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

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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      CAAGCAGAAGACGGCATACGAGATTTTGCCAAGAGTCAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0121      CAAGCAGAAGACGGCATACGAGATAGTAGCGGAAGAAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0122      CAAGCAGAAGACGGCATACGAGATGCAATTAGGTACAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0123      CAAGCAGAAGACGGCATACGAGATCATACCGTGAGTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0124      CAAGCAGAAGACGGCATACGAGATATGTGTGTAGACAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0125      CAAGCAGAAGACGGCATACGAGATCCTGCGAAGTATAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0126      CAAGCAGAAGACGGCATACGAGATTTTCTCTCGACATAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
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      CAAGCAGAAGACGGCATACGAGATGTAGAGGTAGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
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
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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   CAAGCAGAAGACGGCATACGAGATCGTGACAATAGTAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0168   CAAGCAGAAGACGGCATACGAGATCGCTACAACTCGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
ILM_EukBR_0169   CAAGCAGAAGACGGCATACGAGATTTAAGACAGTCGAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
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ILM_EukBR_0172   CAAGCAGAAGACGGCATACGAGATTGGGTCCCACATAGTCAGTCAGCATGATCCTTCTGCAGGTTCACCTAC
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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
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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
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Appendix 2. Index sequences for sample sheet

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I7_Index_ID            index
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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
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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
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