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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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Keywords: pcr master mix, gene specific primer, using gene, pcr strip, pcr, right direction for genotyping, gene specific, sterile p2 pipette tip, specific primer, amplification if gene, transfer cell



Abstract

Ideally use at least two master mixes:

2 vector-specific primers (to determine insert size):

1 vector-specific + 1 gene-specific primer

To save resources one of them could be enough. Is not as neat as it should be but functional. Use Vector + gene specific, because then 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 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

- 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)

