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Colony PCR (E. coli)

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Protocol status: Working
We use this protocol and it's working

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Abstract

Fast and easy PCR to check for cloning inserts. This protocol is not suitable for downstream application of the amplified PCR products.

1 **Set up 1x PCR buffer mix**
5X Green or Colorless GoTaq® Flexi Buffer: 10µl (1x)
PCR Nucleotide Mix, 10mM each: 1µl (0.2mM each dNTP)
upstream primer: 2µl (1.0µM)
downstream primer: 2µl (1.0µM)
GoTaq® G2 Flexi DNA Polymerase (5u/µl): 0.25µl (1.25u)
MgCl2: 2ul (1 mM)
Nuclease-Free Water to: 50µl

Note: Can reduce the final volume to 25µl to save reagents.

2 Using a sterile toothpick or 200µl pipette tips, gently touch a single bacterial colony and dip it into the PCR buffer mix.

3 **PCR reaction**
Initial denaturation: 95°C, 2 mins
*Denaturation: 95°C, 1 min
*Annealing: 55°C, 30 sec
*Extension: 73°C, 1 min for every 1kb of amplification
Final extension: 73°C, 5 mins
Storage: 12°C or room temperature depending on when the products will be analysed
*20 to 30 cycles (Generally 20 cycles is more than enough to amplify)

Note: Increasing the annealing temperature will increase the specificity of the PCR reaction.

Important: Run gel electrophoresis immediately as PCR products will normally be degraded due to the presence of DNAse from the lysed E. coli. PCR products not recommended for long term storage.