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ssDNA2.0: Fill-in mix

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ABSTRACT

Protocol for the preparation of Fill-in 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.

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Note

The volume of Fill-in 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
	Klenow reaction buffer (10x)	Thermo Fisher Scientific	EP0052	-
	25 mM each dNTP	Thermo Fisher Scientific	R1121	-
	Tween-20 †	Thermo Fisher Scientific	11417160	UV
	Extension primer CL128 §	Eurogentec	-	-
	Consumables			
	5 ml screw cap tubes (rack 2d Lp W/barcode)	Thermo Fisher Scientific	NUNC374320-BR	-

* Decontamination of reagents and consumables should be performed as detailed in the Appendix.
† Use to prepare a 2% (vol/vol) solution in water. NOTE: Tween-20 is highly viscous, pipette slowly and with care.
§ Order oligonucleotide CL128 at 10.0 µmol synthesis scale (Eurogentec, HPLC purified). Dissolve in TE buffer (See document in the Appendix for preparation of this buffer) at a concentration of 25 µM. Sequence: 5'-GTGACTGGAGTTCAGACGTGTGCTCTTCC*G*A*T*C*T-3' (* denotes phosphothioate (PTO) linkages).

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 the Fill-in 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	3868.6	
	Klenow reaction buffer (10x)	464	1x
	25 mM each dNTP	46.4	200 µM each
	25 µM CL128 oligonucleotide	116	25 pmol/rct
	2% Tween-20 (v/v)	145	0.05%
	sum	4640	

Note

[Labeling]

Prepare tube labels using Brady printer including name of the mix, date (dd.mm.yyyy), the name of the person who prepared the Fill-in mix and the batch number of the oligo used.

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).

Attention: Batches of oligonucleotides are labelled with Roman numerals (e.g. CL128 - VIII).

Appendix

Document

NAME

TE buffer

CREATED BY

Anna Schmidt

PREVIEW

Document

NAME

UV decontamination of reagents/buffers

CREATED BY

Elena Essel

PREVIEW