IDSEQ – SHALLOW 16S ILLUMINA SEQUENCING FOR ISOLATED COLONIES OR SMALL COMMUNITIES

CREATED/UPDATED BY: L ROSSI

DATE: MAY 2022

Surette Lab, McMaster University
Hamilton, ON, Canada
www.surettelab.ca

NOTES

- The following protocol is for the preparation and sequencing of pure colonies or small communities using Illumina platform sequencing of the v3v4 amplicon.

EQUIPMENT

- thermocycler
- lysis buffer
- 96 well plate or PCR tubes
- Taq polymerase
- Illumina barcoded primers

PROTOCOL

Lysis Buffer	for 50mL
50mM KCL	2.5mL of 1M
10mM Tris-HCl(pH8.3)	0.5mL of 1M
2.5mM MgCl2	125uL of 1M
0.45% Igepal	225uL
0.45% Tween20	225uL

OPTIONAL - before use add Proteinase K to a final of 1mg/mL for as much as you will need that day

Preparation of PCR template from colonies or culture:

For template coming from saturated liquid culture:

Add 30uL of lysis buffer to a thermocycler compatible 96 well plate.

Transfer 5uL of culture to each well

For template coming from arrayed colonies on agar:

Add 50uL of lysis buffer

Pin transfer colonies to buffer.

Boil cells in lysis buffer in a thermocycler at 95C for 15 minutes.

If using Proteinase K, incubate at 55C for 1hour, then at 95C for 15 minutes.

PCR reaction:

	x1 reaction
Buffer	2.5uL
50mM MgCl2	0.75uL
10mM dNTP	0.5uL
1uM v4R primer	2uL
Taq	0.125uL
Template	2uL
dH2O	15uL
1uM V3F* barcoded primers	2uL

25uL final reaction volume, need a different v4R barcoded primer for each set of 96 isolates that are amplified.

PCR program v3v4:

94C – 2min
94C – 30s
47C – 30s
72C – 40s
x 5cycles
94C – 30s
50C – 30s
72C – 40s
x30 cycles
72C – 5min

Run only a subset of reactions on a gel to ensure reaction is good. (need a 600bp amplicon)

Then pool 5uL of each reaction into one tube using 100 to 300 isolates per one well of an Illumina run as prepared in the Surette lab (~0.01% of an Illumina run).