Colony PCR recipe

-37 µl dH20
-5 µl 10X Pfu buffer (see Pfu purification protocol)
-5 µl dNTPs (2.5 mM each)
-1µl 5' primer
-1µl 3' primer
-0.5 µl Pfu DNA polymerase
-0.5 μl Taq DNA polymerase

Works well with this PCR program

a) 94ºC denaturation: 5 minutes

b) 94 ºC denaturation: 30 seconds

c) 55 ºC annealing: 30 seconds

d) 72 ºC extension: 1 min/kb

repeat b-d 35 times

e) 72 ºC final extension: 3 minutes

Pick a decent sized colony with a P200 pipette tip. Touch to an agar plate, 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.