Whole genome colony PCR

21st April 2021

This protocol describes a method to extract and prepare genomic and plasmid DNA from a bacterial colony. This has been verified using gram-negative *E. coli*. A colony was picked from a plate of cultured bacteria and then treated with Proteinase K before preparing libraries for sequencing using the Rapid PCR Barcoding Kit (SQK-RPB004). The sequencing performance of the prepared libraries was assessed using GridION.

Materials

- Plated bacterial colonies
- Rapid PCR Barcoding Kit (SQK-RPB004)
- SFB Expansion (EXP-SFB001)
- Flow Cell Priming Kit (EXP-FLP002)
- 1.5 ml Eppendorf DNA LoBind tubes
- 0.2 ml thin-walled PCR tubes
- Nuclease-free water (e.g. ThermoFisher, cat # AM9937)
- Agencourt AMPure XP beads
- LongAmp Taq 2X Master Mix (e.g. NEB M0287)
- 10 mM Tris-HCl pH 8.0
- 10 mM Tris-HCl pH 8.0, 50mM NaCl (or EB from Sequencing Auxillary Vials (EXP-AUX001 or equivalent)
- (Optional) Thermolabile Proteinase K (e.g. NEB P8111S)
- Thermolabile Exonuclease I (e.g. NEB M0568S)
- Microfuge
- Thermal cycler
- Pipettes and tips for P1000, P200, P100, P20, P10, and P2
- Timer
- Ice bucket with ice
- Magnetic separator, suitable for either 1.5 ml Eppendorf tubes or 0.2 ml PCR tubes
- Hula mixer (or similar gentle rotator mixer)
- DNA QC equipment, e.g. Agilent Bioanalyzer, Qubit fluorometer
- Inoculating loop/needle or sterile toothpick

Method

Step 1

Using either a pipette tip, sterile toothpick, inoculating loop or needle, pick one colony and dip into 50 µl of 10 mM Tris-HCl pH 8.0. Swirl for 10 seconds until the solution becomes turbid.

Step 2 (enriching for plasmid DNA only)

If you are interested in the genomic DNA, proceed straight to step 4. If you are interested in the plasmid DNA, transfer the 50 µl cell suspension to a 0.2 ml thin-walled PCR tube and incubate at 95°C for 5 minutes.

Note: It is observed that pre-treating the colony by heating leads to an enrichment in the observation of plasmid reads in the downstream sequencing, compared with non-heat treated libraries.

Step 3 (enriching for plasmid DNA only)

Add 1 µl of Thermolabile Proteinase K and incubate at 37°C for 15 minutes, then 55°C for 10 minutes.

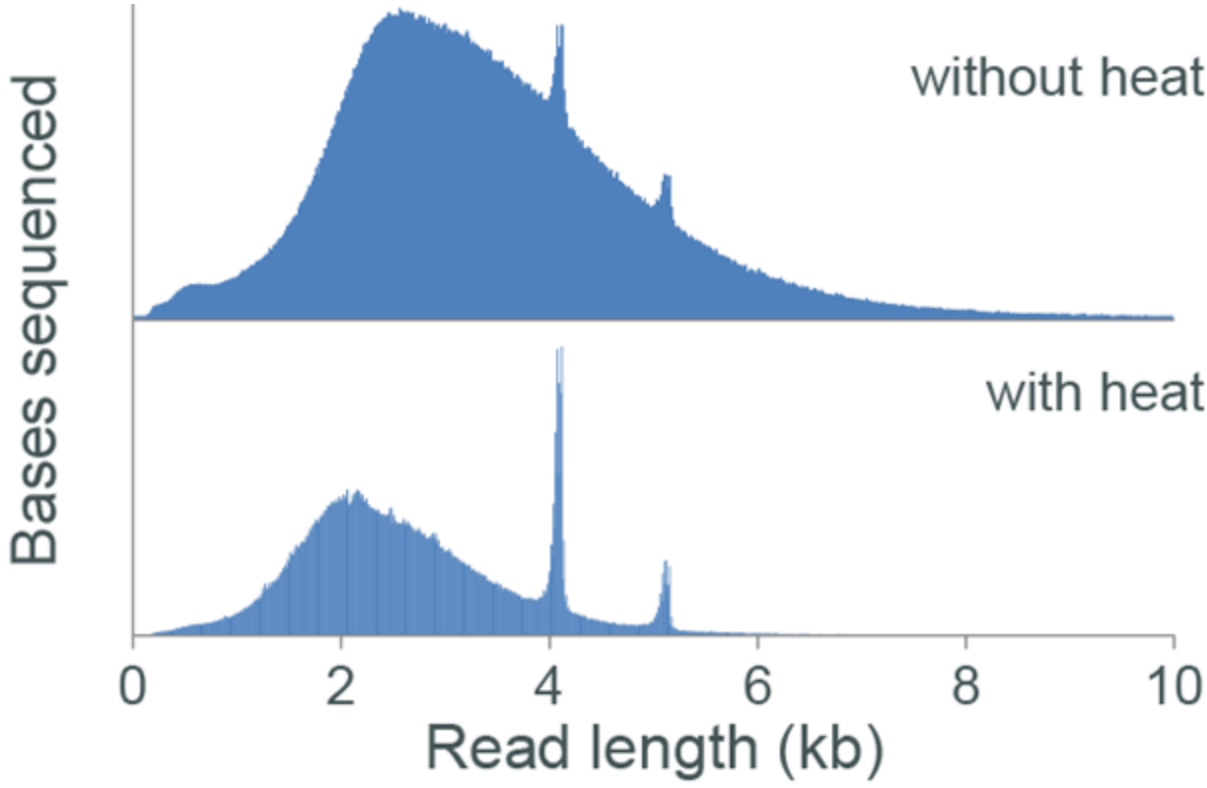
Step 4

Libraries can be prepared for sequencing using the Rapid PCR Barcoding Kit (SQK-RPB004), using 3 µl of the treated cell suspension as the template. If using cells without the heat treatment in step two, it is recommended to PCR for 25 cycles, and if using heat-treated cells, it is recommended to PCR for 30 cycles.

Sequencing performance

Libraries were prepared using the Rapid PCR Barcoding Kit (SQK-RPB004):

- Post PCR yield: 30-60 ng/µl
- Typical output: ★★★. Output from the flow cell may be increased by performing a flow cell wash step at the point where the rate of data acquisition begins to deteriorate due to the accumulation of pores in the “unavailable” or “recovering” state, and then adding a new library.
- Read length profile:



Date	Change note
21st April 2021	Title changed from ‘PCR amplification of

	gram-negative bacterial DNA direct from a colony' to 'Whole genome colony PCR'. Updated step 3 sub-title from '(plasmid DNA only)' to '(enriching for plasmid DNA only)'.