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

		1x Reac tion (Vol ume; μL)	9x Reac tions (Vol ume; μL)
	Steri le Milli Q Wat er	8	72
	Red Taq 2x Mast er Mix(1.5 mM MgC l ₂)	10	90
	F Prim er (10 μM)	1	9
	R Prim er (10 μM)	1	9
	Total Volu me	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.