Version:	1.03			
Number	**Item**	**Recommendation**	**Yes/No/NA**	**Comments or location in manuscript**
Abstract				
1.0	Structured or Unstructured Abstract	Abstract should include information on background, methods, results, and conclusions in structured or unstructured format.	Yes	
1.1	Study Design	State study design in abstract.	Yes	
1.2	Sequencing methods	State the strategy used for metagenomic classification.	Yes	
1.3	Specimens	Describe body site(s) studied.	Yes	
Introduction				
2.0	Background and Rationale	Summarize the underlying background, scientific evidence, or theory driving the current hypothesis as well as the study objectives.	Yes	Pages 2-3
2.1	Hypotheses	State the pre-specified hypothesis. If the study is exploratory, state any pre-specified study objectives.	Yes	Page 3
Methods				
3.0	Study Design	Describe the study design.	Yes	Page 6
3.1	Participants	State what the population of interest is, and the method by which participants are sampled from that population. Include relevant information on physiological state of the subjects or stage in the life history of disease under study when participants were sampled.	Yes	Page 6
3.2	Geographic location	State the geographic region(s) where participants were sampled from.	Yes	Page 6
3.3	Relevant Dates	State the start and end dates for recruitment, follow-up, and data collection.	Yes	Page 6
3.4	Eligibility criteria	List any criteria for inclusion and exclusion of recruited participants.	Yes	Page 6
3.5	Antibiotics Usage	List what is known about antibiotics usage before or during sample collection.	Yes	As per Table 1, half of cohort infants were exposed to maternal intrapartum antibiotic prophylaxis (IAP). IAP was not identified as a confounding factor for hypothesized natural green space-3-year atopy associations in the DAG (directed acyclic graph).
3.6	Analytic sample size	Explain how the final analytic sample size was calculated, including the number of cases and controls if relevant, and reasons for dropout at each stage of the study. This should include the number of individuals in whom microbiome sequencing was attempted and the number in whom microbiome sequencing was successful.	Yes	460 infants with uPLVI map - atopic sensitization data linkage were included in Objective 1 that did not require gut microbiota measures. A post-hoc power analysis for Objective 1 indicated we had 74% power to detect a statistical difference (one-sided alpha, 0.05) between 5.5% (n=204) and 1.6% (n=254) for sensitization to 2+ inhalant allergens. This sample size was reduced to 287 infants with complete gut microbiota profiles to test mediating effects of gut microbiota in Objective 2, for which boot strapping was implemented to improve the reliability of the mediation coefficients.
3.7	Longitudinal Studies	For longitudinal studies, state how many follow-ups were conducted, describe sample size at follow-up by group or condition, and discuss any loss to follow-up.	Yes	Suppl Figure 1

3.8	Matching	For matched studies, give matching criteria.	NA	
3.9	Ethics	State the name of the institutional review board that approved the study and protocols, protocol number and date of approval, and procedures for obtaining informed consent from participants.	Yes	Page 6
4.0	Laboratory methods	State the laboratory/center where laboratory work was done.	Yes	Stool sampling processing and storage (Hamilton Health Sciences Centre Central Storage Facility); Stood DNA isolation and PCR (JA Scott, Univ of Toronto); NGS sequencing (D Guttman, Univ of Toronto)
4.1	Specimen collection	State the body site(s) sampled from and how specimens were collected.	Yes	UBERON_0001988
4.2	Shipping	Describe how samples were stored and shipped to the laboratory.	Yes	Stool samples were aliquoted into cryovials and frozen at -80C immediately following collection. Samples were transferred in batches to a central storage facility using nitrogen vapour shippers to maintain cold chain. Samples were transferred to the laboratory for analysis similarly in nitrogen vapour shippers and maintained at -80 C until analysis.
4.3	Storage	Describe how the laboratory stored samples, including time between collection and storage and any preservation buffers or refrigeration used.	Yes	Stools were frozen as whole mass without suspension in any buffer.
4.4	DNA extraction	Provide DNA extraction method, including kit and version if relevant.	Yes	See attached SOP1.2
4.5	Human DNA sequence depletion or microbial DNA enrichment	Describe whether human DNA sequence depletion or enrichment of microbial or viral DNA was performed.	NA	
4.6	Primer selection	Provide primer selection and DNA amplification methods as well as variable region sequenced (if applicable).	Yes	See attached SOP1.3. Barcodes, adapters and primers listed in mapping_file.pdf (attached).
4.7	Positive Controls	Describe any positive controls (mock communities) if used.	Yes	All NGS runs incorporated a 5% phiX spike
4.8	Negative Controls	Describe any negative controls if used.	Yes	Negative template controls (NTC) used for PCR. See attached SOP1.3
4.9	Contaminant mitigation and identification	Provide any laboratory or computational methods used to control for or identify microbiome contamination from the environment, reagents, or laboratory.	Yes	See attached SOP1.2 - 1.3
4.10	Replication	Describe any biological or technical replicates included in the sequencing, including which steps were replicated between them.	No	
4.11	Sequencing strategy	Major divisions of strategy, such as shotgun or amplicon sequencing.	Yes	16S V4 amplicon sequencing (see attached SOP1.3) sequenced by MiSeq
4.12	Sequencing methods	State whether experimental quantification was used (QMP/cell count based, spike-in based) or whether relative abundance methods were applied.	Yes	150 bp paired-end (MiSeq v2 chemistry); OTUs represented by fewer than 100 sequences in each sample were discarded. Average number of retained reads per sample was 314,302 (range: 22,865 - 836,230).
4.13	Batch effects	Detail any blocking or randomization used in study design to avoid confounding of batches with exposures or outcomes. Discuss any likely sources of batch effects, if known.	NA	
4.14	Metatranscriptomics	Detail whether any mRNA enrichment was performed and whether/how retrotranscription was performed prior to sequencing. Provide size range of isolated transcripts. Describe whether the sequencing library was stranded or not. Provide details on sequencing methods and platforms.	NA	
4.15	Metaproteomics	Detail which protease was used for digestion. Provide details on proteomic methods and platforms (e.g. LC-MS/MS, instrument type, column type, mass range, resolution, scan speed, maximum injection time, isolation window, normalised collision energy, and resolution).	NA	
4.16	Metabolomics	Specify the analytic method used (such as nuclear magnetic resonance spectroscopy or mass spectrometry). For mass spectrometry, detail which fractions were obtained (polar and/or non polar) and how these were analyzed. Provide details on metabolomics methods and platforms (e.g. derivatization, instrument type, injection type, column type and instrument settings).	NA	

5.0	Data sources/measurement	For each non-microbiome variable, including the health condition, intervention, or other variable of interest, state how it was defined, how it was measured or collected, and any transformations applied to the variable prior to analysis.	Yes	Pages 7-8
6.0	Research design for causal inference	Discuss any potential for confounding by variables that may influence both the outcome and exposure of interest. State any variables controlled for and the rationale for controlling for them.	Yes	Page 8 and DAG (directed acyclic graph) in Suppl Figure 4
6.1	Selection bias	Discuss potential for selection or survival bias.	Yes	Page 6
7.0	Bioinformatic and Statistical Methods	Describe any transformations to quantitative variables used in analyses (e.g. use of percentages instead of counts, normalization, rarefaction, categorization).	Yes	Taxon relative abundance and alpha-diversity measures. Original microbiome papers cited to provide full details on normalization.
7.1	Quality Control	Describe any methods to identify or filter low quality reads or samples.	Yes	Qiime v.1.8 default phred quality threshold [q >= 3] and maximum bad run length [r >= 3] was applied during binning and contig assembly. No requirement for minimum number of consecutive high quality base calls to retain. Retained, assembled sequences were then filtered at =>60% similarity against the reference database (Greengenes v.05.2013) using USEARCH6.1 to exclude non-bacterial sequences, chimeric sequences, etc.
7.2	Sequence analysis	Describe any taxonomic, functional profiling, or other sequence analysis performed.	Yes	On a run-by-run basis, filtered sequences (per 7.1) were classified by closed picking using USEARCH6.1 against the Greengenes reference database (v.05.2013) using a 97% similarity threshold. Unclassified sequences were retained and pooled form all sequencing runs and subjected to de novo clustering against the reference database (Greengenes v.05.2013) using USEARCH10. Taxonomies were assigned according to the Greengenes reference database, and closed and de novo picked datasets were merged.
7.3	Statistical methods	Describe all statistical methods.	Yes	Total and phylum-specific microbiota alpha-diversity indices were tested for their mediaiting effect, as described in the paper.
7.4	Longitudinal analysis	If the study is longitudinal, include a section that explicitly states what analysis methods were used (if any) to account for grouping of measurements by individual or patterns over time.	No	Only atopic senstization at 1 year available and of interest to outcome assess
7.5	Subgroup analysis	Describe any methods used to examine subgroups and interactions.		
7.6	Missing data	Explain how missing data were addressed.	Yes	Infants with missing data excluded from analysis
7.7	Sensitivity analyses	Describe any sensitivity analyses.	Yes	Suppl Table 4
7.8	Findings	State criteria used to select findings for reporting.	Yes	Criteria to proceed with mediation analysis on page 8
7.9	Software	Cite all software (including read mapping software) and databases (including any used for taxonomic reference or annotating amplicons, if applicable) used. Include version numbers.	Yes	Qiime 1.8; USEARCH 6.1; USEARCH10; Greengenes v.05.2013; Stata 17.0

8.0	Reproducible research	Make a statement about whether and how others can reproduce the reported analysis.	Yes	R code and output for mediation analyses cited/appended, so all required information available for reproduction.
8.1	Raw data access	State where raw data may be accessed including demultiplexing information.	Yes	CHILD database repository (www.childstudy.ca)
8.2	Processed data access	State where processed data may be accessed.	Yes	CHILD database repository (www.childstudy.ca)
8.3	Participant data access	State where individual participant data such as demographics and other covariates may be accessed, and how they can be matched to the microbiome data.	Yes	CHILD database repository (www.childstudy.ca)
8.4	Source code access	State where code may be accessed.	Yes	
8.5	Full results	Provide full results of all analyses, in computer-readable format, in supplementary materials.	Yes	
Results				
9.0	Descriptive data	Give characteristics of study participants (e.g. dietary, demographic, clinical, social) and information on exposures and potential confounders.	Yes	Table 1, Suppl Tables 1 and 2 and page 3
10.0	Microbiome data	Report descriptive findings for microbiome analyses with all applicable outcomes and covariates.	N/A	Gut microbiota variables were solely tested in mediation analyses, so were neither covariates nor outocmes.
10.1	Taxonomy	Identify taxonomy using standardized taxon classifications that are sufficient to uniquely identify taxa.	N/A	Only alpha-diversity measures of gut microbiota were found to be mediators
10.2	Differential abundance	Report results of differential abundance analysis by the variable of interest and (if applicable) by time, clearly indicating the direction of change and total number of taxa tested.	N/A	
10.3	Other data types	Report other data analyzed--e.g. metabolic function, functional potential, MAG assembly, and RNAseq.	NA	
10.4	Other statistical analysis	Report any statistical data analysis not covered above.	Yes	Gut microbiota mediation analyses on page 8
Discussion				
11.0	Key results	Summarise key results with reference to study objectives	Yes	First paragraph of the Discussion on page 4

12.0	Interpretation	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	Yes	Discussion on page 5
13.0	Limitations	Discuss limitations of the study, taking into account sources of potential bias or imprecision.	Yes	Discussion pn page 6
13.1	Bias	Discuss any potential for bias to influence study findings.	Yes	Page 6
13.2	Generalizability	Discuss the generalisability (external validity) of the study results		Page 6
14.0	Ongoing/future work	Describe potential future research or ongoing research based on the study's findings.	Yes	Page 6
Other information				
15.0	Funding	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Yes	
15.1	Acknowledgements	Include acknowledgements of those who contributed to the research but did not meet critera for authorship.	Yes	
15.2	Conflicts of Interest	Include a conflicts of interest statement.	Yes	
16.0	Supplements	Indicate where supplements may be accessed and what materials they contain.	Yes	
17.0	Supplementary data	Provide supplementary data files of results with for all taxa and all outcome variables analyzed. Indicate the taxonomic level of all taxa.	Yes	

1.1 – *Sy*MBIOTA Sample Tracking Protocol

Purpose: The following provides the guidelines to be followed by all research personnel to ensure proper tracking of all samples received, created, and used during analysis for the *Sy*MBIOTA project.

Responsibility and authority:

Postdoctoral Researcher (PD): responsible for determining the samples to be used for analysis. In this protocol the Postdoctoral Researcher shall:

- Request subject questionnaire and birth chart data from CHILD study
- Determine the subject samples to be used
- Submit request for sample release to CHILD Biologicals Committee

Laboratory Technician (LT): responsible for receiving and tracking stool samples, DNA isolates, and PCR products. In this protocol the Laboratory Technician shall:

- Coordinate the shipment of stool samples from the CRTCL lab
- Track movement of all samples in the lab's possession on Health Diary and/or lab database
- Maintain accurate storage records through Freezerworks Database

Abbreviations and definitions:

CHILD – Canadian Healthy Infant Longitudinal Development Study
CRTCL – Clinical Research and Clinical Trials Laboratory (Hamilton, ON)
LT – Laboratory Technician
PD – Postdoctoral Researcher

Principles:

Equipment:

Analytical balance
Barcode scanner
Computer with internet access
Cryogloves

Consumables:

Procedures:

1. Samples request

   a. The PD will request the birth chart and questionnaire data for all infants enrolled in the CHILD study at the Winnipeg, Edmonton, and Vancouver sites.

   b. Using the data, the PD will select the subjects that represent the best variety of environmental exposures and have the required stool samples for analysis (meconium, 3 month, and 1 year).

   c. The PD will submit a request for samples release to CHILD.

   d. Once the request has been approved, the PD will notify the LT.

2. Samples receipt

   a. The LT will coordinate the shipping of samples to the Scott lab with the CRTCL.

b. Immediately upon receipt of each sample batch at the Scott lab, the LT shall open the parcel to count the number of vials inside and compare this number to the shipping manifest. The LT shall also examine the integrity of the contents to ensure that no vials have become damaged or breached during shipment.

c. Each sample will be weighed on an analytical balance. 2050mg (average mass of a vial) will be subtracted from this amount to gain the estimated amount of stool in the vial. Record the mass on the manifest next to the sample.

d. The LT will “accept” the samples on Health Diary

i. In the “Study Samples” menu select the “Dispatch” tab, click “Inbound”, click “Accept”. Clicking the “Accept” link transfers the ownership of the box from “Shipper” to “Receiver“.

ii. Click the “Receive” link. Received samples will be displayed in the “Expected” tab in the “Study Samples” menu and are ready for processing.

iii. In the “Study Samples” menu select the “Boxes” tab, search the received box, click “Edit Box”, select “Discard”, click “Update”.

e. The LT will scan the samples into Freezerworks.

i. Open Freezerworks and login to the system.

ii. Determine the next box position available in the “Gage 203C” freezer.

iii. To enter the vial into the system, pick it up and scan it using the scanner to accurately enter the sample tracking ID. Enter the other required information: sample date (use today’s date), sample type, site, and study.

iv. Select “Manual Add” under the aliquots section.

v. Select “Gage 203C freezer”

vi. Enter the available position determined for this vial and enter the estimated amount of stool in the vial (from step 2.c).

vii. Click Save to enter the aliquot information.

viii. Click Save to register the information in the Freezerworks database.

ix. Repeat steps 2.e.i. – 2.e.viii. for the remaining samples.

x. Don cryogloves and open the ultralow freezer.

xi. Transfer the vials to their respective places in the appropriate freezer storage box.

xii. Close freezer and remove cryogloves.

3. *Sy*MBIOTA Project Database

a. Basic information

i. Open the form titled “Basic information”

ii. Click on the button “Add New Sample”

iii. Fill in the fields for each sample to be analyzed

(1) The Lab ID will be a sequential numerical/alphabetical system with 12 samples per batch number. For example, Batch 1 will have 12 samples so there will be Lab IDs from 1A through 1L; sample 13 will start batch 2 and be called 2A.

(2) The stool amount will come from the recorded amount in step 1d of the “Symbiota – DNA Isolation” protocol

(3) Notes: Include any unique information about the sample that might be valuable information to know during analysis of the data. For instance, if the stool was still in the diaper liner this needs to be recorded.

iv. When all samples are entered, click Save and close the form. All the entered information will be found in the table “Basic information”

b. Genomic DNA (60ul)

i. Open the table “Basic information”

ii. Find the Lab ID for the sample you want to enter information and click on the + sign at the far left. This will open a new line of cells.

iii. Re-enter the Lab ID

iv. Enter the Quantifluor reading obtained from step 5 of the “Symbiota – DNA Isolation” protocol. The information will be added to the “Genomic DNA (60ul)” table.

v. Click the – sign to hide the extra cells. Repeat steps 3.b.ii – 2.b.iv for all other samples.

vi. Open table “Genomic DNA (60ulA)” to verify the information has been transferred and the Genomic DNA amount has been automatically calculated.

vii. For all samples with >5ng/µl genomic DNA you will need to enter in an amount in the “Amount removed” field to generate the dilution information. Enter an amount that will provide sufficient total volume to perform multiple PCR reactions.

viii. Do not fill in dilution fields for samples with <5ng/µl genomic DNA.

ix. Save the table and close.

c. PCR information

i. Before PCR

(1) Open table “PCR product”

(2) Enter the Lab ID for each sample to be included in the PCR reactions

(3) Enter the next sequential Run# and Barcode# - Each MiSeq run will contain 25 different barcoded samples.

(4) Save the table, close, and perform the PCR reactions following “Symbiota – PCR amplification (Mi-Seq v4)” protocol.

ii. After PCR

(1) Open table “PCR product”

(2) Enter the Estimated product for each sample as determined in step 5.f of the Symbiota – PCR amplification (Mi-Seq v4)” protocol.

(3) The “Total Product (ng)” and “Amount for condensing (ul)” fields will automatically populate.

(4) Save the table and close.

References:

N/A

1.2 – *Sy*MBIOTA DNA Isolation Protocol (Adapted from Qiagen's Isolation of DNA from Stool for Pathogen Detection protocol[1] (Qiagen Inc., Valencia, CA)*)**

Purpose: This protocol describes the DNA isolation steps for stool samples collected as part of the Canadian Healthy Infant Longitudinal Development (CHILD) Study for analysis by the *Sy*MBIOTA project.

Responsibility and authority:

Principal Investigator (PI): lead investigator responsible for the scientific content and financial administration of the study. In this protocol, the PI shall:

- supervise and train the Laboratory Technician
- periodically review log sheets and database for completeness
- authorize the resolution of any inconsistencies on the log sheets or database
- oversee all laboratory procedures
- authorize the ordering of supplies and equipment

Laboratory Technician (LT): responsible for performing DNA isolation, tracking information in the database, maintaining equipment. In the protocol, the LT shall:

- Prepare stool samples for isolation
- Set-up QIAcube for DNA isolation
- Perform pre-extraction steps
- Track samples and results in the *Sy*MBIOTA database
- Confirm DNA isolation with gel electrophoresis and DNA quantification
- Clean and perform regular maintenance on the QIAcube and pipettes

Abbreviations and definitions:

CHILD – Canadian Healthy Infant Longitudinal Development Study
LT – Laboratory Technician
PI – Principal Investigator
QIAcube – Automated sample prep machine

Principles:

These procedures apply to stool samples collected in conjunction with the CHILD study. To avoid accidental confusion of samples, it is imperative that the following procedures are followed precisely at all times. Procedures specifically related to labeling samples and handling of extracts is particularly critical.

Equipment:

Analytical balance
Barcode scanner
Centrifuge
Computer
Float
GelDoc XR+ System (BioRad)
Pipettes
QIAcube (Qiagen cat# 9001292)
QuantiFluor dsDNA system
Rack labeling strip: QIAamp DNA
Reagent bottle rack (Qiagen cat# 990390)
Rotor Adapter holder (Qiagen cat# 990392)
Water bath
Vortex

Consumables:

λHindIII/EcoRI marker
100 base pair DNA ladder
1.5ml microcentrifuge tubes (Fisher)
2ml microcentrifuge tubes (Fisher)
2ml screw-cap microcentrifuge tubes
Agarose, biotechnology grade
Disposable Filter-Tips for QIAcube [1000µl (Qiagen cat# 990352), 200µl (Qiagen cat# 990332)]
Ethanol (70% & 100%)
Flask, 125ml
Lab marker
Loading dye
Pipette tips (1000µl, 200µl)
QIAamp DNA Stool Mini Kit (Qiagen cat# 51504)
QIAamp DNA Accessory Set A (Qiagen cat# 1048142)
Reagent bottles (Qiagen cat# 990393)
Rotor Adapters (Qiagen cat# 990394)
SYBRsafe DNA gel stain
TAE, 1X

Procedures:

1. Before QIAcube

   a. Turn on the water bath to 95°C.

   b. Remove stool samples from the freezer (maximum of 12 samples can be analyzed at a time).

   c. Using the analytical balance, weigh ~200mg of stool in a 2ml screw-cap microcentrifuge tube and place on ice. (Do not allow the sample to thaw until Buffer ASL is added). Continue to aliquot from all samples to be analyzed. ***NOTE****: Stool amount can be 180-220mg. If a sample does not have at least 180mg then use the full sample, as long as there is at least 80mg. Samples less than 80mg will need another aliquot requested in order to reach the minimum amount to be analyzed.*

   d. Record the amount of stool removed in the lab book.

   e. Add 1.4ml of Buffer ASL to each stool sample. Vortex continuously for 1min or until the stool sample is thoroughly homogenized.

   f. Place the samples in a float and place in the heated water bath for 5min.

   g. Remove samples and blot the outside dry with a paper towel

   h. Vortex samples for 15sec, then centrifuge at 14,000x g for 2min to pellet stool.

   i. Pipet 1.2ml of the supernatant into a new 2ml microcentrifuge tube and discard the pellet.

   j. Add 1 InhibitEX Tablet to 2 samples and vortex immediately and continuously for 1min or until the tablet is completely suspended. (Note: Sometimes the liquid at the bottom of the tube does not come in to contact with the tablet. Hold the tube in different positions when

vortexing to make sure all liquid mixes with the tablet.) Repeat until all samples have been combined with the tablet.

k. Incubate at room temperature for 1min to all inhibitors to adsorb to the InhibitEX matrix.

l. Centrifuge samples at 14,000x g for 5min to pellet inhibitors bounds to InhibitEX matrix. Move on to step 2 while the centrifuge is running.

2. QIAcube set-up

a. Press the blue power button to turn on the QIAcube.

b. Select the "QIAamp DNA Stool Human Stool Stool 60µl Elution" protocol on the touch screen by selecting DNA→ 2. QIAamp DNA stool → Human Stool → 3. Stool 60µl Elution. ***NOTE***: This protocol is the Pathogen Detection modified to allow for 60µl elution instead of the factory-set standard elution of 200 µl.

c. Press Start. Press Next

d. Empty the waste drawer. Fill one tip rack with 1000µl disposable filter tips and the other with 200µl disposable filter tips. Press Next.

e. Fill reagent bottles with Buffers AL, AW1, AW2, AE, and 100% ethanol (all obtained from the kits) and place in the correct position in the reagent bottle rack labeled with the QIAamp DNA labeling strip (Note: An error will occur at the start of the program if there is not even liquid in a reagent bottle. Add more buffer to the bottle and restart the program). Press Next.

f. Cut the lid off a 1.5ml microcentrifuge tube. Add appropriate amount of Proteinase K (per Table[2] below) to the tube and place in Position A in the QIAcube. Press Next.

# of Samples	Proteinase K volume (µl)	# of Samples	Proteinase K volume (µl)
2	52	7	133
3	69	8	150
4	85	9	165
5	101	10	182
6	117	12	214

g. Place empty 2ml microcentrifuge tubes onto the shaker in the numbered positions according to the QIAcube Loading Chart. Ensure the lids are folded back and secured in the holder.

h. Place rotor adapters on the rotor adapter holder, in numbered positions that correspond with the proper QIAcube Loading Chart[3]. Write the position numbers in the lab book. Assign a sample ID to each position number. Press Next.

i. Place a QIAamp spin column in each rotor adapter at Position 1. (Note: Discard the collection tube that comes with the spin column before placing the spin column in the rotor adapter)

ii. Load a 1.5ml microcentrifuge tube in Position 3 of each rotor adapter. Ensure the lid is placed in the correct position, according to the sticker on the rotor adapter holder.

iii. Remove the samples from the centrifuge. Transfer 350µl of the supernatant to Position 2 of the correct rotor adapter assigned to each sample. Discard the pellet.

iv. Place rotor adapters in the centrifuge in their assigned position. Press Next.

i. Close the QIAcube door. Press Start to run the protocol.

j. Wait until the protocol begins to run. The QIAcube will take a few minutes to ensure the correct number of pipette tips, amount of buffer, and amount of tubes were placed, as well as the correct positioning of the rotor adapters. The QIAcube will notify if something is wrong. If so, correct the problem and restart the program until the protocol begins to run.

3. QIAcube protocol completion

a. The QIAcube will sound a signal when the protocol has completed.

b. Open the QIAcube door. Carefully remove the 1.5ml microcentrifuge tubes from the rotor adapters. Using a lab marker record the lab ID, sample ID, today's date, and "Genomic DNA" on the tube.

c. Place the tubes in a freezer box and place in the -20°C freezer. Enter the storage information in the database according to SOP 1.1 – *Sy*MBIOTA Sample Tracking Protocol.

d. Open the QIAcube waster drawer.

e. Remove the rotor adapters from the centrifuge and place in the waste drawer. Remove the tube from Position A and place in the waste drawer.

f. Discard all contents of the waste drawer in the yellow biological contaminants bin.

g. Wash the inner liner of the waste drawer with lab grade detergent.

h. Wipe the interior components of the QIAcube with 70% Ethanol.

i. Close the QIAcube door and turn off by pressing the blue power button.

4. Electrophoresis gel of genomic DNA

a. Remove samples from the freezer and allow them to thaw on the bench.

b. Prepare a 1.0% agarose gel using biotechnology grade agarose, 1X TAE, and SYBRsafe stain.

c. Place the gel boat into the electrophoresis chamber filled with 1X TAE.

   d. Flick the bottom of each tube to mix the genomic DNA in the tube. Centrifuge the tubes at 5000rpm for 30sec to ensure all liquid is at the bottom of the tube.

   e. Mix 2µl of loading dye with 5µl of genomic DNA and pipette into a single well in the gel.

   f. Use 100bp DNA ladder and λHindIII/EcoRI as markers

   g. Run for 1hr and 20min at 80V

   h. Take a picture of the gel using the GelDoc XR+ Imaging system to confirm genomic DNA in the eluted sample.

   i. Print the picture and place in the lab book.

5. DNA quantification

   a. Follow procedures in SyMBIOTA SOP 1.5 –Quantification of DNA using Quantifluor dsDNA System

   b. Record the quantity information in the database according to SyMBIOTA SOP 1.1 –Sample Tracking Protocol.

   c. On the side of the tubes write: Lab ID, Sample ID, Stool type, “gDNA”, and the date isolated.

   d. Place the tubes in the appropriately labeled box in the -20 freezer.

6. Genomic DNA dilutions to 5ng/µl

   a. Print the table “Genomic DNA (60ul)” from the SyMBIOTA Samples Database for the select samples you want to dilute.

   b. Pull out the samples that will need to be diluted, from the -20°C freezer and place in a rack.

   c. While waiting for the samples to thaw, place new 1.5ml tubes in a new freezer rack, 1 per sample to be diluted.

   d. Using a blue colored lab marker write:

      i. On the lid: Lab ID, “Dil gDNA”, and “5ng/µl “

      ii. On the side: Lab ID, Sample ID, Stool type, “Diluted gDNA”, and “5ng/µl”

   e. Following the information on the printed out table, pipette the stated amount of genomic DNA and Sigma water in to the appropriately labeled tubes.

   f. When all samples have been diluted, place the tubes in to their appropriately labeled boxes in the -20°C freezer.

References:

1. Isolation of DNA from stool for pathogen detection. *QIAamp DNA Stool Handbook: Second Edition.* Qiagen Inc. Valencia, CA. July 2007

2. Isolation of DNA from stool for pathogen detection. *QIAcube Protocol Sheet*. Qiagen Inc. Valencia, CA. February 2011.

3. QIAcube loading chart. Qiagen Inc. Valencia, CA.

1.3 – SyMBIOTA PCR Amplification Protocol

Purpose: This protocol describes the 16S rDNA PCR amplification steps of DNA obtained from stool samples collected as part of the Canadian Healthy Infant Longitudinal Development (CHILD) Study for analysis by the *Sy*MBIOTA project.

Responsibility and authority:

Principal Investigator (PI): lead investigator responsible for the scientific content and financial administration of the study. In this protocol, the PI shall:

- supervise and train the Laboratory Technician
- periodically review log sheets and database for completeness
- authorize the resolution of any inconsistencies on the log sheets or database
- oversee all laboratory procedures
- authorize the ordering of supplies and equipment

Laboratory Technician (LT): responsible for performing PCR set-up, condensing/cleaning product, quantifying product, and maintaining equipment. In the protocol, the LT shall:

- Set-up PCR reactions
- Program and run Peltier Thermal Cycler
- Condense and clean PCR product
- Confirm DNA amplification with gel electrophoresis and DNA quantification
- Track samples and results in the *Sy*MBIOTA database
- Clean and perform regular maintenance on the pipettes

Abbreviations and definitions:

CHILD – Canadian Healthy Infant Longitudinal Development Study
dNTPs – Deoxyribonucleotide triphosphates
LT – Laboratory Technician
NTC – Negative Template Control
PCR – Polymerase Chain Reaction
PI – Principal Investigator

Principles:

These procedures apply to isolated DNA from stool samples collected in conjunction with the CHILD study. To avoid accidental confusion of samples, it is imperative that the following procedures are followed precisely at all times. Procedures specifically related to labeling tubes and handling of amplified products is particularly critical.

Equipment:

Centrifuge – Allegra X-15R (Beckman Coulter)
Centrifuge – Spectrafuge 14D
Computer
Freezer, -20°C
GelDoc XR+ System (BioRad)
Lab marker
Labtop cooler (Nalgene)
Microcentrifuge
Peltier Thermal Cycler (PTC-200 - MJ Research)
Pipettes
PCR racks
QuantiFluor dsDNA system

Refrigerator, 4°C
Shaker (Microplate Genie SI-0400)

Consumables:

λHindIII/EcoRI marker
100 base pair DNA ladder
Agarose, biotechnology grade
Forward primers (V4+515F)
Barcoded reverse primers (BC-V4-806R)
Ethanol (70% & 100%)
Flask, 125ml
GENECLEAN® Turbo Kit (MP Biomedicals cat# 1102-400)
Kapa2G Robust Hotstart Taq ready mix, 2X
Lab marker
Loading dye
Microcentrifuge tubes, 2ml
PCR tubes (XX)
Pipette tips (200µl, 1000µl)
Sigma water (Molecular Biology grade)
SYBRsafe DNA gel stain
TAE, 1X

Procedures:

1. Barcoded primer assignments - Follow step 3.c.i in "Symbiota – Sample tracking" protocol to assign the barcoded primers for each sample.

2. PCR preparation

   a. Remove isolated genomic DNA samples and PCR reagents (Kapa2G Robust Hotstart Taq ready mix 2X, primers (*as determined from Step 1*), and Sigma water) to be amplified from the freezer and place on the bench to allow them to thaw. ***NOTE:*** *Once the Hotstart ready mix has thawed, place in -20°C benchtop cooler until ready to use.*

   b. Tube prep (While samples and reagents are thawing)

      i. Place PCR tubes in the PCR racks. There will need to be 4 tubes per sample (3 reactions plus a NTC).

      ii. Using a lab marker, label each tube with the appropriate label ID assigned to the sample. The 4th tube for each sample should also be labeled with "NTC".

      iii. Label and rack 1.5ml tubes for the master mixes (1 per barcoded primer).

   c. Flick the bottoms of all the sample and reagent tubes to mix the liquid. Centrifuge the tubes at 5000rpm for 30sec to ensure all liquid is at the bottom of the tube.

   d. Pipette the reagents into the appropriately labeled 1.5ml microcentrifuge tube to create the master mix, by following the amounts in the table below. Place the reagents in the labtop cooler when finished.

Reagent	Concentrations	Amount per reaction	Master Mix (3rx + NTC)

Kapa2G Hotstart mix 2X		12.5	50
V4+515F primer (10µM)	0.6 µM	1.5	6
BC-V4-806R primer (10µM)	0.6 µM	1.5	6
Sigma H_2O		7.5	30
Template DNA (5ng/µl)		10ng in 2µl	8
Total →		**25 µl**	**100 µl**

e. Mix the master mixes by flicking the tube, then centrifuge at 5000rpm for 30sec to ensure no liquid is stuck on the lid.

f. Pipette 23µl of the master mix in to every appropriate PCR tube. Discard the master mix tube.

g. Pipette 2µl of template DNA from the sample tubes into the appropriate tubes. Close the lids.

h. Pipette 2µl of Sigma water in to each of the tubes labeled NTC. Close the lids.

i. Mix the tubes by placing the rack in the Microplate Genie.

j. Spin the liquid to the bottom of the tubes by placing the racks in the Allegra X-15R Centrifuge. Settings:

i. **Rotor**: SX4750
ii. **RCF**: 2000 x g
iii. **Temp**: 4°C
iv. **Time**: 2min
v. **Accel**: MAX
vi. **Decel**: MAX

k. Place the PCR trays in the 4°C fridge until ready to run the PCR program.

3. PCR run

a. Turn on the Peltier Thermal Cycler

b. Locate the program "CAGEF" on the menu of the screen.

c. Verify that the program follows the following amplification profile:

Step	Temperature	Time
1	94°C	3 min
2	94°C	30 sec
3	50°C	30 sec
4	72°C	30 sec
5	Go to Step 2 x 19	
6	10°C	∞

d. Remove the PCR trays from the fridge and place the tubes in the Peltier Thermal Cycler.

e. Close the lid of the thermal cycler carefully, ensuring a snug fit.

f. Start the CAGEF program.

g. At the conclusion of the program, remove the PCR tubes and place them back in the PCR trays. Label the top of the trays with today's date and "SyMBIOTA PCR amplification products".

h. Place the trays in the -20°C freezer until ready to move to condensing and cleaning.

4. Combine and electrophoresis gel of amplified DNA

a. Remove PCR tube trays from the freezer and allow them to thaw on the bench.

b. Prepare a 1.4% agarose gel using biotechnology grade agarose, 1X TAE, and SYBRsafe stain.

c. Spin down the PCR tubes following procedure in step 1.j

d. Rack and label one 1.5ml tube for each sample ID

e. Pipette all liquid from the 3 labeled PCR tubes into appropriately labeled 1.5ml tube.

f. Flick the bottoms of all the 1.5ml sample tubes to mix the liquid. Centrifuge the tubes at 5000rpm for 30sec to ensure all liquid is at the bottom of the tube.

g. Place the gel boat into the electrophoresis chamber filled with 1X TAE.

h. Flick the bottom of each tube to mix the amplified DNA in the tube. Centrifuge the tubes at 5000rpm for 30sec to ensure all liquid is at the bottom of the tube.

i. Mix 2µl of loading dye with 5µl of amplified DNA or NTC and pipette into a single well in the gel.

j. Use 100bp DNA ladder as a marker

k. Run for 1hr and 30min at 80V

l. Take a picture of the gel using the GelDoc XR+ Imaging system to confirm amplified DNA bands are in the correct base pair range, and to verify the NTC has no bands of amplified DNA.

m. Print the picture and place in the lab book.

5. DNA quantification using band brightness

a. Open the image file in Image Lab

b. Click on Image Tools from the Analysis Tool Box on the left of the screen.

i. Use the rotate buttons to ensure the image shows the bands running horizontally across the screen.

ii. Click on the green ↑ to go back to the “Analysis Tool Box”.

c. Click on Lane and Bands

i. With the Lanes tab highlighted, select Automatic. This will detect the lanes based on the bands visible on the gel.

ii. Use the other options in the Lanes tools menu to adjust the number and/or sizes of the lanes to reflect the real data.

iii. Click on the Bands tab, and then select Detect Bands. This will also automatically detect the bands present on the gel.

iv. Use the tools in the Bands menu to select or deselect the bands so only the ones that will be quantified are selected.

v. Click on the green ↑ to go back to the “Analysis Tool Box”.

d. Click on Quantity Tools

i. Click on the Absolute tab and then press the Select button

ii. Click on one of the clearly visible and defined bands in the 100bp DNA ladder.

iii. Enter to numerical quantity of that band. (This will be determined by the amount of DNA ladder placed in the gel and the known amount of DNA in each band from the Product Description Sheet for the specific DNA ladder.) Then click OK.

iv. Repeat steps 4.d.ii-4.d.iii for at least 4 more bands.

v. Use the dropdown box under Units to select the appropriate notation: most likely Nanograms.

vi. Click on the green ↑ to go back to the “Analysis Tool Box”.

e. Click on Analysis table at the top of the menu bar

i. Scroll down the table to verify each band that needs to be quantified has a numerical value under “Abs. Quant.”. Also, verify that the numbers make sense, ie a lighter band should have a smaller number than a brighter band.

ii. Click on the furthest right icon above the table to “Export Analysis table to Excel”. This will open the table in an Excel spreadsheet.

iii. Save the image file and close out of Image Lab.

f. Manipulating the Excel spreadsheet

i. Insert a column to the right of the "Abs. Quant." Column. Title the column "ng/ul". In the first cell for a sample type in the new column type "=". Then click on the Abs. Quant. Cell for that sample. Then type "/5". Hit enter. (This determines the estimated DNA amount per microliter, assuming 5ul were used for the well).

ii. Copy and paste the formula in step 4.f.i for all remaining samples.

iii. Print the table and paste in the lab book.

iv. *NOTE*: Any sample with <100ng of total product will need to be redone. Most likely the sample has PCR inhibitors and it will need to be diluted further in order to achieve maximum yield.

g. Record the quantity information in the database according to SOP 1.1 – *Sy*MBIOTA Sample Tracking Protocol.

Limitations:

N/A

References:

1. Rapid Isolation of DNA from PCR Reactions and Other Enzymatic Solutions. *GENECLEAN Turbo® Kit*. MP Biomedicals Inc. Solon, OH. Revision # 1102-999-3I12.

1.3 – SyMBIOTA Sequencing Preparation Protocol

Purpose: This protocol describes the steps to combine, condense, and gel-cut PCR products from stool samples, collected as part of the Canadian Healthy Infant Longitudinal Development (CHILD) Study for analysis by the *Sy*MBIOTA project, to be sent for sequencing analysis.

Responsibility and authority:

Principal Investigator (PI): lead investigator responsible for the scientific content and financial administration of the study. In this protocol, the PI shall:

- supervise and train the Laboratory Technician
- periodically review log sheets and database for completeness
- authorize the resolution of any inconsistencies on the log sheets or database
- oversee all laboratory procedures
- authorize the ordering of supplies and equipment

Laboratory Technician (LT): responsible for combining/condensing/cleaning product, quantifying product, and maintaining equipment. In the protocol, the LT shall:

- Combine all PCR products for specified sequencing runds
- Condense and clean combined PCR product
- Identify PCR product with gel electrophoresis
- Quantify combined and cleaned PCR product
- Track samples and results in the *Sy*MBIOTA database
- Clean and perform regular maintenance on the pipettes

Abbreviations and definitions:

CAGEF - Centre for Analysis of Genome Evolution & Function (Univ of Toronto)
CHILD – Canadian Healthy Infant Longitudinal Development Study
LT – Laboratory Technician
PCR – Polymerase Chain Reaction
PI – Principal Investigator

Principles:

These procedures apply to 16S DNA v4 PCR product from stool samples collected in conjunction with the CHILD study. To avoid accidental confusion of samples, it is imperative that the following procedures are followed precisely at all times. Procedures specifically related to labeling tubes and handling of amplified products is particularly critical.

Equipment:

Centrifuge – Spectrafuge 14D
Computer
Freezer, -20°C
GelDoc XR+ System (BioRad)
Lab marker
Microcentrifuge – Hanil Micro -12
Pipettes
PCR racks
QuantiFluor dsDNA system
Refrigerator, 4°C
Spatula, stainless steel

Consumables:

100 base pair DNA ladder
Agarose, biotechnology grade
Amicon Ultra Centrifugal Filters – 0.5ml 30K (Millipore UFC503024)
Ethanol (70% & 100%)
Flask, 125ml
GENECLEAN® Turbo Kit (MP Biomedicals cat# 1102-400)
Lab marker
Loading dye – 6X with Xylene cyanol
Microcentrifuge tubes, 1.5ml
PCR tubes (XX)
Pipette tips (200μl, 1000μl)
Sigma water (Molecular Biology grade)
SYBRsafe DNA gel stain
TAE, 1X

Procedures:

1. Combining PCR product
   a. Open the “PCR product” table in the SyMBIOTA Samples Database
   b. Click on the “External Data” tab
   c. Click on the Excel button above “Export”.
   d. Choose the destination you want to save the exported spreadsheet, then click OK.
   e. Find and open the spreadsheet in the exported destination.
   f. Highlight the entire spreadsheet by clicking the triangle in the top left box of the spreadsheet.
   g. Sort the data
      i. Click Data → Sort
         (1) Sort by Run. Sort on Values. Order Smallest to Largest
         (2) Add Level → Sort by Barcode. Sort on Values. Order A to Z.
         (3) Click OK. A warning will pop up, make sure to select “Sort anything that looks like a number, as a number”, and then select OK.
      ii. The data should now be arranged in order of run number with all 25 samples arranged in order of barcode number.
      iii. Print the table containing the rows you plan to combine for sequencing.
   h. Pull the labeled 1.5ml tubes containing the PCR product from the -20°C freezer and allow to thaw on the bench. When thawed, centrifuge in the microcentrifuge to make sure all liquid is at the bottom of the tubes.
   i. Label a new 1.5ml tube with the run number.

j. Following the table from step 1.g.iii, pipette the appropriate amount of liquid from each tube in to the tube labeled with the run number.

k. For balancing purposes it's best to prepare even number of runs at a time.

2. Condensing the combined PCR product

   a. Centrifuge the tubes containing the combined PCR product in the microcentrifuge to get all the liquid at the bottom.

   b. Follow the instructions for using the Amicon Ultra centrifugal filters provided with the filters to condense the combined PCR product to 25-50µl for each run.

   c. **NOTE**: Make sure to label to tubes containing the filter and the final condensed product to avoid mixing of product between runs.

   d. Store the condensed product in the 4°C fridge if you will gel-cut the same day or the -20°F freezer if gel-cutting will occur the next day or more.

3. Gel-cut the combined v4 amplified sequences

   a. Determine the total number of lanes needed based on the volume of condensed product for each run. Estimate 25µl per lane.

   b. Based on the number of lanes needed, prepare a small or large 1.4% agarose gel w/ SYBR safe stain using the large well comb.

   c. Load the condensed product using 6X Xylene cyanol loading dye.

   d. Load 1 well with 100bp ladder to ensure cutting of the correct band later on.

   e. Run the gel at 80 volts for 90min if the gel had 1 comb or 45min if the gel had 2 combs.

   f. While the gel is running, prepare and label a 1.5ml tube and a small metal spatula for each run. Record the weight of each tube after labeling.

   g. Place a layer of plastic wrap over the Xcita Blue Conversion Screen in the GelDoc (smooth out the wrap to remove the bubbles). Remove the gel from the apparatus and place on top of the plastic wrap.

   h. Take a picture using filter 2 to view the bands for cutting. Print a picture to place in the lab book.

   i. Open the GelDoc door and insert the UV protection screen.

   j. Turn off the lights to the room.

   k. Put on the orange UV protection goggles and press the "TRANS UV" button. You will now be able to view the bands within your gel.

   l. Carefully use the spatula to cut the gel to remove the band between 300 and 400 base pairs. Place the removed portion in the appropriately labeled tube. Repeat for all runs.

m. Turn on the room lights. Place the tubes in the 4°C fridge.

n. Take another picture of the gel to verify excision of the bands and place in the lab book.

o. Fold the plastic wrap around the gel and discard in the garbage.

4. Cleaning the cut bands

   a. Remove the tubes containing the gel cuttings from the fridge.

   b. Weigh each tube.

   c. Subtract the pre-weight from the post-weight to determine the amount of gel in the tube.

   d. Follow the steps in the GeneClean Turbo Kit to clean the DNA from the gel using the following modifications:

      i. Elute in 40μl DES for 10 minutes

      ii. Centrifuge the final elution for 2 minutes

   e. Quantify the DNA in each tube following the procedures in SOP 1.5 – DNA Quantification using QuantiFluor dsDNA System. Record in the lab book.

   f. *NOTE*: Each tube needs to contain at least 1μg of DNA as per CAGEF's request to be able to sequence properly.

   g. Label the tubes with: "SyMBIOTA", the run number, the date, and amount of DNA in μg.

   h. Store in the -20°C freezer until ready to be sent to CAGEF for sequencing.

Limitations:

N/A

References:

1.5 – Quantification of dsDNA using the QuantiFluor™ system (Promega)

Purpose:

Abbreviations and definitions:
dsDNA = double stranded DNA including genomic/template DNA, plasmid DNA & PCR product
dH_20 = RNase/DNase-free molecular biology grade distilled water (Cat. #W4502, Sigma)

Principles:

Equipment:

QuantiFluor™ Handheld Fluorometer (Model # E6090, Promega)
QuantiFluor™ PCR Tube Adapter (Cat. # E6101, Promega)
1000µl pipeter (Rainin Inc.
200µl pipeter (Rainin Inc.)
20µl pipeter (Rainin Inc.)

Consumables:

Provided with QuantiFluor™ dsDNA kit (Cat.# E2670, Promega) (all stored at 4°C in covered/opaque box):
- 20X TE buffer (pH 7.5)
- 200X QuantiFluor™ dye (1ml)
- 100µg/ml Lambda DNA Standard (100µg)

RNase/DNase-free molecular biology grade distilled water (Cat.# W4502, Sigma)
0.5ml optically clear PCR tubes (Cat.# PCR-05-C, Axygen)
15ml RNase/DNase-free (sterile) screw-top centrifuge tube
50ml graduated, RNase/DNase-free (sterile) screw-top centrifuge tube
Filtered tips for the above pipeters (sterile, RNase/DNase-free, Rainin Inc.)
5-10ml sterile RNase/DNase-free serological pipet
2ml RNase/DNase-free (sterile) microcentrifuge tube
1.5ml RNase/DNase-free (sterile) microcentrifuge tube

Procedures:
1. Dilute 20X TE Buffer stock to make a 1X TE buffer working solution.
   a. aliquot 2 ml of 20X TE stock to 50ml graduated centrifuge tube
   b. add dH_2O to bring solution up to 40ml
   c. store working solution at 4°C

2. Dilute 200X QuantiFluor™ stock dye to 1X dye working solution.
   a. warm stock solution to room temperature [**Note: be sure that stock has fully thawed and mixed well before taking aliquot. Dye solution is light sensitive and must remained covered while thawing and during storage**.]
   b. aliquot 10µl 200X stock dye into 2ml microcentrifuge tube
   c. add 1990µl 1XTE buffer, cap and mix well
   d. store working solution at 4°C in covered/opaque box

3. Make standard(s) using the Lambda DNA Standard provided with the kit. [**Note: Standards concentration will be halved when diluted 1:1 with sample solutions. Also, make appropriate standards (values comparable to sample values) as based on 1:50 dilutions of the samples to be quantified.**]
   a. for 2000ng/ml standard (for high-cycle number PCR product or cloned-plasmid product) make a 1:50 dilution of Lambda DNA Standard stock

Example, for 5mls of this standard;

i. aliquot 100μl of 100μg/ml (100,000ng/ml) Lambda DNA Standard stock into a 1.5ml microcentrifuge tube

ii. add 4.9ml of 1XTE Buffer, cap and mix well

iii. store working solution at 4°C

b. for 100ng/ml standard for genomic DNA and low cycle-number PCR product dilute 2000ng/μl standard (from Step 3.a.) to a 1:20 solution [**Note: this is a 1:1000 of original concentrated stock as provided by company.**]

Example, for 10mls of this standard;

i. aliquot 500μl of 2000ng/ml standard solution (created in step 3.a) into 15 ml centrifuge tube

ii. add 9.5 mls 1XTE Buffer, cap and mix well

iii. store working solution at 4°C

4. Power on QuantiFluor™ Fluorometer a minimum of 5 minutes before analyzing samples, by pressing the blue "ON/OFF" button once.

5. Place QuantiFluor™ PCR Tube Adapter in cuvette holder as per its manual.

6. Set the fluorometer to discrete mode (if not done previously) by pressing the 'SET' button and using the toggle arrows to select 'DISCRETE'.

7. Take working concentrations solutions (as created above), out of fridge and warm to room temperature. Do not expose dye solution to light until ready to use.

8. Take two 0.5ml PCR tubes and label one with "Blank" and the other with "Standard".

9. Prepare blank by aliquoting 100μl of 1X TE buffer into the 0.5ml PCR tube labeled "Blank".

10. Prepare a standard.
    a. Aliquot 50μl of appropriate standard solution (as created in Step 3) into the 0.5ml PCR tube labeled "Standard". [**Note: choose the standard likely to be closest in value to a 1:50 dilution of your sample.**]
    b. Aliquot 50μl of dye working solution, cap and mix well.
    c. Store at room temperature in covered container until use.

11. Prepare samples dilutions
    a. For each sample, label one 0.5ml PCR tube and aliquot 48μl of 1X TE buffer into it.
    b. Aliquot 2μl of each sample into the corresponding tube.
    c. Aliquot 50μl of dye working solution into each tube, cap and mix well.
    d. Store at room temperature in covered container until read. [**Note: samples should be read shortly after mixing with dye as the signal deteriorates with time.**]

12. Calibrate QuantiFluor™ Fluorometer
    a. Press 'STD VAL' button and toggle to match your selected standard. [**Note: the fluorometer reads in μg/L or ng/ml.**] Once set press 'ENTER'.
    b. Press 'CAL' and then 'ENTER' to start calibration.
    c. Insert your blank solution into PCR tube adapter (as prompted on the devices screen) and press 'ENTER'. Wait while machine reads the blank.
    d. Take out the blank tube, then insert your standard into PCR tube adapter (as prompted on the devices screen) and press 'ENTER'. Wait while machine reads the standard.
    e. Remove the standard tube, then press the 'ENTER' button to accept the calibration (as prompted on the devices screen).

13. Quantify samples.
    a. Insert sample tube and press the 'READ' button.
    b. Once the 'Reading' message disappears from the devices screen disappears, record the value that appears on the screen. [**Note: for large sample sets you can download the results to your computer. See the device manual.**]
    c. Read subsequent sample following Steps 12.a. & 12.b.

14. Calculate dsDNA concentration in original sample.
    a. The above protocol results in 1:50 dilutions of the original sample. Therefore multiply the reading by 50.
    b. The device outputs concentration in ng/ml. Therefore, you must divide by 1000 to get your reading in units of ng/µl.

Limitations:

- The standards listed above may not be appropriate for all your samples. Standards should be similar to the dilutions being measured.
- 1:50 dilutions may not be appropriate for all samples. The approximate limit of detection using the above protocol should be approximately 50pg/ml.
- While the QuantiFluor™ dye is specific to double-stranded DNA, excessive amounts of single stranded DNA (for example, primers and dNTPs) can artificially inflate the reading. Especially problematic are PCR protocols prone to producing pseudo double-stranded artifacts (such as often seen in rDNA-based assays). These contaminants must be cleaned from the sample prior to quantifying using this system.

References:

Manual (Part# TM338) - QuantiFluor™ Handheld Fluorometer (Model # E6090, Promega)
Manual (Part# AP074) - QuantiFluor™ PCR Tube Adapter (Cat. # E6101, Promega)
Manual (Part# TM346) - QuantiFluor™ dsDNA kit (Cat. # E2670, Promega)

Symbiota methods (extended description)

DNA isolation

Following collection and aliquotting, stool samples were maintained frozen at or below –80 C prior to analysis. For the isolation of community DNA, a modification of the Qiagen Isolation of DNA from Stool for Pathogen Detection protocol was used (Qiagen Inc., Valencia CA). In this procedure, a target mass of 200 mg (acceptable range from 80–220 mg, actual mass recorded) of frozen stool was combined with 1.4 mL Qiagen Stool Lysis Buffer (ASL), vortex mixed for 1 min or until the sample appeared thoroughly thawed and homogenized, and placed in a 95 C water bath for 5 min. Samples were then vortex mixed for 15 sec and centrifuged for 2 min at 14 Krpm. A volume of 1.2 mL of supernatant was removed to a new microcentrifuge tube, combined with a tablet of InhibitEX (Qiagen) and vortex mixed continuously for 1 min or until suspended. Samples were then incubated at room temperature for 1 min and centrifuged for 5 min at 14 Krpm. The remainder of the isolation procedure was carried out using a QIAcube robot following the "Pathogen Detection" program modified for a 60 µL elution volume rathewr than the standard 200 µL elution volume. Following completion of the program, DNA quality was evaluated by electrophoresis of 5 µL of isolated DNA in 1.0% agarose in 1×TAE buffer for 80 min at 80 VDV, visualized using SYBR safe stain (Thermo Fisher Scientific / Life Technologies Corp., Carlsbad CA) and recorded using a GelDoc XR+ Imaging system (BioRad Laboratories Inc., Hercules CA). DNA concentration was evaluated using a Quantifluor dsDNA system following the manufacturer's instructions, and adjusted to a final concentration of 5 ng/µL by the addition of 1×TE buffer.

Amplification

Bacterial 16S DNA from hypervariable region V4 was amplified by PCR using the core forward primer V4+515F (5'-TATGGTAATTGTGTGCCAGCMGCCGCGGTAA-3') and the core reverse primer V4-806R (5'-AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-3') synthesized to include Illumina adapers, primer pad and linker sequences, a Golay barcode (forward primer). PCRs were conducted in a final volume of 25 µL, consisting of 10 ng template DNA and 0.6 µM of each primer in Kapa2G Robust Hotstart Taq ready mix (KapaBiosystems, Wilmington MA) at 1× concentration. PCR conditions consisted of an initial denaturation at 94 for 3 min followed by 20 cycles of 94 C for 30 sec (denaturation), 50 C for 30 sec (annealing), and 72 C for 30 sec (extension), with a completion step at 10 C. Amplicon quality was assessed by electrophoresis following the procedure described in the previous section and quantitated based on the summed brightness of bands as determined by the GelDoc XR+ analyser. Samples yielding less 100 ng of total product were reamplified using diluted template DNA to reduce the concentration of PCR inhibitors.

PCR products were combined for multiplex sequencing in batches of 48 up to a maximum of 96. Total volume was reduced using Amicon Ultra centrifugal filter concentrators (Millipore Sigma, Burlington MA) to between 25–50 µL. Concentrated products electrophoresed on a 1.4% agarose gel in 1×TAE containing SYBR safe stain at 80 VDC for 90 min. Bands were excised and purified using a GeneClean Turbo Kit (MP Biomedicals, Santa Ana, CA) following the manufacturer's directions modified to elute the

product in 40 µL DES for 10 min followed by centrifugation of final eluent for 2 min. Final DNA concentration was determined by Quantifluor, as above.

Sequencing and bioinformatics

Samples were sequenced by Illumina MiSeq (San Diego, CA) using 150 bp paired-end (x2) V2 chemistry. Data were outputted in a format consisting of two fastq files containing read 1 and read 2 datasets, and a third fastq file corresponding to barcodes. Following decompression and concatenation of data files, forward and reverse assembled and the resultant contigs binned by barcode using the Qiime (version 1.9.1) open-source bioinformatics pipeline. The analysis pipeline, in brief, consisted of the following; Non-bacterial sequences were excluded as those that failed to cluster against the Greengenes reference database (version May 2013) at 60% similarity. The resulting filtered dataset was subjected to closed reference picking against the Greengenes reference database at 97% similarity using USEARCH6.1. Sequences that failed to cluster were aggregated over the entire dataset (singletons removed) and subjected to de novo clustering using USEARCH10 (64 bit). Taxonomies were assigned according to the Greengenes reference database, and closed- and de novo picked datasets were merged.

Pipeline command sequence summary

```
#MiSeq FASTQ FROM SEQUENCER - UNZIPPING AND CONCATENATING FILES

#note used all files instead of just the 'undefined' files and allowed the
quality parameters to cull for consistency across runs

gunzip *I1*.gz

gunzip *R1*.gz

gunzip *R2*.gz

cat *S1_L001_I1_001.fastq *S2_L001_I1_001.fastq *S3_L001_I1_001.fastq
*S4_L001_I1_001.fastq *S5_L001_I1_001.fastq *S6_L001_I1_001.fastq
*S7_L001_I1_001.fastq *S8_L001_I1_001.fastq *S9_L001_I1_001.fastq
*S10_L001_I1_001.fastq *S11_L001_I1_001.fastq *S12_L001_I1_001.fastq
Undetermined_S0_L001_I1_001.fastq > cat_index.fastq

cat *S1_L001_R1_001.fastq *S2_L001_R1_001.fastq *S3_L001_R1_001.fastq
*S4_L001_R1_001.fastq *S5_L001_R1_001.fastq *S6_L001_R1_001.fastq
*S7_L001_R1_001.fastq *S8_L001_R1_001.fastq *S9_L001_R1_001.fastq
*S10_L001_R1_001.fastq *S11_L001_R1_001.fastq *S12_L001_R1_001.fastq
Undetermined_S0_L001_R1_001.fastq > cat_R1.fastq

cat *S1_L001_R2_001.fastq *S2_L001_R2_001.fastq *S3_L001_R2_001.fastq
*S4_L001_R2_001.fastq *S5_L001_R2_001.fastq *S6_L001_R2_001.fastq
*S7_L001_R2_001.fastq *S8_L001_R2_001.fastq *S9_L001_R2_001.fastq
*S10_L001_R2_001.fastq *S11_L001_R2_001.fastq *S12_L001_R2_001.fastq
Undetermined_S0_L001_R2_001.fastq > cat_R2.fastq

#_________________________________________
```

#MiSeq RUN CLOSED REFERENCE OTU-PICKING PIPELINE

#reference database = Greengenes version May 2013

#count the number of reads for input from MiSeq R1 fastq file

```
count_seqs.py -i cat_R1.fastq -o cat_R1_seq_count.txt
```

#assembling forward and reverse reads

```
join_paired_ends.py -m fastq-join -j 25 -p 5 -b cat_index.fastq -f
cat_R1.fastq -r cat_R2.fastq -o fastq_join/
```

#binning sequences by bar code

```
split_libraries_fastq.py -i fastq_join/fastqjoin.join.fastq -b
fastq_join/fastqjoin.join_barcodes.fastq -m mapping_file.txt -r 3 -p 0.00 -n
0 --rev_comp_mapping_barcodes --barcode_type 12 --max_barcode_errors 1.5 -o
split_seqs/
```

#identify non-bacterial reads to be filtered

```
parallel_pick_otus_usearch61_ref.py -i split_seqs/seqs.fna -r
/home/james/qiime_software/gg_13_8_otus/rep_set/97_otus.fasta --
usearch61_sort_method abundance --sizeorder --similarity 0.6 --jobs_to_start
16 -o prefilter/
```

#count the number of sequences that failed to cluster with reference data set at 60%

```
wc -l prefilter/seqs_failures.txt > prefilter/seqs_failures_count.txt
```

#create filtered data set

```
filter_fasta.py -f split_seqs/seqs.fna -s prefilter/seqs_failures.txt -n -o
prefilter/prefiltered_seqs.fna
```

#parallel closed reference pick OTUs using usearch61

```
parallel_pick_otus_usearch61_ref.py -i prefilter/prefiltered_seqs.fna -r
/home/james/qiime_software/gg_13_8_otus/rep_set/97_otus.fasta --
usearch61_sort_method abundance --sizeorder --similarity 0.97 --jobs_to_start
16 -o closed_ref_OTUs/
```

#count the number of sequences that failed to cluster with reference data set at 97%

```
wc -l closed_ref_OTUs/prefiltered_seqs_failures.txt >
closed_ref_OTUs/prefiltered_seqs_failures_count.txt
```

#pick representative sequence from each OTU cluster

```
pick_rep_set.py -i closed_ref_OTUs/prefiltered_seqs_otus.txt -f
prefilter/prefiltered_seqs.fna -o closed_ref_OTUs/rep_set.fasta
```

#Assign taxonomy using uclust

```
assign_taxonomy.py -i closed_ref_OTUs/rep_set.fasta -m uclust -r
/home/james/qiime_software/gg_13_8_otus/rep_set/97_otus.fasta -t
/home/james/qiime_software/gg_13_8_otus/taxonomy/97_otu_taxonomy.txt --
uclust_min_consensus_fraction 0.51 --uclust_similarity 0.9 --
uclust_max_accepts 3 -o closed_ref_OTUs/uclust_closed_ref_tax_assign/
```

#make biom formatted OTU table

```
make_otu_table.py -i closed_ref_OTUs/prefiltered_seqs_otus.txt -t
closed_ref_OTUs/uclust_closed_ref_tax_assign/rep_set_tax_assignments.txt -o
closed_ref_OTUs/uclust_closed_ref_tax_assign/uclust_closed_ref_picked_OTUs.bi
om
```

#summarize biom table

```
biom summarize-table -i
closed_ref_OTUs/uclust_closed_ref_tax_assign/uclust_closed_ref_picked_OTUs.bi
om -o
closed_ref_OTUs/uclust_closed_ref_tax_assign/uclust_closed_ref_picked_OTUs_bi
om_table_summary.txt
```

#count the number of OTUs per sample

```
alpha_diversity.py -i
closed_ref_OTUs/uclust_closed_ref_tax_assign/uclust_closed_ref_picked_OTUs.bi
om -m observed_species -o
closed_ref_OTUs/uclust_closed_ref_tax_assign/uclust_closed_ref_picked_OTU_cou
nt_per_sample.txt
```

#convert biom-formatted table to tab-delimited text format

#note that this table is not used in downstream pipeline

```
biom convert -i
closed_ref_OTUs/uclust_closed_ref_tax_assign/uclust_closed_ref_picked_OTUs.bi
om -o
closed_ref_OTUs/uclust_closed_ref_tax_assign/uclust_closed_ref_picked_OTUs.tx
t -b --header-key taxonomy
```

#make directory for de novo picking files

```
mkdir denovo_OTUs
```

#pull reads that failed to cluster with reference database for de novo OTU picking

```
filter_fasta.py -f prefilter/prefiltered_seqs.fna -s
closed_ref_OTUs/prefiltered_seqs_failures.txt -o
denovo_OTUs/seqs_for_denovo_pick.fna
```

#count the number of reads for input into denovo-picking step (note will/should be the same number as output in prefiltered_seqs_failures_count.txt)

```
count_seqs.py -i denovo_OTUs/seqs_for_denovo_pick.fna -o
denovo_OTUs/seq_count.txt
```

#______________________________________

 # merge biom tables from sym closed OTU picking and characterize 1) full table 2) table with singletons removed

```
merge_otu_tables.py -i
sym1_130211_uclust_closed_ref_picked_OTUs.biom,sym2_130212_uclust_closed_ref_
picked_OTUs.biom,sym3_130214_uclust_closed_ref_picked_OTUs.biom,sym4_130221_u
clust_closed_ref_picked_OTUs.biom,sym5_130226_uclust_closed_ref_picked_OTUs.b
iom,sym6_130227_uclust_closed_ref_picked_OTUs.biom,sym7_130304_uclust_closed_
ref_picked_OTUs.biom,sym8_130306_uclust_closed_ref_picked_OTUs.biom,sym9_1304
16_uclust_closed_ref_picked_OTUs.biom,sym10_130418_uclust_closed_ref_picked_O
TUs.biom,sym11_130423_uclust_closed_ref_picked_OTUs.biom,sym12_130424_uclust_
closed_ref_picked_OTUs.biom,sym13_130429_uclust_closed_ref_picked_OTUs.biom,s
ym14_130502_uclust_closed_ref_picked_OTUs.biom,sym15_130506_uclust_closed_ref
_picked_OTUs.biom,sym16_130417_uclust_closed_ref_picked_OTUs.biom,sym20_13071
1_uclust_closed_ref_picked_OTUs.biom,sym21_130716_uclust_closed_ref_picked_OT
Us.biom,sym22_130717_uclust_closed_ref_picked_OTUs.biom,sym23_130718_uclust_c
losed_ref_picked_OTUs.biom,sym24_130924_uclust_closed_ref_picked_OTUs.biom,sy
m25_130925_uclust_closed_ref_picked_OTUs.biom,sym26_130930_uclust_closed_ref_
picked_OTUs.biom,sym27_131001_uclust_closed_ref_picked_OTUs.biom,sym28_131009
_uclust_closed_ref_picked_OTUs_corrected.biom,sym29_131106_uclust_closed_ref_
picked_OTUs.biom,sym30_131111_uclust_closed_ref_picked_OTUs_corrected.biom,sy
m31_140225_uclust_closed_ref_picked_OTUs.biom,sym33_140219_uclust_closed_ref_
picked_OTUs.biom,sym34_140220_uclust_closed_ref_picked_OTUs.biom,sym35_140227
_uclust_closed_ref_picked_OTUs.biom,sym36_140916_uclust_closed_ref_picked_OTU
s.biom,sym37_140918_uclust_closed_ref_picked_OTUs.biom,sym38_140818_uclust_cl
osed_ref_picked_OTUs.biom,sym39_140826_uclust_closed_ref_picked_OTUs.biom,sym
40_140826_uclust_closed_ref_picked_OTUs_corrected.biom,sym41_140818_uclust_cl
osed_ref_picked_OTUs.biom,sym42_141103_uclust_closed_ref_picked_OTUs.biom,sym
43_141103_uclust_closed_ref_picked_OTUs.biom,sym44_140916_uclust_closed_ref_p
icked_OTUs.biom,sym45_150113_uclust_closed_ref_picked_OTUs.biom,sym46_150114_
uclust_closed_ref_picked_OTUs.biom,sym47_150224_uclust_closed_ref_picked_OTUs
.biom,sym48_150225_uclust_closed_ref_picked_OTUs.biom,sym49_150225_uclust_clo
sed_ref_picked_OTUs.biom,sym50_150504_uclust_closed_ref_picked_OTUs.biom,sym5
1_150611_uclust_closed_ref_picked_OTUs.biom,sym52_150506_uclust_closed_ref_pi
cked_OTUs.biom,sym53_150507_uclust_closed_ref_picked_OTUs.biom,sym54_150602_u
clust_closed_ref_picked_OTUs.biom,sym55_150603_uclust_closed_ref_picked_OTUs.
biom,sym56_150604_uclust_closed_ref_picked_OTUs.biom,sym57_150604_uclust_clos
ed_ref_picked_OTUs.biom,sym58_150707_uclust_closed_ref_picked_OTUs.biom,sym59
_150708_uclust_closed_ref_picked_OTUs.biom,sym60_150709_uclust_closed_ref_pic
ked_OTUs.biom,sym61_150715_uclust_closed_ref_picked_OTUs.biom,sym62_150817_uc
lust_closed_ref_picked_OTUs.biom,sym63_150812_uclust_closed_ref_picked_OTUs.b
```

```
iom,sym64_150818_uclust_closed_ref_picked_OTUs.biom,sym65_150819_uclust_close
d_ref_picked_OTUs.biom,sym66_151001_uclust_closed_ref_picked_OTUs.biom,sym67_
151006_uclust_closed_ref_picked_OTUs.biom,sym68_160105_uclust_closed_ref_pick
ed_OTUs.biom,sym69_151007_uclust_closed_ref_picked_OTUs.biom,sym70_151109_ucl
ust_closed_ref_picked_OTUs.biom,sym71_151110_uclust_closed_ref_picked_OTUs.bi
om,sym72_151111_uclust_closed_ref_picked_OTUs.biom,sym73_151125_uclust_closed
_ref_picked_OTUs.biom,sym74_151209_uclust_closed_ref_picked_OTUs.biom,sym75_1
51210_uclust_closed_ref_picked_OTUs.biom,sym76_151214_uclust_closed_ref_picke
d_OTUs.biom,sym77_151215_uclust_closed_ref_picked_OTUs.biom,sym78_161114_uclu
st_closed_ref_picked_OTUs.biom,sym79_160216_uclust_closed_ref_picked_OTUs.bio
m,sym80_160217_uclust_closed_ref_picked_OTUs.biom,sym81_160217_uclust_closed_
ref_picked_OTUs.biom,sym82_160329_uclust_closed_ref_picked_OTUs.biom,sym83_16
0329_uclust_closed_ref_picked_OTUs.biom,sym84_160404_uclust_closed_ref_picked
_OTUs.biom,sym85_160404_uclust_closed_ref_picked_OTUs.biom,sym86_160629_uclus
t_closed_ref_picked_OTUs.biom,sym87_160718_uclust_closed_ref_picked_OTUs.biom
,sym88_160705_uclust_closed_ref_picked_OTUs.biom,sym89_160707_uclust_closed_r
ef_picked_OTUs.biom,sym90_160809_uclust_closed_ref_picked_OTUs.biom,sym91_160
809_uclust_closed_ref_picked_OTUs.biom,sym92_160825_uclust_closed_ref_picked_
OTUs.biom,sym93_160825_uclust_closed_ref_picked_OTUs.biom,sym94_160928_uclust
_closed_ref_picked_OTUs.biom,sym95_160929_uclust_closed_ref_picked_OTUs.biom,
sym96_160929_uclust_closed_ref_picked_OTUs.biom,sym97_160929_uclust_closed_re
f_picked_OTUs.biom,sym98_161012_uclust_closed_ref_picked_OTUs.biom,sym99_1610
12_uclust_closed_ref_picked_OTUs.biom,sym100_161013_uclust_closed_ref_picked_
OTUs.biom -o merged_symbiota_closed_picked_table_1to100.biom
```

 # characterize full biom table

```
biom summarize-table -i merged_symbiota_closed_picked_table_1to100.biom -o
merged_symbiota_closed_picked_table_1to100_biom_table_summary.txt
```

```
alpha_diversity.py -i merged_symbiota_closed_picked_table_1to100.biom -m
observed_species -o
merged_symbiota_closed_picked_table_1to100_OTU_count_per_sample.txt
```

#_____________________________________

#COMBINED MiSeq RUN DENOVO OTU-PICKING PIPELINE USING USEARCH10 64 bit

 # concatenate reads that failed to cluster with reference database into single fasta file for denovo picking

```
cat sym1_130211_seqs_for_denovo_pick.fna sym2_130212_seqs_for_denovo_pick.fna
sym3_130214_seqs_for_denovo_pick.fna sym4_130221_seqs_for_denovo_pick.fna
sym5_130226_seqs_for_denovo_pick.fna sym6_130227_seqs_for_denovo_pick.fna
sym7_130304_seqs_for_denovo_pick.fna sym8_130306_seqs_for_denovo_pick.fna
sym9_130416_seqs_for_denovo_pick.fna sym10_130418_seqs_for_denovo_pick.fna
sym11_130423_seqs_for_denovo_pick.fna sym12_130424_seqs_for_denovo_pick.fna
sym13_130429_seqs_for_denovo_pick.fna sym14_130502_seqs_for_denovo_pick.fna
sym15_130506_seqs_for_denovo_pick.fna sym16_130417_seqs_for_denovo_pick.fna
sym20_130711_seqs_for_denovo_pick.fna sym21_130716_seqs_for_denovo_pick.fna
sym22_130717_seqs_for_denovo_pick.fna sym23_130718_seqs_for_denovo_pick.fna
```

```
sym24_130924_seqs_for_denovo_pick.fna sym25_130925_seqs_for_denovo_pick.fna
sym26_130930_seqs_for_denovo_pick.fna sym27_131001_seqs_for_denovo_pick.fna
sym28_131009_corrected_seqs_for_denovo_pick.fna
sym29_131106_seqs_for_denovo_pick.fna
sym30_131111_corrected_seqs_for_denovo_pick.fna
sym31_140225_seqs_for_denovo_pick.fna sym33_140219_seqs_for_denovo_pick.fna
sym34_140220_seqs_for_denovo_pick.fna sym35_140227_seqs_for_denovo_pick.fna
sym36_140916_seqs_for_denovo_pick.fna sym37_140918_seqs_for_denovo_pick.fna
sym38_140818_seqs_for_denovo_pick.fna sym39_140826_seqs_for_denovo_pick.fna
sym40_140826_corrected_seqs_for_denovo_pick.fna
sym41_140818_seqs_for_denovo_pick.fna sym42_141103_seqs_for_denovo_pick.fna
sym43_141103_seqs_for_denovo_pick.fna sym44_140916_seqs_for_denovo_pick.fna
sym45_150113_seqs_for_denovo_pick.fna sym46_150114_seqs_for_denovo_pick.fna
sym47_150224_seqs_for_denovo_pick.fna sym48_150225_seqs_for_denovo_pick.fna
sym49_150225_seqs_for_denovo_pick.fna sym50_150504_seqs_for_denovo_pick.fna
sym51_150611_seqs_for_denovo_pick.fna sym52_150506_seqs_for_denovo_pick.fna
sym53_150507_seqs_for_denovo_pick.fna sym54_150602_seqs_for_denovo_pick.fna
sym55_150603_seqs_for_denovo_pick.fna sym56_150604_seqs_for_denovo_pick.fna
sym57_150604_seqs_for_denovo_pick.fna sym58_150707_seqs_for_denovo_pick.fna
sym59_150708_seqs_for_denovo_pick.fna sym60_150709_seqs_for_denovo_pick.fna
sym61_150715_seqs_for_denovo_pick.fna sym62_150817_seqs_for_denovo_pick.fna
sym63_150812_seqs_for_denovo_pick.fna sym64_150818_seqs_for_denovo_pick.fna
sym65_150819_seqs_for_denovo_pick.fna sym66_151001_seqs_for_denovo_pick.fna
sym67_151006_seqs_for_denovo_pick.fna sym68_160105_seqs_for_denovo_pick.fna
sym69_151007_seqs_for_denovo_pick.fna sym70_151109_seqs_for_denovo_pick.fna
sym71_151110_seqs_for_denovo_pick.fna sym72_151111_seqs_for_denovo_pick.fna
sym73_151125_seqs_for_denovo_pick.fna sym74_151209_seqs_for_denovo_pick.fna
sym75_151210_seqs_for_denovo_pick.fna sym76_151214_seqs_for_denovo_pick.fna
sym77_151215_seqs_for_denovo_pick.fna sym78_xxxxxx_seqs_for_denovo_pick.fna
sym79_151215_seqs_for_denovo_pick.fna sym80_160217_seqs_for_denovo_pick.fna
sym81_160217_seqs_for_denovo_pick.fna sym82_160329_seqs_for_denovo_pick.fna
sym83_160329_seqs_for_denovo_pick.fna sym84_160404_seqs_for_denovo_pick.fna
sym85_160404_seqs_for_denovo_pick.fna sym86_160629_seqs_for_denovo_pick.fna
sym87_160718_seqs_for_denovo_pick.fna sym88_160705_seqs_for_denovo_pick.fna
sym89_160707_seqs_for_denovo_pick.fna sym90_160809_seqs_for_denovo_pick.fna
sym91_160809_seqs_for_denovo_pick.fna sym92_160825_seqs_for_denovo_pick.fna
sym93_160825_seqs_for_denovo_pick.fna sym94_160928_seqs_for_denovo_pick.fna
sym95_160929_seqs_for_denovo_pick.fna sym96_160929_seqs_for_denovo_pick.fna
sym97_160929_seqs_for_denovo_pick.fna sym98_161012_seqs_for_denovo_pick.fna
sym99_161012_seqs_for_denovo_pick.fna sym100_161013_seqs_for_denovo_pick.fna
> concat_sym_1to100_seqs_for_denovo_pick.fna

count_seqs.py -i concat_sym_1to100_seqs_for_denovo_pick.fna -o
concat_sym_1to100_seqs_for_denovo_pick_fna_seq_count.txt
```

 # use filter command to relabel with sample number

 # get unique sequences for clustering

```
usearch10 -fastx_uniques cat_sym_1to100_reads_for_denovo_pick.fna -sizeout -
fastaout cat_sym_1to100_reads_for_denovo_pick_Uniqs.fa
```

```
00:44 4.0Gb   100.0% Reading cat_sym_1to100_reads_for_denovo_pick.fna
00:44 3.9Gb   CPU has 16 cores, defaulting to 10 threads
00:50 6.2Gb   100.0% DF
00:51 6.3Gb   9382875 seqs, 7658500 uniques, 7331721 singletons (95.7%)
00:51 6.3Gb   Min size 1, median 1, max 105358, avg 1.23
01:56 4.8Gb   100.0% Writing cat_sym_1to100_reads_for_denovo_pick_Uniqs.fa
```

 # cluster using usearch10 with n=1 - ONLY FOR TAXON ASSIGNMENT -- TOO LARGE TO MAKE TABLE

```
usearch10 -cluster_otus cat_sym_1to100_reads_for_denovo_pick_Uniqs.fa -
minsize 2 -relabel OTU -otus
cat_sym_1to100_reads_for_denovo_pick_Uniqs_OTUrepSet.fa
```

```
05:13 89Mb    100.0% 5869 OTUs, 46132 chimeras
```

 # assign names with Qiime against Greengenes for consistency with closed picked OTUs (note: need to attach these to OTUs in table using biom command)

```
assign_taxonomy.py -i cat_sym_1to100_reads_for_denovo_pick_Uniqs_OTUrepSet.fa
-m uclust -r /home/james/qiime_software/gg_13_8_otus/rep_set/97_otus.fasta -t
/home/james/qiime_software/gg_13_8_otus/taxonomy/97_otu_taxonomy.txt --
uclust_min_consensus_fraction 0.51 --uclust_similarity 0.9 --
uclust_max_accepts 3 -o uclust_tax_assign/
```

 # cluster using usearch10 with n=100

```
usearch10 -cluster_otus cat_sym_1to100_reads_for_denovo_pick_Uniqs.fa -
minsize 100 -relabel OTU -otus
cat_sym_1to100_reads_for_denovo_pick_Uniqs_OTUrepSet_n10.fa
```

```
usearch10 -otutab cat_sym_1to100_reads_for_denovo_pick.fna -otus
cat_sym_1to100_reads_for_denovo_pick_Uniqs_OTUrepSet_n100.fa -biomout
cat_sym_1to100_denovoOTUtable.json -mapout denovoOTUs_map.txt -notmatched
denovo_unmapped_reads.fa -dbmatched denovo_matched_reads_with_sizes.fa -
sizeout
```

```
usearch10 -otutab cat_sym_1to100_reads_for_denovo_pick.fna -otus
cat_sym_1to100_reads_for_denovo_pick_Uniqs_OTUrepSet_n100.fa -otutabout
cat_sym_1to100_denovoOTUtable_n100.txt
```

 # ____

```
biom convert -i cat_sym_1to100_denovoOTUtable_n100.txt -o
cat_sym_1to100_denovoOTUtable_n100.biom --table-type='OTU table' --process-
obs-metadata taxonomy
```

```
biom add-metadata --sc-separated taxonomy --observation-header OTUID,taxonomy
--observation-metadata-fp
cat_sym_1to100_reads_for_denovo_pick_Uniqs_OTUrepSet_tax_assignments.txt -i
```

```
cat_sym_1to100_denovoOTUtable_n100.biom -o
cat_sym_1to100_denovoOTUtable_n100_wTaxa.biom

biom summarize-table -i cat_sym_1to100_denovoOTUtable_n100_wTaxa.biom -o
cat_sym_1to100_denovoOTUtable_n100_wTaxa_biom_summary.txt

# make .txt version of table to check labels etc

biom convert -i cat_sym_1to100_denovoOTUtable_n100_wTaxa.biom -o
cat_sym_1to100_denovoOTUtable_n100_wTaxa_biom.txt -b --header-key taxonomy

# ________________________________________________________

# filter closed pick table at n100 to match

filter_otus_from_otu_table.py -i
merged_symbiota_closed_picked_table_1to100.biom -n 100 -o
merged_symbiota_closed_picked_table_1to100_n100.biom

# make text version of table to quality check

biom convert -i merged_symbiota_closed_picked_table_1to100_n100.biom -o
merged_symbiota_closed_picked_table_1to100_n100_biom_.txt -b --header-key
taxonomy

biom summarize-table -i merged_symbiota_closed_picked_table_1to100_n100.biom
-o merged_symbiota_closed_picked_table_1to100_n100_biom_summary.txt

# _________________________________________________________

# merge closed picked and denovo picked tables

merge_otu_tables.py -i
cat_sym_1to100_denovoOTUtable_n100_wTaxa.biom,merged_symbiota_closed_picked_t
able_1to100_n100.biom -o
FINAL_merged_closed_and_denovo_picked_OTUtable_filter100.biom

biom summarize-table -i
FINAL_merged_closed_and_denovo_picked_OTUtable_filter100.biom -o
FINAL_merged_closed_and_denovo_picked_OTUtable_filter100_biom_summary.txt

biom convert -i merged_closed_denovo_sym1to100_Feb12_2017.biom -o
merged_closed_denovo_sym1to100_Feb12_2017_biom.txt -b --header-key taxonomy
```

#SampleID	BarcodeSequence	LinkerPrimerSequence	ReversePrimer	Description	Run	CHILD_ID	Age_mo	Stool_amt	Genomic_ngul	NOTES
33A	TCCCTTGTCTCC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	1	20	30497	3-month Stool	188	21.2	
33B	ACGAGACTGATT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	2	20	30678	3-month Stool	161	26.3	
33C	GAATACCAAGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	3	20	30679	3-month Stool	210	27.4	
33D	CGTAATTGCCGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	4	20	30475	3-month Stool	189	12.2	
33E	ATCGCGACTGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	5	20	30608	3-month Stool	211	9.4	
33I	GTGTTGTCGTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	9	20	30732	3-month Stool	181	3.2	Stool in diaper liner
33J	TAGTGATGACCA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	10	20	30808	3-month Stool	562	0.3	
33K	TCGACATCTCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	11	20	30769	3-month Stool	94	0.6	
33L	AGCGGAGGTTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	12	20	30781	3-month Stool	207	1.0	
34A	AGTCGAACGAGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	13	20	30813	3-month Stool	181	9.7	
34B	CGAGCAATCCTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	14	20	30086	3-month Stool	195	17.0	
34C	CATCCCTCTACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	15	20	30177	3-month Stool	192	23.2	
34D	GAACACTTTGGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	16	20	30164	3-month Stool	185	14.3	
34E	GTTCTCTTCTCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	17	20	30162	3-month Stool	186	8.2	
34F	TGGAGTAGGTGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	18	20	30211	3-month Stool	106	4.8	
34G	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	20	30159	3-month Stool	144	4.6	
34H	TCAGAGTAGACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	20	20	30207	3-month Stool	209	4.1	
34I	GTGTAGGTGCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	21	20	30213	3-month Stool	130	6.2	
34K	CACTACGCTAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	23	20	30168	3-month Stool	140	2.9	
35E	CGTAATTGCCGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	4	21	30107	3-month Stool	343	2.3	Stool in diaper liner
35F	ATCGCGACTGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	5	21	30062	3-month Stool	403	10.7	Stool in diaper liner
35G	TTCAGGAACTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	6	21	30101	3-month Stool	581	0.5	Stool in diaper liner
35H	GATTATCGACGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	7	21	30058	3-month Stool	135	8.5	
35I	CGCCTTGATAAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	8	21	30083	3-month Stool	219	6.2	
35J	GTGTTGTCGTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	9	21	30088	3-month Stool	192	21.1	
35K	TAGTGATGACCA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	10	21	30109	3-month Stool	201	25.5	
35L	TCGACATCTCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	11	21	30139	3-month Stool	200	1.6	
36A	AGCGGAGGTTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	12	21	30141	3-month Stool	189	40.2	
36B	AGTCGAACGAGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	13	21	30148	3-month Stool	198	26.8	
36C	CGAGCAATCCTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	14	21	30161	3-month Stool	150	15.2	
36D	CATCCCTCTACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	15	21	30092	3-month Stool	219	11.7	
36E	GAACACTTTGGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	16	21	30486	3-month Stool	212	6.7	
36F	GTTCTCTTCTCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	17	21	30597	3-month Stool	203	26.2	Stool in diaper liner
36G	TGGAGTAGGTGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	18	21	30717	3-month Stool	149	3.2	
36H	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	21	30640	3-month Stool	199	10.5	
36I	TCAGAGTAGACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	20	21	30752	3-month Stool	135	13.6	
36K	CCTACCATTGTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	22	21	30275	3-month Stool	217	12.9	
36L	CACTACGCTAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	23	21	30233	3-month Stool	187	13.7	Stool in diaper liner
37A	CTTGTGCGACAA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	24	21	30235	3-month Stool	394	7.7	
37C	TCCCTTGTCTCC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	1	22	30182	3-month Stool	204	29.2	
37D	ACGAGACTGATT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	2	22	30255	3-month Stool	195	29.1	
37F	CGTAATTGCCGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	4	22	30210	3-month Stool	220	25.1	
37H	TTCAGGAACTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	6	22	30267	3-month Stool	197	15.1	
37I	GATTATCGACGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	7	22	30231	3-month Stool	177	5.3	
37J	CGCCTTGATAAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	8	22	30301	3-month Stool	153	2.3	
37L	TAGTGATGACCA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	10	22	30293	3-month Stool	137	33.6	
38B	AGCGGAGGTTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	12	22	30333	3-month Stool	199	24.3	
38C	AGTCGAACGAGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	13	22	30258	3-month Stool	206	13.9	
38D	CGAGCAATCCTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	14	22	30257	3-month Stool	208	24.4	
38E	CATCCCTCTACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	15	22	30353	3-month Stool	177	2.7	
38F	GAACACTTTGGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	16	22	30294	3-month Stool	162	3.5	
38G	GTTCTCTTCTCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	17	22	30315	3-month Stool	218	10.2	
38I	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	22	30327	3-month Stool	203	25.2	
38J	TCAGAGTAGACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	20	22	30409	3-month Stool	212	1.5	
38K	GTGTAGGTGCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	21	22	30303	3-month Stool	80	4.2	
39C	ACGCTTAACGAC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	25	22	30392	3-month Stool	179	19.8	
39E	ACGAGACTGATT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	2	23	30354	3-month Stool	195	5.9	
39F	GAATACCAAGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	3	23	30382	3-month Stool	180	18.6	
39G	CGTAATTGCCGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	4	23	30314	3-month Stool	115	14.6	
39I	TTCAGGAACTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	6	23	30418	3-month Stool	121	11.9	
39J	GATTATCGACGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	7	23	30285	3-month Stool	215	19.3	
39K	CGCCTTGATAAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	8	23	30358	3-month Stool	186	22.0	Stool in diaper liner
39L	GTGTTGTCGTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	9	23	30434	3-month Stool	150	0.2	
40A	TAGTGATGACCA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	10	23	30250	3-month Stool	191	15.1	
40B	TCGACATCTCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	11	23	30400	3-month Stool	210	24.9	Stool in diaper liner
40D	AGTCGAACGAGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	13	23	30356	3-month Stool	185	2.9	
40E	CGAGCAATCCTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	14	23	30443	3-month Stool	215	16.3	
40F	CATCCCTCTACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	15	23	30324	3-month Stool	218	14.4	
40H	GTTCTCTTCTCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	17	23	30370	3-month Stool	215	33.4	
40I	TGGAGTAGGTGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	18	23	30283	3-month Stool	207	29.1	

#SampleID	BarcodeSequence	LinkerPrimerSequence	ReversePrimer	Description	Run	CHILD_ID	Age_mo	Stool_amt	Genomic_ngul	NOTES
40K	TCAGAGTAGACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	20	23	30491	3-month Stool	86	1.2	
41A	CCTACCATTGTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	22	23	30381	3-month Stool	219	31.1	
41B	CACTACGCTAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	23	23	30447	3-month Stool	195	24.7	
41C	CTTGTGCGACAA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	24	23	30521	3-month Stool	203	24.7	
41E	TCCCTTGTCTCC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	1	24	30506	3-month Stool	205	7.7	
41F	ACGAGACTGATT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	2	24	30493	3-month Stool	205	28.1	
41I	ATCGCGACTGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	5	24	30450	3-month Stool	191	22.3	
41J	TTCAGGAACTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	6	24	30494	3-month Stool	186	28.6	
42A	GTGTTGTCGTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	9	24	30504	3-month Stool	190	15.0	
42B	TAGTGATGACCA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	10	24	30321	3-month Stool	194	24.3	
42D	AGCGGAGGTTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	12	24	30510	3-month Stool	176	2.0	
42H	GAACACTTTGGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	16	24	30639	3-month Stool	197	8.7	
42I	GTTCTCTTCTCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	17	24	30632	3-month Stool	213	5.1	
42J	TGGAGTAGGTGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	18	24	30549	3-month Stool	198	21.7	
42K	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	24	30589	3-month Stool	102	21.0	
43A	GTGTAGGTGCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	21	24	30620	3-month Stool	131	3.3	
43B	CCTACCATTGTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	22	24	30391	3-month Stool	204	18.3	
43C	CACTACGCTAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	23	24	30503	3-month Stool	210	28.5	
43D	CTTGTGCGACAA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	24	24	30554	3-month Stool	212	13.7	
43F	TCCCTTGTCTCC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	1	25	30642	3-month Stool	203	25.4	
43G	ACGAGACTGATT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	2	25	30685	3-month Stool	198	18.3	
43I	CGTAATTGCCGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	4	25	30555	3-month Stool	185	13.7	Stool in diaper liner
43J	ATCGCGACTGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	5	25	30557	3-month Stool	234	20.8	Stool in diaper liner
43K	TTCAGGAACTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	6	25	30707	3-month Stool	113	19.9	
43L	GATTATCGACGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	7	25	30638	3-month Stool	220	7.9	Stool in diaper liner
44A	CGCCTTGATAAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	8	25	30697	3-month Stool	235	21.4	
44B	GTGTTGTCGTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	9	25	30488	3-month Stool	183	20.5	
44C	TAGTGATGACCA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	10	25	30617	3-month Stool	200	24.6	Stool in diaper liner
44D	TCGACATCTCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	11	25	30718	3-month Stool	514	28.0	
44E	AGCGGAGGTTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	12	25	30579	3-month Stool	112	15.0	Stool in diaper liner
44F	AGTCGAACGAGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	13	25	30582	3-month Stool	210	16.6	
44G	CGAGCAATCCTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	14	25	30763	3-month Stool	193	37.1	
44H	CATCCCTCTACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	15	25	30677	3-month Stool	209	14.5	
44I	GAACACTTTGGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	16	25	30746	3-month Stool	205	15.7	
44K	TGGAGTAGGTGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	18	25	30749	3-month Stool	332	8.0	
44L	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	25	30419	3-month Stool	210	3.8	
45A	TCAGAGTAGACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	20	25	30649	3-month Stool	206	17.4	
45B	GTGTAGGTGCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	21	25	30657	3-month Stool	200	21.7	
45C	CCTACCATTGTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	22	25	30766	3-month Stool	201	11.7	Stool in diaper liner
45D	CACTACGCTAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	23	25	30751	3-month Stool	379	2.3	Stool in diaper liner
45E	CTTGTGCGACAA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	24	25	30768	3-month Stool	108	5.6	
45F	ACGCTTAACGAC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	25	25	30709	3-month Stool	193	20.6	Stool in diaper liner
45G	TCCCTTGTCTCC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	1	26	30745	3-month Stool	222	25.2	
45H	ACGAGACTGATT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	2	26	30384	3-month Stool	212	17.0	
45I	GAATACCAAGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	3	26	30501	3-month Stool	142	11.5	
45K	ATCGCGACTGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	5	26	30451	3-month Stool	184	11.3	
45L	TTCAGGAACTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	6	26	30541	3-month Stool	186	21.2	
46A	GATTATCGACGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	7	26	30467	3-month Stool	195	15.9	
46B	CGCCTTGATAAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	8	26	30513	3-month Stool	215	12.8	
46D	TAGTGATGACCA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	10	26	30580	3-month Stool	184	24.3	Stool in diaper liner
46F	AGCGGAGGTTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	12	26	30671	3-month Stool	191	23.6	
46H	CGAGCAATCCTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	14	26	30412	3-month Stool	221	24.8	Stool in diaper liner
46I	CATCCCTCTACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	15	26	30540	3-month Stool	373	9.2	Stool in diaper liner
46J	GAACACTTTGGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	16	26	30631	3-month Stool	209	23.6	
46K	GTTCTCTTCTCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	17	26	30566	3-month Stool	225	34.1	
46L	TGGAGTAGGTGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	18	26	30577	3-month Stool	194	7.7	
47A	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	26	30704	3-month Stool	203	12.8	
47B	TCAGAGTAGACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	20	26	30477	3-month Stool	221	18.9	
47C	GTGTAGGTGCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	21	26	30652	3-month Stool	169	3.6	
47D	CCTACCATTGTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	22	26	30681	3-month Stool	201	21.0	
47E	CACTACGCTAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	23	26	30570	3-month Stool	129	12.2	
47F	CTTGTGCGACAA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	24	26	30740	3-month Stool	213	19.9	
47G	ACGCTTAACGAC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	25	26	30710	3-month Stool	176	14.3	
47I	ACGAGACTGATT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	2	27	30786	3-month Stool	141	14.0	Stool in diaper liner
47J	GAATACCAAGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	3	27	30775	3-month Stool	267	12.8	Stool in diaper liner
47L	ATCGCGACTGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	5	27	30779	3-month Stool	98	0.2	
48A	TTCAGGAACTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	6	27	30795	3-month Stool	204	27.3	Stool in diaper liner
48D	GTGTTGTCGTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	9	27	30502	3-month Stool	188	16.1	Stool in diaper liner
48E	TAGTGATGACCA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	10	27	30712	3-month Stool	332	32.0	
48F	TCGACATCTCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	11	27	30606	3-month Stool	131	22.4	

#SampleID	BarcodeSequence	LinkerPrimerSequence	ReversePrimer	Description	Run	CHILD_ID	Age_mo	Stool_amt	Genomic_ngul	NOTES
48G	AGCGGAGGTTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	12	27	30706	3-month Stool	128	20.9	
48H	AGTCGAACGAGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	13	27	30733	3-month Stool	217	11.0	Stool in diaper liner
48I	CGAGCAATCCTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	14	27	30696	3-month Stool	174	13.7	
48J	CATCCCTCTACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	15	27	30637	3-month Stool	183	30.9	Stool in diaper liner
48K	GAACACTTTGGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	16	27	30687	3-month Stool	239	32.0	Stool in diaper liner
49A	TGGAGTAGGTGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	18	27	30669	3-month Stool	198	11.3	Stool in diaper liner
49B	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	27	30782	3-month Stool	271	31.6	
49D	GTGTAGGTGCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	21	27	30372	3-month Stool	142	1.5	
49E	CCTACCATTGTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	22	27	30456	3-month Stool	183	7.2	
49H	ACGCTTAACGAC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	25	27	30544	3-month Stool	144	6.7	
49I	TCCCTTGTCTCC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	1	29	30158	3-month Stool	80	1.3	
49L	CGTAATTGCCGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	4	29	30096	3-month Stool	233	3.6	Stool in diaper liner
50A	ATCGCGACTGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	5	29	30310	3-month Stool	204	8.1	
50B	TTCAGGAACTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	6	29	30180	3-month Stool	80	1.8	
50C	GATTATCGACGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	7	29	30212	3-month Stool	80	16.3	
50E	GTGTTGTCGTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	9	29	30064	3-month Stool	78	4.5	
50F	TAGTGATGACCA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	10	29	30144	3-month Stool	79	0.9	
50G	TCGACATCTCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	11	29	30318	3-month Stool	196	21.2	
50H	AGCGGAGGTTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	12	29	30126	3-month Stool	200	19.8	
50J	CGAGCAATCCTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	14	29	30099	3-month Stool	207	2.0	
50K	CATCCCTCTACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	15	29	30074	3-month Stool	100	3.6	Stool in diaper liner
51A	GTTCTCTTCTCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	17	29	30263	3-month Stool	184	22.0	
51C	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	29	30256	3-month Stool	209	8.9	
51D	TCAGAGTAGACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	20	29	30189	3-month Stool	207	22.8	
51E	GTGTAGGTGCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	21	29	30115	3-month Stool	191	12.8	
51F	CCTACCATTGTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	22	29	30104	3-month Stool	189	17.3	
51G	CACTACGCTAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	23	29	30133	3-month Stool	94	5.9	Stool in diaper liner
51H	CTTGTGCGACAA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	24	29	30208	3-month Stool	263	18.3	Stool in diaper liner
51I	ACGCTTAACGAC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	25	29	30682	3-month Stool	190	20.7	
51J	TCCCTTGTCTCC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	1	30	30229	3-month Stool	186	26.4	
51K	ACGAGACTGATT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	2	30	30218	3-month Stool	180	2.2	
51L	GAATACCAAGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	3	30	30352	3-month Stool	99	1.5	Stool in diaper liner
52A	CGTAATTGCCGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	4	30	30773	3-month Stool	100	10.3	Stool in diaper liner
52B	ATCGCGACTGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	5	30	30309	3-month Stool	112	1.0	
52C	TTCAGGAACTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	6	30	30355	3-month Stool	192	6.2	
52D	GATTATCGACGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	7	30	30387	3-month Stool	80	3.0	
52E	CGCCTTGATAAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	8	30	30441	3-month Stool	224	25.7	
52F	GTGTTGTCGTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	9	30	30325	3-month Stool	202	5.2	
57A	TAGTGATGACCA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	10	30	30156	3-month Stool	259	25.1	Stool in diaper liner
57B	TCGACATCTCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	11	30	30143	3-month Stool	230	1.2	
57D	AGCGGAGGTTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	12	30	30138	3-month Stool	179	0.5	Stool in diaper liner
57E	AGTCGAACGAGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	13	30	30061	3-month Stool	147	1.4	Stool in diaper liner
57G	CATCCCTCTACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	15	30	30146	3-month Stool	232	2.4	
57H	GAACACTTTGGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	16	30	30128	3-month Stool	159	29.4	
57I	GTTCTCTTCTCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	17	30	30181	3-month Stool	179	0.8	Stool in diaper liner
57K	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	30	30221	3-month Stool	114	15.6	
57L	TCAGAGTAGACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	20	30	30163	3-month Stool	80	12.7	
58B	CCTACCATTGTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	22	30	30380	3-month Stool	80	5.4	
58C	CACTACGCTAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	23	30	30629	3-month Stool	207	27.2	
51J_repPCR	CTTGTGCGACAA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	24	30	30229	3-month Stool	186	26.4	PCR repeated from original gDNA dilution
51J_repIso	ACGCTTAACGAC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	25	30	30229	3-month Stool	192	23.2	Isolation repeated on new 192mg removed from sample
134D	ACTGAGCTGCAT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	40	50	30731	3-month Stool	109	19.1	Stool in diaper liner
134F	TTGGTAAAGTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	42	50	30108	3-month Stool	302	1.3	Stool in diaper liner
134G	CAAAGCGGTATT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	43	50	30179	3-month Stool	202	20.2	
134H	CGAAACTACGTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	44	50	30176	3-month Stool	207	4.7	
135F	TGGAGTAGGTGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	18	51	30054	3-month Stool	202	7.5	
135G	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	51	30066	3-month Stool	193	16.6	
135H	TCAGAGTAGACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	20	51	30069	3-month Stool	214	6.7	
136A	ACGCTTAACGAC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	25	51	30193	3-month Stool	206	31.0	
136B	CAAACAACAGCT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	26	51	30121	3-month Stool	206	25.7	
136D	CTCACCTAGGAA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	28	51	30137	3-month Stool	173	2.0	Stool in diaper liner
136E	CTCACAACCGTG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	29	51	30052	3-month Stool	180	10.0	
136F	GAGAGCAACAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	30	51	30055	3-month Stool	177	1.6	Stool in diaper liner
136G	TCCTCGAGCGAT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	31	51	30057	3-month Stool	95	0.3	Stool in diaper liner
136H	AGTGTTTCGGAC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	32	51	30059	3-month Stool	148	0.5	Stool in diaper liner
138I	GTGTAGGTGCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	21	52	30112	3-month Stool	209	27.1	
138J	CCTACCATTGTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	22	52	30091	3-month Stool	193	25.2	
138K	CACTACGCTAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	23	52	30184	3-month Stool	220	0.5	
139C	TAATACGGATCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	27	52	30067	3-month Stool	208	1.7	
139D	CTCACCTAGGAA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	28	52	30049	3-month Stool	136	1.2	Stool in diaper liner

#SampleID	BarcodeSequence	LinkerPrimerSequence	ReversePrimer	Description	Run	CHILD_ID	Age_mo	Stool_amt	Genomic_ngul	NOTES
139E	CTCACAACCGTG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	29	52	30056	3-month Stool	196	4.6	Stool in diaper liner
139F	GAGAGCAACAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	30	52	30076	3-month Stool	220	24.3	
142D	CTCACCTAGGAA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	28	53	30093	3-month Stool	190	2.0	Stool in diaper liner
142E	CTCACAACCGTG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	29	53	30068	3-month Stool	156	7.1	Stool in diaper liner
142F	GAGAGCAACAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	30	53	30095	3-month Stool	188	8.6	
142G	TCCTCGAGCGAT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	31	53	30111	3-month Stool	244	15.6	Stool in diaper liner
142I	CGCTGTGGATTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	33	53	30047	3-month Stool	185	21.1	
142J	GTGCAACCAATC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	34	53	30124	3-month Stool	186	3.2	
142K	AGGGTGACTTTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	35	53	30063	3-month Stool	199	0.9	
142L	TCACCTCCTTGT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	36	53	30145	3-month Stool	278	48.9	Stool in diaper liner
143L	TGTGCACGCCAT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	48	53	30094	3-month Stool	197	27.2	
144F	GAATACCAAGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	3	54	30147	3-month Stool	193	0.7	
144G	CGTAATTGCCGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	4	54	30046	3-month Stool	191	3.6	
144H	ATCGCGACTGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	5	54	30098	3-month Stool	119	1.3	
145D	AGTCGAACGAGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	13	54	30524	3-month Stool	123	13.7	
147E	CCGAAGATTCTG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	37	54	30195	3-month Stool	201	3.5	
147G	AGCCTGGTACCT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	39	54	30230	3-month Stool	291	5.6	Stool in diaper liner
147H	ACTGAGCTGCAT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	40	54	30167	3-month Stool	213	20.4	
147I	GATCCTCATGCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	41	54	30214	3-month Stool	187	0.8	
147J	TTGGTAAAGTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	42	54	30228	3-month Stool	204	32.0	
149K	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	55	30172	3-month Stool	196	3.6	
149L	TCAGAGTAGACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	20	55	30216	3-month Stool	203	25.5	
151L	CGAAACTACGTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	44	55	30248	3-month Stool	117	0.4	
152A	ACTTTGCTTTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	45	55	30135	3-month Stool	207	6.0	
152B	GCACGTTCTACG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	46	55	30236	3-month Stool	132	0.2	
152C	TGTTGACGATGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	47	55	30247	3-month Stool	114	0.1	
152F	ACGAGACTGATT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	2	56	30220	3-month Stool	163	6.9	
152G	GAATACCAAGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	3	56	30238	3-month Stool	250	2.4	Stool in diaper liner
152H	CGTAATTGCCGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	4	56	30254	3-month Stool	145	4.6	
153C	TCGACATCTCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	11	56	30271	3-month Stool	157	1.3	Stool in diaper liner
153I	GTTCTCTTCTCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	17	56	30307	3-month Stool	206	16.4	
153J	TGGAGTAGGTGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	18	56	30232	3-month Stool	206	0.5	
153K	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	56	30288	3-month Stool	202	5.6	
153L	TCAGAGTAGACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	20	56	30198	3-month Stool	207	0.7	
156B	GCACGTTCTACG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	46	56	30365	3-month Stool	187	1.3	
156C	TGTTGACGATGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	47	56	30244	3-month Stool	194	13.1	
156D	TGTGCACGCCAT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	48	56	30360	3-month Stool	219	2.2	
156E	TCCCTTGTCTCC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	1	57	30336	3-month Stool	203	20.9	
156J	TTCAGGAACTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	6	57	30390	3-month Stool	107	1.5	
157A	GTGTTGTCGTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	9	57	30323	3-month Stool	196	27.4	
157B	TAGTGATGACCA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	10	57	30393	3-month Stool	158	1.7	Stool in diaper liner
157D	AGCGGAGGTTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	12	57	30308	3-month Stool	212	23.6	
157E	AGTCGAACGAGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	13	57	30304	3-month Stool	213	3.0	
157F	CGAGCAATCCTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	14	57	30376	3-month Stool	213	4.5	
157H	GAACACTTTGGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	16	57	30269	3-month Stool	203	19.2	
157I	GTTCTCTTCTCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	17	57	30330	3-month Stool	192	6.1	
159B	GTGCAACCAATC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	34	57	30344	3-month Stool	105	2.6	
159C	AGGGTGACTTTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	35	57	30302	3-month Stool	174	15.5	
159D	TCACCTCCTTGT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	36	57	30616	3-month Stool	204	17.0	
159E	CCGAAGATTCTG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	37	57	30658	3-month Stool	146	26.8	Stool in diaper liner
159H	ACTGAGCTGCAT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	40	57	30516	3-month Stool	229	22.5	Stool in diaper liner
161A	GTGTTGTCGTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	9	58	30433	3-month Stool	139	9.0	Stool in diaper liner
161B	TAGTGATGACCA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	10	58	30342	3-month Stool	199	20.5	
161C	TCGACATCTCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	11	58	30793	3-month Stool	196	4.3	
161D	AGCGGAGGTTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	12	58	30767	3-month Stool	257	9.0	Stool in diaper liner
161E	AGTCGAACGAGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	13	58	30772	3-month Stool	252	0.6	Stool in diaper liner
161F	CGAGCAATCCTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	14	58	30313	3-month Stool	240	3.9	Stool was unusual consistency, perhaps there was part of the liner? Stool red color
161H	GAACACTTTGGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	16	58	30680	3-month Stool	265	17.0	Stool in diaper liner
161I	GTTCTCTTCTCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	17	58	30628	3-month Stool	213	4.8	
161J	TGGAGTAGGTGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	18	58	30792	3-month Stool	193	4.2	
161K	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	58	30700	3-month Stool	218	14.3	Stool in diaper liner
161L	TCAGAGTAGACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	20	58	30790	3-month Stool	238	1.1	Stool in diaper liner
162A	GTGTAGGTGCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	21	58	30719	3-month Stool	218	0.3	
162B	CCTACCATTGTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	22	58	30789	3-month Stool	371	4.3	Stool in diaper liner
204G	ACGAGACTGATT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	2	69	30561	3-month Stool	181	15.3	
204H	GAATACCAAGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	3	69	30568	3-month Stool	221	4.3	Stool in diaper liner
204K	TTCAGGAACTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	6	69	30643	3-month Stool	205	15.9	
204L	GATTATCGACGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	7	69	30750	3-month Stool	201	11.9	Stool in diaper liner
205A	CGCCTTGATAAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	8	69	30734	3-month Stool	246	9.7	Stool in diaper liner
207F	CCGAAGATTCTG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	37	69	30547	3-month Stool	206	2.8	

#SampleID	BarcodeSequence	LinkerPrimerSequence	ReversePrimer	Description	Run	CHILD_ID	Age_mo	Stool_amt	Genomic_ngul	NOTES
207G	ATCGACAACACC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	38	69	30708	3-month Stool	183	0.8	
207H	AGCCTGGTACCT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	39	69	30758	3-month Stool	302	0.3	Stool in diaper liner
207I	ACTGAGCTGCAT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	40	69	30476	3-month Stool	143	0.2	
207J	GATCCTCATGCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	41	69	30716	3-month Stool	203	1.9	
207K	TTGGTAAAGTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	42	69	30480	3-month Stool	192	25.8	
207L	CAAAGCGGTATT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	43	69	30431	3-month Stool	218	6.8	
208A	CGAAACTACGTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	44	69	30685	3-month Stool	189	0.6	
208B	ACTTTGCTTTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	45	69	30509	3-month Stool	201	4.0	
208C	GCACGTTCTACG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	46	69	30535	3-month Stool	198	15.2	
211C	AGTGTTTCGGAC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	32	70	30402	3-month Stool	60	1.7	
211I	ATCGACAACACC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	38	70	30401	3-month Stool	201	4.9	
211J	AGCCTGGTACCT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	39	70	30427	3-month Stool	124	2.4	
213F	TCGACATCTCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	11	71	30548	3-month Stool	145	5.7	
213G	AGCGGAGGTTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	12	71	30405	3-month Stool	207	22.1	
220A	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	72	30698	3-month Stool	228	4.9	Stool in diaper liner
226B	TCAGAGTAGACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	20	72	30774	3-month Stool	231	0.8	Stool in diaper liner
219A	GAGAGCAACAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	30	72	30613	3-month Stool	388	3.6	Stool in diaper liner
219B	TCCTCGAGCGAT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	31	72	30519	3-month Stool	197	20.0	
219E	GTGCAACCAATC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	34	72	30614	3-month Stool	213	1.3	
219L	GATCCTCATGCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	41	72	30576	3-month Stool	209	6.4	
220B	TTGGTAAAGTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	42	72	30630	3-month Stool	124	14.8	
220C	CAAAGCGGTATT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	43	72	30762	3-month Stool	217	14.2	
220D	CGAAACTACGTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	44	72	30703	3-month Stool	115	1.4	Stool in diaper liner
220E	ACTTTGCTTTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	45	72	30641	3-month Stool	180	2.6	
220F	GCACGTTCTACG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	46	72	30626	3-month Stool	213	15.3	
220G	TGTTGACGATGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	47	72	30743	3-month Stool	88	2.6	Stool in diaper liner
220H	TGTGCACGCCAT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	48	72	30728	3-month Stool	277	4.0	Stool in diaper liner
220I	TCCCTTGTCTCC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	1	73	30569	3-month Stool	218	13.3	
220J	ACGAGACTGATT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	2	73	30526	3-month Stool	212	22.9	
220K	GAATACCAAGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	3	73	30757	3-month Stool	219	0.8	Stool in diaper liner
220L	CGTAATTGCCGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	4	73	30523	3-month Stool	210	21.8	
222A	GTTCTCTTCTCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	17	73	30398	3-month Stool	178	3.6	
222C	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	73	30585	3-month Stool	192	8.6	
222D	TCAGAGTAGACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	20	73	30499	3-month Stool	199	20.0	
223A	CTCACAACCGTG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	29	73	30648	3-month Stool	218	8.4	
223B	GAGAGCAACAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	30	73	30723	3-month Stool	219	0.6	
223C	TCCTCGAGCGAT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	31	73	30727	3-month Stool	200	1.5	
223D	AGTGTTTCGGAC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	32	73	30646	3-month Stool	111	2.8	
223F	GTGCAACCAATC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	34	73	30754	3-month Stool	271	13.4	Stool in diaper liner
223G	AGGGTGACTTTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	35	73	30474	3-month Stool	206	5.2	
226C	ATCGCGACTGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	5	74	30722	3-month Stool	169	4.6	Stool in diaper liner
225E	GTGTTGTCGTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	9	74	30735	3-month Stool	190	1.1	
225F	TAGTGATGACCA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	10	74	30432	3-month Stool	204	19.4	
225I	AGTCGAACGAGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	13	74	30587	3-month Stool	192	2.2	Stool in diaper liner
225K	CATCCCTCTACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	15	74	30787	3-month Stool	210	1.7	
226D	GTTCTCTTCTCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	17	74	30778	3-month Stool	194	1.0	
226E	TGGAGTAGGTGG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	18	74	30801	3-month Stool	194	5.2	
226F	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	74	30802	3-month Stool	269	2.9	Stool in diaper liner
226J	CACTACGCTAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	23	74	30788	3-month Stool	161	2.5	Stool in diaper liner
226K	CTTGTGCGACAA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	24	74	30771	3-month Stool	137	0.2	
226L	ACGCTTAACGAC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	25	74	30753	3-month Stool	219	1.5	
228L	TGTTGACGATGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	47	74	30833	3-month Stool	194	24.1	Stool in diaper liner
226A	ACACGACTATAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	19	75	30777	3-month Stool	220	2.5	
230K	GTGTAGGTGCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	21	75	30714	3-month Stool	304	13.2	Stool in diaper liner
230L	CCTACCATTGTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	22	75	30756	3-month Stool	194	1.3	Stool in diaper liner
231D	CAAACAACAGCT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	26	75	30839	3-month Stool	222	2.8	Stool in diaper liner
231E	TAATACGGATCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	27	75	30780	3-month Stool	231	3.4	Stool in diaper liner
231F	CTCACCTAGGAA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	28	75	30821	3-month Stool	221	6.2	Stool in diaper liner
233E	ACGAGACTGATT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	2	76	30832	3-month Stool	183	21.0	Stool in diaper liner
233F	GAATACCAAGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	3	76	30806	3-month Stool	142	8.3	Stool in diaper liner
233G	CGTAATTGCCGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	4	76	30840	3-month Stool	202	46.1	
233H	ATCGCGACTGTC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	5	76	30810	3-month Stool	238	28.4	Stool in diaper liner
233I	TTCAGGAACTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	6	76	30841	3-month Stool	245	6.1	Stool in diaper liner
233J	GATTATCGACGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	7	76	30814	3-month Stool	211	44.6	
233L	GTGTTGTCGTGC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	9	76	30783	3-month Stool	206	3.9	
234A	TAGTGATGACCA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	10	76	30830	3-month Stool	166	3.6	Stool in diaper liner
234B	TCGACATCTCTT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	11	76	30824	3-month Stool	197	1.2	
234C	AGCGGAGGTTAG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	12	76	30713	3-month Stool	185	10.7	Stool in diaper liner
234E	CGAGCAATCCTA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	14	76	30834	3-month Stool	139	0.5	Stool in diaper liner
234F	CATCCCTCTACT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	15	76	30831	3-month Stool	217	7.3	

#SampleID	BarcodeSequence	LinkerPrimerSequence	ReversePrimer	Description	Run	CHILD_ID	Age_mo	Stool_amt	Genomic_ngul	NOTES
234G	GAACACTTTGGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	16	76	30837	3-month Stool	219	1.9	
234H	GTTCTCTTCTCG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	17	76	30765	3-month Stool	155	4.9	Stool in diaper liner
235B	CACTACGCTAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	23	76	30420	3-month Stool	203	7.3	
235D	ACGCTTAACGAC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	25	76	30403	3-month Stool	231	1.6	Stool in diaper liner
235H	CTCACAACCGTG	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	29	76	30699	3-month Stool	201	2.0	Stool in diaper liner
235I	GAGAGCAACAGA	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	30	76	30558	3-month Stool	203	0.4	
235J	TCCTCGAGCGAT	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	31	76	30489	3-month Stool	154	2.4	Stool in diaper liner
236B	GTGCAACCAATC	TATGGTAATTGTGTGCCAGCMGCCGCGGTAA	AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT	34	76	30838	3-month Stool	178	30.3	