# Sample Preparation

# Sample Preparation

Sample type: DNA | RNA

PCR-free: PCR | PCR-Free

Multiplexing: No | Yes

15 results

Clear filters

## Ligation Sequencing Kit

SQK-LSK109

A versatile sequencing kit optimised for throughput

Includes a Flow Cell Priming Kit

**Key features:**

| | |
|---|---|
| Prep time | 60 minutes |
| Input amount | 1000 ng high molecular weight dsDNA 100+ ng DNA if performing fragmentation or PCR |
| Read length | = fragment length |
| Typical throughput | 2-3+ Gb in 6 hours, 8+ Gb in 48 hours per flow cell on MinION/GridION; 10-15+ Gb in 6 hours, 40+ Gb in 48 hours per flow cell on PromethION |

## Ligation Sequencing Kit XL

SQK-LSK109-XL

A versatile sequencing kit optimised for throughput, long reads, and processing multiple samples simultaneously.

Product has a 3 week lead time

**Key features:**

| | |
|---|---|
| Prep time | 110 minutes |
| Input amount | 1000 ng high molecular weight dsDNA 100+ ng DNA if performing fragmentation or PCR |
| Read length | = fragment length |
| Typical throughput | 2-3+ Gb in 6 hours, 8+ Gb in 48 hours per flow cell on MinION/GridION; 10-15+ Gb in 6 hours, 40+ Gb in 48 hours per flow cell on PromethION |

## 16S Barcoding Kit 1-24

SQK-16S024

Genus-level bacterial identification with barcoding for up to 24 samples.

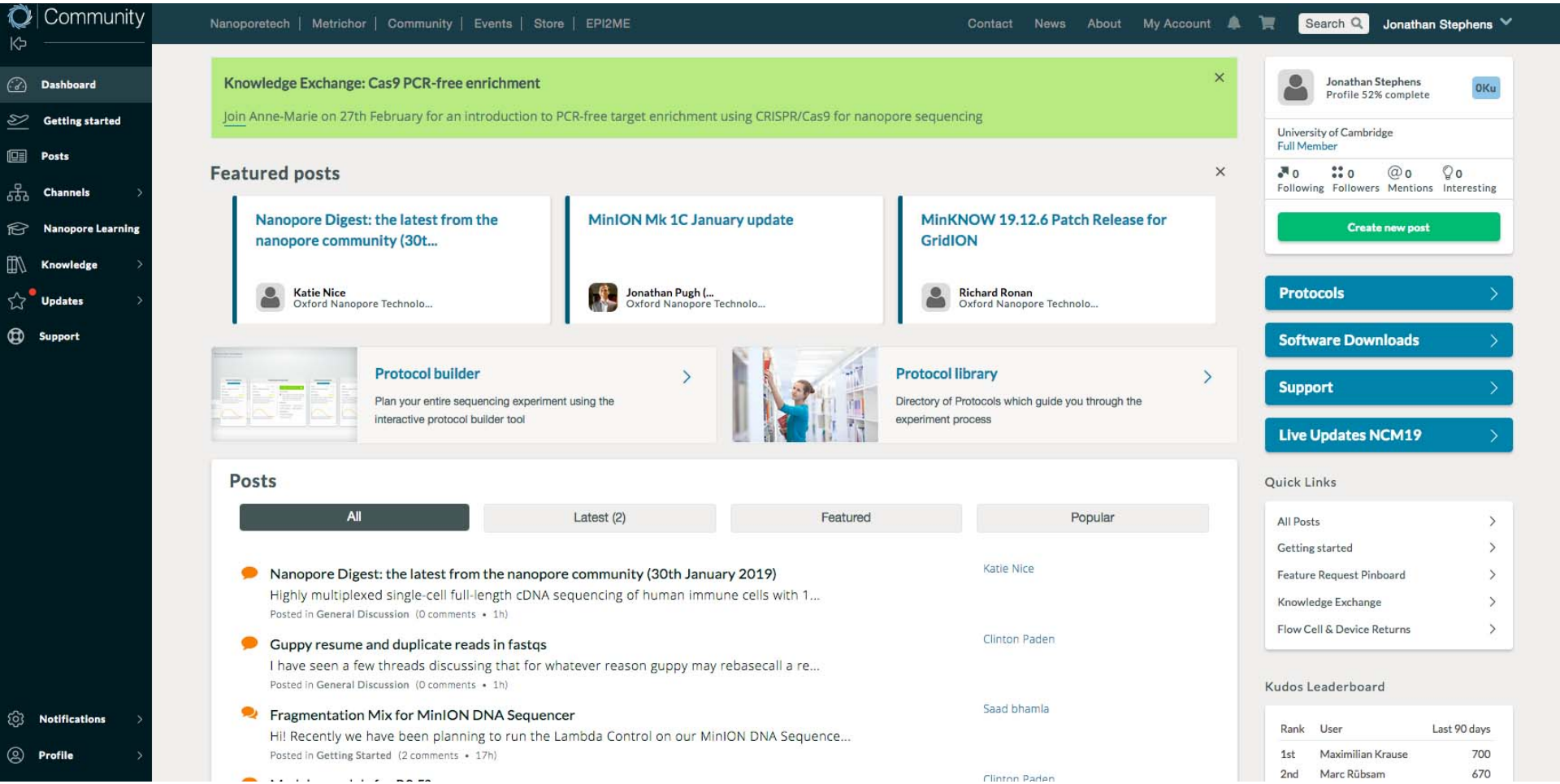
Includes a Flow Cell Priming Kit

Product has a 6 week lead time

**Key features:**

| | |
|---|---|
| Prep time | PCR + 10 minutes |
| Input amount | 10 ng gDNA |
| Read length | = full-length 16S gene (~1.5kb) |
| Typical throughput | 1-2 Gb in 6 hours, 4-8 Gb in 48 hours per flow cell on MinION/GridION |

# Nanopore Community



Nanopore Protocol

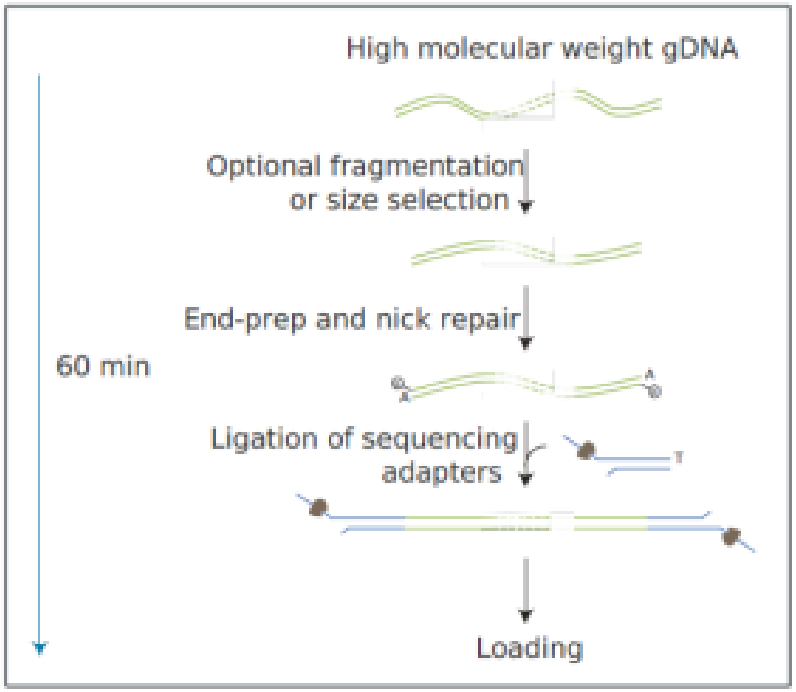
# Genomic DNA by Ligation (SQK-LSK109)

## Equipment and consumables

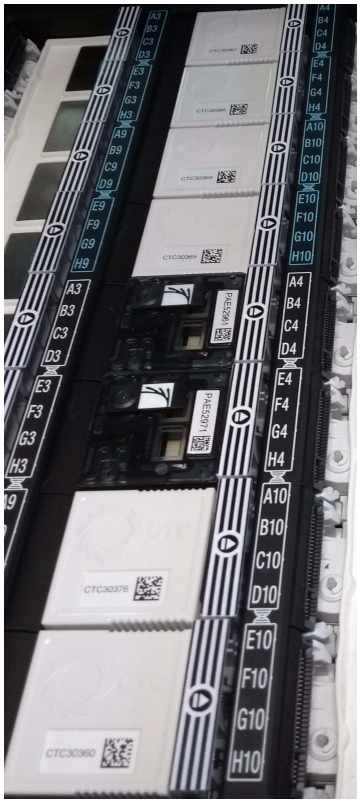
Version: GDE_9063_v109_revS_14Aug2019

- For highest throughput
- High quality DNA
- Fresh > frozen/archived

- 1µg (100-200 fmol) HMW DNA
- 260:280 ~1.8
- 260:230 ~2.0



# PromethION

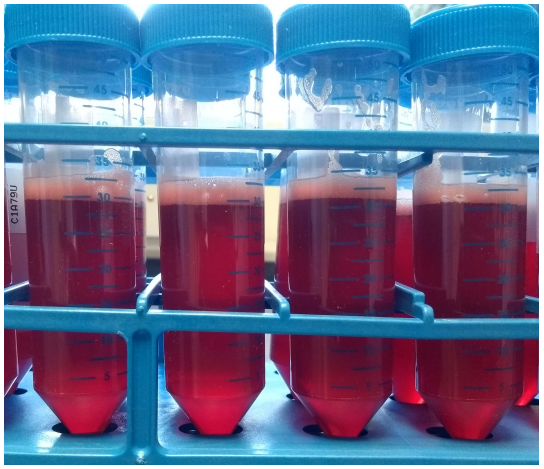


**PromethION Flow Cell**


**MinION Flow Cell**

# *Genomic DNA extraction from Blood*

Red Cell Lysis

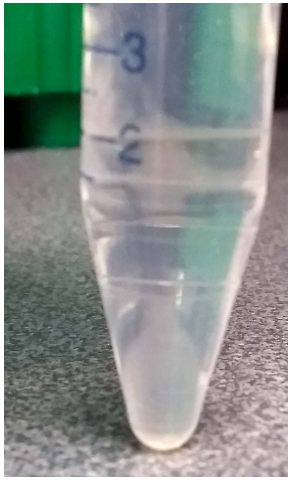


*WBC pellet*

Lysis, Protein Digestion

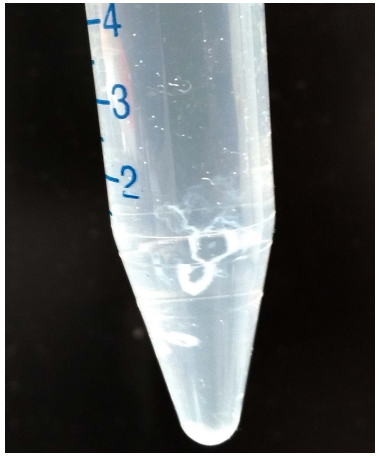
*Guanidine HCl/Sarcosyl/Proteinase K*

Chloroform Extraction



*Aqueous layer*

Ethanol precipitation



70% ethanol wash, dry, resuspend in TE pH 8.0

# *DNA Quality Control*

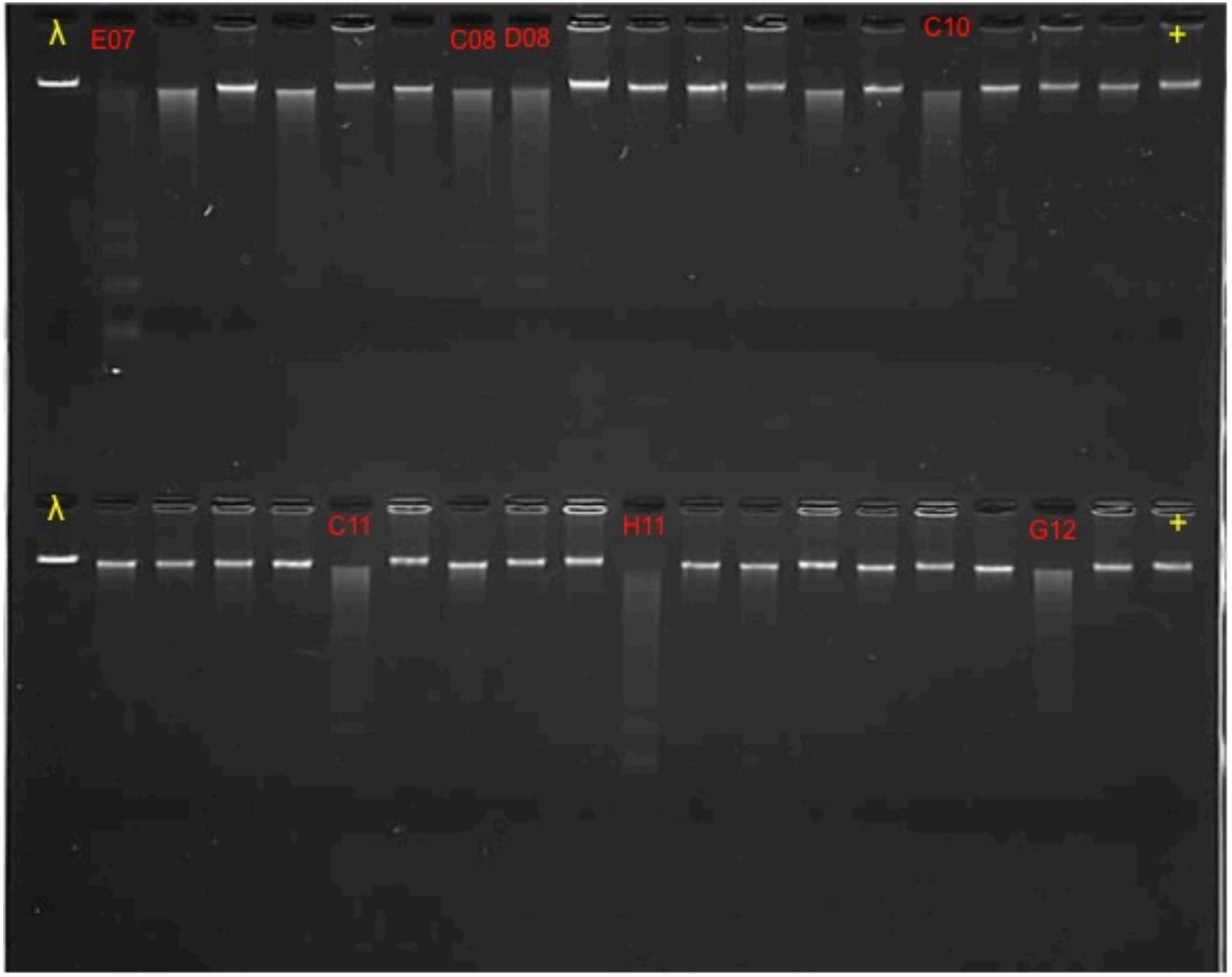
***Quantification***

- *dsDNA specific fluorescent dyes*
- *Qubit, Picogreen*

***DNA Quality***

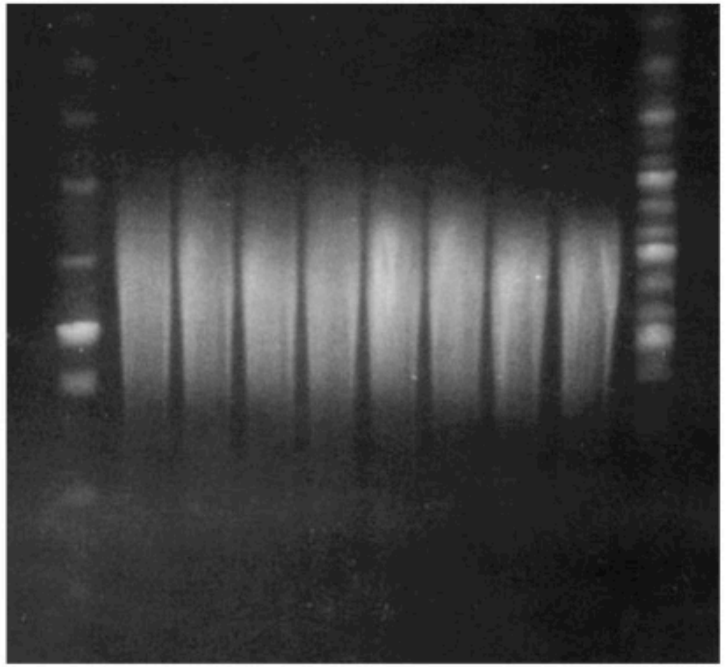
- *Agarose gel electrophoresis*
- *Nanodrop spectrophotometer*
- *Agilent Bioanalyser (12000bp)*
- *Agilent Tapestation (60000bp)*

# *Agarose Gel electrophoresis of Genomic DNA extractions*



# *QIAgen Genomic Tip*

## Pulse Field Gel electrophoresis



— 145.5 kb

— 97.0 kb

— 48.5 kb

QIAGEN Genomic-tip 500/G

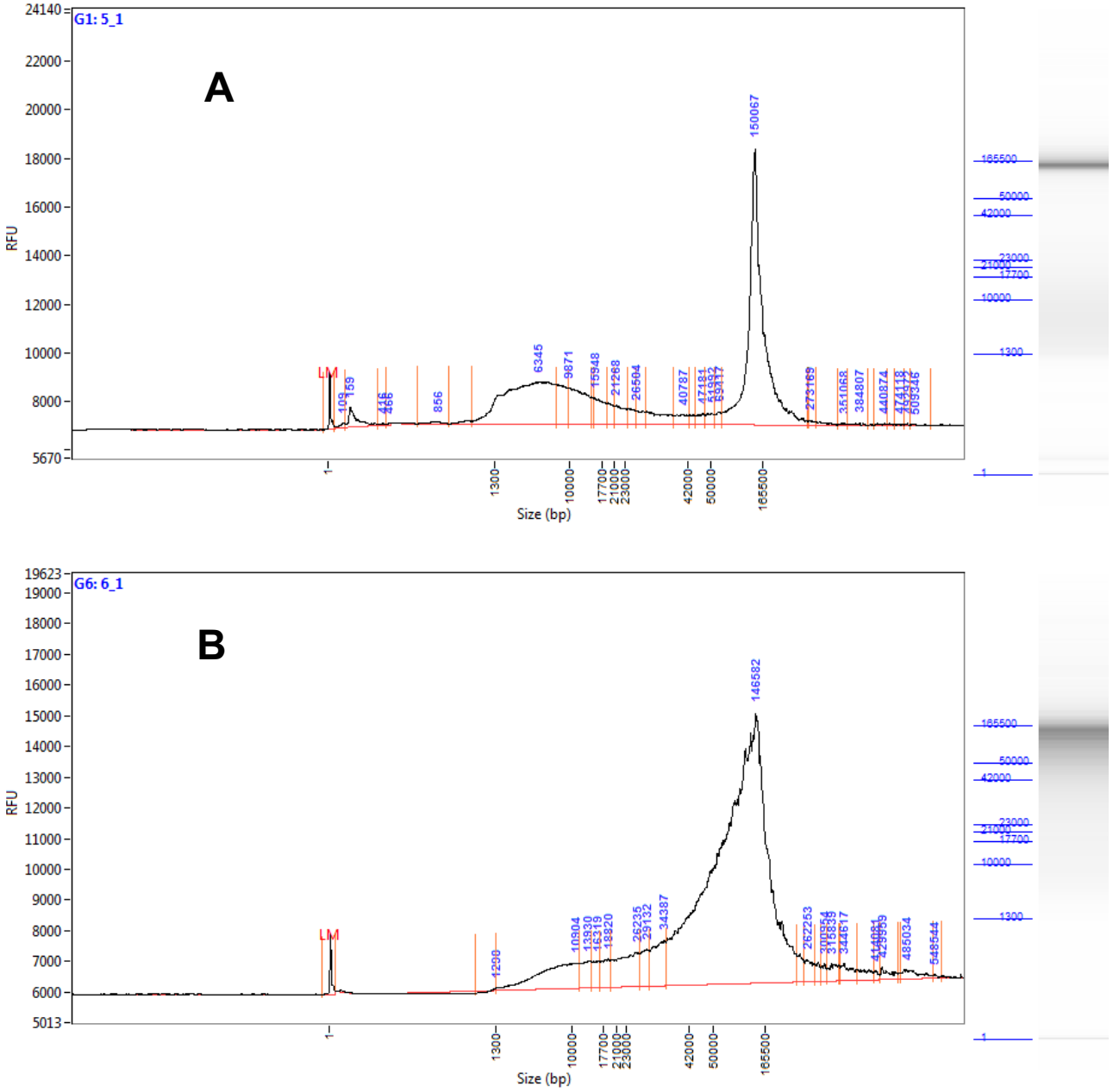
Print

For isolation of up to 500 µg high-molecular-weight DNA from a wide range of samples

- Reliable isolation of DNA up to 150 kb in size
- No phenol or chloroform extractions
- Convenient, parallel processing of multiple samples

QIAGEN Genomic-tips are gravity-flow, anion-exchange tips that allow efficient purification of genomic DNA from a wide range of biological samples. The purified DNA is sized up to 150 kb with an average size of 50–100 kb.

# *Femto pulse sizing of genomic DNA*




# *Nanodrop For DNA Quality Control*

Typical spectral pattern for Nucleic Acid (Figure 1)

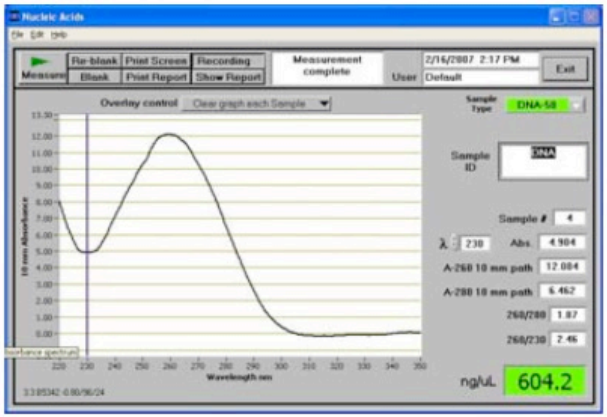
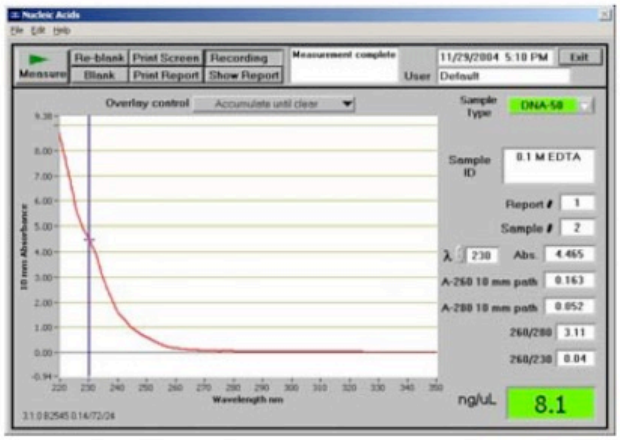
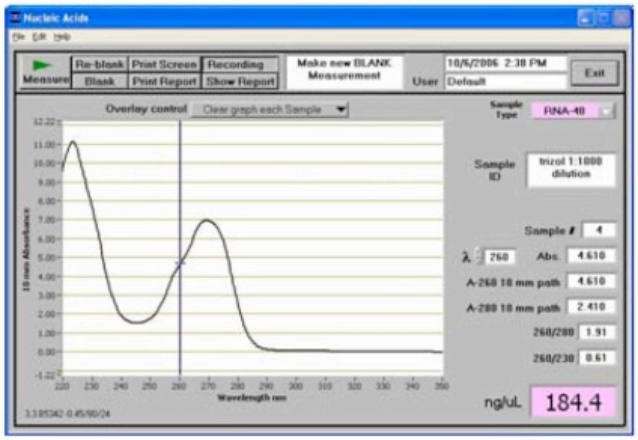



Figure 1.

- 260:280 ~1.8
- 260:230 ~2.0 - 2.2

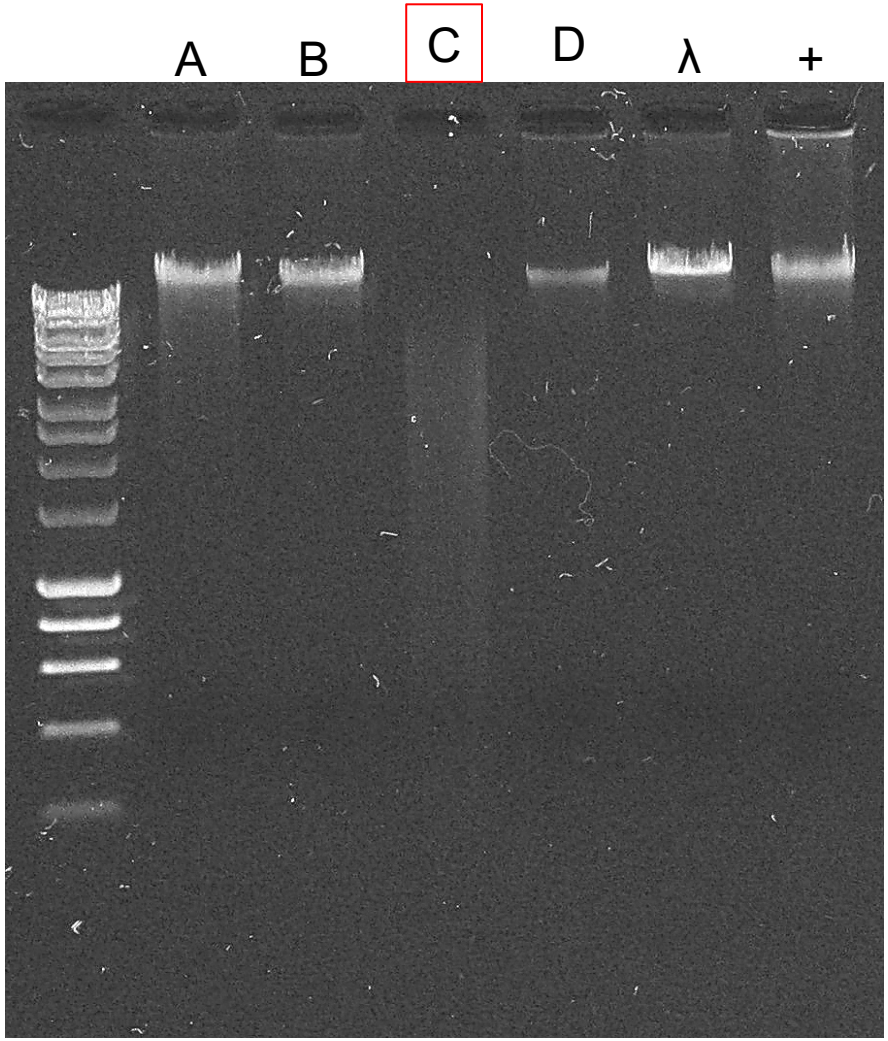
EDTA (Figure 2), carbohydrates and phenol all have absorbance near 230 nm. The TRIzol reagent is a phenolic solution which absorbs in the UV both at 230 nm and ~270 nm (Figure 3).



# *Samples for PromethION Run*

**Sample C**
260/280 1.8
260/230 2.4




*0.7% TBE agarose gel 110V for 60min*

# PCR-free library preparation, for low bias, long fragment native DNA sequencing

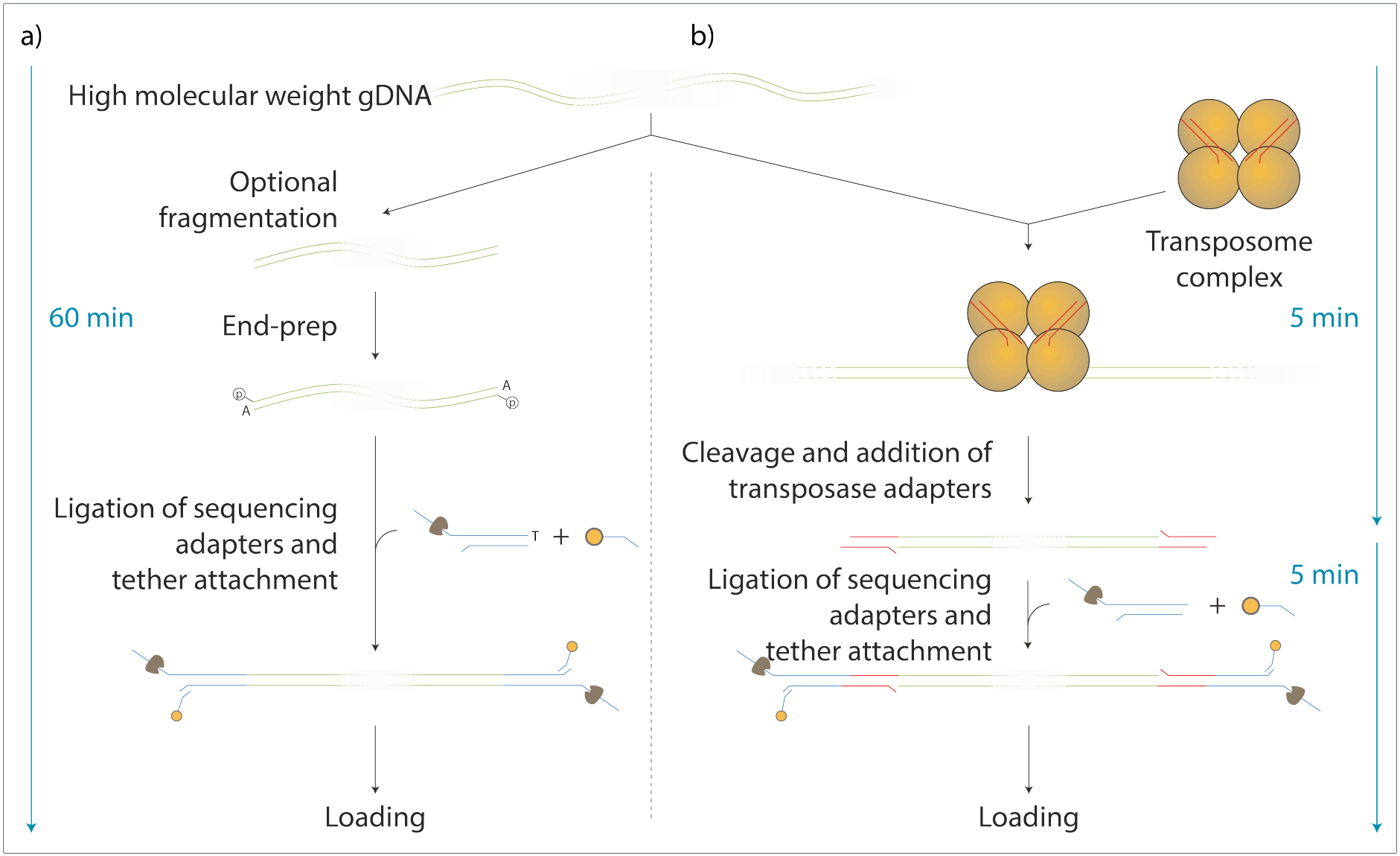
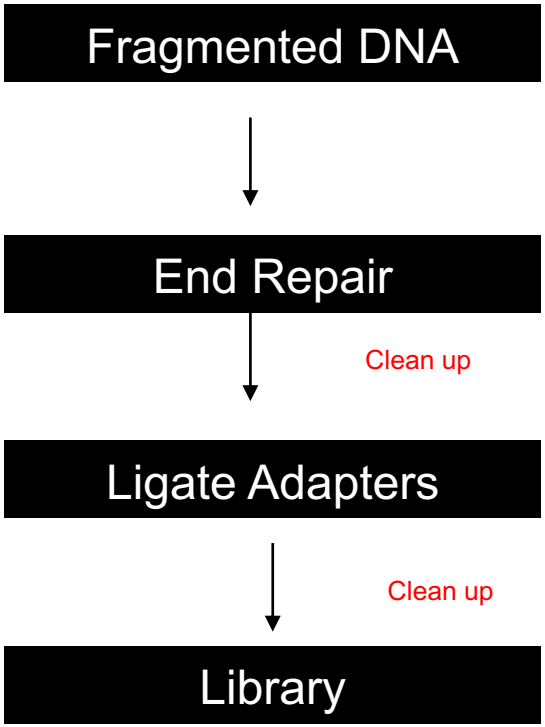
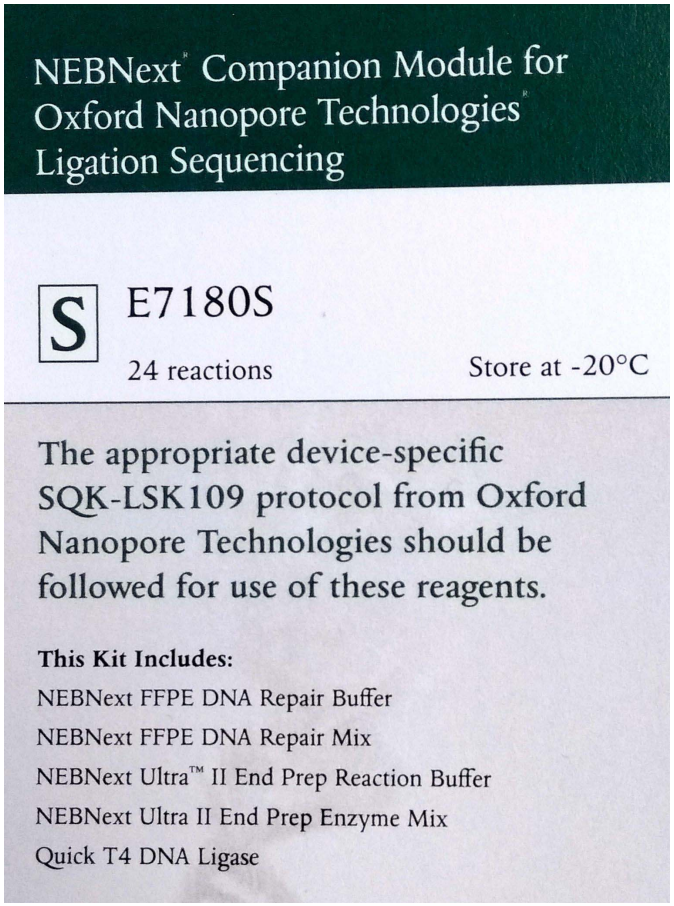



*Fig. 1 Schematic representation of PCR-free library preparation a) ligation b) rapid*

# *Genomic DNA by Ligation (SQK-LSK109)*





NEBNext® Companion Module for Oxford Nanopore Technologies® Ligation Sequencing

S E7180S

24 reactions Store at -20°C

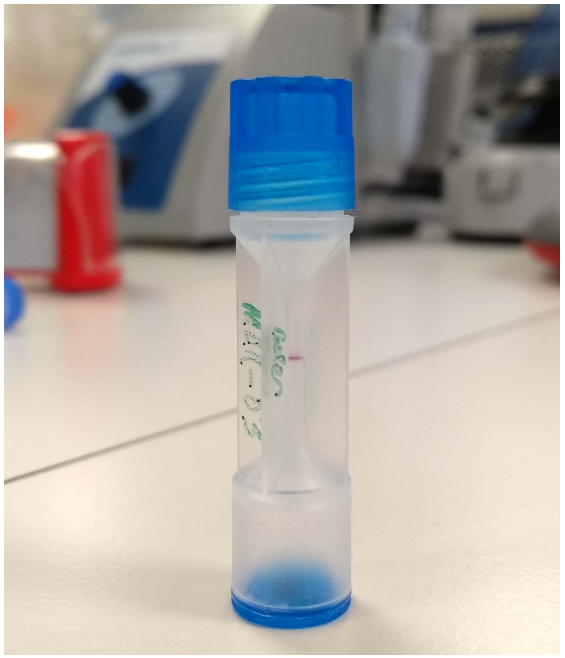
The appropriate device-specific SQK-LSK109 protocol from Oxford Nanopore Technologies should be followed for use of these reagents.

**This Kit Includes:**

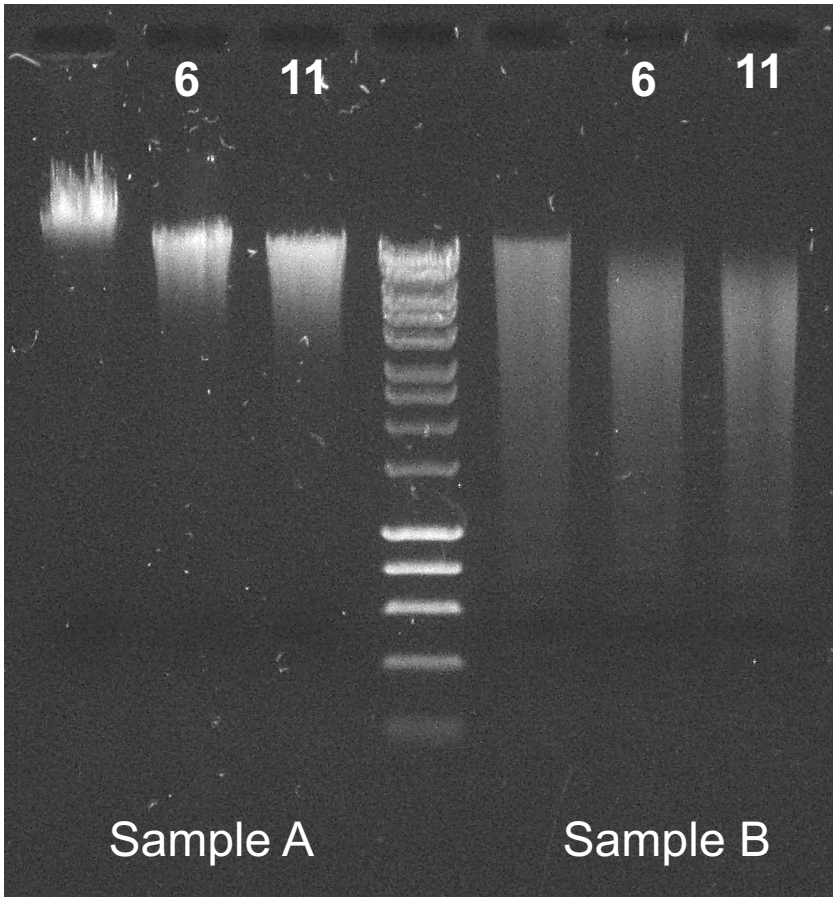
NEBNext FFPE DNA Repair Buffer
NEBNext FFPE DNA Repair Mix
NEBNext Ultra™ II End Prep Reaction Buffer
NEBNext Ultra II End Prep Enzyme Mix
Quick T4 DNA Ligase

# *Genomic DNA by Ligation (SQK-LSK109): Fragmentation*

Covaris g-Tube



- microcentrifuge
- concentration, speed, duration
- 6000rpm 1min for ~10000bp
- 11000rpm 1min for ~4000bp




*0.7% TBE agarose gel*

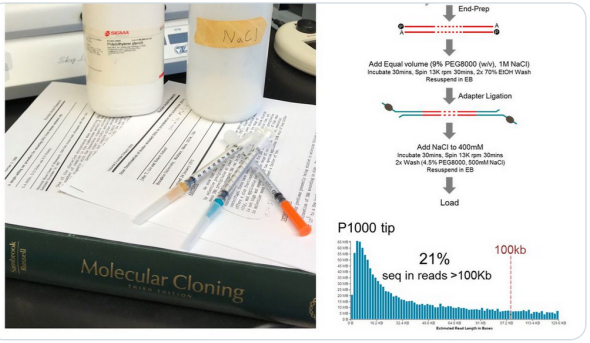
# *Genomic DNA by Ligation (SQK-LSK109): Ultra Long Protocols*

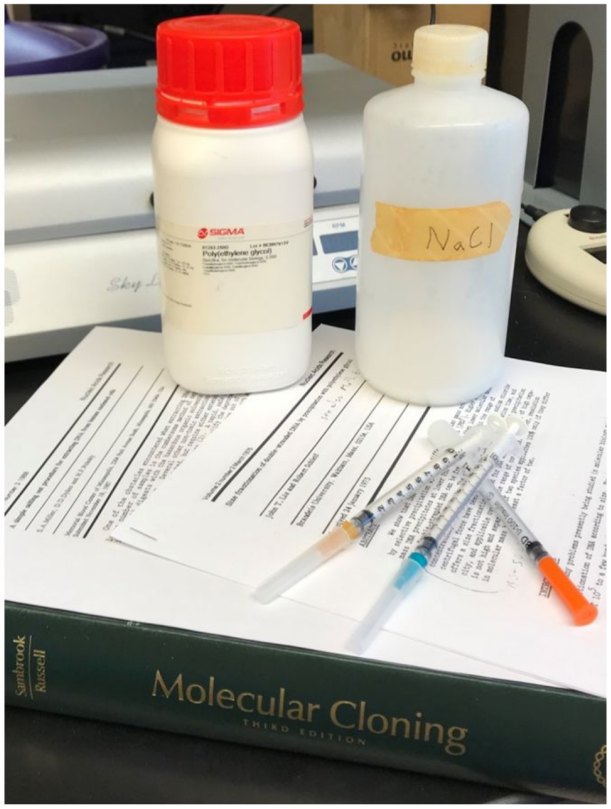
Pinned Tweet

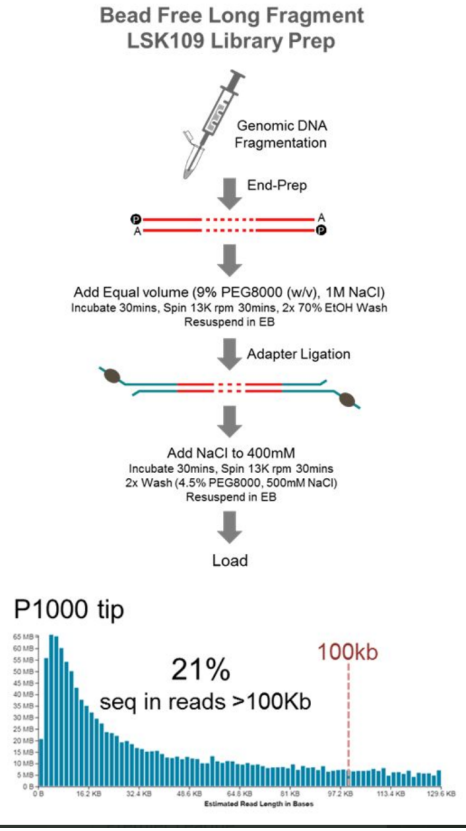
**John Tyson** @DrT1973 · 9 May 2019

Right then.... Posted our methods for HMW extraction, ligation library protocol modifications and bead-free methods for increasing 100Kb+ ultra-long reads on the @nanopore community. community.nanoporetech.com /posts/rocky-mo...

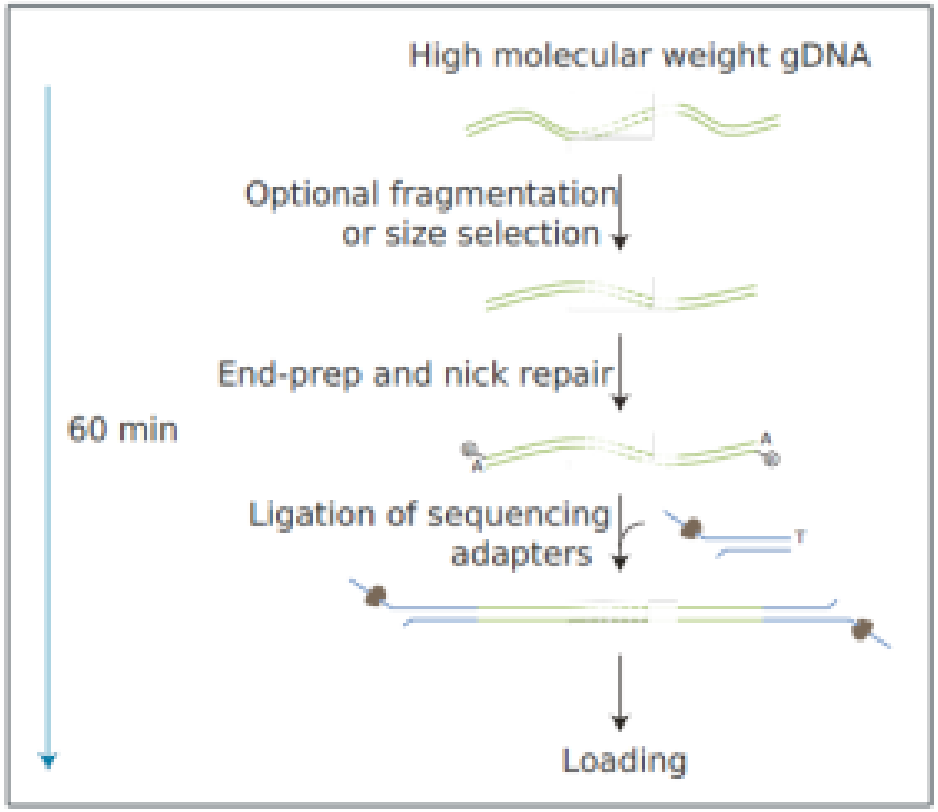
Will also be pushing to @longreadclub and @protocolsIO soon enjoy 😉



# End Repair




NEBNext® Companion Module for Oxford Nanopore Technologies® Ligation Sequencing

S E7180S

24 reactions Store at -20°C

The appropriate device-specific SQK-LSK109 protocol from Oxford Nanopore Technologies should be followed for use of these reagents.

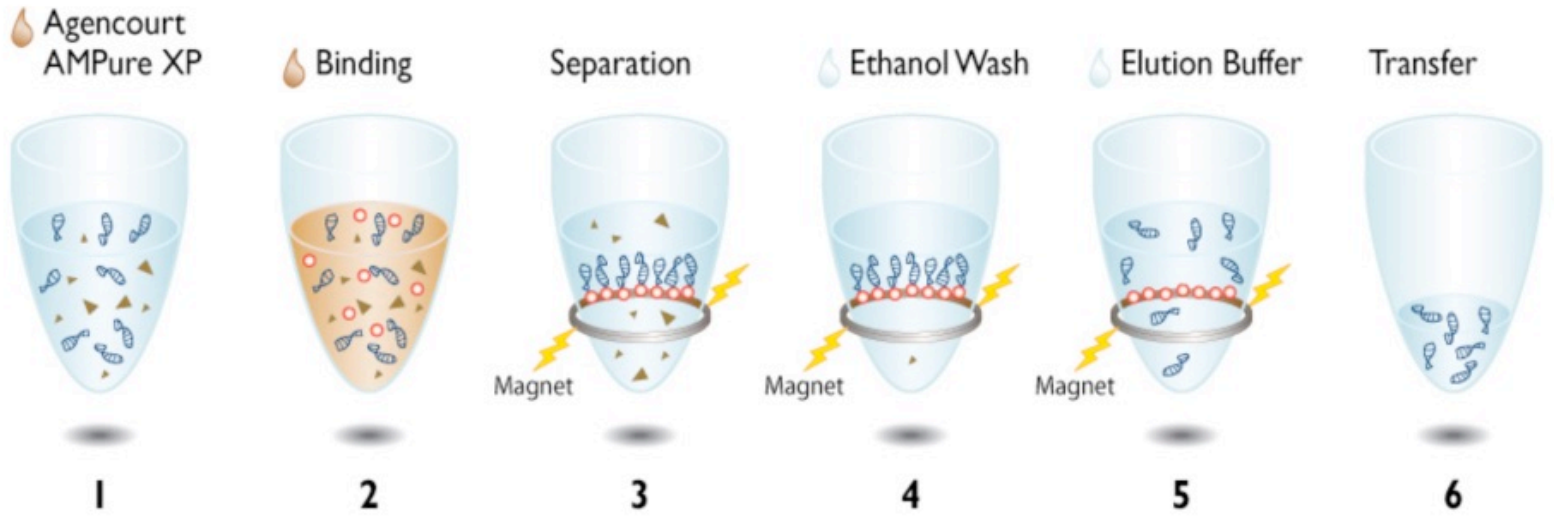
**This Kit Includes:**

NEBNext FFPE DNA Repair Buffer
NEBNext FFPE DNA Repair Mix
NEBNext Ultra™ II End Prep Reaction Buffer
NEBNext Ultra II End Prep Enzyme Mix
Quick T4 DNA Ligase

***FFPE Damage***

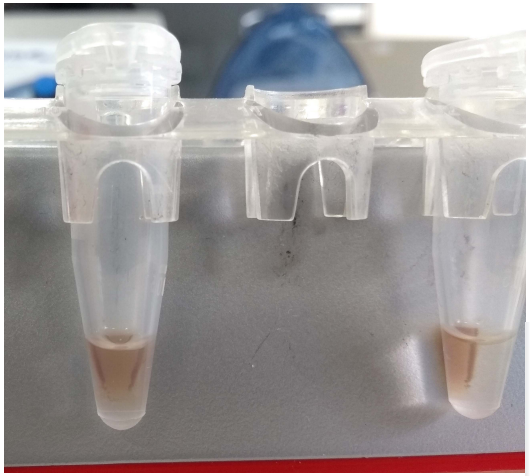
- *Deamination of cytosine to uracil*
- *Nicks and gaps*
- *Oxidized bases*
- *Blocked 3´ ends*

# *AmpureXP Bead Binding of DNA*



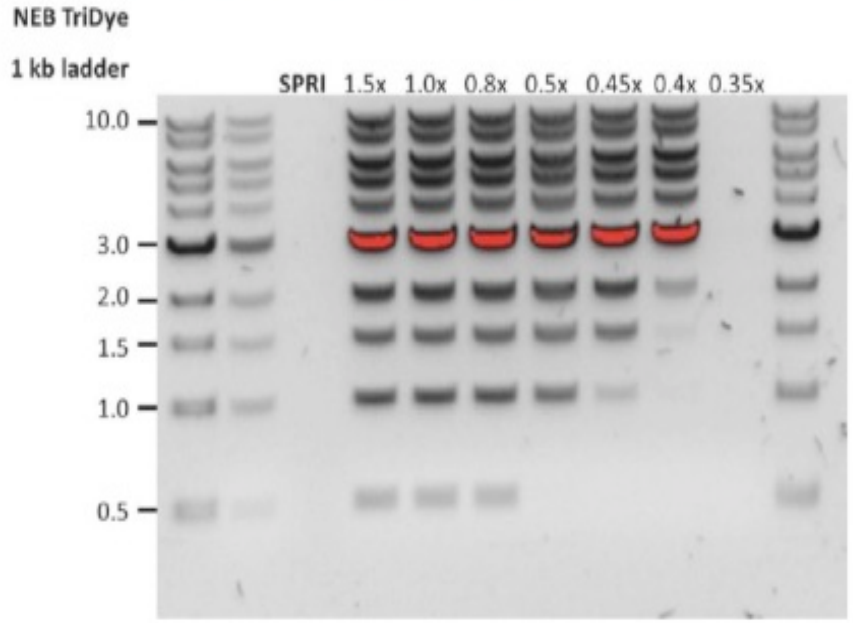

- *Automated gDNA extractions*
- *gDNA purification (buffer exchange)*
- *Size selection*

# *Genomic DNA by Ligation (SQK-LSK109): Library Clean up*



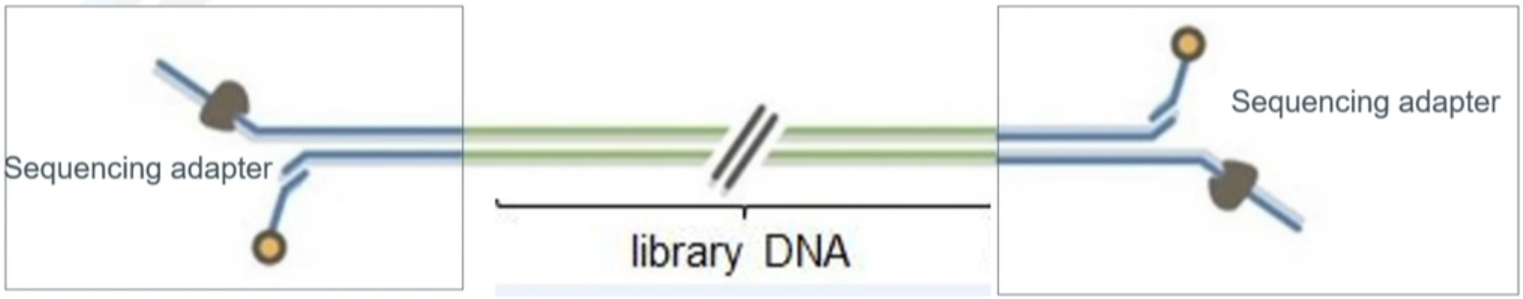
- Long Fragment Buffer
- Enrich >3000bp fragments

The lower the AMPure beads-to-sample ratio, the more stringent the selection against short fragments. Please always determine the input DNA length on an agarose gel (or other gel electrophoresis methods) and then calculate the appropriate amount of AMPure beads to use.



# *Ligation*

- Attachment of “Y-shaped” sequencing adapters
- Comprise of motor protein
- Leader sequence
- Facilitate tethering / capture




- *Quick T4 DNA ligase*
- *Motor protein (helicase)*
- *400 bases/sec*
- *Tethering*
- *Increased throughput e.g 10000x*