

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; RNA later 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

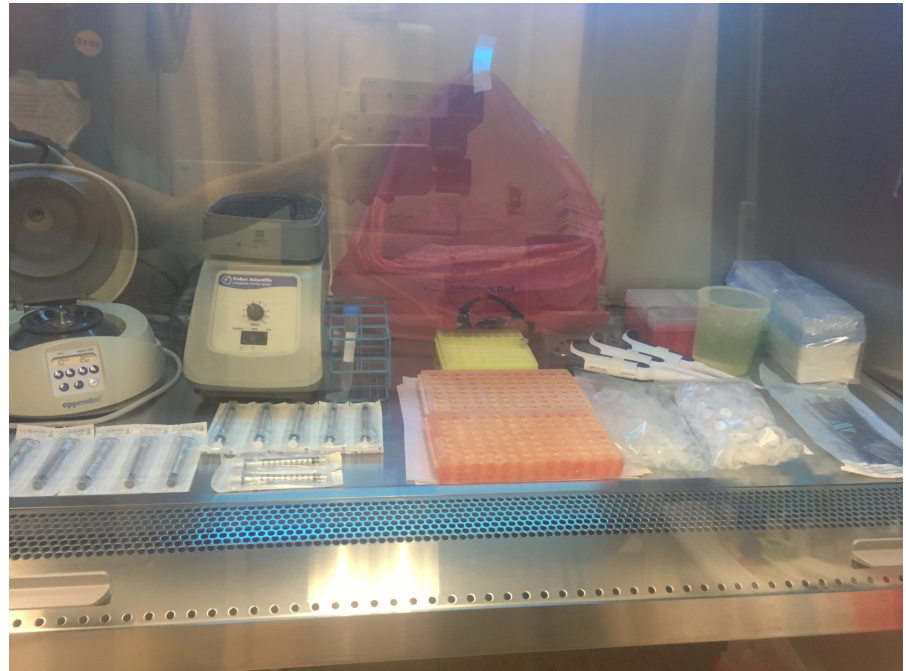
Centrifuge

Filter

DNase/Lysozyme

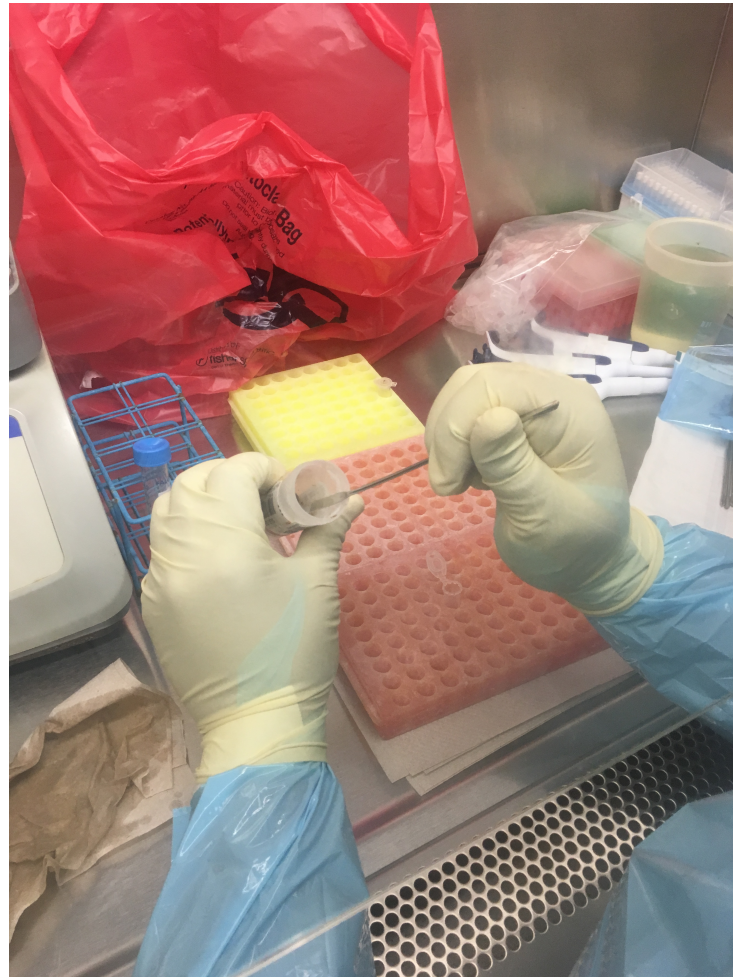
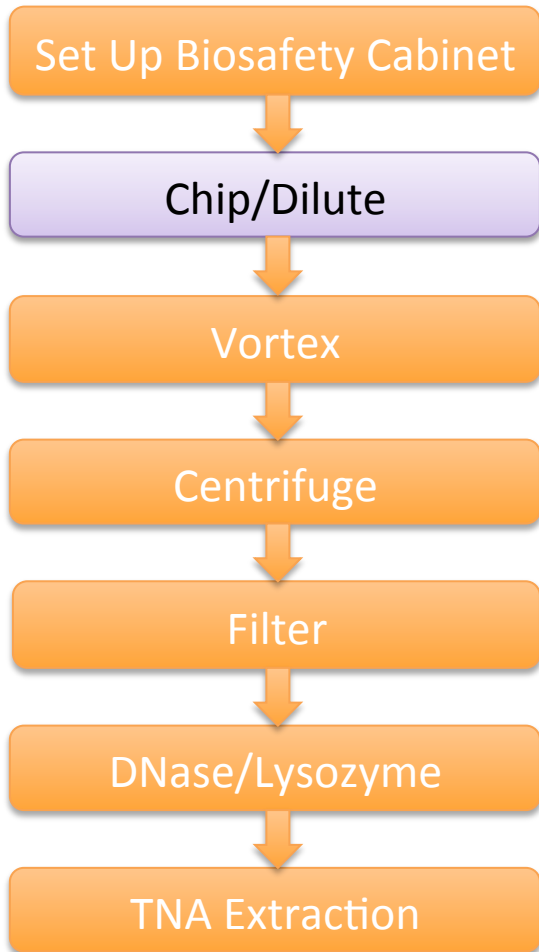
TNA Extraction

- Samples are handled at BSL2+- gown and double glove
- Decon with 10% bleach solution, 70% ethanol, and UV for 30 minutes
- Waste collected in biohazard bags and autoclaved



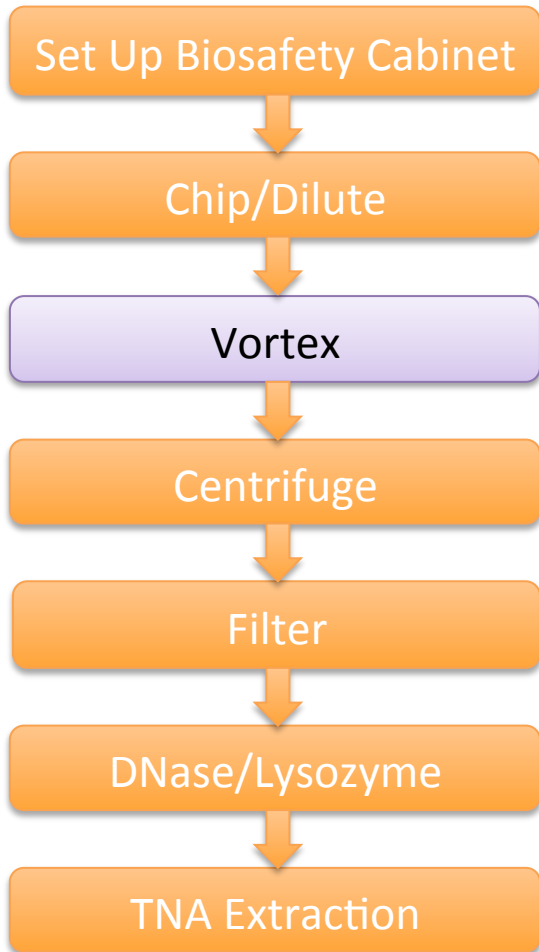
Chip Stool Sample

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



Homogenize Samples

- Vortex for 5 minutes
- Break up stool material



Set Up Biosafety Cabinet

Chip/Dilute

Vortex

Centrifuge

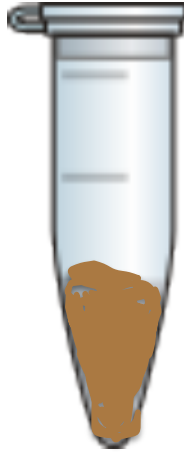
Filter

DNase/Lysozyme

TNA Extraction

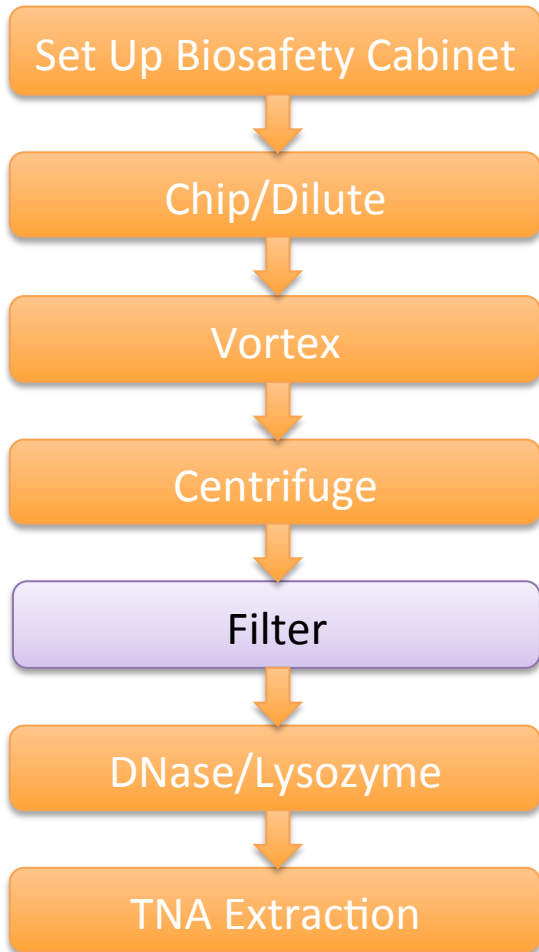
Centrifuge

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

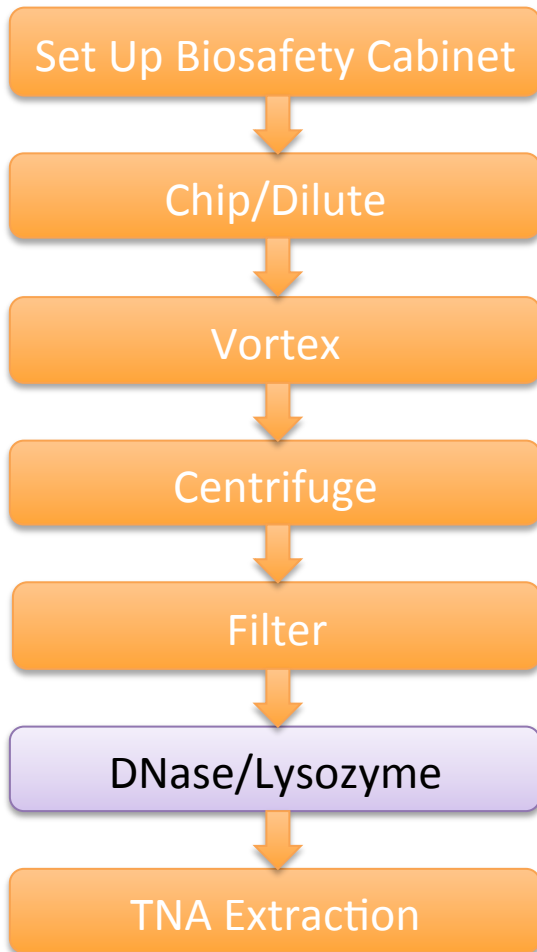


Filter to Remove Bacteria

- .45u filter



Non Encapsulated DNA Removal

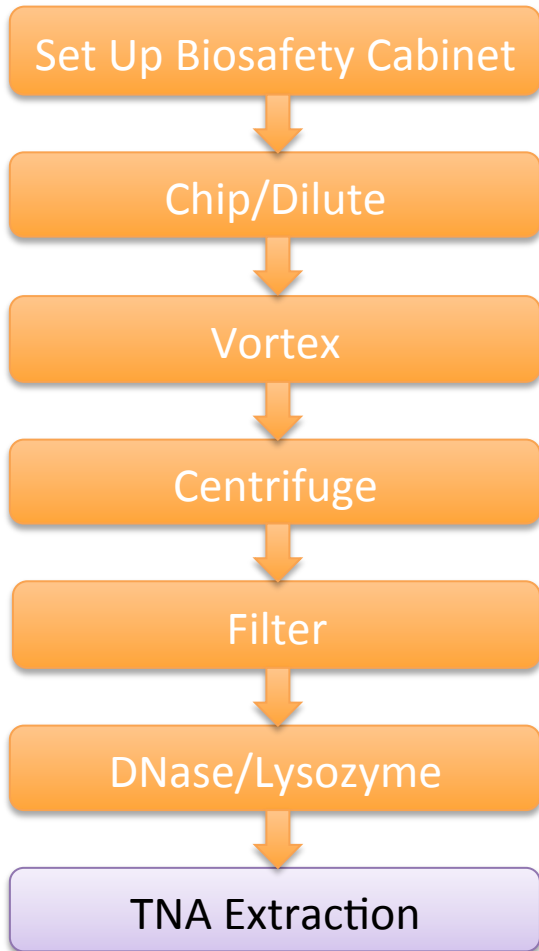


- Lysozyme DNase Enzyme cocktail
- Degrade non-encapsulated DNA

	Per 800ul sample	12 +1 =13 samples
Turbo DNase buffer	108 ul	1,404
TurboDNaseI (2U/ ul)	20 ul	260
Baseline zero (1U/ ul)	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



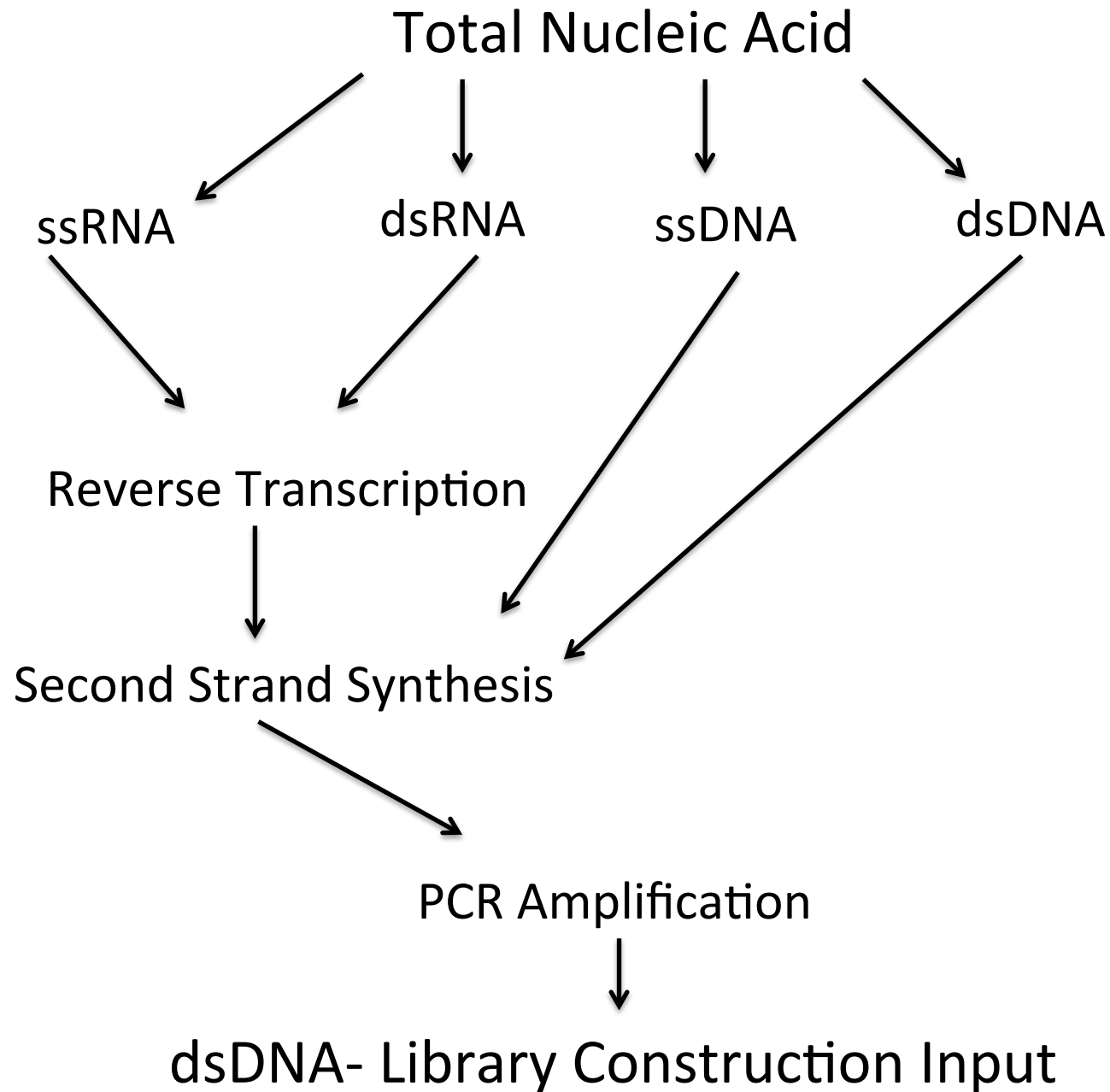
Preparing Samples for Virome Sequencing: 3 Steps

1.) Virus Like Particle (VLP)

Enrichment and Total Nucleic Acid
Extraction

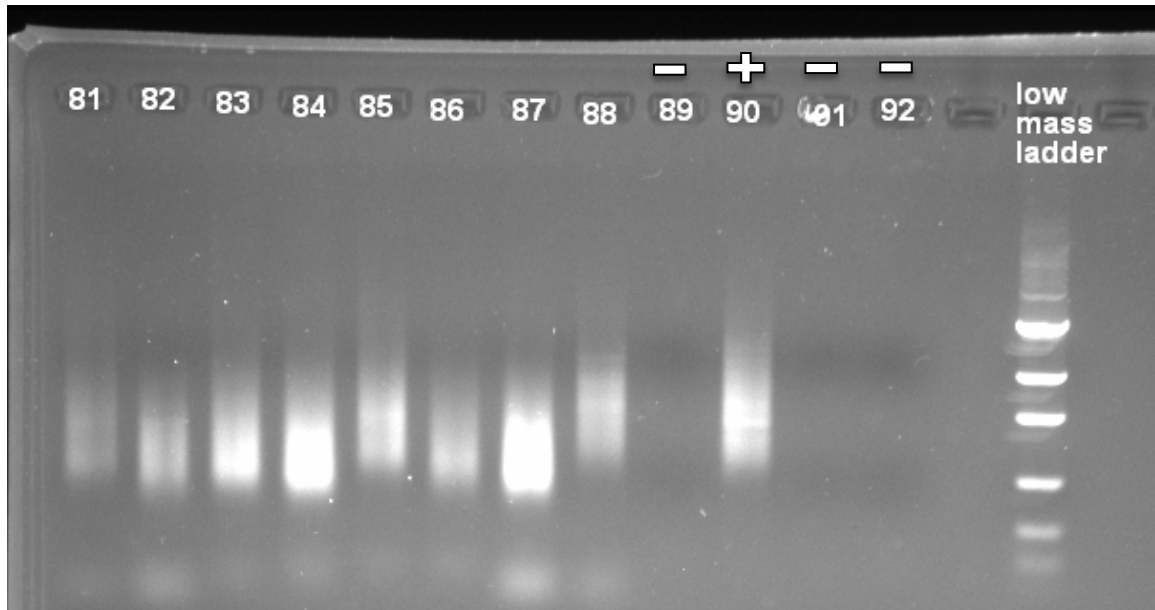
2.) Reverse Transcription, Second
Strand Synthesis and PCR
Amplification

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
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Strand Synthesis, and PCR
Amplification
- 3.) Library Construction



SureSelectXT Reagent Kits

Clean Up/Size Selection



End Repair



Adapter Ligation



Clean Up/Size Selection



PCR Amplification



PCR Clean Up



Quality Control



Pool

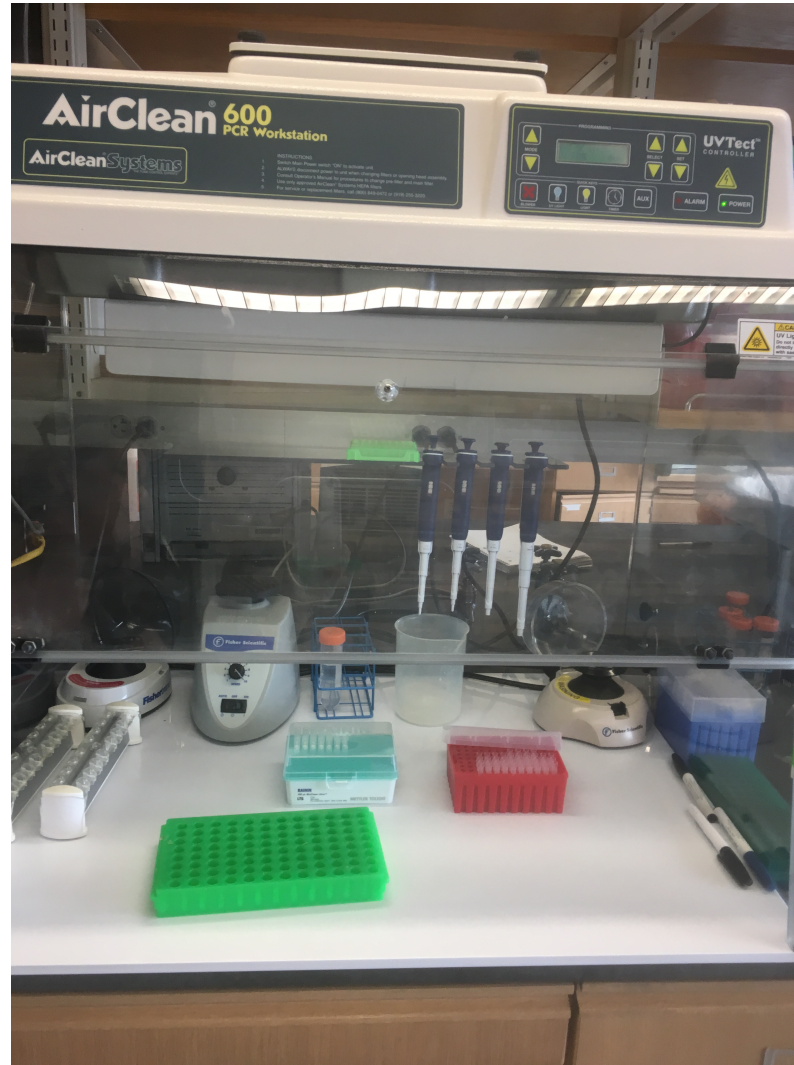


Sequence

New England Biolabs NEB Next DNA Library Construction

PCR Workstation

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



Clean Up/Size Selection

End Repair

Adapter Ligation

Clean Up/Size Selection

PCR Amplification

PCR Clean Up

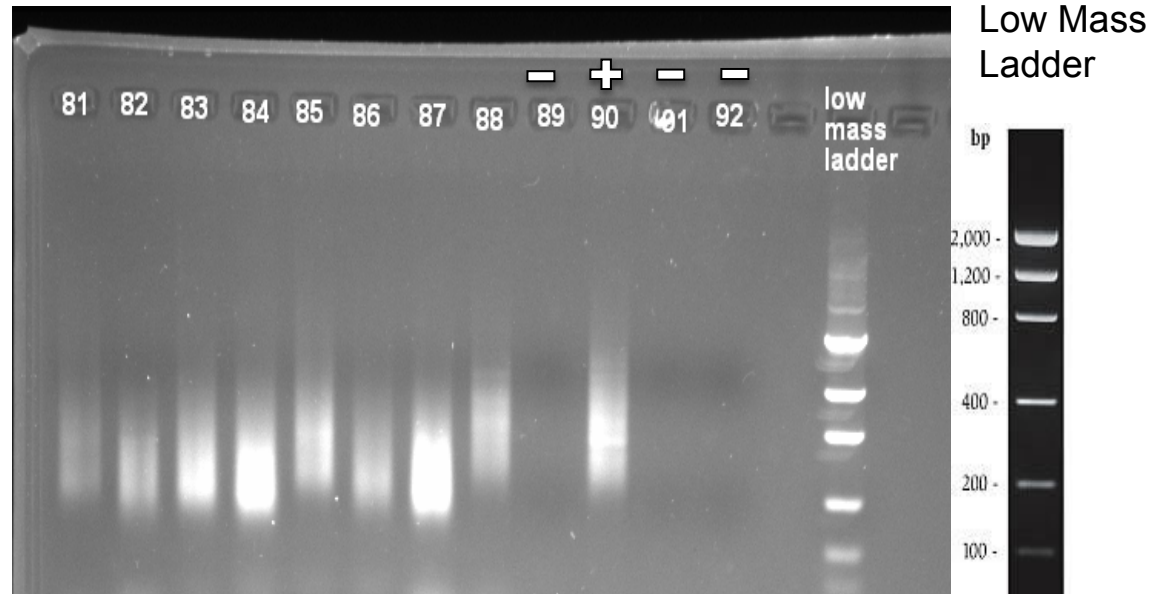
Quality Control

Pool

Sequence

Post PCR Amplification

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



Beckman Coulter- AmPure Bead

Clean Up/Size Selection

End Repair

Adapter Ligation

Clean Up/Size Selection

PCR Amplification

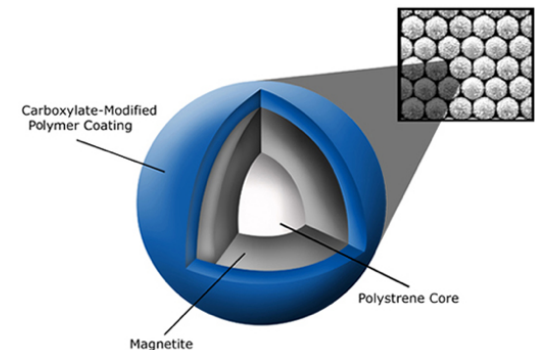
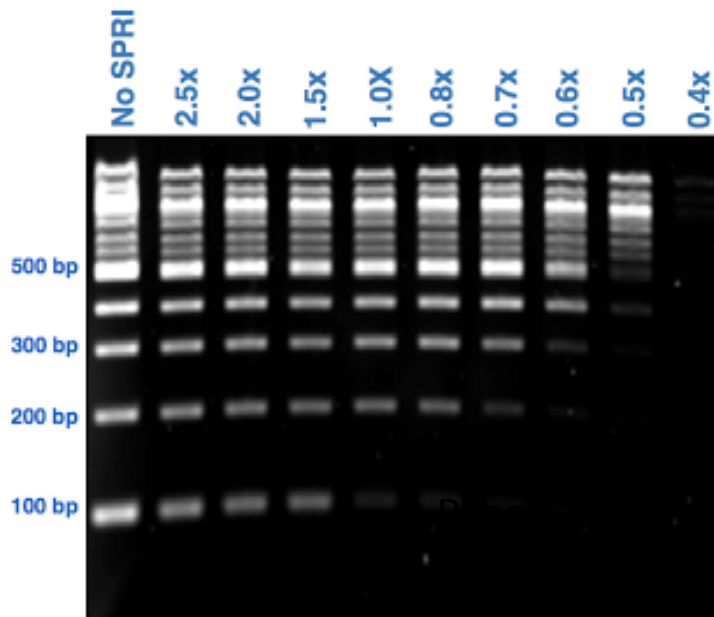
PCR Clean Up

Quality Control

Pool

Sequence

- 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

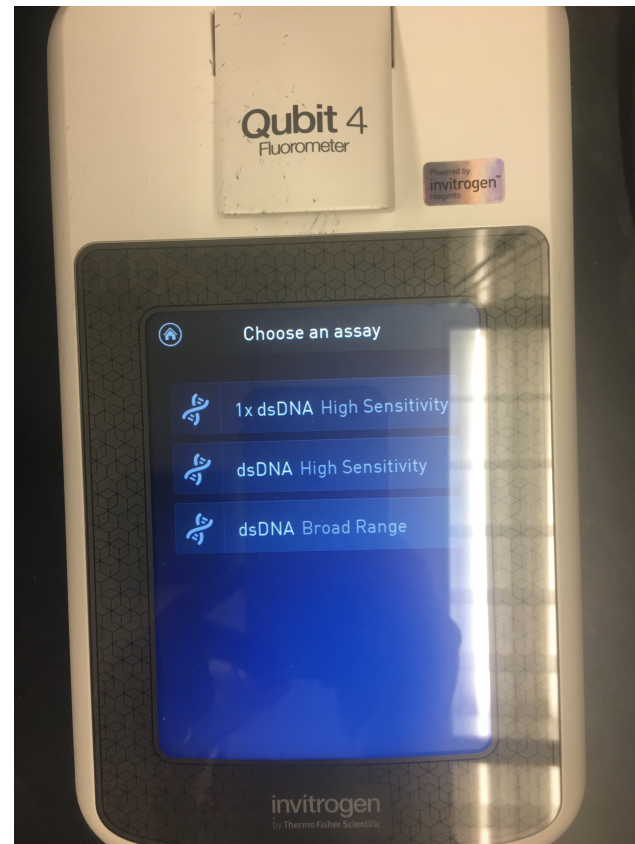
Quality Control

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



Clean Up/Size Selection

End Repair

Adapter Ligation

Clean Up/Size Selection

PCR Amplification

PCR Clean Up

Quality Control

Pool

Sequence

Clean Up/Size Selection

End Repair

Adapter Ligation

Clean Up/Size Selection

PCR Amplification

PCR Clean Up

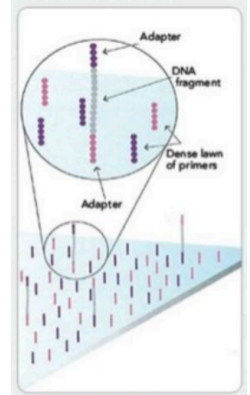
Quality Control

Pool

Sequence

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



```
graph TD; A[Clean Up/Size Selection] --> B[End Repair]; B --> C[Adapter Ligation]; C --> D[Clean Up/Size Selection]; D --> E[PCR Amplification]; E --> F[PCR Clean Up]; F --> G[Quality Control]; G --> H[Pool]; H --> I[Sequence];
```

The flowchart illustrates a 10-step sequencing process. Steps 1 through 9 are represented by blue rounded rectangular boxes, while Step 4 is highlighted with a red background. Each step is connected to the next by a downward-pointing blue arrow. The steps are: 1. Clean Up/Size Selection, 2. End Repair, 3. Adapter Ligation, 4. Clean Up/Size Selection, 5. PCR Amplification, 6. PCR Clean Up, 7. Quality Control, 8. Pool, and 9. Sequence.

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

Library Amplification by PCR

Clean Up/Size Selection

End Repair

Adapter Ligation

Clean Up/Size Selection

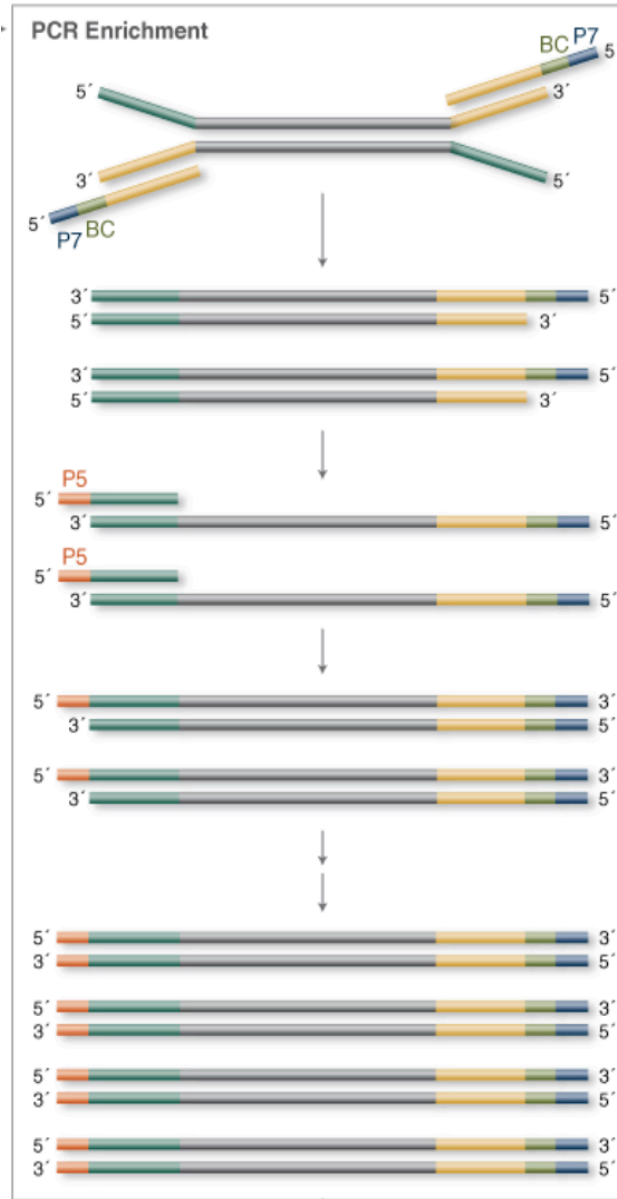
PCR Amplification

PCR Clean Up

Quality Control

Pool

Sequence



- 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



```
graph TD; A[Clean Up/Size Selection] --> B[End Repair]; B --> C[Adapter Ligation]; C --> D[Clean Up/Size Selection]; D --> E[PCR Amplification]; E --> F[PCR Clean Up]; F --> G[Quality Control]; G --> H[Pool]; H --> I[Sequence];
```

The flowchart illustrates the sequencing process. It begins with 'Clean Up/Size Selection', followed by 'End Repair', 'Adapter Ligation', another 'Clean Up/Size Selection', 'PCR Amplification', 'PCR Clean Up' (highlighted in red), 'Quality Control', 'Pool', and finally 'Sequence'. Each step is connected to the next by a downward-pointing arrow.

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

Clean Up/Size Selection

End Repair

Adapter Ligation

Clean Up/Size Selection

PCR Amplification

PCR Clean Up

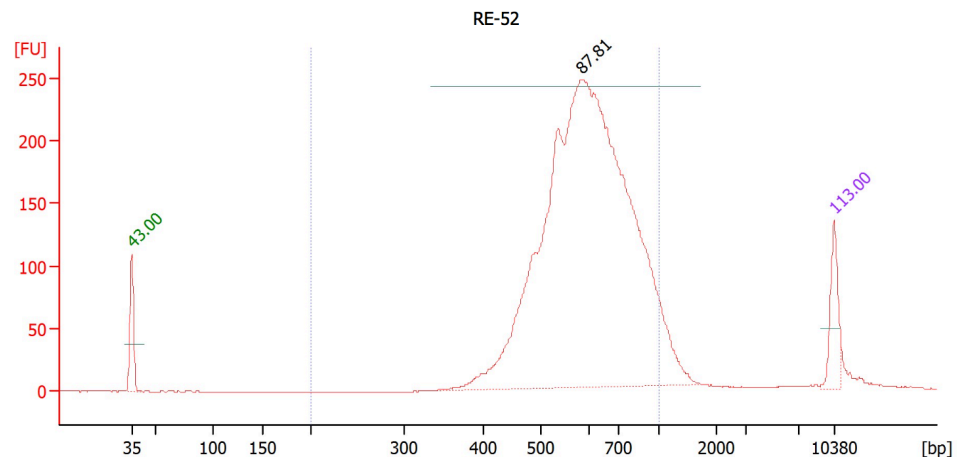
Quality Control

Pool

Sequence

Quality Control

- Agilent Bioanalyzer 2100
- Microfluidics platform for sizing and quantification



Overall Results for sample 9 : RE-52

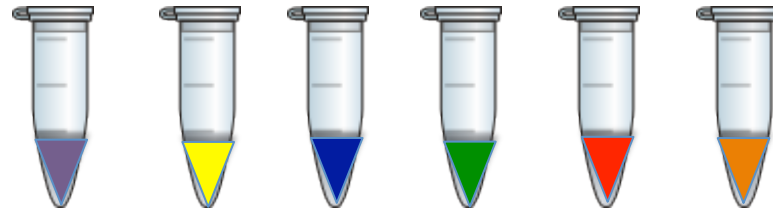
Number of peaks found: 1 Corr. Area 1: 2,893.5
Noise: 0.3

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



Clean Up/Size Selection

End Repair

Adapter Ligation

Clean Up/Size Selection

PCR Amplification

PCR Clean Up

Quality Control

Pool

Sequence

Clean Up/Size Selection



End Repair



Adapter Ligation



Clean Up/Size Selection



PCR Amplification



PCR Clean Up



Quality Control



Pool



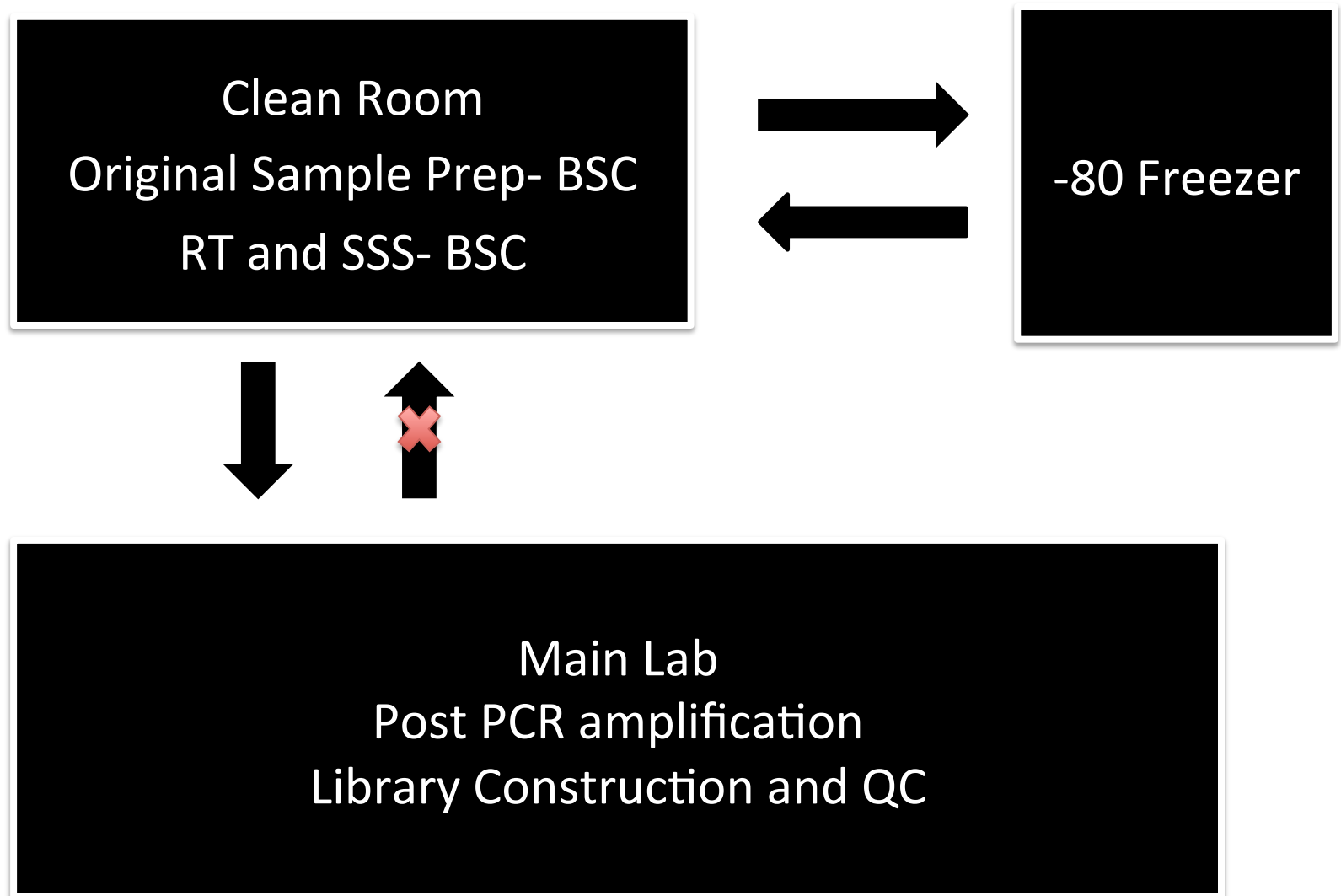
Sequence

MiSeq V2 2X250

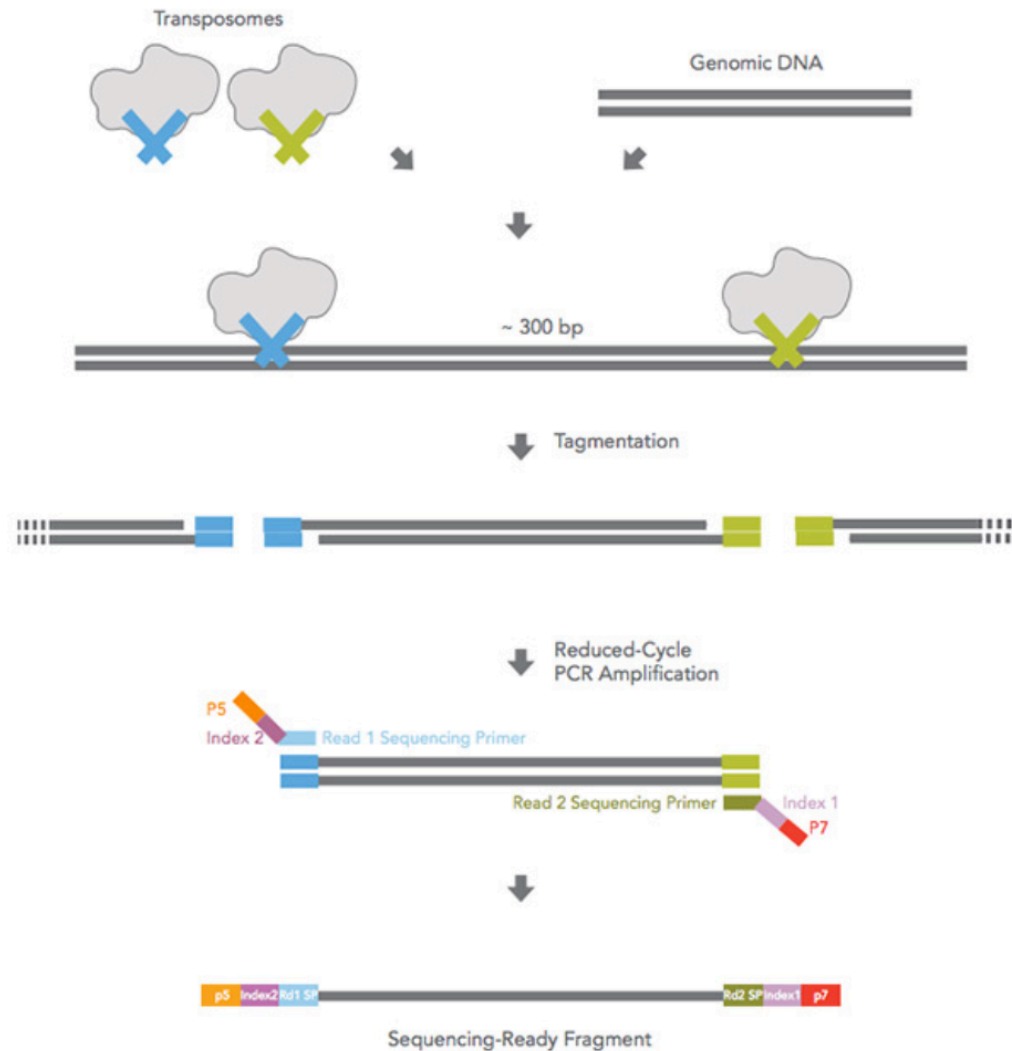


(pick your favorite)

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