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We use this protocol and it's working

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Nanopore amplicon sequencing with DIY adapter

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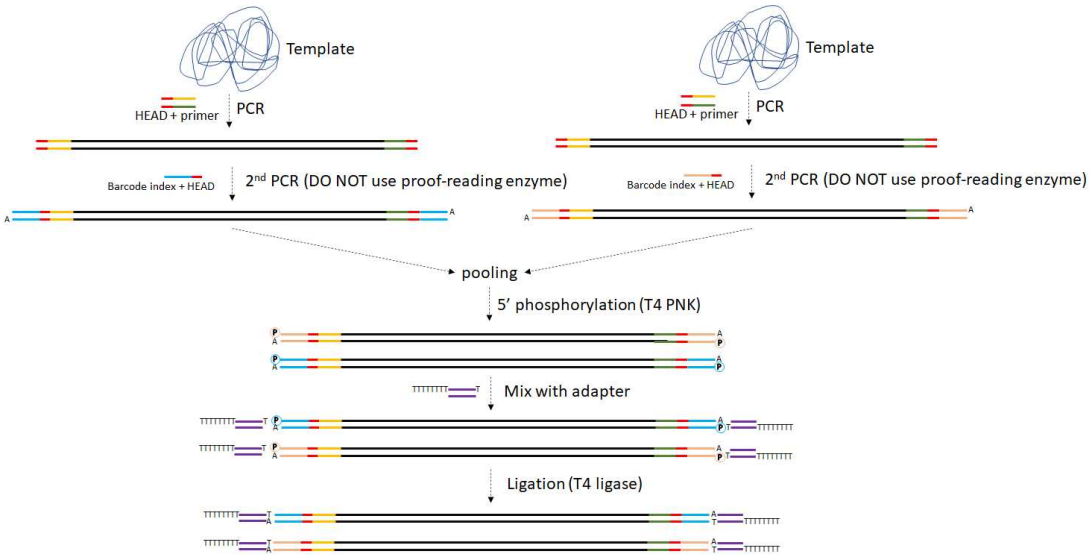
ABSTRACT

Nanopore amplicon sequencing with DIY adapter

Workflow

1



Preparation of amplicons

2 Use a primer with a **HEAD** to make amplicons, as in a regular procedure.
Enzymes **with or without proofreading can be used**, and purification is not necessary.

3 Use a **barcoding index** for the second PCR.
Note:
1. non-proofreading enzyme (Taq) must be used to add a A tail to the PCR product.

4 Purify the PCR product and adjust the final concentration of the purified DNA to ~50 ng/ul.

Pooling & 5' Phosphorylation

5

Mix the following materials in order:
(10 samples as an example)

	A	B
	Sterile water	7 ul
	10X T4 Polynucleotide Kinase Reaction Buffer (with 1 mM ATP)	2 ul
	T4 Polynucleotide Kinase (10U/ul)	1 ul
	PCR product (x10 samples)	1 ul x 10 samples = 10 ul

Note: Some manufacturers' buffers require additional ATP to be added to achieve a final reaction concentration of 0.1 mM.

6 Incubate at 37°C for 30 minutes. 30m

7 Heat inactivate by incubating at 65°C for 20 minutes 20m

8 Purify the DNA and adjust the final concentration to ~50 ng/ul.

Preparation of Adapter 1h 4m

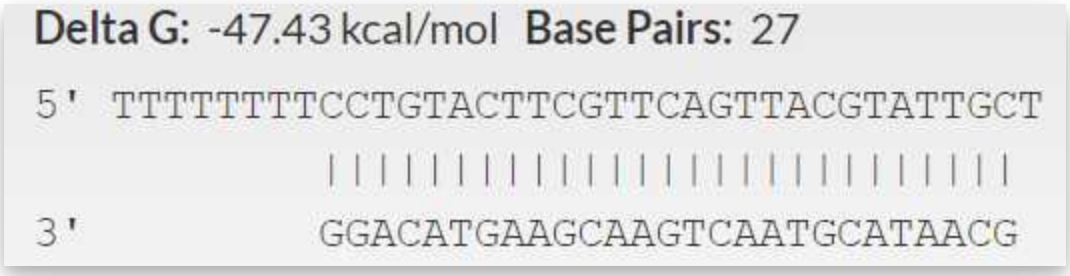
9 Order the following two primers:
Adapter_top: TTTTTTTTCCTGTACTTCGTTCAGTTACGTATTGCT
Adapter_bottom: GCAATACGTAACTGAACGAAGTACAGG
Preferably, choose **OPC or a higher level** of purification method.

10 Dilute both primers to 100uM according to the manufacturer's instructions (TE buffer is recommended) 1m

11 Mix both primers together in equal volumes. 1m

(Now it is 50 uM)

12 Incubate the mixture in a dry bath at 95°C for 2 minutes. 2m



Ideally, Adapter_top and Adapter_bottom would aneal to form a sticky end of double strand DNA

13 Allow the mixture to **slowly cool to room temperature** for one hour. 1h

14 Make 1:10 dilution (5 uM)
Store the mixture at 4°C or in a freezer for long-term storage.
When necessary, divide the mixture into smaller portions to **avoid repeated thawing.**

Attach adapter to PCR products. 1h

15 Mix the following materials in order:

	A	B
	5' Phosphorylated PCR product (50ng/ul)	10 ul
	Sterile water	7 ul
	10X T4 DNA Ligase Buffer (with 1 mM ATP)	2 ul
	Adapter	0.1 ul

	A	B
	T4 ligase	1 ul (300U or 2.5U in Weiss Unit)

Note: Some manufacturers' buffers require additional ATP to be added to achieve a final reaction concentration of 0.1 mM.

16 Incubate at 16-20 °C for at least 01:00:00 1h

17 Purify the DNA and adjust the final concentration to ~50 ng/ul.