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E. coli and B. subtilis Colony PCR

iGEM Dusseldorf[1]

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1 Works for me dx.doi.org/10.17504/protocols.io.8fhhtj6

iGEM Dusseldorf

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

	1x Reaction (Volume; µL)	9x Reactions (Volume; µL)
Sterile MilliQ 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

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.