

Plasmid Sequencing without mini-prep

direct from the colony with TempliPhi whole genome amplification

advantage- no mini-prepping, high throughput, minimal hands-on, good quality

disadvantage – takes a bit longer (if you need it fast) and costs a bit of money (if your current method is free of charge)

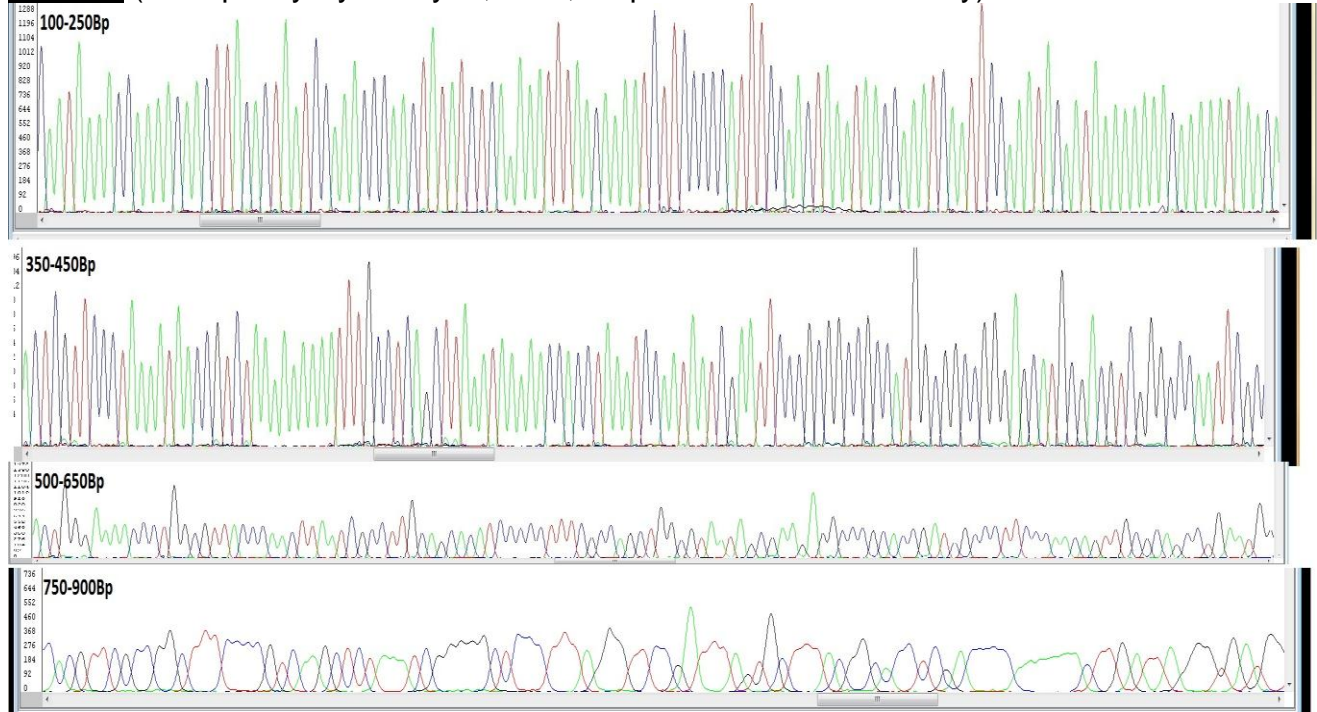
Principle:

This method boils the cells for 3min and amplifies the whole genome overnight with a special kit called TempliPhi (\$1.15/sample) this output is directly used for sequencing

Method:

- With a pipette tip touch the colony (don't transfer too many cells as too much DNA will inhibit the reaction)
- Transfer tip into 20ul of water and mix
- take 0.5ul of the 20ul cell suspension
- add 0.75ul of TempliPhi Sample buffer
- Incubate at 95C for 3min
- make a Mastermix on ice of TempliPhi Reaction Buffer and Enzyme (1.25ul Reaction buffer & 0.05ul enzyme)
- add 1.25ul Mastermix to sample
- incubate at 30C for at least 4h (ideally overnight)
- Heat inactivate 10min at 65C
- add 1ul to 3.2pmol primer and submit to sequence service

Results (example by Kylie Taylor, CEP, sequenced @ ACRF facility)



TempliPhi Amplification Kit (GE Healthcare, catalogue #25-6400-10).
Cat Number: GEHE25-6400-10 1 x 100 Tests for AUD \$464.00