03_01_NEB-protocol-for-TdT-tailing-reaction

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Goal-Setting

- Tailing a primer with particular dNTPs using TdT

Terms / abbreviations

- dNTP = Deoxynucleoside triphosphate
- TdT = Terminal deoxynucleotidyl transferase

Risk areas

- If spilled, always wipe surface with alcohol

Hazard symbols

Required materials and / or information

- Chemicals:
  - 2.5 mM $CoCl_2$ solution, NEB
  - 5.0 pmol DNA Primer
    - 5 pmol in 50 µL is equivalent to 0.1 pmol/µL = 100 nM
  - 10x TdT Reaction Buffer, NEB
    - 50 mM Potassium acetate, 20 mM Tris-acetate, 10 mM Magnesium acetate, pH 7.9 at 25 °C
  - 10 mM dNTPs, ThermoFisher
  - Nuclease free water, ThermoFisher
  - TdT (20 U/µL), NEB
    - 50 mM KPO_4, 100 mM NaCl, 1.43 mM 2-Mercaptoethanol, 0.1 % (v/v) Triton X-100, 50 % (v/v) Glycerol, pH 7.3 at 25 °C
- Material:
  - Gloves
  - PCR tube rack
  - PCR tubes, Sarstedt (autoclaved)
  - Pipettes, Eppendorf
  - Trash bags, Th. Geyer GmbH & Co. KG

Templates, devices, software

- None

Preliminary work

- Thawing frozen ingredients

Operation

1. Prepare the following reaction mixture
2. Add dNTPs and primer last
    a. This will start the incubation immediatly
    b. Stop the time
3. Incubate at X °C for X min (varying depending on experiment)

NEB TdT reaction mixture

	A	B	C	D
1	**Component**	**Stock concentration**	**Desired concentration**	**Volume [mL]**
2	H2O	-	-	38.5
3	TdT Buffer	10x	1x	5
4	CoCl2	2.5 mM	0.25 mM	5
5	TdT	20 U/µL	0.2 U/µL	0.5
6	dNTP	10 mM	100 µM	0.5
7	Primer	10 µM	100 nM	0.5
8	total			50

Disposal

- Autoclave trash bags, discard in S1 waste

Troubleshooting

- Wear gloves to reduce the risk of DNAse and RNAse contamination
- Always keep TdT cooled until usage

Follow-up work

- 03_02_Heat-inactivation-of-TdT-reaction
- 03_03_EDTA-inactivation-of-TdT-reaction
- If materials are empty care about new order