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E. coli Colony PCR

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

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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 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

- 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)