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ssDNA2.0: Ligation mix II

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ABSTRACT

Protocol for the preparation of Ligation mix II 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

This protocol describes the preparation of five (or multiples of five) tubes containing Ligation mix II. Each tube suffices for a 96-well library preparation plate (containing 96 + 20 reactions to account for dead volumes and loss of reagent). It is advisable to prepare 2-4 batches (10-20 tubes) at once.

Materials

	Reagent/consumable	Supplier	Catalogue number	Decontamination *
	Reagents			
	Water, HPLC-grade	Sigma Aldrich/Merck	11553332500	UV
	5M NaCl	Sigma Aldrich/Merck	S5150-1L	UV
	TE buffer ¶	self		UV
	T4 DNA ligase buffer (10x)	Thermo Fisher Scientific	EL0013	-
	50% PEG-4000 (w/v)	Jena Bioscience	CSS-253	UV
	Tween-20 †	P5927-100ML	P5927-100ML	UV
	Adapter oligonucleotide CL53 ‡	Sigma-Aldrich (Merck)	-	-
	Adapter oligonucleotide TL178 §	Eurogentec	-	-
	Consumables			
	1.5-ml LoBind Safe-lock tubes	neoLab Migge	VB-0285	UV
	0.2-ml PCR eight-tube strips	neoLab Migge	VB-0357	UV
	5 ml screw cap tubes (rack 2d Lp W/barcode)	VWR	NUNC374320-BR	UV

* Decontamination of reagents and consumables should be performed as detailed in the documents in the Appendix.
¶ See document in the Appendix for preparation of TE buffer
† Use to prepare a 2% (vol/vol) solution in water. NOTE: Tween-20 is highly viscous, pipette slowly and with care.
‡ Order oligonucleotide CL53 at 0.2 µmol synthesis scale (Sigma-Aldrich/Merck, desalted). Dissolve in TE buffer at a concentration of 500 µM. Sequence: 5'-CGACGCTCTTC-ddC-3' (ddC denotes a dideoxy cytidine)
§ Order oligonucleotide TL178 at 10 µmol synthesis scale (Eurogentec, desalted). Dissolve in TE buffer at a concentration of 500 µM. Sequence: 5'-phosphate-GGAAGAGCGTCGTGTAGGGAAAGAGTGTA-3'

Equipment

- Benchtop thermo mixer (e.g. ThermoMixer C; Eppendorf, cat. no. 5382000015)
- 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)

Protocol

1. Prepare the double-stranded adapter by combining the reagents below in a 1.5 ml Eppendorf Safe-Lock tube. Mix properly, spin down and aliquot the mix to four wells of a PCR strip-tube (75 µl each well).

	Reagent	Volume (µl)	Final concentration in reaction
	TE buffer	57 µl	
	5 M NaCl	3 µl	50 mM
	500 µM CL53	120 µl	200 µM
	500 µM TL178	120 µl	200 µM
	Sum	300 µl	

Note

[Note]

The specified volumes in the table suffice for five tubes of master mix. It is advisable to prepare 2-4 batches of the double-stranded adapter at once, corresponding to the number of master mix tubes that will be prepared.

2. Incubate PCR strip-tube(s) in thermal cycler using the following profile: 95 °C 10 sec, ramp to 14 °C at 0.1°C/s.

Note

[Documentation]

Note the lot/batch numbers of the reagents used for double-stranded adapter preparation in Labfolder (orange fields).

Attention: Batches of oligonucleotides are labelled with Roman numerals (e.g. TL178 - VIII) or letters (e.g. CL53 - A).

3. Combine the contents of all four wells into a 1.5 ml Eppendorf Safe-Lock tube.

4. Add 300 µl TE buffer to obtain 600 µl of 100µM CL53/TL178. Mix by vortexing and spin down.

5. Prepare the Ligation mix II in 5 ml screw-cap tubes by combining the following reagents. Mix thoroughly by vortexing and spin tubes briefly in a microcentrifuge.

Note

[Note]

CRITICAL STEP: White precipitate may be present in the ligation buffer after thawing. Heat the buffer vial briefly in a thermo mixer to 37 °C and vortex until the precipitate has dissolved.

	Reagent	Volume (µl)	Final concentration in reaction
	Water	3364	
	T4 DNA ligase buffer (10x)	464	1x
	50% PEG-4000 (w/v)	464	5%
	2% Tween-20 (v/v)	116	0.05%
	100 µM adapter CL53/TL178	116	2.5 µM
	sum	4524	

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 Ligation mix II and the batch number of the Ligation mix II.

Attention: Since each tube of hybridized oligonucleotides suffices for the preparation of five tubes of Ligation mix II, a batch number is assigned to every unit of five Ligation mix II tubes using Roman numerals (e.g. batch I, batch II, etc.).

6. 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).

6. Freeze at -20 °C until used.

Appendix

Document

NAME

TE buffer

CREATED BY

Anna Schmidt

PREVIEW

Document

NAME

UV decontamination of materials

CREATED BY

Elena Essel

PREVIEW

Document

NAME

UV decontamination of reagents/buffers

CREATED BY

Elena Essel

PREVIEW