

Sample Preparation

Sample Preparation

Sample type

PCR-free

Multiplexing

15 results

DNA RNA PCR PCR-Free No Yes Clear filters

[Ligation Sequencing Kit](#)

SQK-LSK109



A versatile sequencing kit optimised for throughput

i 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.

i 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.

i Includes a Flow Cell Priming Kit

i 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

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Knowledge Exchange: Cas9 PCR-free enrichment

Join Anne-Marie on 27th February for an introduction to PCR-free target enrichment using CRISPR/Cas9 for nanopore sequencing

Featured posts

Nanopore Digest: the latest from the nanopore community (30t...

Katie Nice
Oxford Nanopore Technolo...

MinION Mk 1C January update

Jonathan Pugh (...
Oxford Nanopore Technolo...

MinKNOW 19.12.6 Patch Release for GRIDION

Richard Ronan
Oxford Nanopore Technolo...



Protocol builder

Plan your entire sequencing experiment using the interactive protocol builder tool



Protocol library

Directory of Protocols which guide you through the experiment process

Posts

All

Latest (2)

Featured

Popular

- Nanopore Digest: the latest from the nanopore community (30th January 2019)**
Highly multiplexed single-cell full-length cDNA sequencing of human immune cells with 1...
Posted in General Discussion (0 comments • 1h)
- Guppy resume and duplicate reads in fastqs**
I have seen a few threads discussing that for whatever reason guppy may rebasecall a re...
Posted in General Discussion (0 comments • 1h)
- Fragmentation Mix for MinION DNA Sequencer**
Hi! Recently we have been planning to run the Lambda Control on our MinION DNA Sequence...
Posted in Getting Started (2 comments • 17h)

Katie Nice

Clinton Paden

Saad bhamla

Clinton Paden

Jonathan Stephens
Profile 52% complete

University of Cambridge
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Rank	User	Last 90 days
1st	Maximilian Krause	700
2nd	Marc RübSam	670

Nanopore Protocol

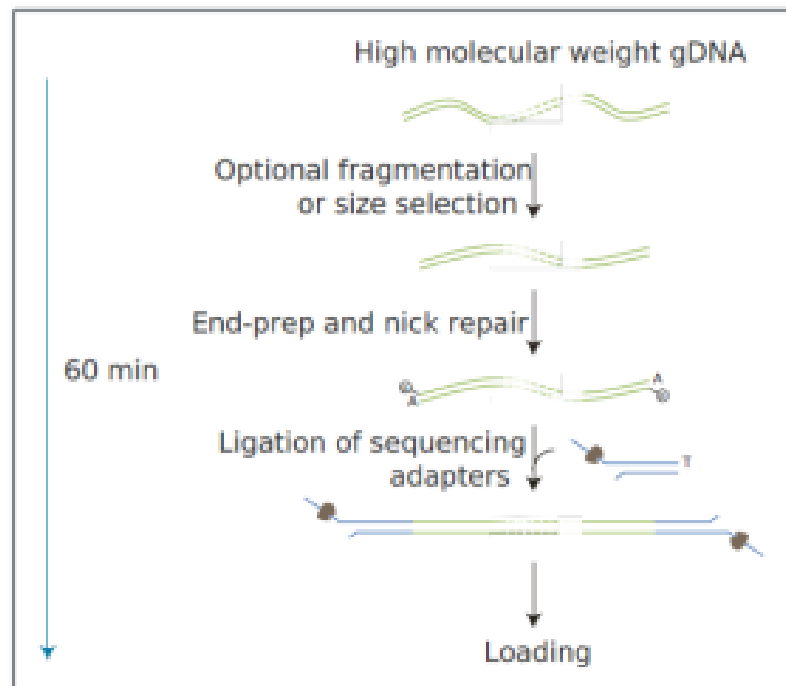
Genomic DNA by Ligation (SQK-LSK109)

Equipment and consumables

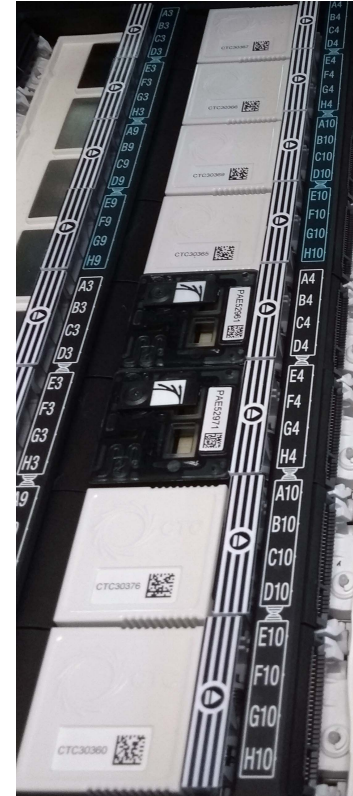
Version: GDE_9063_v109_revS_14Aug2019

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

- 1 μ g (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

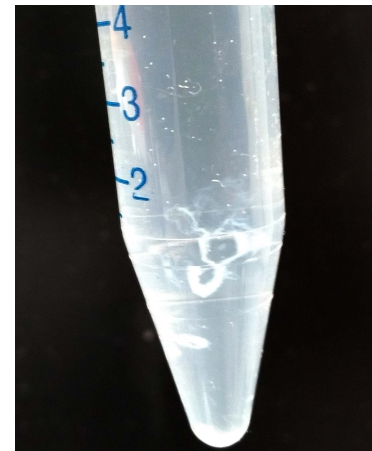
Chloroform
Extraction



Aqueous layer

Ethanol
precipitation

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



DNA Quality Control

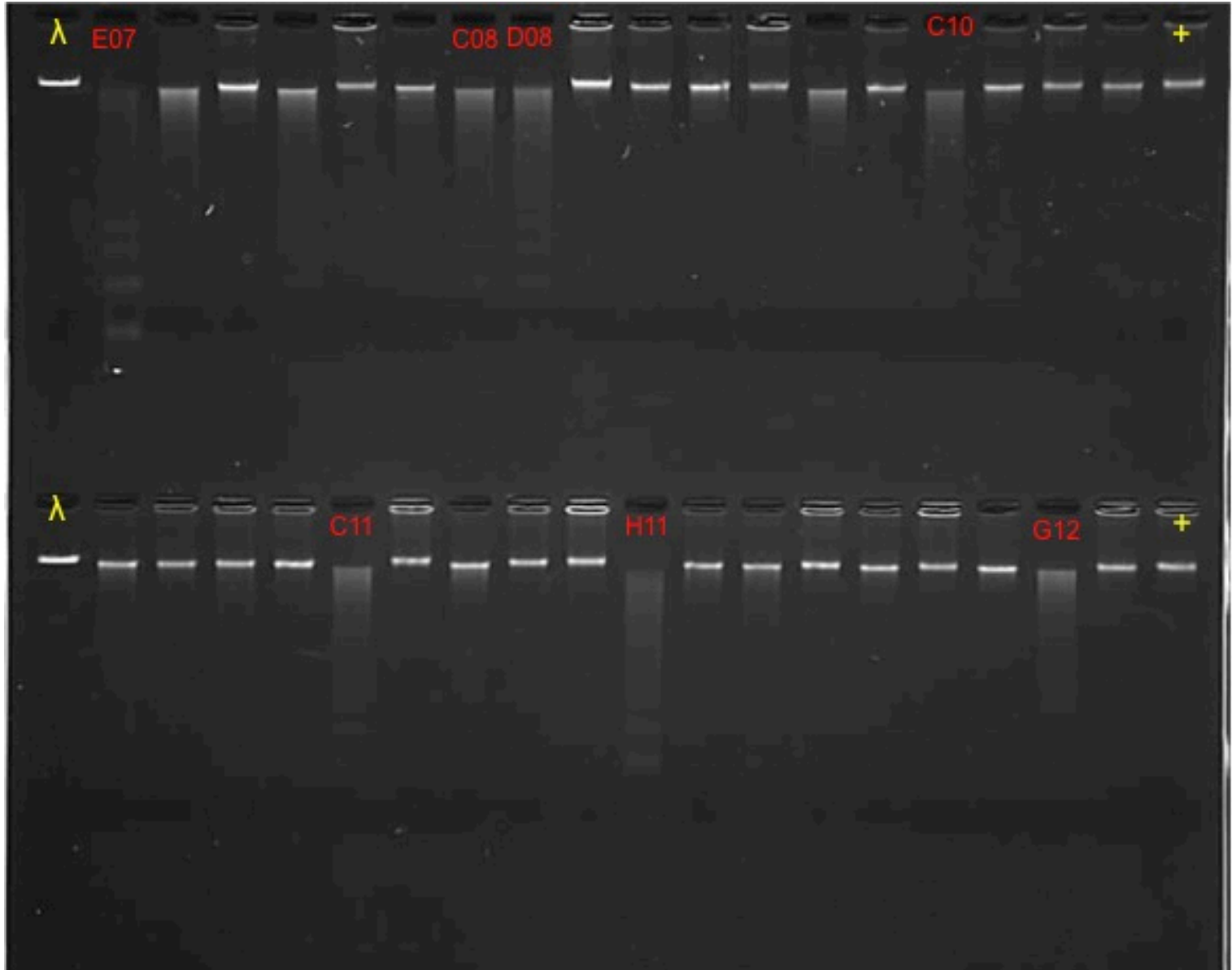
Quantification

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

DNA Quality

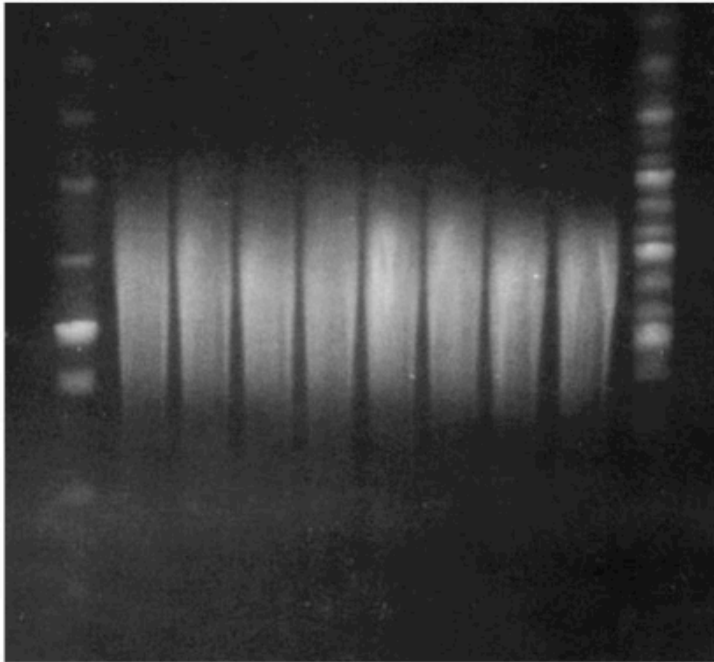
- *Agarose gel electrophoresis*
- *Nanodrop spectrophotometer*
- *Agilent Bioanalyser (12000bp)*
- *Agilent Tapestation (60000bp)*

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



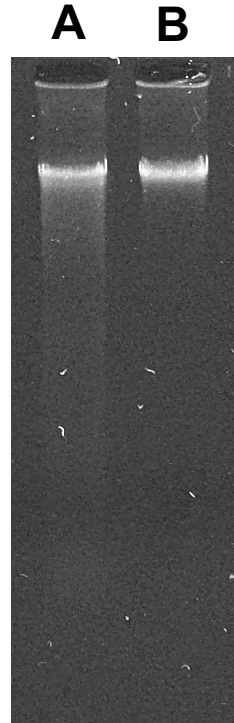
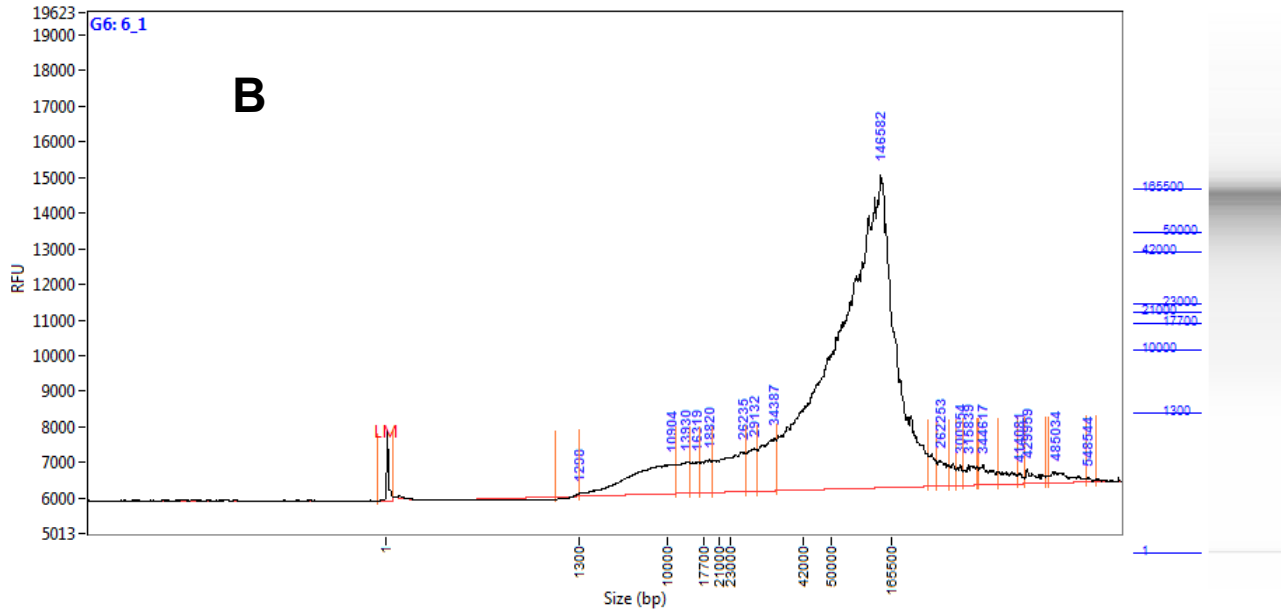
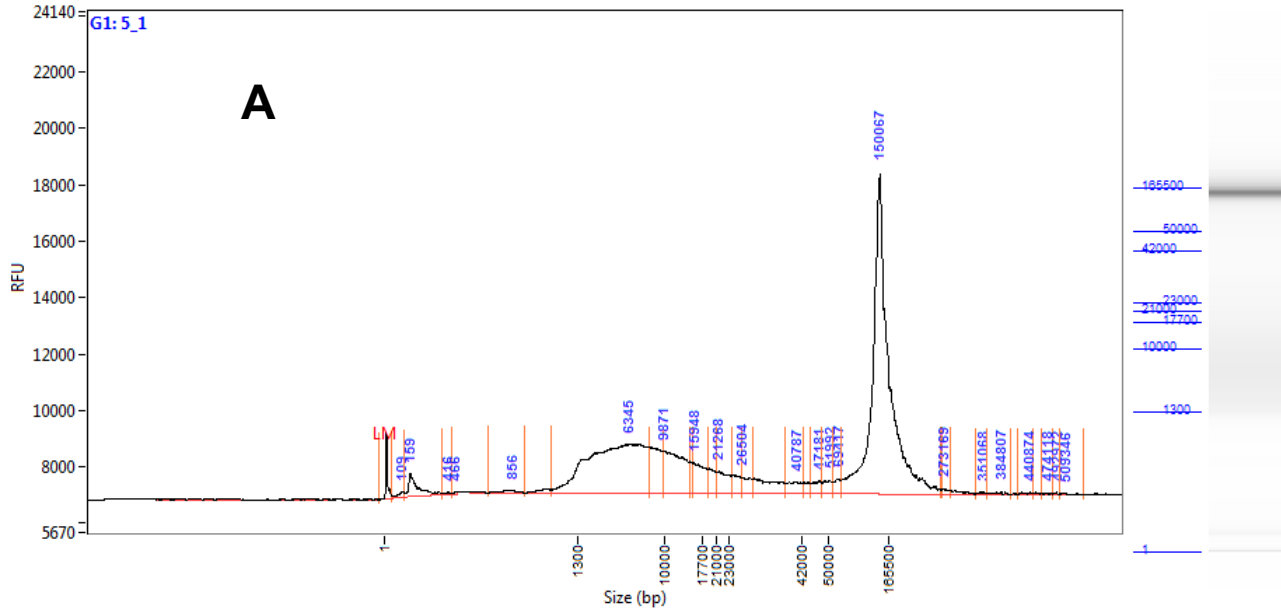
For isolation of up to 500 µg high-molecular-weight DNA from a wide range of samples

- 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

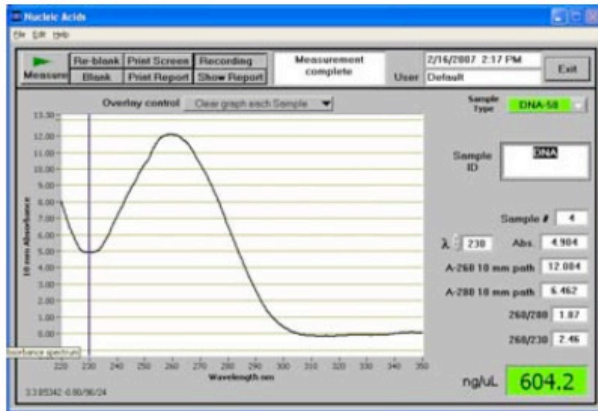
average size of 50–100 kb.

Femto pulse sizing of genomic DNA



Nanodrop For DNA Quality Control

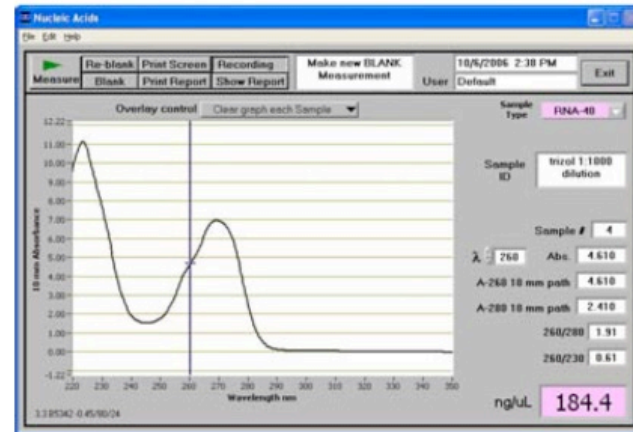
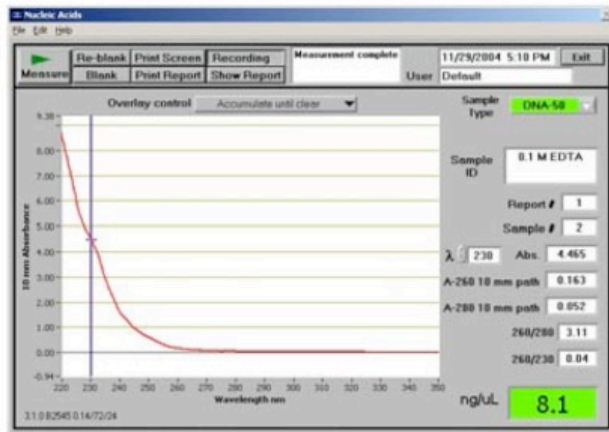
Typical spectral pattern for Nucleic Acid (Figure 1)



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

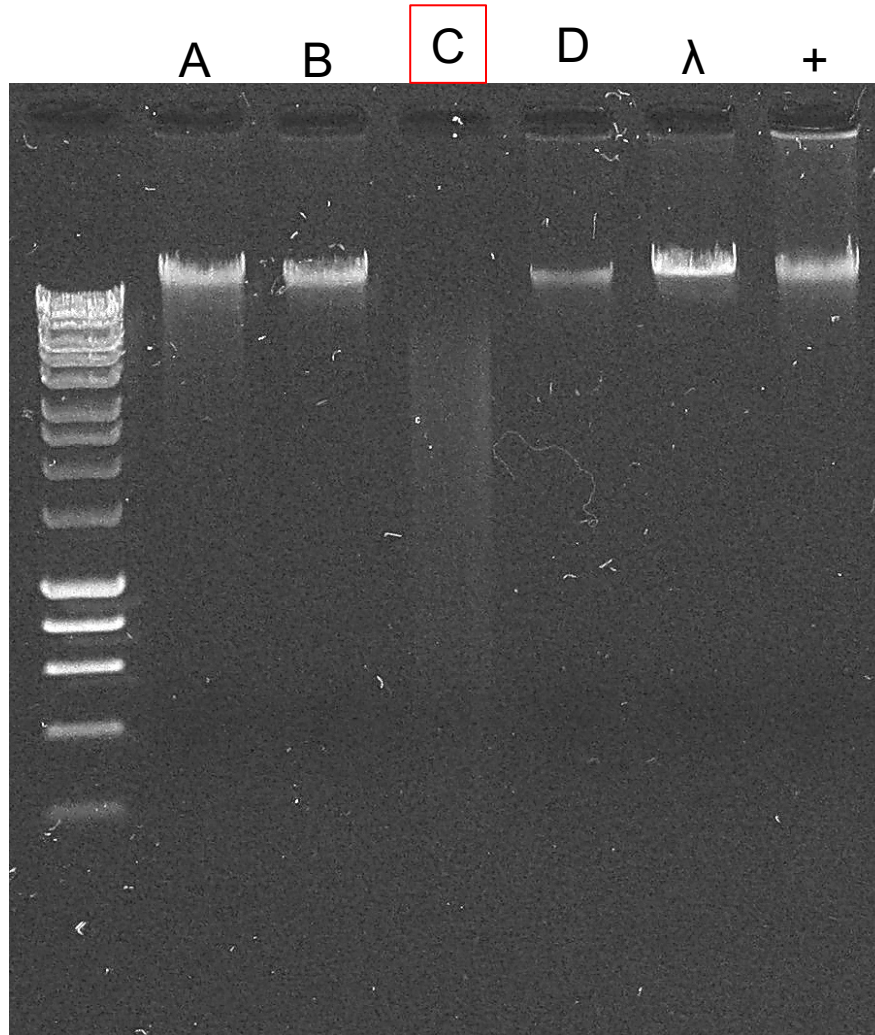
Figure 1.

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

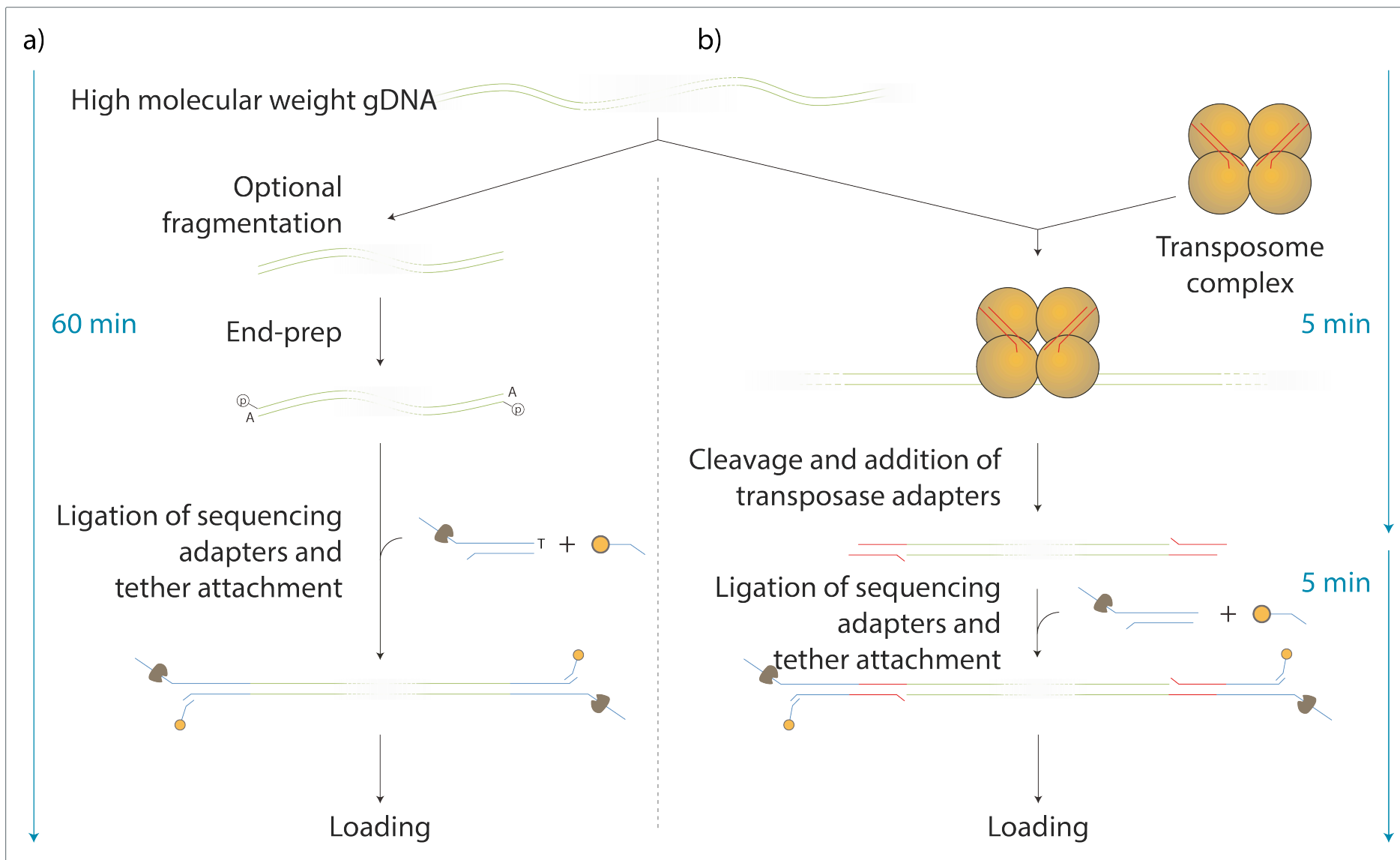
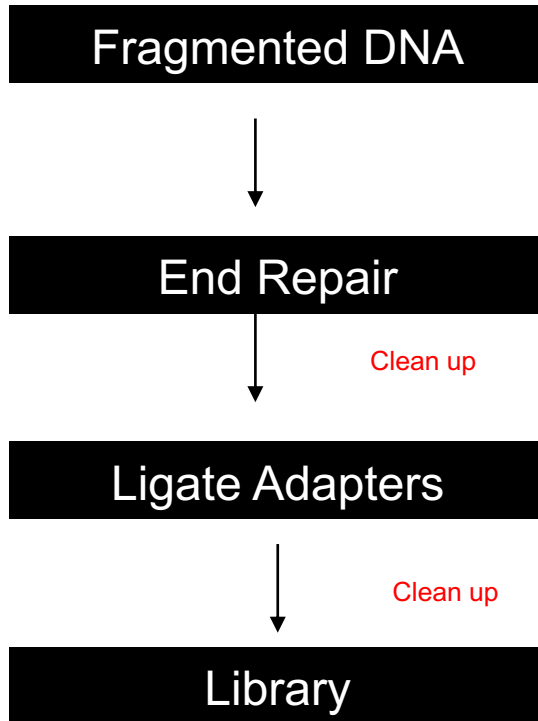


Fig. 1 Schematic representation of PCR-free library preparation a) ligation b) rapid

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:

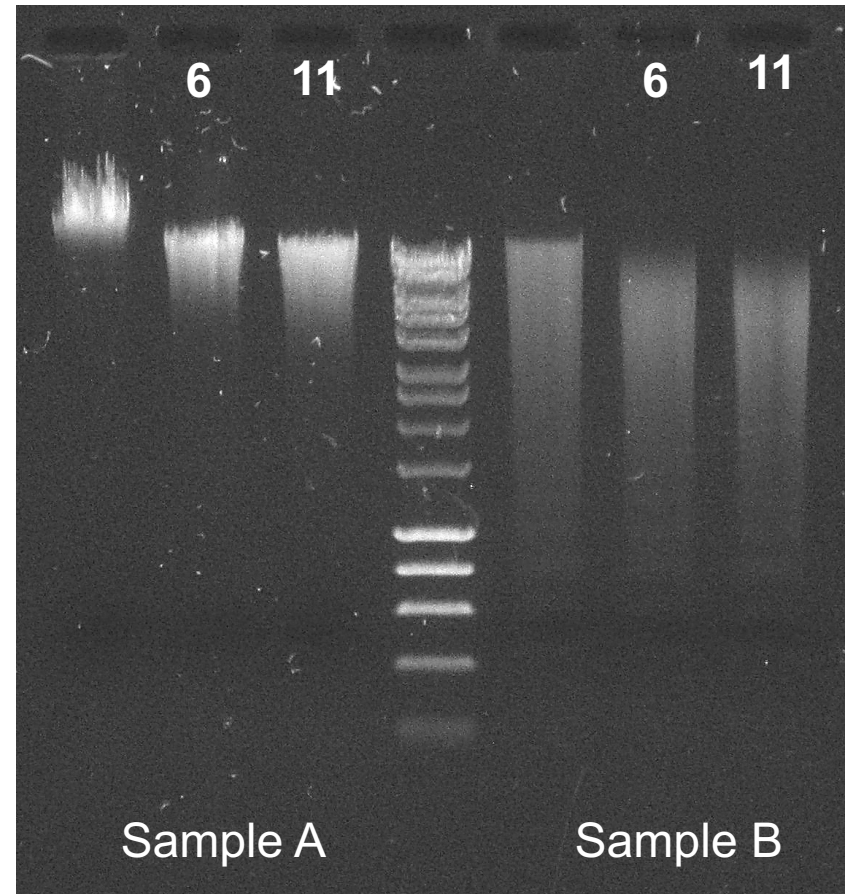
- NEBNext FFPE DNA Repair Buffer
- NEBNext FFPE DNA Repair Mix
- NEBNext Ultra™ II End Prep Reaction Buffer
- NEBNext Ultra II End Prep Enzyme Mix
- Quick T4 DNA Ligase

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

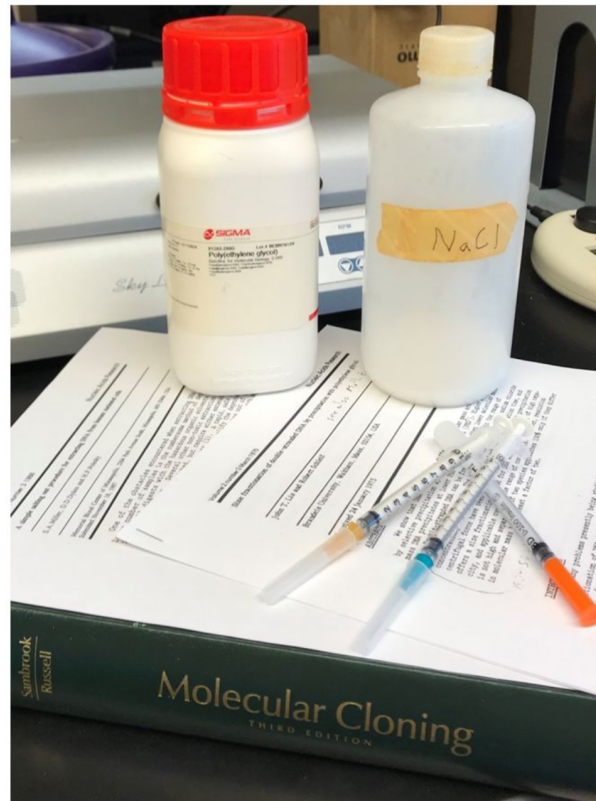
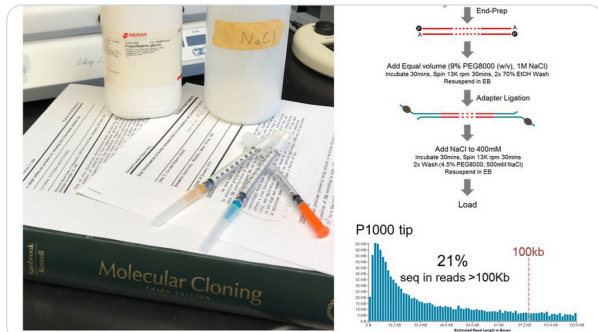
Pinned Tweet



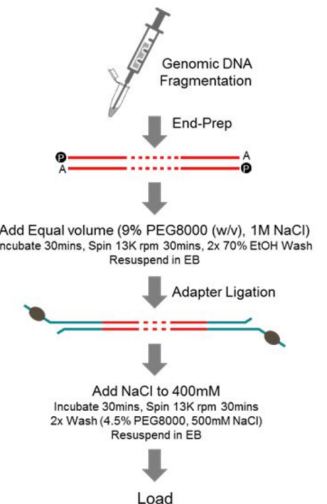
John Tyson @DrT1973 · 9 May 2019

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...

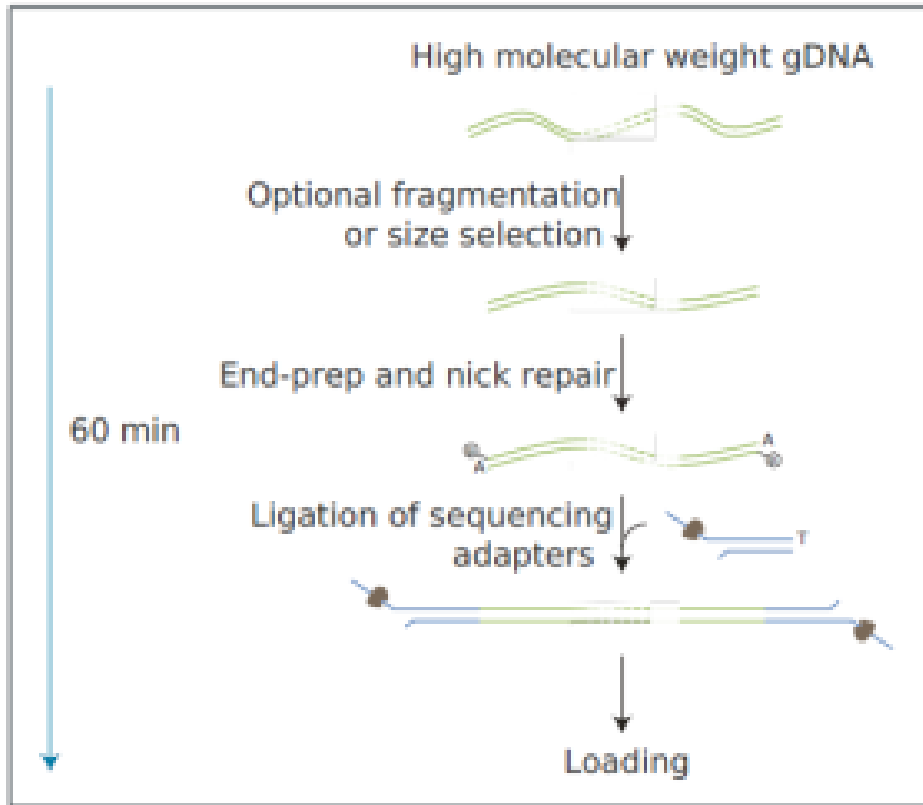
Will also be pushing to @longreadclub and @protocolsIO soon enjoy 😊



Bead Free Long Fragment LSK109 Library Prep



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

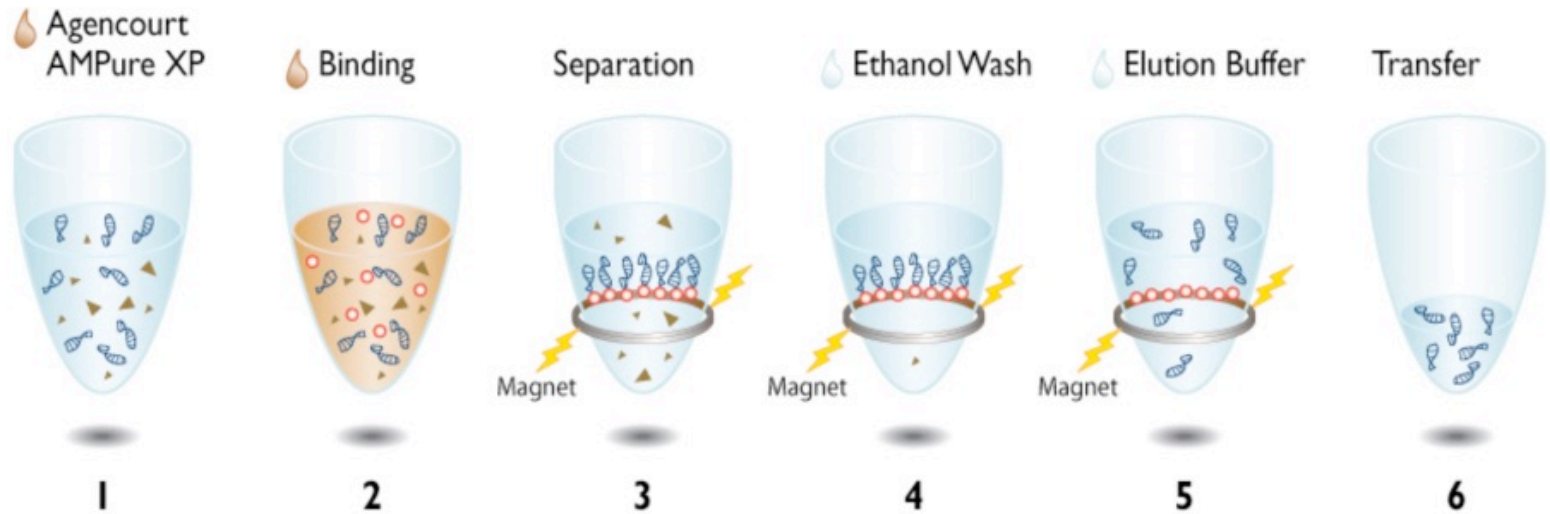
NEBNext Ultra II End Prep Enzyme Mix

Quick T4 DNA Ligase

FFPE Damage

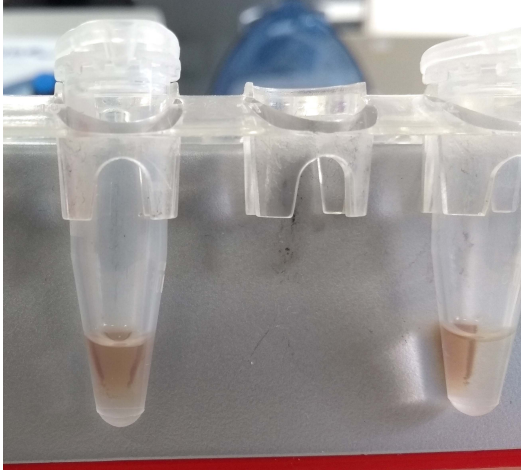
- *Deamination of cytosine to uracil*
- *Nicks and gaps*
- *Oxidized bases*
- *Blocked 3' ends*

AmpureXP Bead Binding of DNA



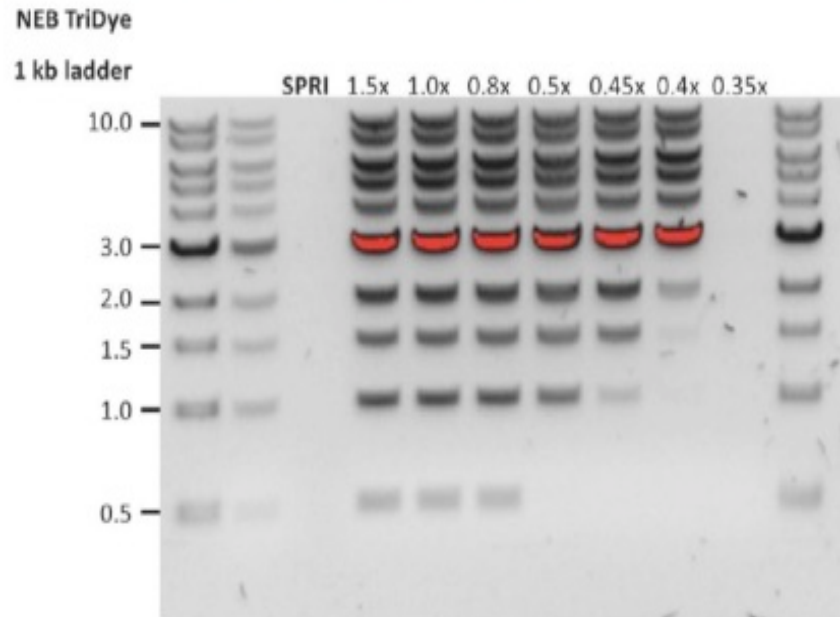
- *Automated gDNA extractions*
- *gDNA purification (buffer exchange)*
- *Size selection*

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



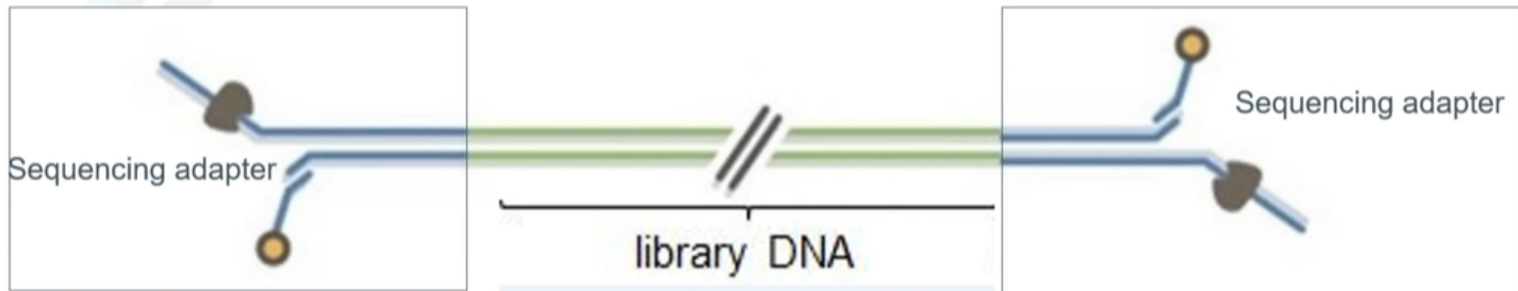
- Long Fragment Buffer
- Enrich >3000bp fragments

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
- Comprised of motor protein
- Leader sequence
- Facilitate tethering / capture



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