Protocol for colony PCR

We use TaKaRa EX Taq premix to amplify target fragment.

Isolate a single colony from a freshly streaked selective plate, and inoculate a culture of 1- 5 mL LB medium containing the appropriate selective antibiotic. Incubate for 2 hours at 37°C with vigorous shaking (~ 220 rpm).

EX Taq premix: 5ul

Forward primer (10 uM): 0.4ul

Reverse primer (10 uM):0.4ul

dd H_2O: 3.8ul

DNA template (Culture medium containing the colony): 0.4 ul

The reaction condition settings are as follows:

95°C 2min (98 °C 10s 55 °C 30s 72 °C 1kb/min) 72 °C 10min

Cycles: 30

Protocol for PCR with PrimeStar HS DNA polymerase

PrimeSTAR® Premix: 25ul

Forward primer (10 uM): 0.8ul

Reverse primer (10 uM):0.8ul

DNA template: 0.4 ul

dd H2O: 8ul

The reaction condition settings are as follows:

98 °C 3min (98 °C 10s 55 °C 5s 72 °C 1kb/min) 72 °C 10min

Cycles: 30