

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 immediately
 - 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	H ₂ O	-	-	38.5
3	TdT Buffer	10x	1x	5
4	CoCl ₂	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