WCSB, Wageningen University	IBISBA-SOP-WU13
	Version 1.0

EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: Colony PCR of *S. cerevisiae*, *P. putida*, *E. coli*

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Instruction

Colony PCR of *S. cerevisiae*, *P. putida*, *E. coli*

1. Introduction / Purpose

In this SOP, colony PCR of *S. cerevisiae*, *P. putida*, and *E. coli* is described. There are alternative methods available for both bacterial strains that are not described here.

Keywords: Colony – PCR – *S. cerevisiae* – *P. putida* – *E. coli*

2. Equipment and chemicals

2.1. Equipment

- PCR machine

2.2. Chemicals

- Phire Plant Direct PCR Kit (Thermo Fisher Scientific)
- Phire Green Hot Start II DNA Polymerase (Thermo Fisher Scientific)
- MQ water

2.3 Other materials

- Primers (forward and reverse)
- The colonies have to be analysed by PCR

3. Procedures

The template DNA in a colony PCR reaction is prepared as follows:

- Put 30 µl of Dilution Buffer (from Phire Plant Direct PCR Kit) in a 1.5 ml eppendorf tube
- Transfer a colony from an agar plate to the tube, resuspend
- Incubate 2 minutes @ 70°C in a heat block
- Use 1 µl of this mixture as template

Prepare PCR reaction mixture with Phire Green Hot Start II DNA Polymerase kit:

- MQ water: enough to make a total volume of 20µl
- 4 µl 5x Phire Green Reaction Buffer
- 0.4 µl 10 mM dNTP’s

- 1 µl Primer forward(stock of 10 pmol/µl
- 1 µl Primer reverse (stock of 10 pmol/µl)
- 0.4 µl Phire Hot Start II DNA Polymerase
- 1 µl DNA template

Volume can be scaled up (to 50 µl) or down (to 15 µl), change all volumes accordingly.

Cycling protocol:

Cycling Step	**Temperature**	**Time**	**Cycles**
Initial denaturation	98°C	30 s	1
Denaturation Annealing Extension	98°C	5 s	25-35
	X°C	5 s	
	72°C	10-15 s/kb	
Final extension	72°C	1 min	1
Hold	16°C	hold	1

Annealing temperature depends on primers.

5. Remarks / troubleshooting

Because of the use of 5x Phire Green Reaction Buffer, addition of loading dye is not necessary before running an agarose gel.

Troubleshooting: see manuals of kits on website of Thermo Fisher Scientific.

6. Biosafety

No biosafety issues are associated with this protocol.

7. Acknowledgements


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