

Sample Types

Stool
Urine
Tissue
Cerebral Spinal Fluid
Cervicovaginal Swab
Nasopharyngeal Swab
Bronchoalveolar Lavage
Sputum
Serum
Synovial Fluid
Whole Blood
Cerebral Spinal Fluid
Allantoic Fluid
Amniotic Fluid
Breastmilk
Oral Wash
Saliva
Pericardial Fluid
Semen
Sewage

How much sample do you need?

- Stool at least 200mg, <1500mg 
- Fluid type samples eg; serum, sputum, etc: 1mL

Storage Conditions

- Frozen at -80 degrees as quickly as possible
- No medium eg; RNAlater etc

Original Sample



Chip/Aliquot



Lyse



Extract DNA



Amplification



Pool



Final Library

Preparing Samples for Virome Sequencing: 3 Steps

- 1.) Virus Like Particle (VLP)
Enrichment and Total Nucleic Acid Extraction
- 2.) Reverse Transcription, Second Strand Synthesis and PCR Amplification
- 3.) Library Construction

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Biosafety Cabinet Set Up

Set Up Biosafety Cabinet



Chip/Dilute



Vortex



Centrifuge



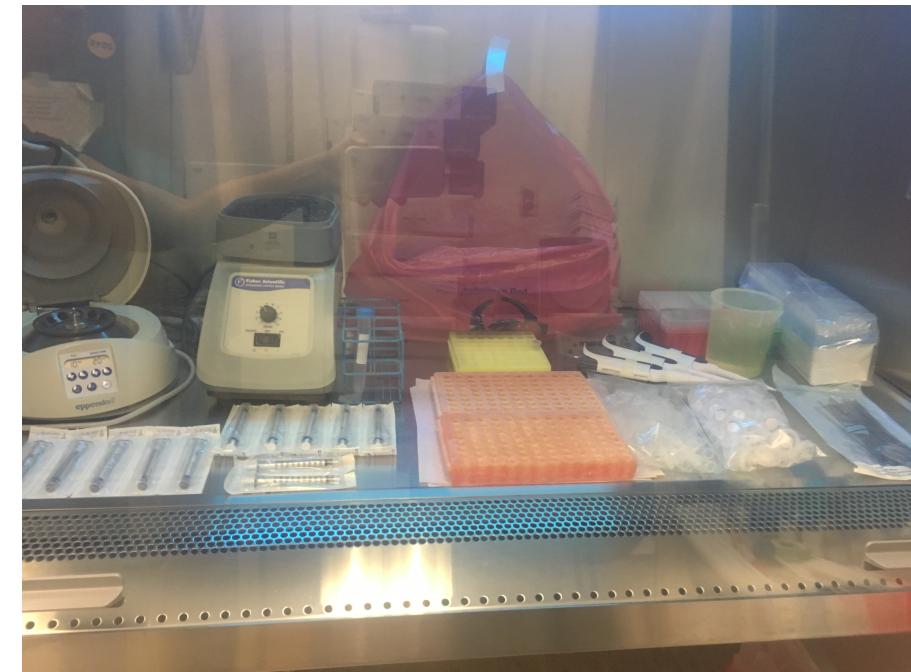
Filter



DNase/Lysozyme



TNA Extraction



- Samples are handled at BS_L2+- gown and double glove
- Decon with 10% bleach solution, 70% ethanol, and UV for 30 minutes
- Waste collected in biohazard bags and autoclaved

Chip Stool Sample

Set Up Biosafety Cabinet

Chip/Dilute

Vortex

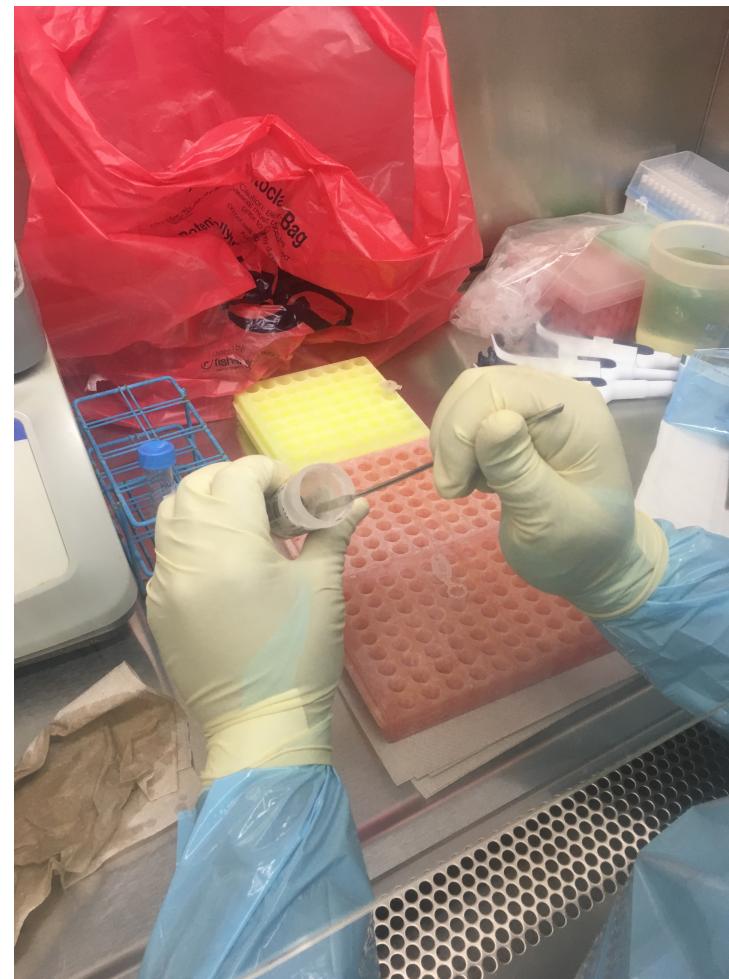
Centrifuge

Filter

DNase/Lysozyme

TNA Extraction

- Keep sample frozen
- Chip ~200mg of stool
- Add SM Buffer (NaCl, Tris, MgSO₄)



Homogenize Samples

Set Up Biosafety Cabinet

Chip/Dilute

Vortex

Centrifuge

Filter

DNase/Lysozyme

TNA Extraction

- Vortex for 5 minutes
- Break up stool material



Centrifuge

Set Up Biosafety Cabinet

Chip/Dilute

Vortex

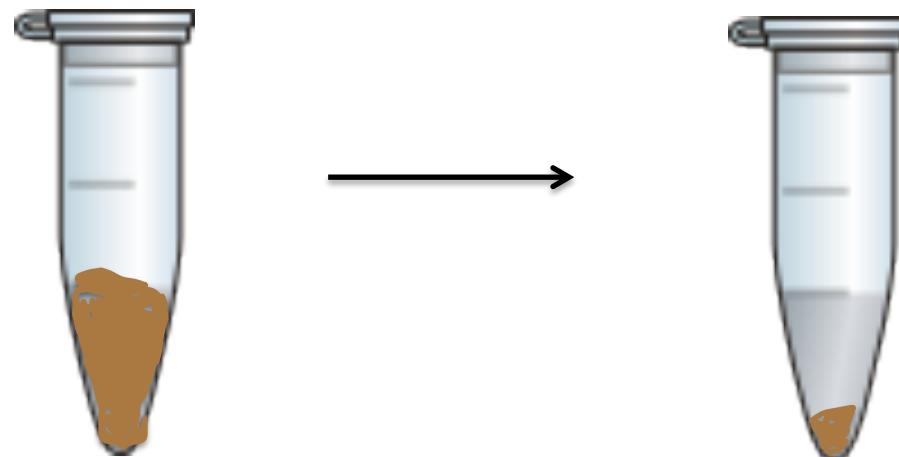
Centrifuge

Filter

DNase/Lysozyme

TNA Extraction

- 7,000G for 10 minutes
- Pellet stool particles



Filter to Remove Bacteria

Set Up Biosafety Cabinet



Chip/Dilute



Vortex



Centrifuge



Filter



DNase/Lysozyme



TNA Extraction

- .45 μ filter



Non Encapsulated DNA Removal

Set Up Biosafety Cabinet



Chip/Dilute



Vortex



Centrifuge



Filter



DNase/Lysozyme

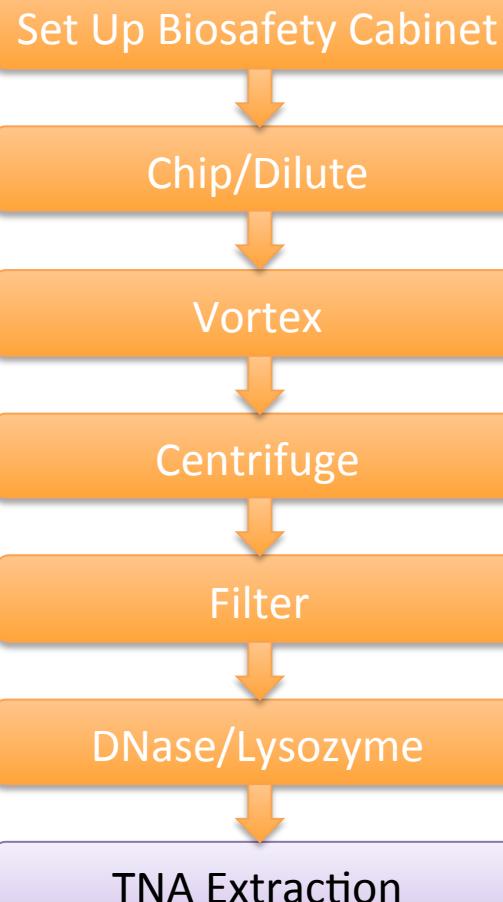


TNA Extraction

- Lysozyme DNase Enzyme cocktail
- Degrade non-encapsulated DNA

	Per 800ul sample	12 +1 =13 samples
Turbo DNase buffer	108 ul	1,404
TurboDNaseI (2U/ml)	20 ul	260
Baseline zero (1U/ml)	4 ul	52
Lysozyme (10mg/ml)	80 ul	1,040
H2O	68 ul	884
	280ul	

Total Nucleic Acid Extraction



- Extract both DNA and RNA
- Automated systems
- Manual Kits- Qiagen DNeasy



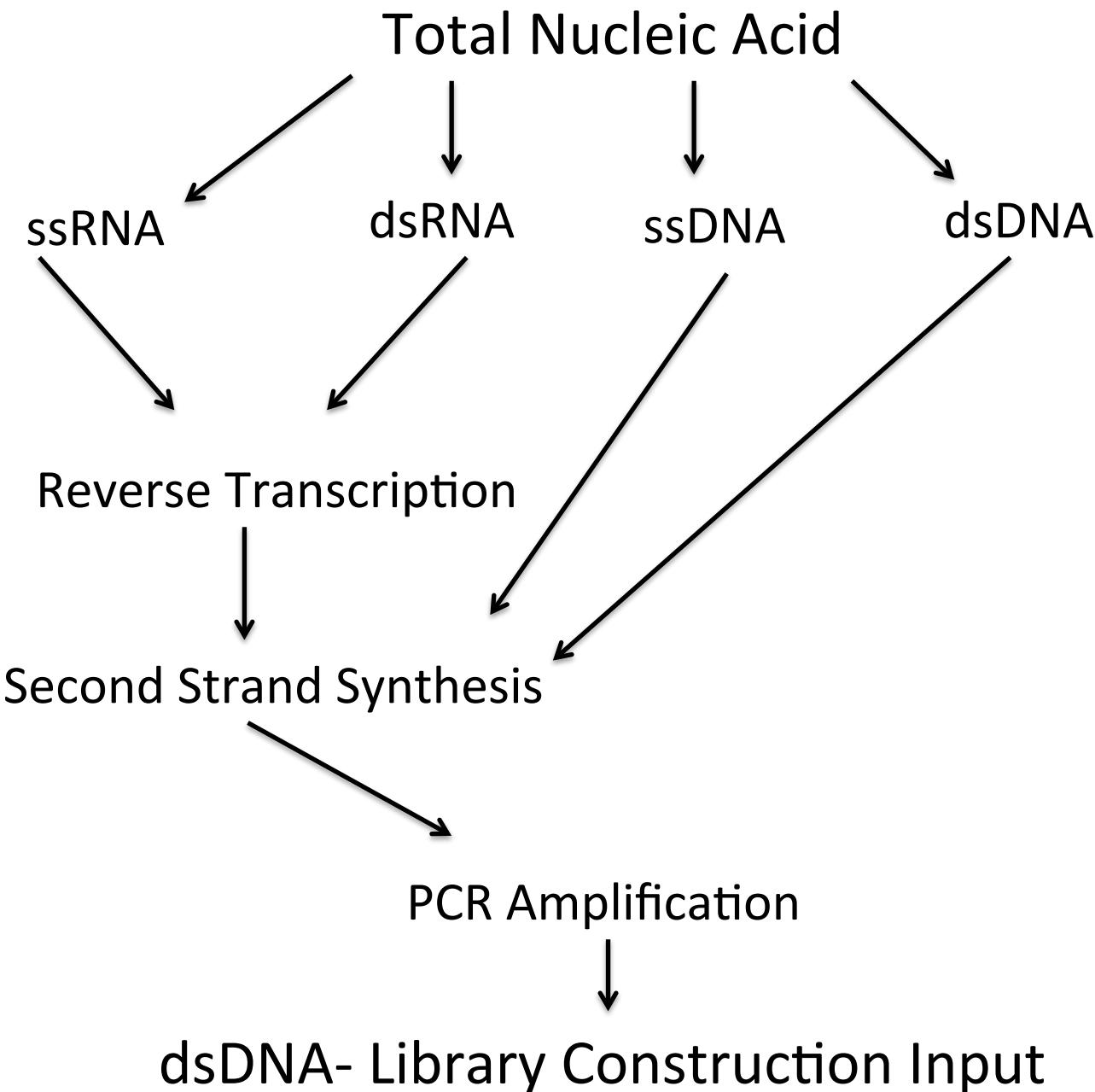
Preparing Samples for Virome Sequencing: 3 Steps

1.) Virus Like Particle (VLP)

Enrichment and Total Nucleic Acid Extraction

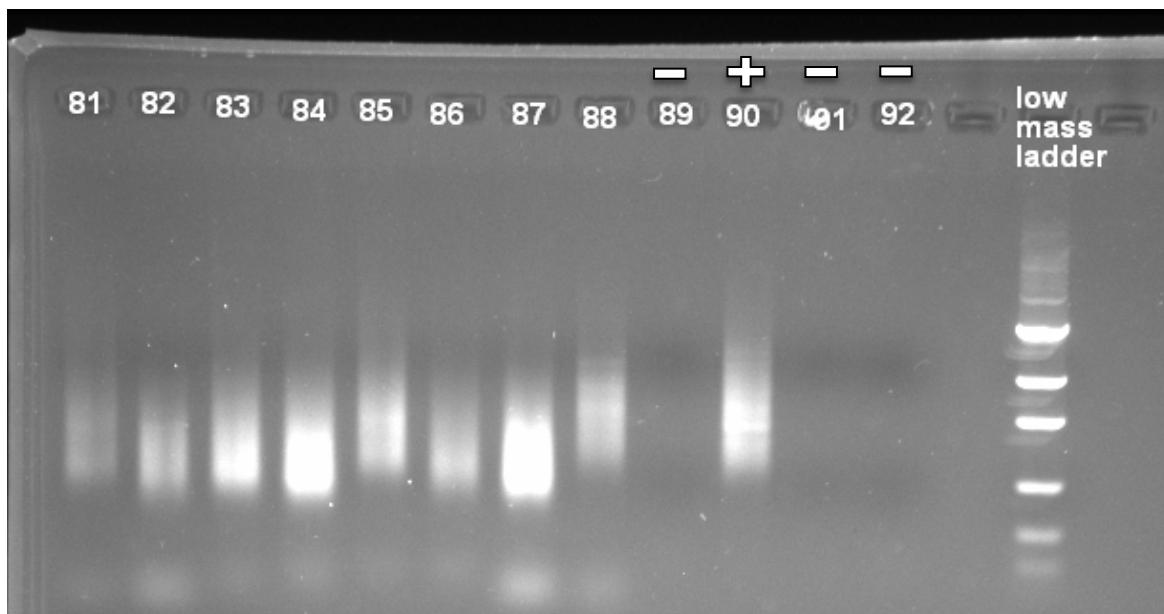
2.) Reverse Transcription, Second Strand Synthesis and PCR Amplification

3.) Library Construction

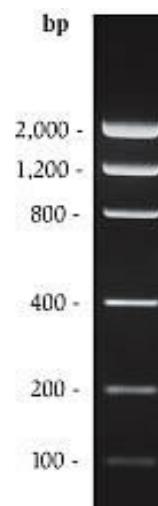


Post PCR Amplification

- 1% Agarose gel- 10ul of amplified DNA
- Expected smear 200bp-1kb
- Failure rate averages 8-10%



Invitrogen Low Mass Ladder



Preparing Samples for Virome Sequencing: 3 Steps

1.) Virus Like Particle (VLP)

Enrichment and Total Nucleic Acid Extraction

2.) Reverse Transcription, Second

Strand Synthesis, and PCR

Amplification

3.) Library Construction



Clean Up/Size Selection

End Repair

Adapter Ligation

Clean Up/Size Selection

PCR Amplification

PCR Clean Up

Quality Control

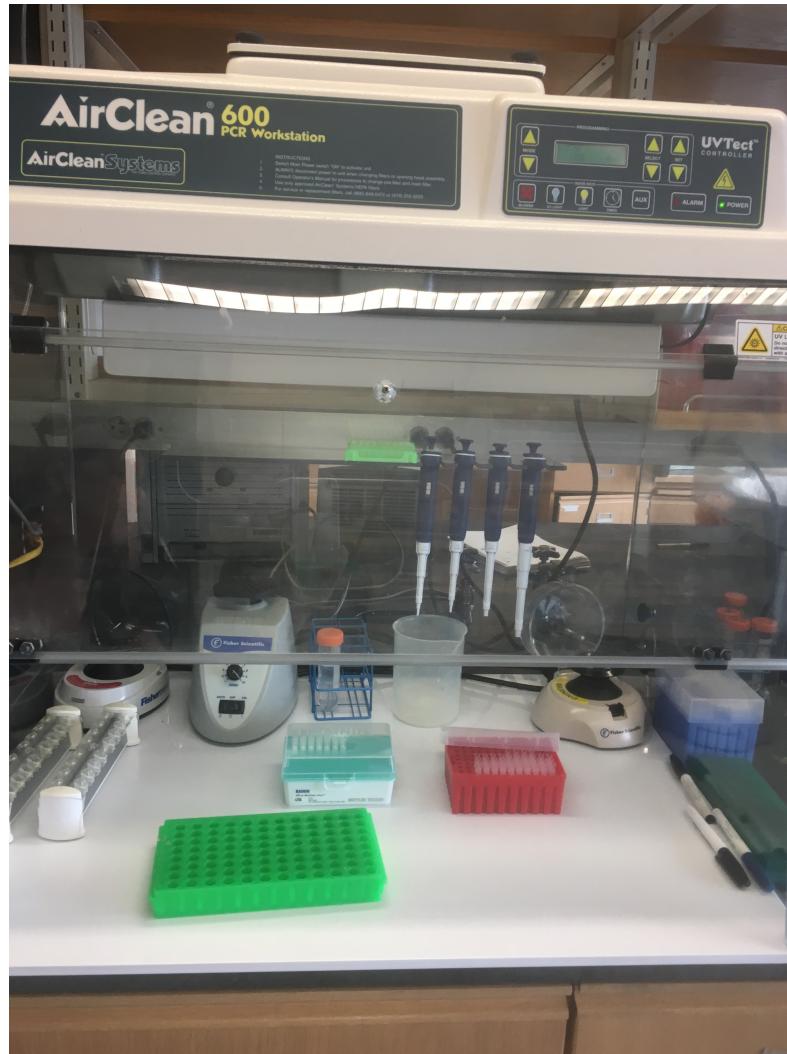
Pool

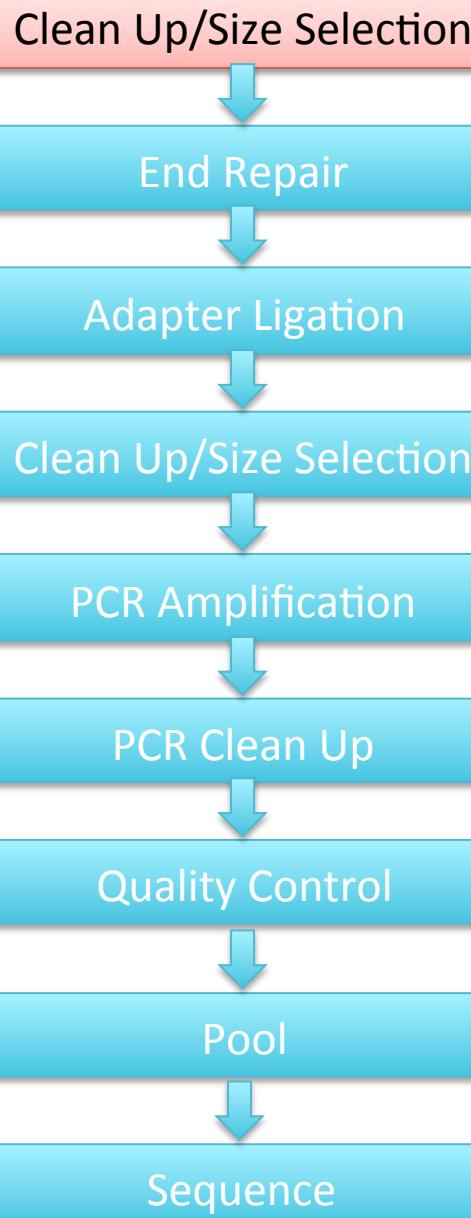
Sequence

New England Biolabs NEB Next DNA Library Construction

PCR Workstation

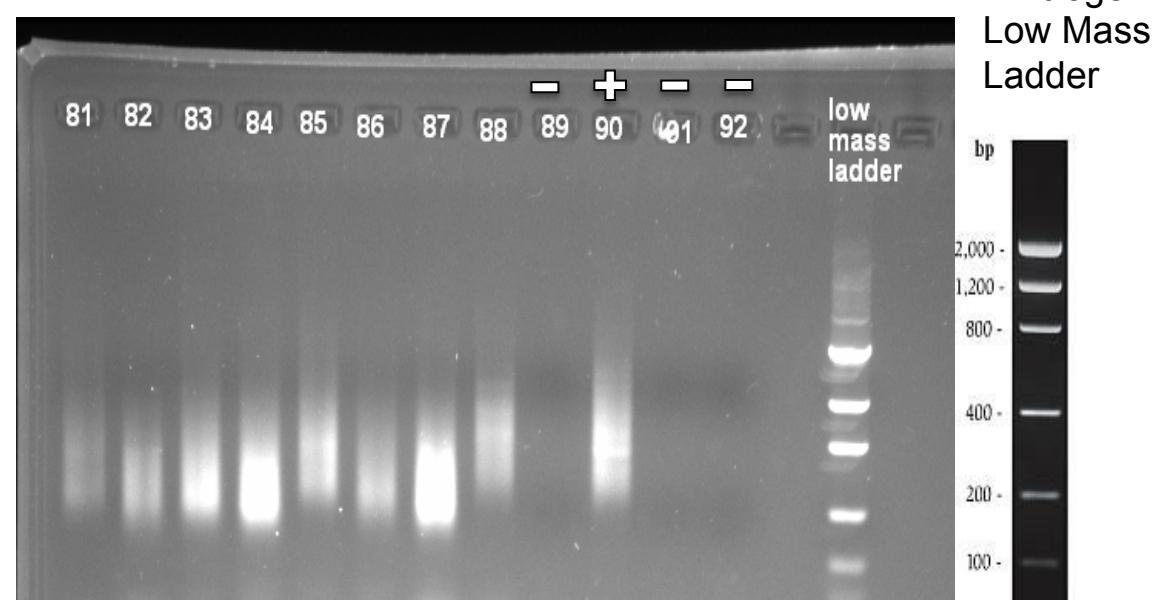
- Vertical Laminar air flow
- Hepa filtration system
- Built in UV





Post PCR Amplification

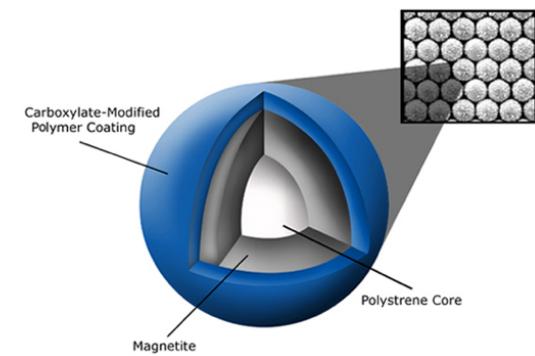
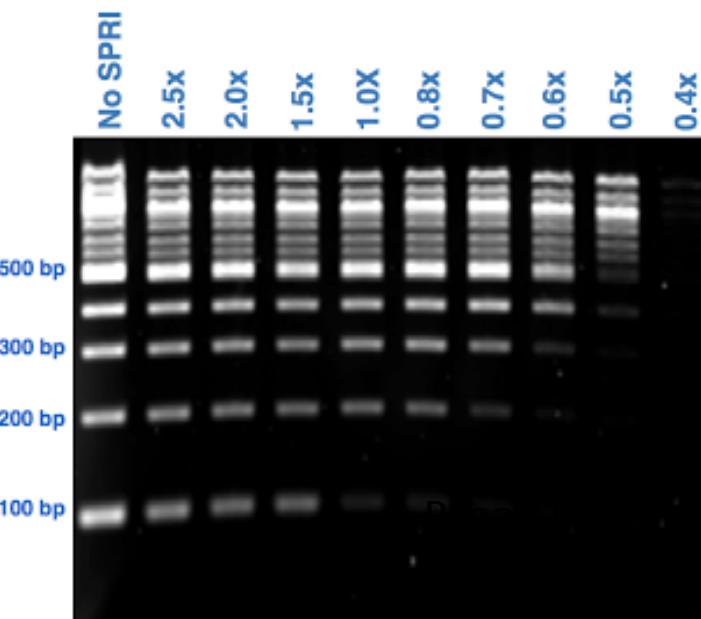
- 1% Agarose gel- 10ul of amplified DNA
- Expected smear 200bp-1kb
- Target 400-600bp for library construction



Beckman Coulter- AmPure Bead



- SPRI Bead (Solid Phase Reversible Immobilization)
- Uses Paramagnetic beads to selectively bind nucleic acid by size
- PEG (polyethylene glycol) causes the negatively charged DNA to bind to the carboxyl molecules on bead surface
- Lower the ratio of SPRI:DNA= larger final fragments at elution



<https://youtu.be/zGV0SjCe0CU>

Clean Up/Size Selection



End Repair



Adapter Ligation



Clean Up/Size Selection



PCR Amplification



PCR Clean Up



Quality Control



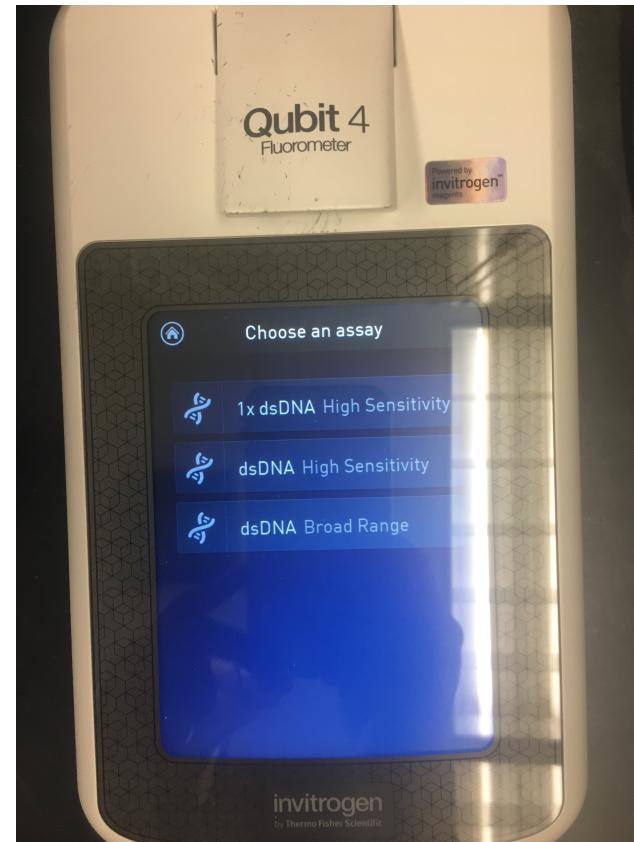
Pool



Sequence

Sample Quantification

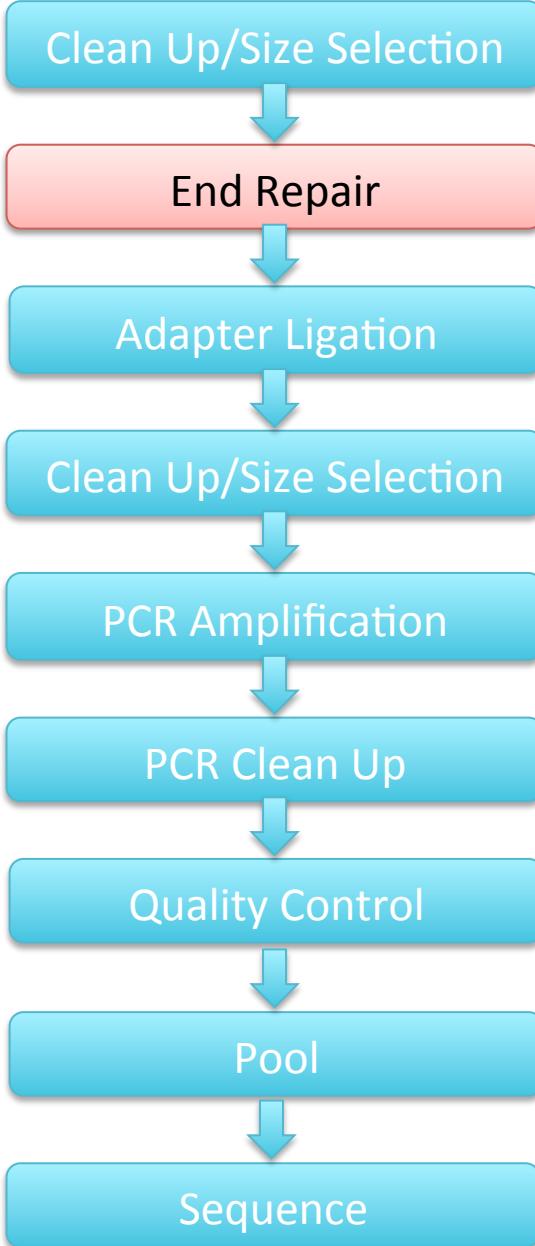
- Library input DNA 20-100ng
- Can go as low as 5ng
- Knowing input is critical for downstream steps- adapter concentration and PCR amplification cycle number



End Repair

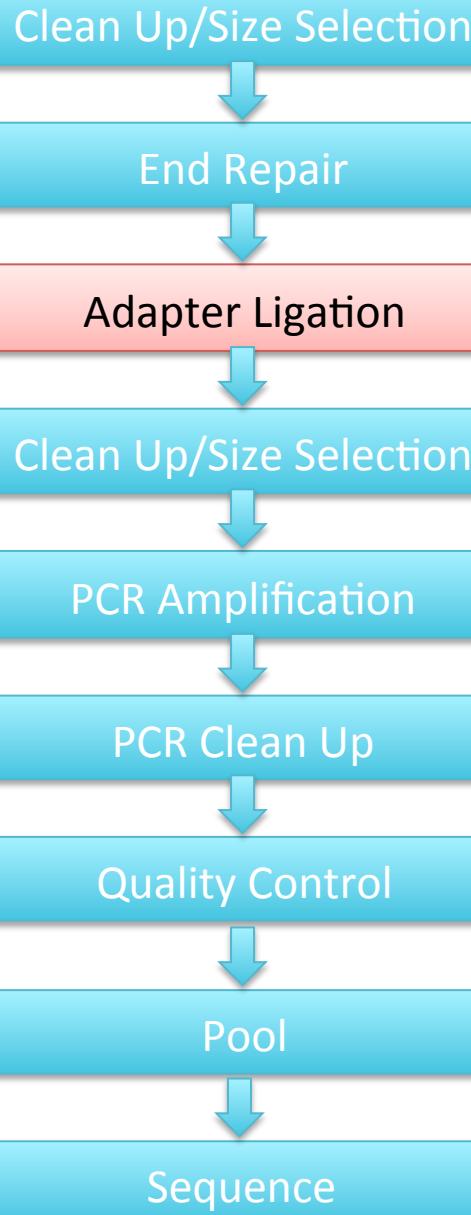
5' Phosphorylation and dA-Tailing

- Strands are blunted and phosphorylated
- Adding an A to 3' ends



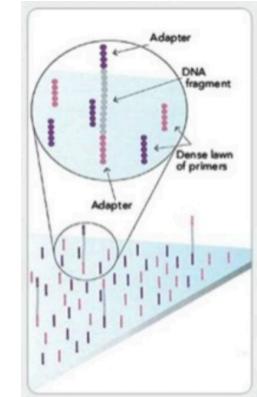
End Repair, 5' Phosphorylation and dA-Tailing





Adapter Ligation

- Adapters with single T overhang ligated on the end repair dA fragment
- Amount of adapter is critical
- USER enzyme used to cleave hairpin loop



Adaptor Ligation with optional NEBNext Adaptor



U Excision



Clean Up/Size Selection



End Repair



Adapter Ligation



Clean Up/Size Selection



PCR Amplification



PCR Clean Up



Quality Control



Pool



Sequence

Clean Up- Post Adapter Ligation

- Size Selection 400-600bp
- Remove unused ligation reaction components, adapter dimers, and concatemers

Clean Up/Size Selection

End Repair

Adapter Ligation

Clean Up/Size Selection

PCR Amplification

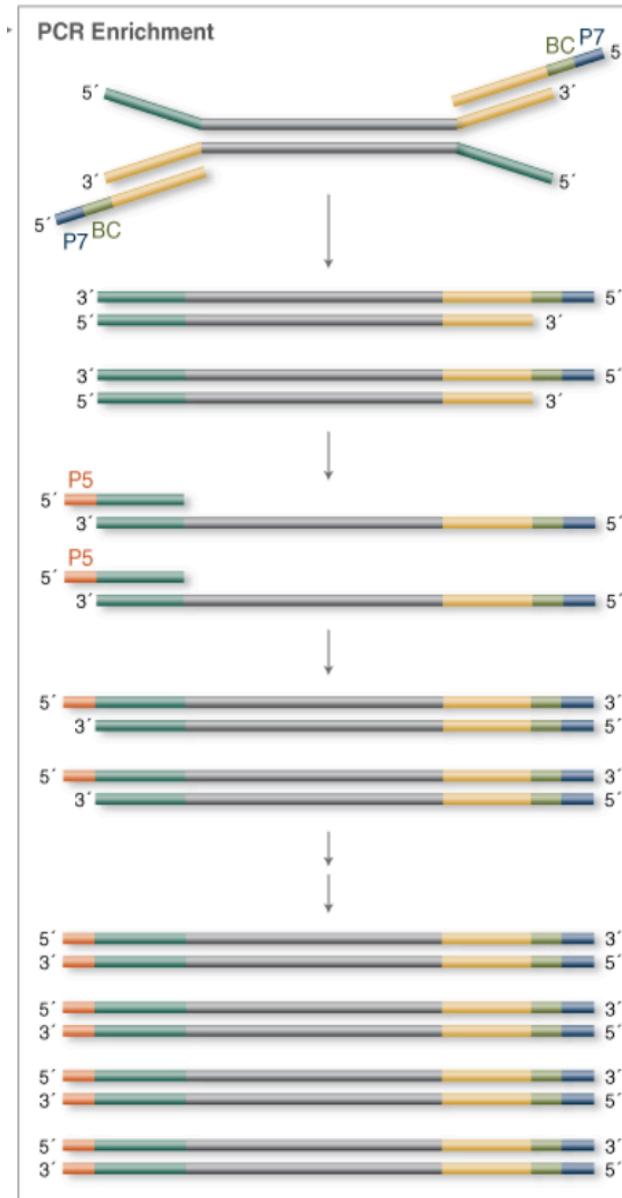
PCR Clean Up

Quality Control

Pool

Sequence

Library Amplification by PCR



- Increase the amount of library
- Select for libraries with adapters on each end
- Indexes can be added for multiplexing- 24 unique indexes

Clean Up/Size Selection



End Repair



Adapter Ligation



Clean Up/Size Selection



PCR Amplification



PCR Clean Up



Quality Control



Pool



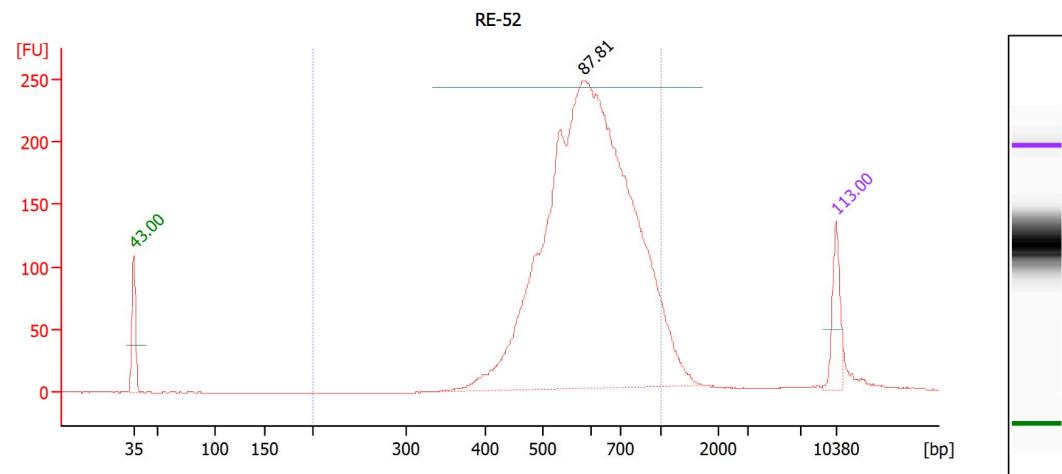
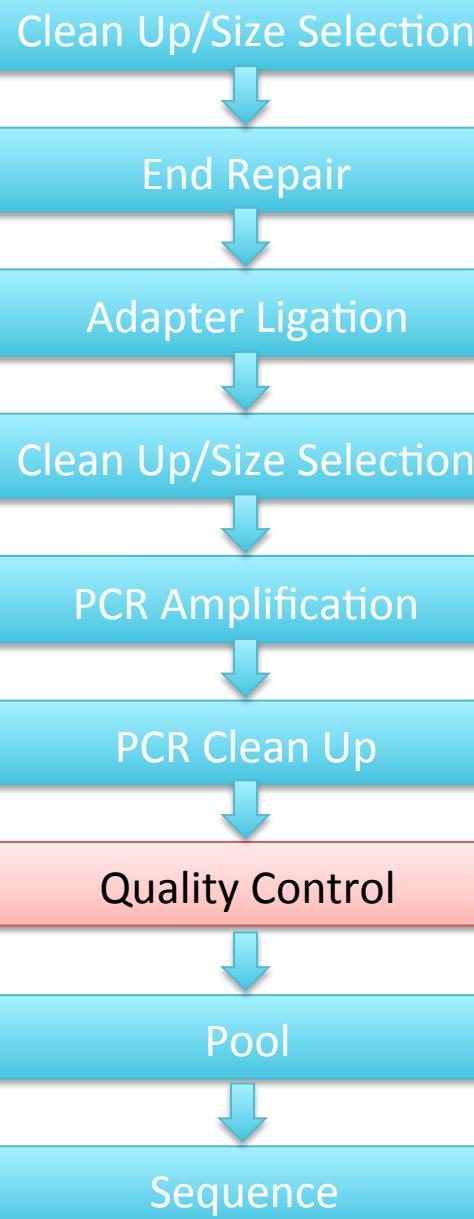
Sequence

Clean Up- Post PCR Amplification

- Remove free barcodes, nucleotides
- Remove adapter dimers

Quality Control

- Agilent Bioanalyzer 2100
- Microfluidics platform for sizing and quantification



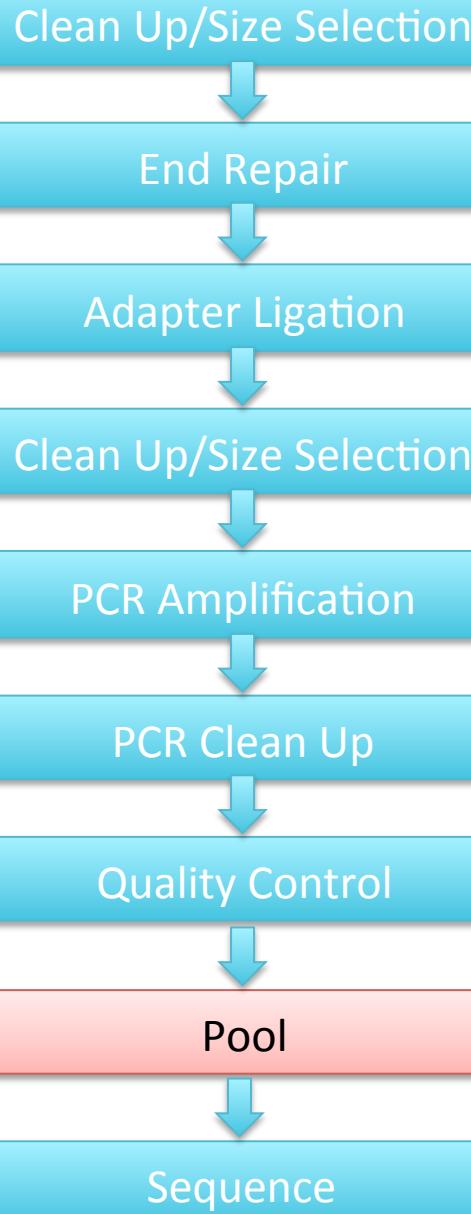
Overall Results for sample 9 : RE-52

Number of peaks found: 1 Corr. Area 1: 2,893.5
Noise: 0.3

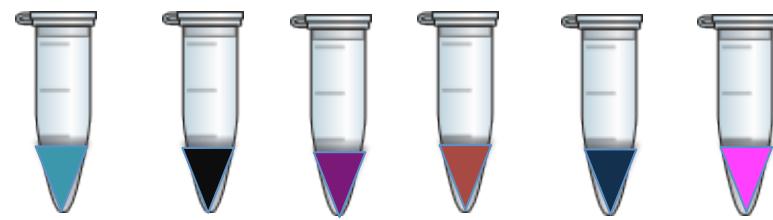
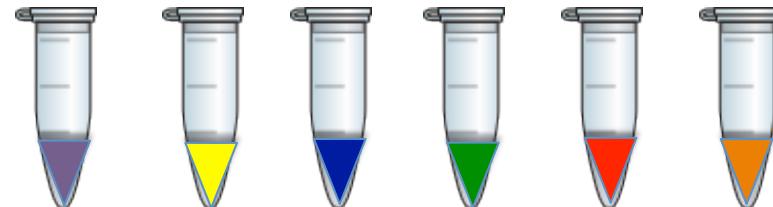
Peak table for sample 9 : RE-52

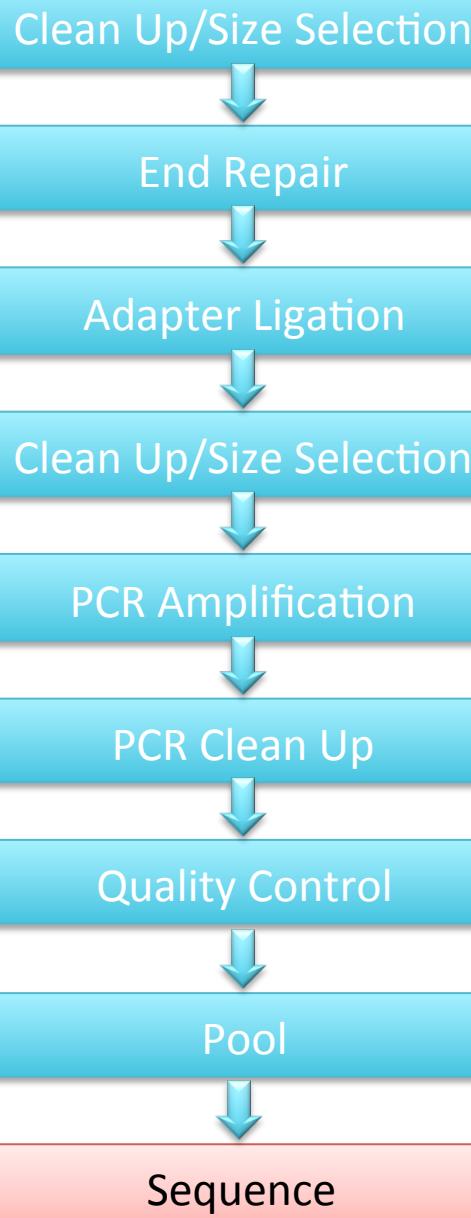
Peak	Size [bp]	Conc. [pg/μl]	Molarity [pmol/l]	Observations
1	35	125.00	5,411.3	Lower Marker
2	585	2,754.89	7,140.6	
3	10,380	75.00	10.9	Upper Marker

Pool Final Libraries



- Individual Barcode for multiplexing
- Pool equal molar concentration
- Sequencing Core requires 20ul at 2-10nM



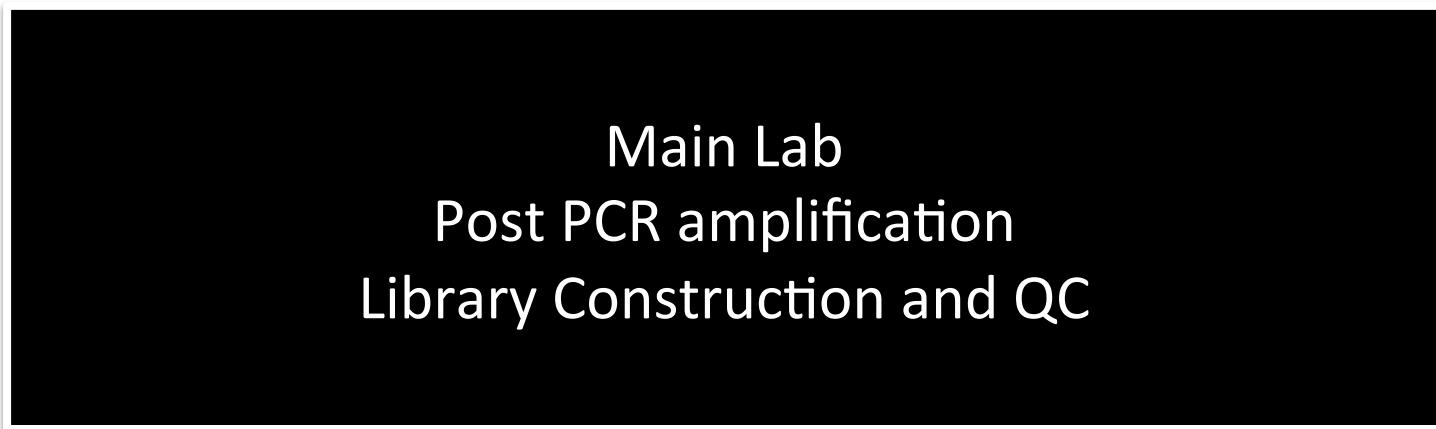
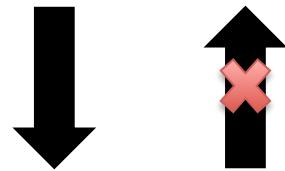


MiSeq V2 2X250

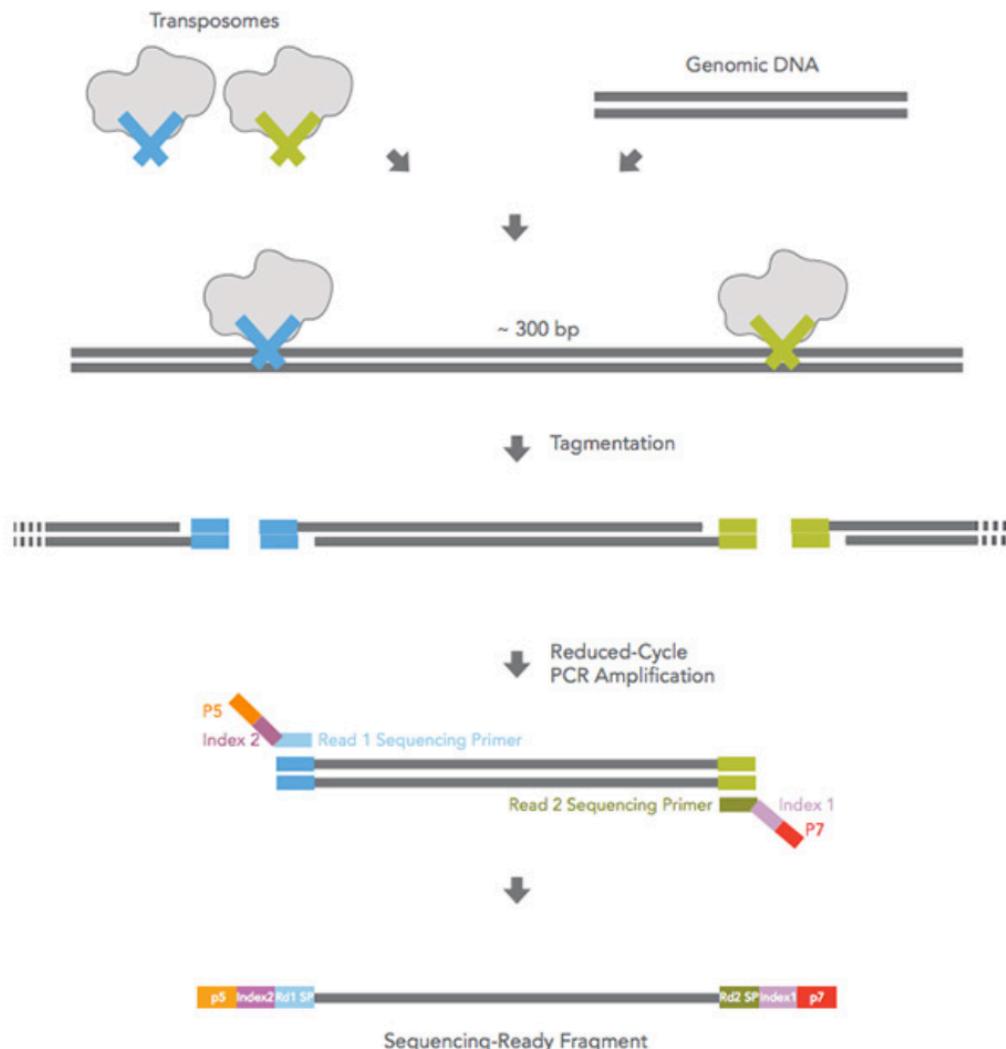


(pick your favorite)

Laboratory Layout



Nextera Library Preparation Biochemistry



Nextera chemistry simultaneously fragments and tags DNA in a single step. A simple PCR amplification then appends sequencing adapters and sample indexes to each fragment.

16S/ITS vs Virome

16S/18S/ITS- DNA only

- Chip-20mg/Buffer A
- Bead Beat- Lyse
- Extraction
- PCR Amplification/
Library Construction
- QC
- Sequence

Virome- DNA + RNA

- Chip-200mg/SM Buffer
- Vortex- Homogenize
- VLP enrichment
- Extraction
- Reverse Transcription,
Second Strand Synthesis,
PCR Amplification
- Library Construction
- QC
- Sequence

Shotgun

- Chip
- Bead Beat- Lyse
- Extraction
- Library Construction
- QC
- Sequence