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Indexing PCR and purification of dsDNA libraries

In 1 collection

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol for the indexing PCR and purification of dsDNA libraries, optimized for ultra-short ancient DNA molecules, modified from Meyer & Kircher (2010) Cold Spring Harb. Protoc. (doi: **10.1101/pdb.prot5448**).

Guidelines

Please read the general guidelines for working in the Ancient DNA protocol collection – University of Tartu, Institute of Genomics.

Materials

Reagents:

	A	B	C	D	E	F	G
	Step	Reagents	Conc.	Unit	Manufacturer	Kit/full description	Product number
	Purification	PB Buffer	N/A	N/A	Qiagen	MinElute PCR Purification Kit	19066
	Purification	PE Buffer	N/A	N/A	Qiagen	MinElute PCR Purification Kit	19065
	Purification	EB Buffer	N/A	N/A	Qiagen	MinElute PCR Purification Kit	28006

Equipment and consumables:

	A	B
	Number	Equipment and consumables
	1	0.2 ml tube rack
	1	1.5 ml tube rack
	1	50 ml Falcon rack
		100 µl filter tips
		200 µl filter tips
		1000 µl filter tips
	[# of samples]+1	1.5 ml tubes
	[# of samples]	MinElute columns
	1	50 ml Falcon (waste)

Lab equipment:
Dead Air Hood
Centrifuge (1.5/2 ml)
Heat block
Mini table centrifuge/vortexer

Other consumables:
DNA ExitusPlus

Paper towels

Troubleshooting

Safety warnings

Reagents

DNA ExitusPlus
H319 Causes serious eye irritation.

Guanidinium hydrochloride (GuHCl) (in PB buffer of Qiagen MinElute kit)
- H302 Harmful if swallowed.
- H332 Harmful if inhaled.
- H315 Causes skin irritation.
- H319 Causes serious eye irritation.

Ethanol
- H225 Highly flammable liquid and vapor.
- H319 Causes serious eye irritation.

Equipment
UV radiation

- UV radiation can damage eyes and can be carcinogenic in contact with skin. Do not look directly at unshielded UV radiation. Do not expose unprotected skin to UV radiation.
- UV emitters generate ozone during operation. Use only in ventilated rooms.

Before start

Previous step:
This protocol follows the library preparation protocols.

Following step:
After the purification, the libraries are ready for quality control.

Equipment and consumables:

	A	B
	Number	Equipment and consumables
	1	0.2 ml tube rack
	1	1.5 ml tube rack
	1	50 ml Falcon rack
		100 µl filter tips
		200 µl filter tips
		1000 µl filter tips
	[# of samples]+1	1.5 ml tubes
	[# of samples]	MinElute columns
	1	50 ml Falcon (waste)

[# of samples] includes the blank(s).

PCR

1 In the modern lab, place the PCR strips in the cycler and run the following program:

	A	B	C	D
	Step	Time [min:sec]	Temperature [°C]	Cycles
	Preincubation	5:00	94	1
	Denaturation	0:30	94	15
	Annealing	0:30	60	
	Elongation	0:30	68	
	Final elongation	7:00	72	1
	Hold	infinite	4	1

2

Note

Continue immediately with the purification or stop here and purify later.
To continue with purification, take MinElute columns out of the fridge while the PCR is running.

To stop and purify later, put the strips into the fridge (if stored for max. 1 day) or freezer (if stored for multiple days) after the PCR is done. For the purification, take the MinElute columns out of the fridge in time so they can reach room temperature until PB buffer and libraries are added.

Purification

2m

3 Turn on the heat block 37 °C for the elution.

4 Label the 1.5 ml EB tube and aliquot: [# of samples]×35 µl plus 10%.

5 Prepare PE (wash) buffer by adding ethanol and aliquoting to 50 ml tubes.

6 Label MinElute columns.
Label the 50 ml waste tube.

7 Label tubes:

	A	B	C
	Top	Project ID	PROJ
		Library ID	ABC001A 1 SG1
		indices	NEB1 (single) i701 / i501 (double)
	Side	Project ID	PROJ
		Library ID	ABC001A 1 SG1
		indices	NEB1 (single) i701 / i501 (double)
		date	01.01.2021
		initials	XY

8 Add 500 µL PB buffer (binding buffer) to the MinElute column.

9 Add the (first) PCR reaction (100 µL) and pipette-mix.

10 Spin 13 rpm, 00:01:00 . 1m

11 Discard flowthrough into your waste tube.

12

Note

Steps 12 to 14 only apply if you have 2x split the PCR reaction.

13 Add 500 µL PB buffer (binding buffer) to the MinElute column.

14 Add the second PCR reaction (100 µL) and pipet-mix.

15 Spin 13 rpm, 00:01:00 .

1m

16 Discard flowthrough into your waste tube.

17 Add 690 µL µl PE buffer (wash buffer), change tip for every sample.

18 Spin 13 rpm, 00:01:00 .

19 Discard flowthrough into your waste tube.

20 Spin 13 rpm, 00:01:00 (dry spin).

21 Put column in labeled tube.

22 Elute in 35 µL EB buffer (elution buffer). Change tip for every sample.

23 Incubate at 37 °C for 00:10:00 .

10m

24 Spin 13 rpm, 00:02:00 .

25 Check that there is liquid in your tube, throw away the column and close your tube.

26 Put the tubes into the fridge (if stored for max. 1 day) or freezer (if stored for multiple days).