between CLJ and control groups. Therefore, the abundance of bacterial pathogens typically connected with UTI: *E. coli, Klebsiella* spp., *Pseudomonas aeruginosa, Enterococcus* spp., *Proteus* spp., *Enterobacter faecalis, Staphylococcus saprophyticus*, *Streptococcus agalactiae* and *Acinobacter* were searched for from taxonomic data done with Silva -, Greengenes - and HOMD- reference databases. From those, *E. coli* and *S. agalactiae* and at genus level *Enterococcus, Pseudomonas* and *Staphylococcus* were identified by using HOMD- reference database.

After deployment of 80 % compliance limit, the three months urine and feces data were used to build boxplots that show relative abundances of identified taxa (Figure 15). *S. agalactiae* was identified only in CLJ group urine samples.

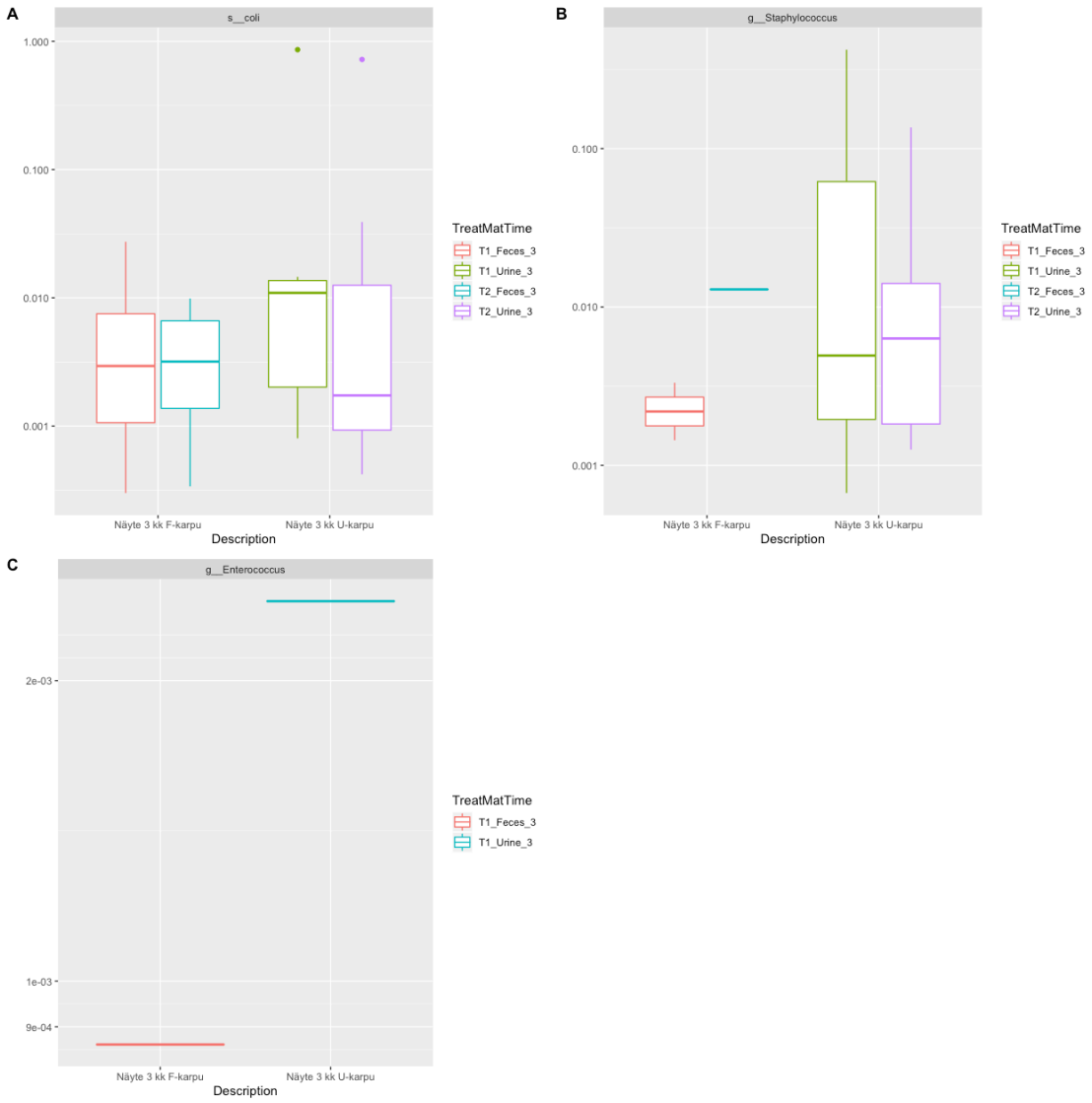

Figure 15 UTI connected pathogens relative abundance. A= *E. coli,* B= *Staphylococcus* & C=*Enterococcus*

Heatmap was used to visualize differences in abundance of UTI-related *E. coli, Streptococcus, Enterococcus, Pseudomonas* and *Staphylococcus* strains in urine and feces samples of patients in both treatment groups. Results in Figure 16 show that CLJ group patients have less *E. coli* strains in feces and urine samples than those in control group. There are also more other UTI connected bacterial strains, such as *Enterococcus*, in control group feces and urine samples than in CLJ group (Figure 17).

Alpha and Beta diversity was measured from data limited with UTI related ASVs. UTI related ASVs in feces samples was not normal distributed and in urine samples was normal distributed. Alpha diversity measures are shown in Figure 16. There were no significant differences in urine or feces samples in Alpha or Beta diversity.

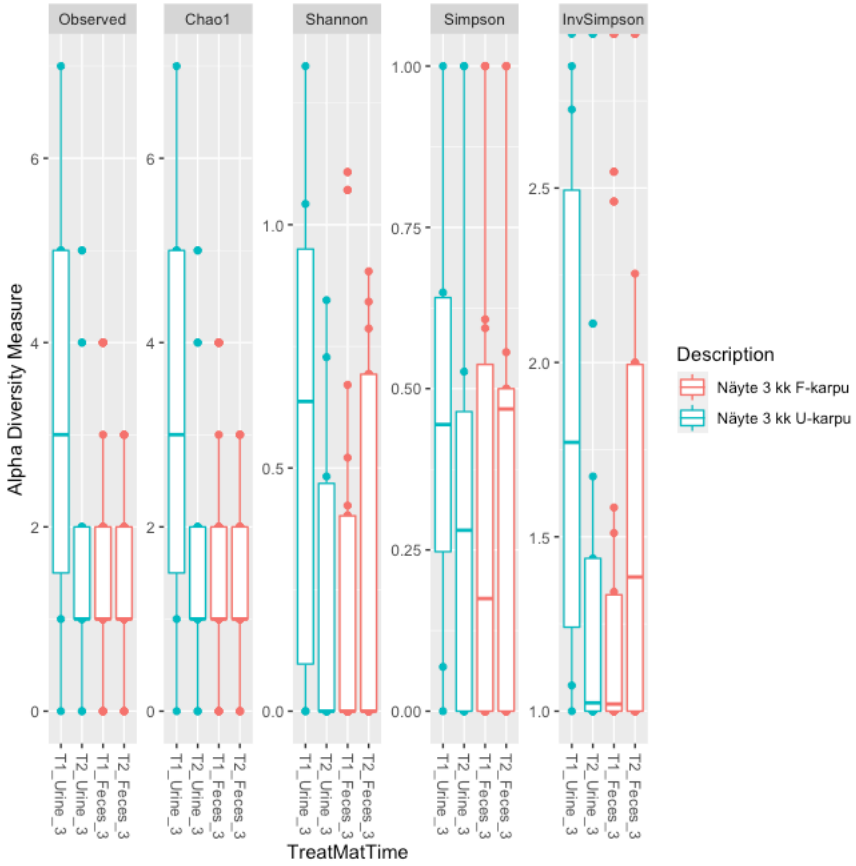

Figure 16 Alpha diversity metrics. Each measure is listed above each plot, and x-axel contains groups. Groups are divided by treatment, sample material and collection time in months. Last two are shown also as a color code: Turquoise indicates urine samples and red indicates 3 months feces samples.Y-axel contains abundance metrics values.

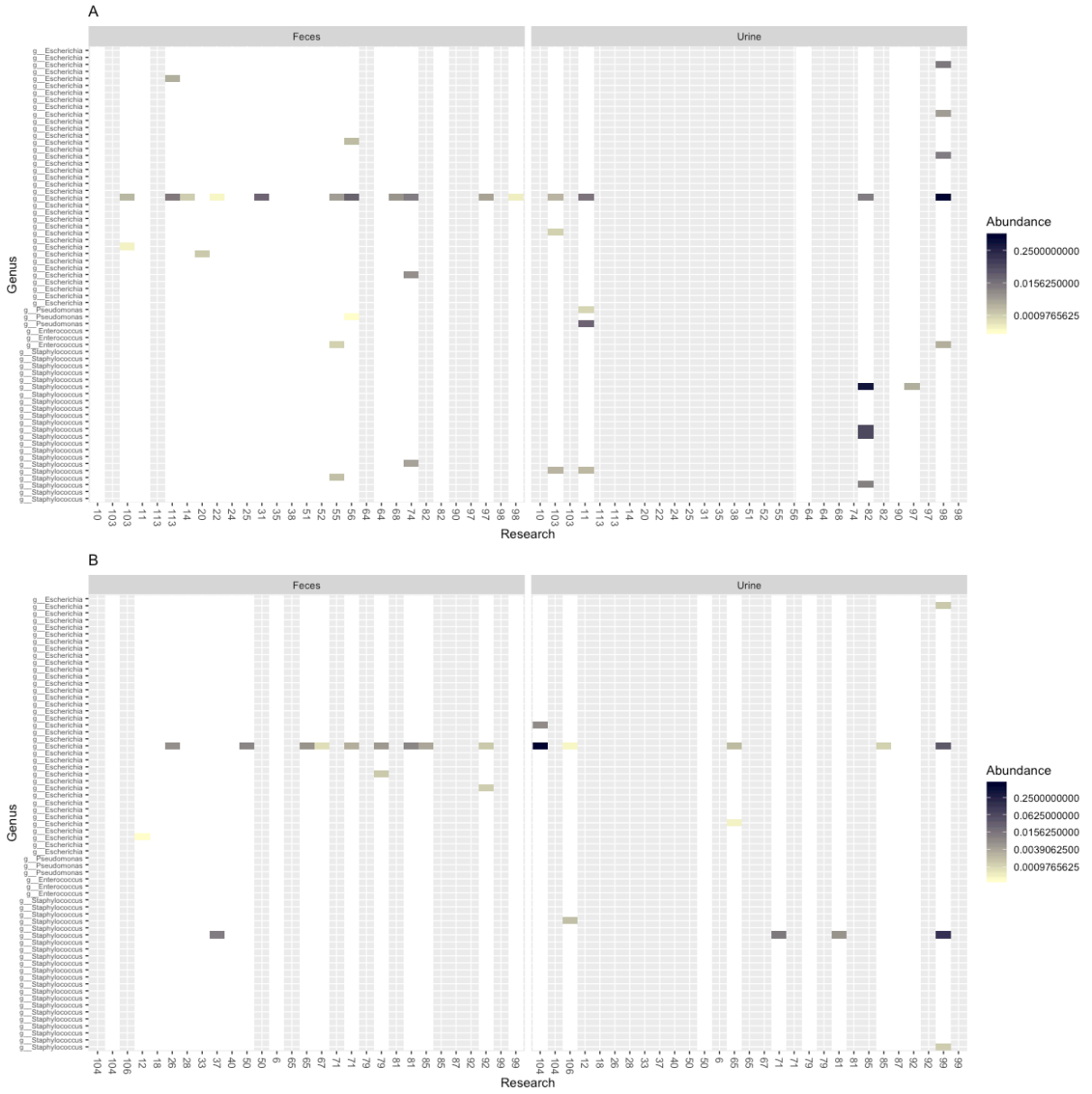

Figure 17 Heatmap shows differences between sample material in patients at different treatment groups. Darker color indicates higher relative abundance. A= Control; B=CLJ.

5. Discussion

5.1. Microbiome sequencing and analysis

The aim of this research was to study effect of Cranberry-Lingonberry juice consumption on gut and urinary tract microbiome. The microbiome was analyzed by sequencing the V1-V8 variable regions of the ribosomal 16S RNA gene. The 16S ribosomal RNA barcodes based on V1-V8 regions have produced good results in earlier research (Chakravorty, 2007). Those have been successfully used to identify many bacterial species from small extracted amount of genetic material.

Healthy gut microbiome composition varies between sex, age groups and even between individuals, but most dominant bacterial phyla are often the same. Typically, Firmicutes and Bacteroidetes represent 90 % of gut microbiome. Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia are also common to gut microbiome. In different conditions and under specific diet, those ratios can change (Huttenhower et al., 2012). The biggest problem in my study was finding the best alignment reference database so that the required taxa could be identified. Silva, Greengenes and HOMD have their pros and cons. Silva and Greengenes identified much more ASVs, but UTI related bacteria identification was poor. HOMD identified UTI related bacteria but had overall poor identification level.

For alpha and beta diversity differences, the null hypothesis remained valid, and they were in many ways similar between groups. According to our results, this is good because patients involved in the research were the same aged children with a similar background. If something significant had been discovered, reason would have been something more than just CLJ-effect (Huttenhower et al., 2012; Quinn et al., 2018). However, there were differences in relative abundances at species, phylum and family levels between treatments, which may serve as a basis for deeper research.

5.2. Microbiome composition in cranberry-lingonberry consumption group and control group

Biggest difference in this study was observed between sample materials (feces or urine). This was expected, as according to current knowledge our body has different microbial composition between different body parts. Urine samples indicate urinary tract area and feces samples gut environment (Huttenhower et al., 2012).

In most of the samples, Bacteroidetes and Firmicutes dominated the microbiome. Other studies have shown that age and body mass index (BMI) can affect the Bacteroidetes and Firmicutes ratio

(Mariat et al., 2009; Koliada et al., 2017). The effect of antibiotics on Bacteroidetes/Firmicutes ratio has also been studied, where the antibiotic treatment increased the ratio between the two phyla (Dubourg, et al., 2013). Proteobacteria relative abundance also changed between samples regardless of treatment in this study. Typically, healthy individuals have low abundance of Proteobacteria, and disease state correlates with its relative abundance (Shin et al., 2015).

The cranberry-lingonberry juice (CLJ) had no such effect on relative abundance of gut microbiome as has been shown earlier on red wine polyphenols, increase in abundance of the phyla Proteobacteria, Actinobacteria, Fusobacteria, Firmicutes, and Bacteroidetes (Queipo-Ortuño et al., 2012). There were differences between samples, but cranberry-lingonberry juice consumption was not clearly causing an increasing trend in those phyla. However, in our randomized trial, there were 77 children, and in Queipo-Ortuño et al. (2018) randomized trial, there was only ten male participants, which could explain the differences. Also, the time period between trials was different. In our study samples were collected in one-year period, their study period was only 20 days. Our experimental setup allowed also other sources of polyphenols for the children. Therefore, our research data cannot be regarded to show polyphenol overall effect on gut microbiome at phylum level. As has been shown, polyphenols include a wide variety of compounds, of which most have effect on microbiomes at genus and species level.

5.2.1. Urinary tract microbiome

Heat tree shows that with most species, the log2 ratio between treatments was near zero, but every treatment group also contained some extremes. CLJ group urine samples had higher abundance, at order-level, Clostridiales and at class-level Bacteroidia. Control group contained more Firmicutes and Proteobacteria at phylum level. Many UTI causing pathogens belong to Proteobacteria, so this finding might indicate that CLJ has some effect to bacterial adhesion in urinary tract (Behzadi et. all, 2010; Garout et al. ,2015).

After deployment of 80 % compliance limit results confirmed the finding, although taxa- or ASV-based differential abundance tests after FDR correction did not produce statistically significant differences. Urine krona plot shows that there are 7 % less Proteobacteria and 5 % less *E. coli* in CLJ group than in control group. The relative abundance of *E. coli* varies a lot between urine samples regardless of the treatment, i.e. there are ASVs for which relative abundance is less than 0.001 and others for which relative abundance is over 0.9. Typically, UTI and other illnesses cause *E. coli* to peak in urine and fecal samples (Garout et al., 2015). *E. coli* peak in urine samples could indicate that patients still has UTI. *E. coli* relative abundance median for urine samples is much lower in CLJ

group. Also, Kruskal Wallis test showed that phylum Proteobacteria difference was significant before FDR-correction. Regardless of losing significance after FDR correction, the results support the hypothesis that CLJ decreases amount of *E. coli* in urinary tract microbiota and affects against bacterial adhesion (Sobota et al. 1984; Zafriri et al., 1989; Raz et al., 2004; Kylli et al. 2011; Tapiainen et al., 2012; Kline et. al., 2016; Wojnicz, 2016; Paalanne et al., 2018).

Interesting finding was that Firmicutes relative abundance was lower in control group (< 11 %) with deployment of 80 % compliance limit than without limitation. Heat tree- plot showed reverse results. Firmicutes and Bacteroidetes are typically the most dominant phylas in the human microbiota (Huttenhower et al., 2012). Relative abundance plot also showed that there are relative huge differences in samples regardless of the treatment.

Even so, further analysis using HOMD- alignment database showed interesting results although differential abundance test did not show those to be significant. CLJ group contained more genus *Lactobacillus* members. There was 9 % *Lactobacillus* out of total bacteria. In control group the *Lactobacillus* relative abundance in urinary tract microbiome was minimal. This bacterial genus is typical to normal urinary tract microbiome (Hilt et al., 2014). There is also evidence that berries flavan-3-ols favor *Lactobacillus* (Cardona et al., 2016). Members of phylum Firmicutes could be colonizing the free space or the difference could be caused by another factor. More research should be done according to these findings.

Krona plot of urine three months samples also showed 6 % difference in genus *Staphylococcus* between CLJ- and control-treatments. CLJ group relative abundance was lower, but boxplot showed that median in groups was nearly same. Also, the differences were not statistically different in taxa or ASV based tests. Heatmap shows that different members or strains of genus *Staphylococcus* are found in patients of different treatment groups. Interesting would be to find out which of those are pathogenic to human. It has been shown that *Staphylococcus* causes UTI and berry extracts can inhibit it *(*Imirzalioglu et al, 2008; Puupponen-Pimiä *et al., 2005).* Although, genus *Staphylococcus* members are part of healthy urinary tract microbiome (Hilt et al., 2014).

Mann Whitney test results showed that the genus *Finegoldia* had significantly differential abundance between treatments in urine samples, but there was no available literature about its connection to UTI or cranberry and lingonberry polyphenols. Also, there were significant differences in genera *Fastidiosipila* and *Negativicoccus* before BH-FDR correction. Even so, in literature those genera have no any clear connection to UTI or polyphenols.

5.2.2. Gut microbiome

Three months feces samples had significant differences in phylum Cyanobacteria. Closer look showed that the identified phylum included two classes, chloroplast and 4C0d-2. Of those, chloroplast (cp) was more abundant in CLJ group and 4C0d-2 in control group. Even so, relative amount of phylum Cyanobacteria was under 0.3 % in both treatment groups and there is no literature about connections to UTI. Still, interesting is that urine from CLJ group contained seemingly more chloroplast than control group. Berries cells include chloroplasts, and the cpDNA might be present in berry juice, so those can be originated from CLJ.

There was also significant difference in genus *Escheria-Shigella* before BH-FDR correction. By Krona plot, genus was identified to contain only *E. coli* members. In CLJ group with deployment of 80 % compliance limit *E. coli* relative abundance was 0.3 % and in control group 0.5 %. This finding could indicate that CLJ has effect on gut microbiome and possible UTI related pathogen strains (Paalanne et al., 2018).

For many biochemical compounds, microbial metabolism is needed before their antioxidant properties can be utilized in the human body (Cassidy & Minihane, 2016; Chen et al., 2014). From our data such bacteria, *Eggerthella lenta* and *Coprococcus eutactus* were identified. *E. lenta* is member of phylum Actinobacteria and can catalyze the dihydroxylation of flavonoids in human gut (Braune & Blaut, 2016). Its relative abundance after deployment of 80 % compliance limit in CLJ group feces samples in three months timepoint was 0.09 % and in control group 0.04 %. This could suggest abundance rising for bacteria that can catabolize cranberry polyphenols, however the difference was not significant. *C. eutactus* can produce butyrate metabolite from polysaccharides in gut (Wang, et al., 2019). Other taxa earlier connected with polyphenol effects on gut microbiome (Cardona et al., 2013; Braune & Blaut, 2016) were not identified. Even so, polyphenols are shown to affect microbiome in other studies, so these results should be taken under closer examination in the future *(*Cardona et al., 2013).

Akkermansia and *Faecalibacterium* were also identified in three months fecal and urine data alignment by Silva reference database. Abundance rise of both genera has earlier been shown to be connected to lingonberry polyphenols (Heyman-Lindén et al., 2016). There were not significant differences between treatments in this study. CLJ group contained genus *Akkermansia* in 0.8 % of total bacterial abundance in feces samples and 0.07 % in urine samples. Abundance of *Akkermansia* was much less in control group, as fecal samples contained 0.4 % and urine samples 0.02 % of *Akkermansia* out of total bacterial abundance. Although the results were not significant, this suggests that CLJ polyphenols might change gut microbiome.

5.2.3. PACs longtime effect to gut microbiome

In three-month fecal treatment group, highly different abundant species were found in Bacteroidales and Clostridiales according to heat tree-plot. Both orders contained members that were more common under both treatments. Earlier studies have shown that polyphenol-rich diet caused ecological shifts in gut microbiome of pigs, and Lachnospiraceae- and Ruminococcaccea-families were ones with increased abundances. On the other hand, that study used PACs from grape seeds, which contain more B-type PACs (Choy, Y. et al, 2014) compared to the A-type PACs of cranberry and lingonberry. Krona plots done using Silva alignment database were used to get closer look for differences. After deployment of 80 % compliance limit, Lachnospiraceae-family members were more abundant in CLJ group. There was 5 % difference between treatment groups in bacterial relative abundances. ASV based differential abundance test also showed that family Ruminococcaccea member, genus *Oscillospira*, reached level of significance before FDR correction in three months sample group with deployment of 80 % compliance limit. Connection of *Oscillospira* to polyphenols has not been identified.

Lachnospiraceae and Ruminococcacceae -family abundance increased across time in fecal samples under CLJ-treatment. According to the study design, the CLJ effect should be visible from three months samples on, so this finding might be connected to CLJ effect on gut microbiome even though the result was not statistically significant (Choy et al, 2014).

Overall the heat tree analysis results supported hypothesis that CLJ could influence gut microbiome, because the species occurrence was different between treatment groups. There were ecological shifts in the order Clostridiales, and phyla Proteobacteria and Verrucomicrobia across time in the fecal samples. There were families and genera for which abundance under treatments changed across time. E.g. one Enterobacteriales member, for which the abundance was higher in CLJ group than in the control group in fecal samples of twelve-month timepoint, has higher abundance in control group in six-month timepoint. This is interesting, because many Enterobacteriales members, *E. coli, Klebsiella* sp*., Proteus* sp. and *Enterobacter* sp., are connected to UTI and typically related to urinary tract microbiome (Behzadi et. all, 2010; Garout et al. ,2015). There could also be other reasons for such fluxes besides treatment, such as natural variation and study design (Huttenhower et al., 2012). To get statistically significant results, the differences could be further analyzed in six - and twelve months feces samples with deployment of 80 % compliance limit and by using HOMD- alignment database.

5.3. Gut – and urinary tract microbiome connection

To examine the hypothesis that UTI-causing pathogenic strains enter urethra from gut microbiome, which is potentially affected by CLJ, heatmaps on potentially UTI-causing strains were built and compared between patients' urine and gut samples. Heatmap showed that there mainly are different bacterial strains of *E. coli* in research patients' urine and feces samples. However, some strains were only found from control group feces and urine samples. This finding shows that PACs might affect bacterial conditions and favor specific *E. coli* strains over others. Many earlier studies show that cranberry proanthocyanidins may inhibit the adhesion of type I and P-fimbriated uropathogens (Gupta K et al., 2007; Lavigne et al., 2008; Zafriri et al., 1989). Members in the family Enterobacteriaceae typically have such an adhesion mechanism, for example, *E. coli*. *E. coli* strains are also a common part of normal microbiome, typically found in feces. Therefore, differentiating a pathogenic version from "normal inhabitants" is hard. More studies should be done to find out which of the strains are uropathogenic.

Differences were also found among strains of *Streptococcus* and *Staphylococcus* genera. Both consist UTI related pathogen strains and *Streptococcus agalactiae,* which is related to UTI, was identified. Interestingly, it was found only in CLJ group urine samples.

Genus *Streptococcus* was less abundant in CLJ group (0.3 %) than in control group (0.7 %) feces samples, and in urine samples, the situation was opposite. In CLJ group the abundance was 2 % and in control group 0.2 %. On the other hand, *Staphylococcus* was more abundant in CLJ group (0.08 %) than in control group (0.02 %) feces samples, but in urine samples, the situation was opposite. In CLJ group the abundance was 1 % and in control group 6 %. Even though differential abundances between CLJ and control group in these genera were not statistically significant, differences could be caused by PACs effect to bacterial adhesion.

There was also a couple of patients in control group that had members of *Pseudomonas* genus in their gut and urinary tract microbiota. Also, *Enterococcus* strains were found in patients' samples. An earlier study has shown that cranberry extract inhibits *Enterococcus faecalis* growth and enzymatic activities and limits biofilm formation *in vitro* (Wojnicz et al., 2016). Our results showed that there were a couple of patients in control groups containing relative high abundance of genus *Enterococcus* strains, but in other patients its abundance was limited. Therefore, there could be some connection between treatment and abundance of this bacterium.

6. Conclusions and outlook

There were significant differences in microbial abundance at phylum, genera and even ASVs level between CLJ and control group treatments, and some findings reached level of significance, but lost it after BH-FDR correction. In urine samples with deployment of 80 % compliance limit genus *Finegoldia* was differentially abundant between CLJ and control group. However, there was not literature about its connection to UTI or polyphenols. Difference in phylum Proteobacteria reached level of significant before BH-FDR correction. As our hypothesis is that CLJ PACs might affect UTI related pathogen adhesion in urinary tract, this finding is the most important. Many UTI-related pathogens including *E. coli* belong to phylum Proteobacteria. In feces with deployment of 80 % compliance limit there was significantly differentially abundant taxa before BH-FDR. Most important was *Escheria-Shigella* which was later identified to present *E. coli* at species level. This finding indicates that CLJ might affect UTI related bacteria in gut environment.

After application of compliance limit relative abundance results became more rational although Alpha and Beta diversity analysis results had no change. The most interesting, although statistically non-significant, finding was that there were clearer differences in the relative abundance levels of *E. coli* in the urine samples after application of compliance limit. With compliance limit applied, the relative abundance median of *E. coli* in samples was much lower in CLJ treatment group samples. The relative abundances of *E. coli* were also lower, potentially indicating that CLJ has an effect on bacterial adhesion.

Abundance of another UTI causing genus *Staphylococcus*, which belongs to Firmicutes phylum, was also lower in the CLJ group, even though levels of Firmicutes overall were higher in the CLJ group. On the other hand, control group samples contained such abundances of *Enterococcus* and *Pseudomonas* genus that were not identified in the CLJ group samples. These results could also suggest that cranberry-lingonberry juice consumption changes gut and urine microbiota, specifically on potentially pathogenic strains, and may through these mechanisms affect occurrence of UTI. More accurate reference alignment data might help to identify taxa at species level and show more interesting differences.

Many factors affect gut microbiome, and many factors are also connected with effects of polyphenols on the body and on the microbiome. E.g. UTI is more common in women than in men, and *E. coli* is more common UTI causing organism in boys than in girls. Therefore, there can be differences between boys and girls on their urinary tract inhabitants and relative abundances of

pathogens under various treatments. Furthermore, gram-positive UTI causing bacteria are common in patients with other UTI risk factors (Kline & Lewis, 2016). Medication and diet might also influence the results on gut microbiome (Chen et al, 2014; Choy et al., 2014; Mariat et al., 2009; Koliada et al., 2017).

These factors should be mapped out in participants of the research. Information about past antibiotics uses, dietary and medical history could be utilized in statistical analysis to provide more information about CLJ effect on gut microbiomes at Phylum- level ratios. Wider hospital metadata information might be good for subgroup studies.

Medical history of the patients recruited for this study would enable comparisons of groups shared by similar UTI history. Urine samples of patients with similar UTI history under CLJ treatment should contain similar uropathogens. It would be interesting to study more which microbial groups that are tolerant to CLJ polyphenols utilize and colonize the space freed by sensitive species. Other studies have shown that the combination of probiotics and AC-PACs give best results against UTI (Polewski, 2016). Our findings support those. Could a healthy diet itself drive ecological shift towards higher mutualism in gut microbiome without added medication?

Studies have shown that fecal and urine samples typically contain colonic degradation products of major PACs (Cassidy & Minihane, 2016). PAC and other phenolic metabolites concentration should be measured in samples. Earlier study done with animal models has shown that there might be connection between fecal iron and food-borne pathogenic bacteria in the gut. Iron deficiency reduces pathogenic bacterial strains in the gut (Kortman et al., 2015). Paalanne et al. (2018) studied fecal iron and lactoferrin concentrations in research where UTI patient's microbiome was compared to control group microbiome. Results were not statistically significant and further research would be needed (Paalanne et al., 2018). Chemical information combined with microbial data from samples could give more information about CLJ long-time effects on gut and urinary tract microbiome and microbial colonization. A-type PACs have also reduced *Candida albicans* adhesion properties, therefore, fungal analysis could also be done (Rauf et al., 2019).

In the future, when more information about microbial metabolism is gathered, this data could be used for analyzing the role of bacteria in the decomposition of polyphenols. Work is still in progress, and information from *in vitro* studies cannot be fully utilized until more *in vivo* studies have been done. And more *in vitro* studies are needed for understanding what takes place in the microbiome of a higher organism, so dialogue between these research fields shall continue

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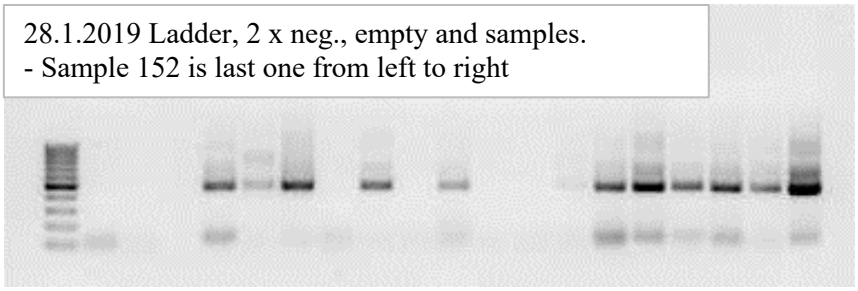
Pictures

Vaccinium (Ericaceae): Ethnobotany and pharmacological potential - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Cranberry-A-type-proanthocyanidin_fig1_261878995 [accessed 30 Oct, 2019]

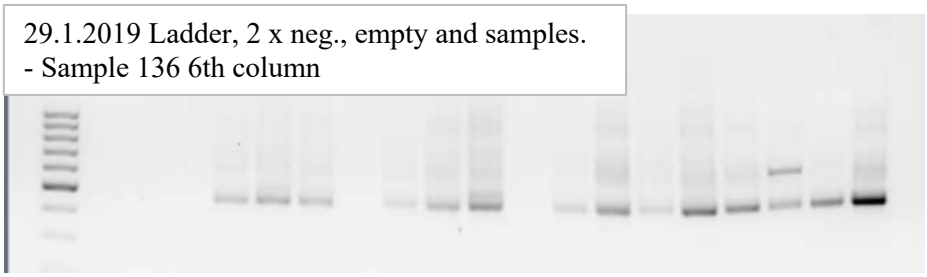
Appendix

Electrophoresis pictures

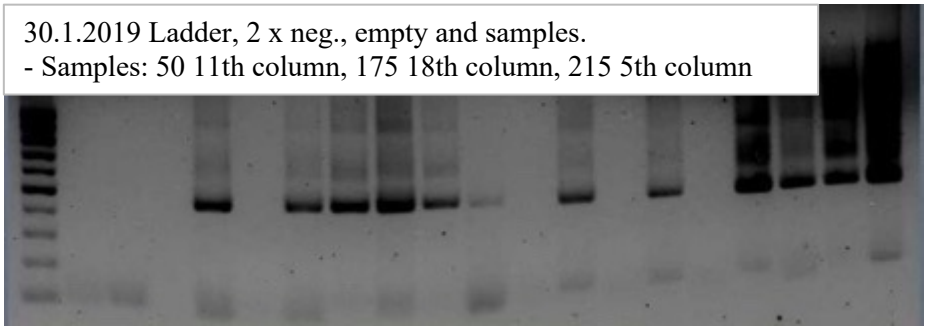
28.1.2019 Ladder, 2 x neg., empty and samples.
- Sample 152 is last one from left to right



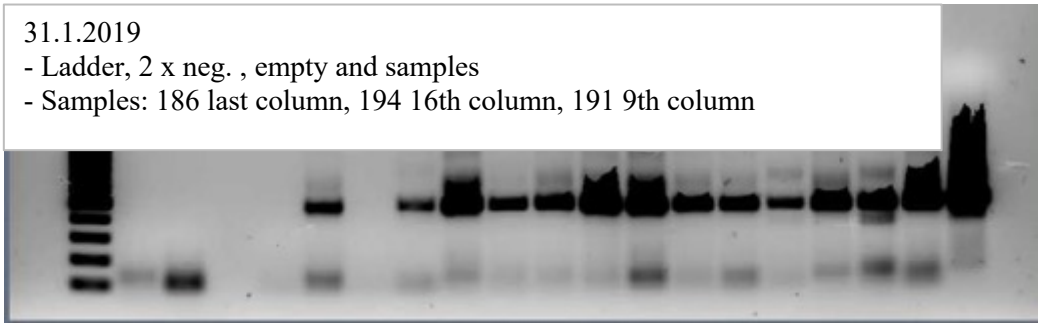
29.1.2019 Ladder, 2 x neg., empty and samples.
- Sample 136 6th column



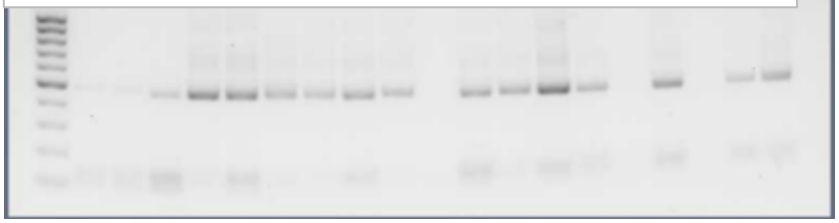
30.1.2019 Ladder, 2 x neg., empty and samples.
- Samples: 50 11th column, 175 18th column, 215 5th column



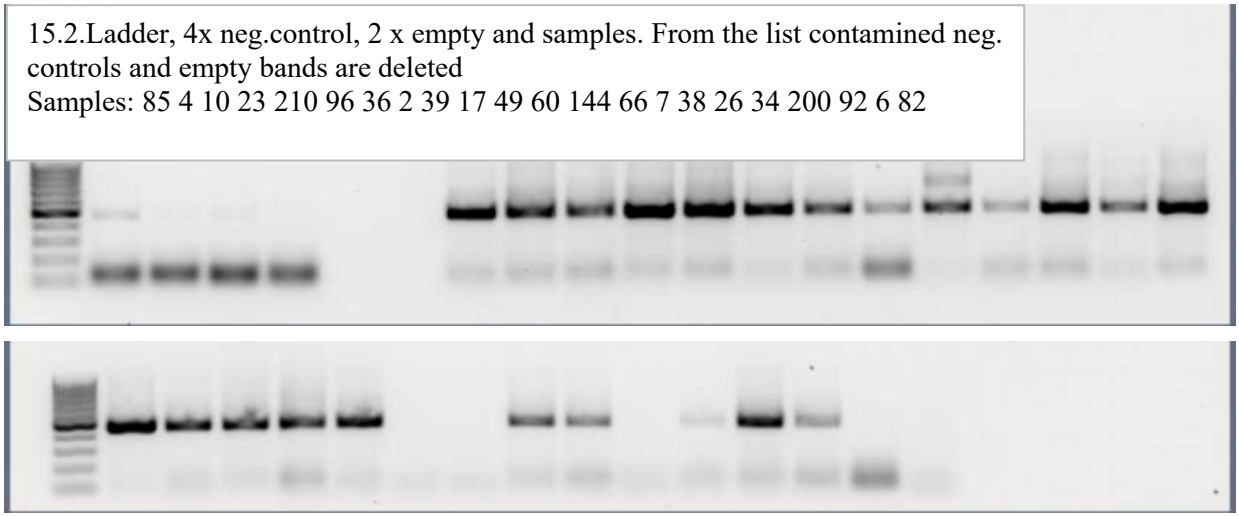
31.1.2019
- Ladder, 2 x neg. , empty and samples
- Samples: 186 last column, 194 16th column, 191 9th column



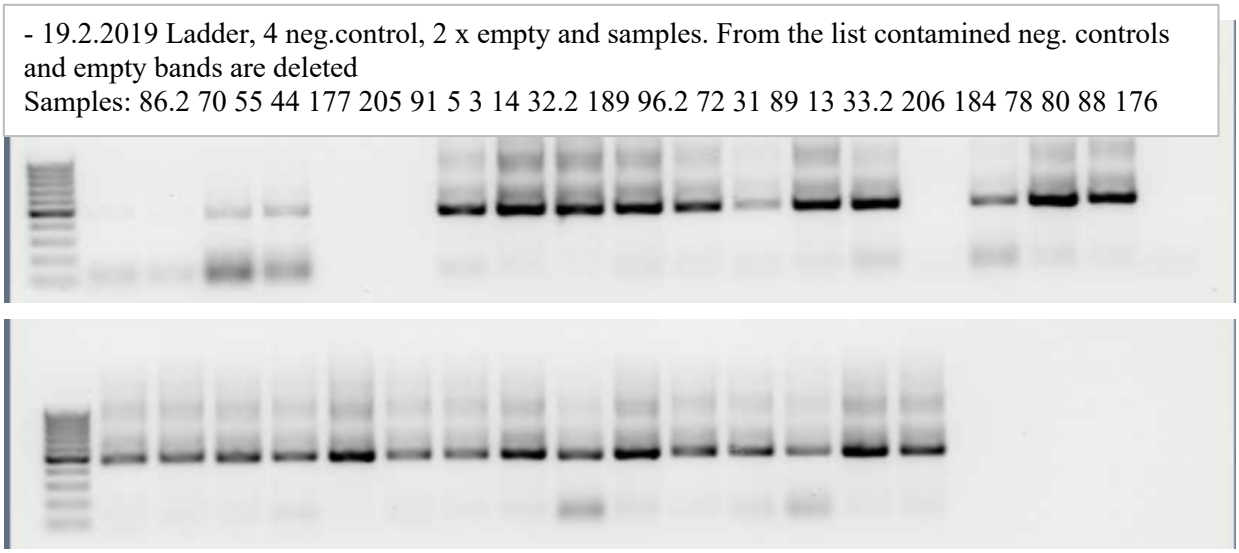
11.2.2019 Ladder, 3 x neg. and samples
- Samples: 7 19th column, 38 14th column



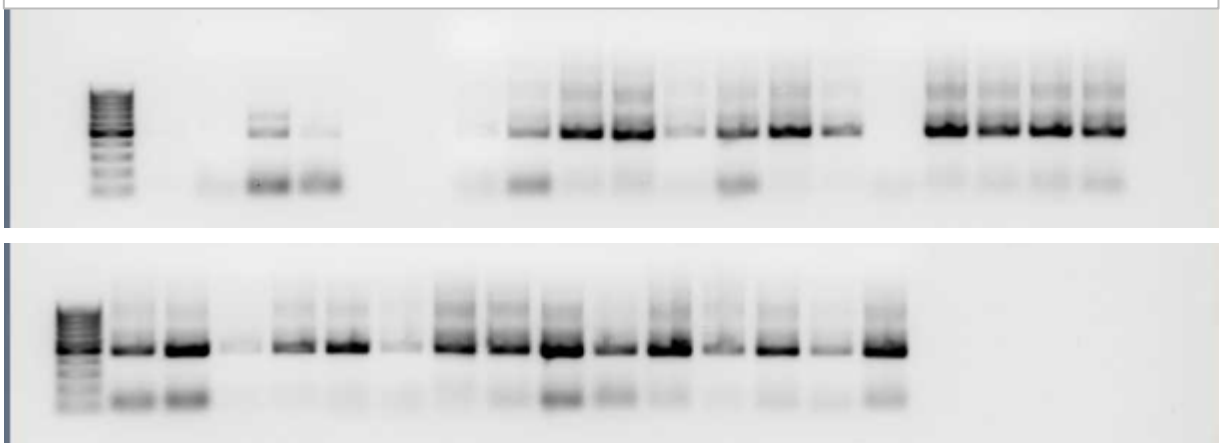
15.2.Ladder, 4x neg.control, 2 x empty and samples. From the list contamined neg. controls and empty bands are deleted
Samples: 85 4 10 23 210 96 36 2 39 17 49 60 144 66 7 38 26 34 200 92 6 82



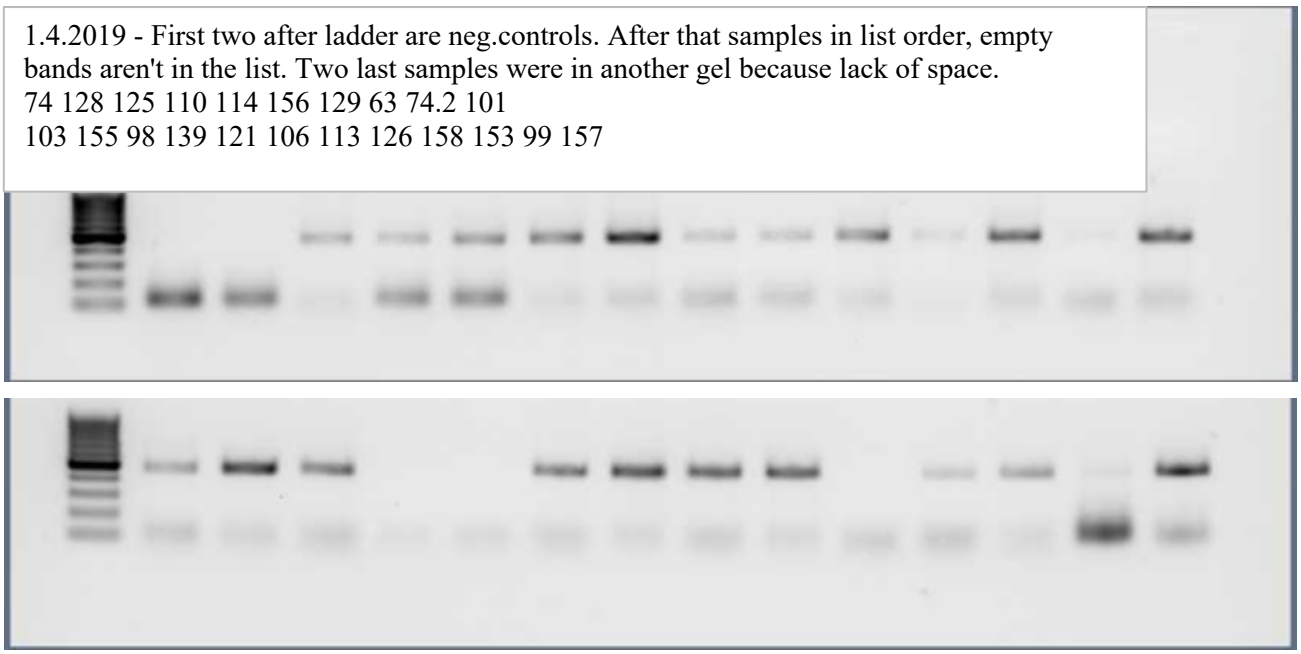
- 19.2.2019 Ladder, 4 neg.control, 2 x empty and samples. From the list contamined neg. controls and empty bands are deleted
Samples: 86.2 70 55 44 177 205 91 5 3 14 32.2 189 96.2 72 31 89 13 33.2 206 184 78 80 88 176



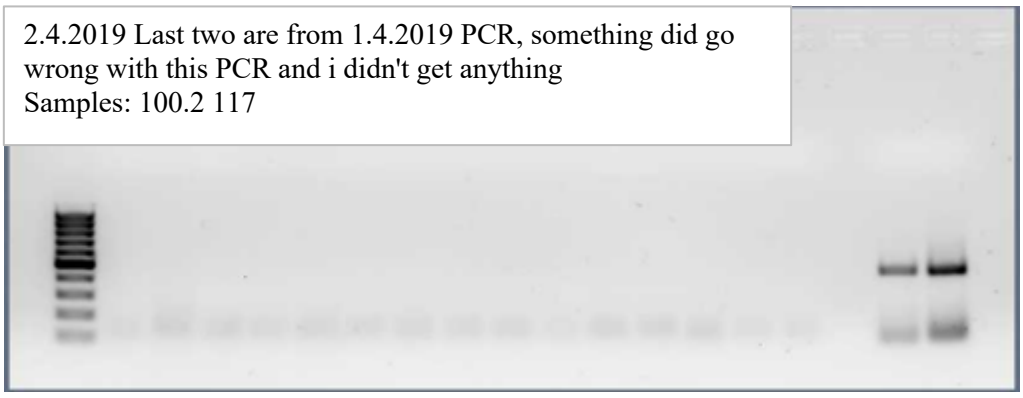
25.2.2019 Ladder, 4 neg.control, 2 x empty and samples. From the list contamined neg. controls and empty bands are deleted
Samples:46 51 57 53 138 199 15 9 48 11 54 193 28 90 95 8 47 20 203 207 83 81 140 201



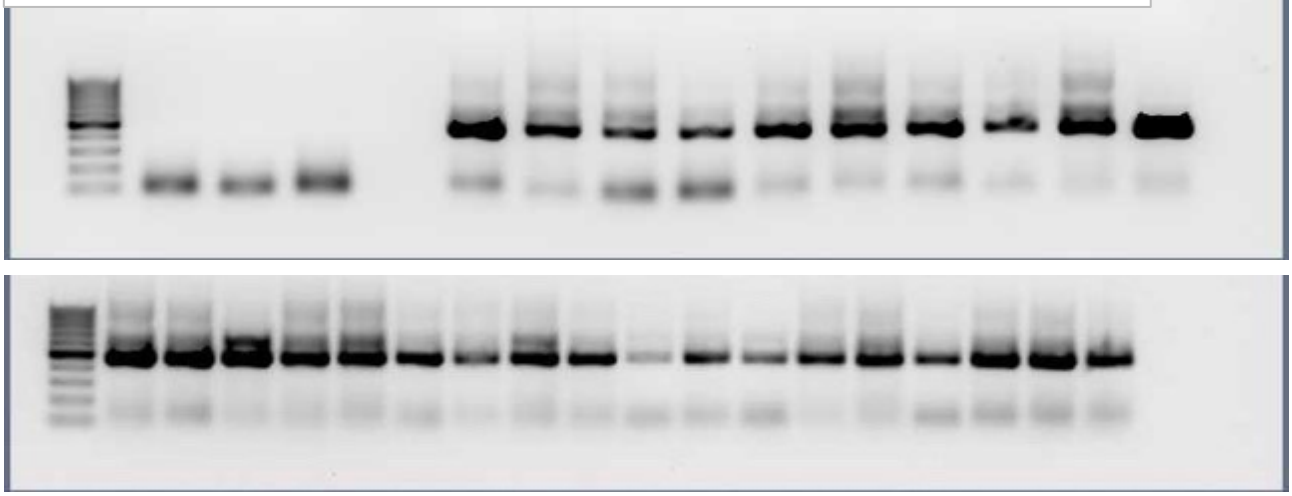
1.4.2019 - First two after ladder are neg.controls. After that samples in list order, empty bands aren't in the list. Two last samples were in another gel because lack of space.
74 128 125 110 114 156 129 63 74.2 101
103 155 98 139 121 106 113 126 158 153 99 157



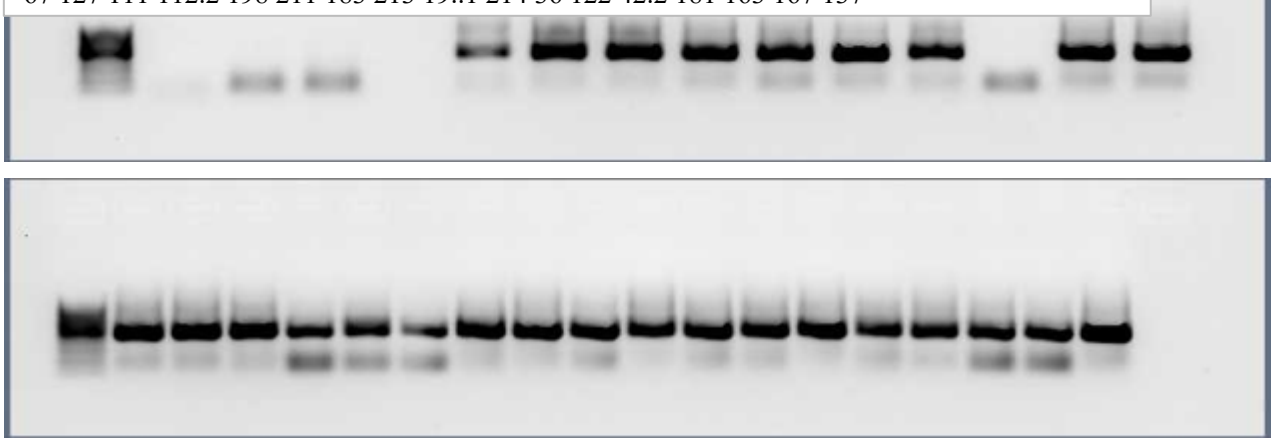
2.4.2019 Last two are from 1.4.2019 PCR, something did go wrong with this PCR and i didn't get anything
Samples: 100.2 117



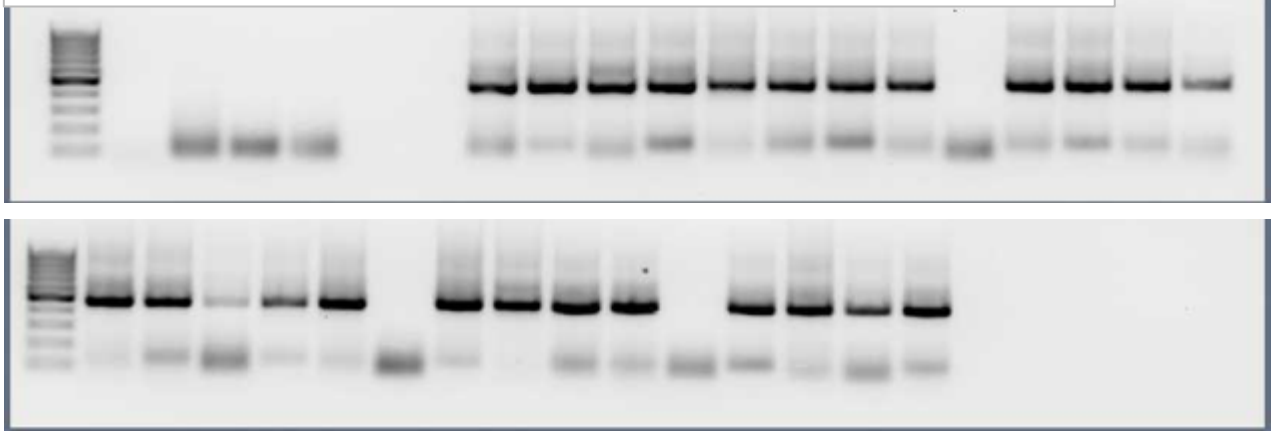
2.4.2019 -After ladder three neg. controls, empty and then samples in the list order
Samples: 168 190 35 119 149 137 212 165 118 97.2
178 41 109 75 37 20 86 102 92 120 9 108 105 18 204.2 69.2 180 172



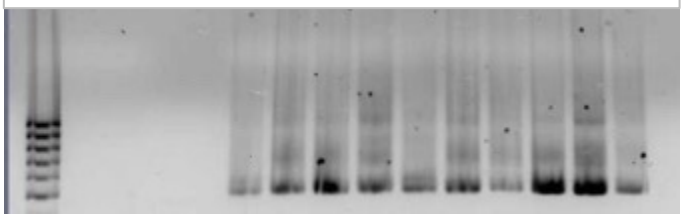
3.4.2019 - After ladder three neg. controls, empty and then samples/primers in list order
Samples: 93 79 76 168 45 77 150 24 94 58
67 127 111 112.2 198 211 183 213 19..1 214 36 122 42.2 181 163 167 137



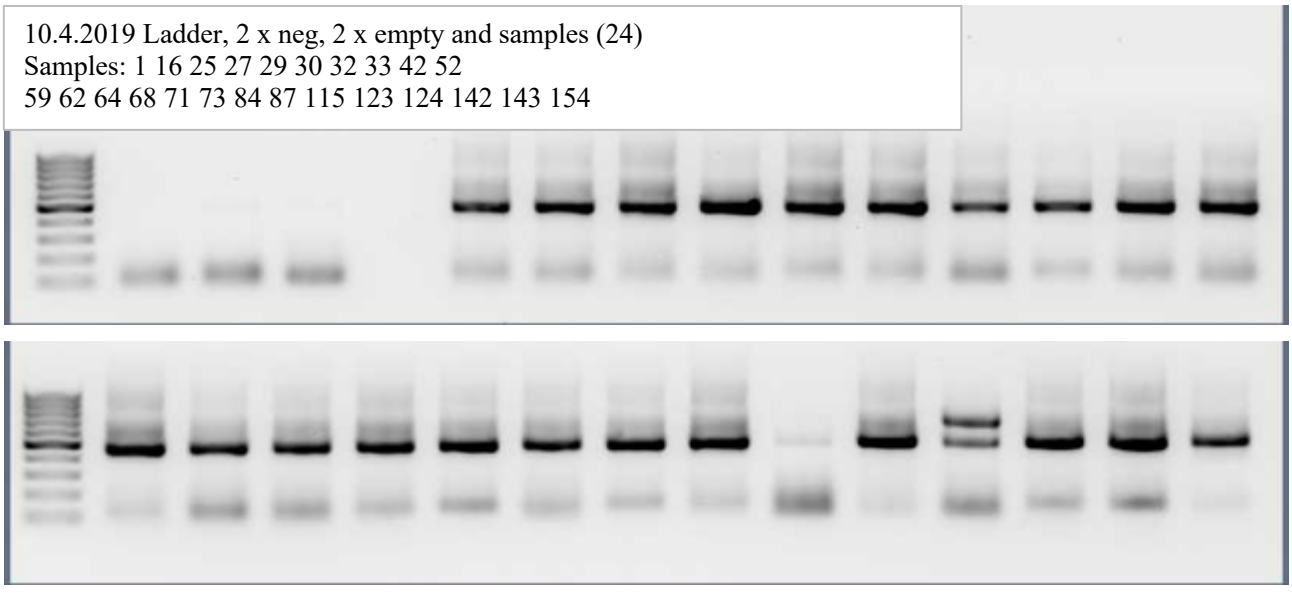
5.4.2019 Ladder, empty (primer run out), 3 x neg, 2 x empty and samples (24)
Samples: 208 12 169 146 22 165 141 151 192 21 166 204 164
182 179 40 171 145 116 161 197 147 170 43 148



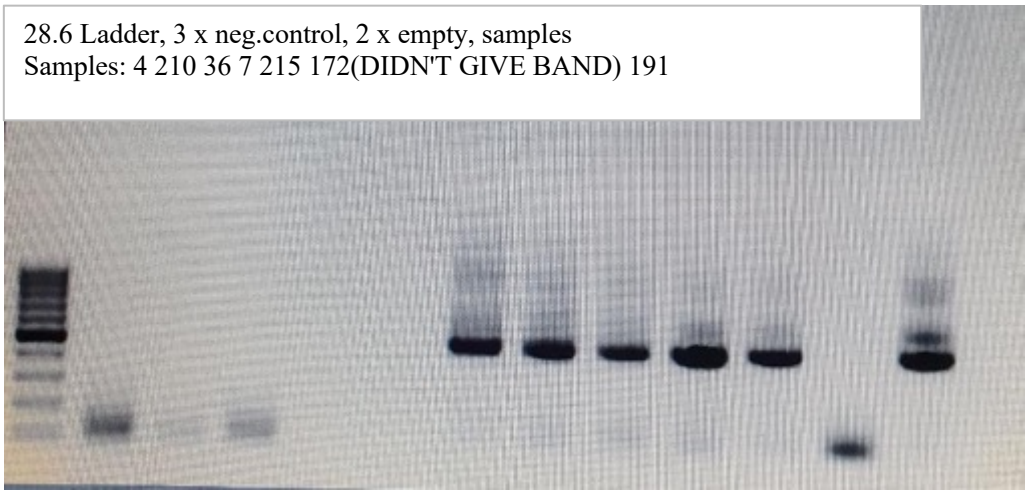
9.4.2019 Ladder, 2 x neg, 2 x empty and samples (10)
Samples:159 160 162 173 174 187 188 195 209 107



10.4.2019 Ladder, 2 x neg, 2 x empty and samples (24)
Samples: 1 16 25 27 29 30 32 33 42 52
59 62 64 68 71 73 84 87 115 123 124 142 143 154



28.6 Ladder, 3 x neg.control, 2 x empty, samples
Samples: 4 210 36 7 215 172(DIDN'T GIVE BAND) 191



Metadata

#SampleID	sample	fPrimer	barcode-sequence	LinkerPrimerSequence		Research	
	TreatmentGroup	Description	DaysSinceExperimentStart		SampleMaterial	TotalSamples	
	TreatmentGroup2	FecesTotal	TreatMatTime	TreatGroup			
1	1	132	GACACATTC	CAGCMGCCGCGGTAATWC		39	1
	Näyte 6 kk F-karpu		180	Feces	3	1	3
	T1_Feces_6	First					
2	2	22	CACACTATC	CAGCMGCCGCGGTAATWC		56	1
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
3	3	45	TATATCACT	CAGCMGCCGCGGTAATWC		55	1
	Näyte 3 kk F-karpu		90	Feces	4	1	4
	T1_Feces_3	First					
4	4	28	CACGTCGTC	CAGCMGCCGCGGTAATWC		31	1
	Näyte 12 kk F-karpu		360	Feces	4	1	4
	T1_Feces_12	First					
5	5	44	CATATCATC	CAGCMGCCGCGGTAATWC		10	1
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
6	6	5	CTATATGTC	CAGCMGCCGCGGTAATWC		1	1
	Näyte 3 kk F-karpu		90	Feces	2	1	2
	T1_Feces_3	First					
7	7	42	TATGCTGCT	CAGCMGCCGCGGTAATWC		54	2
	Näyte 3 kk F-karpu		90	Feces	3	2	3
	T2_Feces_3	Second					
8	8	81	CCGTACATC	CAGCMGCCGCGGTAATWC		20	1
	Näyte 6 kk F-karpu		180	Feces	2	1	2
	T1_Feces_6	First					
9	9	205	TAACTCTCT	CAGCMGCCGCGGTAATWC		6	2
	Näyte 3 kk F-karpu		90	Feces	3	2	3
	T2_Feces_3	Second					
10	10	27	CGCATCACT	CAGCMGCCGCGGTAATWC		15	2
	Näyte 3 kk F-karpu		90	Feces	3	2	3
	T2_Feces_3	Second					
11	11	74	GACGTGCTC	CAGCMGCCGCGGTAATWC		20	1
	Näyte 3 kk F-karpu		90	Feces	2	1	2
	T1_Feces_3	First					
12	12	9	ACATGATCT	CAGCMGCCGCGGTAATWC		10	1
	Näyte 6 kk F-karpu		180	Feces	3	1	3
	T1_Feces_6	First					
13	13	55	CAATCTCTC	CAGCMGCCGCGGTAATWC		18	2
	Näyte 3 kk F-karpu		90	Feces	1	2	1
	T2_Feces_3	Second					

14	14	47	ACTCTCTCT	CAGCMGCCGCGGTAATWC	24	1	
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
15	15	70	TACTGTAGG	CAGCMGCCGCGGTAATWC	11	1	
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
16	16	103	ATTAGAGTC	CAGCMGCCGCGGTAATWC	28	2	
	Näyte 3 kk F-karpu		90	Feces	3	2	3
	T2_Feces_3	Second					
17	17	20	AGCGCACTC	CAGCMGCCGCGGTAATWC	41	1	
	Näyte 6 kk F-karpu		180	Feces	3	1	3
	T1_Feces_6	First					
18	18	208	CAGAGAGTC	CAGCMGCCGCGGTAATWC	14	1	
	Näyte 6 kk F-karpu		180	Feces	3	1	3
	T1_Feces_6	First					
19	19	236	CTGCACGCT	CAGCMGCCGCGGTAATWC	51	1	
	Näyte 3 kk F-karpu		90	Feces	2	1	2
	T1_Feces_3	First					
20	20	200	TGCATCATC	CAGCMGCCGCGGTAATWC	26	2	
	Näyte 3 kk F-karpu		90	Feces	3	2	3
	T2_Feces_3	Second					
21	21	114	TCACAGCAA	CAGCMGCCGCGGTAATWC	3	2	
	Näyte 6 kk F-karpu		180	Feces	3	2	3
	T2_Feces_6	Second					
22	22	108	CGTGTCGCT	CAGCMGCCGCGGTAATWC	7	1	
	Näyte 3 kk F-karpu		90	Feces	2	1	2
	T1_Feces_3	First					
23	23	26	TACTGATCT	CAGCMGCCGCGGTAATWC	11	1	
	Näyte 6 kk F-karpu		180	Feces	3	1	3
	T1_Feces_6	First					
24	24	225	ACATGTAGG	CAGCMGCCGCGGTAATWC	22	1	
	Näyte 3 kk F-karpu		90	Feces	2	1	2
	T1_Feces_3	First					
25	25	102	TATCGACCT	CAGCMGCCGCGGTAATWC	1	1	
	Näyte 6 kk F-karpu		180	Feces	2	1	2
	T1_Feces_6	First					
26	26	12	TCATGTACT	CAGCMGCCGCGGTAATWC	14	1	
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
27	27	97	TGTCATAGG	CAGCMGCCGCGGTAATWC	43	1	
	Näyte 6 kk F-karpu		180	Feces	3	1	3
	T1_Feces_6	First					
28	28	78	GATGCACTC	CAGCMGCCGCGGTAATWC	3	2	
	Näyte 3 kk F-karpu		90	Feces	3	2	3
	T2_Feces_3	Second					

29	29	90	TCGATACCT	CAGCMGCCGCGGTAATWC	12	2	
	Näyte 3 kk F-karpu		90	Feces	2	2	2
	T2_Feces_3	Second					
30	30	89	TTGTGTATC	CAGCMGCCGCGGTAATWC	28	2	
	Näyte 12 kk F-karpu		360	Feces	3	2	3
	T2_Feces_12	Second					
31	31	52	ATAGCACGG	CAGCMGCCGCGGTAATWC	6	2	
	Näyte 6 kk F-karpu		180	Feces	3	2	3
	T2_Feces_6	Second					
34	34	11	TCATGCGTC	CAGCMGCCGCGGTAATWC	43	1	
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
35	35	185	CATATGTGG	CAGCMGCCGCGGTAATWC	7	1	
	Näyte 12 kk F-karpu		360	Feces	2	1	2
	T1_Feces_12	First					
36	36	238	GCGCGGTCT	CAGCMGCCGCGGTAATWC	48	2	
	Näyte 3 kk F-karpu		90	Feces	3	2	3
	T2_Feces_3	Second					
37	37	199	ACAACTACT	CAGCMGCCGCGGTAATWC	38	1	
	Näyte 3 kk F-karpu		90	Feces	4	1	4
	T1_Feces_3	First					
38	38	98	AGTACATTC	CAGCMGCCGCGGTAATWC	11	1	
	Näyte 12 kk F-karpu		360	Feces	3	1	3
	T1_Feces_12	First					
39	39	21	AGCGCGTCT	CAGCMGCCGCGGTAATWC	32	2	
	Näyte 3 kk F-karpu		90	Feces	1	2	1
	T2_Feces_3	Second					
40	40	120	GCCGAGATC	CAGCMGCCGCGGTAATWC	35	1	
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
41	41	196	GTAGCCATC	CAGCMGCCGCGGTAATWC	40	2	
	Näyte 3 kk F-karpu		90	Feces	1	2	1
	T2_Feces_3	Second					
43	43	137	GCCAGAGTC	CAGCMGCCGCGGTAATWC	41	1	
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
44	44	39	AGTCATATC	CAGCMGCCGCGGTAATWC	37	2	
	Näyte 6 kk F-karpu		180	Feces	3	2	3
	T2_Feces_6	Second					
45	45	221	GTACGACCT	CAGCMGCCGCGGTAATWC	3	2	
	Näyte 12 kk F-karpu		360	Feces	3	2	3
	T2_Feces_12	Second					
46	46	60	ACAGTATGG	CAGCMGCCGCGGTAATWC	15	2	
	Näyte 6 kk F-karpu		180	Feces	3	2	3
	T2_Feces_6	Second					

47	47	83	TTGCATACT	CAGCMGCCGCGGTAATWC	35	1	
	Näyte 6 kk F-karpu		180	Feces	3	1	3
	T1_Feces_6	First					
48	48	73	GACGTATCT	CAGCMGCCGCGGTAATWC	39	1	
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
49	49	18	CGCTAGTCT	CAGCMGCCGCGGTAATWC	14	1	
	Näyte 12 kk F-karpu		360	Feces	3	1	3
	T1_Feces_12	First					
50	50	62	CGCTACAGG	CAGCMGCCGCGGTAATWC	38	1	
	Näyte 6 kk F-karpu		180	Feces	4	1	4
	T1_Feces_6	First					
51	51	61	ACAGTTATC	CAGCMGCCGCGGTAATWC	31	1	
	Näyte 6 kk F-karpu		180	Feces	4	1	4
	T1_Feces_6	First					
52	52	46	TGTGTCGTC	CAGCMGCCGCGGTAATWC	28	2	
	Näyte 6 kk F-karpu		180	Feces	3	2	3
	T2_Feces_6	Second					
53	53	66	ATCTCCTCT	CAGCMGCCGCGGTAATWC	22	1	
	Näyte 6 kk F-karpu		180	Feces	2	1	2
	T1_Feces_6	First					
54	54	75	GTGACTACT	CAGCMGCCGCGGTAATWC	37	2	
	Näyte 3 kk F-karpu		90	Feces	3	2	3
	T2_Feces_3	Second					
55	55	38	TGTCAGCTC	CAGCMGCCGCGGTAATWC	15	2	
	Näyte 12 kk F-karpu		360	Feces	3	2	3
	T2_Feces_12	Second					
57	57	65	AACACATCT	CAGCMGCCGCGGTAATWC	10	1	
	Näyte 12 kk F-karpu		360	Feces	3	1	3
	T1_Feces_12	First					
58	58	227	CGCATTGTC	CAGCMGCCGCGGTAATWC	33	2	
	Näyte 3 kk F-karpu		90	Feces	1	2	1
	T2_Feces_3	Second					
59	59	69	AACTGTATC	CAGCMGCCGCGGTAATWC	6	2	
	Näyte 12 kk F-karpu		360	Feces	3	2	3
	T2_Feces_12	Second					
60	60	17	AGCTACACT	CAGCMGCCGCGGTAATWC	25	1	
	Näyte 6 kk F-karpu		180	Feces	3	1	3
	T1_Feces_6	First					
62	62	87	ACGCGACCT	CAGCMGCCGCGGTAATWC	50	2	
	Näyte 3 kk F-karpu		90	Feces	4	2	4
	T2_Feces_3	Second					
63	63	156	CAGGAGATC	CAGCMGCCGCGGTAATWC	50	2	
	Näyte 3 kk U-karpu		90	Urine	4	2	4
	T2_Urine_3	Second					

64	64	100	CATGCCACT	CAGCMGCCGCGGTAATWC	31	1	
	Näyte 3 kk F-karpu		90	Feces	4	1	4
	T1_Feces_3	First					
66	66	15	GCAGTCGTC	CAGCMGCCGCGGTAATWC	7	1	
	Näyte 6 kk F-karpu		180	Feces	4	1	4
	T1_Feces_6	First					
67	67	228	TAGAGGATC	CAGCMGCCGCGGTAATWC	12	2	
	Näyte 6 kk F-karpu		180	Feces	2	2	2
	T2_Feces_6	Second					
68	68	101	AGTACGCCT	CAGCMGCCGCGGTAATWC	52	1	
	Näyte 3 kk F-karpu		90	Feces	1	1	1
	T1_Feces_3	First					
70	70	35	ATGACTATC	CAGCMGCCGCGGTAATWC	41	1	
	Näyte 12 kk F-karpu		360	Feces	3	1	3
	T1_Feces_12	First					
71	71	104	AATCGCACT	CAGCMGCCGCGGTAATWC	74	1	
	Näyte 3 kk F-karpu		90	Feces	4	1	4
	T1_Feces_3	First					
72	72	51	GCACAGCTC	CAGCMGCCGCGGTAATWC	73	2	
	Näyte 3 kk F-karpu		90	Feces	4	2	4
	T2_Feces_3	Second					
73	73	105	AATCGTGTC	CAGCMGCCGCGGTAATWC	71	2	
	Näyte 3 kk F-karpu		90	Feces	3	2	3
	T2_Feces_3	Second					
75	75	198	GTAGCGTGG	CAGCMGCCGCGGTAATWC	51	1	
	Näyte 6 kk F-karpu		180	Feces	2	1	2
	T1_Feces_6	First					
76	76	219	AGACCGACT	CAGCMGCCGCGGTAATWC	59	1	
	Näyte 12 kk F-karpu		360	Feces	3	1	3
	T1_Feces_12	First					
77	77	222	TGAAGAGTC	CAGCMGCCGCGGTAATWC	68	1	
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
78	78	30	TCGTACACT	CAGCMGCCGCGGTAATWC	67	2	
	Näyte 3 kk F-karpu		90	Feces	1	2	1
	T2_Feces_3	Second					
79	79	218	TGAGAGATC	CAGCMGCCGCGGTAATWC	62	2	
	Näyte 3 kk F-karpu		90	Feces	3	2	3
	T2_Feces_3	Second					
80	80	31	ATGCAGCTC	CAGCMGCCGCGGTAATWC	62	2	
	Näyte 6 kk F-karpu		180	Feces	3	2	3
	T2_Feces_6	Second					
81	81	92	TCGATGTTC	CAGCMGCCGCGGTAATWC	48	2	
	Näyte 12 kk F-karpu		360	Feces	3	2	3
	T2_Feces_12	Second					

82	82	4	TCACATATC	CAGCMGCCGCGGTAATWC	50	2	
	Näyte 6 kk F-karpu		180	Feces	4	2	4
	T2_Feces_6	Second					
83	83	91	CTGGTATCT	CAGCMGCCGCGGTAATWC	56	1	
	Näyte 6 kk F-karpu		180	Feces	3	1	3
	T1_Feces_6	First					
84	84	106	TCTCTAACT	CAGCMGCCGCGGTAATWC	54	2	
	Näyte 6 kk F-karpu		180	Feces	3	2	3
	T2_Feces_6	Second					
85	85	29	TACGTCGCT	CAGCMGCCGCGGTAATWC	64	1	
	Näyte 3 kk F-karpu		90	Feces	2	1	1
	T1_Feces_3	First					
87	87	112	GCACACGAA	CAGCMGCCGCGGTAATWC	56	1	
	Näyte 12 kk F-karpu		360	Feces	3	1	3
	T1_Feces_12	First					
88	88	32	ACGTAGTCT	CAGCMGCCGCGGTAATWC	54	2	
	Näyte 12 kk F-karpu		360	Feces	3	2	3
	T2_Feces_12	Second					
89	89	53	AGACCAGTC	CAGCMGCCGCGGTAATWC	37	2	
	Näyte 12 kk F-karpu		360	Feces	3	2	3
	T2_Feces_12	Second					
90	90	79	GATGCGTCT	CAGCMGCCGCGGTAATWC	43	1	
	Näyte 12 kk F-karpu		360	Feces	3	1	3
	T1_Feces_12	First					
91	91	43	AGTGTATCT	CAGCMGCCGCGGTAATWC	50	2	
	Näyte 12 kk F-karpu		360	Feces	4	2	4
	T2_Feces_12	Second					
92	92	203	ATAATCATC	CAGCMGCCGCGGTAATWC	55	1	
	Näyte 6 kk F-karpu		180	Feces	4	1	4
	T1_Feces_6	First					
93	93	217	TCACACGCT	CAGCMGCCGCGGTAATWC	59	1	
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
94	94	226	AGATTAGCT	CAGCMGCCGCGGTAATWC	35	1	
	Näyte 12 kk F-karpu		360	Feces	3	1	3
	T1_Feces_12	First					
95	95	80	TACGTTATC	CAGCMGCCGCGGTAATWC	39	1	
	Näyte 12 kk F-karpu		360	Feces	3	1	3
	T1_Feces_12	First					
98	98	162	GAGAGCCTC	CAGCMGCCGCGGTAATWC	59	1	
	Näyte 3 kk U-karpu		90	Urine	3	1	3
	T1_Urine_3	First					
99	99	140	GACTGCGTC	CAGCMGCCGCGGTAATWC	85	2	
	Näyte 3 kk U-karpu		90	Urine	4	2	4
	T2_Urine_3	Second					

101	101	158	CCGTATGCT	CAGCMGCCGCGGTAATWC	87	2	
	Näyte 3 kk U-karpu		90	Urine	1	2	1
	T2_Urine_3	Second					
102	102	202	TTACGTGTC	CAGCMGCCGCGGTAATWC	79	2	
	Näyte 3 kk U-karpu		90	Urine	3	2	3
	T2_Urine_3	Second					
103	103	160	TTGACATCT	CAGCMGCCGCGGTAATWC	81	2	
	Näyte 3 kk U-karpu		90	Urine	4	2	4
	T2_Urine_3	Second					
105	105	207	ACGTACAGG	CAGCMGCCGCGGTAATWC	75	1	
	Näyte 3 kk U-karpu		90	Urine	4	1	4
	T1_Urine_3	First					
106	106	170	ACGATTGTC	CAGCMGCCGCGGTAATWC	80	1	
	Näyte 3 kk U-karpu		90	Urine	2	1	1
	T1_Urine_3	First					
107	107	190	TGAGAAGCT	CAGCMGCCGCGGTAATWC	90	1	
	Näyte 3 kk U-karpu		90	Urine	4	1	4
	T1_Urine_3	First					
108	108	206	GCAGTTACT	CAGCMGCCGCGGTAATWC	97	1	
	Näyte 3 kk U-karpu		90	Urine	4	1	4
	T1_Urine_3	First					
109	109	197	ATAGCCACT	CAGCMGCCGCGGTAATWC	98	1	
	Näyte 3 kk U-karpu		90	Urine	3	1	3
	T1_Urine_3	First					
110	110	151	CACGTTACT	CAGCMGCCGCGGTAATWC	99	2	
	Näyte 3 kk U-karpu		90	Urine	3	2	3
	T2_Urine_3	Second					
111	111	230	GCGATGTGG	CAGCMGCCGCGGTAATWC	92	2	
	Näyte 3 kk U-karpu		90	Urine	4	2	4
	T2_Urine_3	Second					
113	113	171	TATTAGTCT	CAGCMGCCGCGGTAATWC			
	Unknown	None	Unknown	Unknown	Unknown	Unknown	
	Unknown		First				
114	114	152	TGCATTGCT	CAGCMGCCGCGGTAATWC	95	2	
	Näyte 3 kk U-karpu		90	Urine	4	2	4
	T2_Urine_3	Second					
115	115	107	AATATACTC	CAGCMGCCGCGGTAATWC	68	1	
	Näyte 3 kk U-karpu		90	Urine	3	1	3
	T1_Urine_3	First					
116	116	125	CTCAATCTC	CAGCMGCCGCGGTAATWC	96	2	
	Näyte 3 kk U-karpu		90	Urine	2	2	1
	T2_Urine_3	Second					
117	117	146	TACGTATGG	CAGCMGCCGCGGTAATWC			
	Unknown	None	Unknown	Unknown	Unknown	Unknown	
	Unknown		First				

118	118	191	GCACATAGG	CAGCMGCCGCGGTAATWC	102	1	
	Näyte 3 kk U-karpu		90	Urine	3	1	3
	T1_Urine_3	First					
119	119	186	CGTGTTATC	CAGCMGCCGCGGTAATWC	106	2	
	Näyte 3 kk U-karpu		90	Urine	1	2	1
	T2_Urine_3	Second					
120	120	204	GTAATCACT	CAGCMGCCGCGGTAATWC	109	1	
	Näyte 3 kk U-karpu		90	Urine	4	1	4
	T1_Urine_3	First					
121	121	168	GTGGTCGCT	CAGCMGCCGCGGTAATWC	104	2	
	Näyte 3 kk U-karpu		90	Urine	4	2	4
	T2_Urine_3	Second					
122	122	239	TTGTGCGCT	CAGCMGCCGCGGTAATWC	103	1	
	Näyte 3 kk U-karpu		90	Urine	4	1	4
	T1_Urine_3	First					
123	123	122	CACCAGCTC	CAGCMGCCGCGGTAATWC	74	1	
	Näyte 3 kk U-karpu		90	Urine	4	1	4
	T1_Urine_3	First					
124	124	159	ATGACATGG	CAGCMGCCGCGGTAATWC	73	2	
	Näyte 3 kk U-karpu		90	Urine	4	2	4
	T2_Urine_3	Second					
125	125	150	TGCATGTGG	CAGCMGCCGCGGTAATWC	71	2	
	Näyte 3 kk U-karpu		90	Urine	3	2	3
	T2_Urine_3	Second					
126	126	172	TCTAATCTC	CAGCMGCCGCGGTAATWC	76	2	
	Näyte 3 kk U-karpu		90	Urine	3	2	3
	T2_Urine_3	Second					
127	127	229	ACGATCACT	CAGCMGCCGCGGTAATWC	78	1	
	Näyte 3 kk U-karpu		90	Urine	4	1	4
	T1_Urine_3	First					
128	128	149	AGCATGTTC	CAGCMGCCGCGGTAATWC	64	1	
	Näyte 3 kk U-karpu		90	Urine	2	1	1
	T1_Urine_3	First					
129	129	155	TTGCACGTC	CAGCMGCCGCGGTAATWC	65	2	
	Näyte 3 kk U-karpu		90	Urine	4	2	4
	T2_Urine_3	Second					
136	136	2	CGAGAGACT	CAGCMGCCGCGGTAATWC	109	1	
	Näyte 6 kk F-karpu		180	Feces	4	1	4
	T1_Feces_6	First					
137	137	216	GCTCTCTTC	CAGCMGCCGCGGTAATWC	110	2	
	Näyte 6 kk F-karpu		180	Feces	4	1	4
	T1_Feces_6	First					
138	138	67	ACCAGAGCT	CAGCMGCCGCGGTAATWC	95	2	
	Näyte 12 kk F-karpu		360	Feces	4	2	4
	T2_Feces_12	Second					

139	139	163	CAGAGGACT	CAGCMGCCGCGGTAATWC	111	1	
	Näyte 3 kk U-karpu		90	Urine	3	1	3
	T1_Urine_3	First					
140	140	94	TGTCAATCT	CAGCMGCCGCGGTAATWC	111	1	
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
141	141	110	CATATTGCT	CAGCMGCCGCGGTAATWC	102	1	
	Näyte 12 kk F-karpu		360	Feces	3	1	3
	T1_Feces_12	First					
142	142	174	TATGCGTGG	CAGCMGCCGCGGTAATWC	105	2	
	Näyte 3 kk F-karpu		90	Feces	2	2	1
	T2_Feces_3	Second					
143	143	164	GTGTGTAGG	CAGCMGCCGCGGTAATWC	102	1	
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
144	144	16	TCAGTGCTC	CAGCMGCCGCGGTAATWC	104	2	
	Näyte 3 kk F-karpu		90	Feces	4	2	4
	T2_Feces_3	Second					
145	145	124	GACCATATC	CAGCMGCCGCGGTAATWC	85	2	
	Näyte 12 kk F-karpu		360	Feces	4	2	4
	T2_Feces_12	Second					
147	147	133	CACACATGG	CAGCMGCCGCGGTAATWC	99	2	
	Näyte 6 kk F-karpu		180	Feces	3	2	3
	T2_Feces_6	Second					
148	148	139	CACTGATTC	CAGCMGCCGCGGTAATWC	91	1	
	Näyte 6 kk F-karpu		180	Feces	3	1	3
	T1_Feces_6	First					
149	149	187	TGTGTTACT	CAGCMGCCGCGGTAATWC	98	1	
	Näyte 6 kk F-karpu		180	Feces	3	1	3
	T1_Feces_6	First					
150	150	223	CCATGCGCT	CAGCMGCCGCGGTAATWC	103	1	
	Näyte 3 kk F-karpu		90	Feces	4	1	4
	T1_Feces_3	First					
151	151	111	GTATACAGG	CAGCMGCCGCGGTAATWC	97	1	
	Näyte 6 kk F-karpu		180	Feces	4	1	4
	T1_Feces_6	First					
152	152	96	CGTCAGCCT	CAGCMGCCGCGGTAATWC	82	1	
	Näyte 12 kk F-karpu		360	Feces	4	1	4
	T1_Feces_12	First					
153	153	178	AGTTGCGTC	CAGCMGCCGCGGTAATWC	84	1	
	Näyte 12 kk F-karpu		360	Feces	4	1	4
	T1_Feces_12	First					
154	154	167	ATGGTCGTC	CAGCMGCCGCGGTAATWC	95	2	
	Näyte 6 kk F-karpu		180	Feces	4	2	4
	T2_Feces_6	Second					

155	155	161	TTGACGCTC	CAGCMGCCGCGGTAATWC	112	2	
	Näyte 3 kk U-karpu		90	Urine	2	2	1
	T2_Urine_3	Second					
156	156	154	ATGCAATCT	CAGCMGCCGCGGTAATWC	113	1	
	Näyte 3 kk U-karpu		90	Urine	3	1	3
	T1_Urine_3	First					
157	157	143	GGCATACTC	CAGCMGCCGCGGTAATWC	108	1	
	Näyte 3 kk U-karpu		90	Urine	2	1	1
	T1_Urine_3	First					
158	158	176	CGTTGATCT	CAGCMGCCGCGGTAATWC	108	1	
	Näyte 3 kk F-karpu		90	Feces	2	1	1
	T1_Feces_3	First					
159	159	14	TCAGTATCT	CAGCMGCCGCGGTAATWC	113	1	
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
160	160	71	GACTGTACT	CAGCMGCCGCGGTAATWC	103	1	
	Näyte 12 kk F-karpu		360	Feces	4	1	4
	T1_Feces_12	First					
161	161	241	GCGCGCAGG	CAGCMGCCGCGGTAATWC	104	2	
	Näyte 12 kk F-karpu		360	Feces	4	2	4
	T2_Feces_12	Second					
162	162	84	TCGGCACTC	CAGCMGCCGCGGTAATWC	110	2	
	Näyte 3 kk F-karpu		90	Feces	2	2	2
	T2_Feces_3	Second					
163	163	214	AGTCACGCT	CAGCMGCCGCGGTAATWC	92	2	
	Näyte 12 kk F-karpu		360	Feces	4	2	4
	T2_Feces_12	Second					
164	164	117	ATATAGTTC	CAGCMGCCGCGGTAATWC	90	1	
	Näyte 12 kk F-karpu		360	Feces	4	1	4
	T1_Feces_12	First					
165	165	109	AATATGTCT	CAGCMGCCGCGGTAATWC	103	1	
	Näyte 6 kk F-karpu		180	Feces	3	2	3
	T2_Feces_6	Second					
166	166	115	ACACAGCCT	CAGCMGCCGCGGTAATWC	97	1	
	Näyte 12 kk F-karpu		360	Feces	4	1	4
	T1_Feces_12	First					
167	167	215	ATTGAGATC	CAGCMGCCGCGGTAATWC	109	1	
	Näyte 3 kk F-karpu		90	Feces	4	1	4
	T1_Feces_3	First					
168	168	220	GCAACTATC	CAGCMGCCGCGGTAATWC	99	2	
	Näyte 3 kk F-karpu		90	Feces	2	2	2
	T2_Feces_3	Second					
170	170	134	TACACCGTC	CAGCMGCCGCGGTAATWC	104	2	
	Näyte 6 kk F-karpu		180	Feces	4	2	4
	T2_Feces_6	Second					

171	171	121	ACCGAGACT	CAGCMGCCGCGGTAATWC	81	2	
	Näyte 12 kk F-karpu		360	Feces	4	2	4
	T2_Feces_12	Second					
172	172	212	GAGTTAGCT	CAGCMGCCGCGGTAATWC	92	2	
	Näyte 6 kk F-karpu		180	Feces	4	2	4
	T2_Feces_6	Second					
173	173	23	TACACTACT	CAGCMGCCGCGGTAATWC	96	2	
	Näyte 3 kk F-karpu		90	Feces	2	2	1
	T2_Feces_3	Second					
174	174	13	CTACGTGCT	CAGCMGCCGCGGTAATWC	84	1	
	Näyte 6 kk F-karpu		180	Feces	4	1	4
	T1_Feces_6	First					
175	175	63	TGCTAGTTC	CAGCMGCCGCGGTAATWC	95	2	
	Näyte 3 kk F-karpu		90	Feces	4	2	4
	T2_Feces_3	Second					
176	176	33	CTGCATATC	CAGCMGCCGCGGTAATWC	90	1	
	Näyte 6 kk F-karpu		180	Feces	4	1	4
	T1_Feces_6	First					
177	177	40	TGTACTATC	CAGCMGCCGCGGTAATWC	98	1	
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
178	178	195	TTATATGCT	CAGCMGCCGCGGTAATWC	97	1	
	Näyte 3 kk F-karpu		90	Feces	4	1	4
	T1_Feces_3	First					
179	179	119	CTCAACTCT	CAGCMGCCGCGGTAATWC	75	1	
	Näyte 12 kk F-karpu		360	Feces	4	1	4
	T1_Feces_12	First					
180	180	211	CTGTGTACT	CAGCMGCCGCGGTAATWC	78	1	
	Näyte 12 kk F-karpu		360	Feces	4	1	4
	T1_Feces_12	First					
181	181	213	CTGGTGCTC	CAGCMGCCGCGGTAATWC	85	2	
	Näyte 6 kk F-karpu		180	Feces	4	2	4
	T2_Feces_6	Second					
182	182	118	GACCACGCT	CAGCMGCCGCGGTAATWC	73	2	
	Näyte 12 kk F-karpu		360	Feces	4	2	4
	T2_Feces_12	Second					
183	183	234	TCTGGCTTC	CAGCMGCCGCGGTAATWC	91	1	
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
184	184	59	CCATGTATC	CAGCMGCCGCGGTAATWC	82	1	
	Näyte 6 kk F-karpu		180	Feces	4	1	4
	T1_Feces_6	First					
185	185	128	CTCTCAATC	CAGCMGCCGCGGTAATWC	92	2	
	Näyte 3 kk F-karpu		90	Feces	4	2	4
	T2_Feces_3	Second					

186	186	10	CTACGCATC	CAGCMGCCGCGGTAATWC	90	1	
	Näyte 3 kk F-karpu		90	Feces	4	1	4
	T1_Feces_3	First					
187	187	88	GCGCGACTC	CAGCMGCCGCGGTAATWC	65	2	
	Näyte 12 kk F-karpu		360	Feces	4	2	4
	T2_Feces_12	Second					
188	188	7	ATAGCTGTC	CAGCMGCCGCGGTAATWC	81	2	
	Näyte 6 kk F-karpu		180	Feces	4	2	4
	T2_Feces_6	Second					
189	189	49	CGAGAAGTC	CAGCMGCCGCGGTAATWC	85	2	
	Näyte 3 kk F-karpu		90	Feces	4	2	4
	T2_Feces_3	Second					
190	190	184	TCTCTGGTC	CAGCMGCCGCGGTAATWC	76	2	
	Näyte 6 kk F-karpu		180	Feces	3	2	3
	T2_Feces_6	Second					
191	191	127	GGCTATGCT	CAGCMGCCGCGGTAATWC	81	2	
	Näyte 3 kk F-karpu		90	Feces	4	2	4
	T2_Feces_3	Second					
192	192	113	CCACACGTC	CAGCMGCCGCGGTAATWC	74	1	
	Näyte 6 kk F-karpu		180	Feces	4	1	4
	T1_Feces_6	First					
193	193	76	GTGTGATCT	CAGCMGCCGCGGTAATWC	74	1	
	Näyte 12 kk F-karpu		360	Feces	4	1	4
	T1_Feces_12	First					
194	194	99	TGTACATGG	CAGCMGCCGCGGTAATWC	78	1	
	Näyte 6 kk F-karpu		180	Feces	4	1	4
	T1_Feces_6	First					
195	195	188	ACTCTTCTC	CAGCMGCCGCGGTAATWC	75	1	
	Näyte 6 kk F-karpu		180	Feces	4	1	4
	T1_Feces_6	First					
197	197	242	ATGACCGCT	CAGCMGCCGCGGTAATWC	68	1	
	Näyte 6 kk F-karpu		180	Feces	3	1	3
	T1_Feces_6	First					
198	198	232	CATCGCAGG	CAGCMGCCGCGGTAATWC	82	1	
	Näyte 3 kk U-karpu		90	Urine	4	1	4
	T1_Urine_3	First					
199	199	68	TACTGGCTC	CAGCMGCCGCGGTAATWC	82	1	
	Näyte 3 kk F-karpu		90	Feces	4	1	4
	T1_Feces_3	First					
200	200	8	ATACGACTC	CAGCMGCCGCGGTAATWC	65	2	
	Näyte 6 kk F-karpu		180	Feces	4	2	4
	T2_Feces_6	Second					
201	201	95	TCTAACTCT	CAGCMGCCGCGGTAATWC	80	1	
	Näyte 3 kk F-karpu		90	Feces	2	1	1
	T1_Feces_3	First					

203	203	85	CTGACATTC	CAGCMGCCGCGGTAATWC	84	1	
	Näyte 3 kk F-karpu		90	Feces	4	1	4
	T1_Feces_3	First					
205	205	41	CGTACTACT	CAGCMGCCGCGGTAATWC	78	1	
	Näyte 3 kk F-karpu		90	Feces	4	1	4
	T1_Feces_3	First					
206	206	58	TTACGCACT	CAGCMGCCGCGGTAATWC	73	2	
	Näyte 6 kk F-karpu		180	Feces	4	2	4
	T2_Feces_6	Second					
207	207	86	TGGTCTCTC	CAGCMGCCGCGGTAATWC	71	2	
	Näyte 6 kk F-karpu		180	Feces	3	2	3
	T2_Feces_6	Second					
208	208	6	CTAGCACTC	CAGCMGCCGCGGTAATWC	75	1	
	Näyte 3 kk F-karpu		90	Feces	4	1	4
	T1_Feces_3	First					
209	209	179	CATCGGTCT	CAGCMGCCGCGGTAATWC	72	1	
	Näyte 6 kk F-karpu		180	Feces	1	1	1
	T1_Feces_6	First					
210	210	25	TGCGCTGTC	CAGCMGCCGCGGTAATWC	76	2	
	Näyte 3 kk F-karpu		90	Feces	3	2	3
	T2_Feces_3	Second					
211	211	233	TATATACGG	CAGCMGCCGCGGTAATWC	105	2	
	Näyte 3 kk U-karpu		90	Urine	2	2	1
	T2_Urine_3	Second					
212	212	189	TATATTGTC	CAGCMGCCGCGGTAATWC	113	1	
	Näyte 6 kk F-karpu		180	Feces	3	1	3
	T1_Feces_6	First					
213	213	235	CTGACGCCT	CAGCMGCCGCGGTAATWC	109	1	
	Näyte 12 kk F-karpu		360	Feces	4	1	4
	T1_Feces_12	First					
214	214	237	CCGCGTGTC	CAGCMGCCGCGGTAATWC	111	1	
	Näyte 6 kk F-karpu		180	Feces	3	1	3
	T1_Feces_6	First					
215	215	36	TCGCGCATC	CAGCMGCCGCGGTAATWC	112	2	
	Näyte 6 kk F-karpu		180	Feces	2	2	1
	T2_Feces_6	Second					
100_2	100_2	144	GTCTCTCCT	CAGCMGCCGCGGTAATWC	84	1	
	Näyte 3 kk U-karpu		90	Urine	4	1	4
	T1_Urine_3	First					
112_2	112_2	231	CGTACCGTC	CAGCMGCCGCGGTAATWC	91	1	
	Näyte 3 kk U-karpu		90	Urine	3	1	3
	T1_Urine_3	First					
204_1	204_1	116	CTATAGTAA	CAGCMGCCGCGGTAATWC	79	2	
	Näyte 3 kk F-karpu		90	Feces	3	2	1
	T2_Feces_3	Second					

204_2	204_2	209	ATGTGATTC	CAGCMGCCGCGGTAATWC	79	2	
	Näyte 3 kk F-karpu		90	Feces	3	2	1
	T2_Feces_3	Second					
32_1	32_1	37	TCGCGTGCT	CAGCMGCCGCGGTAATWC	25	1	
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
32_2	32_2	48	AGTGTGCTC	CAGCMGCCGCGGTAATWC	25	1	
	Näyte 3 kk F-karpu		90	Feces	3	1	3
	T1_Feces_3	First					
33_1	33_1	3	GTATAGTCT	CAGCMGCCGCGGTAATWC	26	2	
	Näyte 12 kk F-karpu		360	Feces	3	2	3
	T2_Feces_12	Second					
33_2	33_2	57	CGAAGAGCT	CAGCMGCCGCGGTAATWC	26	2	
	Näyte 12 kk F-karpu		360	Feces	3	2	3
	T2_Feces_12	Second					
42_1	42_1	1	CTATACACT	CAGCMGCCGCGGTAATWC	24	1	
	Näyte 12 kk F-karpu		360	Feces	3	1	3
	T1_Feces_12	First					
42_2	42_2	240	CCGCGCACT	CAGCMGCCGCGGTAATWC	24	1	
	Näyte 12 kk F-karpu		360	Feces	3	1	3
	T1_Feces_12	First					
69_2	69_2	210	GTGTGGCTC	CAGCMGCCGCGGTAATWC	48	2	
	Näyte 6 kk F-karpu		180	Feces	3	2	3
	T2_Feces_6	Second					
74_1	74_1	147	GTCCTCTCT	CAGCMGCCGCGGTAATWC	38	1	
	Näyte 12 kk F-karpu		360	Feces	4	1	4
	T1_Feces_12	First					
74_2	74_2	157	TAGGAGACT	CAGCMGCCGCGGTAATWC	38	1	
	Näyte 12 kk F-karpu		360	Feces	4	1	4
	T1_Feces_12	First					
86_1	86_1	201	GCATGATTC	CAGCMGCCGCGGTAATWC	65	2	
	Näyte 3 kk F-karpu		90	Feces	4	2	4
	T2_Feces_3	Second					
86_2	86_2	34	TCGTATGTC	CAGCMGCCGCGGTAATWC	65	2	
	Näyte 3 kk F-karpu		90	Feces	4	2	4
	T2_Feces_3	Second					
96_1	96_1	24	ATCTCTCTC	CAGCMGCCGCGGTAATWC	55	1	
	Näyte 12 kk F-karpu		360	Feces	4	1	4
	T1_Feces_12	First					
96_2	96_2	50	TTATACATC	CAGCMGCCGCGGTAATWC	55	1	
	Näyte 12 kk F-karpu		360	Feces	4	1	4
	T1_Feces_12	First					
97_2	97_2	192	CCACATACT	CAGCMGCCGCGGTAATWC	62	2	
	Näyte 3 kk U-karpu		90	Urine	3	2	3
	T2_Urine_3	Second					