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One-pot ligation protocol for Oxford Nanopore libraries

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1 Works for me dx.doi.org/10.17504/protocols.io.k9acz2e

MinION user group for high molecular weight DNA extraction from all kingdoms

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ABSTRACT

This is our 'one-pot ligation' protocol for Oxford Nanopore ligation libraries. It benefits from increased recovery of library and faster preparation time by using the Ultra II ligation module in conjunction with the Ultra II end repair/dA-tailing module therefore removing a clean-up step. It can be used with or without the optional FFPE DNA repair step which shoudl help to improve read-lengths in nicked DNA. The incubation times given here are sufficient to generate high quality libraries in under one hour. In the past we have cut these times in half without noticably impacting performance but we will leave this up to you.

MATERIALS

NAME	CATALOG #	VENDOR
NEBNext FFPE DNA Repair Mix - 96 rxns	M6630L	New England Biolabs
Agencourt AmPure XP beads	A63880	
NEBNext Ultra II End Repair/dA-Tailing Module - 96 rxns	E7546L	New England Biolabs
NEBNext Ultra II Ligation Module - 96 rxns	E7595L	New England Biolabs
Ligation Sequencing Kit 1D	SQK-LSK108	

1 Set up either the following end-prep reaction:

DNA (200-400 fmol)	25 µl
Ultra II End Prep Reaction Buffer	3.5 µl
Ultra II End Prep Enzyme Mix	1.5 µl
Total	**30 µl**

Or to include FFPE DNA repair set up the following combined reaction:

DNA (200-400 fmol)	24 µl
Ultra II End Prep Reaction Buffer	1.75 µl
FFPE DNA Repair Buffer	1.75 µl
Ultra II End Prep Enzyme Mix	1.5 µl
FFPE DNA Repair Mix	1 µl
Total	**30 µl**

2 Incubate at RT for 10 minutes.

00:10:00

20 °C

3 Then incubate at 65°C for 10 minutes.

00:10:00

65 °C

4 Place on ice for 30 seconds.

00:00:30

5 Add the following directly to the previous reaction:

AMX 1D	20 µl
Ultra II Ligation Master Mix	40 µl
Ligation Enhancer	1 µl
Total	**91 µl**

6 Incubate at RT for 20 minutes.

00:20:00

20 °C

7 Add 45.5 µl Ampure XP beads.

45.5 µl **Ampure XP beads**

8 Incubate at room temperature for 10 minutes.

00:10:00

20 °C

9 Spin down briefly and place on a magnetic rack until solution clears.

10 Taking care to avoid the pellet remove the supernatant.

11 Add 150 µl ABB and resuspend by gently flicking (wash 1/2).

150 µl **ABB**

12 Spin down briefly and place on a magnetic rack until solution clears (wash 1/2).

13 Taking care to avoid the pellet remove the supernatant (wash 1/2).

14 Add 150 µl ABB and resuspend by flicking (wash 2/2).

150 µl **ABB**

15 Spin down briefly and place on a magnetic rack until solution clears (wash 2/2).

16 Taking care to avoid the pellet remove the supernatant (wash 2/2).

17 Spin down again and remove all residual ABB with a P10 pipette.

18 Add 12 µl ELB and resuspend beads by flicking.
12 µl ELB

19 Incubate at RT for 10 minutes.
00:10:00

20 Spin down briefly and place on a magnetic rack until solution clears.

21 In a new tube prepare library dilution for sequencing:

	With LLB	Without LLB
RBF	35 µl	35 µl
Nuclease-free water	2.5 µl	28 µl
LLB	25.5 µl	-
Library	12 µl	12 µl
Total	75 µl	75 µl

N.B. I would generally include LLB unless working with HMW DNA, which can cause them to clump, or if I plan to flush/reload the flowcell, in which case they would get flushed out anyway.

22 Mix by gently flicking before removing 1 µl to assess concentration by Qubit (wait until beads have settled before measuring).
1 µl Library

Expected recovery is 50-80% of starting material, lower recovery is indicative of presence of short fragments or inaccurate quantification of the input material. Loading insufficient library into a flowcell will significantly impact yield.