03_04 PCR for ssDNA Samples

MITTWOCH, 2.6.2021

Goal setting

- Performing a PCR reaction using the Phusion™ High–Fidelity DNA Polymerase

Terms / abbreviationsTerms / abbreviations

- DMSO = Dimethyl sulfoxide
- PCR = Polymerase chain reaction

Risk areas

- If spilled, always wipe surface with alcohol

Hazard symbols

Required materials/ information

- Chemicals:
  - 5x Phusion HF buffer, ThermoFisher
  - 5x Phusion GC buffer, ThermoFisher
  - 10 mM dNTP, ThermoFisher
  - 10 µM Forward primer, Ella Biotech
  - 10 µM Reverse primer, Ella Biotech
  - 50 mM $MgCl_2$ solution, ThermoFisher
  - Autoclaved MilliQ water, Sartorius arium pro VF
  - DMSO, ThermoFisher
  - Phusion DNA Polymerase (2 U/µL), ThermoFisher
  - Template DNA
- Material:
  - Ice or cooling rack
  - PCR tubes, Sarstedt
  - Trash bags, Th. Geyer GmbH & Co. KG

Templates, devices, software

- Nanodrop spectrophotometer, ThermoFisher
- Thermocycler, Eppendorf
- Pipettes, Eppendorf

Preliminary work

- Any reaction creating DNA that should be amplified
- 04_01 Spectralphotometer NanoDrop to detected desired amount of template DNA

Operation

1. Put the PCR tube on ice or the cooling rack
2. Add the components in the order listed in the following table

Composition of Master Mix

	A	B	C	D	E
1	**Component**	**20 [µL]**	**50 [µL]**	**Final concentration**	**Unit**
2	MilliQ water	add to 20 µL	add to 50 µL		
3	5X Phusion HF Buffer	4	10	1	x
4	10 mM dNTPs	0.4	1	200	µM
5	Forward primer 10 µM	1	2.5	0.5	µM
6	Reverse primer 10 µM	1	2.5	0.5	µM
7	Template DNA *	x	x	0.2	
8	Phusion High-Fidelity DNA Polymerase	0.2	0.5	0.02	U/µl

* use 1 µL from standard TdT reaction as template

3. Run the PCR program in the thermocycler according to the following table

PCR Program for Thermocycler

	A	B	C	D
1	**Temperature [°C]**	**Time**	**Unit**	**Repeats**
2	98	30	s	1x
3	98	5	s	30x
4	63	10	s	
5	72	30	s	
6	72	5	min	1x

Increase elongation time for fragments > 1 kb

Disposal

- Autoclave trash bags, discard in S1 waste

Troubleshooting

- Wear gloves to reduce the risk of DNase and RNase contamination
- The Phusion DNA Polymerase should be pipetted carefully and gently as the high glycerol content (50%) in the storage buffer may otherwise lead to pipetting errors

Sources

MAN0012393_Phusion_HighFidelity_DNAPolymerase_UG.pdf

Follow-up work

- Purify fragments with a cleanup-kit
- If materials are empty, care about new order