

Mar 21, 2024

Version 1

0.1xBWT+SDS buffer V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.5qpvo311zv4o/v1>

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MPI EVA Ancient DNA C...



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Document Citation: Anna Schmidt, Sarah Nagel, Matthias Meyer 2024. 0.1xBWT+SDS buffer. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.5qpvo311zv4o/v1>

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Created: January 09, 2024



Last Modified: April 10, 2024

Document Integer ID: 93164

Keywords: stranded dna library preparation, dna library preparation, stranded dna library, dna libraries for the sequencing, dna from ancient biological remain, degraded dna, sds buffer, dna, protocol for the preparation, wash buffer, ancient biological remain

Funders Acknowledgements:

Max Planck Society

Grant ID: -

Abstract

Protocol for the preparation of 0.1x BWT+SDS buffer (Bind and wash buffer I) 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.

Note

This protocol describes the preparation of 500 ml buffer.

Materials

	Reagent/consumable	Supplier	Catalogue number
	Reagents		
	Water	Sigma Aldrich/Merck	1153332500
	5 M NaCl	Sigma Aldrich/Merck	S5150-1L
	1 M Tris-HCl, pH 8.0	AppliChem	A4577,1000
	0.5 M EDTA, pH 8.0	AppliChem	A4892,1000
	Tween-20	Thermo Fisher Scientific	11417160
	20% SDS	Thermo Fisher Scientific	AM9820
	Consumables		
	Square media bottle 500 ml	VWR	391-0630
	50 ml serological pipet	Corning BV	357550
	10 ml serological pipet	Corning BV	357551
	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	471.25 ml	



	Reagent	Volume	Final concentration in reaction
	5 M NaCl	10 ml	2 M
	1 M Tris-HCl, pH 8.0	5 ml	10 mM
	0.5 M EDTA, pH 8.0	1 ml	1 mM
	100% Tween-20	0.25 ml	0.05%
	20% SDS solution	12.5 ml	0.50%
	<i>sum</i>	<i>500 ml</i>	

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 ~71 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

[Documentation]

Note the lot numbers, date and initials written on the reagents used for buffer preparation in Labfolder (orange fields).



Appendix

Document

NAME

UV decontamination of reagents/buffers

CREATED BY

Elena Essel

Preview