Amplification and Illumina Sequencing of 27F and 519R region of the 16S rRNA gene

Introduction
The protocol detailed here is designed to amplify the 27F and 519 region of the 16S rRNA gene Primers for paired-end 16s community sequencing on the Illumina MiSeq platform using.

Primers for amplification of 27F (Lane 1991) and 519R (Lane et al. 1993) region of the 16S rRNA gene

**ILM\_27F\_Uv3** – forward primer
Field number (space-delimited), description:
1. 5' illumina adapter
2. Forward primer pad
3. Forward primer linker
4. Forward primer (1391f)

AATGATACGGCGACCACCGAGATCTACAC TATGGCGAGT GA AGAGTTTGATCMTGGCTCAG

**ILM\_519R\_NNNN** – reverse primer
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 GG GWATTACCGCGGCKGCTG

* This primer includes a 12 base Golay barcode as described by Caporaso et al.

Preparation of master mix for amplification of 27F and 519R region of the 16S rRNA gene

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume 1 rxn</th>
<th>Final Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10x ImmoBuffer (^{(a)})</td>
<td>2.5</td>
<td>1 x</td>
</tr>
<tr>
<td>10 mM dNTP</td>
<td>0.5</td>
<td>200 nM</td>
</tr>
<tr>
<td>50 mM MgCl2</td>
<td>1.25</td>
<td>2.5 mM</td>
</tr>
<tr>
<td>ILM_27F_Uv3 (forward) (5 (\mu)M)</td>
<td>2.5</td>
<td>500 nM</td>
</tr>
<tr>
<td>ILM_519R_NNNN (5(\mu)M)</td>
<td>2.5</td>
<td>500 nM</td>
</tr>
<tr>
<td>Immolase DNA Polymerase (5U/(\mu)L) (^{(a)})</td>
<td>0.2</td>
<td>1 Unit</td>
</tr>
<tr>
<td>(\text{H}_2\text{O})</td>
<td>14.55</td>
<td>-</td>
</tr>
<tr>
<td>Template</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Total Volume</td>
<td>25</td>
<td>-</td>
</tr>
</tbody>
</table>
Thermocycler Conditions for amplification of 27F and 519R region of the 16S rRNA gene (96 well thermocyclers)

<table>
<thead>
<tr>
<th></th>
<th>Temperature</th>
<th>Time (mm:ss)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation</td>
<td>95°C</td>
<td>10:00</td>
</tr>
<tr>
<td>Amplification (35 cycles)</td>
<td>94°C</td>
<td>00:30</td>
</tr>
<tr>
<td></td>
<td>55°C</td>
<td>00:10</td>
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<tr>
<td></td>
<td>72°C</td>
<td>00:45</td>
</tr>
<tr>
<td>Final Extension</td>
<td>72°C</td>
<td>10:00</td>
</tr>
</tbody>
</table>

Process

1. Dilute DNA 1:10.
2. Amplify samples with conditions outlined above
3. Clean amplicon using AMpure XP according to the manufacturer protocol (Beckman Item # 5067-1504)
4. Run amplicons on an agarose gel. Expected band size for 27F/519R is approx. 530 bp.
5. Quantify amplicons with Picogreen (see manufacturers protocol; Invitrogen Item # P11496).
6. Run on Agilent Bioanalyzer chip, Screen Tape, or Lab Chip to get accurate sizing information.
7. Combine equimolar amounts of amplicons into a single, sterile tube.
8. Optional: If spurious bands were present on gel (in step 3), ½ of the final pool can be run on a gel and then gel extracted to select only the target bands.
9. Measure concentration using Qubit or picogreen of final pool that has been cleaned and check the 260/280 ratio. For best results the 260/280 should be between 1.8-2.0.

Sequencing of 27F and 519R region of the 16S rRNA gene

Sequencing Primers

Read 1 Primer
ACACTATGGCGAGTAGAGTTTGATCMTGGCTCAG
Sequencing Setup

1. Dilute pool prepared in step 2.3.7 to 4nM.

Dilute according to Illumina protocol. See Preparing Libraries for Sequencing on the MiSeq (part #15039740).

2. Prepare MiSeq Reagent Cartridge. See MiSeq Reagent Preparation Guide (part #15044983).

3. Add custom sequencing primers into reservoirs 12-14. See Using Custom Primers on the MiSeq (part #15041638).

4. Load 600 μl of library pool into the MiSeq reagent cartridge in designated reservoir.

5. Modify sample sheet to include custom primer’s sequence/indexes (see index sequences in appendix 2) – Optional. Set Workflow to “Metagenomics”


References

