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Indexing of ancient DNA libraries

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

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Abstract

This protocol describes how to index ancient DNA sequencing libraries with the P5 and P7 index primers mix. All the reagents and PCR mixture should be prepared in the clean lab, and then the PCR must be run in the modern lab. The output of this protocol is indexed ancient DNA libraries.

Guidelines

If it is an ancient sample, prepare the PCR mixture in the clean lab and run in the modern lab.

Protocol materials

NEBNext Q5U Master Mix – 250 rxns **New England Biolabs Catalog #**M0597L

KAPA HiFi HotStart Uracil ReadyMix (2X) **Roche Catalog #**07959079001

Before start

- Make sure you have the P5+P7 index primers mix (10 uM) ready before you start.
- If you are going to sequence in the following steps, avoid using the same index for different libraries in one sequencing pool.

Prepare the PCR master mix

1

> Note
>
> Prepare the reagents and mixture in the clean lab and run the PCR in the modern lab.

Index PCR master mix setup:

- N samples = No. of libraries (incl. extraction and library build negative controls) + index PCR negative control (no library template - use your ddH2O as the "template").
- Required reagents:

1. NEBNext Q5U Master Mix – 250 rxns **New England Biolabs Catalog #**M0597L or KAPA HiFi HotStart Uracil ReadyMix (2X) **Roche Catalog #**07959079001 or any **uracil-tolerant polymerase**.
2. ddH2O.
3. P5 and P7 indexing primers mix (10 µM).

	Reagent	Stock Conc.	Volume (µL)	N sample
	ddH2O		8	8 * (n*1.1)
	Q5U Master Mix		25	25 * (n*1.1)
	Total volume		33	33 * (n*1.1)
	Aliquot 33 uL of the master mix into PCR tubes, then only add the unique primer mix for each tube.			
	P5+P7 index primer mix	10 µM	2	
	DNA template		15	
	Total volme		50	

2 Add 33 µL of indexing PCR master mix to a PCR tube (as stated in the table above).

3 Add 2 µL of the P5+P7 index primer mix to each PCR tube (as stated in the table above). **Make sure that each sample gets a unique index combination.**

4 Add 15 µL of the prepared DNA (library) template to the PCR tube. For the indexing negative control, use ddH2O. The final volume of each PCR tube is 50 µL.

5 Put the PCR tubes in a ziploc bag and label it with your name, date, sample IDs, etc. to take it to the modern lab.

Running PCR

6 Run the PCR in the modern lab.

For Q5U:

	Step	Temp	Time	Cycles
	A*	98 °C	60 s	* Pre-Heat Step
	B	98 °C	45 s	
	C	98 °C	15 s	** xCycles (12-18)
		60 °C	30 s	
		72 °C	20 s	

* Pre-Heat Step: Step A is for Q5U specifically, as the enzyme's documentation says that the PCR machine should be pre-heated to 98°C before the samples go in, so the samples should be placed into the PCR machine quickly just before the end of this step, which is then followed by the 45s 98°C step.

** The number of cycles depends on your libraries. (If you want to accurately determine the number of cycles, you can do a qPCR first.)
12-18 cycles are sufficient for ancient DNA amplification. A higher number of cycles will lead to higher duplication rates in your sequencing data. If the libraries will be neriched for mitochondrial DNA, this is not usually a problem, as you will have a high duplication in the post-capture libraries, and you want a high concentration to do the capture/enrichment, so many indexing cycles are a good idea. If you are not capturing and instead doing shotgun for nuclear deep sequencing, then do a qPCR to accurately determine the number of indexing cycles required in order to limit PCR duplicates in your library and retain as much complexity as possible.Clean

7 After the index PCR, clean the indexed libraries using the SPRI bead cleanup protocol, in the modern lab.

Protocol

NAME

Clean up of Index PCRs using SPRI Beads

CREATED BY

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Preview