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 µM)
  - Nuclease free water, ThermoFisher
  - Reverse Primer, Ella Biotech (M13 rev 10 µM)
- Materials:
  - Ice
  - PCR tubes, Sarstedt
  - Pipettes, Eppendorf

Templates, devices, software

- NanoDrop spectraphotometer, 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 µL 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/µL 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 [µL]**	**x**
2	2x Taq PCR Mastermix	500	50
3	M13 fwd 10 µM	100	
4	M13 rev 10 µM	100	
5	Nuclease free water	300	
6	Total	1000	

- Fill 20 µL 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	**°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