COLONY PCR FOR BACTERIAL 16S RRNA GENE

Created/updated by: L. Rossi Date: May 31 2016
Surette Lab, McMaster University
Hamilton, ON, Canada
www.surettelab.ca

EQUIPMENT

- Thermocycler
- PCR tubes or 96 well plate
- PCR standard reagents
- Primers

PROTOCOL

1. Pick half to two fresh colonies, depending on the quality of growth, from agar surface with a sterile pipette tip and resuspend in 50 µl of sterile dH_20 or 5% autoclaved Chelex in a tube.

2. Boil suspensions for 15 minutes then centrifuge to remove debris at 13 000 rpm for 5 minutes at RT.

3. Use 5 µl of supernatant as temple DNA for a 50 µl PCR reaction

Primers
16S_8f **(MS7) –** AGAGTTTGATCCTGGCTCAG
16S_926r **(MS14) –** CCGTCAATTCCTTTRAGTTT
16S_926rb **–** CCGTCAATTYMTTTRAGT better for *Bifidobacterium* (Sim et al. 2012)

Reaction setup	**1X**
10X PCR Buffer (Invitrogen)	5 µl
$MgCl_2$ (50mM)	1.5 µl
dNTPs (10mM)	1 µl
Forward primer (10µM)	1 µl
Reverse primer (10µM)	1 µl
Template, genomic DNA	5 µl
Taq polymerase (Invitrogen)	0.25 µl
Water	35.25 µl
Total	50 µl

4. Prepare a master mix with everything except the template (mix well then spin down to avoid variability). Aliquot the mastermix into all wells then pipette the template to the bottom of each well. No mixing of the tubes/plate is necessary.

5. Following the amplification, run 5 μl on a 1% agarose gel.

6. Sequence in forward direction using 8f primer.

Thermocycler set up

1) 94^{0}C – 2min
2) 94^{0}C – 30sec
3) 56^{0}C – 30sec
4) 72^{0}C – 1min
5) Go to 2 for 29 cycles
6) 72^{0}C – 10min

REFERENCES

Sim, K. et al., 2012. Improved Detection of Bifidobacteria with Optimised 16S rRNA-Gene Based Pyrosequencing. *PLOS ONE*, 7(3), p.e32543.