

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

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...)** by Katie Nice
- MinION Mk 1C January update** by Jonathan Pugh (...)
- MinKNOW 19.12.6 Patch Release for GridION** by Richard Ronan

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)** by Katie Nice
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** by Clinton Paden
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** by Saad bhamia
Hi! Recently we have been planning to run the Lambda Control on our MinION DNA Sequence...
Posted in Getting Started (2 comments • 17h)

Jonathan Stephens
Profile 52% complete
University of Cambridge Full Member
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Flow Cell & Device Returns

Kudos Leaderboard

Rank	User	Last 90 days
1st	Maximilian Krause	700
2nd	Marc Rübsam	670

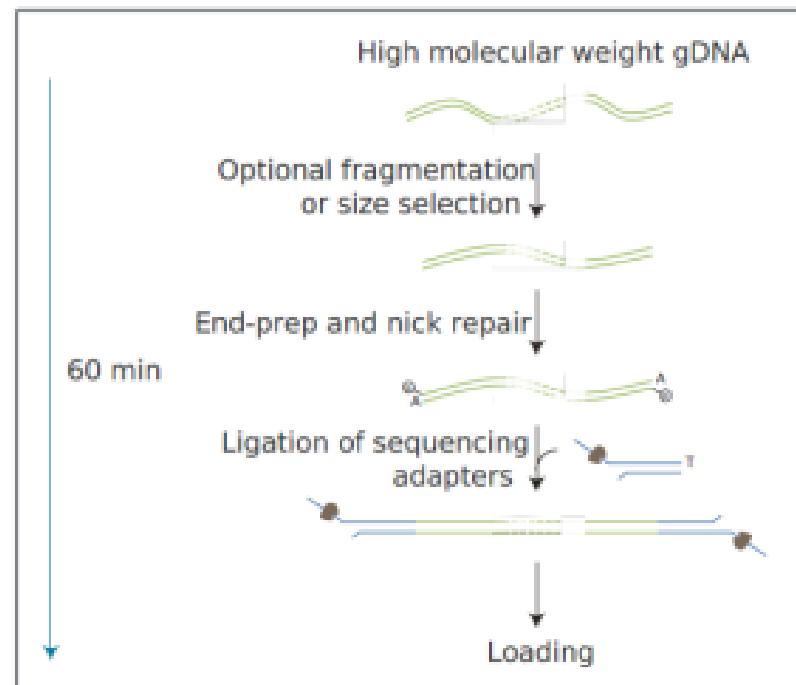
Nanopore Protocol

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



MinION Flow Cell



**PromethION
Flow Cell**



Genomic DNA extraction from Blood

Red Cell Lysis



*WBC
pellet*

**Lysis,
Protein
Digestion**

Guanidine HCl/Sarcosyl/Proteinase K

**Chloroform
Extraction**



Aqueous layer

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



**Ethanol
precipitation**

DNA Quality Control

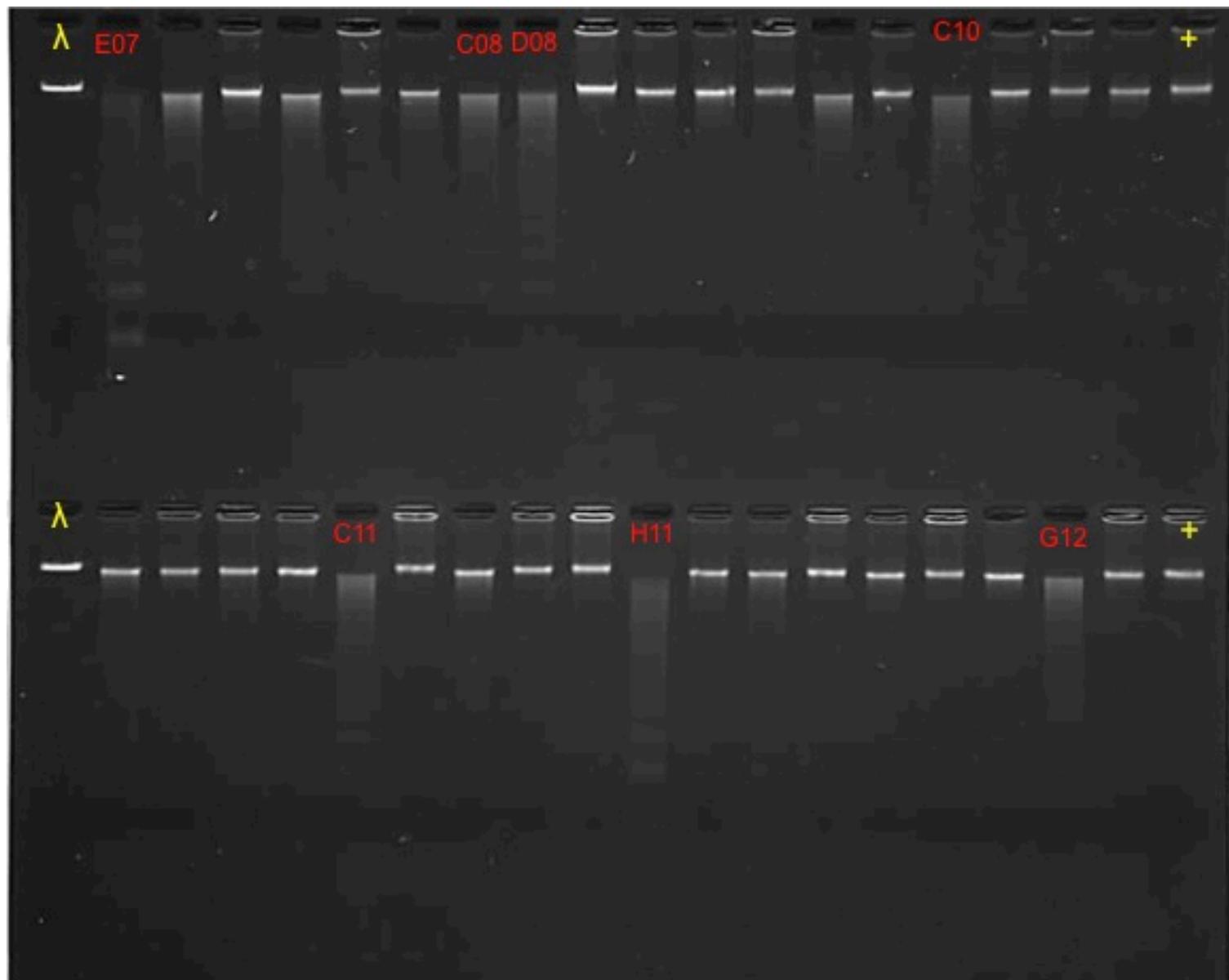
Quantification

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

DNA Quality

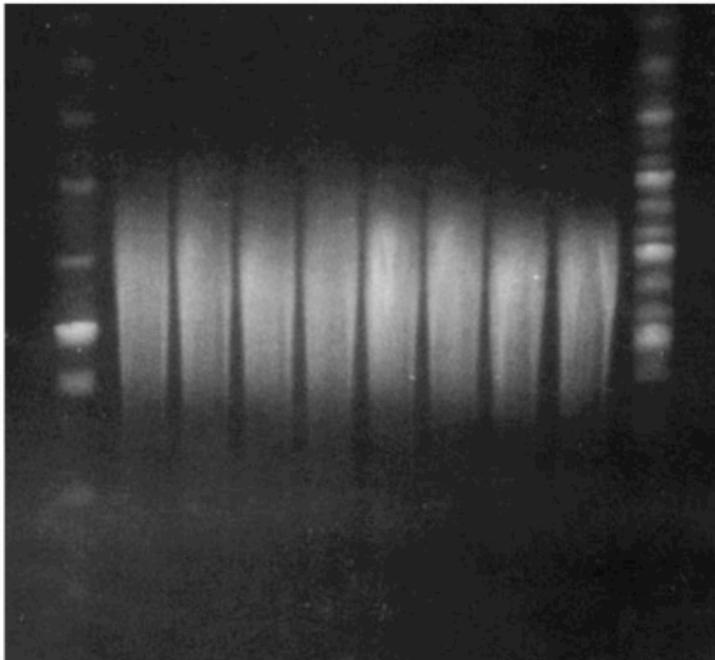
- *Agarose gel electrophoresis*
- *Nanodrop spectrophotometer*
- *Agilent Bioanalyser (12000bp)*
- *Agilent Tapestation (60000bp)*

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



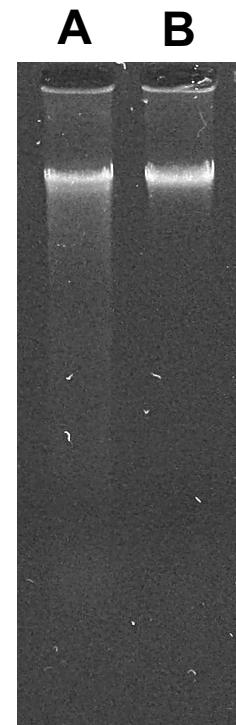
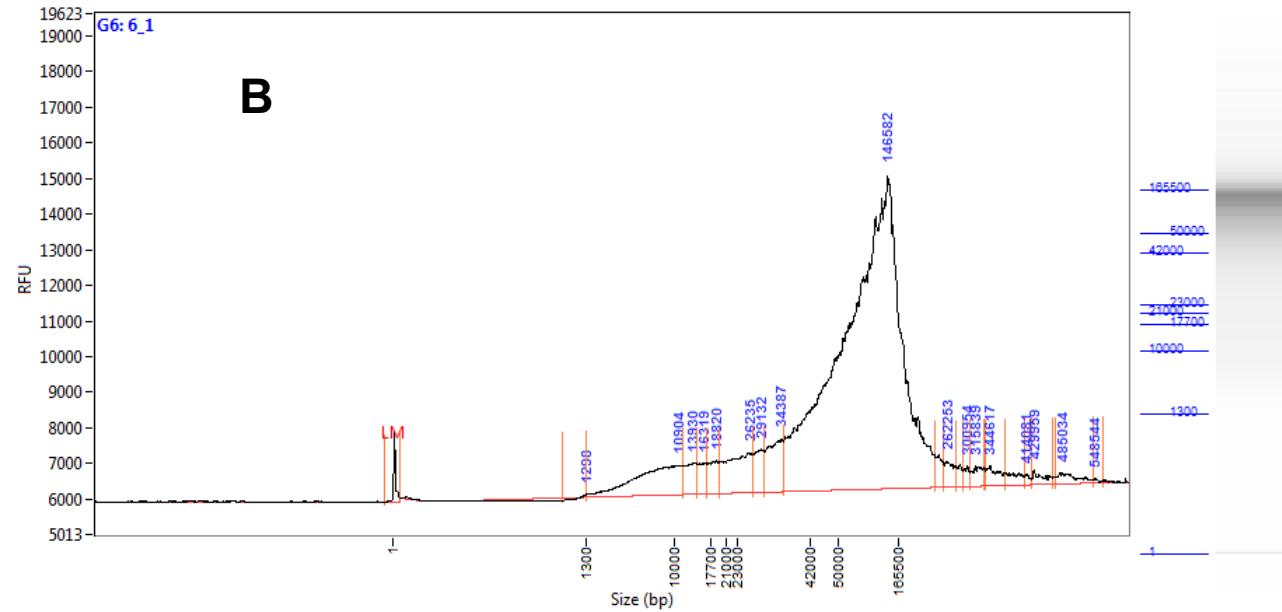
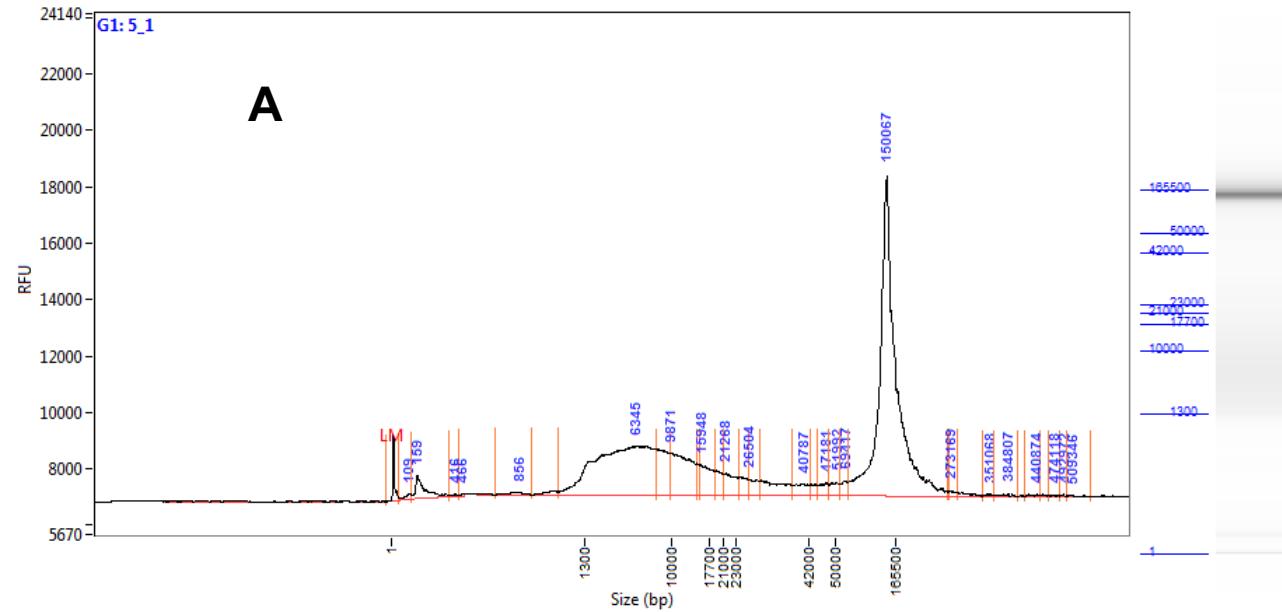
average size of 50–100 kb.

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

Femto pulse sizing of genomic DNA



Nanodrop For DNA Quality Control

Typical spectral pattern for Nucleic Acid (Figure 1)

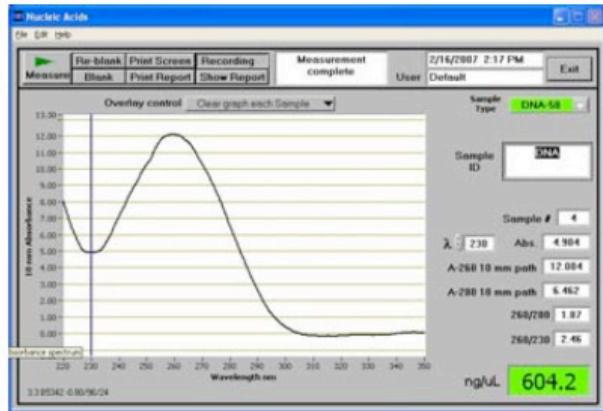
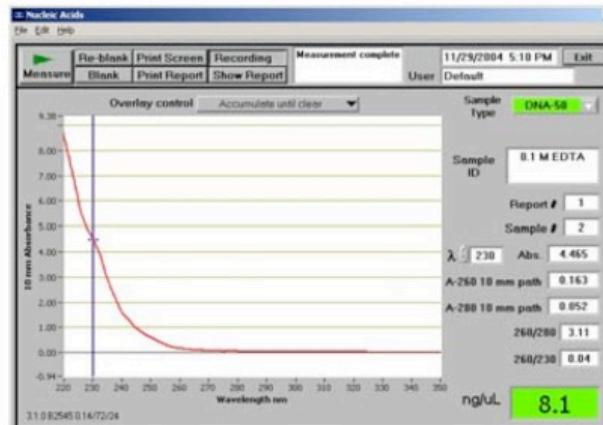
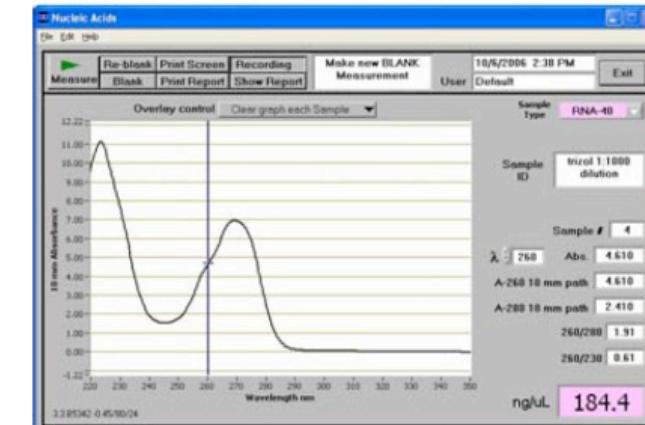


Figure 1.

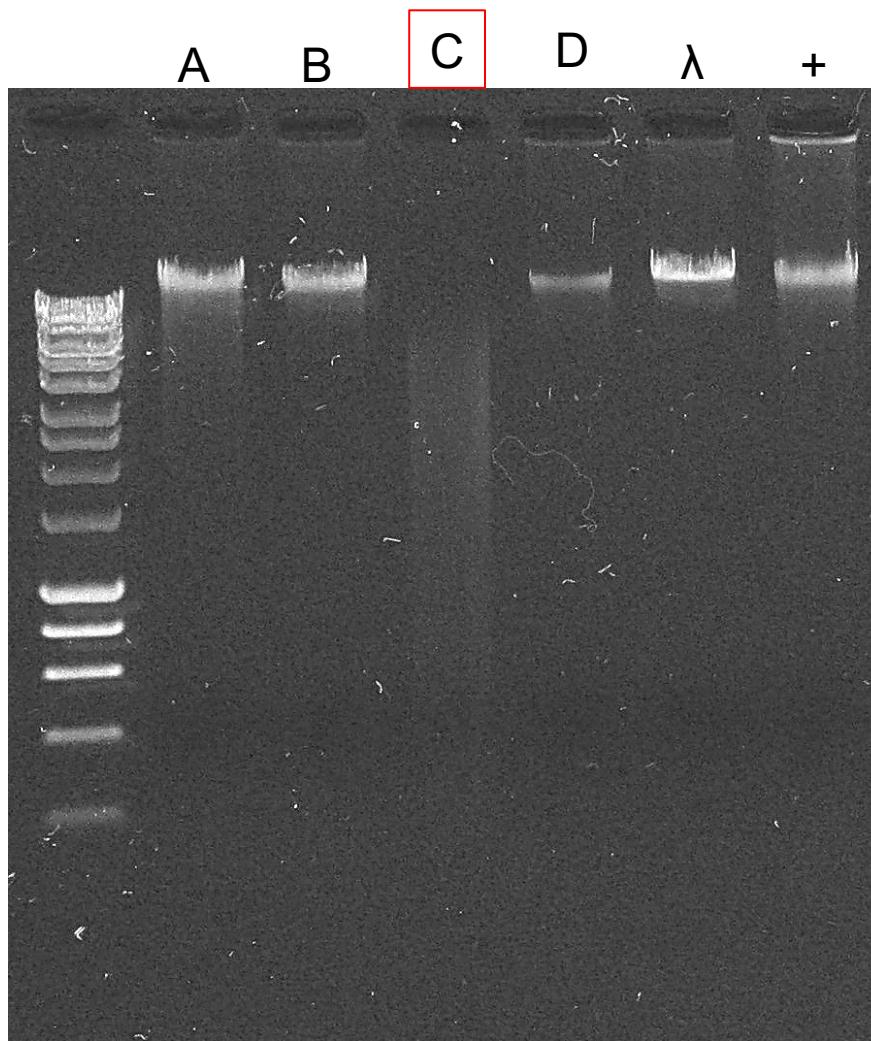
EDTA (Figure 2), carbohydrates and phenol all have absorbance near 230 nm. The TRIzol reagent is a phenolic solution which absorbs in the UV both at 230 nm and ~ 270 nm (Figure 3).



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



Samples for PromethION Run

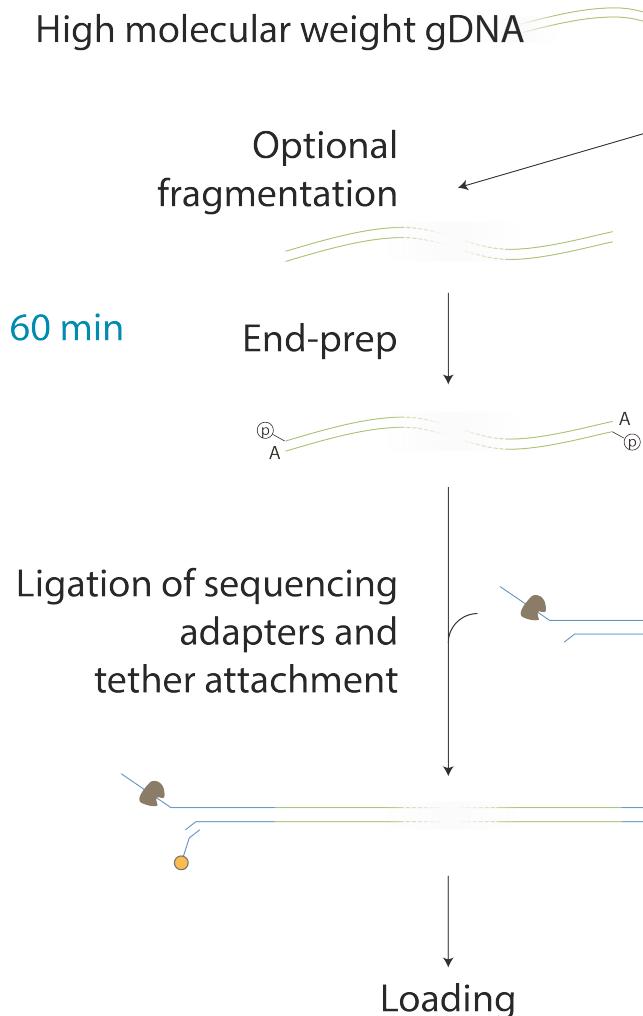


Sample C
260/280 1.8
260/230 2.4

0.7% TBE agarose gel 110V for 60min

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

a)



b)

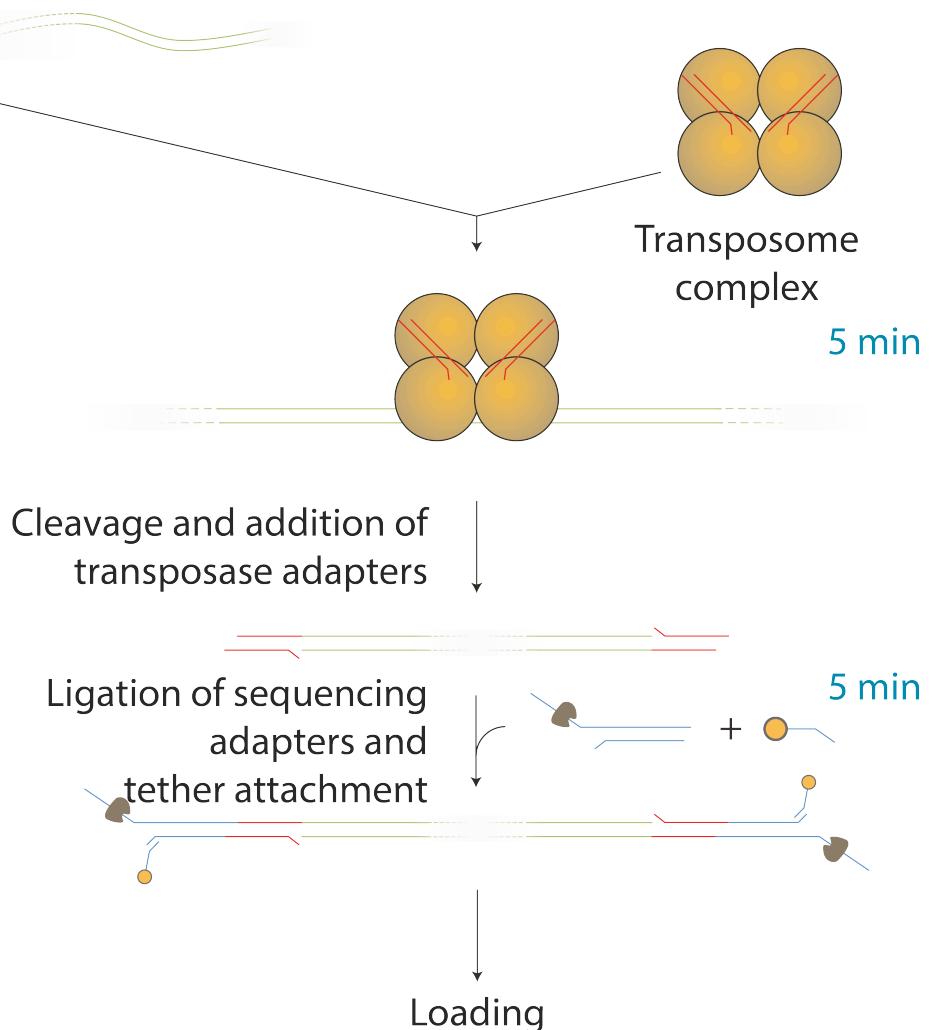
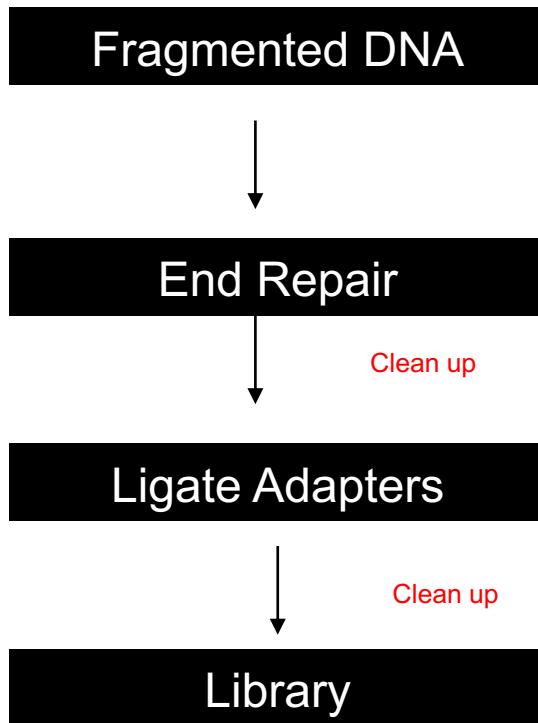


Fig. 1 Schematic representation of PCR-free library preparation a) ligation b) rapid

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:

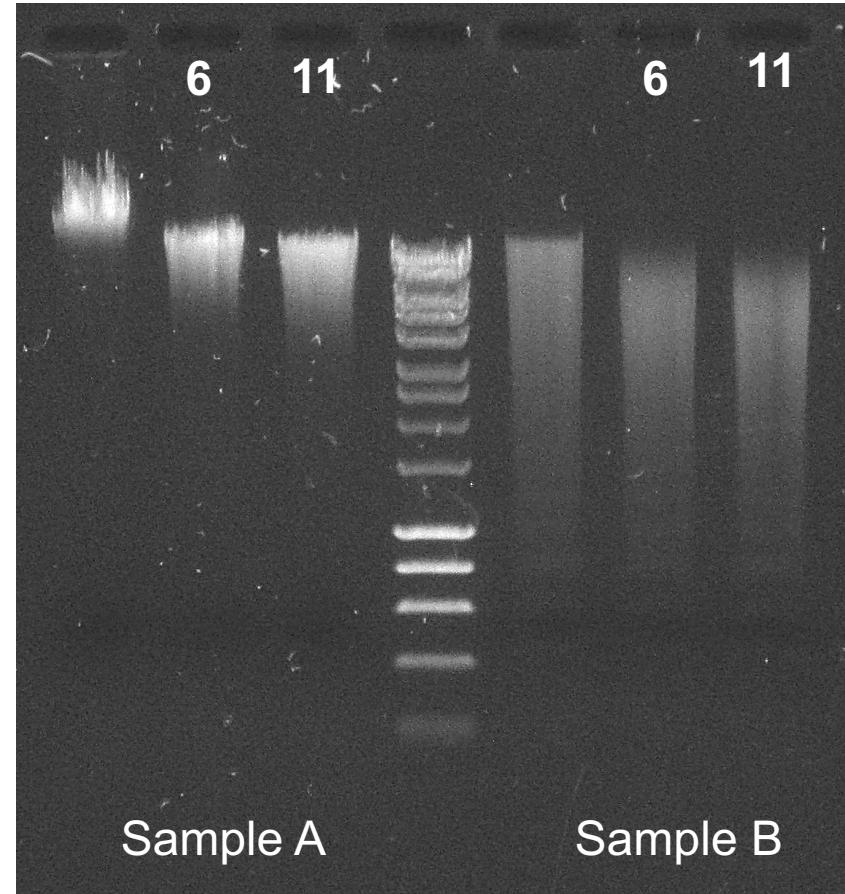
NEBNext FFPE DNA Repair Buffer
NEBNext FFPE DNA Repair Mix
NEBNext Ultra™ II End Prep Reaction Buffer
NEBNext Ultra II End Prep Enzyme Mix
Quick T4 DNA Ligase

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

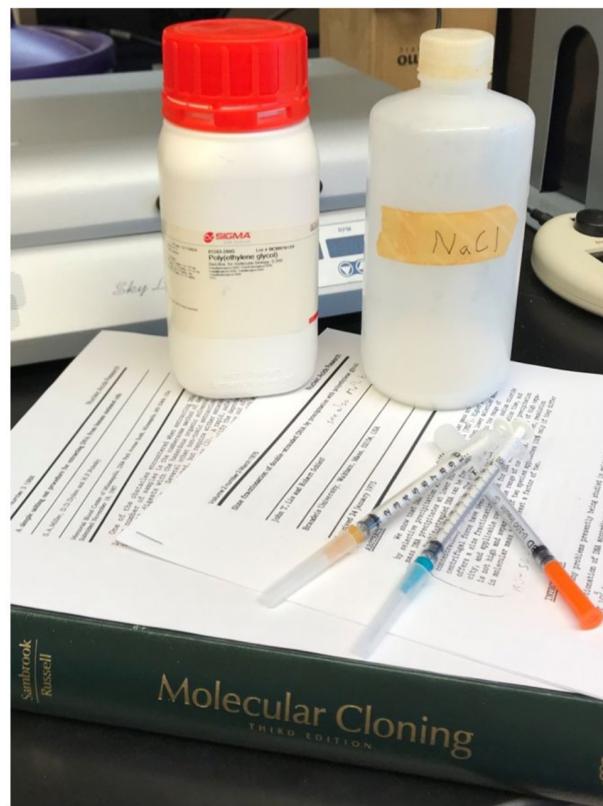
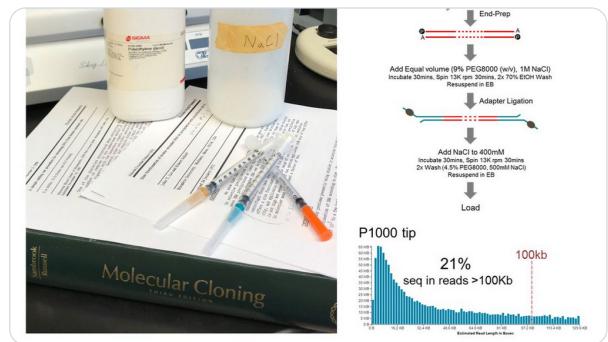
Pinned Tweet



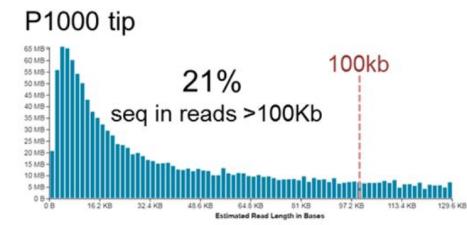
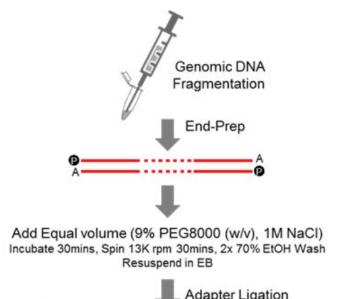
John Tyson @DrT1973 · 9 May 2019

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...](http://community.nanoporetech.com/posts/rocky-mo...)

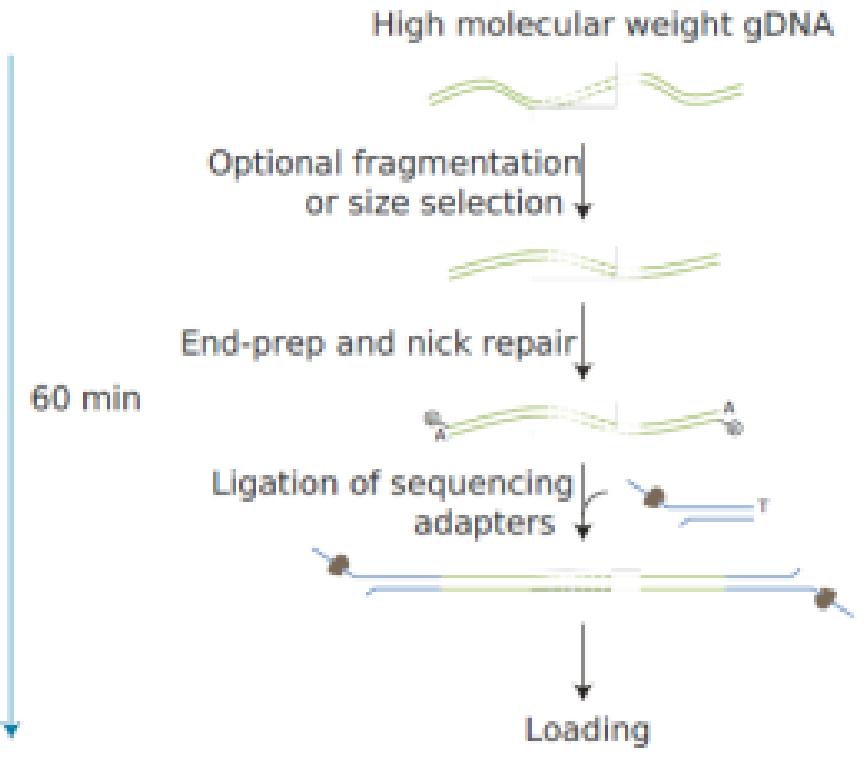
Will also be pushing to @longreadclub and @protocolsIO soon enjoy 😊



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.

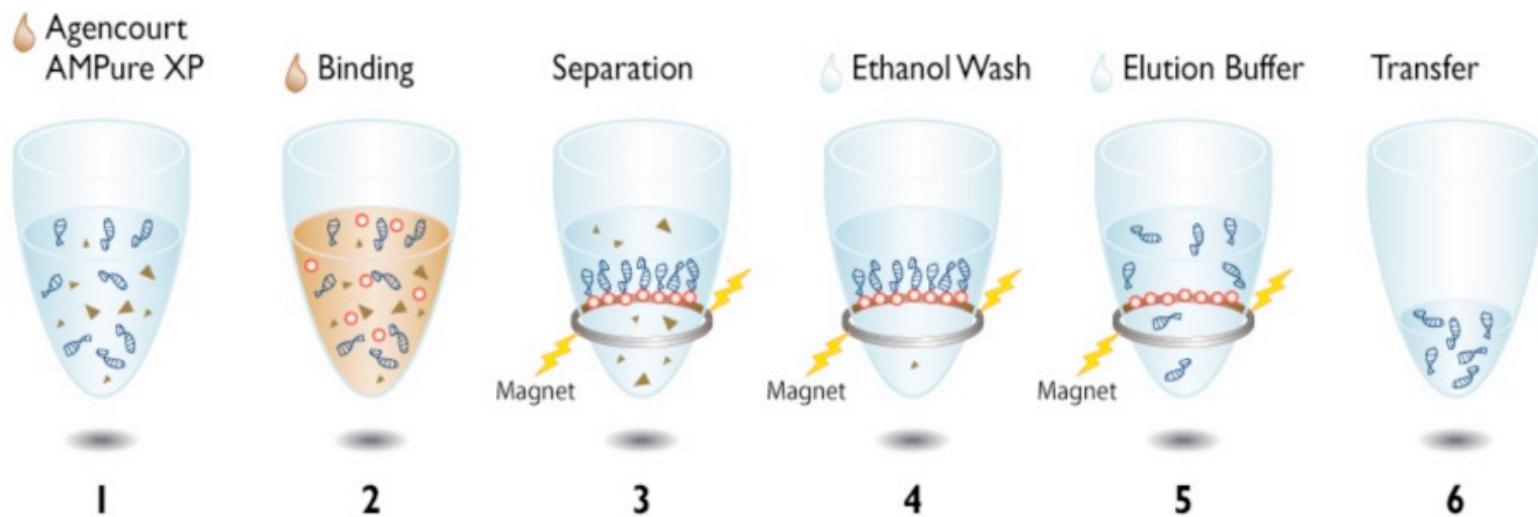
This Kit Includes:

- 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

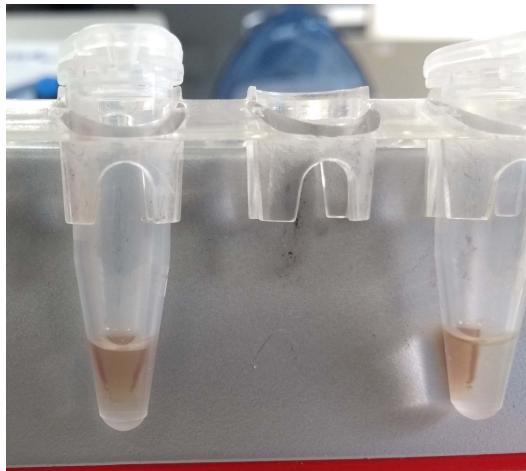
- *Deamination of cytosine to uracil*
- *Nicks and gaps*
- *Oxidized bases*
- *Blocked 3' ends*

AmpureXP Bead Binding of DNA



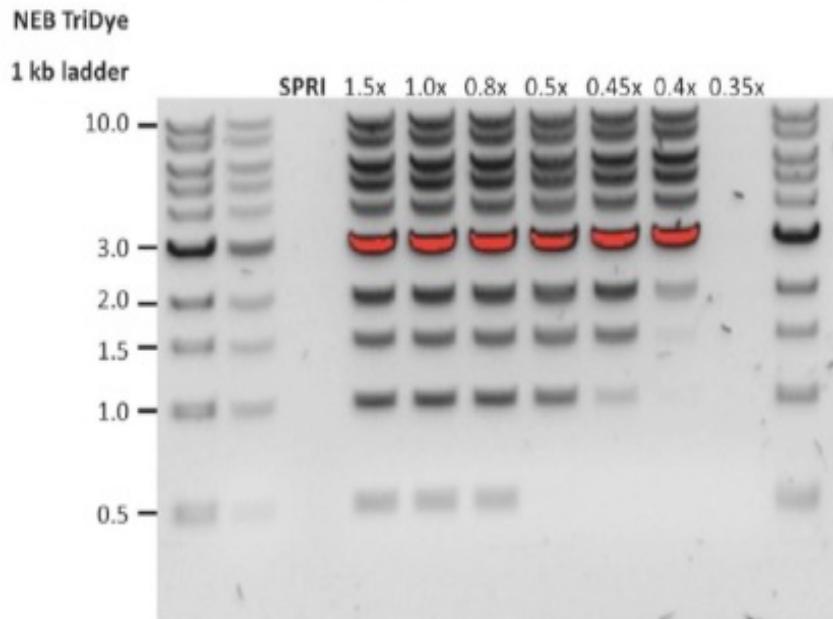
- *Automated gDNA extractions*
- *gDNA purification (buffer exchange)*
- *Size selection*

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



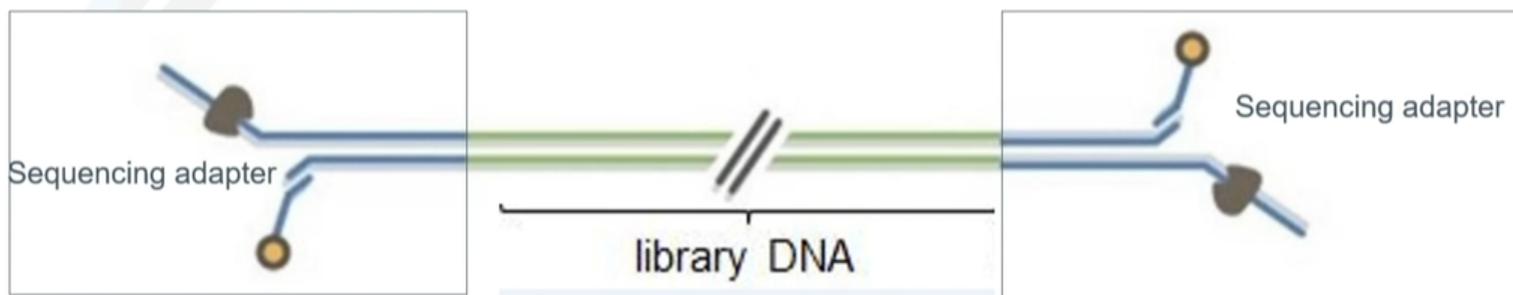
- Long Fragment Buffer
- Enrich >3000bp fragments

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
- 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*