Materials List for:

A Method to Define the Effects of Environmental Enrichment on Colon Microbiome Biodiversity in a Mouse Colon Tumor Model

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Materials

Name	Company	Catalog Number	Comments
Teklad Diets/Harlan Labs Chow	Harlan Labs	3980X	Standard irradiated chow formulated by Dr. Mario Capecchi in collaboration with Harlan Labs.
Cell-Sorb Plus bedding	Fangman Specialties	82010	Autoclave prior to use.
AIMS Tattooing System For Neonates	AIMS	NEO-9	https://animalid.com/neonate-rodent-tattoo-identification/32. Other animal grade tattoo systems and inks can be used with similar results including the Aramis Micro Tattoo Kit.
Zyfone One Cage 2100 AllerZone Mouse Micro-Isolator System Complete with cage, AllerZone filter top and modular diet delivery system	Lab Products	82120ZF	Each EE cage requires one of each catalog # 82120ZF, 82100ZF, and 82101ZF, as well as two of 82109ZF. Food is only in one side.
Zyfone One Cage 2100 Life Span Enrichment Device	Lab Products	82109ZF	Each EE cage requires one of each catalog # 82120ZF, 82100ZF, and 82101ZF, as well as two of 82109ZF. Food is only in one side.
Zyfone One Cage 2100 Cage 13-7/8" Length X 19-1/16" Width X 7-3/4" Depth	Lab Products	82100ZF	Each EE cage requires one of each catalog # 82120ZF, 82100ZF, and 82101ZF, as well as two of 82109ZF. Food is only in one side.
Zyfone One Cage 2100 AllerZone Micro-Isolator filter top	Lab Products	82101ZF	Each EE cage requires one of each catalog # 82120ZF, 82100ZF, and 82101ZF, as well as two of 82109ZF. Food is only in one side.
Tunnel	Bio-Serv	K3323 or K3332	Connect cages together and use for enrichment
Grommet to connect Tunnel to cages	Fabricated by the University of Utah Machine Shop	n/a	Be certain the material is resistant to chewing and autoclavable
Fast-track wheel	Bio-Serv	K3250 or K3251	Use with mouse igloo and floor
Mouse Igloo	Bio-Serv	K3328, K3570 or K3327	Use with Fast-track wheel and floor
Mouse Igloo floor	Bio-Serv	K3244	Use with mouse Igloo and Fast-Track
Mouse Hut	Bio-Serv	K3272, K3102 or K3271	
Crawl Ball	Bio-Serv	K3330 or K3329	
Bio-hut	Bio-Serv	K3352	Wood pulp hut used for sheltering and nesting

Adhesive film	VWR	60941-072	Use to temporarily cover drilled hole in large cage to prevent mice from escaping
Laminar Flow Ventilated Rack	Techniplast	Bio-C36	The cabinet we used in this study is not currently supplied. The Bio-C36 is very similar.
1.5 mL Microfuge Tube- RNAse and DNAse free	Any supplier		
QIAamp DNA Stool MiniKit	Qiagen	51504	This kit supplies reagents for 50 DNA preparations. Stool Lysis Buffer=ASL; Guanidinium Chloride Lysis Buffer= AL; Wash Buffer 1 with Guanidinium Chloride= AW1; Wash Buffer 2= AW2; Elution Buffer with EDTA=AE
Waterbath (capable of heating to 95)	Any supplier		For 94 degree incubation of stool samples to lyse cells.
Waterbath (capable of heating to 70 degrees)	Any supplier		For 70 degree incubation of stool samples
Ethanol (200 proof)	Sigma Aldrich	E7023	
Fluorometer: Qubit	ThermoFisher Scientific	Q33216	
Qubit dsDNA broad Range Assay Kit	ThermoFisher Scientific	Q32850	
EB Buffer or 10 mM Tris pH 8.5	Qiagen	19086	
Experiment specific primers	Any Supplier		
PCR grade water	Any supplier		
2X KAPA HiFi HotStart Ready Mix	Kapa Biosystems	KK2601	For Amplicon Amplification (1.25 mL allows 100 rxns).
Agarose for running diagnostic gels	Any supplier		
TapeStation High Sensitivity D1000 Screen Tape Trace	Agilent	5067-5583	TapeStation or Bioanalyzer instruments are common in Institutional Genomics Cores to analyze library quality . Alternatively a Bioanalyzer DNA1000 Chip (Agilent, 5067-1504) can be used.
Agencourt AMPure XP Magnetic Beads	Beckman Coulter	A63880	Magentic beads For PCR cleanup- 5 mL will clean 250 PCR reactions
Magnetic stand	Life Technologies	AM10027	
Library Preparation Guide	Illumina		Illumina. 16S Metagenomic Sequencing Library Preparation: Preparing 16S ribosomal RNA Gene Amplicons for the Illumina MiSeq System. https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf.
Unique Dual Indexing	Illumina	Illumina Experiment Manager Software	Freely available at: https://support.illumina.com/sequencing/sequencing_software/experiment_manager/downloads.html
Nextera XT 96 Index Kit	Illumina	FC-131-1002	Used to add barcodes to amplicons
MicroAmp Optical 96-well reaction plate	Applied Biosystems/ThermoFisher	N8010560	

TruSeq Index Plate Fixture	Illumina	FC-130-1005	
Adhesive clear plate seal	Applied Biosystems /ThermoFisher	4360954	Applied Biosystems/ThermoFisher Microamp adhesive film
Sequencing by MiSeq with v3 reagents and dual 300 bp reads	Illumina	MS-102-3003	
PhiX Control Kit	Illumina	FC-110-3001	
Proteinase K (600 mAU/ml)	Qiagen	19131	Equivalent to 20 mg/ml of proteinase K. Supplied with QiaAmp kit
Data Analysis Tools	Qiime	QIIME software Tools	Installation may differ based on your system and the QIIME website describes several options (http://qiime.org/install/install.html). For this study, MacQIIME software package 1.9.1 was utilized (compiled by Werner Lab, SUNY, http://www.wernerlab.org/software/macqiime
Step 13.2.	Qiime	FastQ Join method	(http://code.google.com/p/ea-utils). For this study Multiple join paired ends was used http://qiime.org/scripts/multiple_join_paired_ends.html. Aronesty, E. ea-utils: Command-line tools for processing biological sequencing data. Expression Analysis, Durham, NC. (2011).
Step 13.3.	Qiime	De-Novo OTU picking protocol	http://qiime.org/scripts/pick_de_novo_otus.html.
Step 13.3.1.		Open Taxonomic Units (OTUs) using Uclust	Edgar, R.C. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 26 (19), 2460-2461, doi:10.1093/bioinformatics/btq461 (2010).
Step 13.3.1.	Pynast	Pynast	Caporaso, J.G. et al. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics. 26 (2), 266-267, doi:10.1093/bioinformatics/btp636 (2010).
Step 13.3.1.	Pynast	Pynast_Greengenes	DeSantis, T.Z. et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol. 72 (7), 5069-5072, doi:10.1128/AEM.03006-05 (2006). Greengenes version 13_8 was used in this study
13.3.1. Note:	Qiime	Multiple Split Libraries	http://qiime.org/scripts/multiple_split_libraries_fastq.html.
13.3.1. Note:	Qiime	Pick de novo OTUs script	http://qiime.org/scripts/pick_de_novo_otus.html
Step 13.2.2.	Qiime	Create a mapping file	http://qiime.org/documentation/file_formats.html.
Step 13.2.2.	Qiime	Validate a mapping file	http://qiime.org/scripts/validate_mapping_file.html.
Step 13.3.3.	Qiime	Link the OTU to sample description to mapping file	http://qiime.org/scripts/make_otu_network.html.
Step 13.3.4.	Qiime	Summarize Taxa through plots	http://qiime.org/scripts/summarize_taxa_through_plots.html.

Step 13.3.5.	Qiime	Biome Summarize table	http://biom-format.org/documentation/summarizing_biom_tables.html In this study, all samples were rarified to 20,000 OTUs followed by analysis using alpha rarefaction script in QIIME.