03_01 Thermofisher 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:
  - 5.0 pmol DNA Primer, Ella Biotech
    - 5 pmol in 50 µL is equivalent to 0.1 pmol/µL = 100 nM
  - 10 mM dNTPs, ThermoFisher
  - 5x TdT Buffer, ThermoFisher
    - 1 M Potassium cacodylate, 0.125 M Tris, 0.05% (v/v) Triton X-100, 5 mM $CoCl_2$, pH 7.2 at 25°C
  - Nuclease free water, ThermoFisher
  - TdT (20 U/µL), ThermoFisher
    - 100 mM Potassium acetate, 2 mM 2-Mercaptoethanol, 0.01 % (v/v) Triton X-100, 50 % (v/v) Glycerol, pH 6.8
- 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)

ThermoFisher TdT reaction mixture

	A	B	C	D	E	F
1	**Component**	**Stock concentration**	**Unit**	**Volume needed [µL]**	**Desired concentration**	**Unit**
2	H2O	-	-	**38**	-	-
3	TdT Buffer	5	x	**10**	1	x
4	TdT	20	U/µL	**0.5**	0.2	U/µL
5	dNTP	10	mM	**0.5**	0.1	mM
6	Primer	1000	nM	**1**	20	nM
7	Total			**50**		
8	**Desired Total**			**50**		

- Amount of H_2O is calculated automatically when e.g. the primer volume changes
- Formula used to calculate the amount of Primer or dNTP needed: $c_0V_0 = cV \rightarrow V_0 = (cV)/c_0$
- Calculation only valid if stock and desired concentration have the same unit!

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_03 EDTA Inactivation of TdT Reaction
- 03_03 EDTA Inactivation of TdT Reaction
- If materials are empty care about new order