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# E. coli and B. subtilis Colony PCR

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

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**Keywords:** pcr

1 Prepare a PCR master mix for 9 reactions, as follows:

| | | 1x Reaction (Volume; µL) | 9x Reactions (Volume; µL) |
|---|---|---|---|
| | Sterile Milli Q Water | 8 | 72 |
| | Red Taq 2x Master Mix(1.5 mM MgCl2) | 10 | 90 |
| | F Primer (10 µM) | 1 | 9 |
| | R Primer (10 µM) | 1 | 9 |
| | Total Volume | 20 | 180 |

2 Aliquot 20 µL of the master mix in 8 tubes of a PCR strip.

3 Transfer cells from a single colony (8 in total) using a sterile P2 pipette tip or inoculating loop

4 Run thermocycler using the following program (extension for 1.5 kb insert):

| | Step | Temp (°C) | Time (mm:ss) | Purpose |
|---|---|---|---|---|
| | 1 | 95 | 3:00 | Initial Denaturation |
| | 25 cycles of Steps 2 to 4: | | | |
| | 2 | 95 | 0:20 | Denaturation |
| | 3 | 57 | 0:30 | Annealing |
| | 4 | 72 | 1:00 | Extension |
| | 5 | 72 | 5:00 | Final Extension |
| | 6 | 4 | ∞ | Storage |

Note

*Extension time may vary depending on insert size*

5 Run samples on a gel at 80-150 V until the dye line is approximately 75-80% of the way down the gel. A typical run time is about 1-1.5 hours, depending on the gel concentration

and voltage.