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Version 1

ssDNA2.0: Dephosphorylation mix V.1

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Sarah Nagel¹, Anna Schmidt¹, Matthias Meyer¹

¹Max Planck Institute for Evolutionary Anthropology

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Abstract

Protocol for the preparation of Dephosphorylation mix for automated single-stranded DNA library preparation using the ssDNA2.0 method (Gansauge et al. 2020).

References

Gansauge, M.-T., Aximu-Petri, A., Nagel, S., & Meyer, M. (2020). Manual and automated preparation of single-stranded DNA libraries for the sequencing of DNA from ancient biological remains and other sources of highly degraded DNA. *Nature Protocols*, 15, 2279-2300.

Note

The volume of Dephosphorylation mix suffices for one 96-well library preparation plate (96 + 20 reactions to account for dead volumes and loss of reagent). It is advisable to prepare 10-20 mixes at once.

Materials

Reagent/consumable	Supplier	Catalogue number	Decontamination *
Reagents			
Water, HPLC-grade	Sigma Aldrich/Merck	1153332500	UV
10x T4 RNA Lig buffer	New England Biolabs	B0216B-OTCS	-
Tween-20 †	Thermo Fisher Scientific	11417160	UV
Consumables			
5 ml screw cap tubes (rack 2d Lp W/barcode)	VWR	NUNC374320-BR	-

* Decontamination of reagents should be performed as detailed in the document in the Appendix.

† Use to prepare a 2% (vol/vol) solution in water. NOTE: Tween-20 is highly viscous, pipette slowly and with care.

Equipment

- Label printer (e.g. Brady M611, cat. no. M611-EU-LABS) and tube labels (e.g. Labels for TLS2200/TLS PC Link/Polyester, cat. no. PTL-82-499)

Protocol

1. Prepare Dephosphorylation mix in a 5 ml screw-cap tube by combining the following reagents. Mix thoroughly by vortexing. Spin tube briefly in a microcentrifuge.

Reagent	Volume (µl)	Final concentration in reaction
Water	588.8	
T4 RNA Lig buffer (10x)	1024	1x

	Reagent	Volume (μl)	Final concentration in reaction
	2% (v/v) Tween-20	256	0.05%
	<i>sum</i>	<i>1868.8</i>	

Note

[Labeling]

Prepare tube labels using Brady printer including name of the mix, date (dd.mm.yyyy) and the name of the person who prepared the Dephosphorylation Mix.

2. Freeze at -20 °C until used.

Note

[Documentation]

Note the lot/batch numbers of the reagents used for master mix preparation in Labfolder (orange fields).

Appendix

Document

NAME

UV decontamination of reagents/buffers

CREATED BY

Elena Essel

Preview