

05_05 Colony PCR

MITTWOCH, 2.6.2021

Goal setting

- Extract and amplify the fragment from the picked colonies as preparation for Sanger sequencing

Terms / abbreviations

- cPCR = Colony polymerase chain reaction
- PCR = Polymerase chain reaction

Risk areas

- None

Required materials/ information

- Chemicals:
 - 2x Taq PCR Mastermix, Qiagen
 - Forward Primer, Ella Biotech (M13 fwd 10 μ M)
 - Nuclease free water, ThermoFisher
 - Reverse Primer, Ella Biotech (M13 rev 10 μ M)
- Materials:
 - Ice
 - PCR tubes, Sarstedt
 - Pipettes, Eppendorf

Templates, devices, software

- NanoDrop spectrophotometer, ThermoScientific NanoDrop 2000
- Thermocycler, Eppendorf

Preliminary work

- [05_04 pGEM T Easy Vector System](#)

Operation

Detecting the right template DNA amount:

- General guidelines for low complexity DNA (e.g. plasmid, lambda or BAC DNA) are: 1 pg–10 ng per 50 μ L reaction volume
- If cDNA synthesis reaction mixture is used as a source of template, the volume of the template should not exceed 10% of the PCR reaction volume.
- To detect the desired amount, measure concentration in ng/ μ L according to [04_01 Spectralphotometer NanoDrop](#)
- Then calculate how much is needed

Prepare a Mastermix for the cPCR for needed amount of reactions:

- Always prepare at least for 1-5 reactions more than needed!

Composition of Mastermix



	A	B	C
1	Component	Volume for x reactions [μ L]	x
2	2x Taq PCR Mastermix	500	50
3	M13 fwd 10 μ M	100	
4	M13 rev 10 μ M	100	
5	Nuclease free water	300	
6	Total	1000	

- Fill 20 μ L of the Mastermix into the PCR tubes (on ice!)
- Pick the colonies from the plates, streak them on a numbered master plate, and put the pipetting tips into the PCR tubes (on ice!)
- Shake the PCR tubes carefully and remove the pipette tips

Run the following cPCR program:

cPCR Program



	A	B	C
1	$^{\circ}$ C	Time	Cycles
2	95	2'	
3	95	30"	30x
4	55	30"	
5	72	45"	
6	72	5'	

Troubleshooting

- None

Follow-up work

- Purify fragments with a cleanup-kit