One-pot ligation protocol for Oxford Nanopore libraries

Josh Quick

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.

Citation: Josh Quick One-pot ligation protocol for Oxford Nanopore libraries. **protocols.io** dx.doi.org/10.17504/protocols.io.k9acz2e
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Materials

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

Protocol

Step 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**

Step 2.

Incubate at RT for 10 minutes.

TEMPERATURE
20 °C Additional info:

Step 3.

Then incubate at 65°C for 10 minutes.

TEMPERATURE
65 °C Additional info:

Step 4.

Place on ice for 30 seconds.

Step 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**

ANNOTATIONS
Tommy Au 12 Jan 2018

Thanks for sharing the protocol!

Do you think NEB Blunt/TA Ligase Master Mix would work?

Step 6.

Incubate at RT for 20 minutes.

TEMPERATURE
20 °C Additional info:

Step 7.

Add 45.5 µl Ampure XP beads.

AMOUNT
45.5 µl Additional info: Ampure XP beads

Step 8.

Incubate at room temperature for 10 minutes.

TEMPERATURE
20 °C Additional info:

Step 9.

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

Step 10.

Taking care to avoid the pellet remove the supernatant.

Step 11.

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

AMOUNT
150 µl Additional info: ABB

Step 12.

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

Step 13.

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

Step 14.

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

AMOUNT
150 µl Additional info: ABB

Step 15.

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

Step 16.

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

Step 17.

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

Step 18.

Add 12 µl ELB and resuspend beads by flicking.

AMOUNT
12 µl Additional info: ELB

Step 19.

Incubate at RT for 10 minutes.

Step 20.

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

Step 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

NOTES
Josh Quick 13 Dec 2017

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.

Step 22.

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

AMOUNT
1 µl Additional info: Library

EXPECTED RESULTS

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.

ANNOTATIONS

Alan Tourancheau 12 Feb 2018

What's your typical loading molarity? From your recommended input of 200-400 fmol and considering 50-80% recovery, I'd say 100 fmol to 320 fmol?

Ken CHO 14 Feb 2018

Have you tried this approach on a flowcell? What was the throughput?