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E. coli Colony PCR

iGEM Dusseldorf[1]

[1]Heinrich-Heine Universität Düsseldorf

1 Works for me dx.doi.org/10.17504/protocols.io.76ihrce

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ABSTRACT

Ideally use at least two master mixes:
2 vector-specific primers (to determine insert size):
1 vector-specific + 1 gene-specific primer
To safe resources one of them sould be enough. Is not as neat as it should be but functional. Use Vector + gene specific, because than you can be sure, that the gene is inserted in the right direction

For genotyping of putative colonies

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

- Aliquot 20 µL of the master mix in the 8 tubes of a PCR strip.
- Transfer cells from a single colony (8 in total) using a sterile P2 pipette tip.
- 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

- Analyze bands on a 1% Agarose gel:

(no amplification if Gene is missing or in wrong direction when using gene specific primer)
(Smaller than expected band if no insert is present when using two vector specific primer)

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