One-pot native barcoding protocol

Scope:

This is our ‘one-pot ligation’ protocol for Oxford Nanopore native barcoded ligation libraries using shearing. We have seen no reduction in performance compared to standard libraries yet it can be made faster by using the Ultra II ligation module which is compatible with the Ultra II end repair/dA-tailing module removing a clean-up step. It can be used with or without the optional FFPE DNA repair step. If you have the time I would recommend using the double incubation times in blue, if you are in a hurry the times in red are a good compromise between speed and efficiency.

Required:

g-TUBEs (optional)
SQK-LSK108 1D ligation kit
Native barcoding kit
Ultra II End Repair/dA-Tailing Module
Ultra II Ligation Module
FFPE DNA Repair Mix (optional)
Ampure XP beads
80% ethanol
EB (10 mM Tris-HCl pH 8)

Protocol:

Dilute 1 ug gDNA to 50 µl with EB and pipette into Covaris g-TUBE
Centrifuge g-TUBE at 5000 rpm for 2 x 30 sec spins, invert tube and repeat
Transfer newly sheared DNA into a clean microcentrifuge tube
Remove 1 µl and asses concentration by Qubit
Set up either the following end-prep reaction for each sample:

Sheared DNA (500 ng)	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 (500 ng)	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**

Incubate at RT for 5 mins or 10 mins then 65°C for 5 mins or 10 mins
Place on ice for 30 secs
Add the following directly to the previous reactions:

NBXX barcode	2.5 µl
Ultra II Ligation Master Mix	20 µl
Ligation Enhancer	1 µl
Total	**53.5 µl**

Incubate at RT for 10 mins or 20 mins, 70°C for 5 mins then place on ice
Pool all barcoded fragments together into a clean 1.5 ml Eppendorf tube
Add 26.75 µl Ampure XP beads per sample
Incubate for 5 mins or 10 mins
Place on a magnet rack until clear
Remove supernatant
Add 200 µl 80% ethanol to the tube still on the magnetic rack
Incubate 30 secs
Remove supernatant
Repeat last three steps
Spin down and remove residual 70% ethanol
Air dry 1 min
Resuspend in 31 µl EB
Incubate off the magnetic rack for 5 mins or 10 mins
Replace on magnetic rack
Remove 1 µl and asses concentration by Qubit
Set up the following adapter ligation reaction:

Cleaned-up barcoded fragments (~3 µg)	30.0 µl
BAM 1D	20.0 µl
Ultra II Ligation module	40.0 µl
Ultra II Ligation enhancer	1.0 µl
Total volume	**91.0 µl**

Incubate at RT for 10 mins or 20 mins
Add 45.5 µl Ampure XP beads
Incubate for 5 mins or 10 mins mins
Place on a magnetic rack until clear
Remove supernatant
Add 150 µl ABB and resuspend by flicking (CAUTION: do not use 80% ethanol)
Place on magnetic rack until clear
Remove supernatant
Repeat ABB wash
Spin down and remove residual ABB
Add 12 µl ELB and resuspend by flicking
Incubate at RT for 5 mins or 10 mins
Place on magnetic rack
In a new tube prepare library dilution for sequencing:

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

Mix well
Remove 1 µl and asses concentration by Qubit (wait until beads have settled before measuring)