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Stringency wash buffer

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ABSTRACT

Protocol for the preparation of Stringency wash buffer 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 500 ml buffer.

Materials

	Reagent/consumable	Supplier	Catalogue number
	Reagents		
	Water	Sigma Aldrich/Merck	1153332500
	20x SSC buffer	Thermo Fisher Scientific	AM9770
	20% SDS solution	Thermo Fisher Scientific	AM9820
	Consumables		
	Square media bottle 500 ml	VWR	391-0630
	50 ml serological pipet	Corning BV	357550
	5 ml serological pipet	Corning BV	357543

Equipment

- Serological pipette controller (e.g. battery-powered pipetting aid ROTILABO, cat. no. TC16.1)

Protocol

1. Prepare the buffer in a 500 ml square media bottle by adding the following reagents. Use the glass pipette for transfer of large volumes (> 1 ml). Mix reagents by shaking the bottle.

	Reagent	Volume	Final concentration in reaction
	Water	495 ml	
	20x SSC buffer	2.5 ml	0.1x
	20% SDS solution	2.5 ml	0.1%
	sum	500 ml	

Note

[Note]

It is also acceptable to use the scale of the bottle to fill up to 400 ml with water, then adding the remaining 95 ml using the glass pipette.

2. Review the protocol in which the buffer is used to determine whether the buffer should be decontaminated using UV treatment. Instructions for UV-decontamination are provided in the Appendix.

Note

[Labeling]

Label the bottle with the buffer name, batch ID, date and the initials of the person who prepared the buffer.

Attention: Every single bottle prepared at the same day gets a new batch ID. Name the batches with Roman numerals (e.g. batch I, batch II, etc.)

3. Store the buffer at room temperature until used. Shelf life is at least two months from preparation.

Note

[Note]

Note the lot numbers and the date and initials of the reagents used for the preparation in Labfolder (orange fields).

Appendix

Document

NAME

UV decontamination of reagents/buffers

CREATED BY

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PREVIEW