



NGS Library Construction

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Talk Outline

- » What is a NGS sequencing library?
- » How are they made? *Options and considerations.*
- » Problems



Sequencers



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www.illumina.com





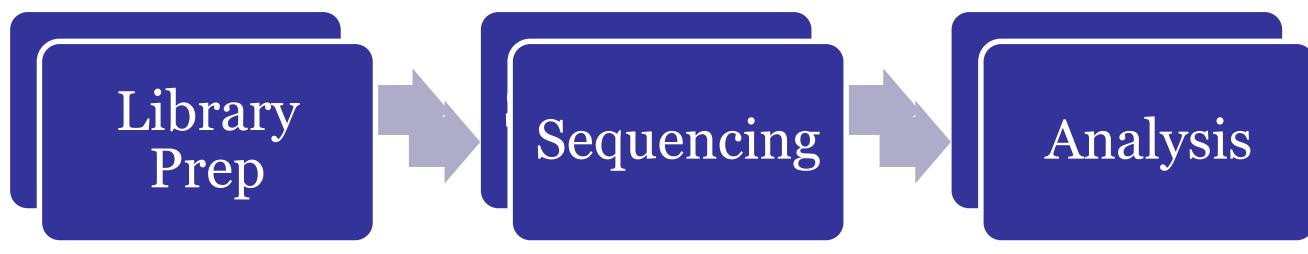


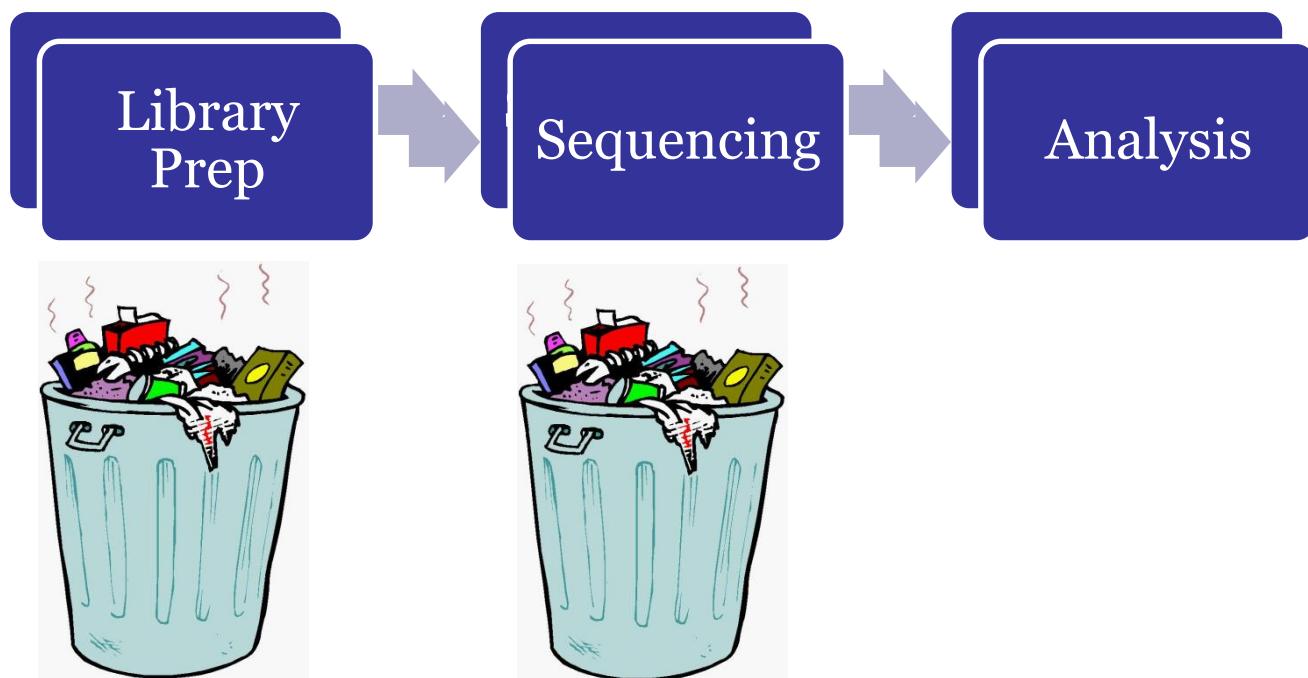
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1 tcctggcatc agttactgtg ttgactcact cagtgttggg atcactcact ttccccctac
61 aggactcaga tctgggaggc aattaccttc ggagaaaaac gaataggaaa aactgaagtg
121 ttacttttt taaagctgct gaagttgtt ggtttctcat tgtttttaag cctactggag
181 caataaaagtt tgaagaacctt ttaccaggtt ttttttatcg ctgccttgat atacactttt
241 caaaatgctt tggtgggaag aagtagagga ctgttatgaa agagaagatg ttcaaaagaa
301 aacattcaca aaatgggtaa atgcacaatt ttctaagttt gggaaagcagc atattgagaa
361 cctcttcagt gacctacagg atgggaggcg cctcctagac ctcctcgaag gcctgacagg
421 gcaaaaactg cccaaagaaa aaggatccac aagagttcat gccctgaaca atgtcaacaa
481 ggcactgcgg gtttgcaga acaataatgt tgatttagtg aatattggaa gtactgacat
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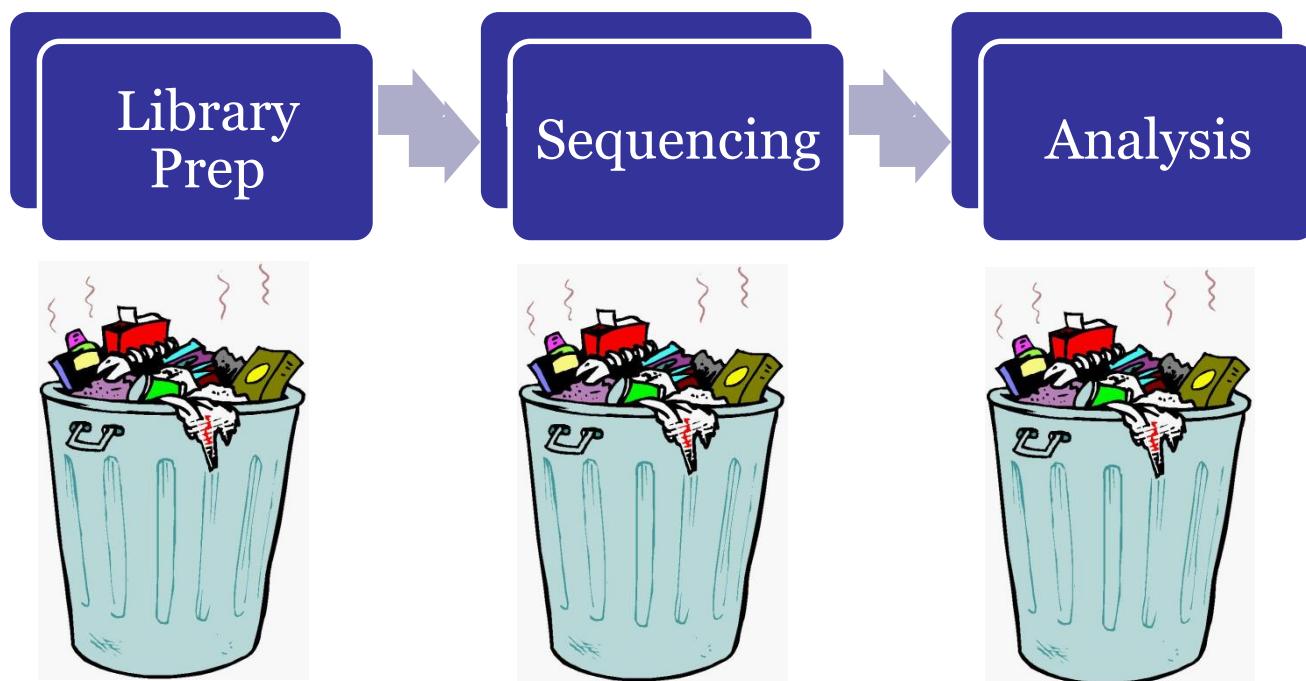


Sequencing workflow

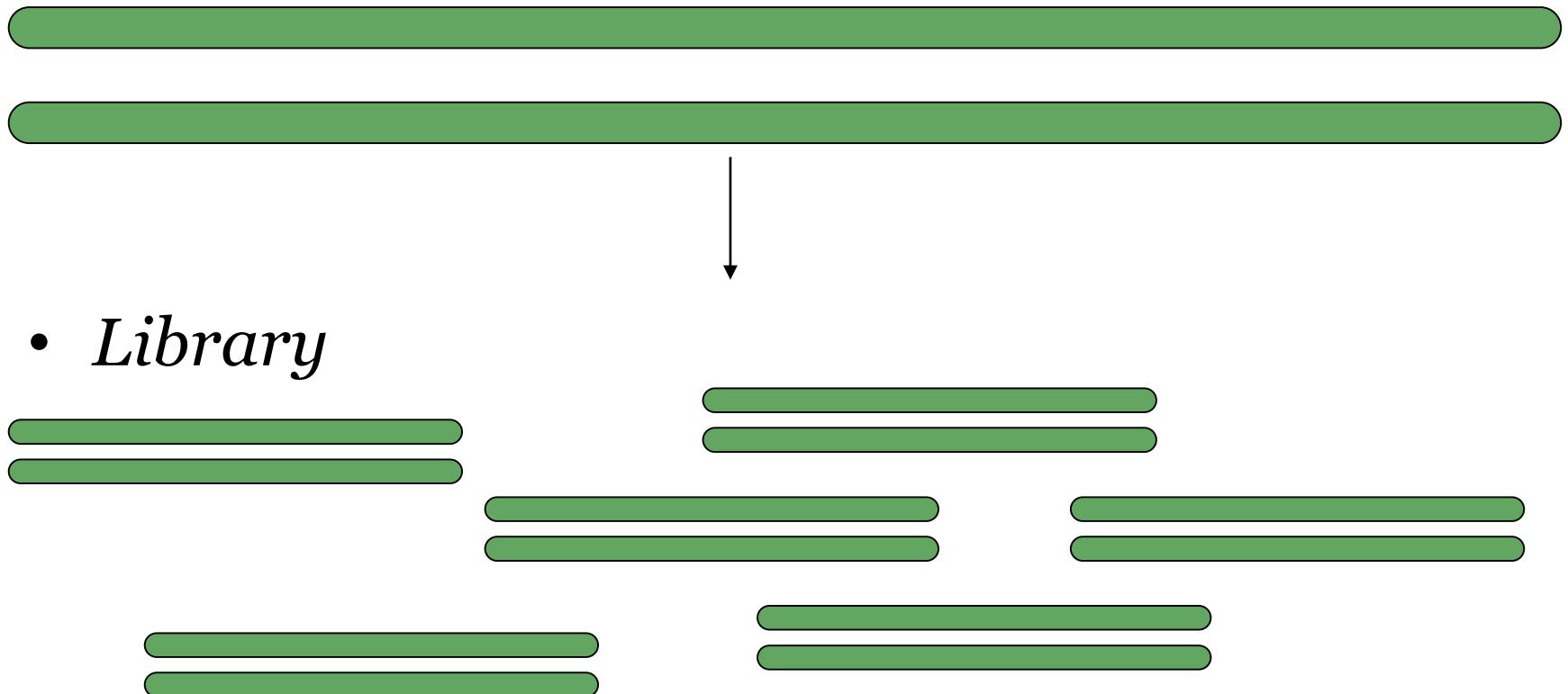




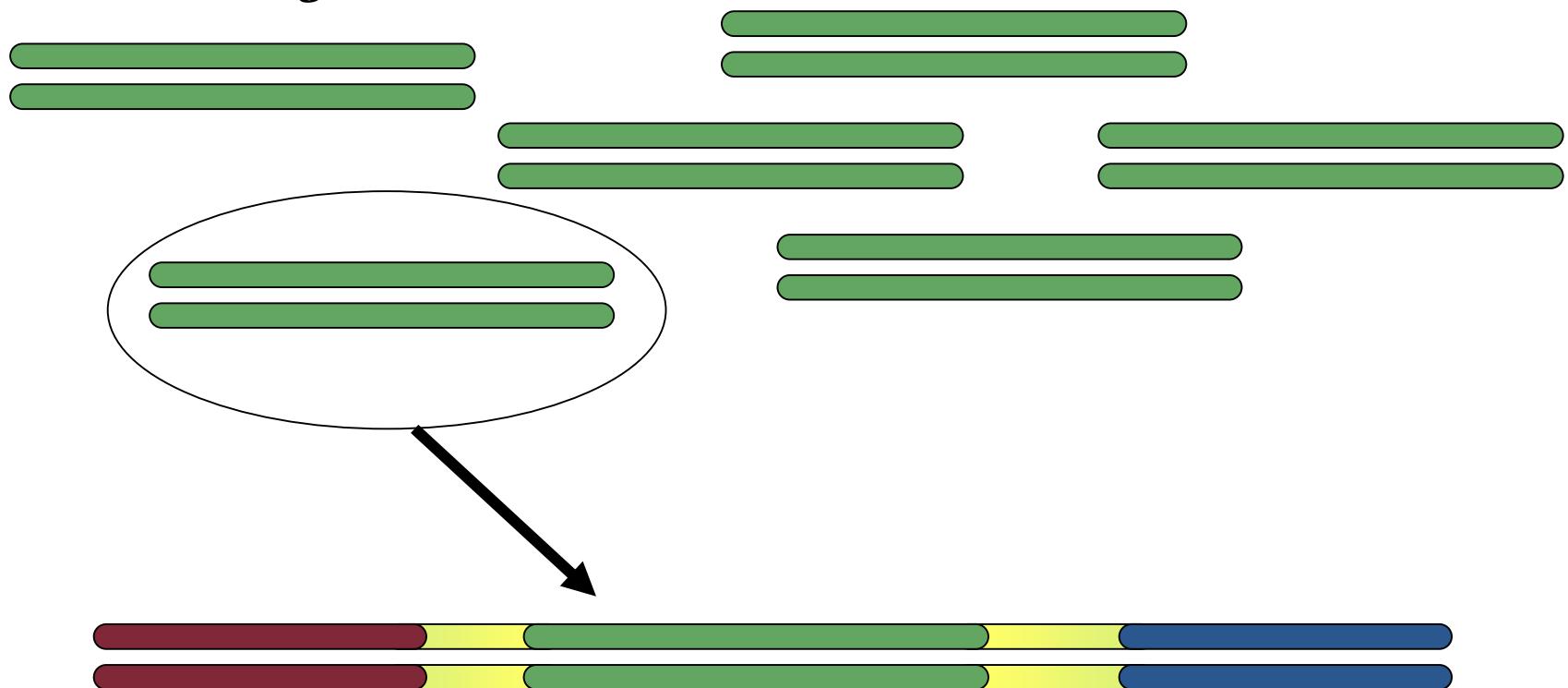




» Original DNA

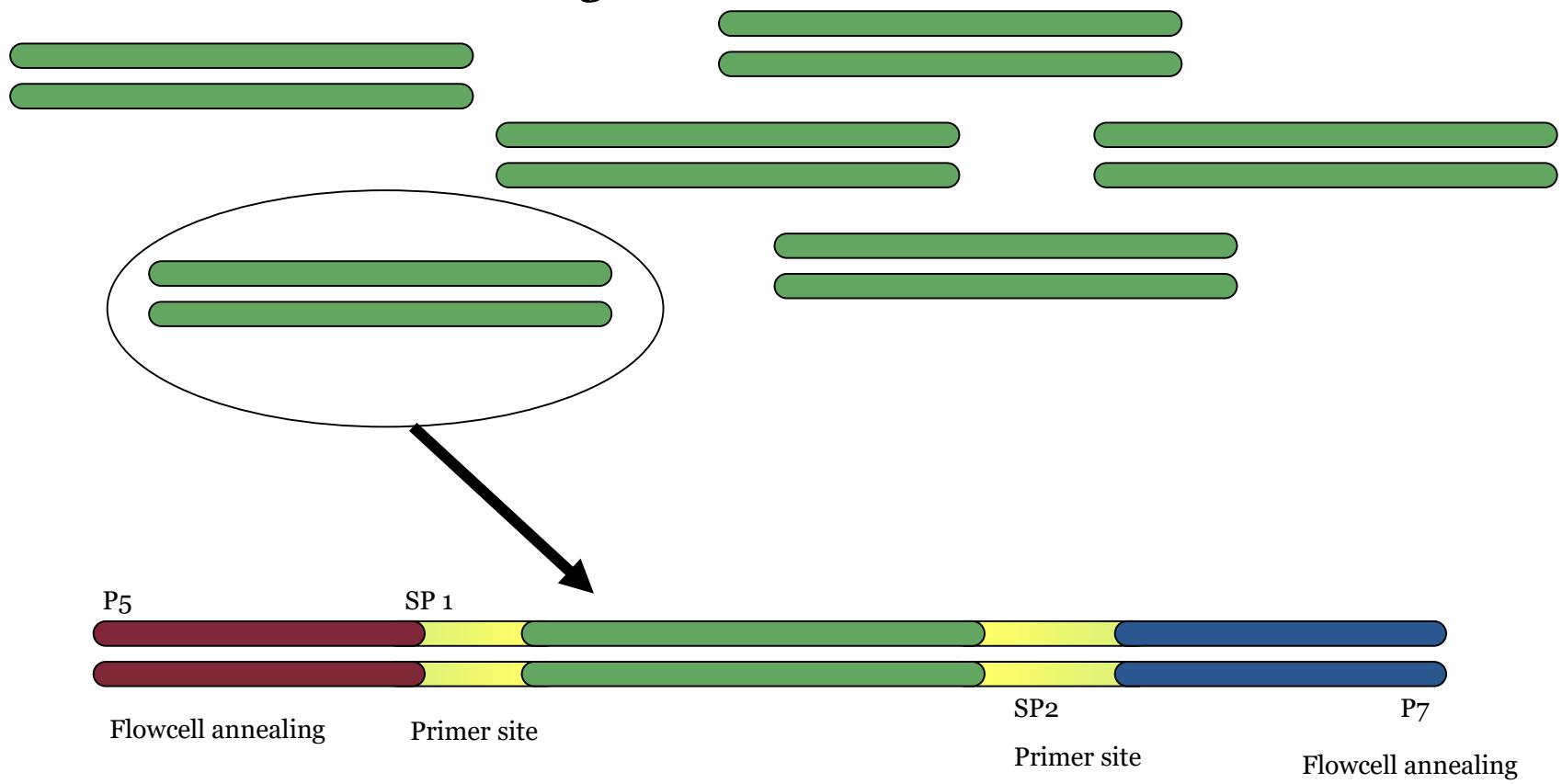


- *Library*



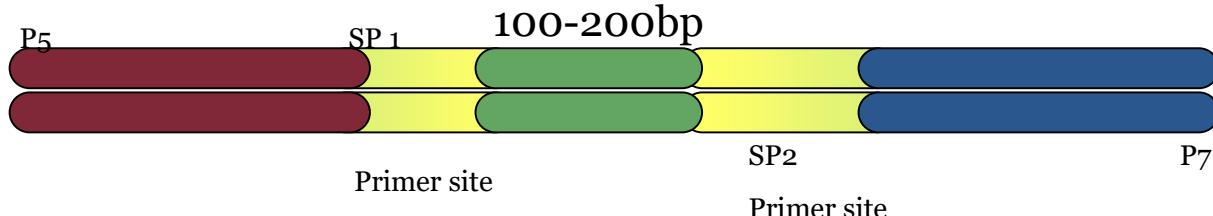


- *Illumina Library*



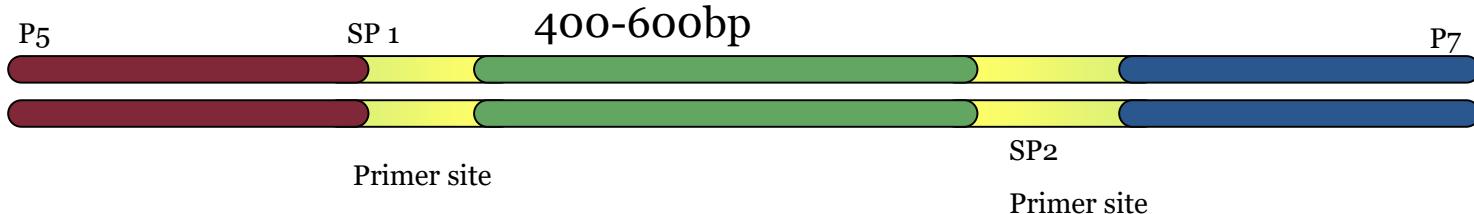
Illumina Library Insert sizes

Exome, targeted, RNAseq, ChIP, ATAC



Reads need to be just long enough to map and ideally not to overlap exon boundaries

Whole Genome

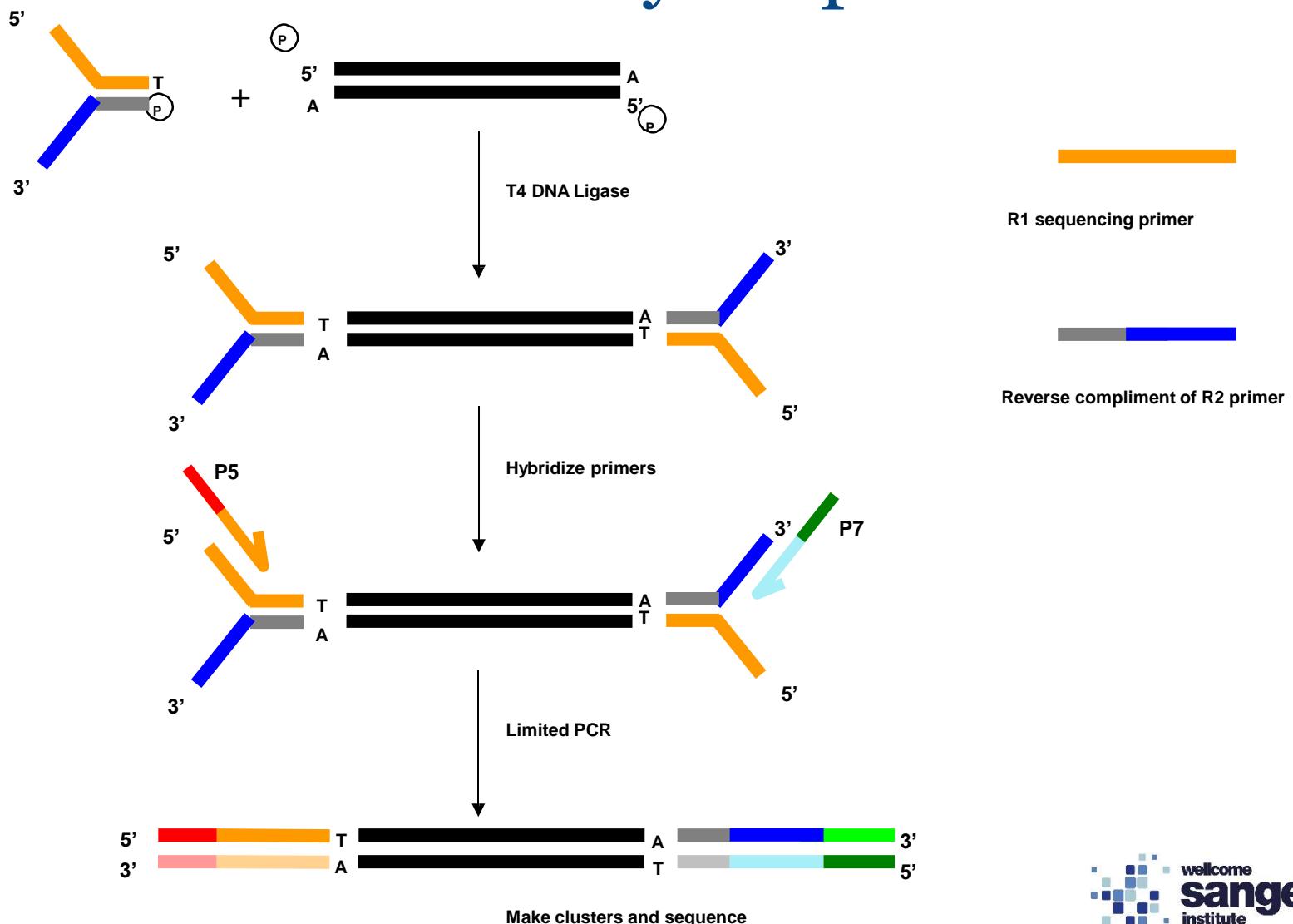


Reads need to be just long to span common repeat elements eg AluI with unique sequence on either side so can map

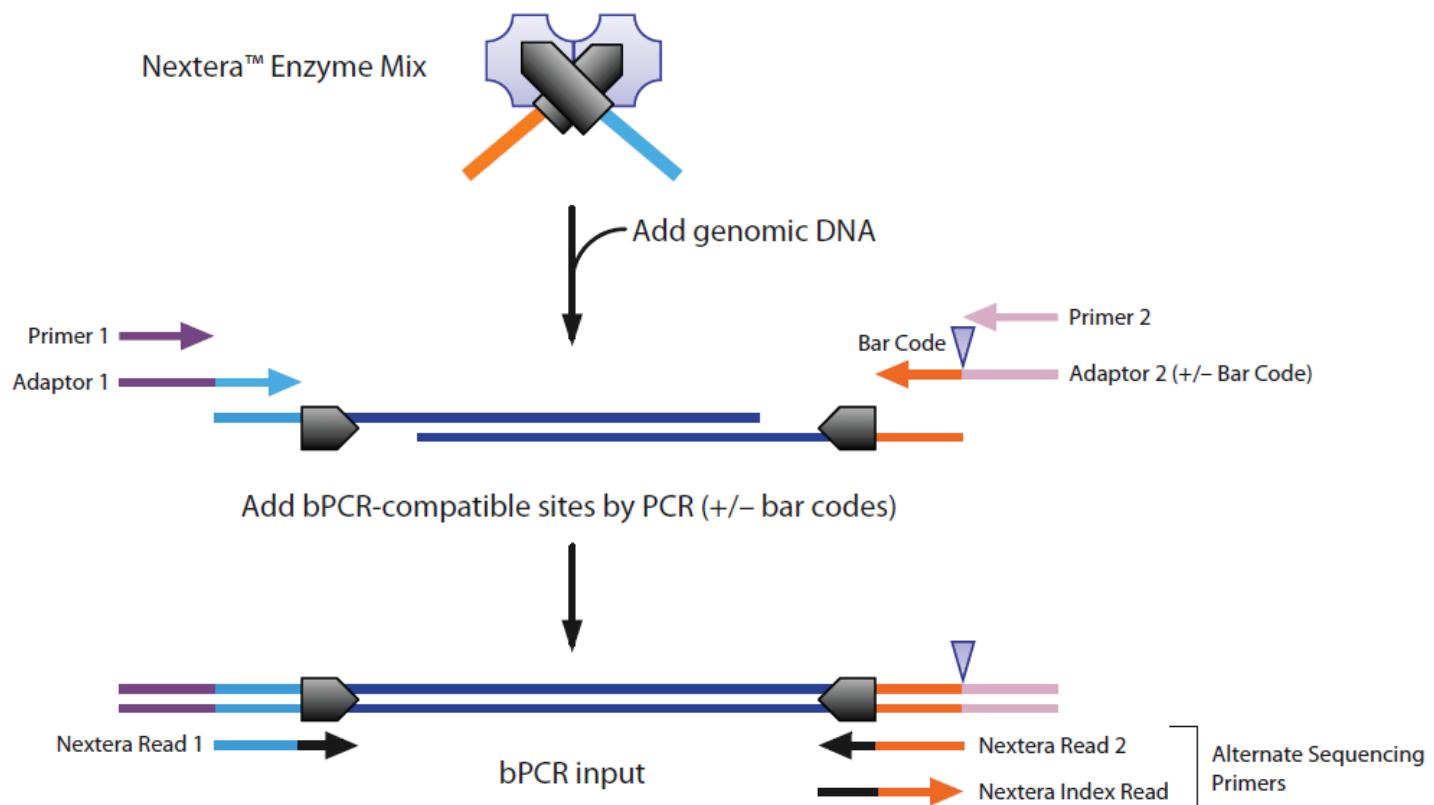
Library Prep Approaches

1. Classical adapter ligation method
2. Transposon mediated
3. Inclusion of adapter sequences during PCR

Illumina Paired End Library Prep

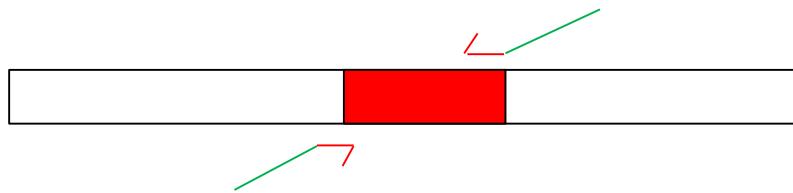


Nextera



Amplicon sequencing

add adapter sequences to 5' end of primers





Illumina Library Prep



How do you go about making a
good library?



QC



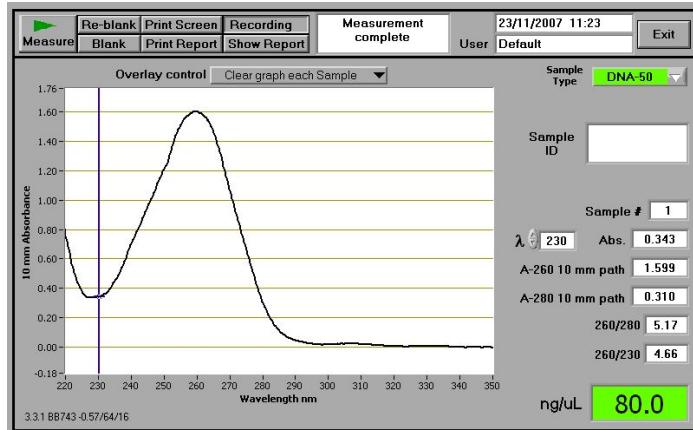
Input DNA for NGS

- Properly quantified. **Flourimetric methods preferred**
- DNA from appropriate source
- DNA >40kb required for long read applications
 - Pure DNA 260:280 close to 1.8
 - Nanodrop reading should be approx. 2x qubit
- RNA RIN>8



Initial Characterisation/QC

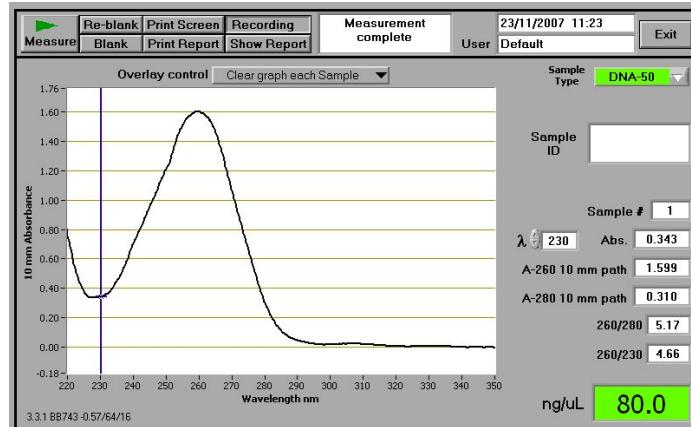
- Spectrophotometry (inc nanodrop)





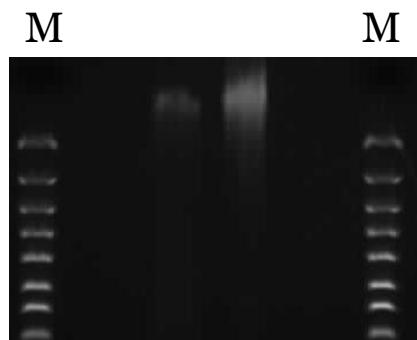
Initial Characterisation/QC

- Spectrophotometry (inc nanodrop)



Initial Characterisation/QC

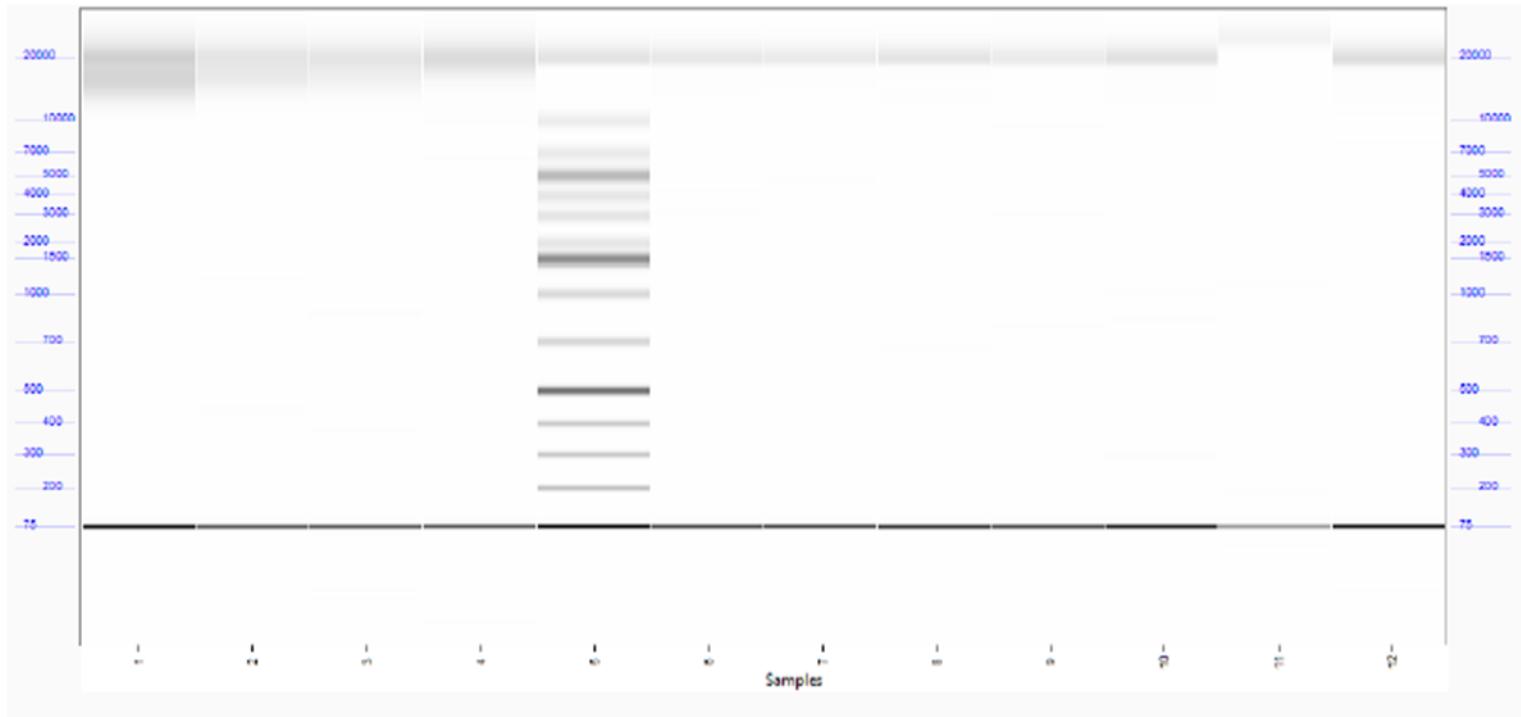
- Gel



- Qubit



Advanced Analytical Fragment Analyzer

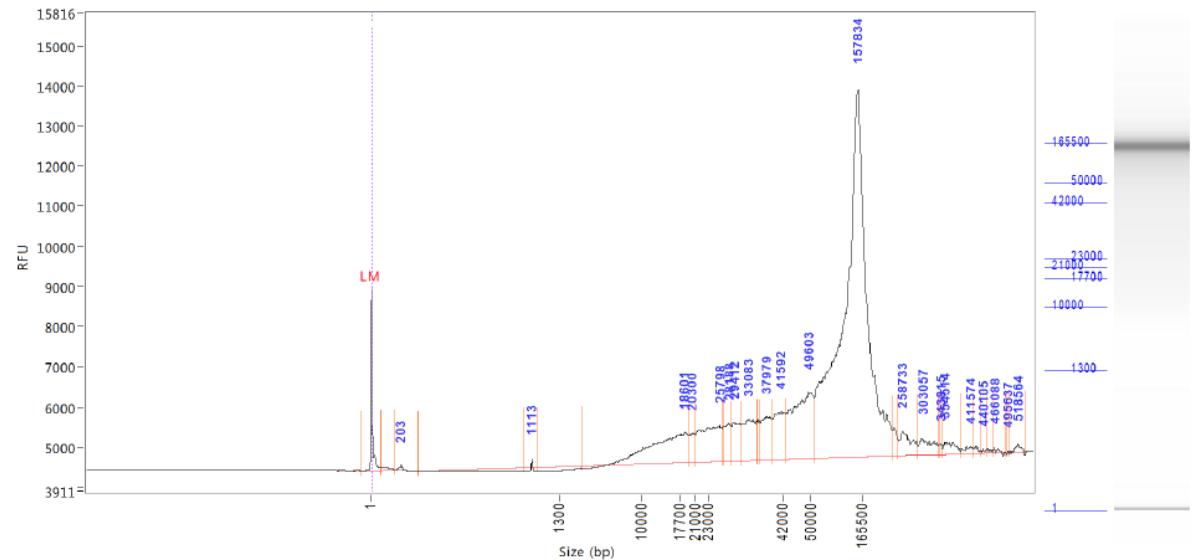


Advanced Analytical Femto Pulse

Sample: Giant Hogweed -2 mls

Well Location: G5

Created: 3/26/2018 7:46 PM



Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	CV%	RFU	Corr. Peak Area
------	--------------	------------------	--------------	------------	-------------------	-----	-----	-----------------

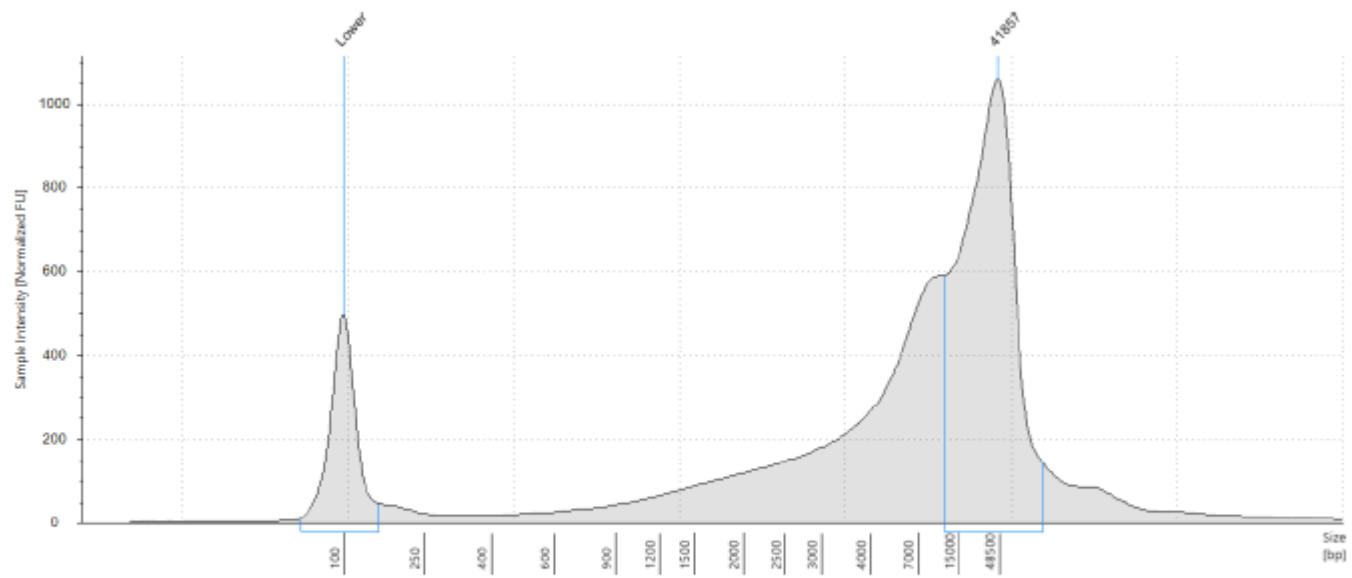
TIM: 0.0111 nmole/L

Total Conc.: 0.2301 ng/uL

DQN: N/A (Size threshold is less than lower marker end point)

Threshold: 0

Agilent Tapestation



Sample Table

Well	DIN	Conc. [ng/ μ l]	Sample Description	Alert	Observations
B1	6.9	85.3		⚠️	Caution! Expired ScreenTape device

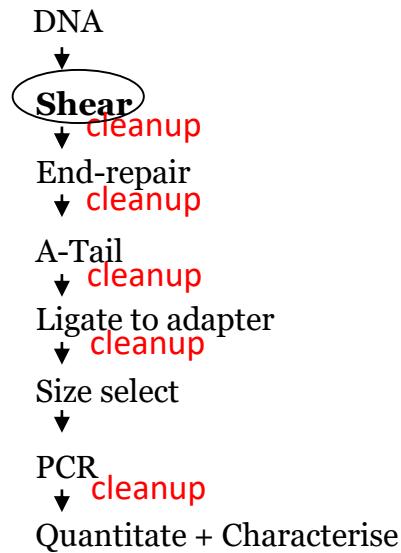
Agilent Tapestation for sequencing library QC

Offer range of kits for different input and size ranges



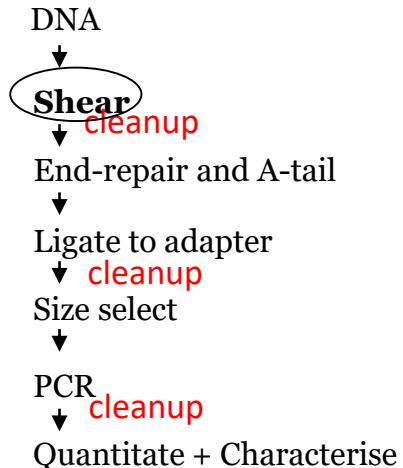
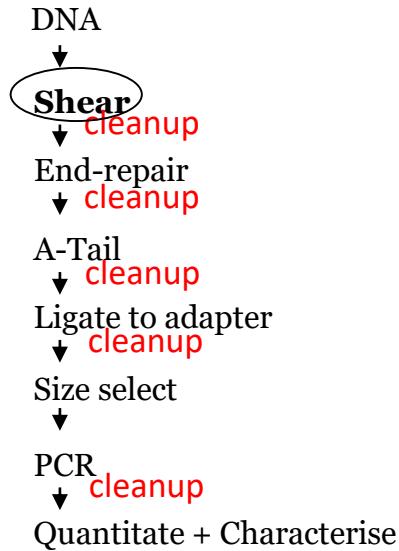


Generic library prep workflow 2006



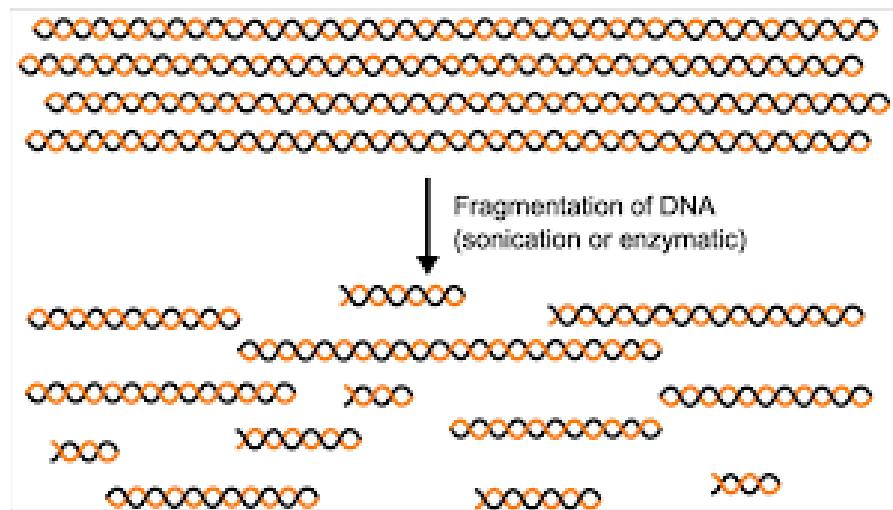


Streamlining generic library prep workflows





Shearing

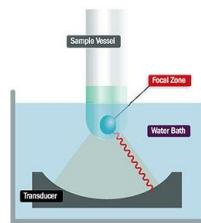




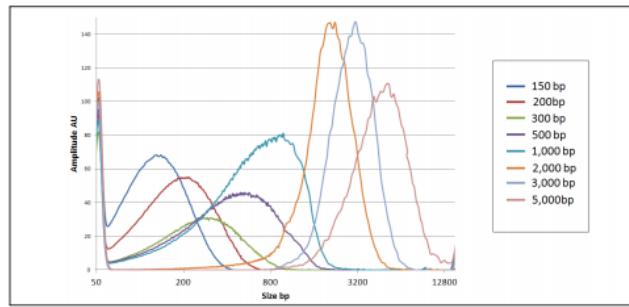
DNA Shearing by Acoustics



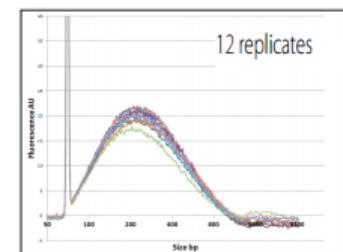
AFA tube



Focused acoustics



Programmable shearing

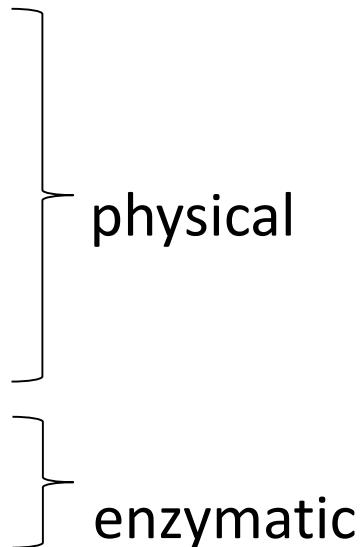


Reproducible

Shearing

Other options

- Matrical sonicman (96 well sonication)
www.matrical.com
- Bioruptor www.diagenode.com
- Episonic. www.epigentek.com
- Nebulisation (single sample)
- Fragmentase (NEB)
- Nextera (Epicentre)



physical

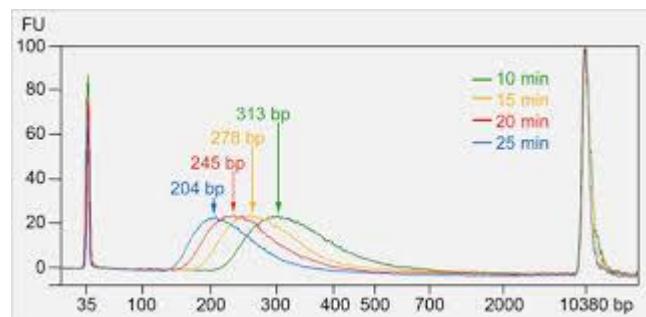
enzymatic

Shearing

Other options

- Matrical sonicman (96 well sonication)
www.matrical.com
- Bioruptor www.diagenode.com
- Episonic. www.epigentek.com
- Nebulisation (single sample)
- Fragmentase (NEB)
- Nextera (Epicentre)

} physical
}
enzymatic

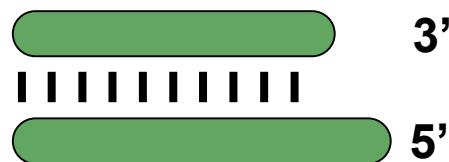
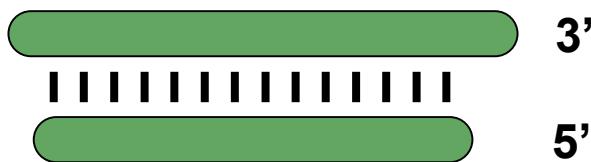
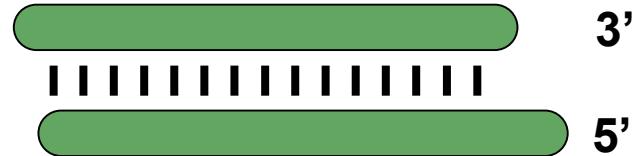
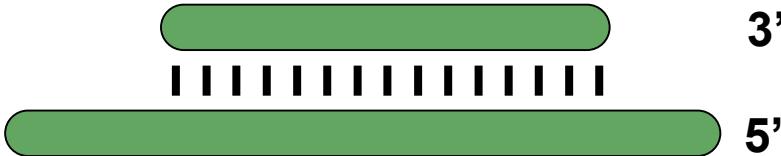


Shearing Problems

- Enzymatic shearing can result in variation in fragment size between samples
 - Can be dependent on amount, GC content or presence of contaminants
- Sonication based methods can generate heat leading to AT dropout
- Fragments smaller than the read length can give rise to low ratio of mapped reads.
- Smaller fragments cluster more efficiently so if multiplexing libraries they should be sheared to similar fragment size ranges else some will be represented more than others in final sequence output.

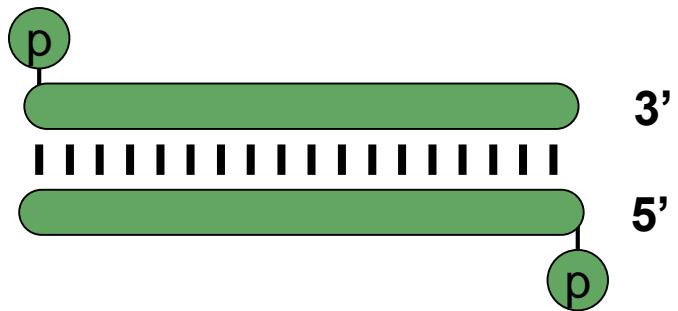


After shearing



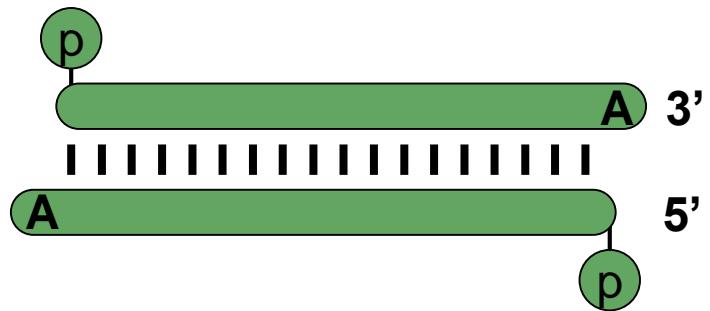


End-Repair

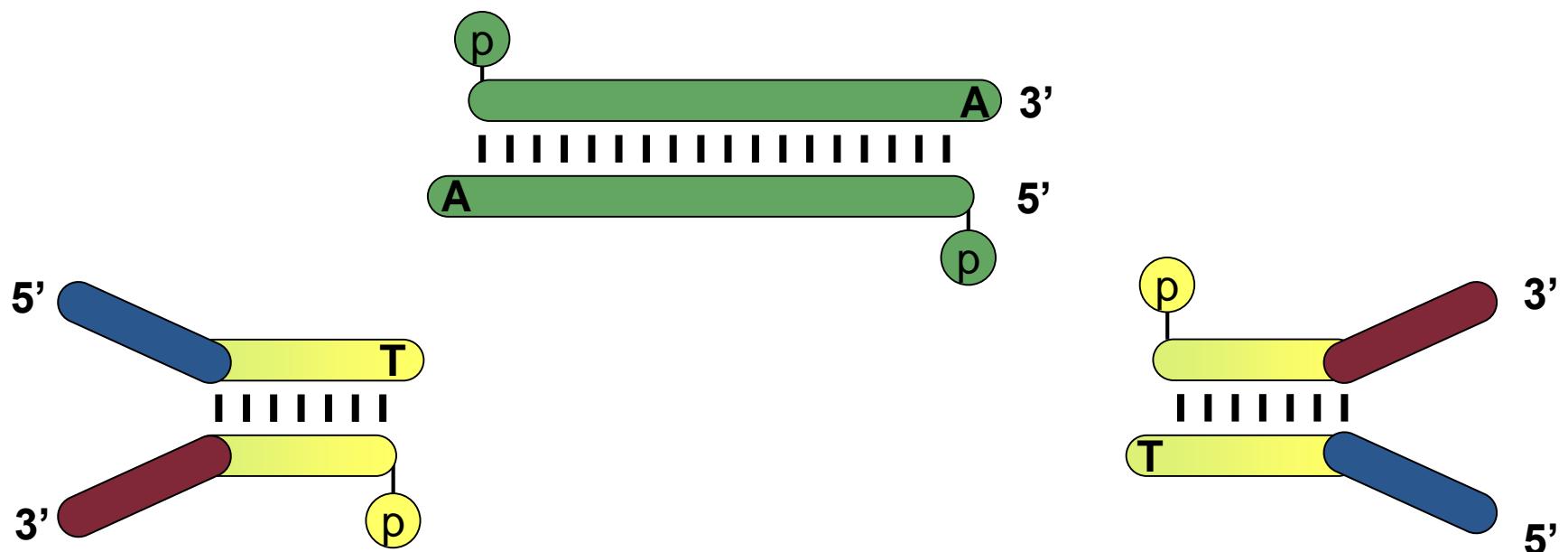




Tailing

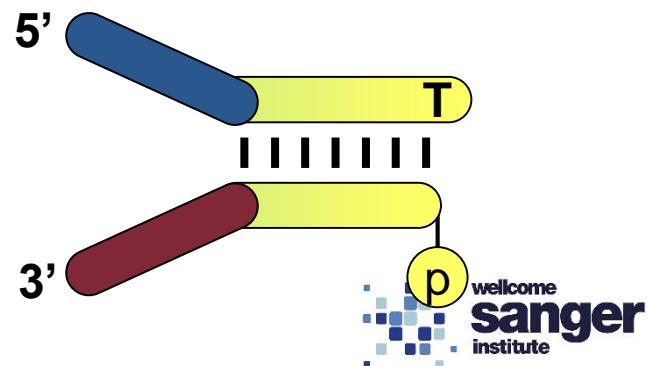
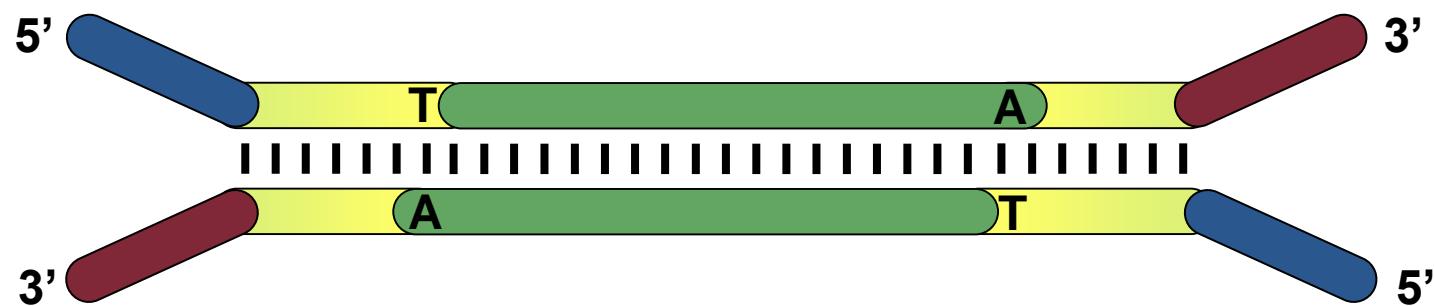


Ligation of Illumina Adapters





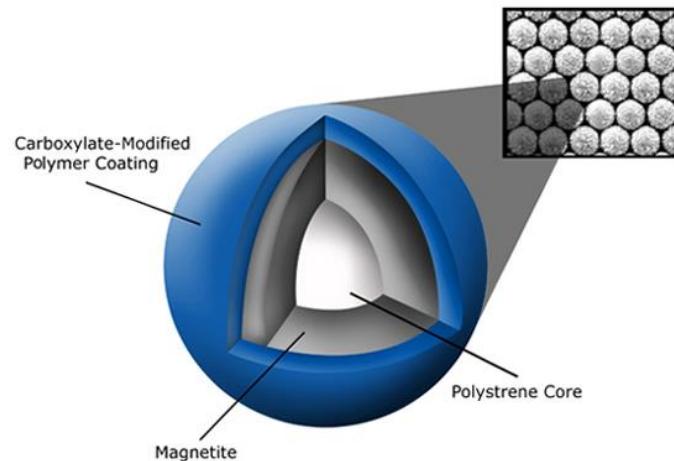
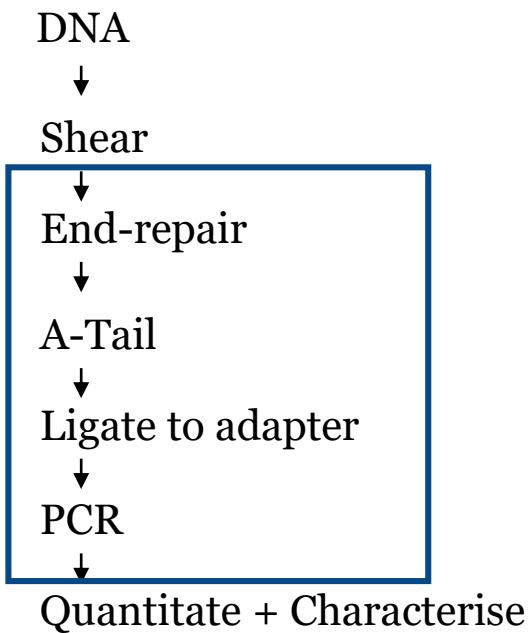
After Ligation





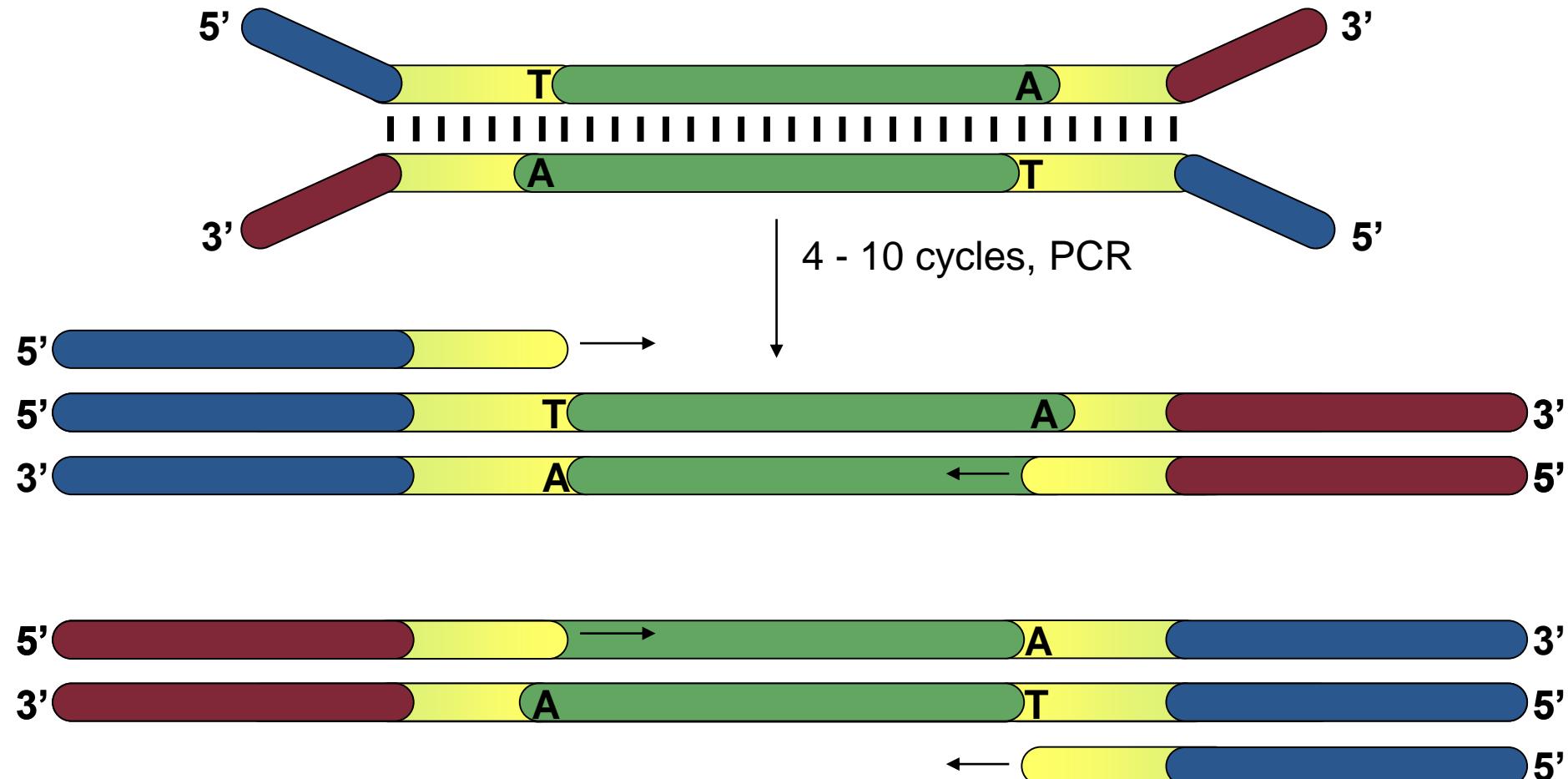
SPRI: Ampure

» <http://www.beckmangenomics.com/products.html>



- » Advantages:
Easy to automate, some size selection properties
- » Disadvantages:
A bit fiddly to do manually

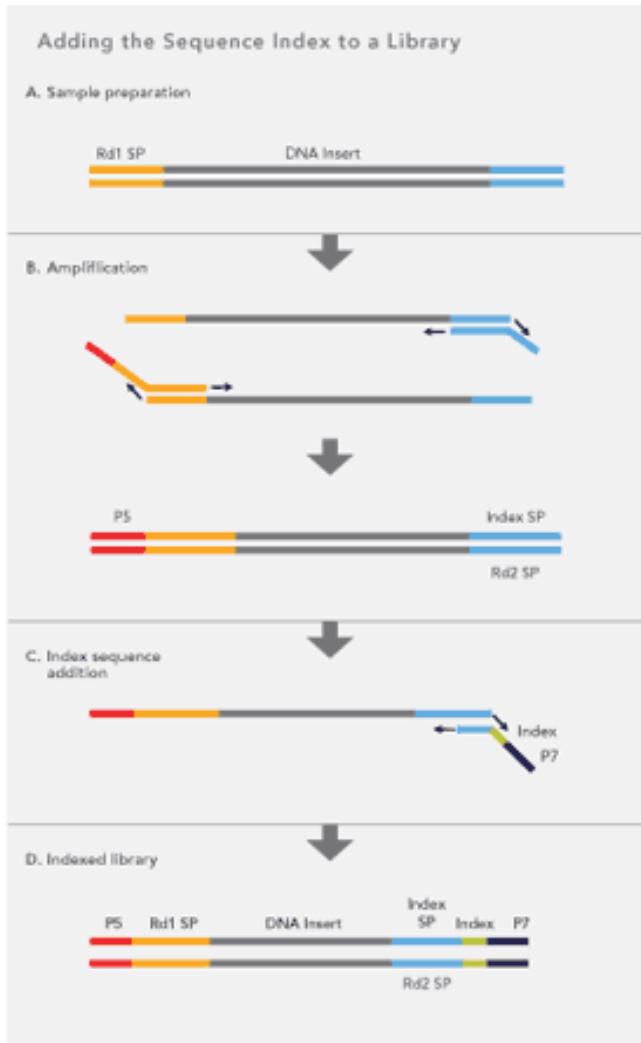
Amplification





Multiplexing

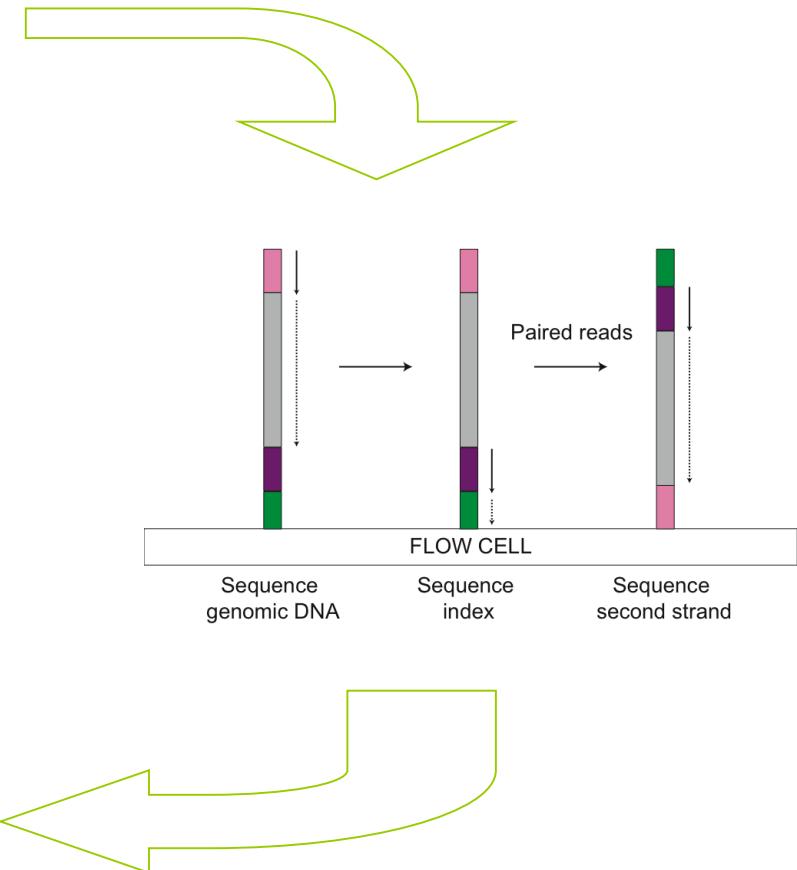
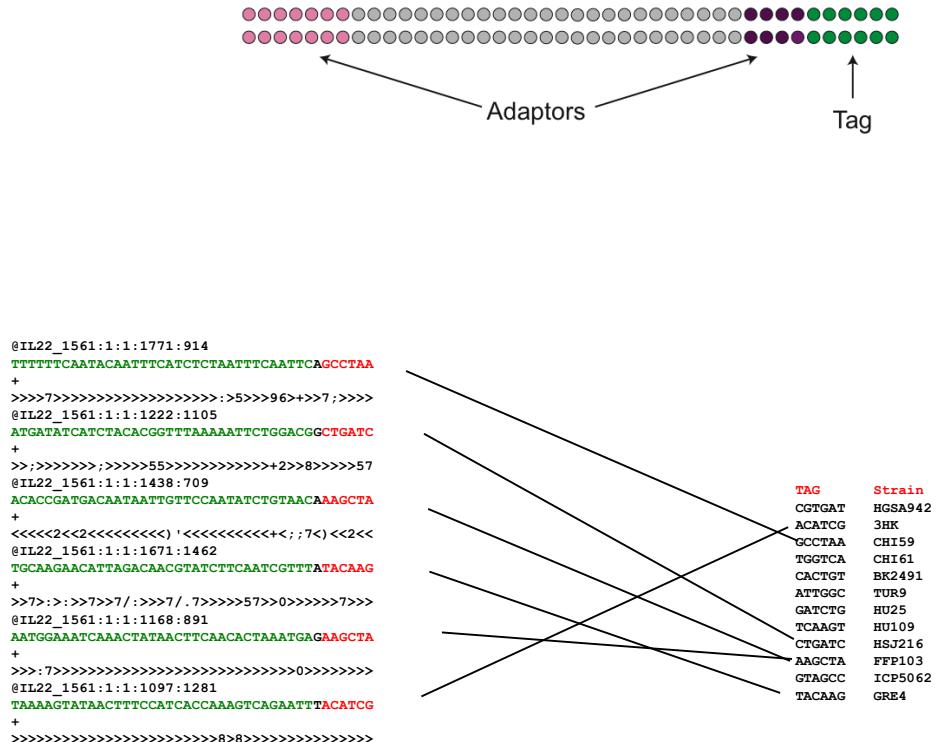
Multiplexing



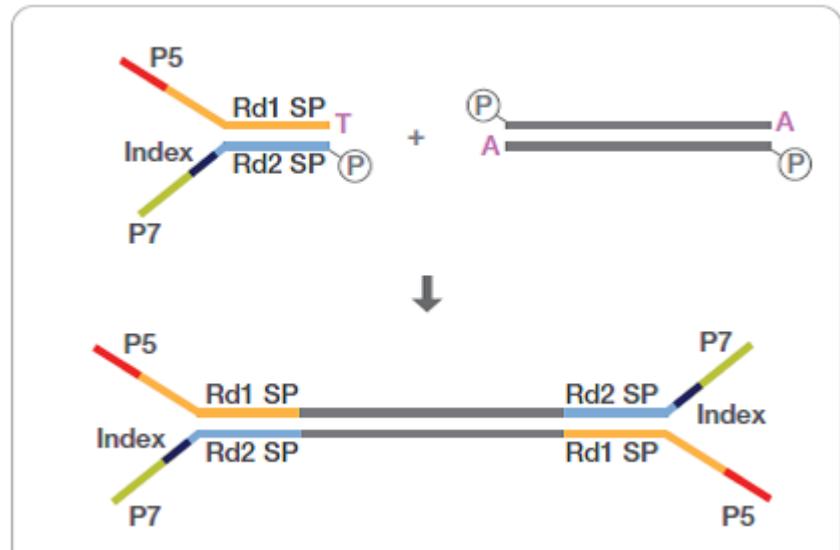
Tag sequences

Tag 1	CGTGAT
Tag 2	ACATCG
Tag 3	GCCTAA
Tag 4	TGGTCA
Tag 5	CACTGT
Tag 6	ATTGGC
Tag 7	GATCTG
Tag 8	TCAAGT
Tag 9	CTGATC
Tag 10	AAGCTA
Tag 11	GTAGCC
Tag 12	TACAAG

Multiplexing on Illumina



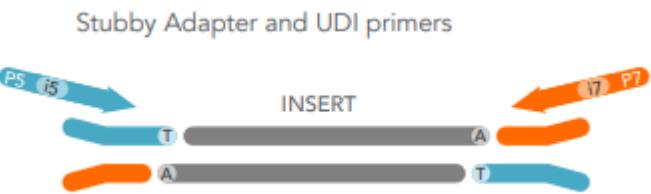
Multiplexing Options



Full length Adapters

Available from:

Illumina
IDT
NEB
Roche
Quantabio
Tecan
Perkin Elmer
etc



Truseq stubby Adapters

Stubby Adapters available from IDT

Indexing primers from:
Illumina
IDT
NEB
Roche
Quantabio
Tecan
Perkin Elmer
Eurofins
etc

NGS Library Prep Kits

- Enzymatic or physical shearing options
- With or without adapters/primers
- PCR or PCRfree



NGS library kit manufacturers

- » NEB
- » Illumina
- » IDT
- » Twist bioscience
- » Roche/Kapa
- » Qiagen
- » Agilent
- » Tecan
- » Agilent
- » Tecan
- » Thermo
- » Watchmaker
- » Meridian
- » Quantabio
- » Thermo

NEB UltraExpress FS DNA library kit E3340L





Size Selection



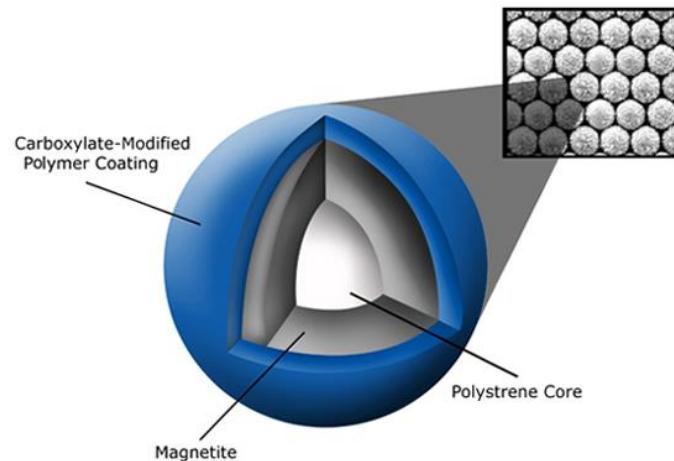
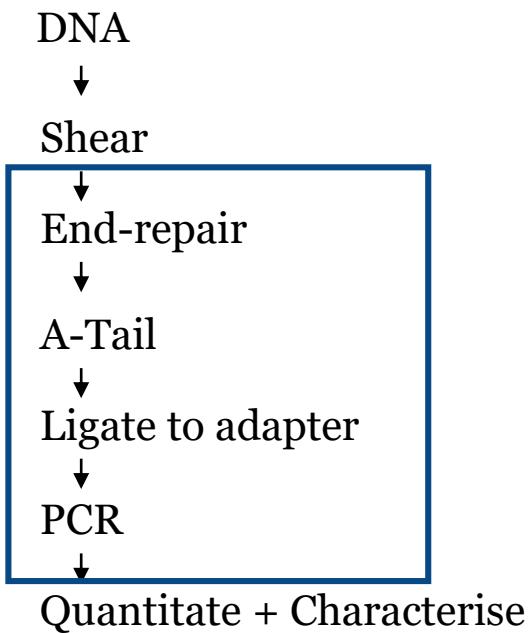
Size Selection

- » Gel
- » Pippin Prep
- » Ampure



SPRI: Ampure

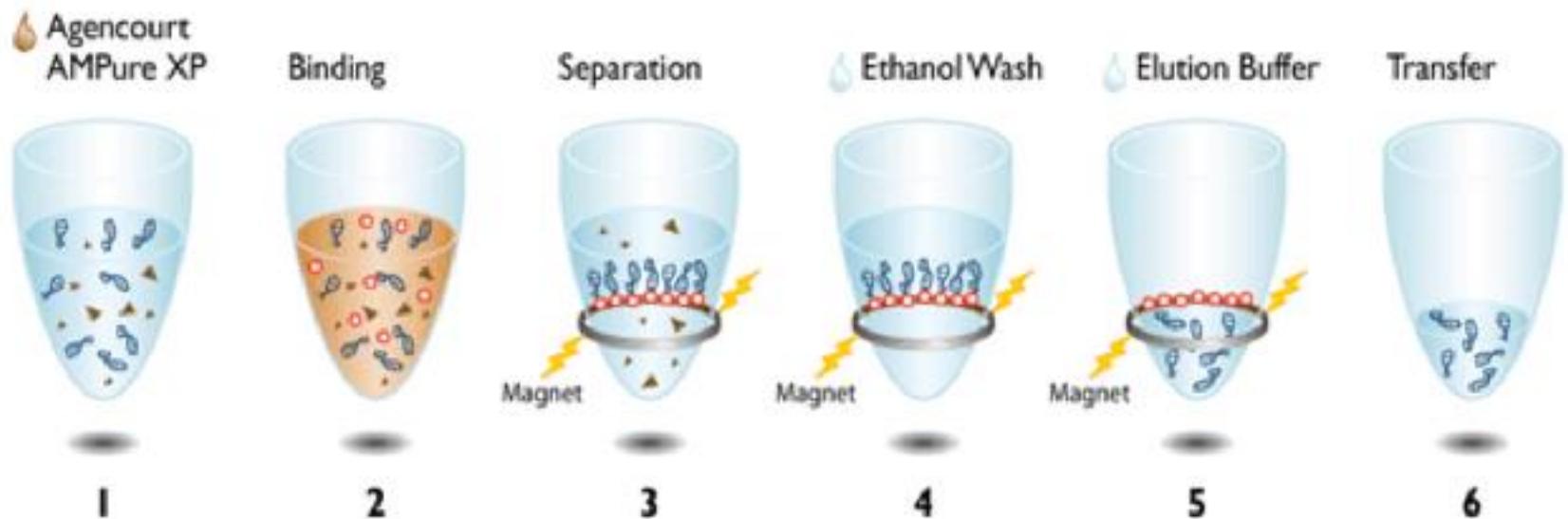
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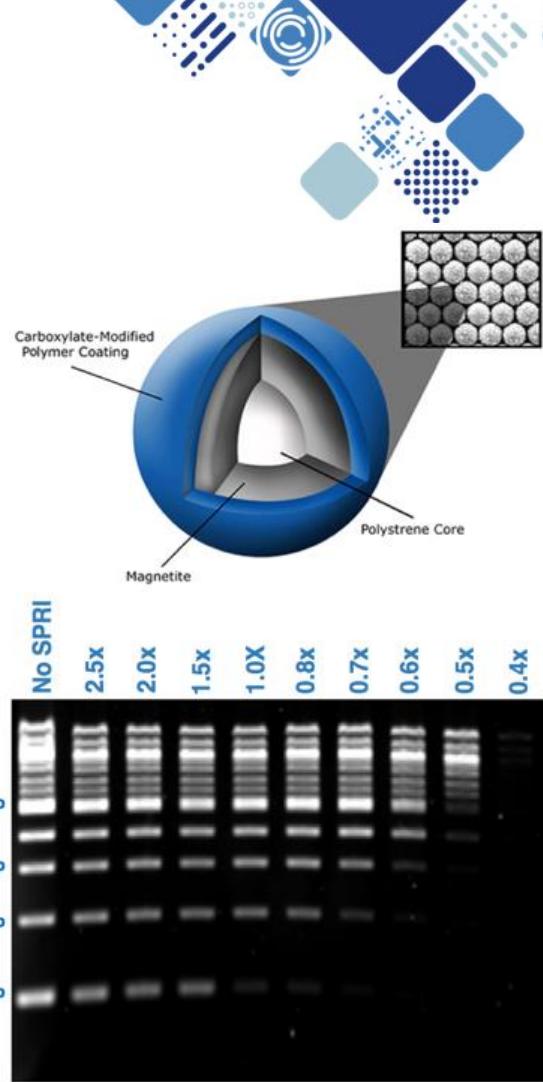


SPRI purification



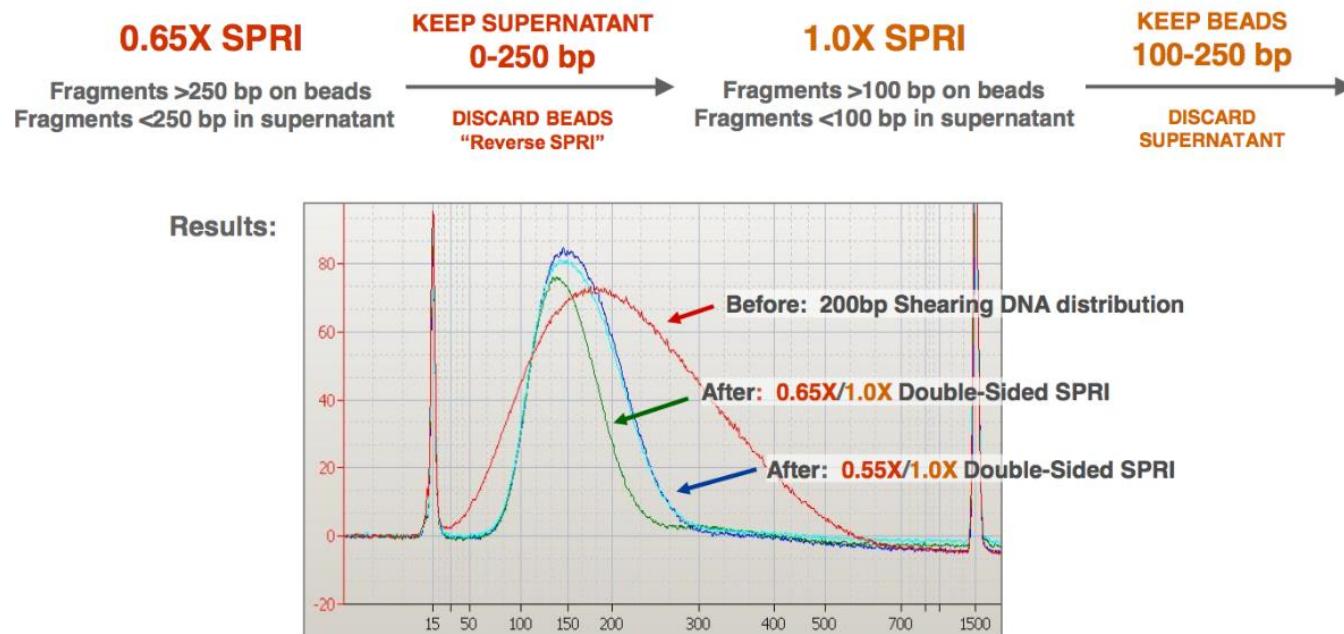
Working with SPRI Beads

- » SPRI (Solid Phase Reversible Immobilization) Beads
- » Paramagnetic beads made of polystyrene surrounded by a layer of magnetite, which is coated with carboxyl molecules
- » Reversibly bind DNA in the presence of the “crowding agent” polyethylene glycol (PEG) and salt (20% PEG, 2.5M NaCl is the magic mix).
- » PEG causes the negatively-charged DNA to bind with the carboxyl groups on the bead surface.
- » DNA fragment size affects the total charge per molecule with larger DNAs having larger charges; this promotes their electrostatic interaction with the beads and displaces smaller DNA fragments.
- » **The size of fragments eluted from the beads** is determined by the concentration of PEG, which is determined by the mix of SPRI:DNA at a given ratio.
- » **The lower the ratio of SPRI:DNA the higher the molecular weight of the fragments bound to the beads.**



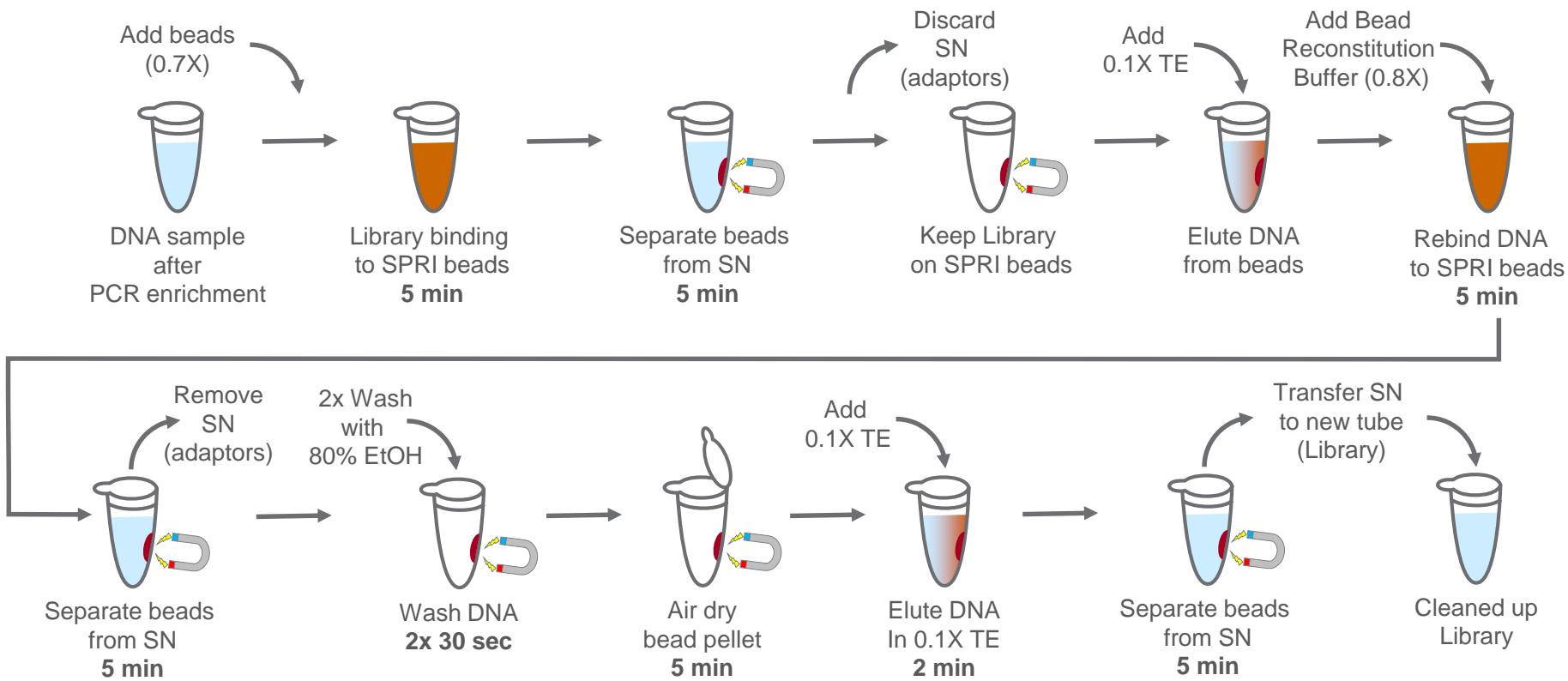
Double sided SPRI

- By implementing a combination of good shearing with SPRI and “reverse” SPRI, one can select a size range:



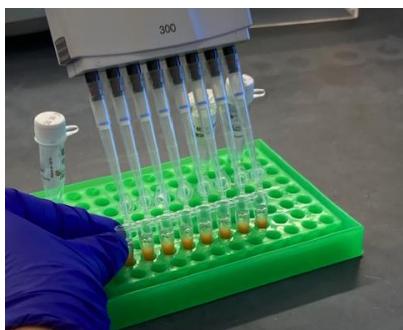
Source: Enseqlopedia from Broad Institute and Illumina

Phased Cleanup: removal of adaptors

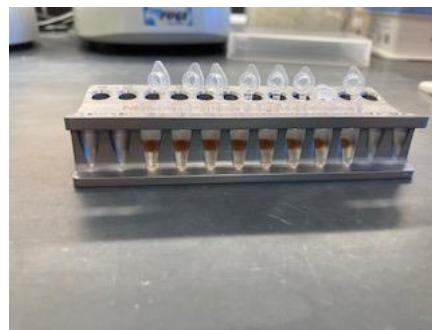


Phased Bead Cleanup for UltraExpress RNA workflow

Add 70 µl (0.7X)
resuspended beads to
the 100 µl PCR reaction



- Pipet mix or quick vortex/centrifuge
- Room Temp 5 minutes

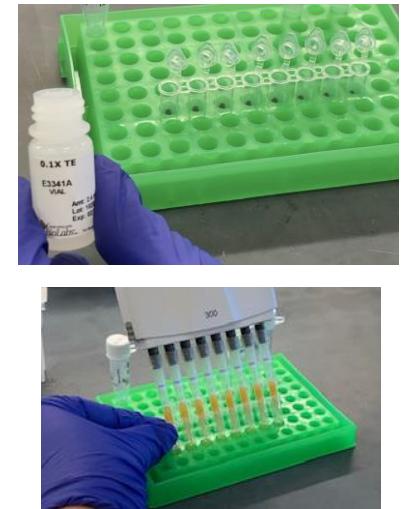


Incubate on magnet
for 5 minutes



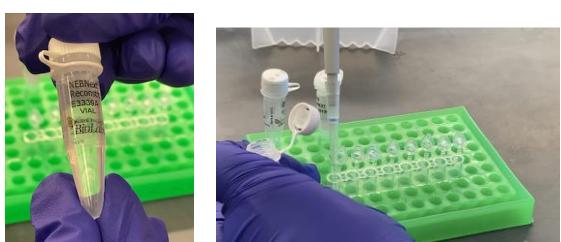
Without disturbing the
beads carefully remove
and discard supernatant

Remove tubes from magnet

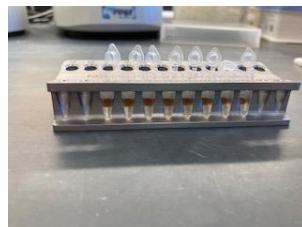
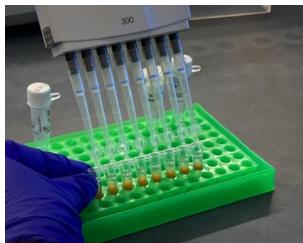


Add 100 µl of 0.1X
TE and pipet to mix

Phased Bead Cleanup for UltraExpress RNA workflow



Add 80 μ l (0.8X) NEBNext Bead Reconstitution Buffer to the 100 μ l resuspended beads



Pipet mix and
incubate at RT 5 min

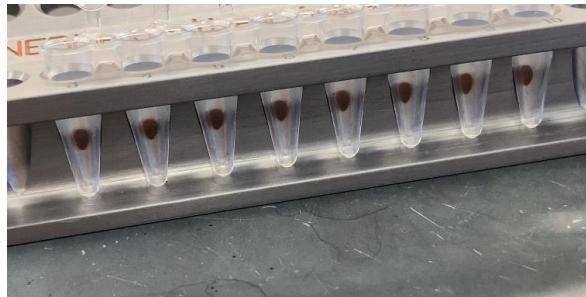
Incubate on magnet
5 min

Discard
supernatant

200 μ l (80% ETOH)
wash 2X for 30
seconds

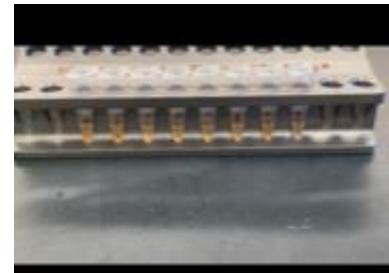
Phased Bead Cleanup for UltraExpress RNA workflow

Be sure to remove all residual 80% ETOH and incubate up to 5 min



Note: Do not allow beads to over dry or crack

When beads are ready elute with 0.1X TE and incubate at RT



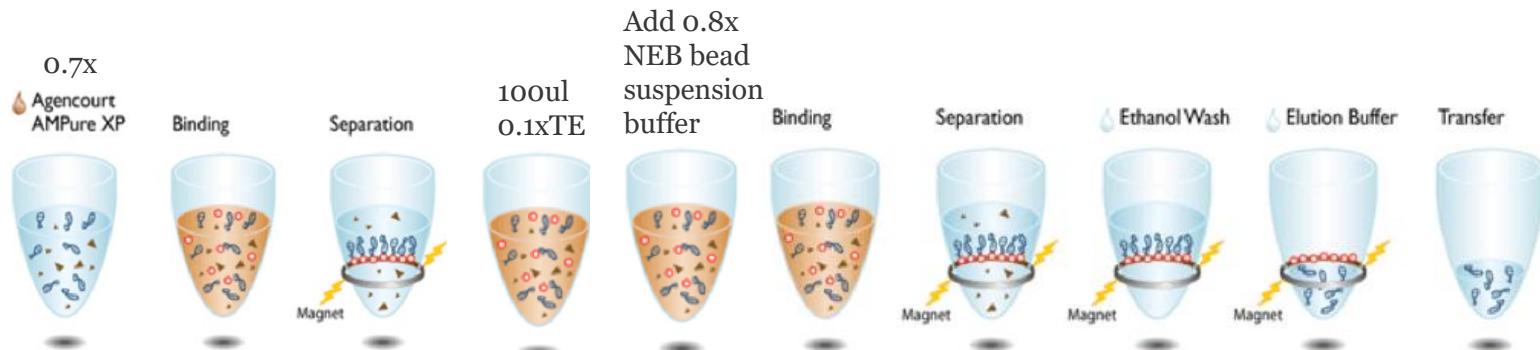
Separate
on magnet



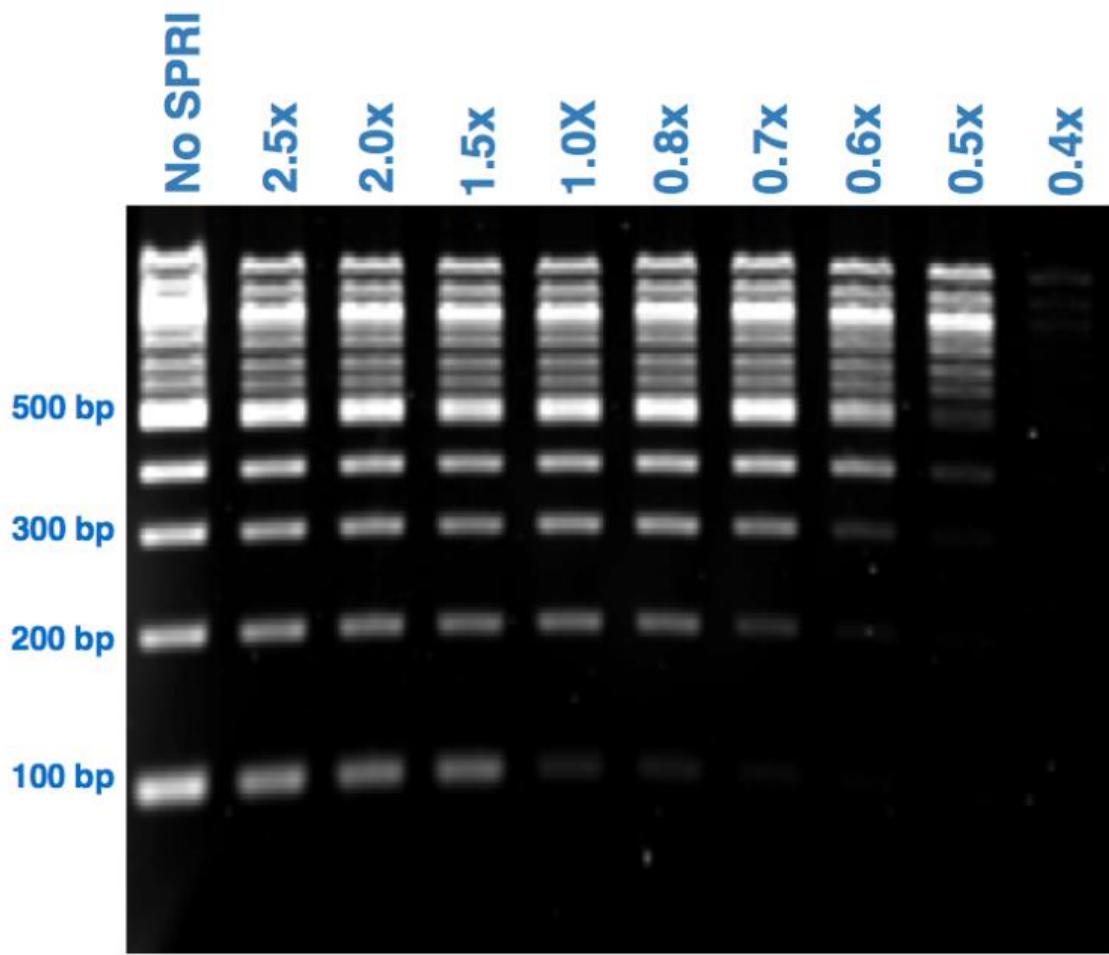
Transfer eluate
to a new tube



NEB phased cleanup from NEBNext UltraExpress™ FS DNA Library Prep Kit



Ampure Size Selection



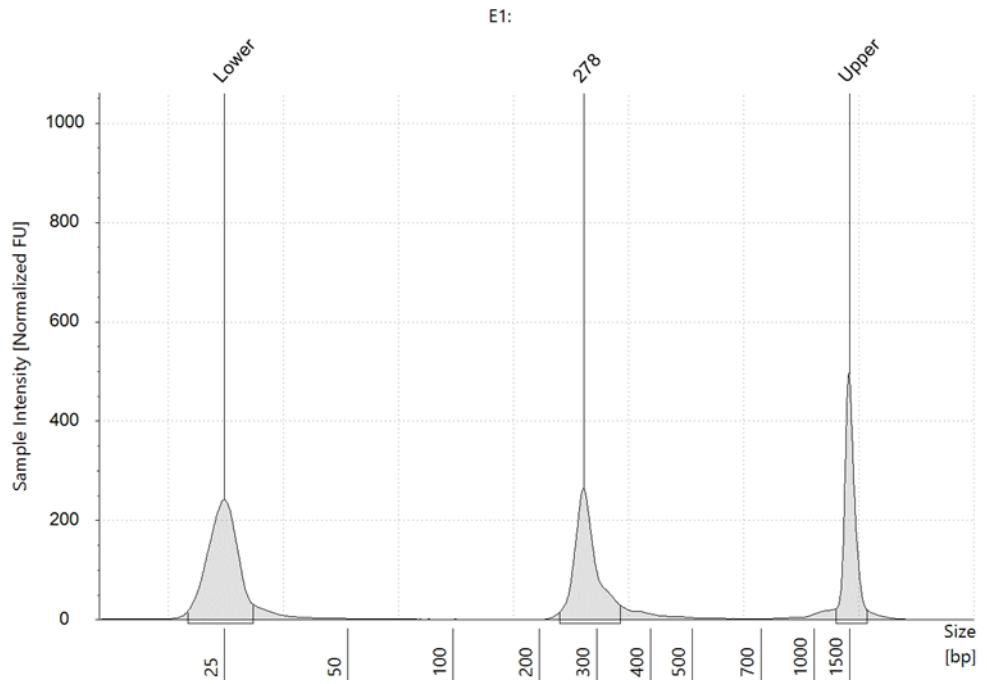


Analysis / Library QC





Final Analysis





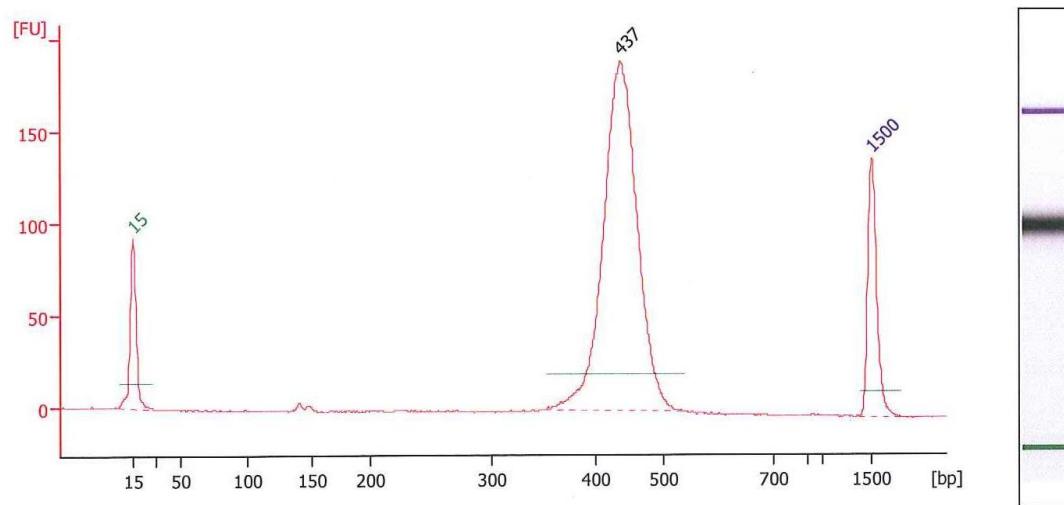
Problems



What can possibly go wrong!!

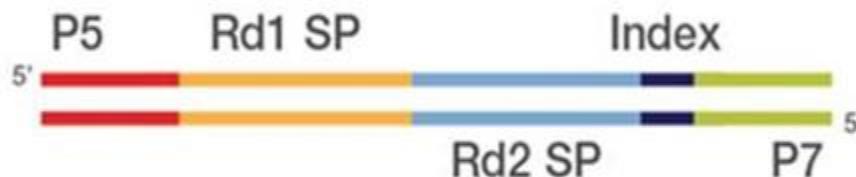


Adapter Dimer



Review of Adapter Dimers and Their Sources

- Adapter dimers are formed when the P5 and P7 adapters ligate to one another rather than to an inserted library



Adapter Dimers Sources

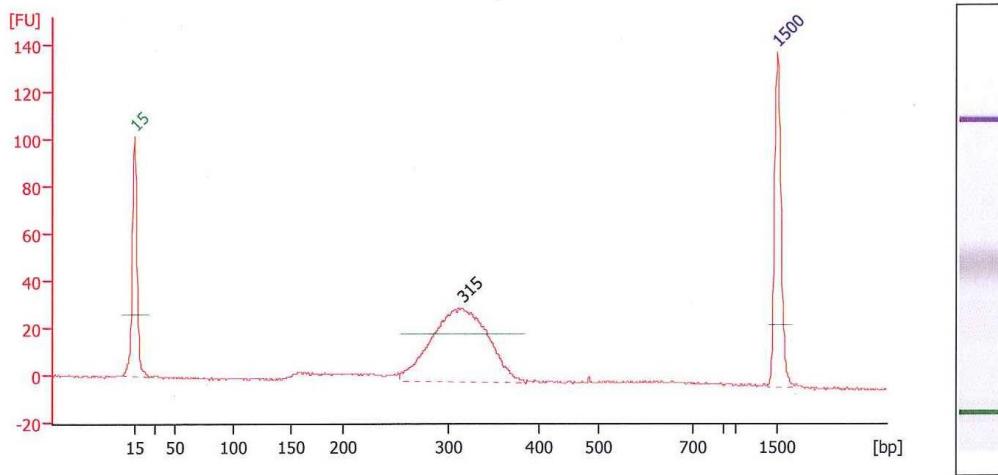
Poor quality input

Bead Handling Error

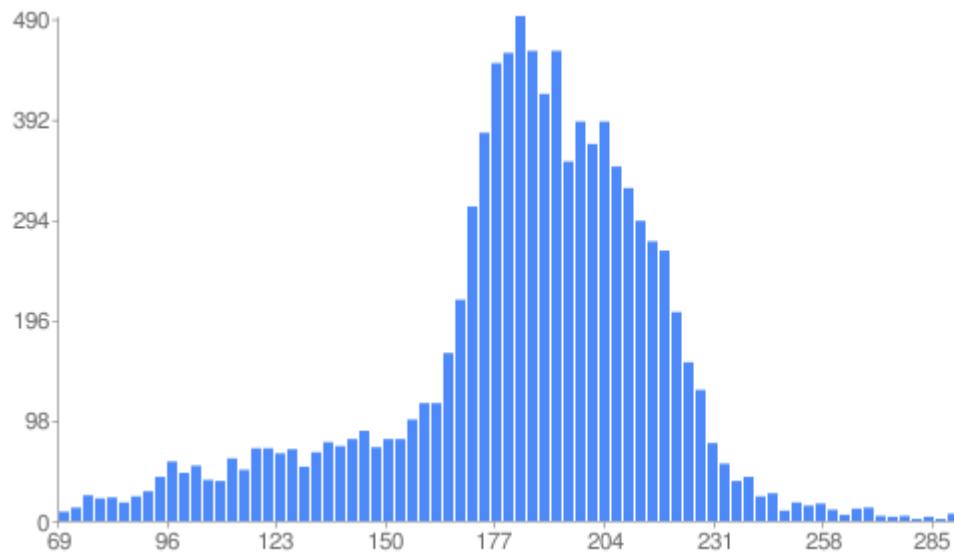
Insufficient input amount

Enzymatic failure

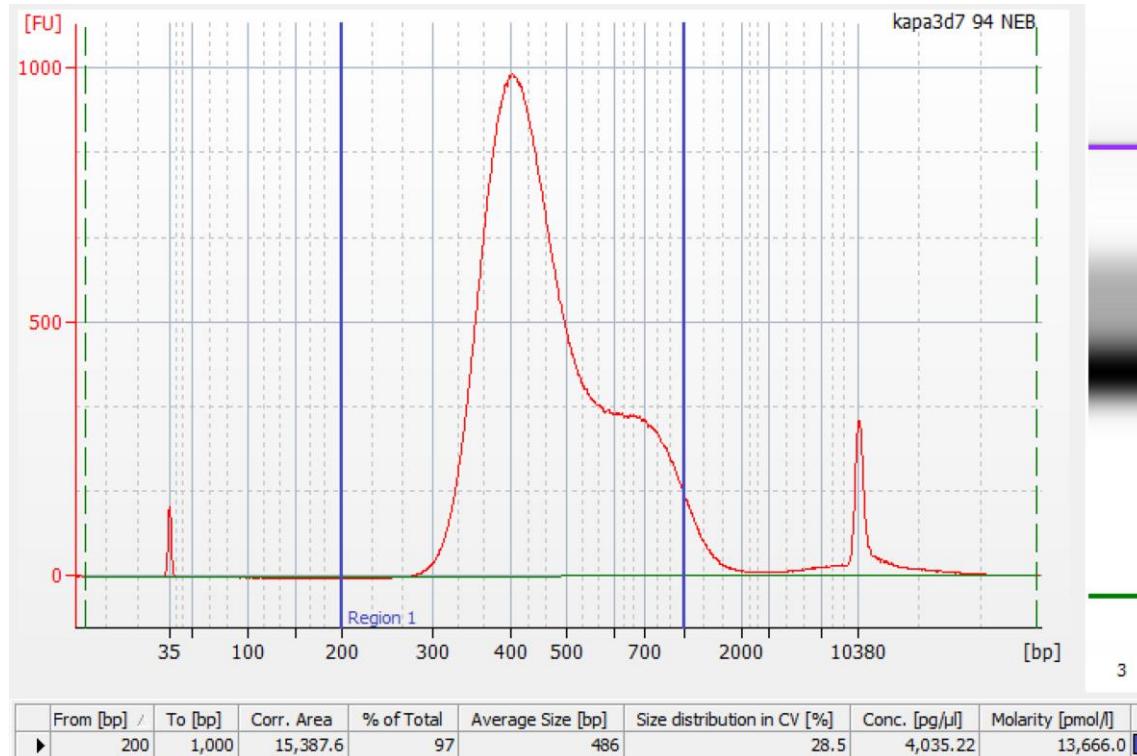
Small Insert Contamination



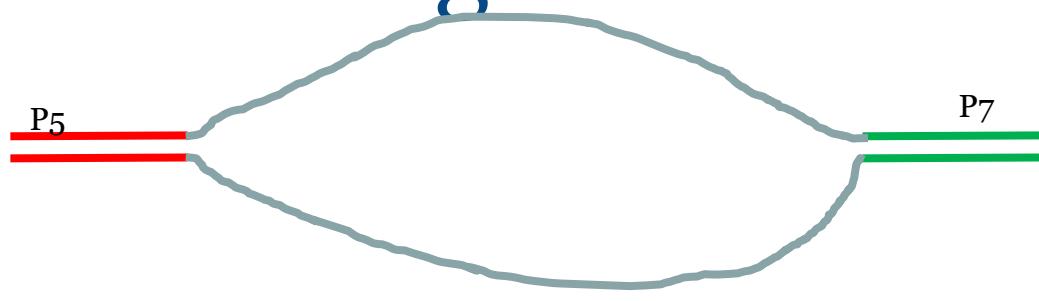
Small Insert Contamination



Larger fragments??



When primers deplete P5 and P7 can anneal but internal fragments don't match up so are single stranded

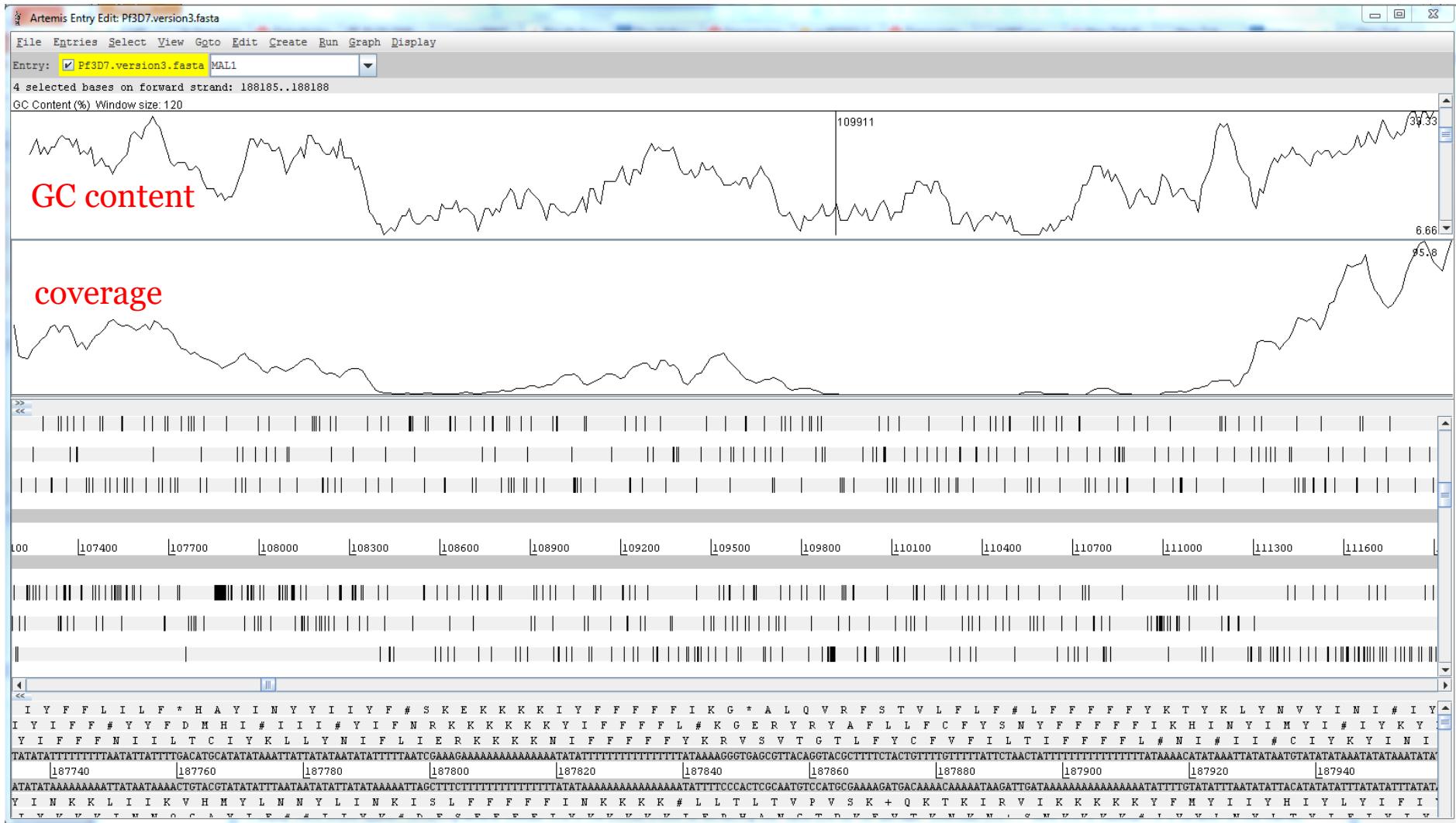


Problem: DNA binding dyes used in quantitation eg Qubit and in tapestation/bioanalyzer bind ds DNA so don't properly quantify
Need to qPCR to properly quantify



Bias

P. falciparum with original Illumina sample prep





Required Sequence Coverage

Coverage Histogram [i](#)





Required Sequence Coverage

Coverage Histogram [i](#)

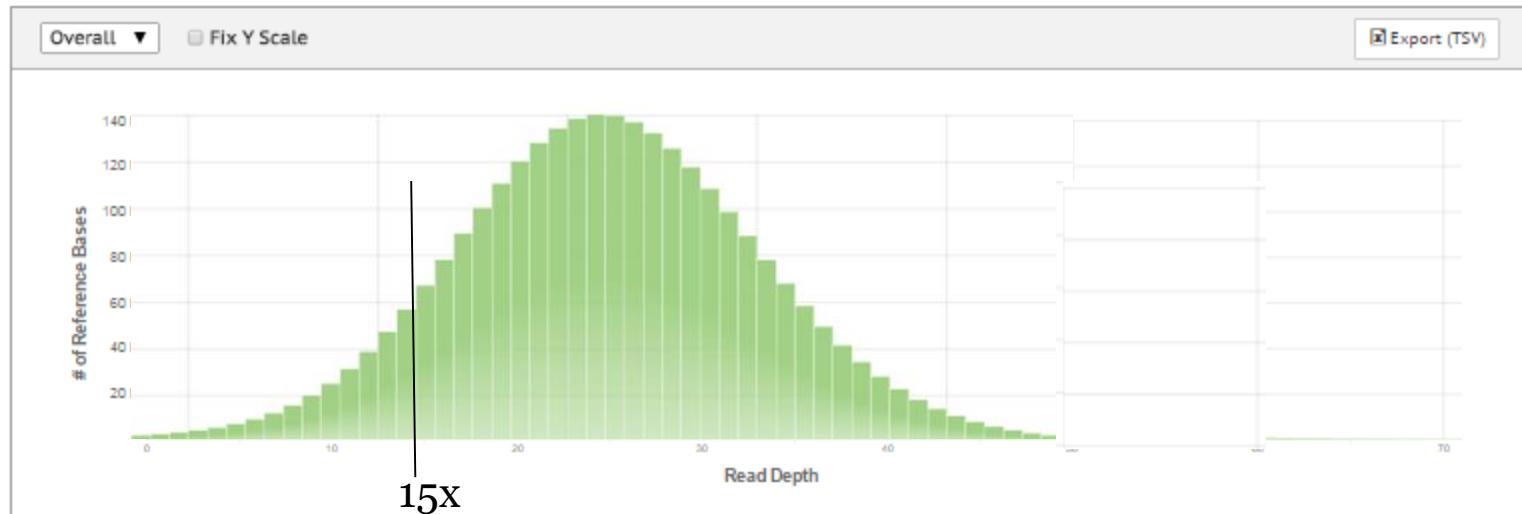


30x sequencing depth required for efficient calling of heterozygous variants
Aim for 95% > 15x so you have sufficient coverage over most positions to call variants



Required Sequence Coverage

Coverage Histogram [i](#)



If we get bias the amount of regions with low coverage will increase and we wont be able to call variants so we then have a choice of whether to fail those samples or sequence again



Contributors to bias

- » Heat
- » Shearing method
- » PCR
- » Target enrichment

Contributors to bias

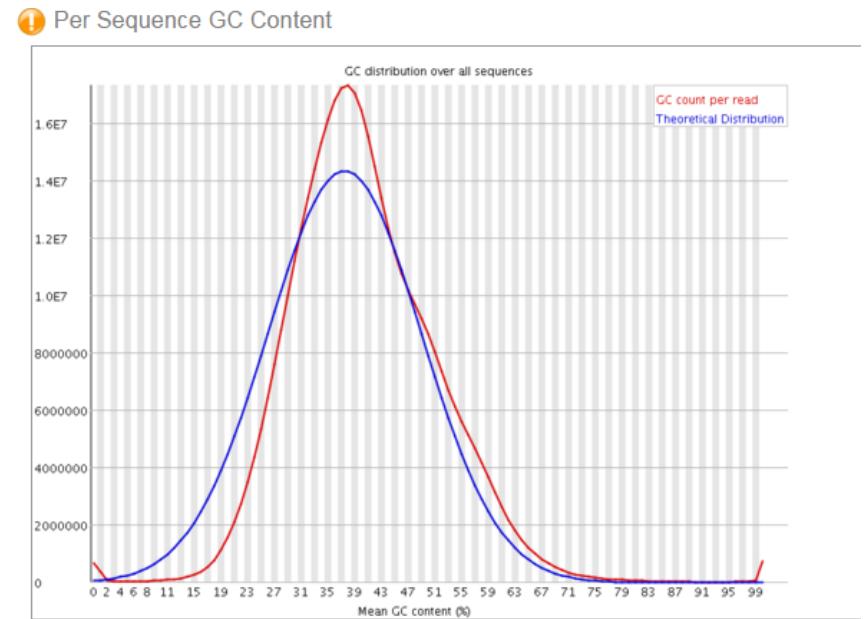
- » Heat

- » Heat steps >65C can irreversibly denature AT rich regions

- » Shearing method

- » PCR

- » Target enrichment



Contributors to bias

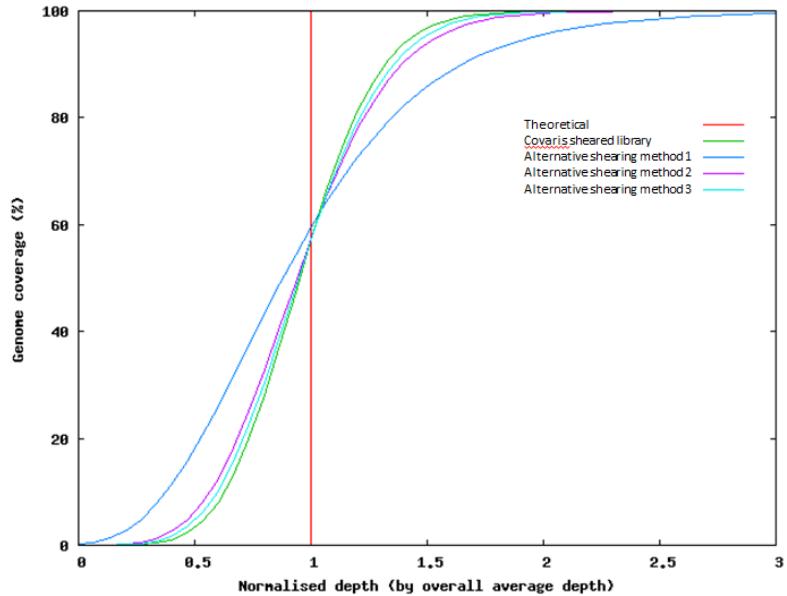
- » Heat

- » Heat steps >65C can irreversibly denature AT rich regions

- » Shearing method

- » PCR

- » Target enrichment

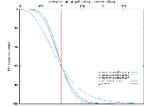


Contributors to bias

» Heat

» Heat steps >65C can irreversibly denature AT rich regions

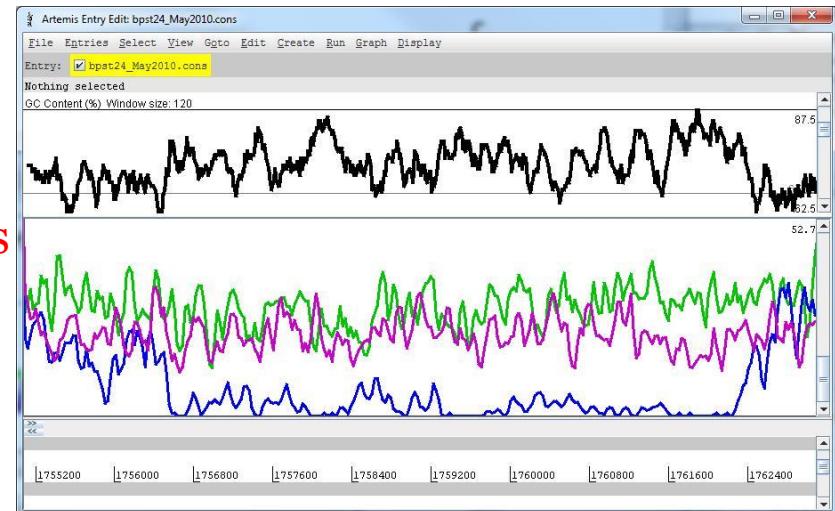
» Shearing method



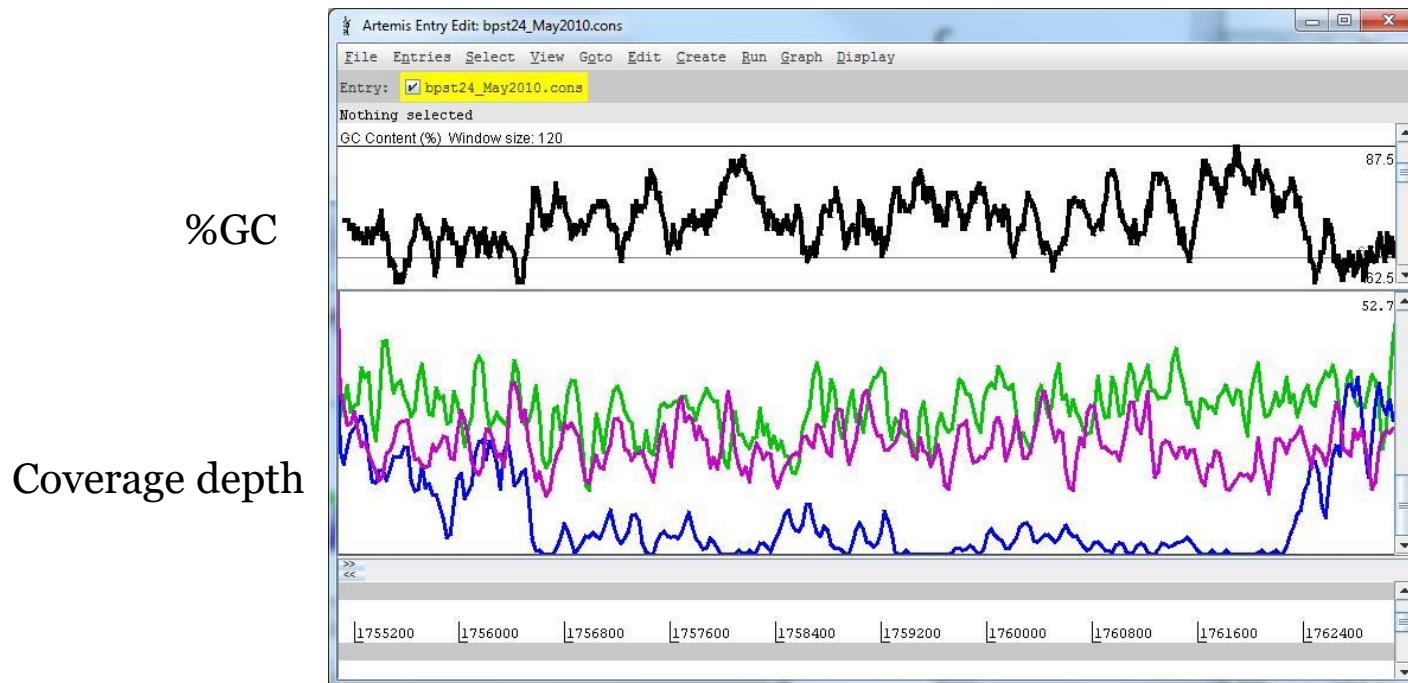
» PCR

» Need right Enzyme and conditions

» Target enrichment



Coverage across a section of *B. pertussis* genome



— Kapa HiFi
— Phusion
— noPCR

See <https://www.nature.com/articles/nmeth.1814>
and <https://doi.org/10.1093/mgen.0.001228>

Recommended PCR enzymes for NGS library prep

- » Quantabio repliQa
- » Watchmaker Equinox
- » Takara Premier plus

For Long Read

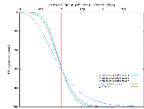
- » Quantabio repliQa
- » Merck KOD Extreme

Contributors to bias

» Heat

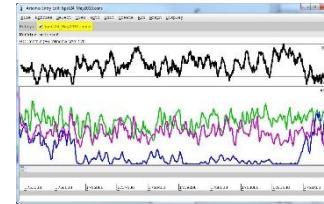
- » Heat steps >65C can irreversibly denature AT rich regions

» Shearing method



» PCR

- » Need right Enzyme and conditions



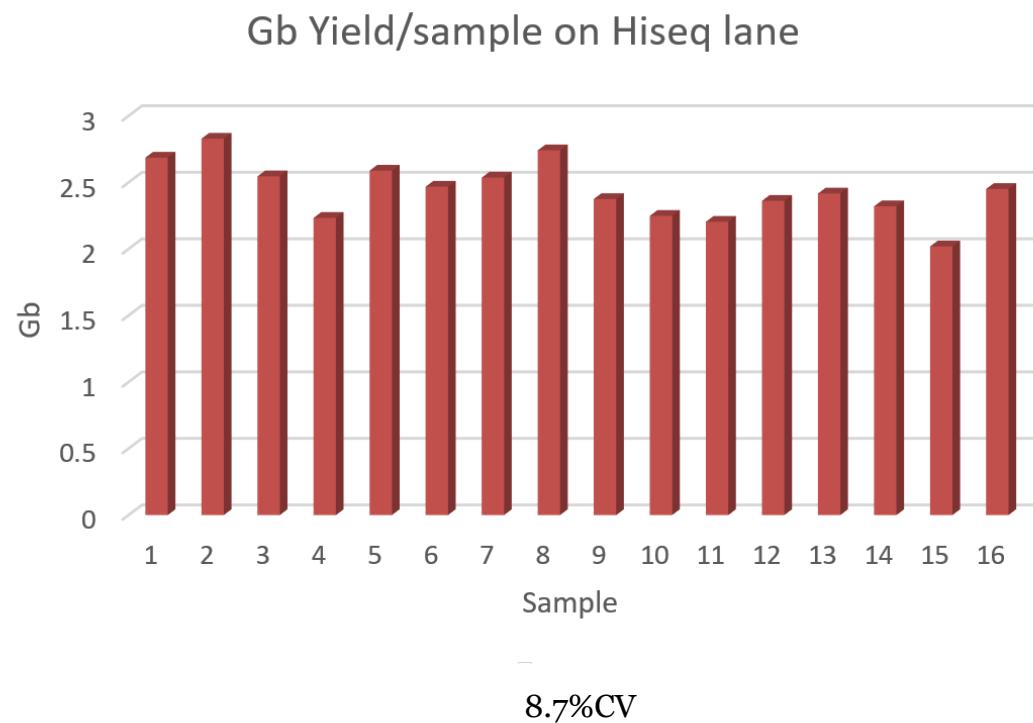
» Target enrichment

- » 100x coverage required for hybrid capture target enrichment
- » 1000x coverage required for PCR based targeted sequencing

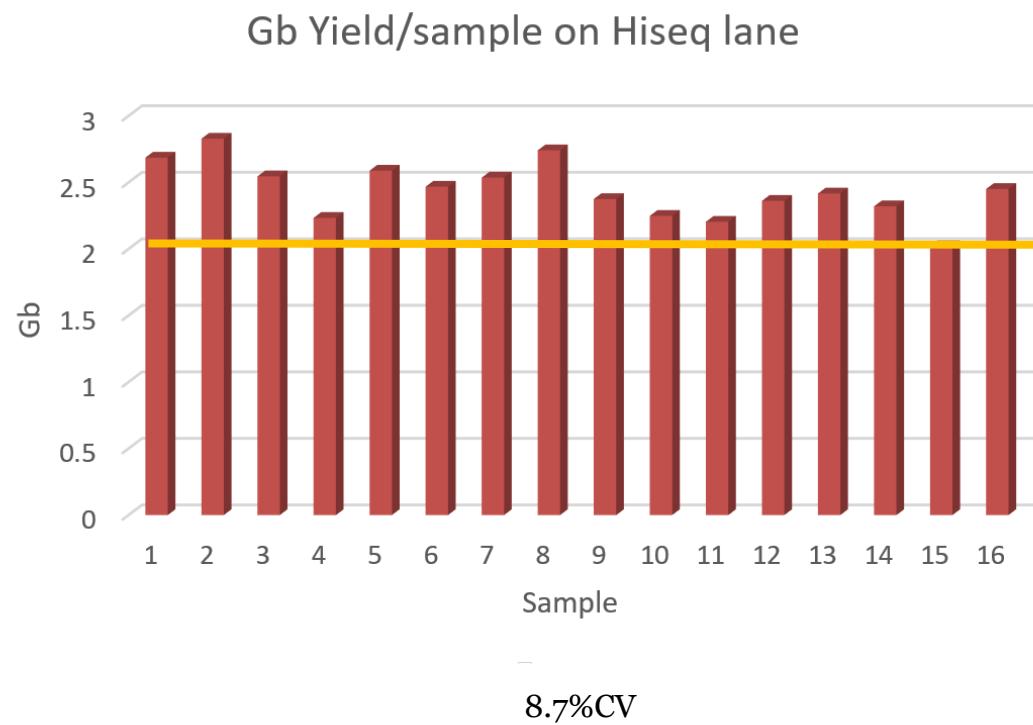


Sample Representation Bias

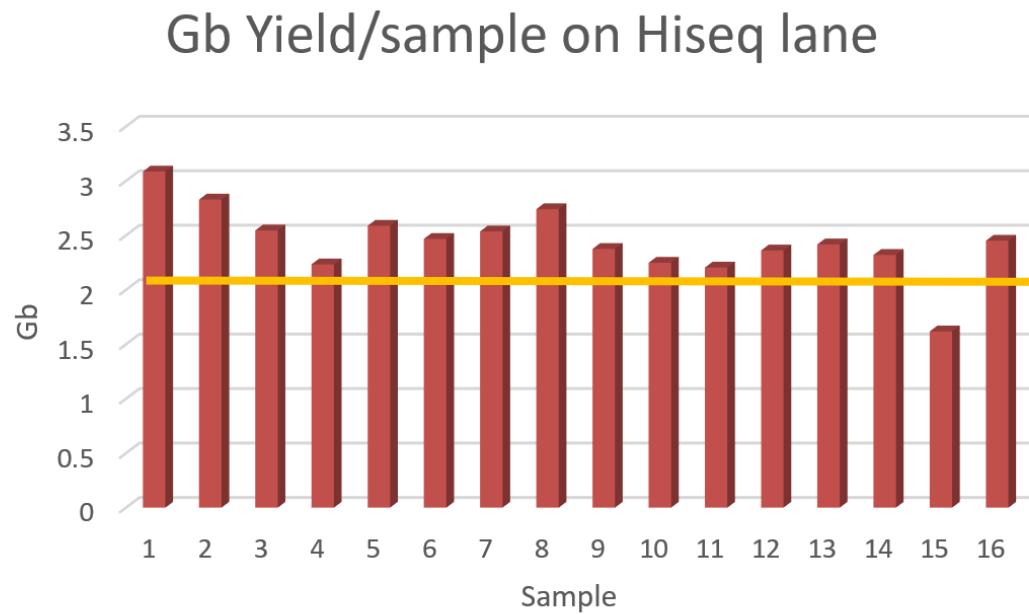
Sample representation



Sample representation

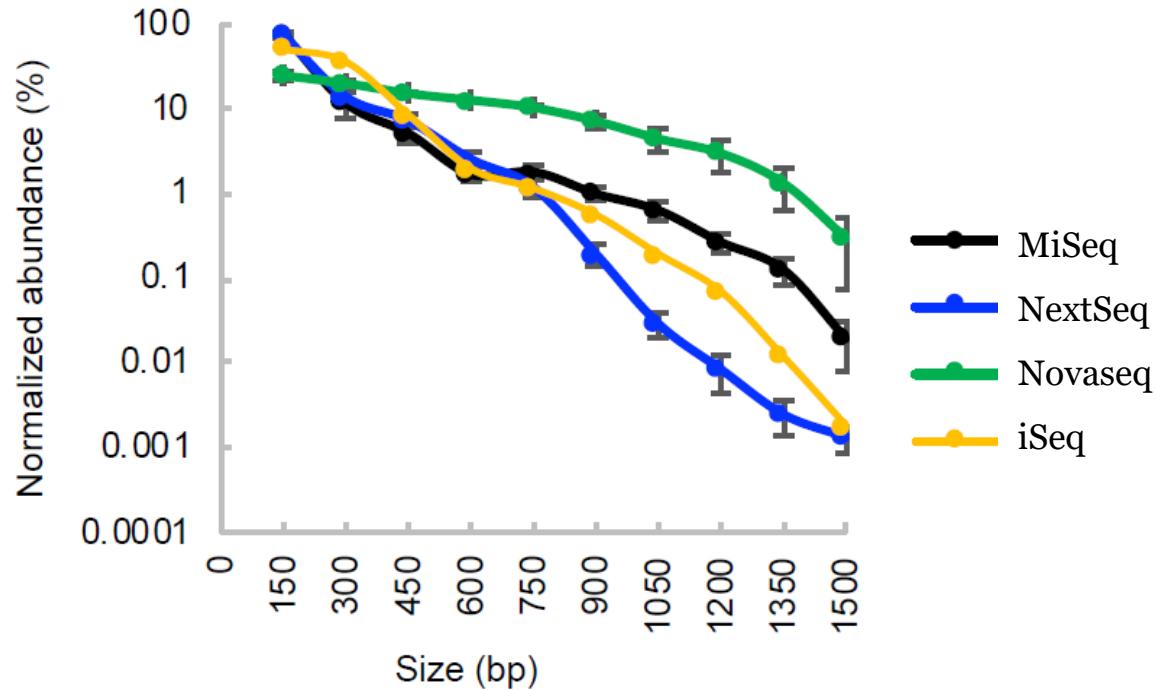


Sample representation



13%CV

Measuring Sequencer Size Bias Using REcount: A Novel Method for Highly Accurate Illumina Sequencing-Based Quantification





The secret to a low sample representation CV

- » Standardise as much as possible
 - » Automation
 - » Reliable quantification step
 - » Standardise DNA input amounts
 - » Accurate pipetting
 - » Control fragment sizes



Efficiency

Library Prep Efficiency

Aigrain et al. BMC Genomics (2016) 17:458
DOI 10.1186/s12864-016-2757-4

BMC Genomics

METHODOLOGY ARTICLE

Open Access

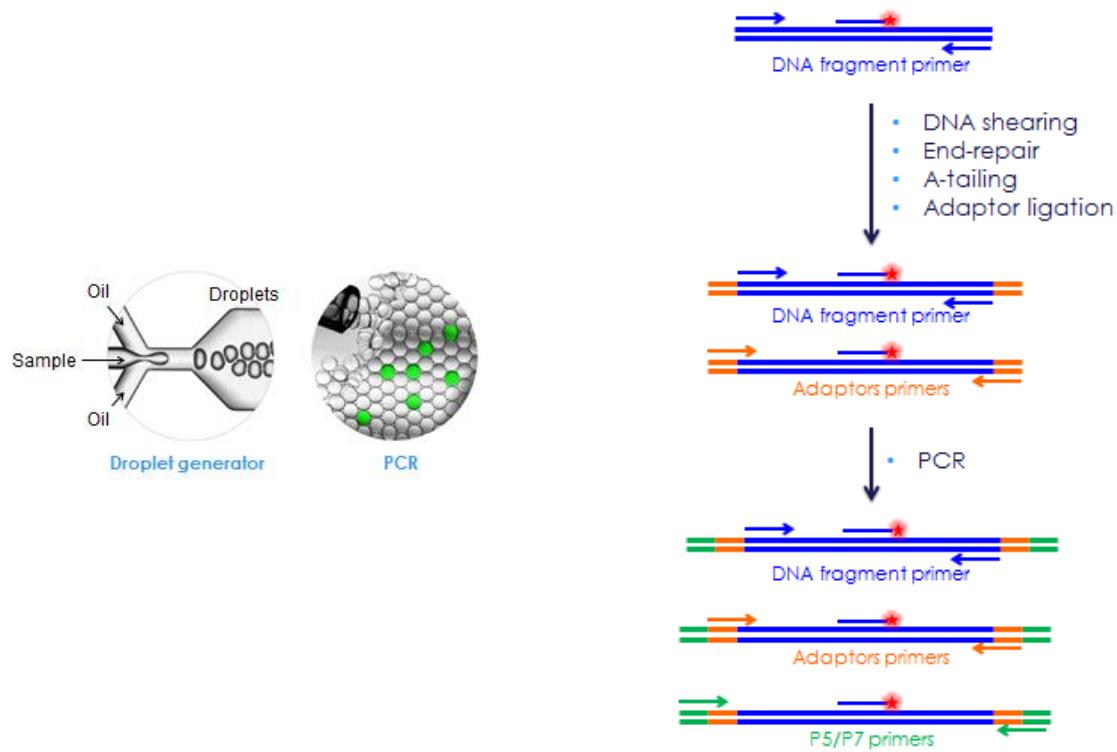


CrossMark

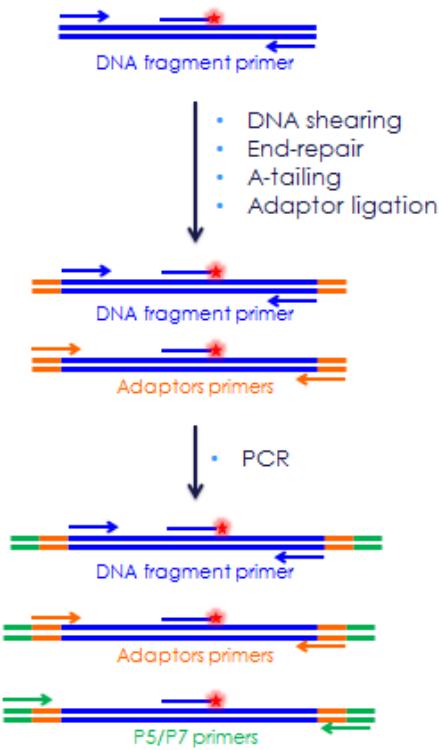
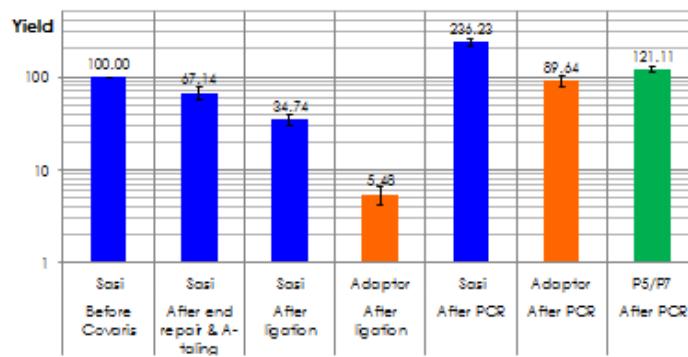
Quantitation of next generation sequencing library preparation protocol efficiencies using droplet digital PCR assays - a systematic comparison of DNA library preparation kits for Illumina sequencing

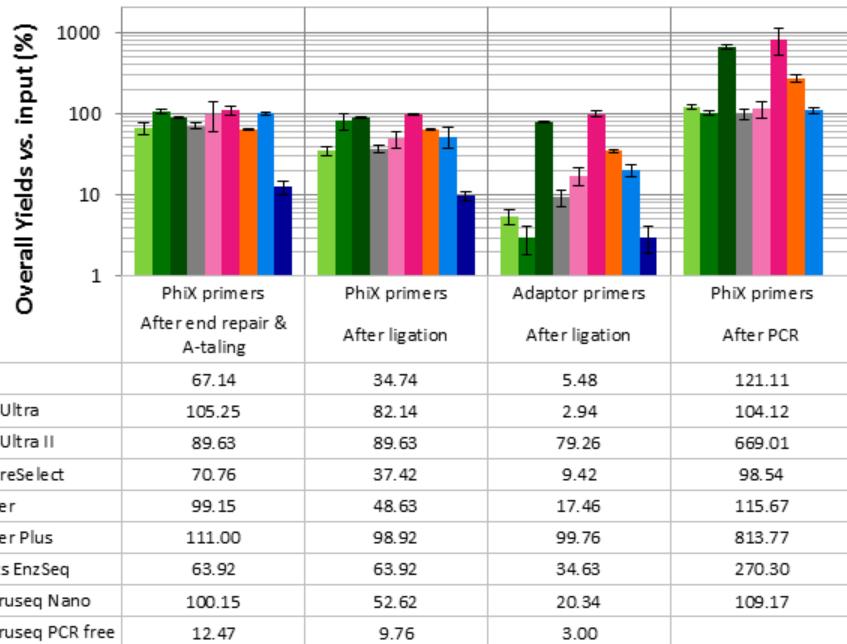
Louise Aigrain*, Yong Gu and Michael A. Quail

Illumina library efficiency



Illumina library efficiency







Library quantification prior to sequencing



Library quantification prior to sequencing

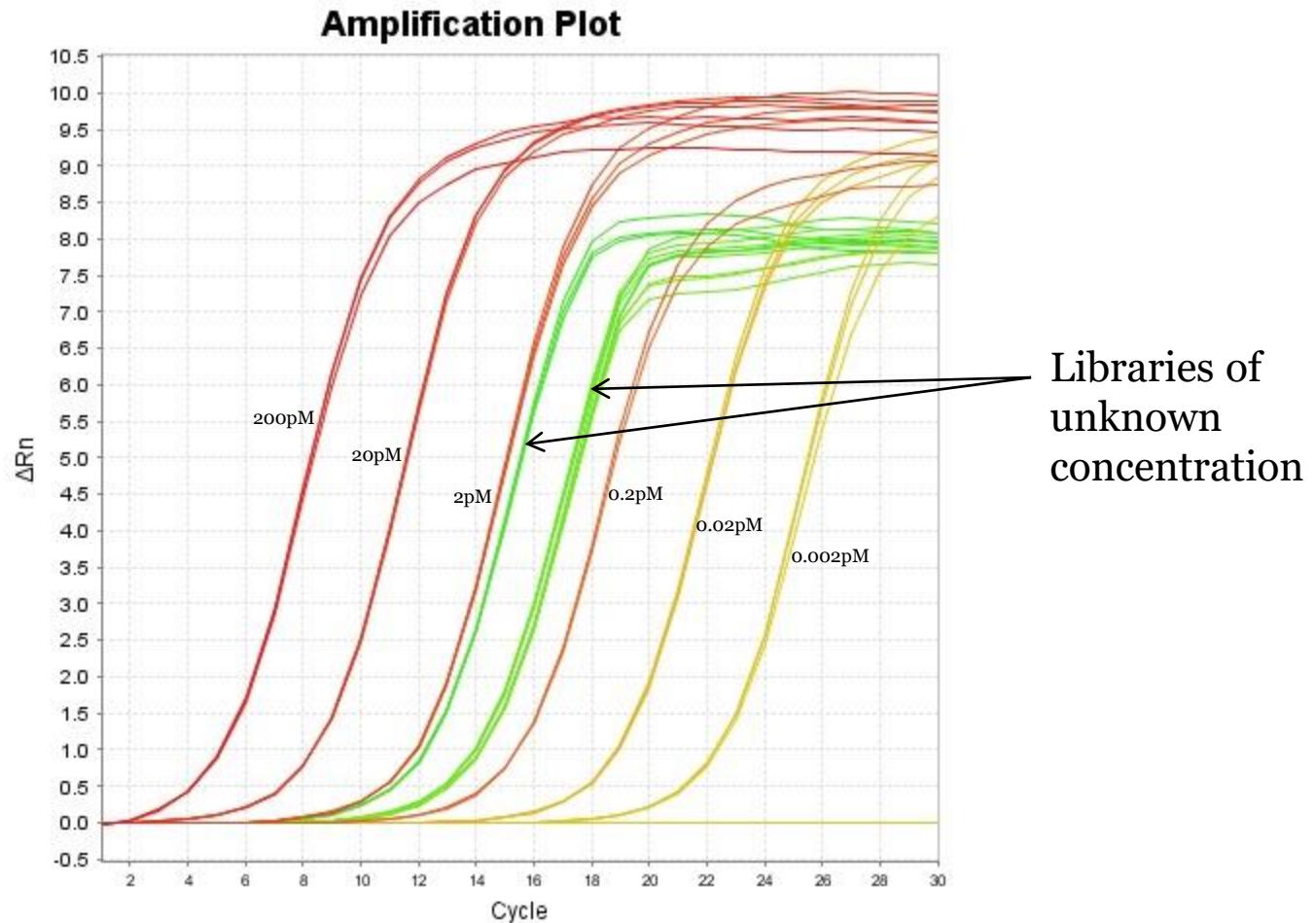
Options:

qPCR

Agilent bioanalyser, Caliper
Labchip GX

Qubit

qPCR quantification



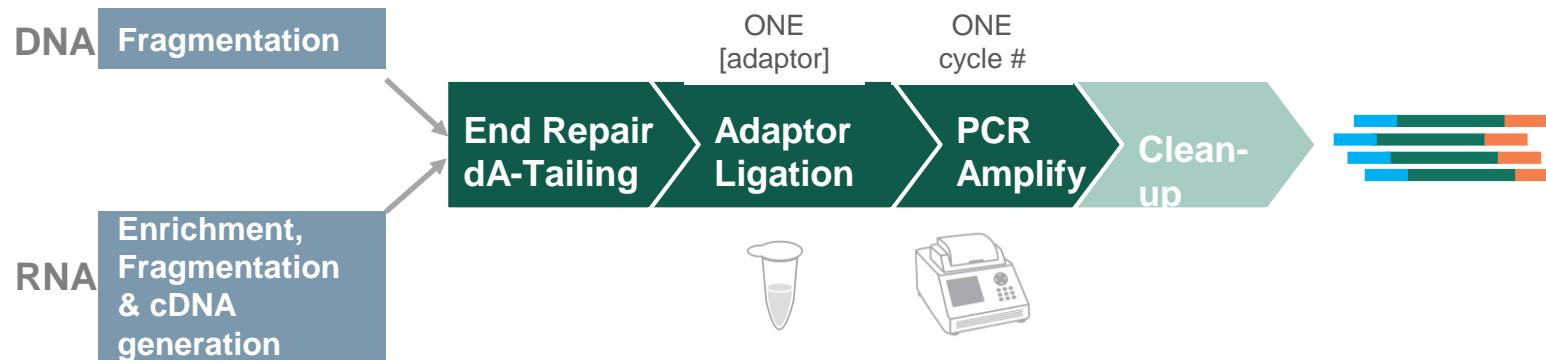
Introduction to the Library Prep Workflow

NEBNext UltraExpress® DNA

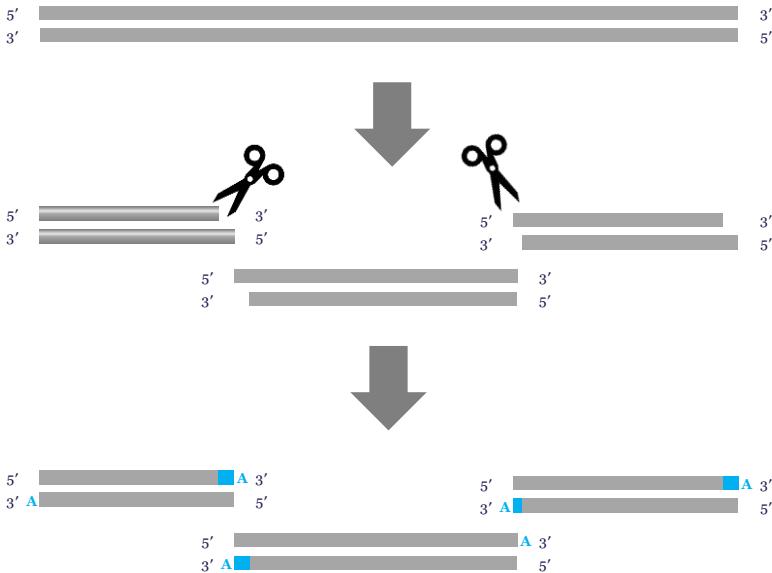
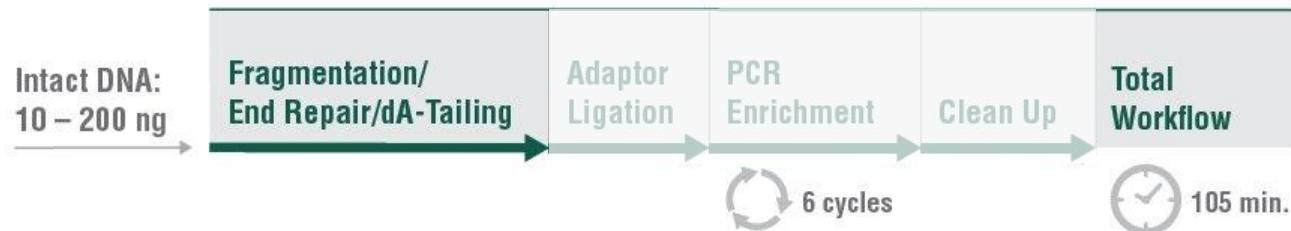
Max Fritsch | Field Application Scientist



Streamlining the library prep workflow



NEBNext UltraExpress® DNA Library Prep Workflow



Fragmentation

- Random fragmentation overhangs can be 1-4 bases on the 5' end, blunt ends or 3' overhangs
- Incubation time determines fragment size

End repair

- Enzymatic activity that fills in 5' overhangs and chews back 3' overhangs to create blunt ends

dA-Tailing

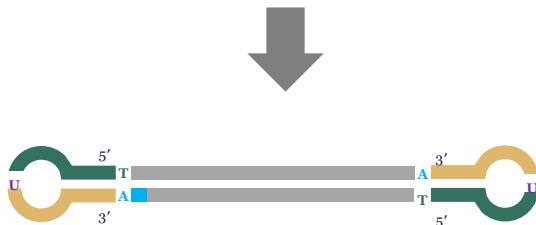
- Enzymatic activity to 'A'-tail the blunt-ended DNA fragments to prepare them for adaptor ligation

NEBNext UltraExpress® DNA Library Prep Workflow



Adaptor Ligation

- Ligation of the T overhang of the hairpin adaptor to the A overhang of the fragmented DNA



Hairpin Adaptor

- Higher conversion rates
- Greater complexity
- Minimizes adaptor dimer formation



NEBNext UltraExpress® DNA Library Prep Workflow

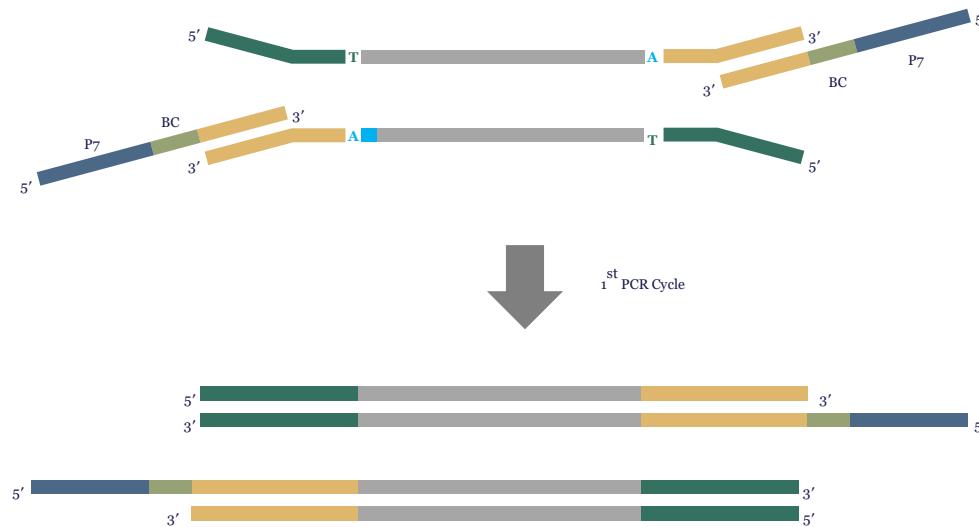
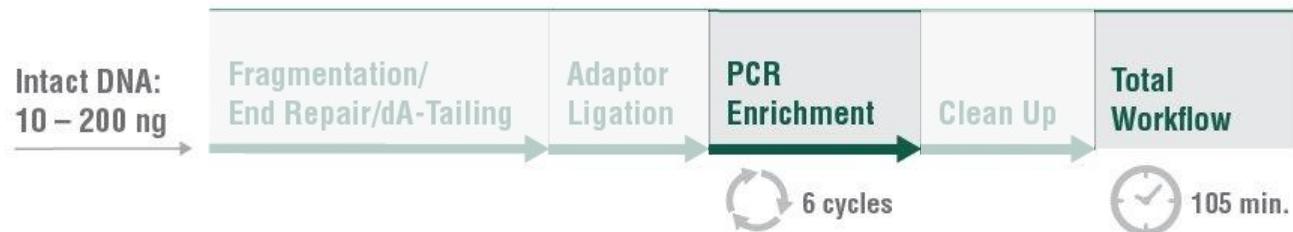


'U' excision

- USER enzyme removes in a series of enzymatic reactions the uracil base to open the NEBNext hairpin adaptor in preparation for PCR



NEBNext UltraExpress® DNA Library Prep Workflow

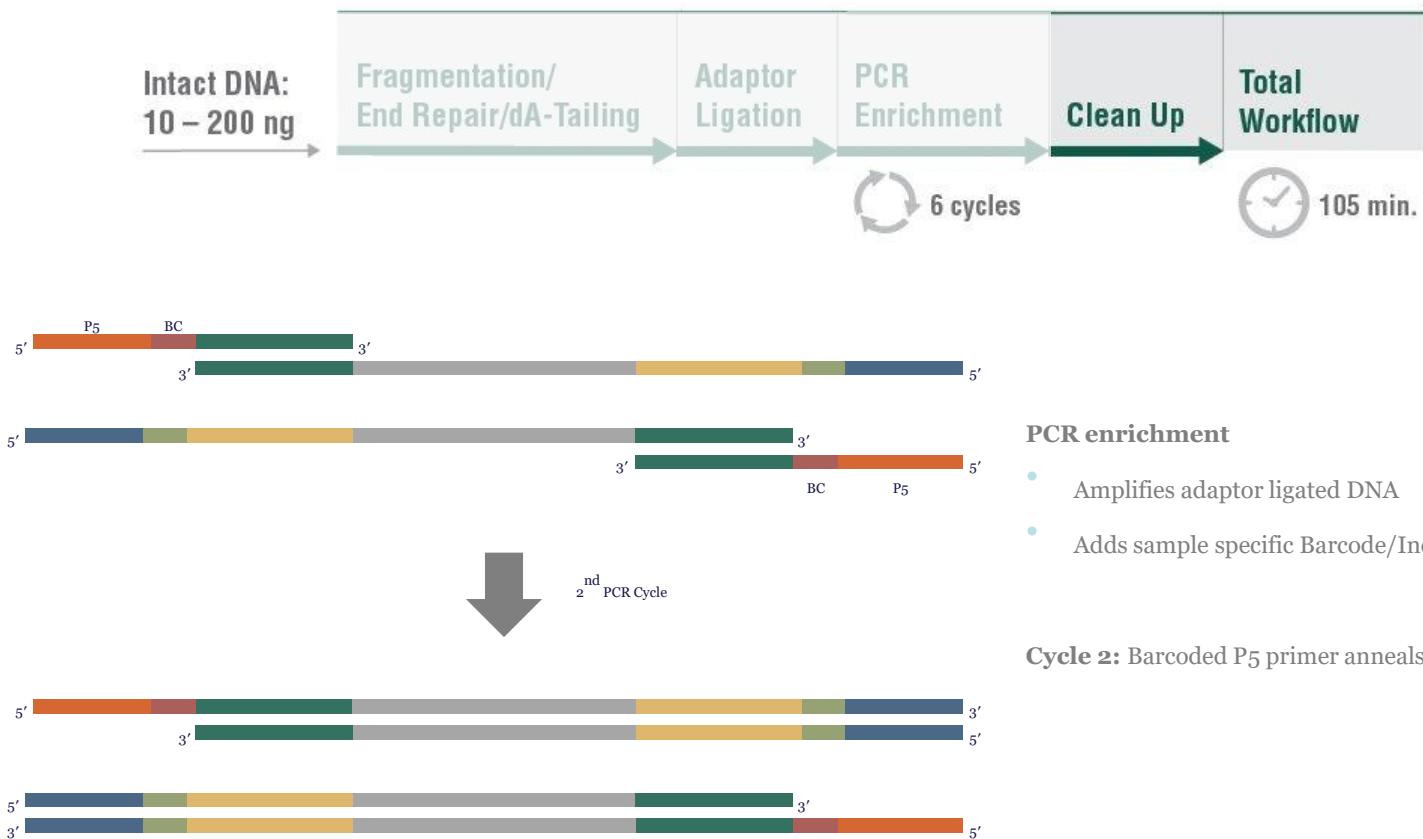


PCR enrichment

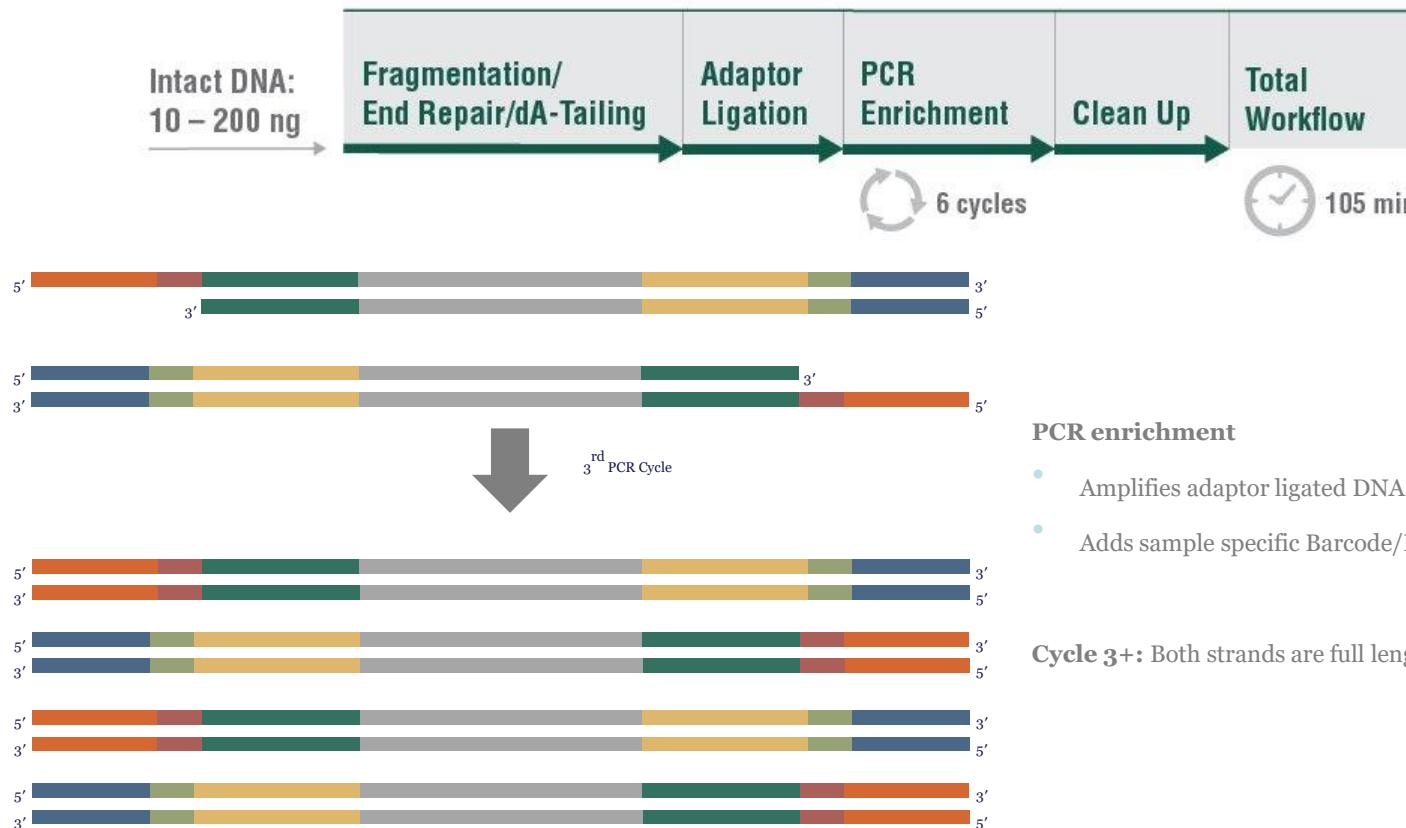
- Amplifies adaptor ligated DNA
- Adds sample specific Barcode/Index

Cycle 1: the barcoded P7 PCR primer anneals, but there is still sequence missing on both strands

NEBNext UltraExpress® DNA Library Prep Workflow



NEBNext UltraExpress® DNA Library Prep Workflow



NEBNext UltraExpress® DNA Library Prep Workflow



Library cleanup

- Removes enzymes and buffer from the PCR
- Removes any leftover adaptor dimer
- Removes PCR primers
- Phased Clea-up: double clean-up

Quick Reminder: Best Practices in the Lab

1. Thaw the buffers on top of the ice or in your hand and vortex to mix.
2. DO NOT vortex enzymes! 
3. Mix enzymes by flicking the tube gently and inverting the tube.
4. Quick spin down the tubes containing the enzymes and buffers.
5. Keep enzymes and thawed buffers on ice. 
6. Keep reactions on ice unless it is specified to stay at room temp (RT) 
7. Mix reactions thoroughly by pipetting up and down as indicated in protocol. 



Part Two



Talk Outline

- » Libraries for other sequencing platforms.
- » Types of NGS sequencing library?
- » Automation of library prep



Sequencers



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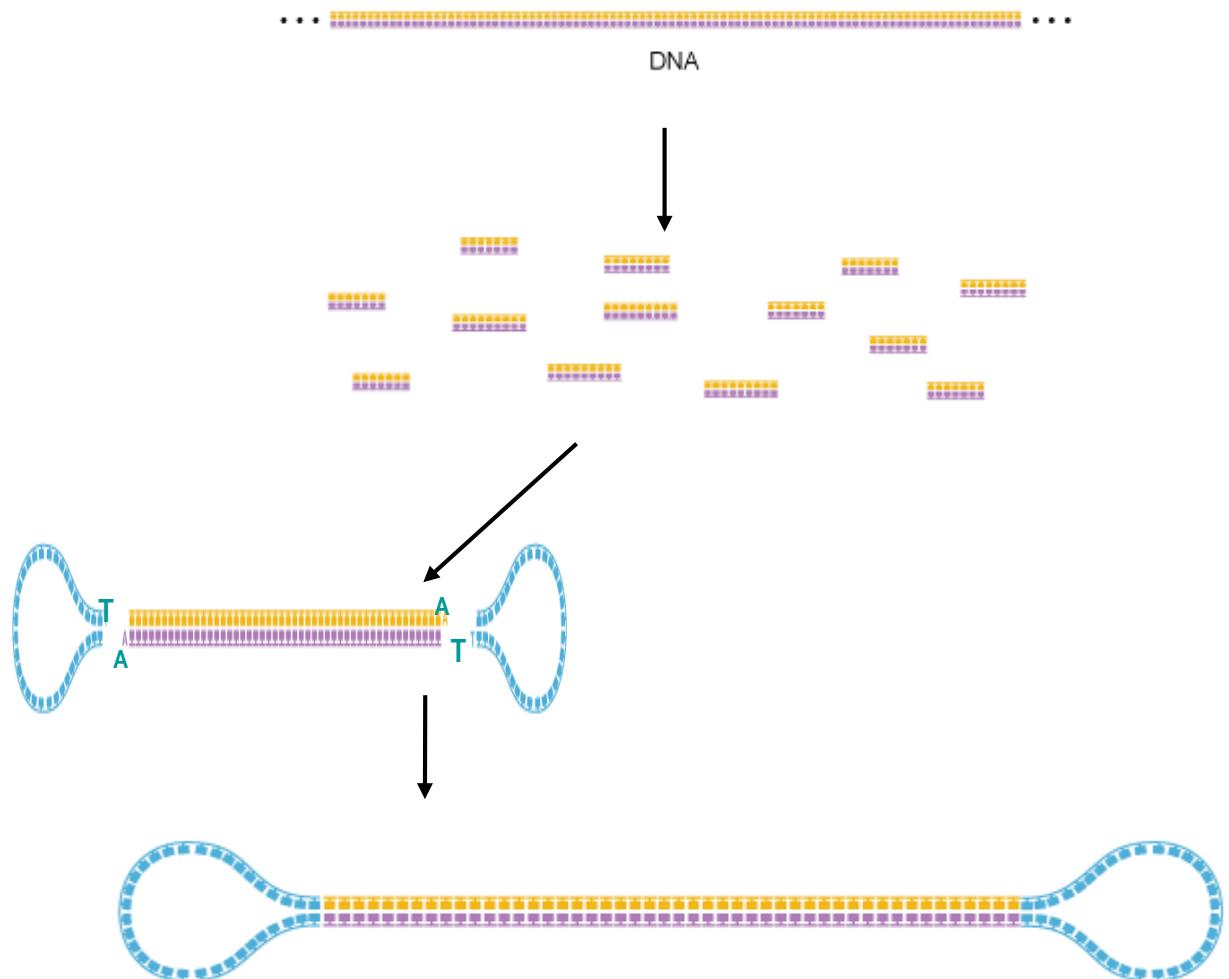
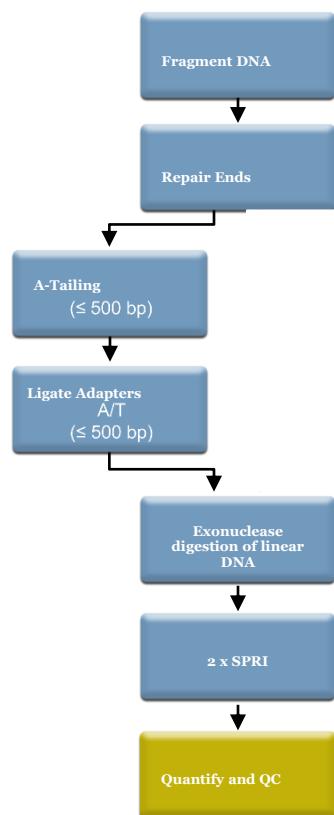
Sequencers



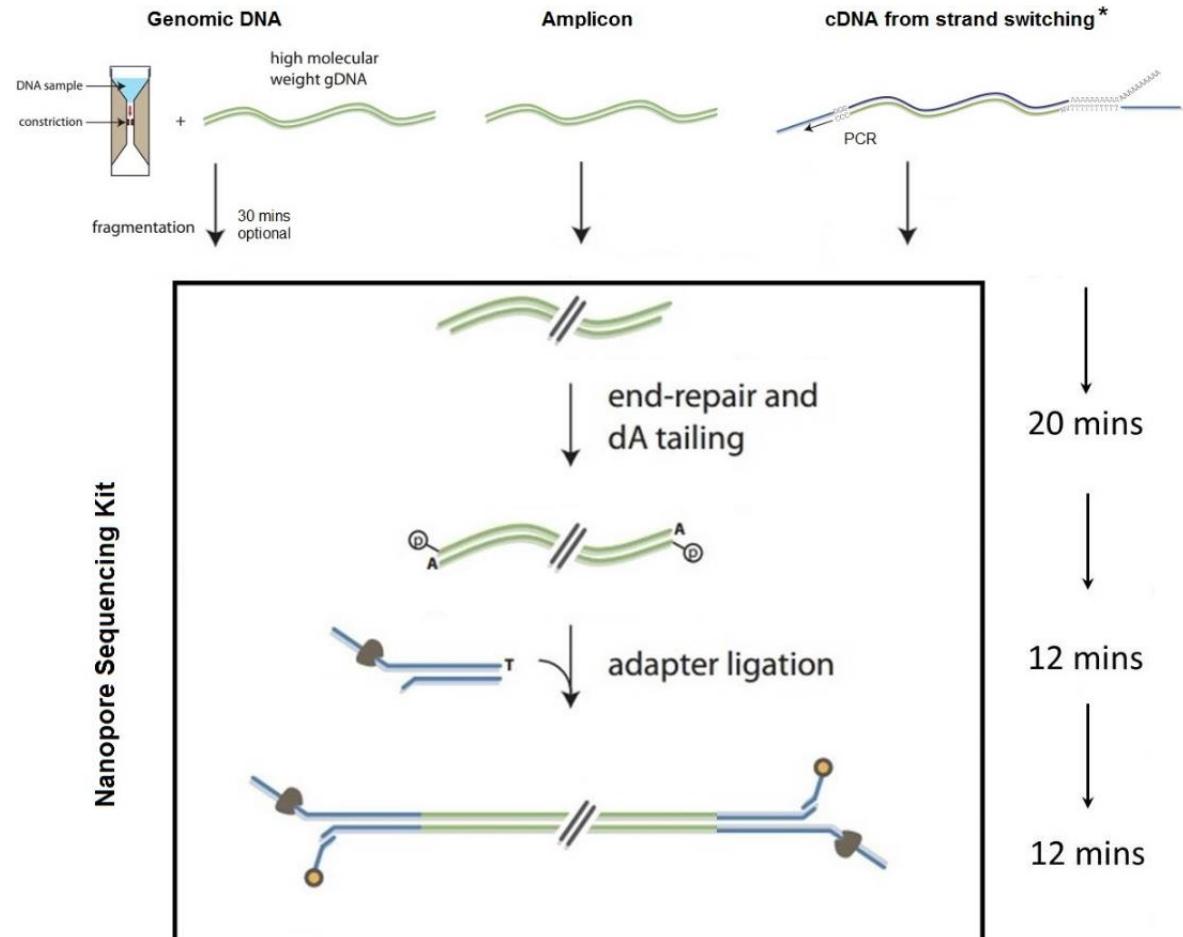


PacBio Library Prep

Library Preps



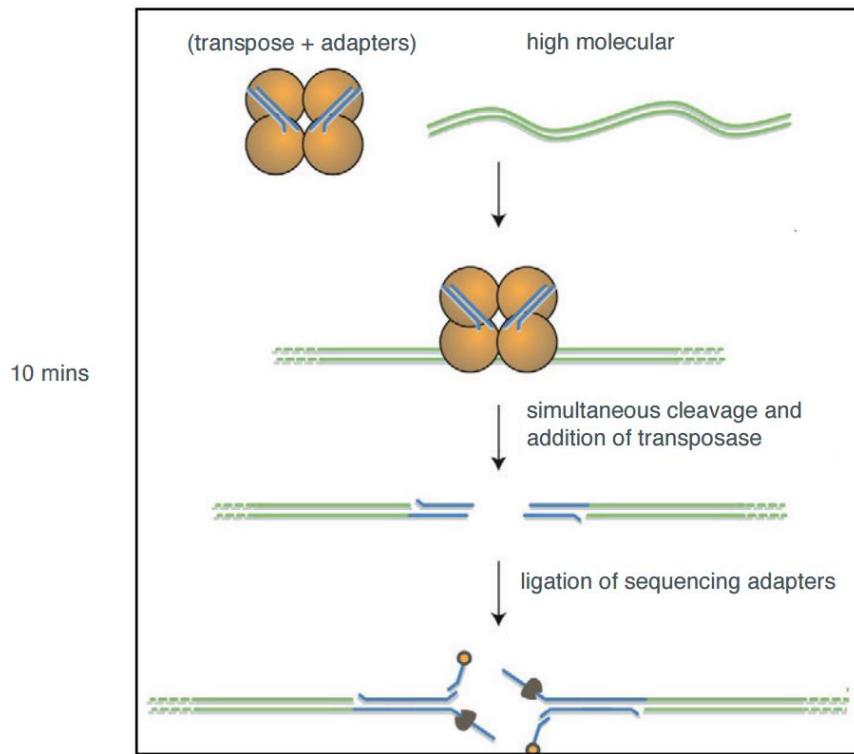
ONT 1D Library prep





RAPID SEQUENCING KIT

A two-step, 10 minute protocol



Starting material will be fragmented; recommended starting size >30 kb for genomic DNA



Sequencers



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Alternative standard library prep methods

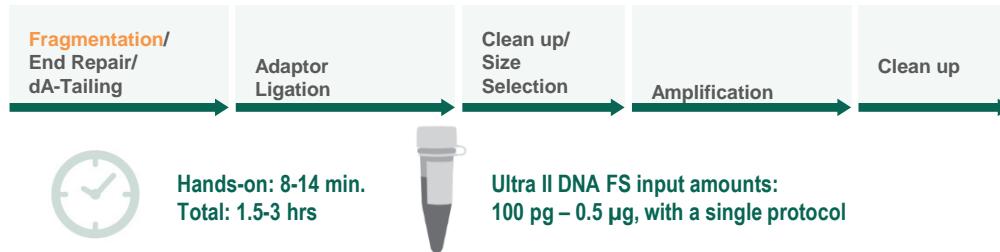


Next generation enzymatic shearing



Ultra II NEB FS

Kit: Enzymatic fragmentation, combined with Ultra II DNA Library Prep reagents



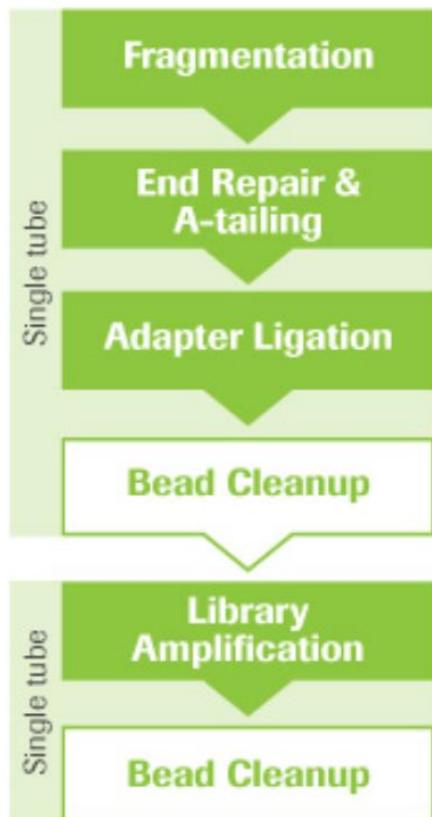
- ❖ Robust, easy to use and compatible with input DNA in water and standard DNA buffers (TE, Tris-HCl).
- ❖ Capable of generating a wide range of insert sizes, by varying incubation time.
- ❖ Uses the same fragmentation protocol, for all input amounts and GC contents.
- ❖ Produces high yields of high quality libraries.

UltraII FS. Shearing relatively unaffected by contaminants, GC content and input amount. Controllable fragment sizes. Can use for low input.



KAPA HyperPlus Kit

Total time: ~2.5 hours



Roche Sequencing

Search Product

Product Type

- Assays and Reagents
- Techniques
 - DNA Library Preparation
 - Sample Preparation
- Applications
 - Whole Genome Sequencing
- Product Family
 - KAPA

KAPA HyperPlus Kits →
KAPA HyperPlus Kits provide a streamlined workflow with integrated, low-bias enzymatic...

KAPA HyperPrep Kits →
As the second-generation option, KAPA HyperPrep Kits provide a novel one-tube chemistry and a...

KAPA EvoPlus V2 Kits →
KAPA EvoPlus V2 Kits boost fragmentation, conversion efficiency, and sequencing with a...

KAPA EvoPrep Kits →
KAPA EvoPrep Kits are the latest high-performance, streamlined, and automation-friendly library...



Simple, automatable workflows for all sample types

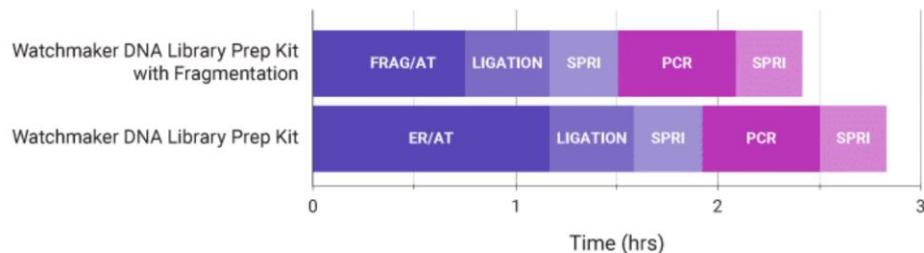
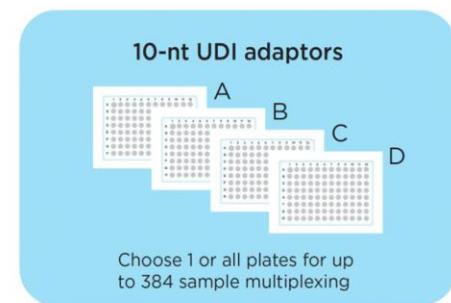
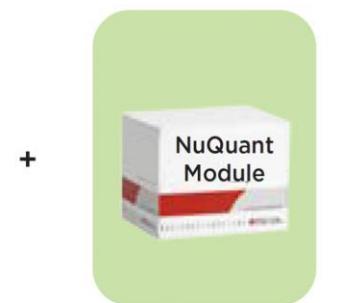


Figure 1. Simple DNA library prep solutions. Watchmaker's DNA portfolio offers highly streamlined workflows to address all DNA sample types. Samples requiring fragmentation use a consolidated enzymatic fragmentation and A-tailing step (Frag/AT), while pre-fragmented samples (e.g., cfDNA or mechanically sheared DNA) use a combined end-repair and A-tailing step (ER/AT). Amplifiable libraries may be prepared in approximately 1.25 to under 2 hours, respectively. For applications requiring PCR, [Equinox polymerase](#) provides excellent fidelity and yields while only adding about 1 hour to the overall workflow.

Celero DNA-Seq Library Prep Kit Components



Core Module: Celero DNA-Seq Enz and Celero DNA-Seq Mech

The core module for enzymatic workflow contains the reagents for the enzymatic fragmentation and ligation. Alternatively, the core module for mechanical workflow contains reagents to be used with sheared DNA, for end repair and ligation.

NuQuant Module

The NuQuant module contains the necessary PCR enzyme mix and primers for library amplification and integration of NuQuant.

Adaptor Plates: 10-nt UDI adaptor plates for upto 384-plex multiplexing

The adaptor plates provide the unique dual index adaptors for multiplexing samples for sequencing.



EN SUPPORT MY ORDERS SIGN IN

Products / NGS / Library Preparation / Enzymatic Fragmentation Kit

Library Preparation Enzymatic Fragmentation Kit 2.0

Sequence With Confidence

GET QUOTE

OVERVIEW DATA ORDERING RESOURCES

Enhanced Enzymatic Fragmentation

The Twist Library Preparation Enzymatic Fragmentation (EF) Kit 2.0 is built to help you achieve a more efficient sequencing pipeline and obtain more accurate Next-Generation Sequencing (NGS) results. Compared to the original Twist Library Preparation EF Kit 1.0, this updated kit has several benefits. In addition to new fragmentation and ligation modules, the upgraded kit now includes the Equinox Library Prep Amp Mix. This hot-start enzyme formulation has a lower upper rate* and high efficiency at low input volumes – making it ideal

New Human Sample ID

Ensure the accuracy of whole exome sequencing by tracking samples from whole blood. Twist Human Sample ID utilizes established Twist Library Prep and multiplexed PCR to identify and track

Nextera

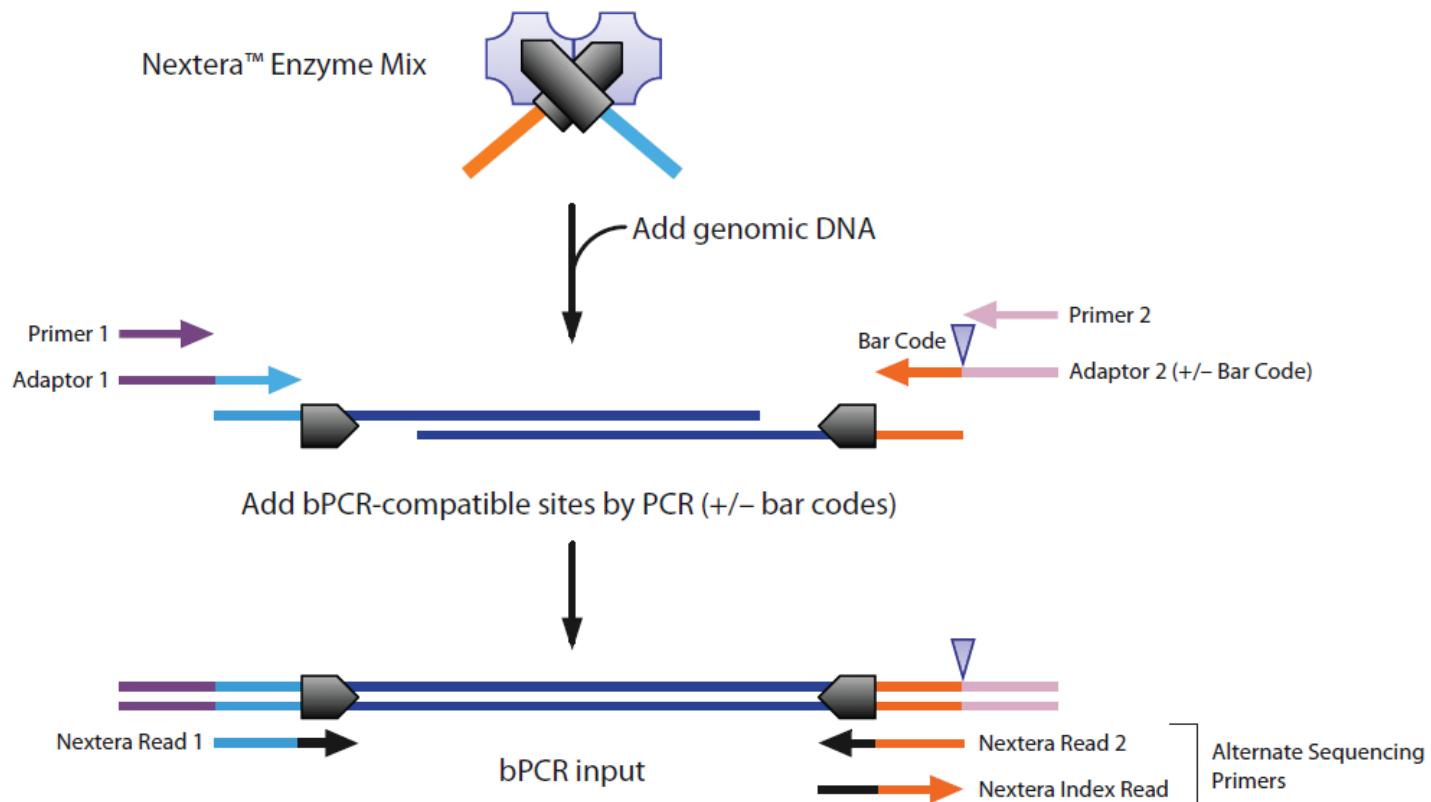


Figure 1. Generating Illumina®-compatible libraries.

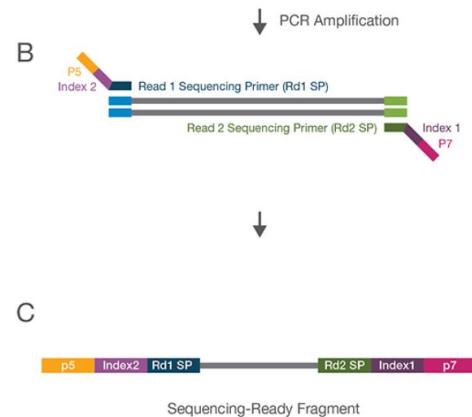
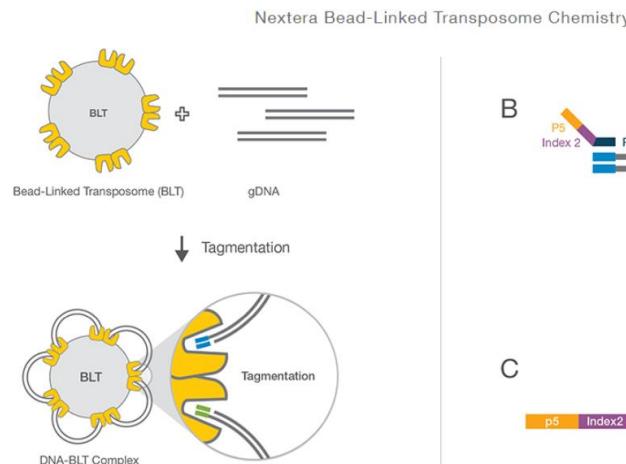
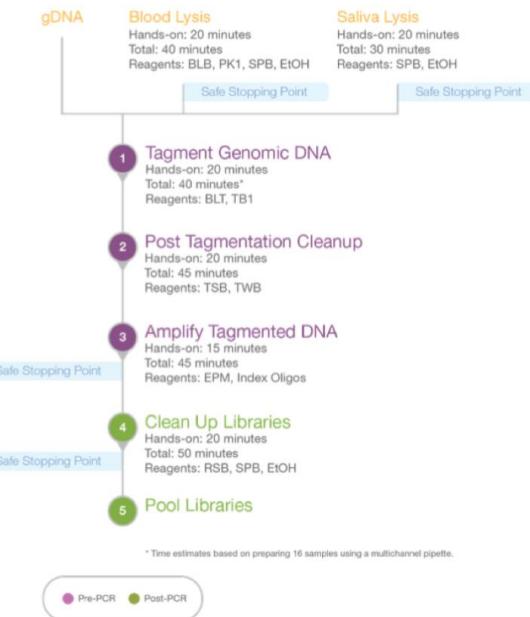


Nextera XT

- » It works but
 - » Can be expensive
 - » Size of fragments sensitive to DNA amount, GC content and contaminants
 - » Small inserts. Adapter. Lower mappability
 - » DNA not in correct size range
 - » More variable insert sizes
 - » More variable coverage when multiplexing

Illumina NextFlex

Okay but insert sizes 300bp



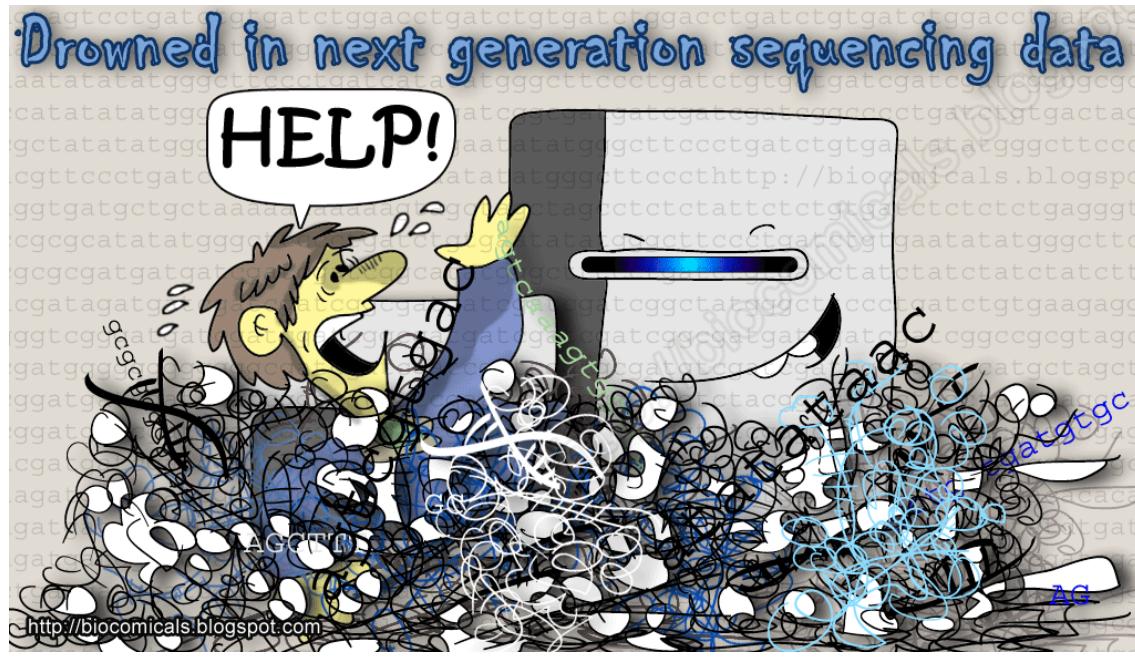
Nextera Flex. Much much better than old Nextera and XT. Shearing relatively unaffected by contaminants, GC content and input amount. Expensive and not too easy to tune fragment sizes.



Other library types

What is Targeted Sequencing??

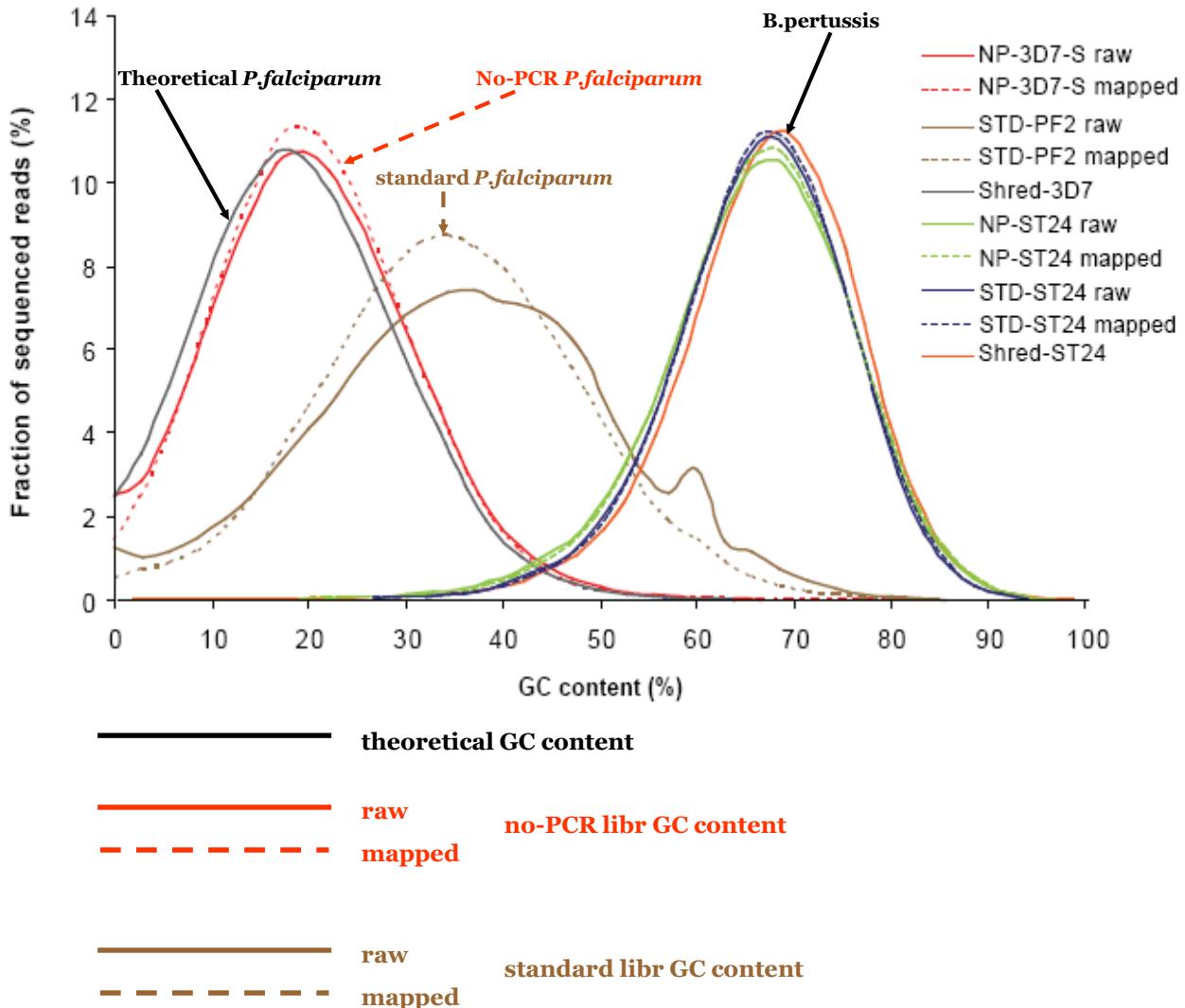
“Targeted resequencing is a variation of re-sequencing where only a small subset of the genome is sequenced, such as the exome, a particular chromosome, a set of genes or a region of interest”





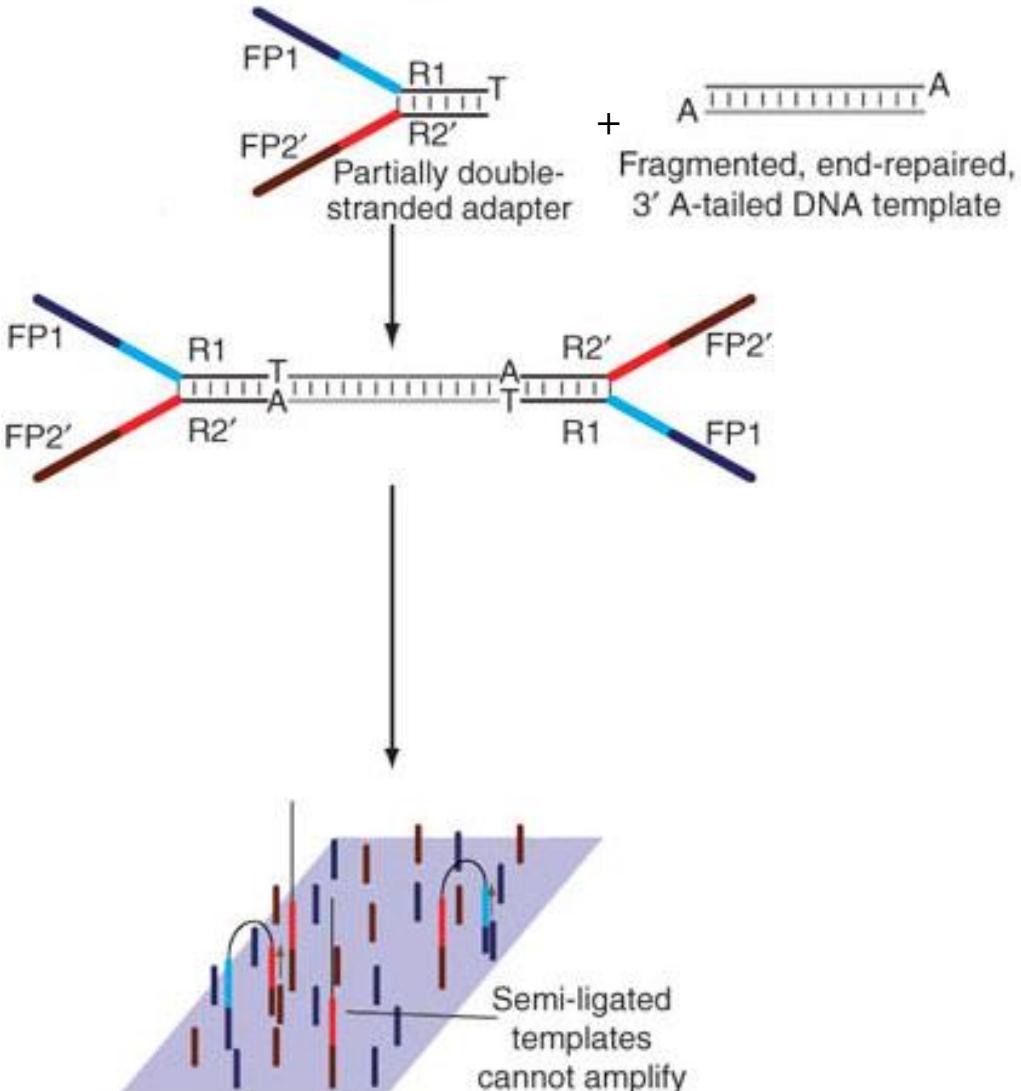
NoPCR (PCRfree)

Amplification bias



Sequence data from both raw and mapped no-PCR datasets represent much closer the base composition of the *P.falciparum* 3D7 genome than the standard datasets.

No-PCR library preparation :



FP1 and FP2': part of the adapter sequence

Enrichment for fully ligated templates during the cluster generation stage

- ✓ Non-ligated templates cannot attach to the flowcell
- ✓ Semi-ligated templates cannot form a bridge and thus clusters

More crucial than for the standard library preparation are:

- ✓ Sample DNA quality and quantity
- ✓ Quantification of libraries with lower concentration



RNA seq



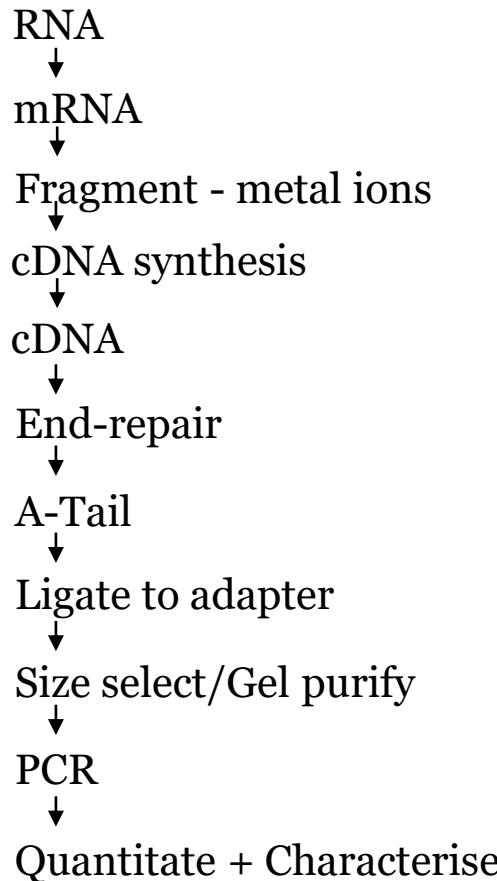
RNA seq Approaches

- » 3' gene expression via oligo dT priming
- » Full length cDNA
- » Single cell 3', 5' or probe based
- » Enrichment and rRNA depletion
 - » Typically oligodT
 - » Probe based for globin, RNA or other depletion

RNA seq

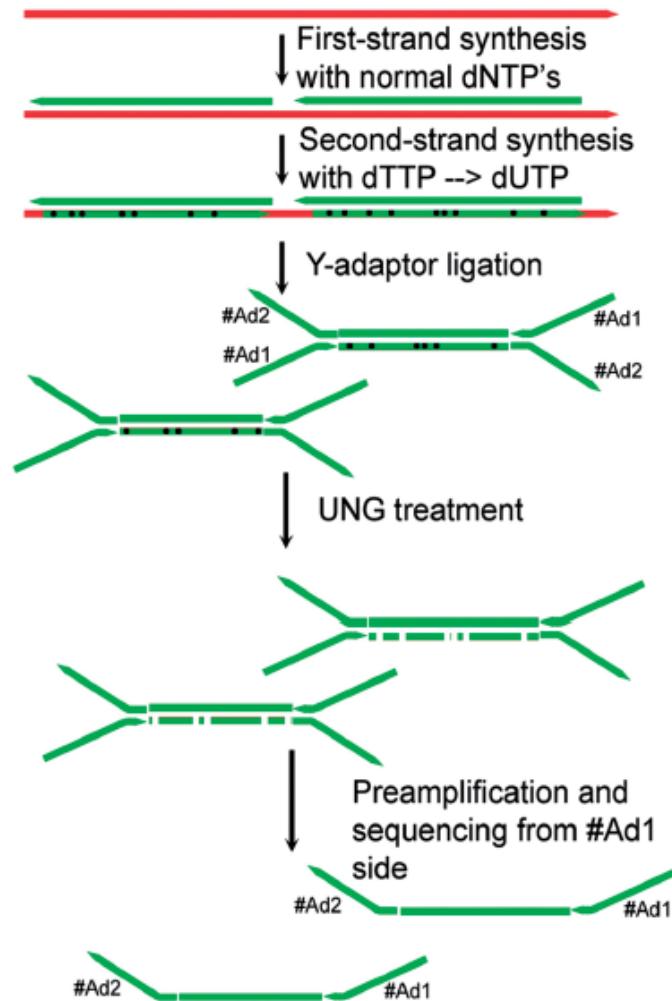
Illumina cDNA protocol

needs good quality DNA free RNA
Ideally RIN > 8



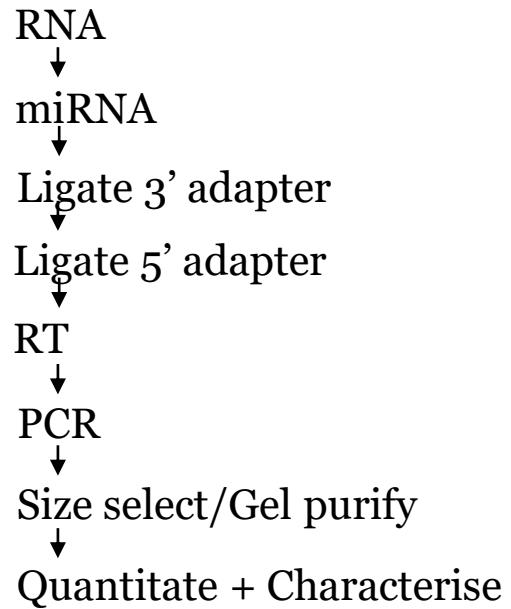
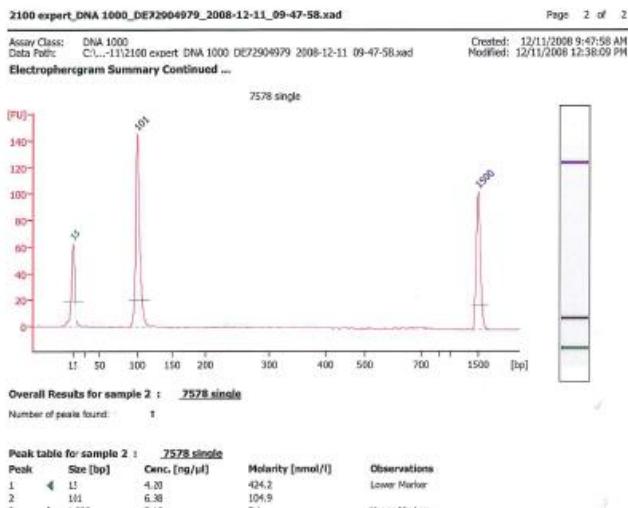
Directional RNA seq dUTP/USER protocol

Parkhomchuk et al.,
NAR. 2009, 37 (18)





small RNA

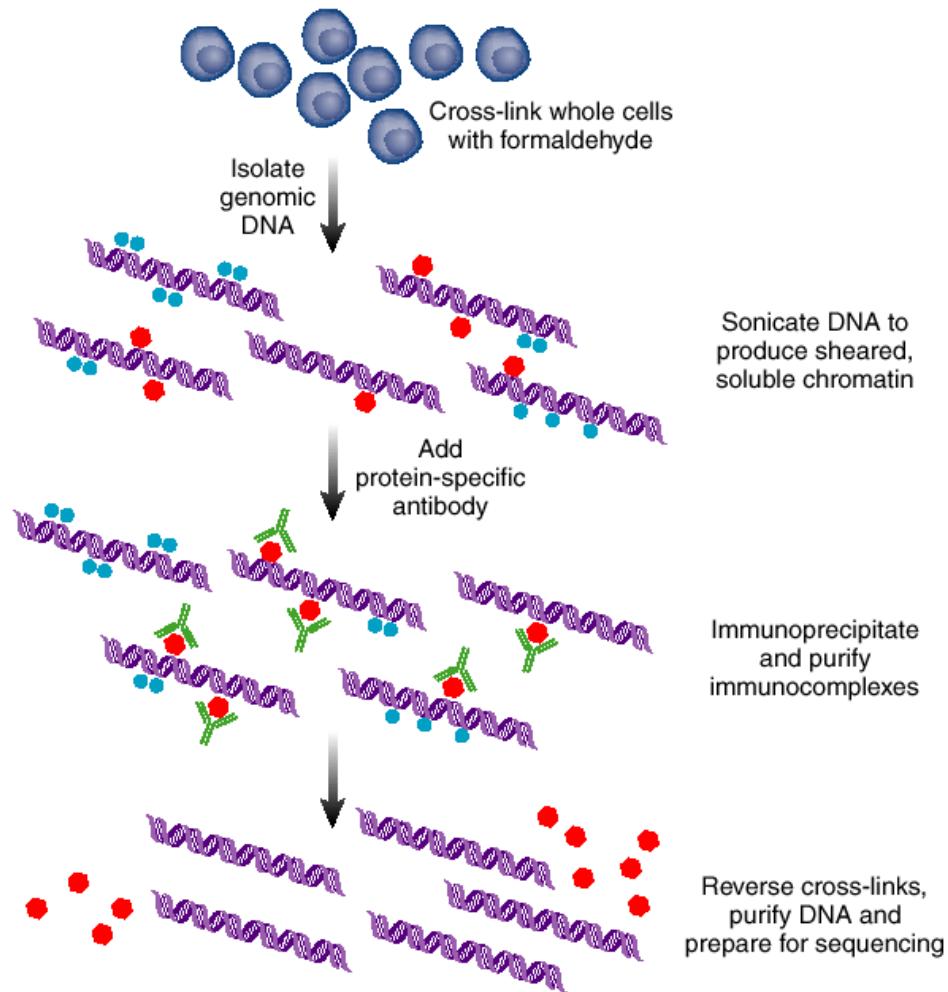


» Notes

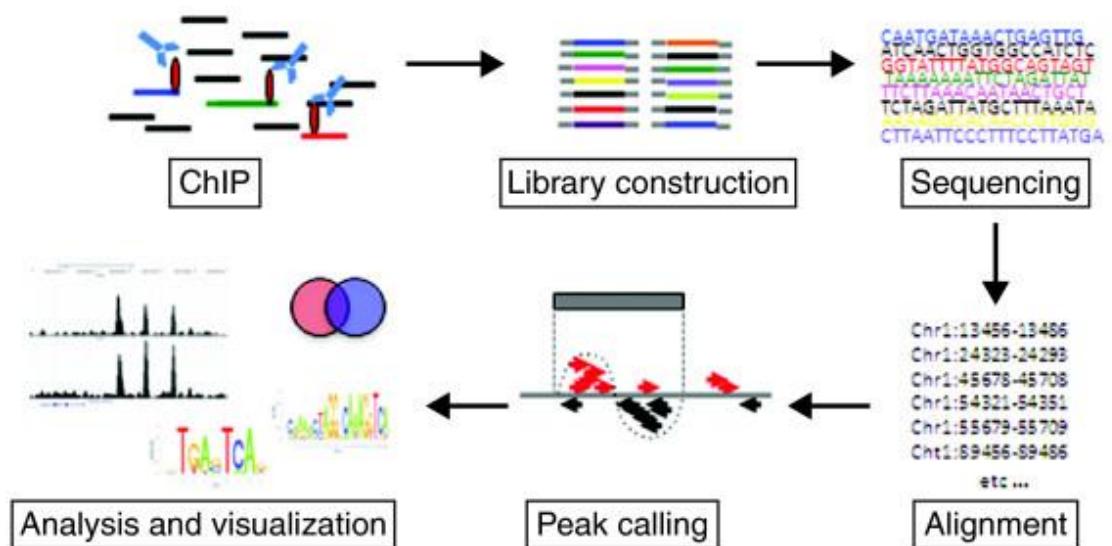
-need 2ug total RNA

ChIP

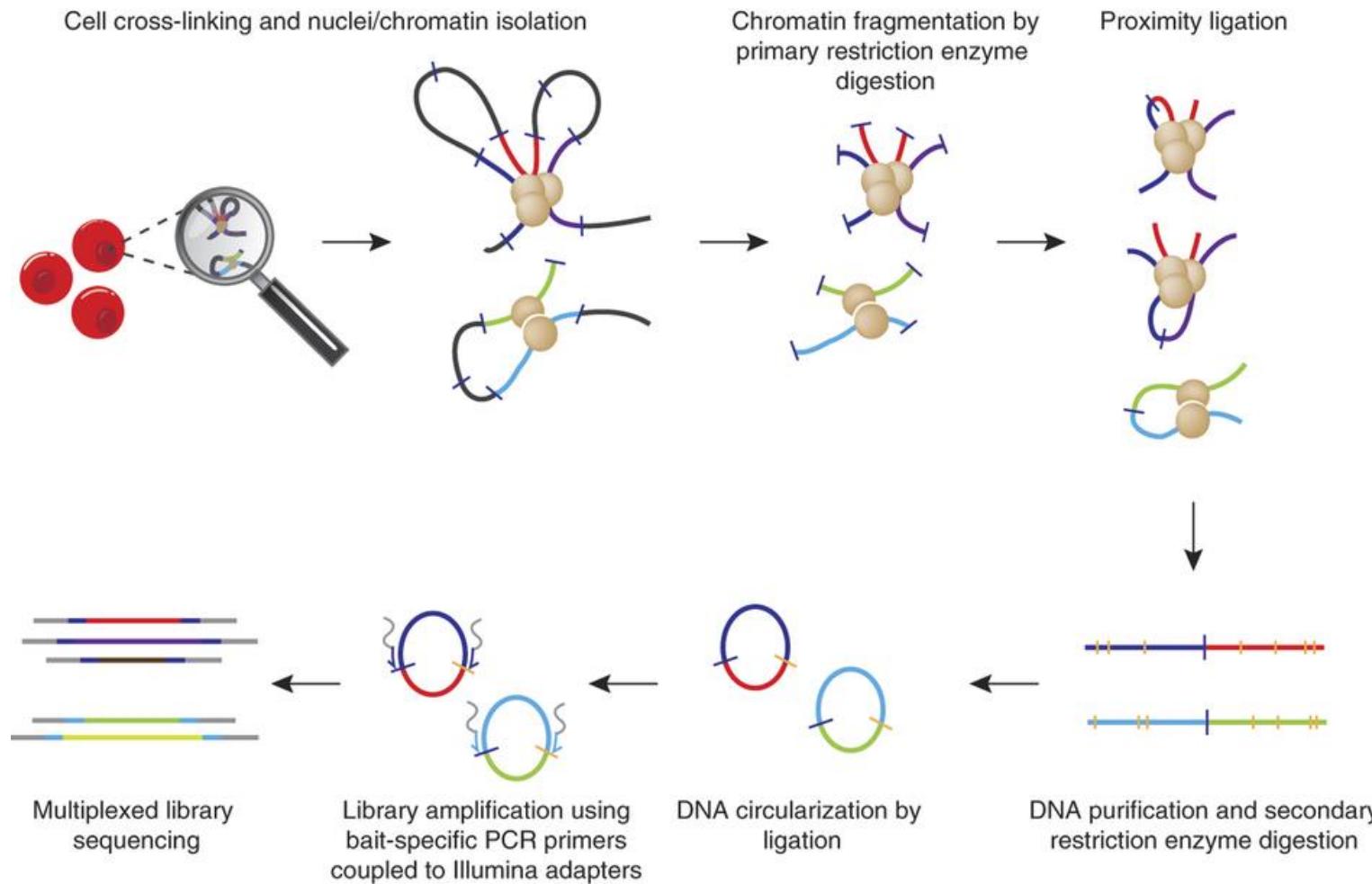
Chromatin Immunoprecipitation



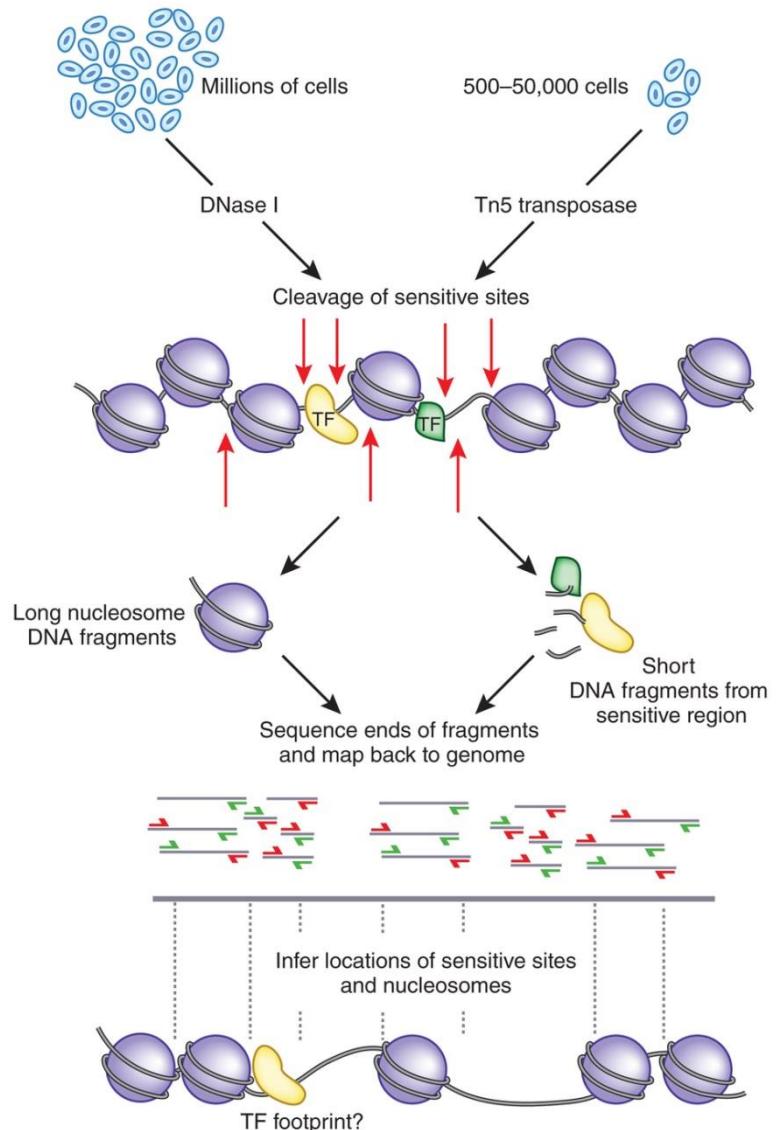
Katie Ris



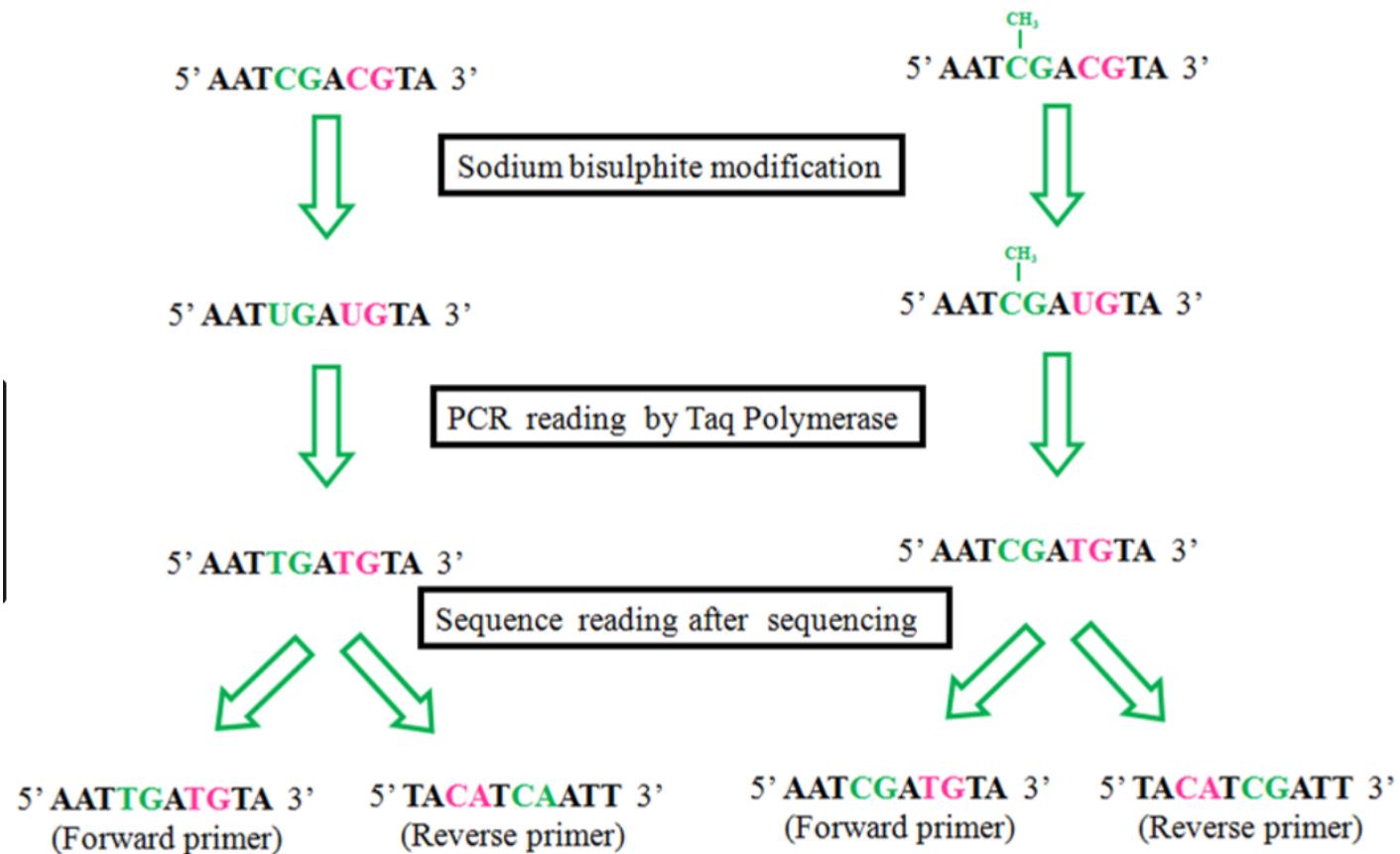
HiC



ATAC and DNase Seq



Methyl Seq



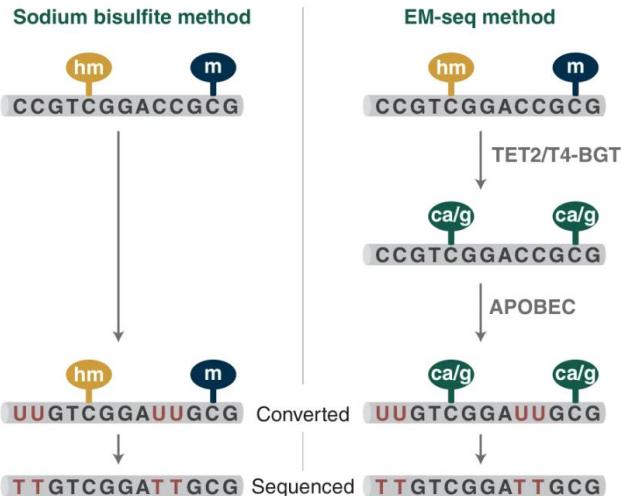
NEB EM-Seq



NEBNext UltraShear (NEB #M7634) has been optimized for enzymatic fragmentation of DNA compatible with EM-seq workflows.

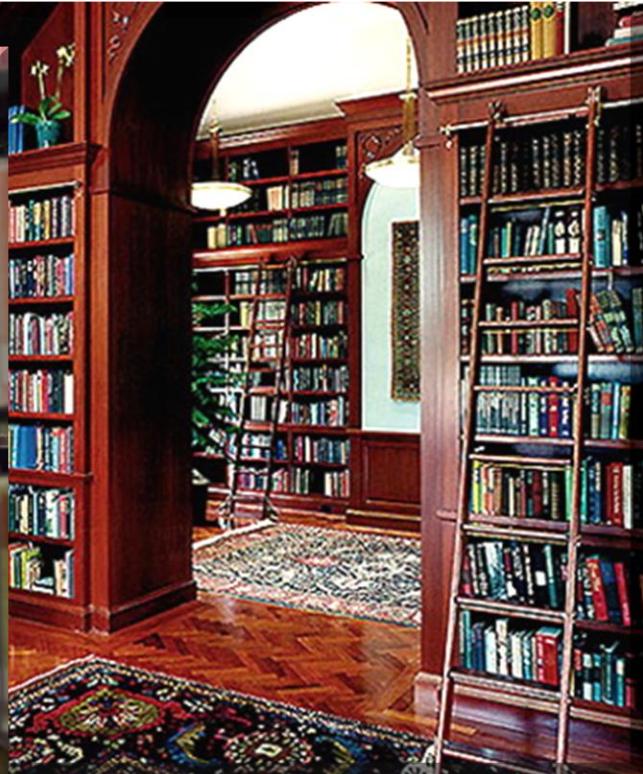
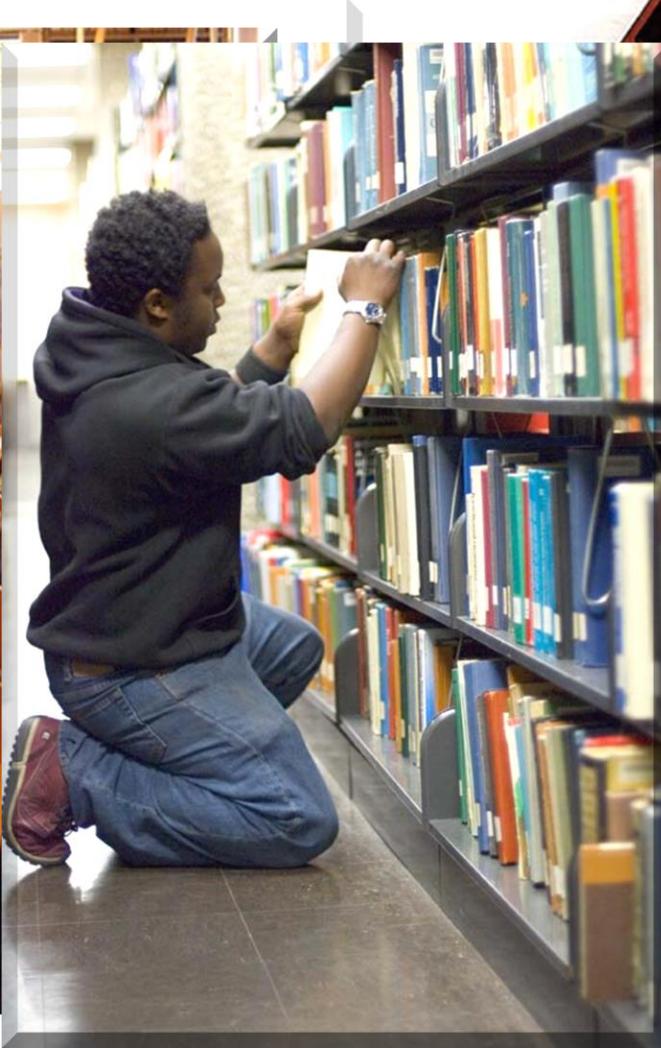
For specific detection of 5hmC, the NEBNext Enzymatic E5hmC-seq Kit (NEB #E3350) is now also available.

Figure 1: EM-seq™ conversion method



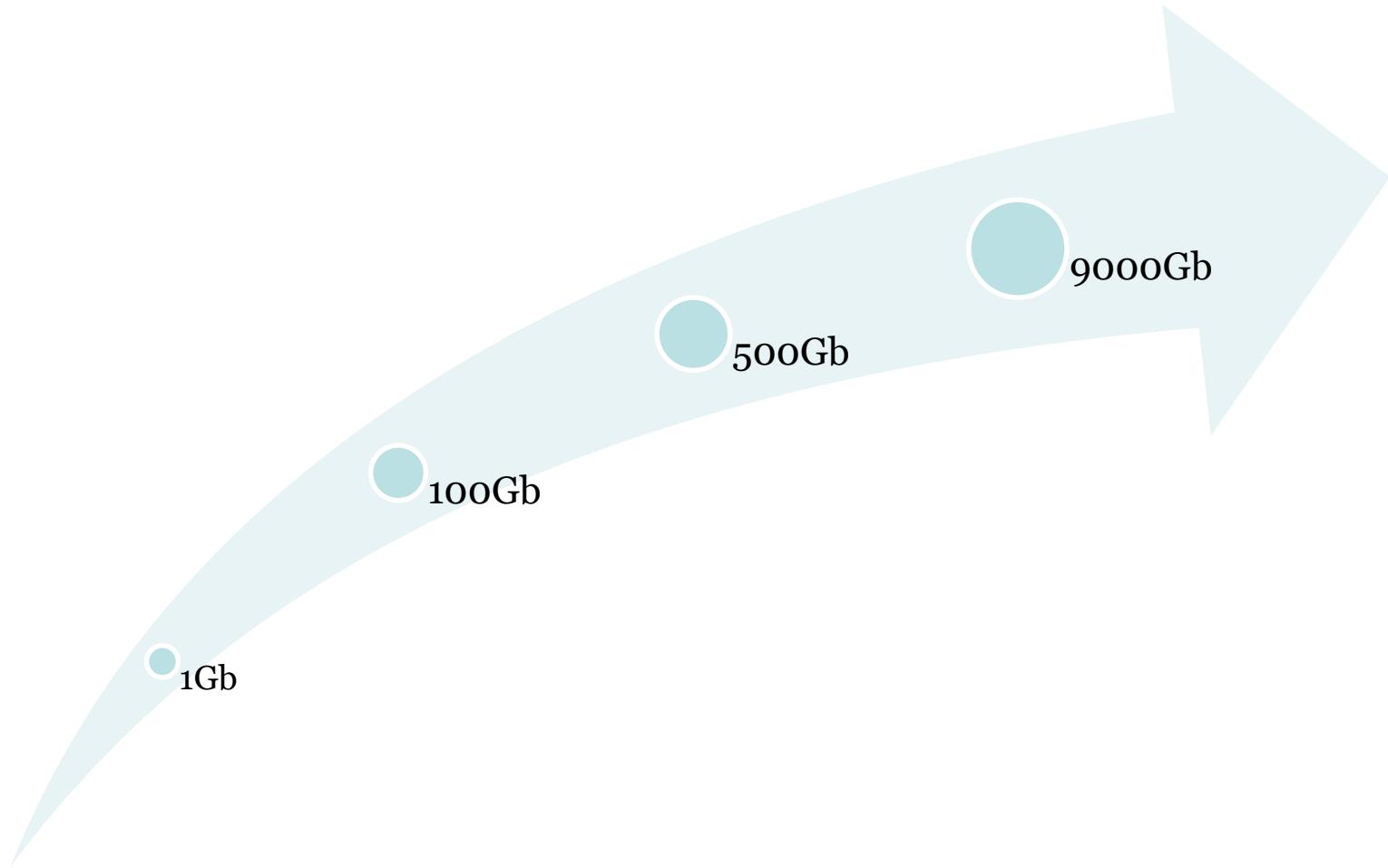
The EM-seq™ v2 workflow accommodates a wider input range than the original EM-seq workflow, with a 100-fold lower minimum input amount. The v2 workflow is more streamlined, has one fewer cleanup step and is faster. Note that NEBNext LV UDI primers are not included in the kit and are available separately.

How Many Libraries?





Sequence yield



To make all these **LIBRARIES** is

lot of work

Samples from different sources
Different levels of complexity
Large sample sets





Headache!!

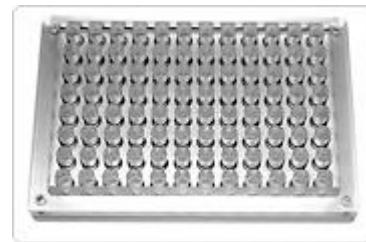
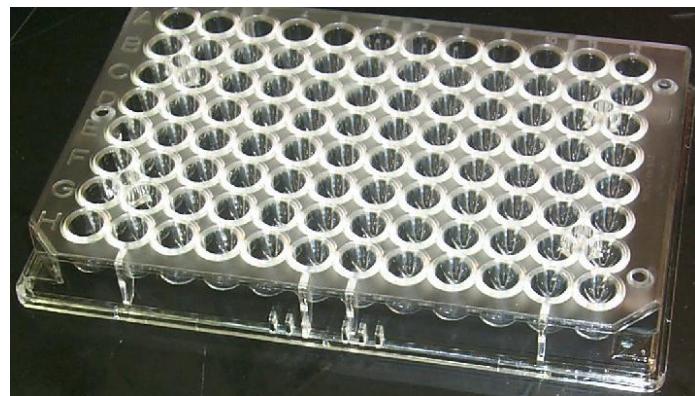
Automation





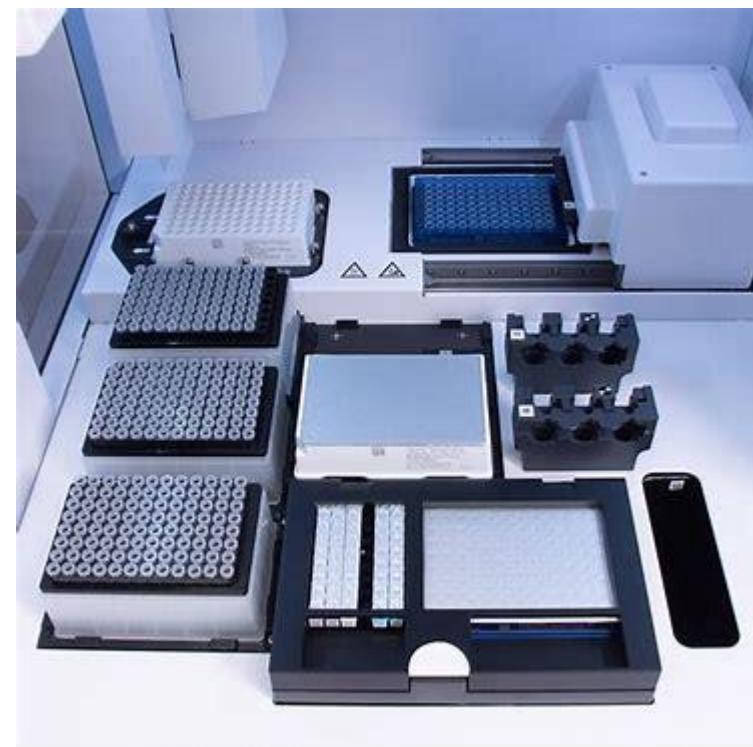
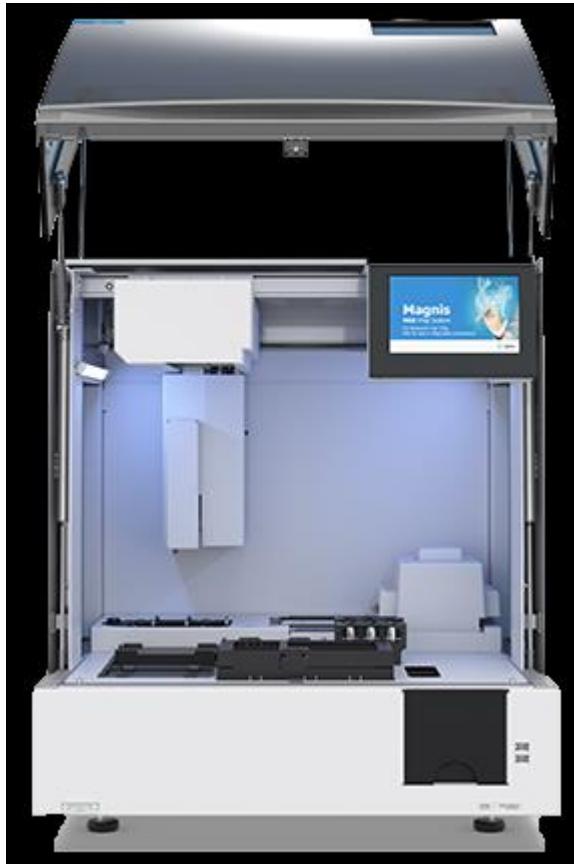
Approaches to Automation

High throughput manual



Low throughput automation

Agilent Magnis – 8 sample automation



Low throughput automation



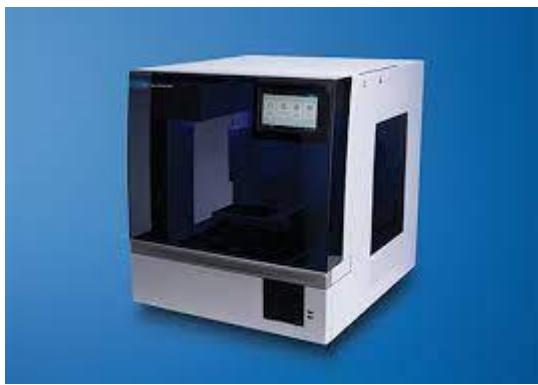
Apollo 324

<http://www.wafergen.com/products/apollo-324>



Tecan MagicPrep NGS

<http://www.tecan.com/magic>



Agilent Magnis
<http://www.agilent.com>



PE BioCule



High throughput automation



Hamilton Star



Tecan fluent



Beckman i7



Revvity Sciclone NGS



Agilent Bravo

Introductory systems?



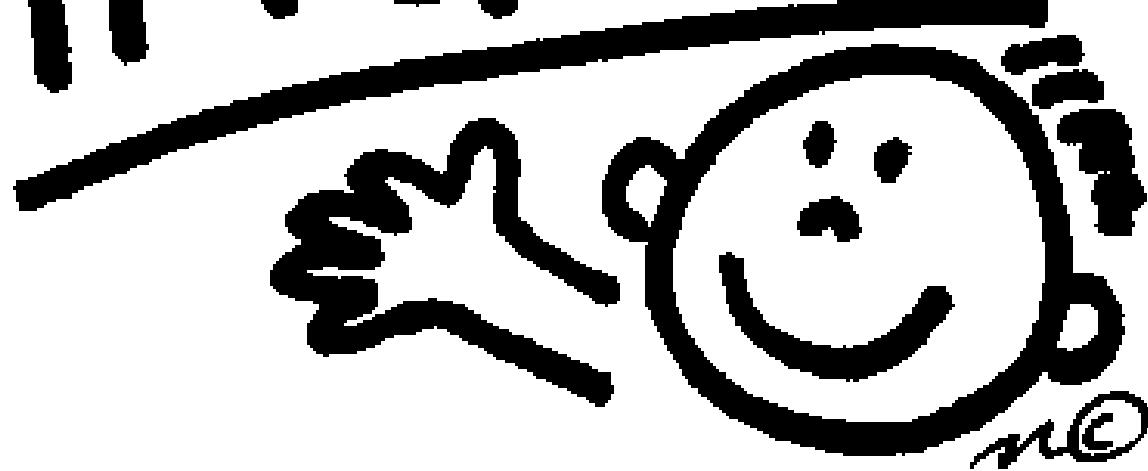
Opentrons flex



SPT Firefly



Thanks!





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Any Questions ?

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