

Additional File 1 : Preparation kits and DNA extractions

Table S1. Kit summary

| Kit name | Ref | DNA | Time ¹ | Barcode Adapter | Sequencing Adapter | Consumables | Study name |
|---|-------------------|---------|-------------------|--------------------|-----------------------|--------------------------|---------------|
| "Ligation Sequencing" | SQK-LSK110 | 1000 ng | 140 min | NA ^{2,3} | AMII | FFPE, ER/dA | LIG |
| + "Native barcoding ^{2,3"} | NA ^{2,3} | | | | | LIG1, LIG2 | |
| "Ligation Sequencing" | SQK-LSK110 | 1000 ng | 85 min | BCA + BC01-BC12 | AMX-F | FFPE, ER/dA ⁴ | PCR |
| + "PCR barcoding" | + EXP-PBC001 | | + PCR | | | LIG1, LIG2, LR-Taq | |
| "Rapid Barcoding" (="Native tagmentation") | SQK-RBK004 | 400 ng | 10 min | RB01-RB12 | RAP | -- | TAG |

Kits available to produce up to 6 libraries of 12 samples using ligation, tagmentation or amplification at the time of the study. Magnetic beads are required for all kits and thus are not listed in consumables. The library names used in the present study are given in the last column as LIG, TAG or PCR (see manuscript introduction).

¹ONT-indicated minimum time

²Native Barcoding expansion NOT AVAILABLE for SQK-LSK110. The required expansion for the previous kit SQK-LSK109 was named EXP-NBD104 and contained adapters NBD01-NBD12.

³A pooled library without barcodes was prepared via ligation (SQK-LSK110) and sorted bioinformatically (see methods for further information)

⁴End-repair is conducted twice (prior to PCR adapter ligation and prior to sequencing adapter ligation)

Abbreviations :

AMII = Adapter Mix II

AMX-F = Adapter Mix F

BC = Barcode

BCA = Barcode Adapter

ER/dA = NEBNext Ultra II End Repair / dA-Tailing Module

FFPE = NEBNext FFPE DNA Repair Mix

LIG1 = NEB Blunt/TA DNA Ligase

LIG2 = NEBNext Quick T4 DNA ligase

LR-Taq = Long Range Taq

RAP = Rapid Sequencing Adapter Attachment

RB = Rapid Barcode