MAR 25, 2024

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.e6nvwdnwwlmk/v1

Document Citation: Matthias Meyer, Anna Schmidt, Sarah Nagel 2024. ssDNA2.0: Adapter/splinter mix. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwdnwwlmk/v1

Created: Jan 10, 2024

Last Modified: Mar 25, 2024

ssDNA2.0: Adapter/splinter mix

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ABSTRACT

Protocol for the preparation of decontaminated (i.e., nuclease-treated) Adapter/splinter mix 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.

DOCUMENT integer ID: 93286

Funders Acknowledgement:
Max Planck Society
Grant ID: -

Note

This protocol describes the preparation of five (or multiples of five) tubes containing Adapter/splinter mix. Each tube 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 2-4 batches (10-20 mixes) at once.

Materials

	Reagent/consumable	Supplier	Catalogue number	Decontamination *
	Reagents			
	Water, HPLC-grade	Merck	270733	UV
	T4 RNA ligase reaction buffer (10x)	New England Biolabs	B0216B-OTCS	-
	Klenow fragment (10 U/µl)	Thermo Fisher Scientific	EP0052	-
	T4 polynucleotide kinase (PNK) (10 U/µl)	Thermo Fisher Scientific	EK0031	-
	Tween-20 ¶	Thermo Fisher Scientific	11417160	UV
	Adapter oligonucleotide TL181 †	IDT	-	-
	Splinter oligonucleotide TL159 ‡	Eurogentec	-	-
	Consumables			
	0.2-ml PCR eight-tube strips	neoLab Migge	VB-0357	UV
	1.5-ml Safe-lock LoBind tubes	neoLab Migge	VB-0285	UV
	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 TL181 at 1µmol synthesis scale (Integrated DNA Technologies, desalted). Dissolve in TE buffer

(See document in the Appendix for preparation of this buffer) at a concentration of 100 µM. Sequence: 5'-phosphate-AGATCGGAAGAAA[A][A][A][A][A][A][A]-TEG-biotin-3' ([A] denotes 2'-O-Methyl nucleotides)
‡ Order oligonucleotide TL159 at 1µmol synthesis scale (Eurogentec, desalted). Dissolve in TE buffer (See document in the Appendix for preparation of this buffer) at a concentration of 100 µM. Sequence: 5'-[A][A][A]CTTCCGATCTNNNNNNNN[A]-AmC6-3' ([A] denotes 2'-O-Methyl nucleotides)

Equipment

- Thermal cycler for PCR strip tubes (e.g. Veriti 96-Well fast Thermal Cycler, cat. no. 4375305)
- 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)
- centrifuge for 5 ml tubes (e.g. MyFuge 5 Microcentrifuge, cat. no. 55C1005-E)

Protocol

1. Prepare the following reaction mix for decontamination of adapter oligonucleotide TL181 in a 1.5 ml Eppendorf Safe-lock tube, mix properly by flipping the tube with a finger and briefly spin down.

	Reagent	Volume (µl)	Final concentration in reaction
	Water	210	
	100 µM TL181 splinter oligo	70	20 µM
	T4 RNA Lig buffer (10x)	35	1x
	Klenow fragment (10U/µl)	17.5	0.5 U/µl
	PNK (10U/µl)	17.5	0.5 U/µl
	sum	350	

Note

[Note]
The specified volumes in this table and the table below suffice for five tubes of Adapter/splinter mix. It is advisable to prepare 2-4 batches at once.

2. Prepare the following reaction mix for decontamination of splinter oligonucleotide TL159 in a 1.5 ml Eppendorf Safe-lock tube, mix properly by flipping the tube with a finger and briefly spin down.

	Reagent	Volume (µl)	Final concentration in reaction
	Water	140	
	100 µM TL159 splinter oligo	140	40 µM
	T4 RNA Lig buffer (10x)	35	1x
	Klenow fragment (10U/µl)	17.5	0.5 U/µl
	PNK (10U/µl)	17.5	0.5 U/µl
	sum	350	

3. Distribute reaction mix from step 1 to seven wells of a PCR 8-strip tube, adding 50 µl to each well.

4. Distribute reaction mix from step 2 to seven wells of another PCR 8-strip tube, adding 50 µl to each well.

5. In a thermal cycler, incubate both PCR 8-strip tubes for 20 min at 37°C, followed by 1 min at 95°C to inactivate the enzymes.

6. Transfer the content of strip-tube 1 to strip-tube 2 (100 µl final volume each well) and mix by vortexing. Briefly spin tubes down.

7. In a thermal cycler, incubate the strip-tube at 95°C for 10 s and cool down to 10°C at a rate of 0.1°C/s.

8. Combine the content of all wells in a 5 ml screw-cap tube (final volume 700 µl).

9. Add 2,660 µl of water and 140 µl of 2% Tween-20 solution to obtain Adapter/splinter mix at a concentration of 2/4 µM in a final volume of 3.5 ml. Mix well by vortexing. Briefly spin tubes down in a 5 ml table centrifuge.

10. Distribute Adapter/splinter mix from step 9 to five 5 ml screw-cap tubes by adding 690 µl to each tube.

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 Adapter/splinter mix and the batch number of the Adapter/splinter mix.

Attention: The hybridization of the oligos suffices for the preparation of five separate Adapter/splinter mixes. A batch number is assigned to every unit of five Adapter/splinter mixes using Roman numerals (e.g. batch I, batch II, etc.).

11. 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. TL181-IV) or letters (e.g. TL159-x).

Appendix

Document

NAME

UV decontamination of materials

CREATED BY

Elena Essel

PREVIEW

Document

NAME

UV decontamination of reagents/buffers

CREATED BY

Elena Essel

PREVIEW

Document

NAME

TE buffer

CREATED BY

Anna Schmidt

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