Colony PCR protocol

Aim: To identify positive colonies from cloning experiments.

I always find that colony PCR protocols can be very hit and miss, some people will say it depends on how many bacteria cells you are loading in to your PCR reaction. I think this protocol is quite simple and has two main advantages. (1) Normally you "save" a little of the colony by streaking on a separate agar plate to be able to grow up for plasmid extraction later on, this allows you to skip that step. (2) It somewhat standardises how much bacteria you are loading into your reactions, providing your transformed bacteria grow to a similar density each time.

Method:

- After transformation of your cloning reaction pick colonies as normal and grow in 5 mL of LB with antibiotic and incubate at 37 ºC overnight with shaking
- The next day (16 hours of growth) take 1 µL of each 5 mL culture and dilute in 100 µL of PCR grade dH_2O (These diluted samples can be frozen until PCR or used straight away)
- Then with the remaining culture, centrifuge at 13,000rpm, label and freeze the cell pellets in microcentrifuge tubes for later
- Next add 10 µL of the 1:100 dilution into the below 25 µL PCR reaction with taq DNA polymerase

E.coli culture 1:100	10 µL	☐
10x Taq polymerase buffer	2.5 µL	☐
50 mM $MgCl_2$	0.75 µL	☐
10 mM dNTPs	0.5 µL	☐
10 µM Forward primer	1.25 µL	☐
10 µM Reverse primer	1.25 µL	☐
Taq polymerase	0.2 µL	☐
dH_2O	8.55 µL	☐
TOTAL	25 µL	

- Run your PCR as manufacturer's instructions, I have a 5 minute 94 ºC step at the beginning to ensure the cells are broken up to liberate the plasmid – I usually anneal at 50 – 55 ºC
- Then add 5 µL of 6 x DNA loading dye and load 15 µL of the reaction for agarose gel electrophoresis
- Once you have identified your positive colonies perform mini preps on the cell pellets from the 5 mL culture that was previously frozen
- Then perform Sanger sequencing to confirm the insert is of the correct sequence.

Notes:

- This protocol is best for cloning reactions that are somewhat efficient, I tend to pick 5 colonies to start with and perform colony PCR on all of them often at least 4/5 will have a PCR product of the correct size. Then I just sequence one colony, depending on the urgency needed of the particular vector.
- For forward primers I tend to use a primer specific to the plasmid promoter (CMV EF1a etc.) and then the reverse primer either the same one used for the cloning or another one within the actual insert of interest.
- Always include a positive and negative control for your PCR