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ssDNA2.0: Ligation mix I V.1

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MPI EVA Ancient DNA C...

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Abstract

Protocol for the preparation of Ligation mix I for the first adapter ligation in 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.

Troubleshooting

Note

The volume of Ligation mix I 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
	50% PEG-8000 (w/v)	Jena Bioscience	CSS-256	UV
	100 mM ATP	Thermo Fisher Scientific	R0441	-
	Consumables			
	5 ml screw cap tubes (rack 2d Lp W/barcode)	VWR	NUNC374320-BR	-

* Decontamination of reagents and consumables should be performed as detailed in the documents below.

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 a 5 ml tube with Ligation mix I by adding the following reagents. Pre-mix reagents by slowly inverting the tube at least two times and spin down (full mixing is performed later, after enzyme addition).

	Reagent	Volume (µl)	Final concentration in reaction
	Water	116	
	50% PEG-8000 (w/v)	3712	20%

	Reagent	Volume (µl)	Final concentration in reaction
	100 mM ATP	46.4	0.5 mM
	sum	*3874.4*	

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 Ligation mix I.

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

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Preview