

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



Required materials and / or information

- Chemicals:
 - 5.0 pmol DNA Primer, Ella Biotech
 - 5 pmol in 50 μ L is equivalent to 0.1 pmol/ μ L = 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/ μ L), 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 immediately
 - 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	H ₂ O	-	-	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 H₂O 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