Colony PCR-Phusion

-33 µl dH20
-10 µl homemade 5X buffer Phusion buffer
-5 µl dNTPs (2.5 mM each)
-1µl 5' primer
-1µl 3' primer
-1 μl Phusion polymerase

This recipe is enough for two colony PCR reactions.

Works well with this PCR program

Colony program

a) 94ºC denaturation: 5 minutes

b) 94 ºC denaturation: 30 seconds

c) 55 ºC annealing: 30 seconds

d) 72 ºC extension: 15 sec/kb

repeat b-d 30-36 times

e) 72 ºC final extension: 3 minutes

Pick a decent sized colony with a P200 pipette tip. Touch to an agar plate with appropriate antibiotic, put tip into a PCR tube or well of a 96-well PCR plate containing 24.5 μl of PCR mix and leave. Repeat with desired number of colonies. For positive control, add 0.5 μl of an appropriate template, for negative control do not add anything.

Shake tips in PCR mix vigorously, remove tips and run on colony PCR program.