Protocol for 18S rRNA amplification and sequencing on the Illumina MiSeq

1.0 Introduction

The 18S protocol detailed here is designed to amplify eukaryotes broadly with a focus on microbial eukaryotic lineages. The primers are based on those of Amaral-Zettler et al 2009 and designed to be used with the Illumina platform. The protocol is based on that used by the Earth Microbiome Project (EMP), found here: http://www.earthmicrobiome.org/emp-standard-protocols/18s/

Amaral-Zettler, LA, EA McCliment, HW Ducklow, SM Huse. 2009. A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. PLoS ONE 4:e6372.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 2012 Aug; 6(8): 1621–1624.

2.0 Amplification of 1391f and EukBr region of the 18S rRNA gene

2.1 Primers for amplification of 1391f and EukBr region of the 18S rRNA gene

Amplification primers

The primer sequences in this protocol are always listed in the 5’ -> 3’ orientation. See Section 4 for more information on ordering, concentration, and resuspension. Primer constructs designed by Laura Wegener Parfrey.

ILM_Euk_1391f PCR Primer Sequence – Forward primer

Field number (space-delimited), description:
1. 5' Illumina adapter
2. Forward primer pad
3. Forward primer linker
4. Forward primer (1391f)

AATGATACGGCGACCACCGAGATCTACAC TATCGCCGTT CG GTACACACCGCCCGTC

ILM_EukBr PCR primer sequence – Reverse primer, barcoded

Each sequence contains different 12 base Golay barcode as described by Caporaso et al.

1. Reverse complement of 3' Illumina adapter
2. Golay barcode
3. Reverse primer pad
4. Reverse primer linker
5. Reverse primer (EukBr)

CAAGCAGAAGACGGCATACGAGAT XXXXXXXXXXXX AGTCAGTCAG CA TGATCCTTCTGCAGGTTCACCTAC

Full list of primer sequences is provided in Appendix 1.

2.2 Preparation of master mix for amplification of 1391f and EukBr region of the 18S rRNA gene

Component	Volume 1 rxn
PCR Grade H2O (note a, below)	13.0 µL
5 Primer Hot MM (note b, below)	10.0 µL
Forward primer (10µM)	0.5 µL
Reverse primer (10µM)	0.5 µL
Template DNA	1.0 µL
Total reaction volume	25.0 µL

Notes:

(a) PCR grade water was purchased from MoBio Laboratories (MoBio Labs: Item#17000-11)
(b) 5 PRIME HotMasterMix (5 PRIME: Item# 2200410)
(c) Final primer concentration of mastermix: 0.2 µM

2.3 Thermocycler Conditions for amplification of 1391f and EukBr region of the 18S rRNA gene (96 well thermocyclers)

	Temperature	Time (mm:ss)
Activation	94°C	3:00
Amplification (35 cycles)	94°C	00:45
	57°C	01:00
	72°C	01:30
Final Extension	72°C	10:00
HOLD	4°C	∞

2.4 Process

2.4.1 Dilute DNA 1:10.
2.4.2 Amplify samples with conditions outlined above.
2.4.3 Run amplicons on an agarose gel. Expected band size for 1391f/Eukbr is roughly 200 bp.
2.4.4 If there is no band present, repeat PCR using either the undiluted DNA or a 1:100 dilution. Use the concentration of the DNA extract to determine if the DNA should be further diluted or used neat.
2.4.5 Clean and normalize the PCR products. For this step, both Agencourt AMPure XP bead-clean-up and SequalPrep Normalization plates (Invitrogen, A1051001) are acceptable.
If using Agencourt AMPure XP beads:
a. Perform a bead clean-up following manufacturer's instructions.
b. Quantify amplicon yields. Acceptable methods include Picogreen (see manufacturers protocol; Invitrogen Item #P11496) or the high sensitivity Quant-iT™ DNA Assay Kit (Life Technologies, Q-33120).

c. Run clean amplicons on Agilent Bioanalyzer, LabChip GX, or 2200 TapeStation to get accurate sizing information.
d. Normalise amplicons by diluting to 10nM with variable volume of buffer (EB buffer, or 10mM Tris pH 8.5) and a set volume of the amplicons.
e. Pool equal volumes of each normalized amplicon.

If using SequalPrep Normalization plates:

f. Perform a plate normalization following manufacturer's instructions.
g. Pool equal volumes of each normalized amplicon.
h. Run clean amplicon pool on Agilent Bioanalyzer, LabChip GX, or 2200 TapeStation to get accurate sizing information.

2.4.6 Optional: If spurious bands were present on gel (in step 2.4.3), or in the clean amplicons (step 2.4.5c) or amplicon pool (step 2.4.5h), a portion of the final amplicon pool can go through a gel extraction or a second round of bead clean up. A lower bead to template ratio will get rid of small bands.

2.4.7 Measure concentration of the final clean pool using Qubit or picogreen.

3.0 Sequencing of 1391f and EukBr region of the 18S rRNA gene

3.1 Sequencing Primers

ILM_Euk_R1: Read 1 Sequencing Primer

Field description (space-delimited):
1. Forward primer pad
2. Forward primer linker
3. Forward primer

TATCGCCGTT CG GTACACACCGCCCGTC

ILM_Euk_R2: Read 2 Sequencing Primer

Field description (space-delimited):
1. Reverse primer pad
2. Reverse primer linker
3. Reverse primer

AGTCAGTCAG CA TGATCCTTCTGCAGGTTCACCTAC

ILM_Euk_INDEX: Index Read Sequencing Primer

Field description (space-delimited):
1. Reverse complement of reverse primer
2. Reverse complement of reverse primer linker
3. Reverse complement of reverse primer pad

GTAGGTGAACCTGCAGAAGGATCA TG CTGACTGACT

3.2 Sequencing Setup

3.2.1 Dilute pool prepared in step 2.4.5 to 4nM.
3.2.2 Denature according to Ilumina protocol, with increased PhiX control spike-in as recommended for low diversity libraries. See *Preparing Libraries for Sequencing on the MiSeq (part #15039740)*.
3.2.3 Prepare MiSeq Reagent Cartridge (v2 300-cycles). See *MiSeq Reagent Kit v2 - Reagent Preparation Guide (part # 15034097).*
3.2.4 Using an extra long pipette tip set to 75 µL, add 3.4 µL of Read1 sequencing primer (100 µM) into well 12 of the MiSeq Reagent Cartridge and mix 10 times. Repeat adding the Index Primer into well 13 and the Read2 sequencing primer into well 14.
3.2.5 Load 600 µL of library pool into the MiSeq reagent cartridge in designated sample well.
3.2.6 Modify sample sheet to include the custom index sequences (see index sequences in Appendix 2).
3.2.7 Start sequencing run following *MiSeq System User Guide (part # 15027617).*

4.0 Primer ordering and resuspension: tips and getting started (From EMP protocol)

Resuspension of primers is a critical step and must be done with Ultra pure water under sterile conditions. It is recommended that resuspension be done in a hood.

- Stock plates are generally 100 µM and are aliquoted and diluted to 10 µM for use.
- When making stock plates it is a good idea to make multiple replicate plates so that there are back up plates of primers.
- The primer sequences in this protocol are always listed in the 5' -> 3' orientation. This is the orientation that should be used for ordering.
- We use the standard desalting cleanup option.
- If you are using a robot the volume in the plates should be 30 µL per well or greater. 15µL per well is the absolute minimum.

Ordering barcoded primers

- Contact IDT or your primer provider to get a quote prior to ordering.
- Barcoded primers are ordered in 96 well plates at the 100 nmole scale.
- Make sure to select the option of receive the same amount per well of each primer (do not get full yield). This is generally the 8 nmole/well option.
- Select the rows option for load scheme: [Rows [A01,A02,A03]]
- If you will be archiving plates of primers for later use consider selecting the option for replicate plates.

Appendix 1. Primers Sequences

Primer Name	Primer Sequence
ILM_Euk_R1	TATCGCCGTTCGGTACACACCGCCCGTC
ILM_Euk_R2	AGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_Euk_INDEX	GTAGGTGAACCTGCAGAAGGATCATGCTGACTGACT
ILM_Euk_1391F_U	AATGATACGGCGACCACCGAGATCTACACTATCGCCGTTCGGTACACACCGCCCGTC
ILM_EukBR_0097	CAAGCAGAAGACGGCATACGAGATTACCGCTTCTTCAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0098	CAAGCAGAAGACGGCATACGAGATTGTGCGATAACAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0099	CAAGCAGAAGACGGCATACGAGATGATTATCGACGAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0100	CAAGCAGAAGACGGCATACGAGATGCCTAGCCCAATAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0101	CAAGCAGAAGACGGCATACGAGATGATGTATGTGGTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0102	CAAGCAGAAGACGGCATACGAGATACTCCTTGTGTTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0103	CAAGCAGAAGACGGCATACGAGATGTCACGGACATTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0104	CAAGCAGAAGACGGCATACGAGATGCGAGCGAAGTAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0105	CAAGCAGAAGACGGCATACGAGATATCTACCGAAGCAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0106	CAAGCAGAAGACGGCATACGAGATACTTGGTGTAAGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0107	CAAGCAGAAGACGGCATACGAGATTCTTGGAGGTCAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0108	CAAGCAGAAGACGGCATACGAGATTCACCTCCTTGTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0109	CAAGCAGAAGACGGCATACGAGATGCACACCTGATAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0110	CAAGCAGAAGACGGCATACGAGATGCGACAATTACAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0111	CAAGCAGAAGACGGCATACGAGATTCATGCTCCATTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0112	CAAGCAGAAGACGGCATACGAGATAGCTGTCAAGCTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0113	CAAGCAGAAGACGGCATACGAGATGAGAGCAACAGAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0114	CAAGCAGAAGACGGCATACGAGATTACTCGGGAACTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0115	CAAGCAGAAGACGGCATACGAGATCGTGCTTAGGCTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0116	CAAGCAGAAGACGGCATACGAGATTACCGAAGGTATAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0117	CAAGCAGAAGACGGCATACGAGATCACTCATCATTCAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0118	CAAGCAGAAGACGGCATACGAGATGTATTTCGGACGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0119	CAAGCAGAAGACGGCATACGAGATTATCTATCCTGCAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0120	CAAGCAGAAGACGGCATACGAGATTTGCCAAGAGTCAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0121	CAAGCAGAAGACGGCATACGAGATAGTAGCGGAAGAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0122	CAAGCAGAAGACGGCATACGAGATGCAATTAGGTACAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0123	CAAGCAGAAGACGGCATACGAGATCATACCGTGAGTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0124	CAAGCAGAAGACGGCATACGAGATATGTGTGTAGACAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0125	CAAGCAGAAGACGGCATACGAGATCCTGCGAAGTATAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0126	CAAGCAGAAGACGGCATACGAGATTTCTCTCGACATAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0127	CAAGCAGAAGACGGCATACGAGATGCTCTCCGTAGAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0128	CAAGCAGAAGACGGCATACGAGATGTTAAGCTGACCAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0129	CAAGCAGAAGACGGCATACGAGATATGCCATGCCGTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0130	CAAGCAGAAGACGGCATACGAGATGACATTGTCACGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0131	CAAGCAGAAGACGGCATACGAGATGCCAACAACCATAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0132	CAAGCAGAAGACGGCATACGAGATATCAGTACTAGGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0133	CAAGCAGAAGACGGCATACGAGATTCCTCGAGCGATAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0134	CAAGCAGAAGACGGCATACGAGATACCCAAGCGTTAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0135	CAAGCAGAAGACGGCATACGAGATTGCAGCAAGATTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0136	CAAGCAGAAGACGGCATACGAGATAGCAACATTGCAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0137	CAAGCAGAAGACGGCATACGAGATGATGTGGTGTTAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0138	CAAGCAGAAGACGGCATACGAGATCAGAAATGTGTCAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0139	CAAGCAGAAGACGGCATACGAGATGTAGAGGTAGAGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0140	CAAGCAGAAGACGGCATACGAGATCGTGATCCGCTAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0141	CAAGCAGAAGACGGCATACGAGATGGTTATTTGGCGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0142	CAAGCAGAAGACGGCATACGAGATGGATCGTAATACAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0143	CAAGCAGAAGACGGCATACGAGATGCATAGCATCAAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0144	CAAGCAGAAGACGGCATACGAGATGTGTTAGATGTGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0145	CAAGCAGAAGACGGCATACGAGATTTAGAGCCATGCAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0146	CAAGCAGAAGACGGCATACGAGATTGAACCCTATGGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC

ILM_EukBR_0147	CAAGCAGAAGACGGCATACGAGATAGAGTCTTGCCAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0148	CAAGCAGAAGACGGCATACGAGATACAACACTCCGAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0149	CAAGCAGAAGACGGCATACGAGATCGATGCTGTTGAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0150	CAAGCAGAAGACGGCATACGAGATACGACTGCATAAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0151	CAAGCAGAAGACGGCATACGAGATACGCGAACTAATAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0152	CAAGCAGAAGACGGCATACGAGATAGCTATGTATGGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0153	CAAGCAGAAGACGGCATACGAGATACGGGTCATCATAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0154	CAAGCAGAAGACGGCATACGAGATGAAACATCCCACAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0155	CAAGCAGAAGACGGCATACGAGATCGTACTCTCGAGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0156	CAAGCAGAAGACGGCATACGAGATTCAGTTCTCGTTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0157	CAAGCAGAAGACGGCATACGAGATTCGTGCGTGTTGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0158	CAAGCAGAAGACGGCATACGAGATGTTATCGCATGGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0159	CAAGCAGAAGACGGCATACGAGATGATCACGAGAGGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0160	CAAGCAGAAGACGGCATACGAGATGTAAATTCAGGCAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0161	CAAGCAGAAGACGGCATACGAGATAGTGTTTCGGACAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0162	CAAGCAGAAGACGGCATACGAGATACACGCGGTTTAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0163	CAAGCAGAAGACGGCATACGAGATTGGCAAATCTAGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0164	CAAGCAGAAGACGGCATACGAGATCACCTTACCTTAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0165	CAAGCAGAAGACGGCATACGAGATTTAACCTTCCTGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0166	CAAGCAGAAGACGGCATACGAGATTGCCGTATGCCAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0167	CAAGCAGAAGACGGCATACGAGATCGTGACAATGTAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0168	CAAGCAGAAGACGGCATACGAGATCGCTACAACTCGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0169	CAAGCAGAAGACGGCATACGAGATTTAAGACAGTCGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0170	CAAGCAGAAGACGGCATACGAGATTCTGCACTGAGCAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0171	CAAGCAGAAGACGGCATACGAGATCGCAGATTAGTAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0172	CAAGCAGAAGACGGCATACGAGATTGGGTCCCACATAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0173	CAAGCAGAAGACGGCATACGAGATCACTGGTGCATAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0174	CAAGCAGAAGACGGCATACGAGATAACGTAGGCTCTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0175	CAAGCAGAAGACGGCATACGAGATAGTTGTAGTCCGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0176	CAAGCAGAAGACGGCATACGAGATTCGTCAAACCCGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0177	CAAGCAGAAGACGGCATACGAGATTAATCGGTGCCAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0178	CAAGCAGAAGACGGCATACGAGATTTGATCCGGTAGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0179	CAAGCAGAAGACGGCATACGAGATCGGGTGTTTGCTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0180	CAAGCAGAAGACGGCATACGAGATTTGACCGCGGTTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0181	CAAGCAGAAGACGGCATACGAGATGTGCAACCAATCAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0182	CAAGCAGAAGACGGCATACGAGATGCTTGAGCTTGAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0183	CAAGCAGAAGACGGCATACGAGATCGCTGTGGATTAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0184	CAAGCAGAAGACGGCATACGAGATCTGTCAGTGACCAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0185	CAAGCAGAAGACGGCATACGAGATACGATTCGAGTCAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0186	CAAGCAGAAGACGGCATACGAGATGGTTCGGTCCATAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0187	CAAGCAGAAGACGGCATACGAGATCTGATCCATCTTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0188	CAAGCAGAAGACGGCATACGAGATTATGTGCCGGCTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0190	CAAGCAGAAGACGGCATACGAGATTGTAAGACTTGGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0191	CAAGCAGAAGACGGCATACGAGATCGGATCTAGTGTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0192	CAAGCAGAAGACGGCATACGAGATCGATCTTCGAGCAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0193	CAAGCAGAAGACGGCATACGAGATGTCGAATTTGCGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC

Appendix 2. Index sequences for sample sheet

I7_Index_ID	index
ILM_EukBR_0097	GAAGAAGCGGTA
ILM_EukBR_0098	TGTTATCGCACA
ILM_EukBR_0099	TCGTCGATAATC
ILM_EukBR_0100	ATTGGGCTAGGC
ILM_EukBR_0101	ACCACATACATC
ILM_EukBR_0102	AACACAAGGAGT
ILM_EukBR_0103	AATGTCCGTGAC
ILM_EukBR_0104	TACTTCGCTCGC
ILM_EukBR_0105	GCTTCGGTAGAT
ILM_EukBR_0106	CTTACACCAAGT
ILM_EukBR_0107	TGACCTCCAAGA
ILM_EukBR_0108	ACAAGGAGGTGA
ILM_EukBR_0109	TATCAGGTGTGC
ILM_EukBR_0110	TGTAATTGTCGC
ILM_EukBR_0111	AATGGAGCATGA
ILM_EukBR_0112	AGCTTGACAGCT
ILM_EukBR_0113	TCTGTTGCTCTC
ILM_EukBR_0114	AGTTCCCGAGTA
ILM_EukBR_0115	AGCCTAAGCACG
ILM_EukBR_0116	ATACCTTCGGTA
ILM_EukBR_0117	GAATGATGAGTG
ILM_EukBR_0118	CGTCCGAAATAC
ILM_EukBR_0119	GCAGGATAGATA
ILM_EukBR_0120	GACTCTTGGCAA
ILM_EukBR_0121	TCTTCCGCTACT
ILM_EukBR_0122	GTACCTAATTGC
ILM_EukBR_0123	ACTCACGGTATG
ILM_EukBR_0124	GTCTACACACAT
ILM_EukBR_0125	ATACTTCGCAGG
ILM_EukBR_0126	ATGTCGAGAGAA
ILM_EukBR_0127	TCTACGGAGAGC
ILM_EukBR_0128	GGTCAGCTTAAC
ILM_EukBR_0129	ACGGCATGGCAT
ILM_EukBR_0130	CGTGACAATGTC
ILM_EukBR_0131	ATGGTTGTTGGC
ILM_EukBR_0132	CCTAGTACTGAT
ILM_EukBR_0133	ATCGCTCGAGGA
ILM_EukBR_0134	TAACGCTTGGGT
ILM_EukBR_0135	AATCTTGCTGCA
ILM_EukBR_0136	TGCAATGTTGCT
ILM_EukBR_0137	TAACACCACATC
ILM_EukBR_0138	GACACATTTCTG
ILM_EukBR_0139	CTCTACCTCTAC
ILM_EukBR_0140	TAGCGGATCACG
ILM_EukBR_0141	CGCCAAATAACC
ILM_EukBR_0142	GTATTACGATCC
ILM_EukBR_0143	TTGATGCTATGC
ILM_EukBR_0144	CACATCTAACAC
ILM_EukBR_0145	GCATGGCTCTAA
ILM_EukBR_0146	CCATAGGGTTCA

ILM_EukBR_0147	TGGCAAGACTCT
ILM_EukBR_0148	TCGGAGTGTTGT
ILM_EukBR_0149	TCAACAGCATCG
ILM_EukBR_0150	TTATGCAGTCGT
ILM_EukBR_0151	ATTAGTTCGCGT
ILM_EukBR_0152	CCATACATAGCT
ILM_EukBR_0153	ATGATGACCCGT
ILM_EukBR_0154	GTGGGATGTTTC
ILM_EukBR_0155	CTCGAGAGTACG
ILM_EukBR_0156	AACGAGAACTGA
ILM_EukBR_0157	CAACACGCACGA
ILM_EukBR_0158	CCATGCGATAAC
ILM_EukBR_0159	CCTCTCGTGATC
ILM_EukBR_0160	GCCTGAATTTAC
ILM_EukBR_0161	GTCCGAAACACT
ILM_EukBR_0162	TAAACCGCGTGT
ILM_EukBR_0163	CTAGATTTGCCA
ILM_EukBR_0164	TAAGGTAAGGTG
ILM_EukBR_0165	CAGGAAGGTTAA
ILM_EukBR_0166	TGGCATACGGCA
ILM_EukBR_0167	ACTATTGTCACG
ILM_EukBR_0168	CGAGTTGTAGCG
ILM_EukBR_0169	CGACTGTCTTAA
ILM_EukBR_0170	GCTCAGTGCAGA
ILM_EukBR_0171	TACTAATCTGCG
ILM_EukBR_0172	ATGTGGGACCCA
ILM_EukBR_0173	TATGCACCAGTG
ILM_EukBR_0174	AGAGCCTACGTT
ILM_EukBR_0175	CGGACTACAACT
ILM_EukBR_0176	CGGGTTTGACGA
ILM_EukBR_0177	TGGCACCGATTA
ILM_EukBR_0178	CTACCGGATCAA
ILM_EukBR_0179	AGCAAACACCCG
ILM_EukBR_0180	AACCGCGGTCAA
ILM_EukBR_0181	GATTGGTTGCAC
ILM_EukBR_0182	TCAAGCTCAAGC
ILM_EukBR_0183	TAATCCACAGCG
ILM_EukBR_0184	GGTCACTGACAG
ILM_EukBR_0185	GACTCGAATCGT
ILM_EukBR_0186	ATGGACCGAACC
ILM_EukBR_0187	AAGATGGATCAG
ILM_EukBR_0188	AGCCGGCACATA
ILM_EukBR_0190	CCAAGTCTTACA
ILM_EukBR_0191	ACACTAGATCCG
ILM_EukBR_0192	GCTCGAAGATCG
ILM_EukBR_0193	CGCAAATTCGAC