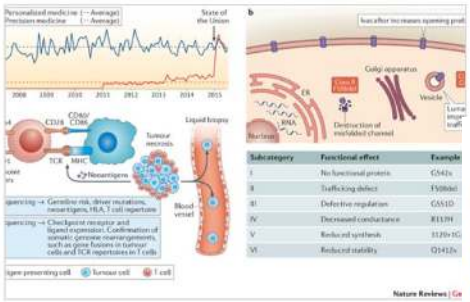
From Sample to Seq

Lindsay Droit
Washington University School of Medicine
St. Louis, Missouri

Next Gen Sequencing Applications

Nature Reviews Genetics | Review Article

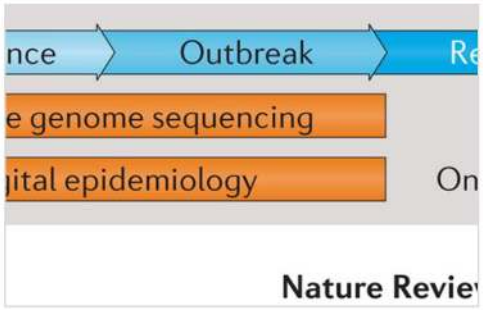


Towards precision medicine

Precision medicine is a strategy for tailoring clinical decision making to the underlying genetic causes of disease. This Review describes how, despite the straightforward overall principles of precision medicine, adopting it responsibly into clinical practice will require many technical and conceptual hurdles to be overcome. Such challenges include optimized sequencing strategies, clinically focused bioinformatics pipelines and reliable metrics for the disease causality of genetic variants. show less

Euan A. Ashley

Nature Reviews Genetics | Review Article

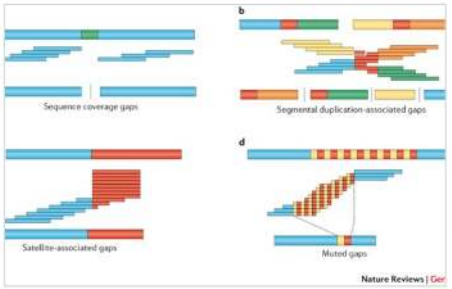


Towards a genomics-informed, real-time, global pathogen surveillance system

Next-generation sequencing has the potential to support public health surveillance systems to improve the early detection of emerging infectious diseases. This Review delineates the role of genomics in rapid outbreak response and the challenges that need to be tackled for genomics-informed pathogen surveillance to become a global reality. show less

Jennifer L. Gardy & Nicholas J. Loman

Nature Reviews Genetics | Review Article

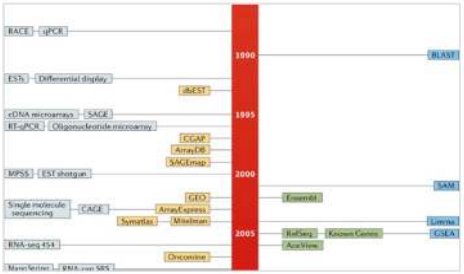


Genetic variation and the *de novo* assembly of human genomes

The wealth of existing and emerging DNA-sequencing data provides an opportunity for a comprehensive understanding of human genetic variation, including the discovery of disease-causing variants. This Review describes how the limitations of current reference-genome assemblies confound the characterization of genetic variation and how this can be mitigated by important advances in algorithms and sequencing technology that facilitate the *de novo* assembly of genomes. show less

Mark J. P. Chaisson, Richard K. Wilson & Evan E. Eichler

Nature Reviews Genetics | Review Article



Cancer transcriptome profiling at the juncture of clinical translation

Although cancer genome sequencing is becoming routine in cancer research, cancer transcriptome profiling through methods such as RNA sequencing (RNA-seq) provides information not only on mutations but also on their functional cellular consequences. This Review discusses how technical and analytical advances in cancer transcriptomics have provided various clinically valuable insights into gene expression signatures, driver gene prioritization, cancer microenvironments, immuno-oncology and prognostic biomarkers. show less

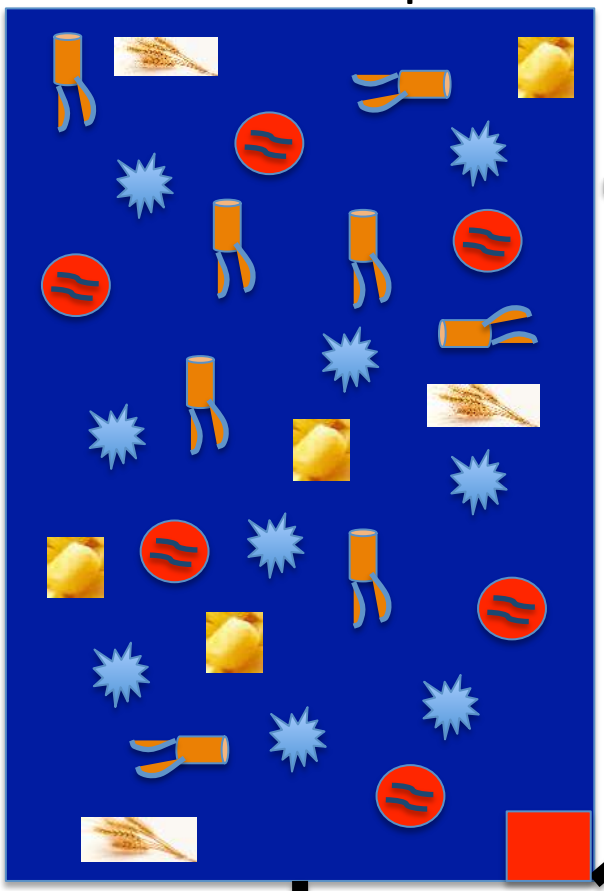
Marcin Cieślik & Arul M. Chinnaiyan

Using Next Generation Sequencing to Define the Microbiome

1.) Amplicon Surveys- targeted amplicons
- 16S (bacteria)
- 18S (bacteria)
- ITS (fungi)

2.) Shotgun Metagenomics-
    -entire metagenome: sacrifice resolution
    -enriched metagenome- Virome

Stool Sample



Amplicon Sequencing

16S/18S/ITS
-DNA
-targeted Amplicons

Enriched Metagenome- Virome
Virus Like Particle (VLP)
-DNA, RNA
-remove bacteria
-remove host

Shotgun Sequencing
Kwon Lab- Matt

Sample Types

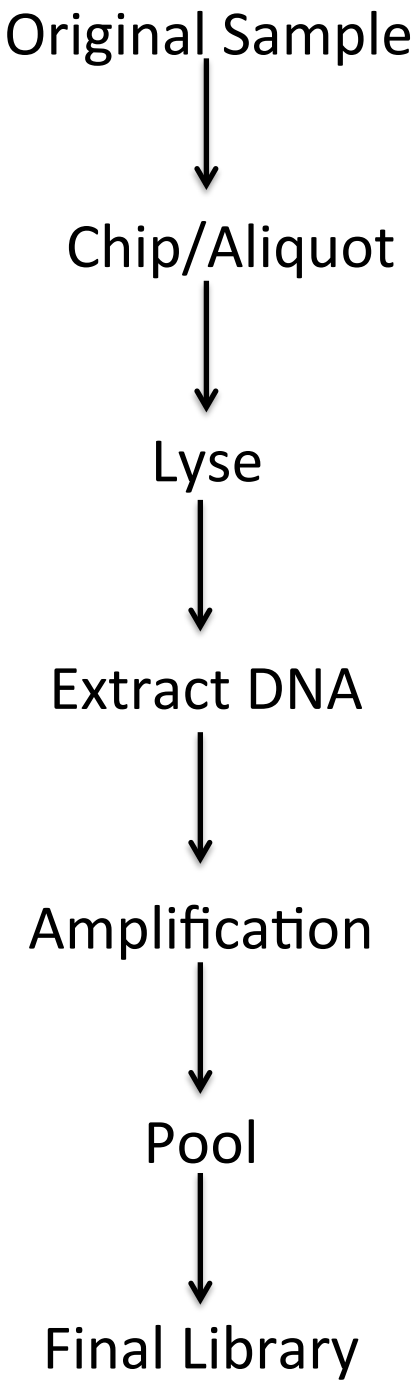
Stool
Urine
Tissue
Cerebral Spinal Fluid
Cervicovaginal Swab
Nasopharyngeal Swab
Bronchoalveolar Lavage
Sputum
Serum
Synovial Fluid
Whole Blood
Cerebral Spinal Fluid
Allantoic Fluid
Amniotic Fluid
Breastmilk
Oral Wash
Saliva
Pericardial Fluid
Semen
Sewage

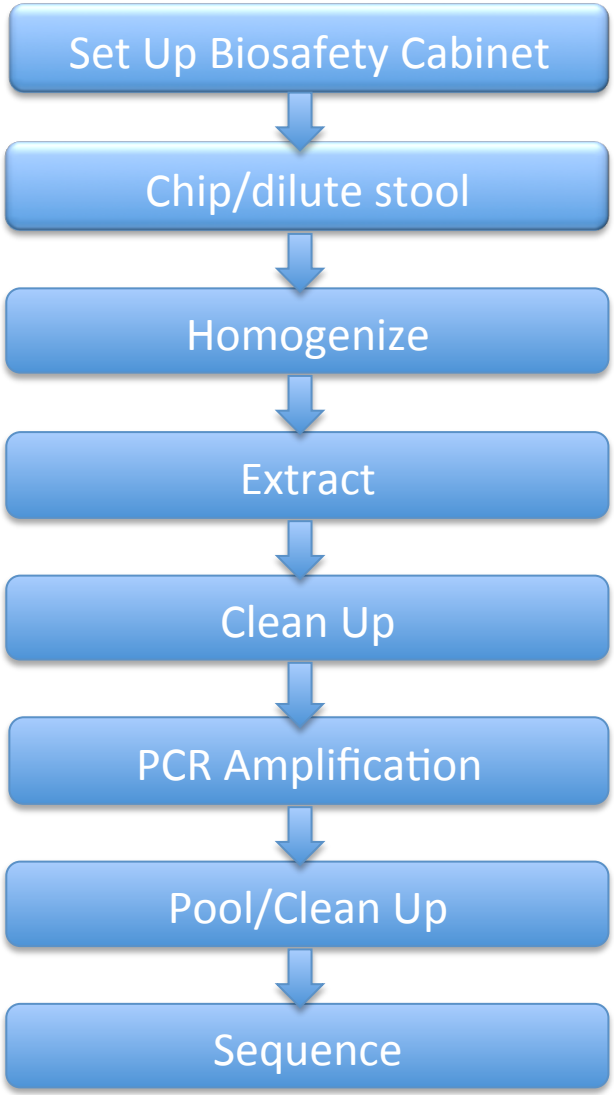
How much sample do you need?

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

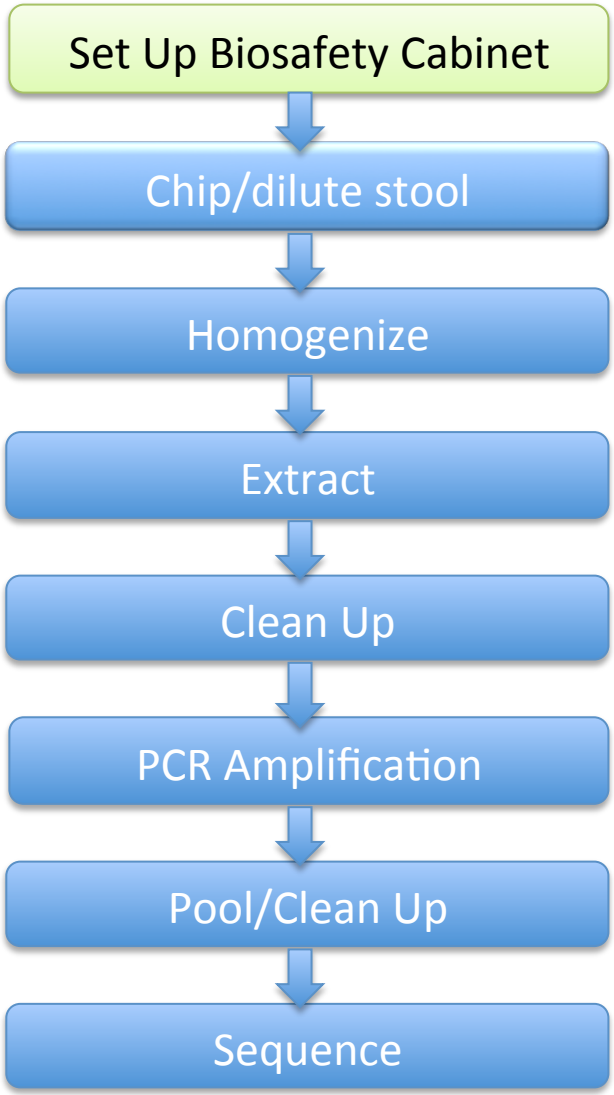
Storage Conditions

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


Original Sample
Chip/Aliquot
Lyse
Extract DNA
Amplification
Pool
Final Library

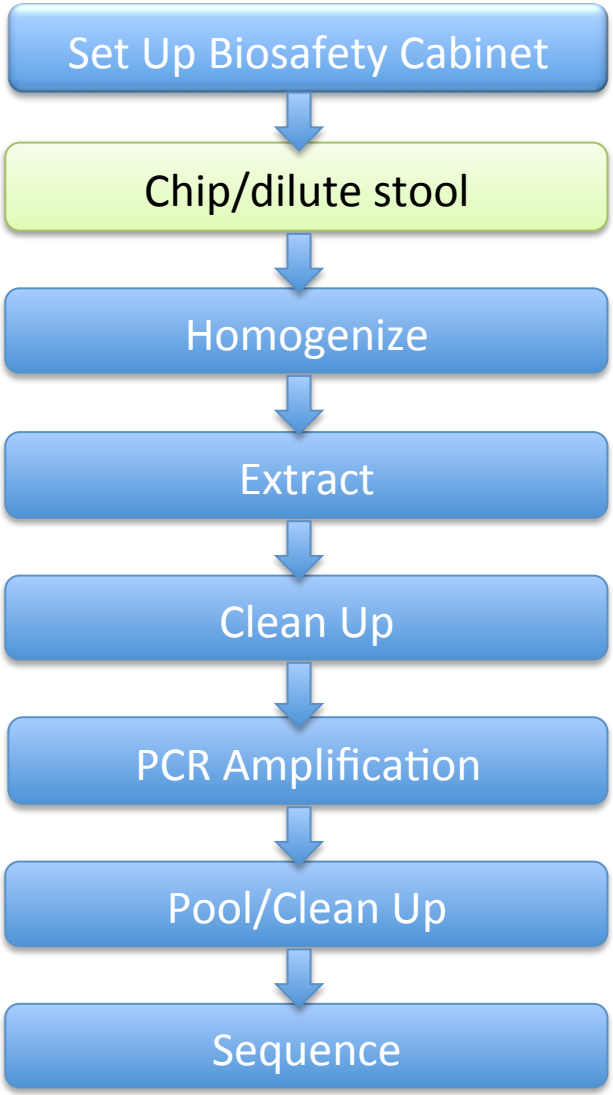


Preparing Samples for 16S/ITS/18S Amplicon Sequencing



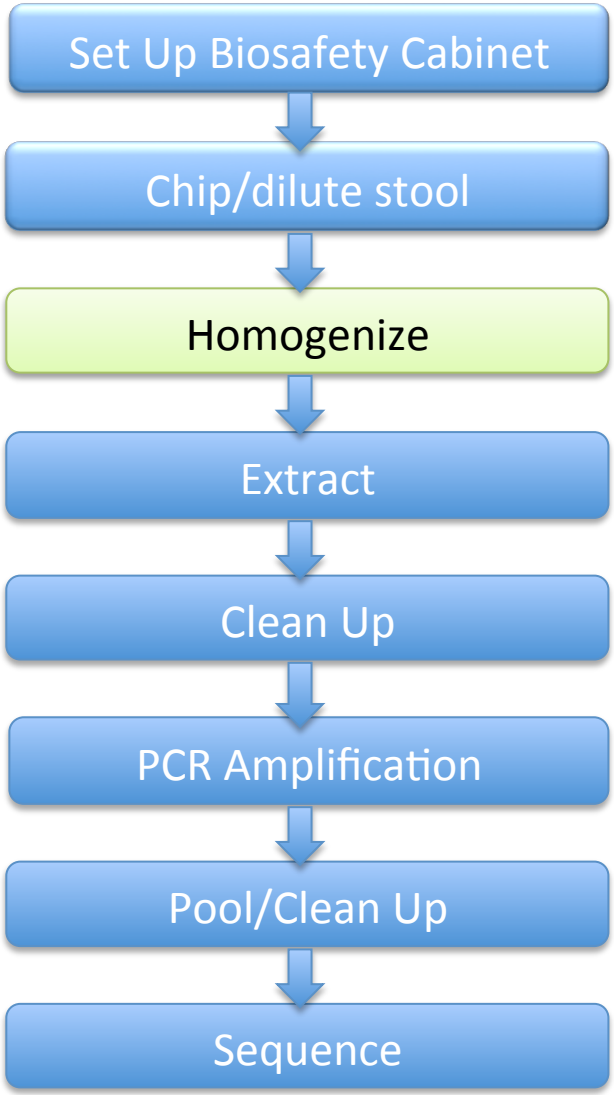
Biosafety Cabinet Set Up

- 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



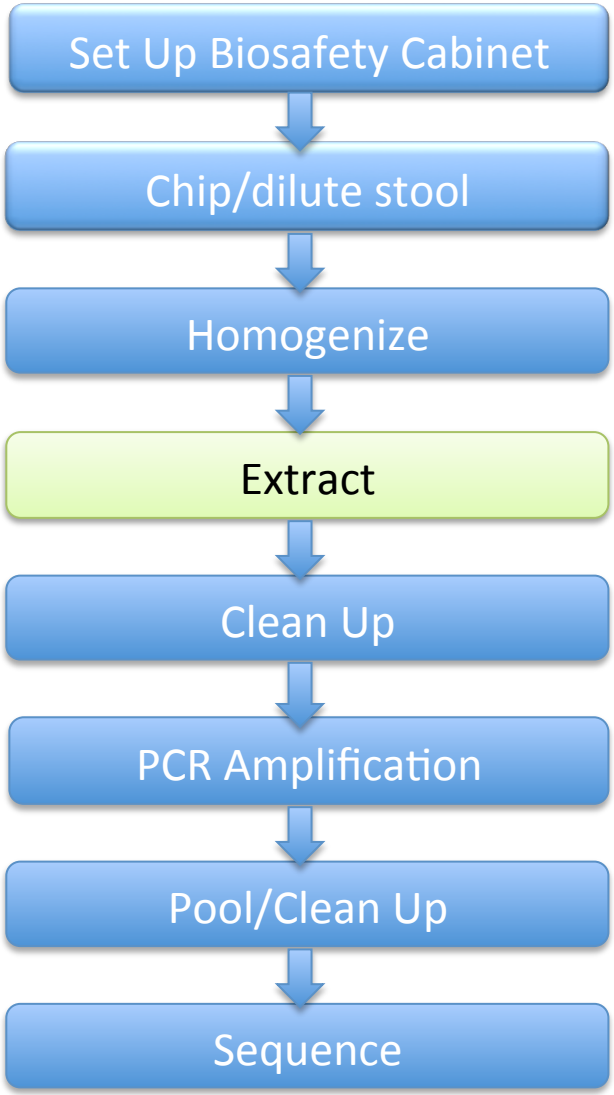
Chip Stool Sample

- Keep sample frozen
- Chip ~20mg of stool
- 200ul of autoclaved 1.0mm silica beads
- Add 20% SDS, Buffer A (NaCl, Tris, EDTA), PCI



Homogenize Samples

- Bead beat for 1 minute
- Lyse cells to release bacterial DNA



Crude DNA Extraction

Aqueous solution of homogenized stool

+

Equal volume phenol:chloroform:IAA

↓

Vortex

↓

Centrifuge

↓

Pipette Aqueous Layer

↓

Isopropanol Precipitation

↓

Resuspend in 50ul of H2O

Set Up Biosafety Cabinet

Chip/dilute stool

Homogenize

Extract

Clean Up

PCR Amplification

Pool/Clean Up

Sequence

Qiagen DNeasy

- P:C:I extracted nucleic acid is not pure enough for PCR
- Samples arranged in 96 well format for remainder of protocol

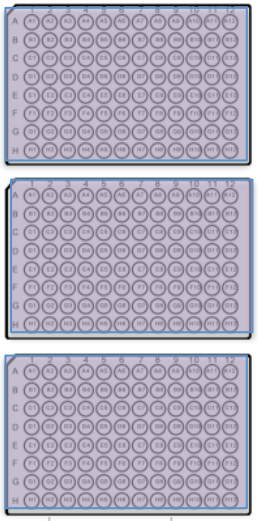
PCR Workstation

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

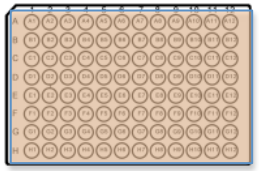
PCR Amplification Plate Set Up

- Set Up Biosafety Cabinet
- Chip/dilute stool
- Homogenize
- Extract
- Clean Up
- PCR Amplification
- Pool/Clean Up
- Sequence

Triplicate Sample Plates



Negative Control Plate



Sample Plate

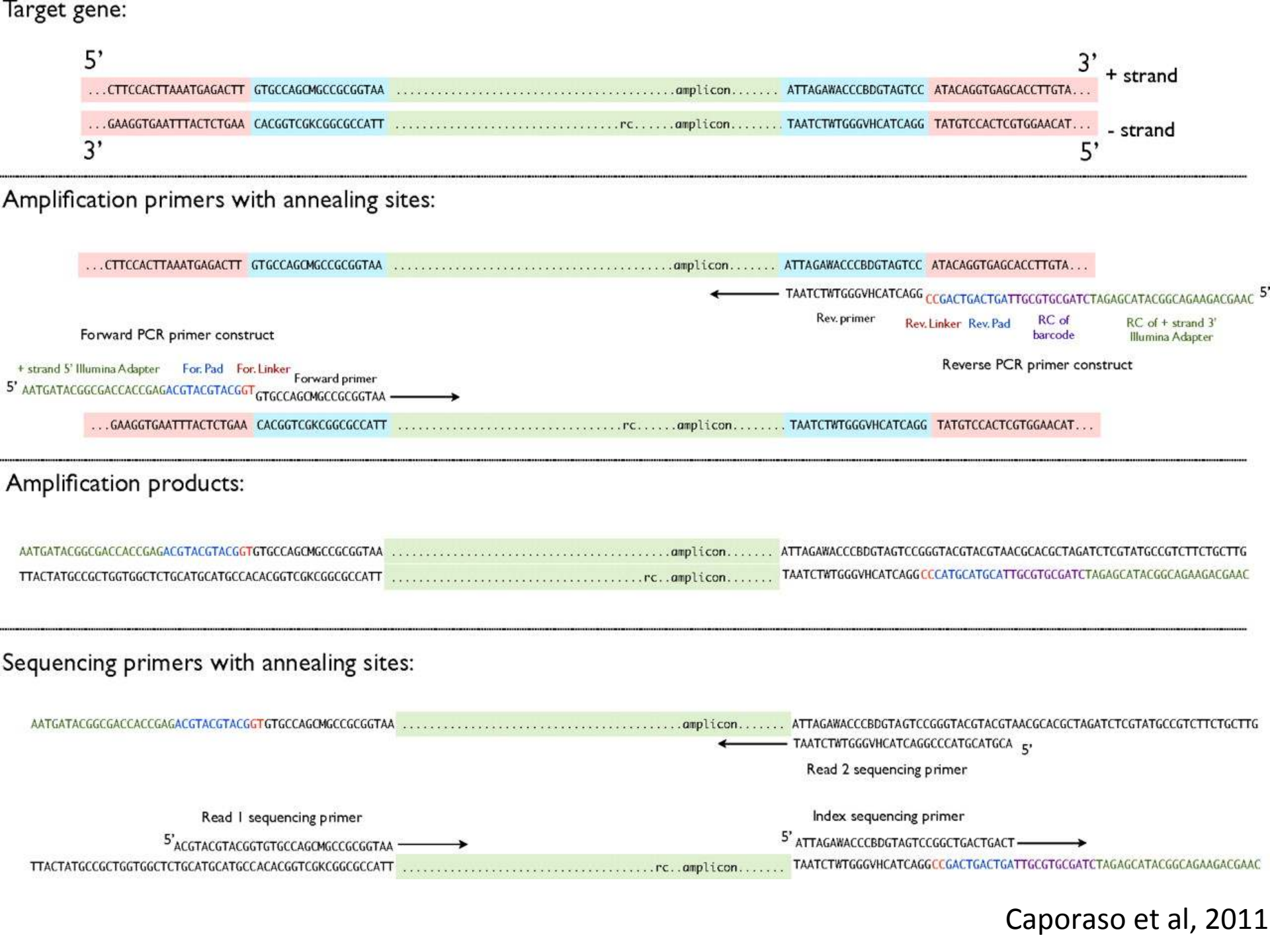
	1	2	3	4	5	6
A		1322	1346	1348	1434	1332
B	1329		1334	1352	1393	1316
C	1330	1331		1333	1350	1351
D	1382	1383	1384		1385	1388

.....

Primer Plate

	1	2	3	4	5	6
A	806rcbc0	806rcbc1	806rcbc2	806rcbc3	806rcbc4	806rcbc5
B	806rcbc12	806rcbc13	806rcbc14	806rcbc15	806rcbc16	806rcbc17
C	806rcbc24	806rcbc25	806rcbc26	806rcbc27	806rcbc28	806rcbc29
D	806rcbc36	806rcbc37	806rcbc38	806rcbc39	806rcbc40	806rcbc41

.....



Caporaso et al, 2011

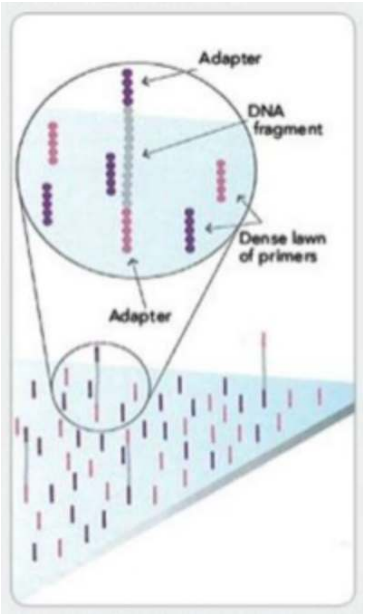
Primer Design

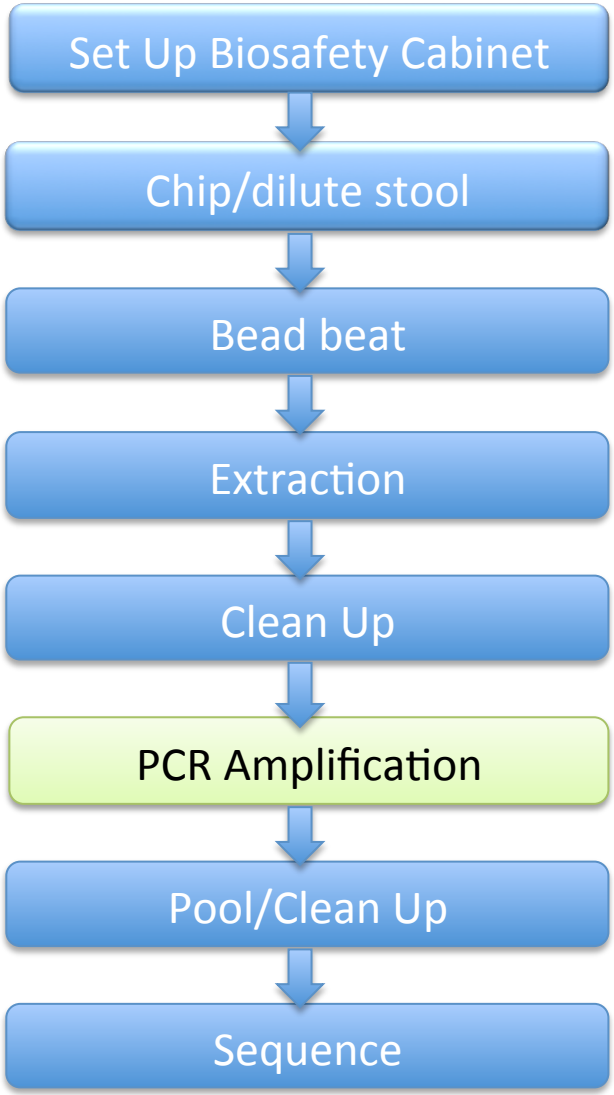
Conserved Sequence

Forward/Reverse Primer

Barcode

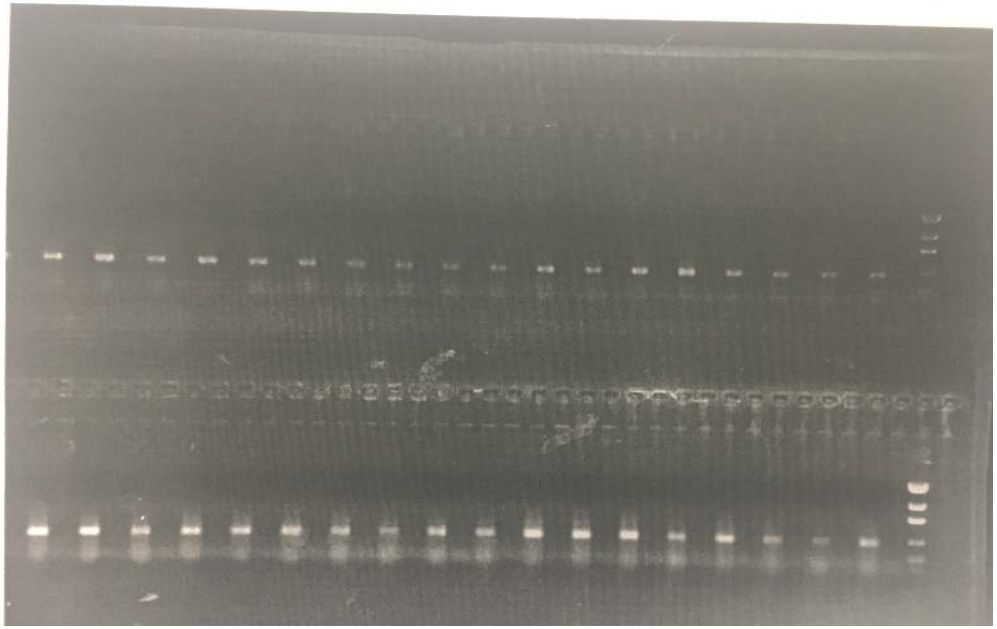
Illumina Adapter





Post PCR Amplification

- Triplicate reactions are pooled into single plate
- 1% Agarose gel- 5ul of amplified DNA
- Expected band 16S=390bp, 18S=260bp +/- 50bp, ITS= ~230bp



Failure Rate: up to 40% for some projects
Troubleshooting No Band:
-Antibiotics treated samples often don't show a band
-PCR inhibitors- sometimes a 1:10 or 1:100 dilution of the sample will work

Set Up Biosafety Cabinet

Chip/dilute stool

Homogenize

Extract

Clean Up

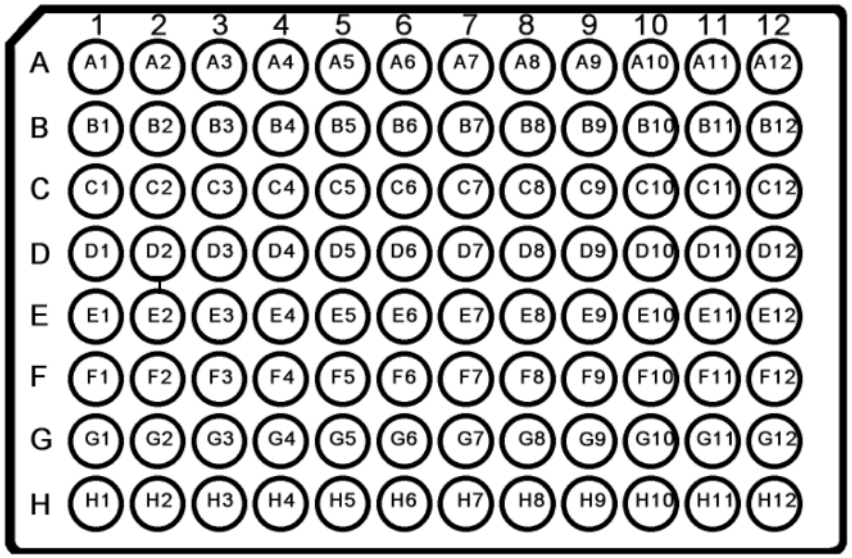
PCR Amplification

Pool/Clean Up

Sequence

Sample Pooling and Clean Up

- Pool 5ul per sample. Including H2O negative controls
- Clean Up
- Quantify Using Nanodrop



Pool 5ul/sample

Set Up Biosafety Cabinet

↓

Chip/dilute stool

↓

Homogenize

↓

Extract

↓

Clean Up

↓

PCR Amplification

↓

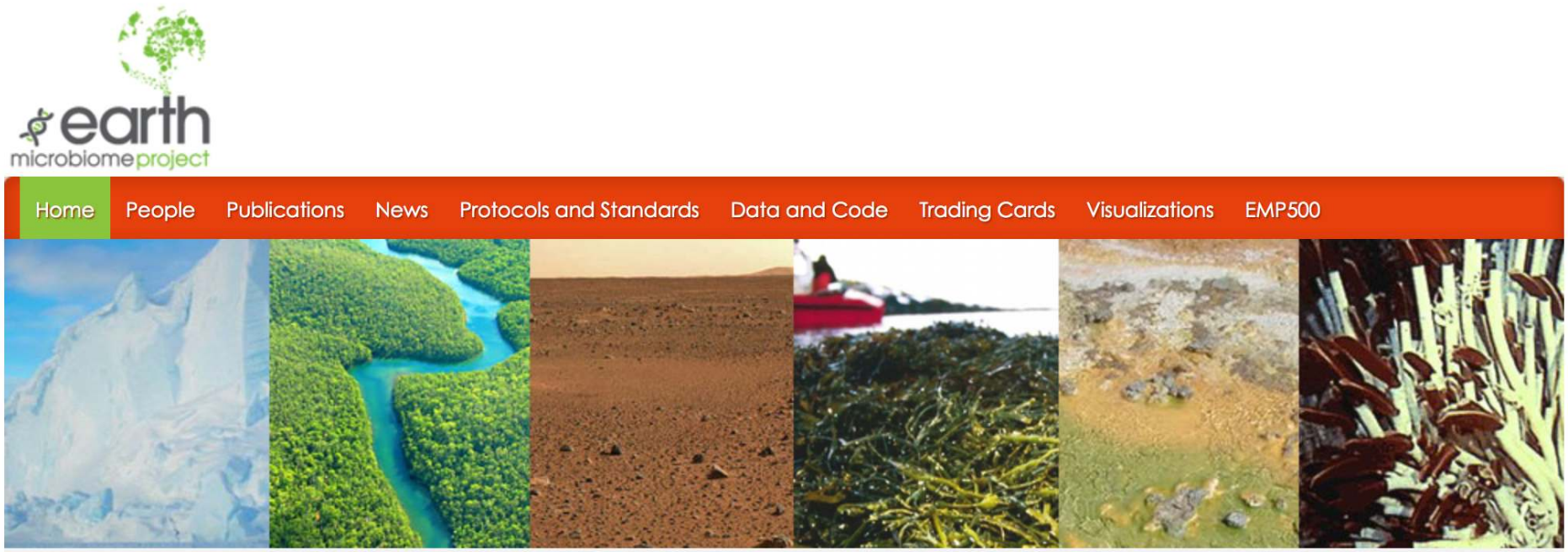
Pool/Clean Up

↓

Sequence

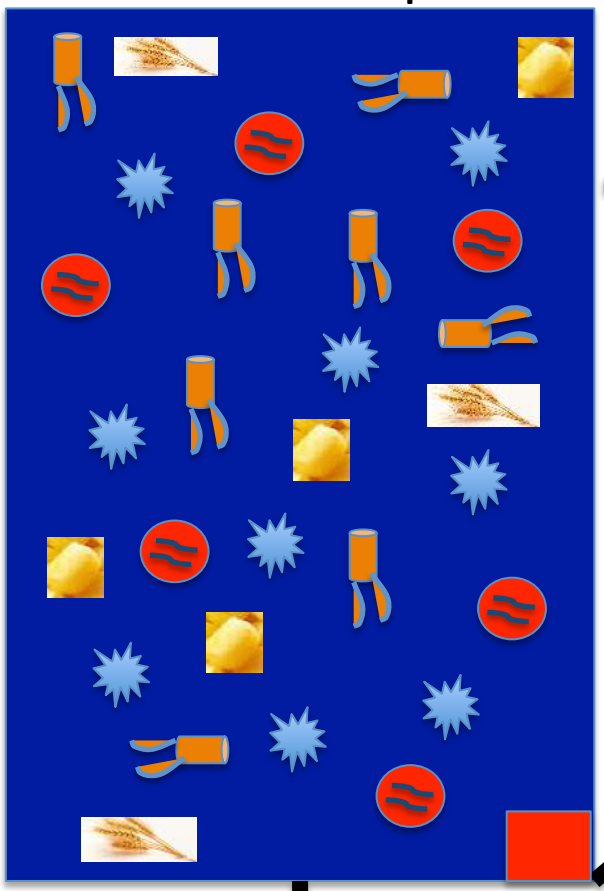
MiSeq V2 2X250

(pick your favorite)



http://www.earthmicrobiome.org

Stool Sample



Amplicon Sequencing

16S/18S/ITS
-DNA
-targeted Amplicons

Enriched Metagenome- Virome

Virus Like Particle (VLP)
-DNA, RNA
-remove bacteria
-remove encapsulated DNA

Shotgun Sequencing

Kwon Lab- Matt

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

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

Set Up Biosafety Cabinet

Chip/Dilute

Vortex

Centrifuge

Filter

DNase/Lysozyme

TNA Extraction

Biosafety Cabinet Set Up

- 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

- 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

- Vortex for 5 minutes
- Break up stool material

Set Up Biosafety Cabinet

Chip/Dilute

Vortex

Centrifuge

Filter

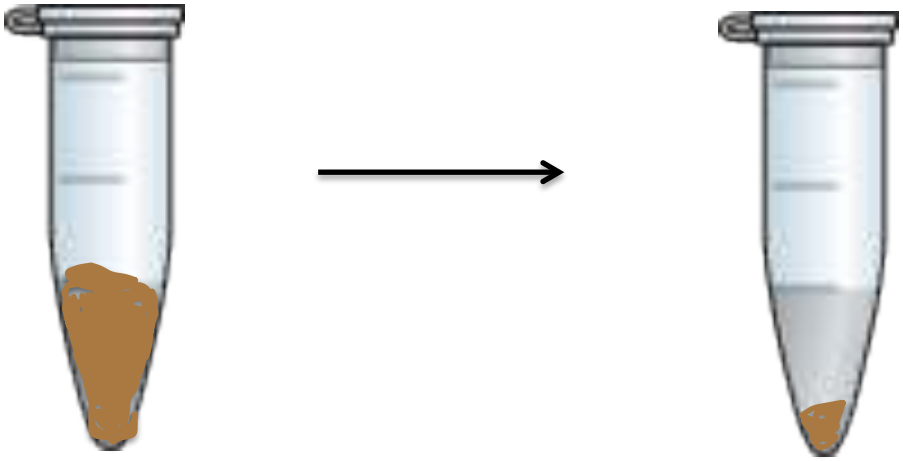
DNase/Lysozyme

TNA Extraction

- Set Up Biosafety Cabinet
- Chip/Dilute
- Vortex
- **Centrifuge**
- Filter
- DNase/Lysozyme
- TNA Extraction

Centrifuge

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



Set Up Biosafety Cabinet

Chip/Dilute

Vortex

Centrifuge

Filter

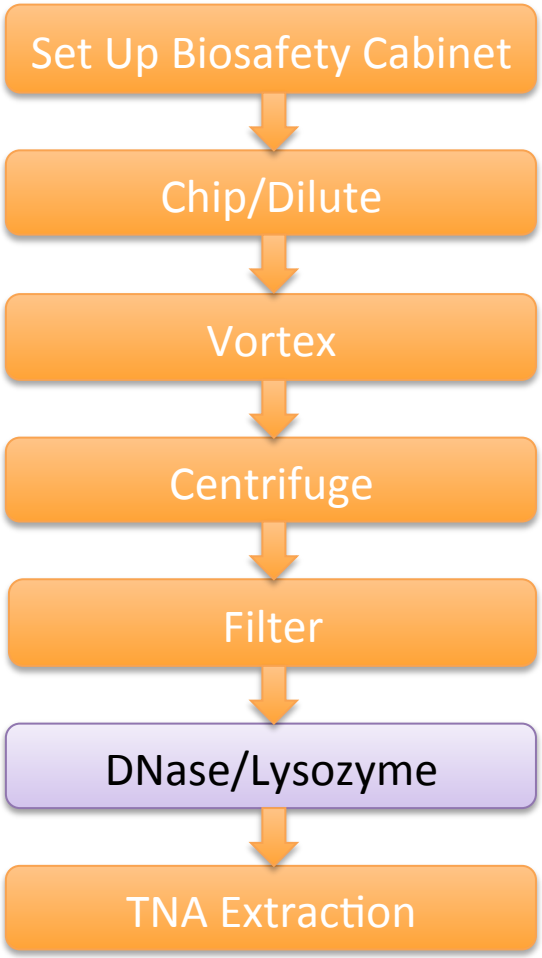
DNase/Lysozyme

TNA Extraction

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

- Set Up Biosafety Cabinet
- Chip/Dilute
- Vortex
- Centrifuge
- Filter
- DNase/Lysozyme
- TNA Extraction

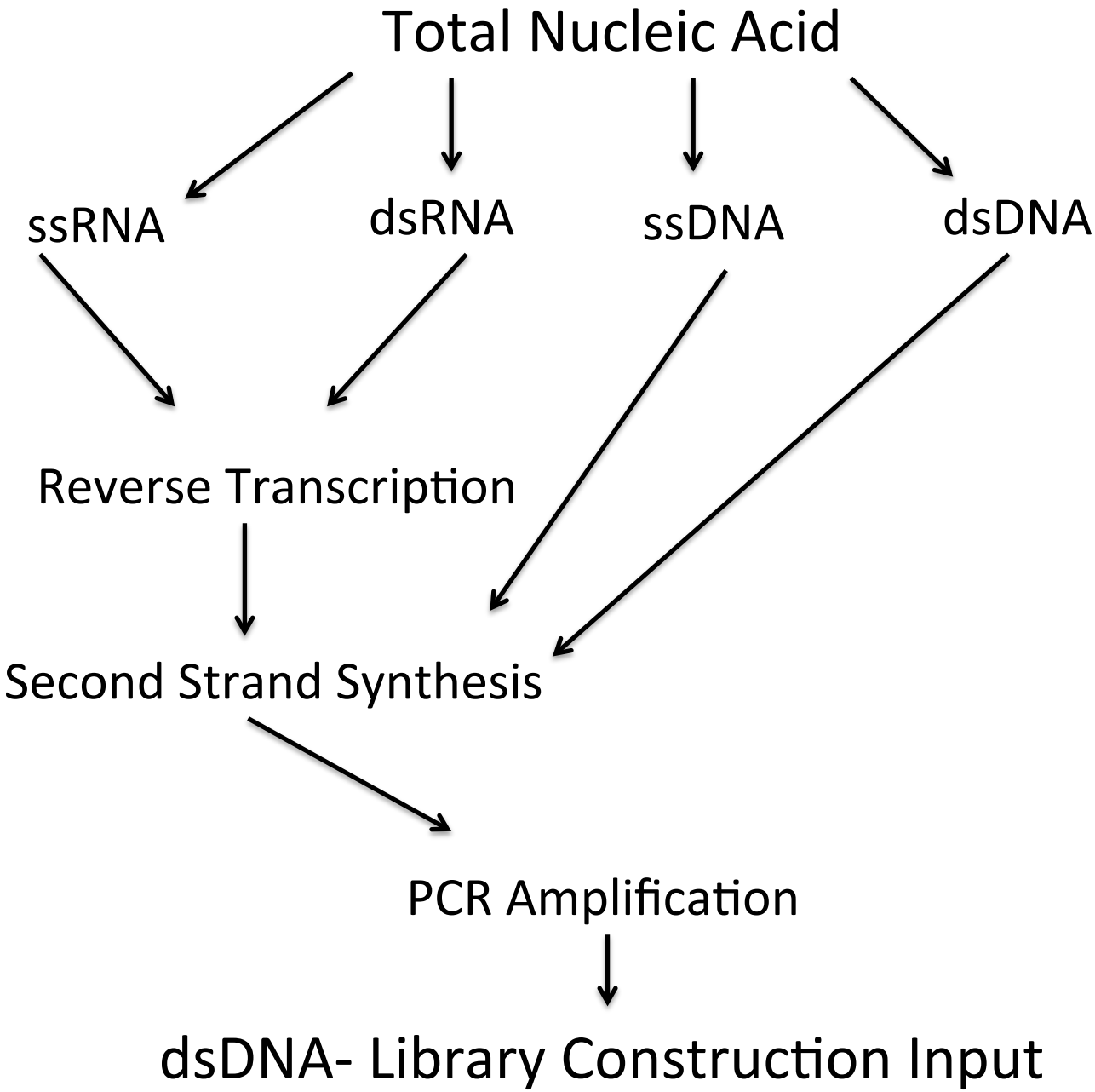
- Extract both DNA and RNA
- Automated systems
- Manual Kits- Qiagen DNeasy

Preparing Samples for Virome Sequencing: 3 Steps

1.) Virus Like Particle (VLP) Enrichment and Total Nucleic Acid Extraction

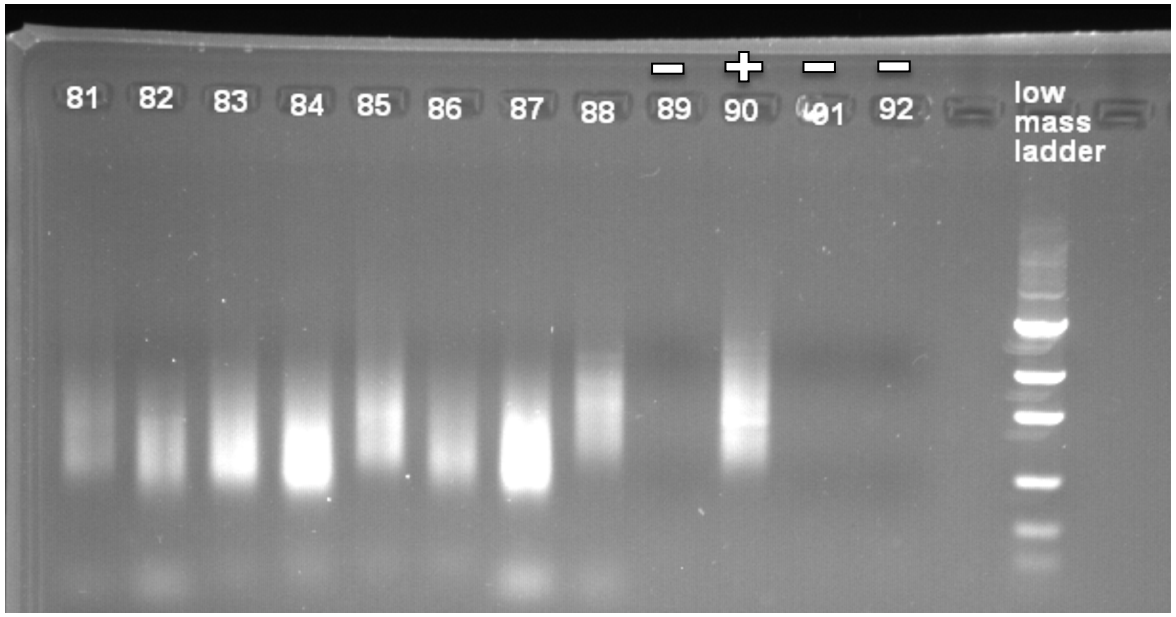
2.) Reverse Transcription, Second Strand Synthesis and PCR Amplification

3.) Library Construction


Total Nucleic Acid
ssRNA
dsRNA
ssDNA
dsDNA
Reverse Transcription
Second Strand Synthesis
PCR Amplification
dsDNA- Library Construction Input

Post PCR Amplification

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



Invitrogen Low Mass Ladder

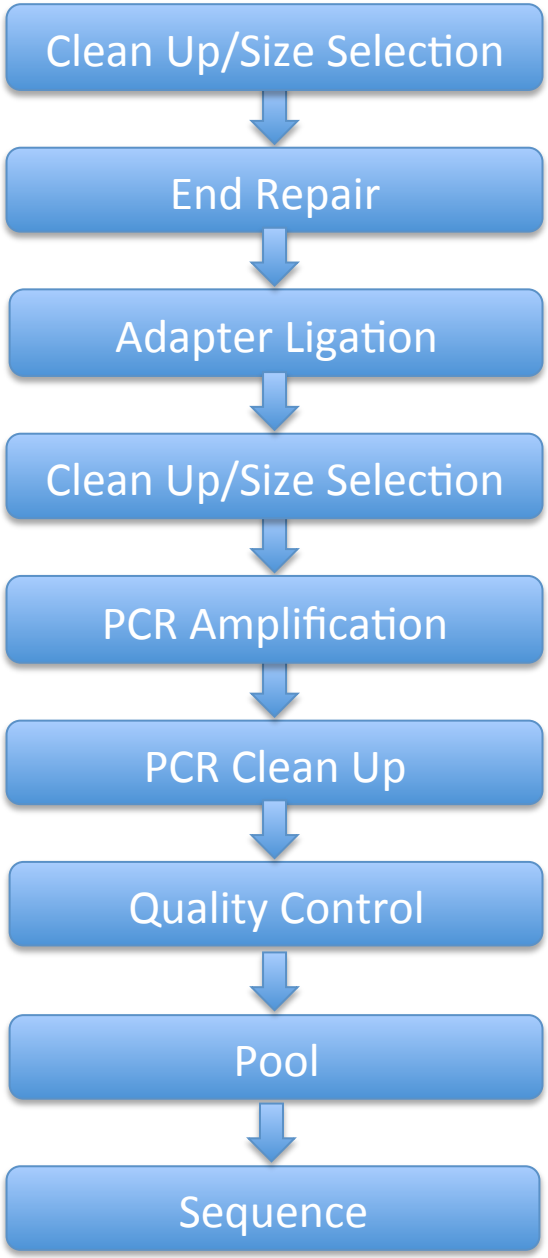
Preparing Samples for Virome Sequencing: 3 Steps

1.) Virus Like Particle (VLP) Enrichment and Total Nucleic Acid Extraction

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

3.) Library Construction

SureSelectXT Reagent Kits



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

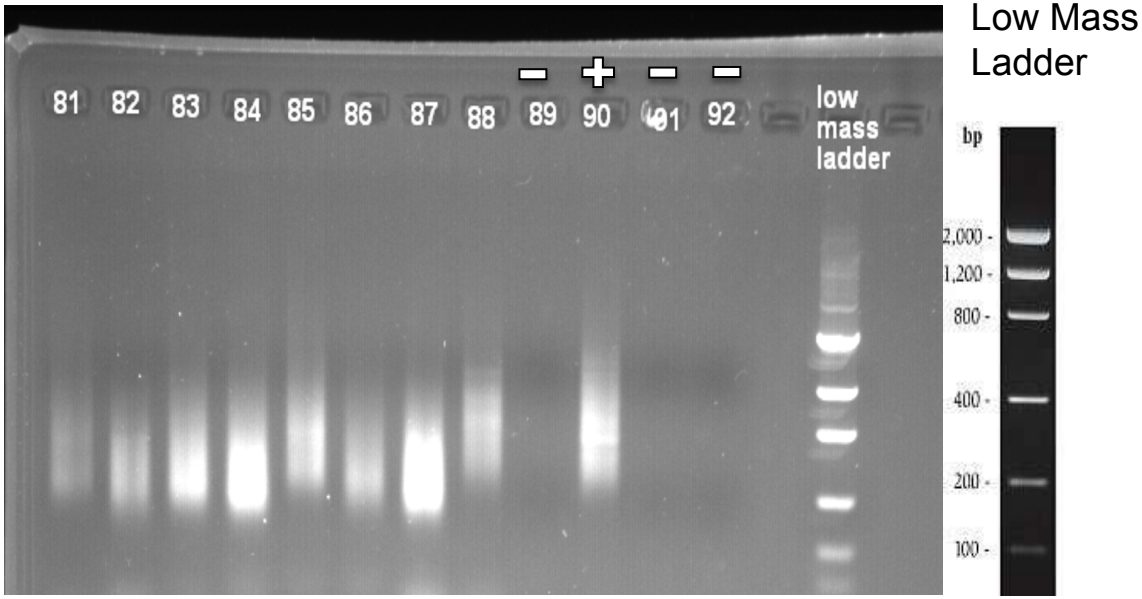
Quality Control

Pool

Sequence

Post PCR Amplification

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

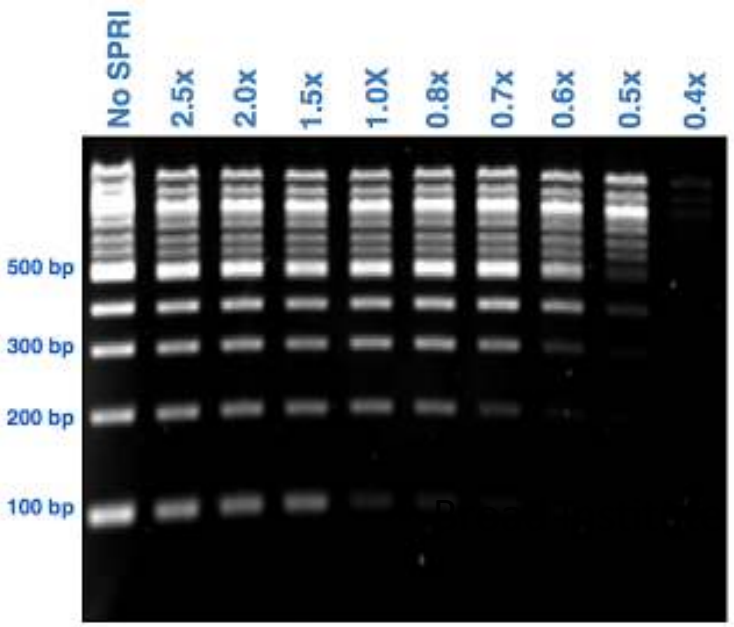


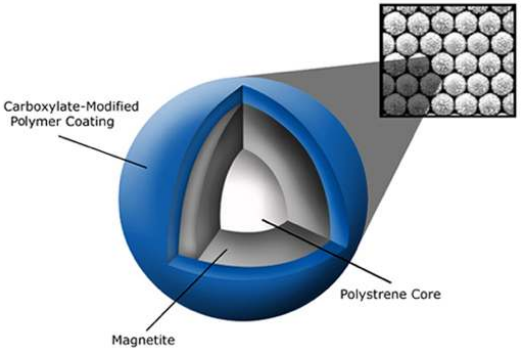
Invitrogen Low Mass Ladder

Beckman Coulter- AmPure Bead

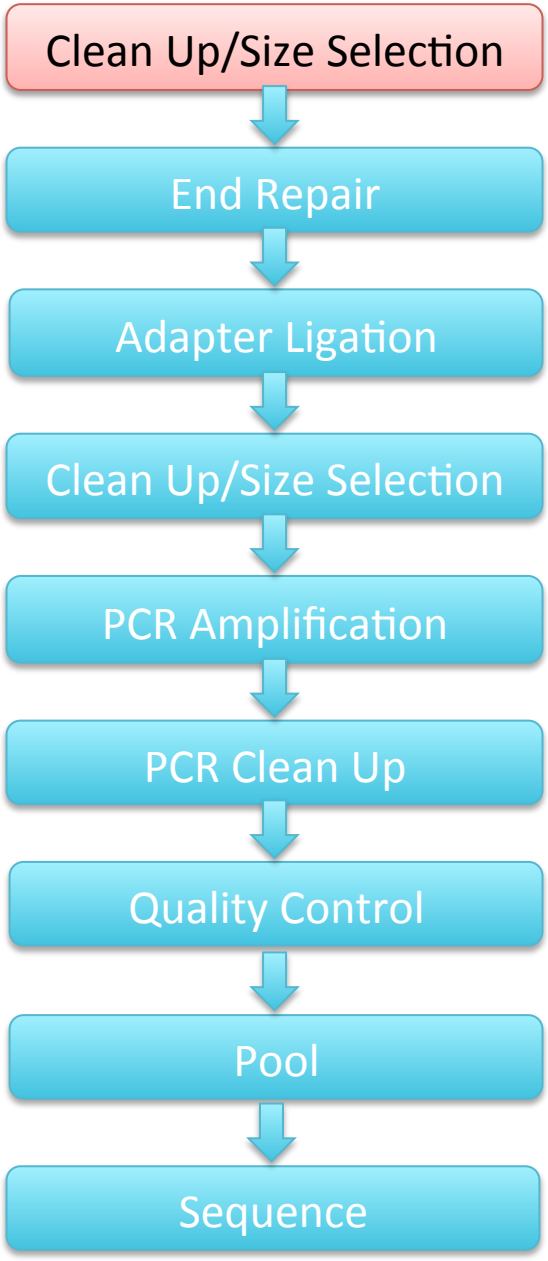
- Clean Up/Size Selection
- End Repair
- Adapter Ligation
- Clean Up/Size Selection
- PCR Amplification
- PCR Clean Up
- Quality Control
- Pool
- Sequence

- SPRI Bead (Solid Phase Reversible Immobilization)
- Uses Paramagentic 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



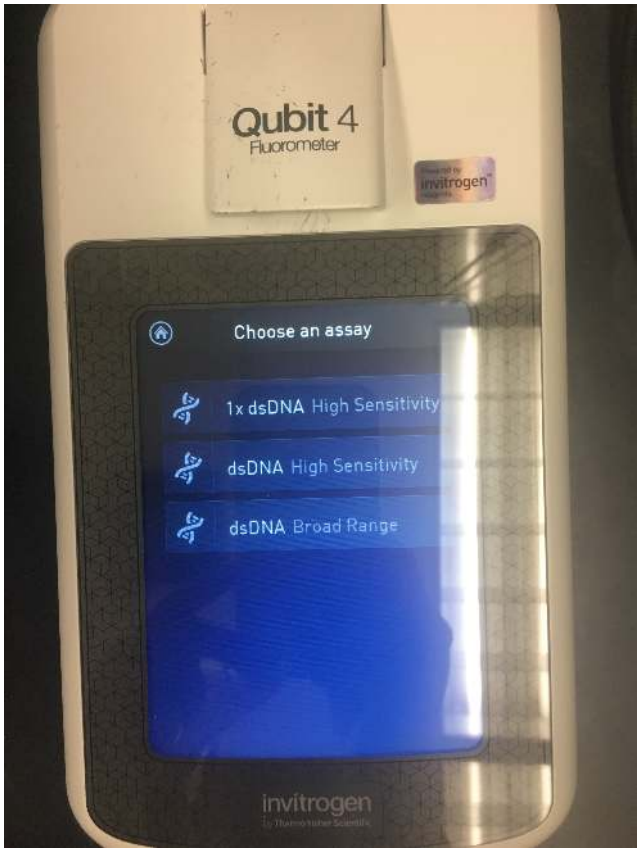


https://youtu.be/zGV0SjCe0CU



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



Clean Up/Size Selection

End Repair

Adapter Ligation

Clean Up/Size Selection

PCR Amplification

PCR Clean Up

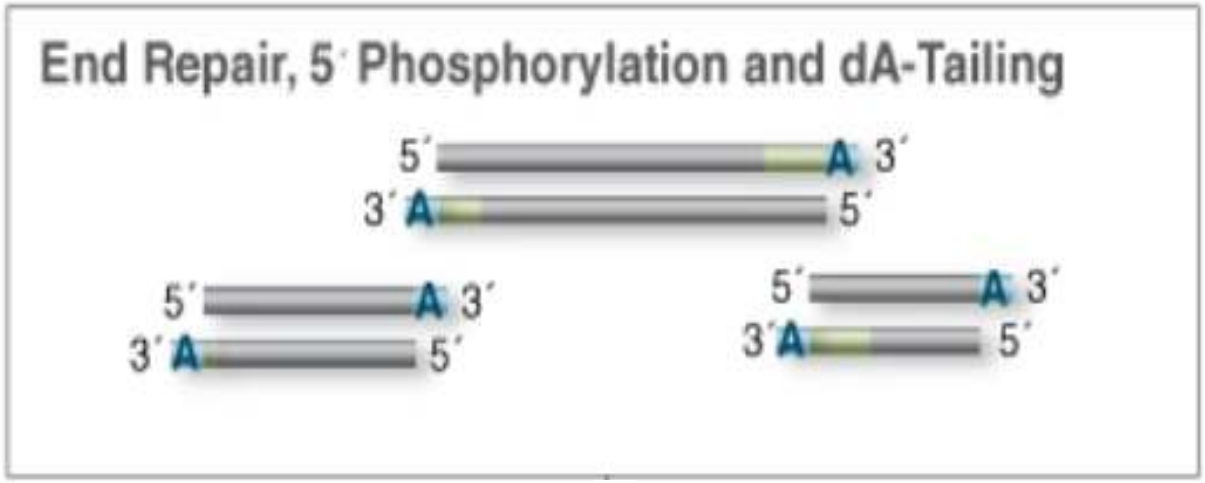
Quality Control

Pool

Sequence

End Repair
5’ Phosphorylation and dA-Tailing

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



Clean Up/Size Selection

End Repair

Adapter Ligation

Clean Up/Size Selection

PCR Amplification

PCR Clean Up

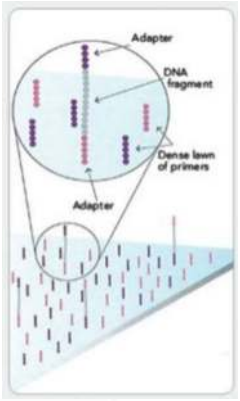
Quality Control

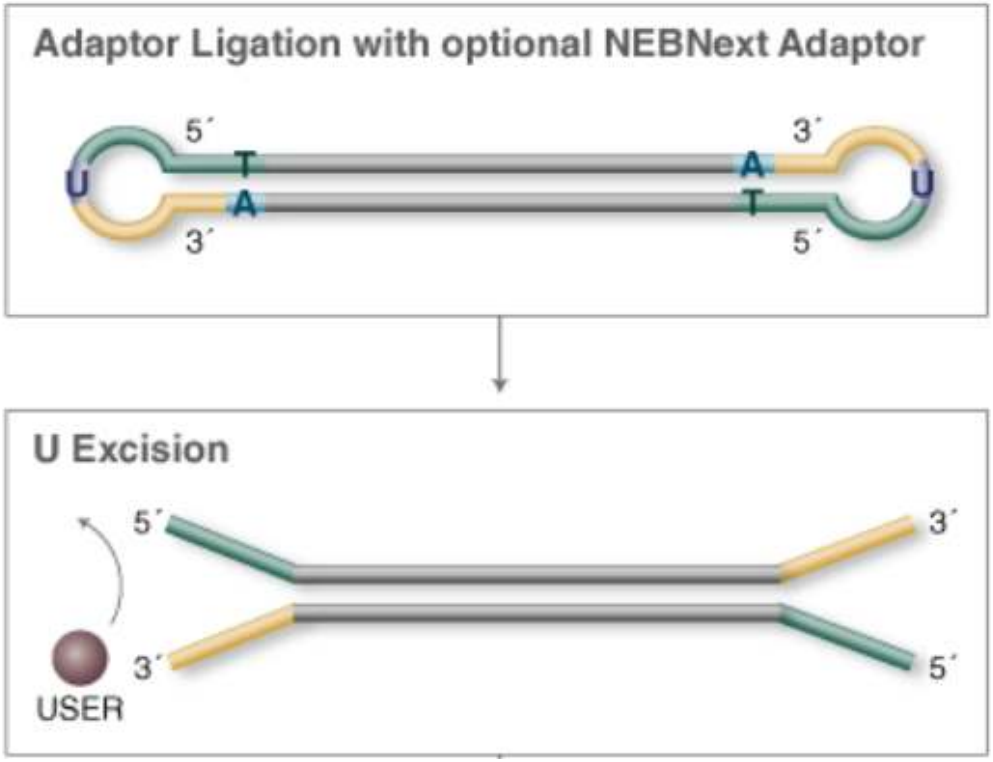
Pool

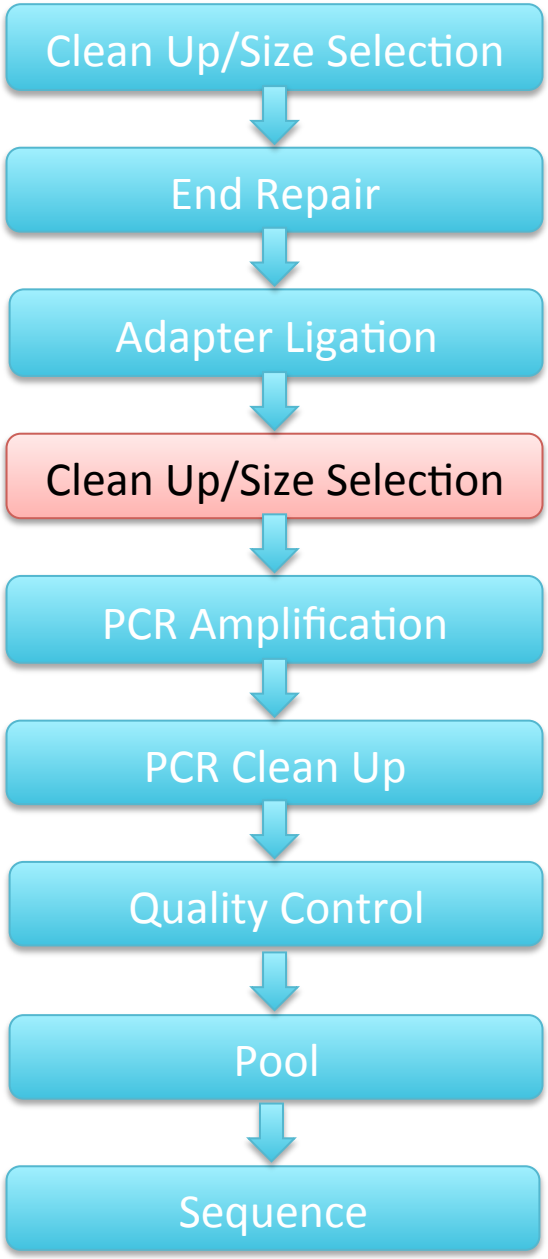
Sequence

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





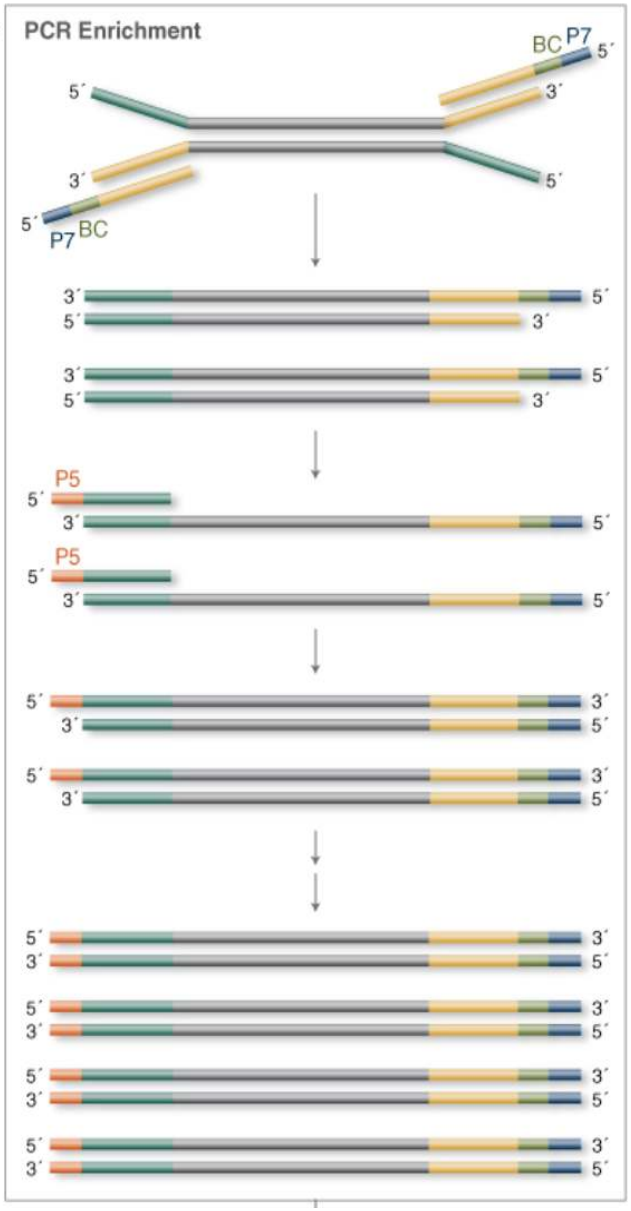


Clean Up- Post Adapter Ligation

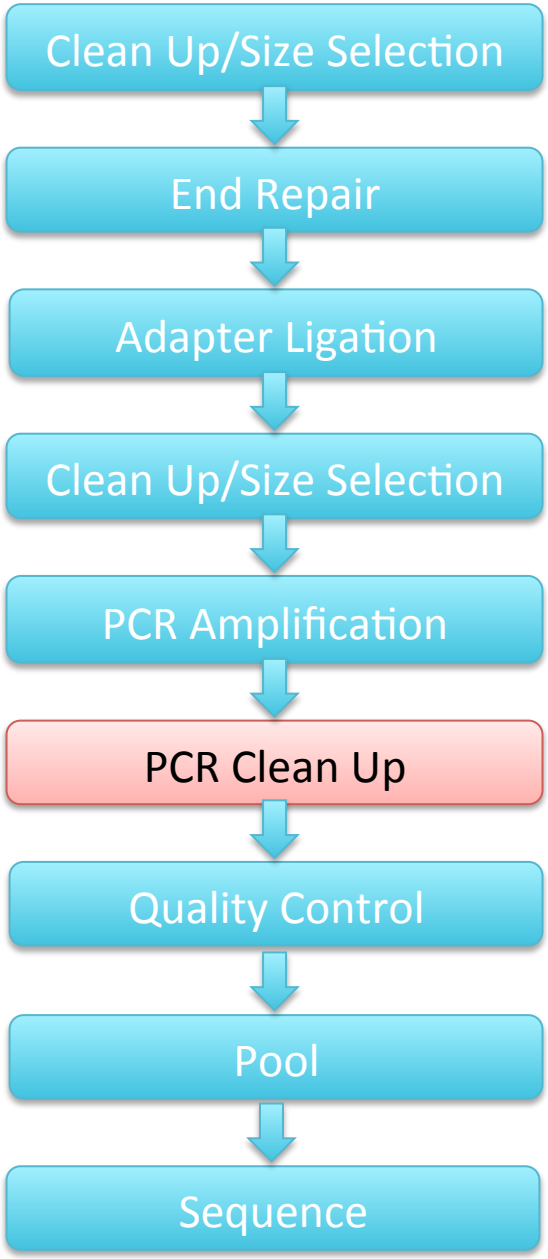
- Size Selection 400-600bp
- Remove unused ligation reaction components, adapter dimers, and concatemers

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



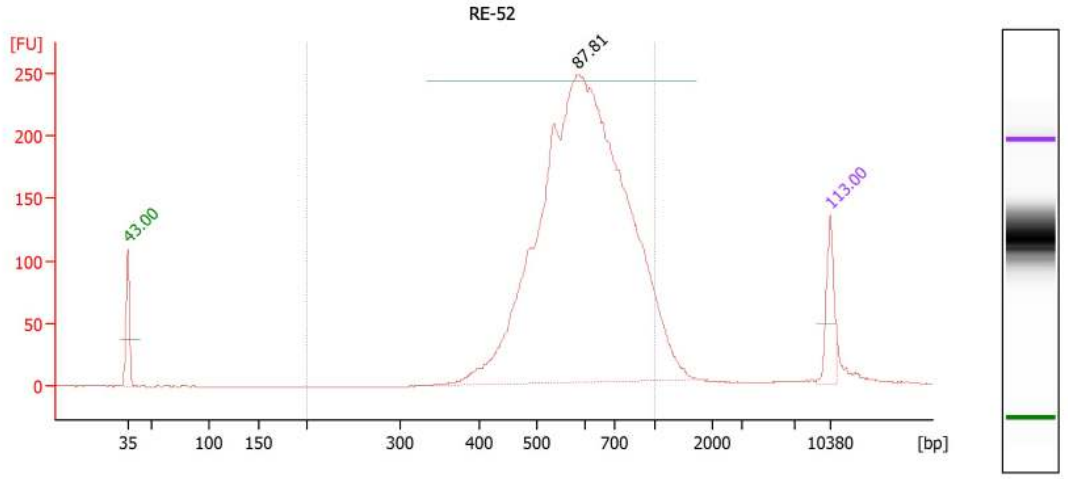
Clean Up- Post PCR Amplification

- 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 Bioanalzyer 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

Clean Up/Size Selection

End Repair

Adapter Ligation

Clean Up/Size Selection

PCR Amplification

PCR Clean Up

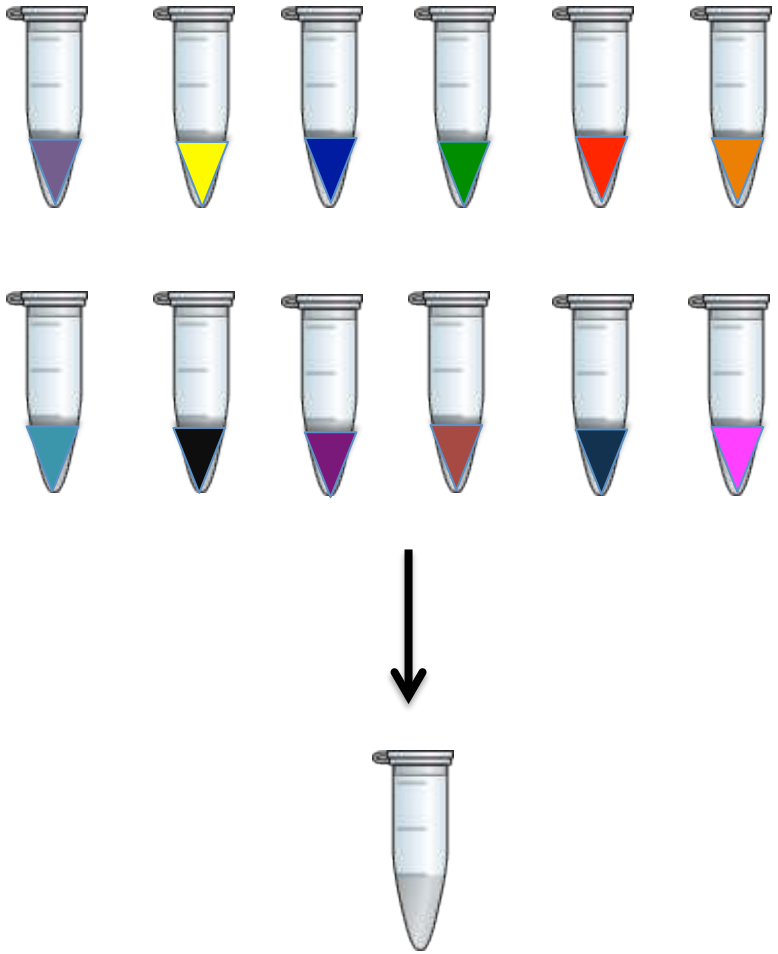
Quality Control

Pool

Sequence

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

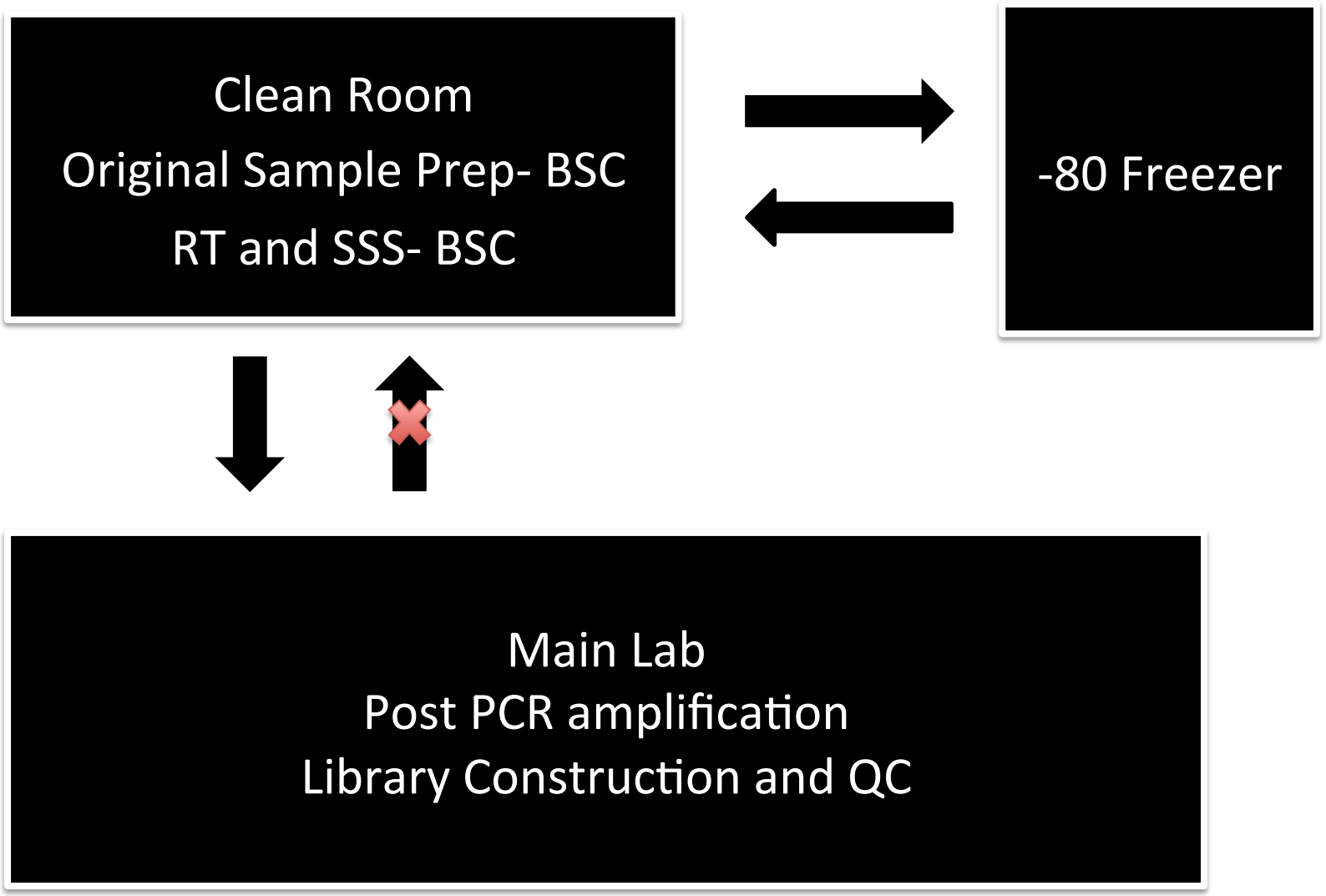
↓

Sequence

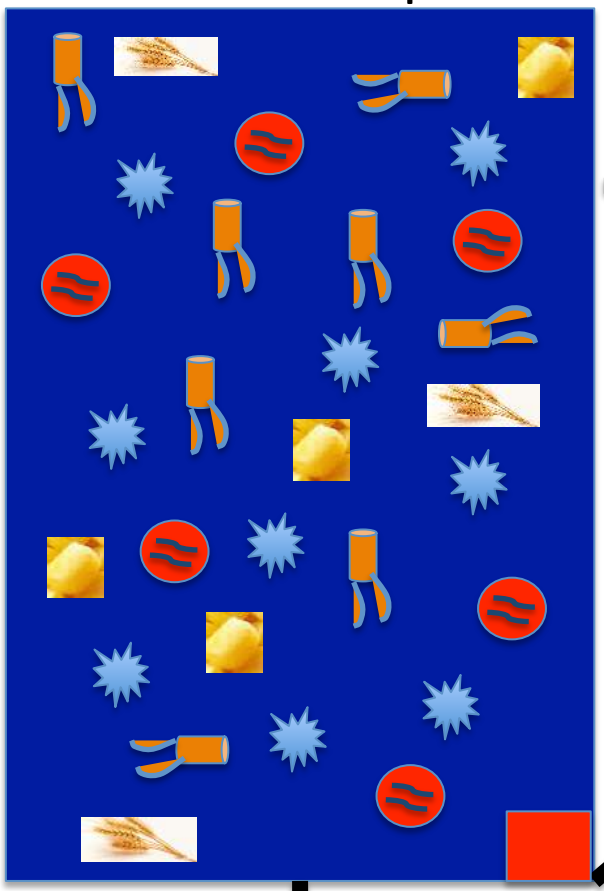
MiSeq V2 2X250

(pick your favorite)

Laboratory Layout



Stool Sample



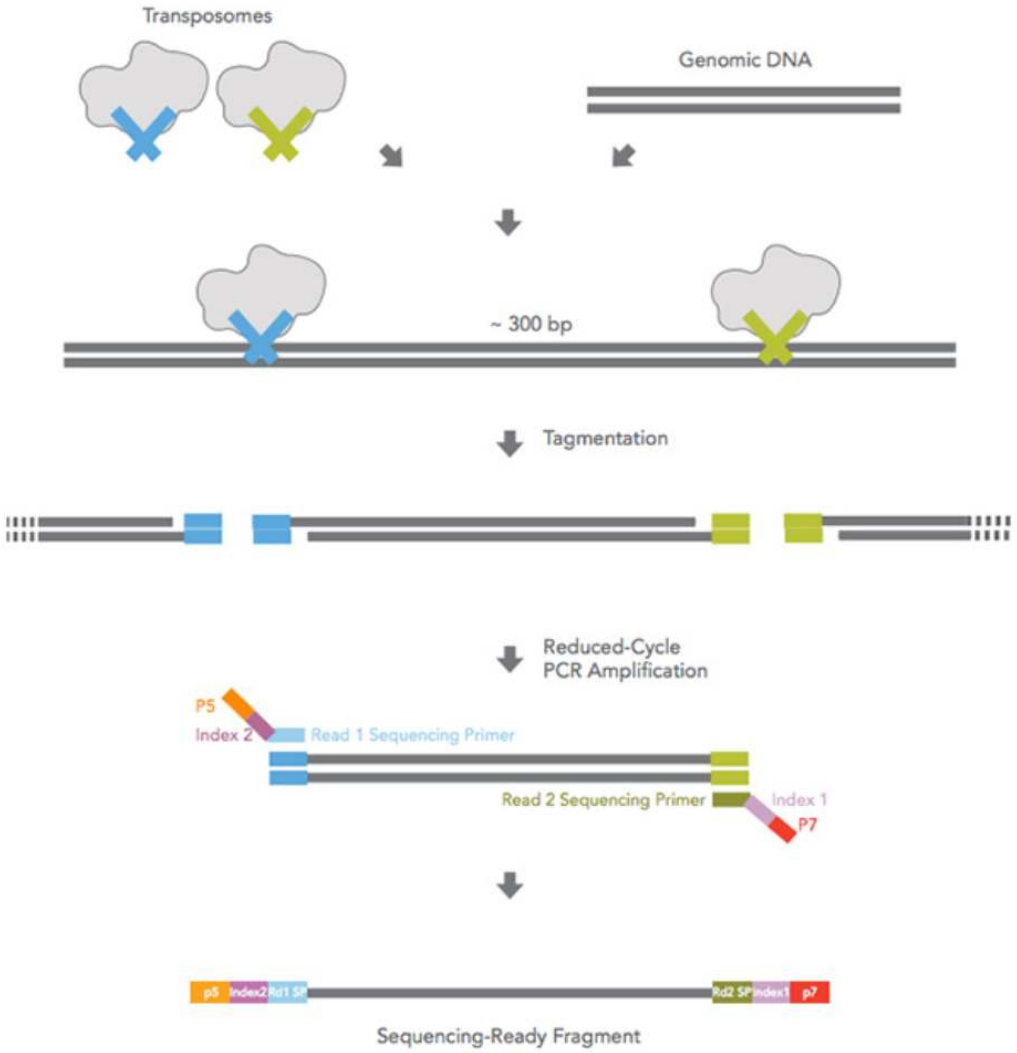
Amplicon Sequencing

16S/18S/ITS
-DNA
-targeted Amplicons

Enriched Metagenome- Virome
Virus Like Particle (VLP)
-DNA, RNA
-remove bacteria
-remove host

Shotgun Sequencing
Kwon Lab- Matt

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

RNAseq for Expression Analysis

A Plethora of Biological Sequence Analyses Enabled by RNA-Seq

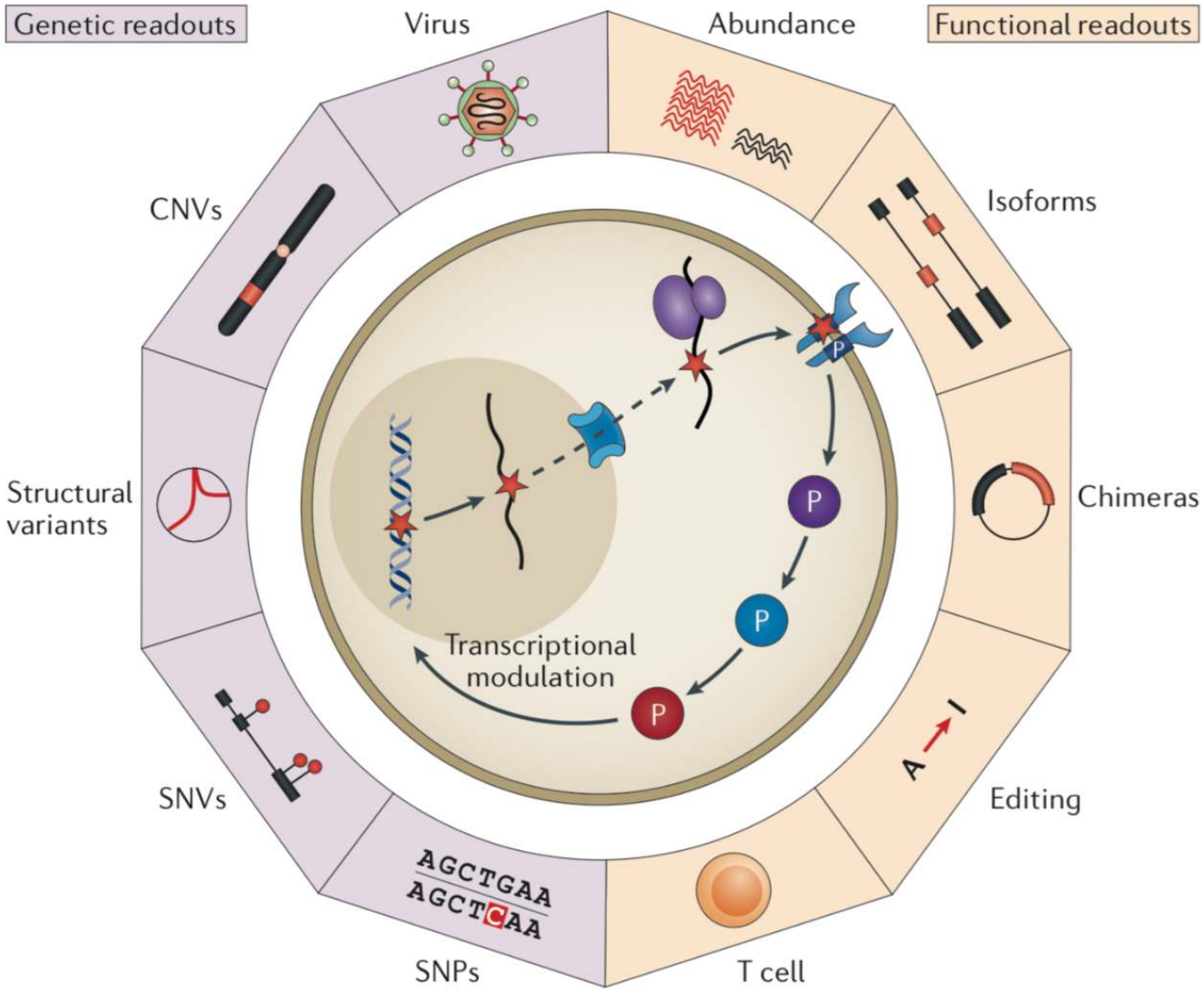
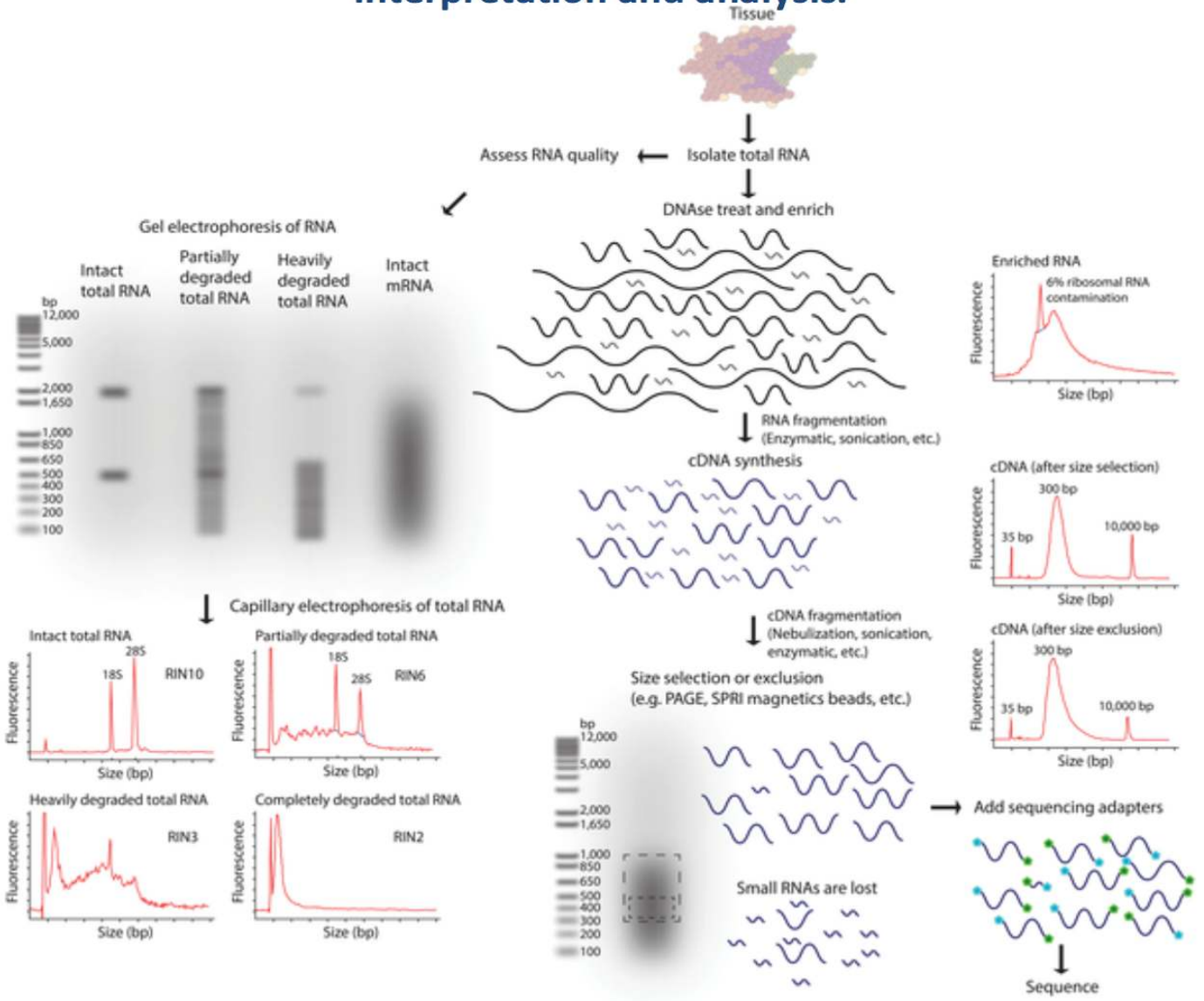


Figure 2 | **Transcriptome profiling for genetic causes and functional phenotypic readouts.**

From Cieslik and Chinnaiyan, NRG, 2017

RNA-seq library fragmentation and size selection strategies that influence interpretation and analysis.



http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393

PLOS | COMPUTATIONAL BIOLOGY

rRNA Depletion/Fragment

Clean Up Size Selection

First Strand cDNA

Second Strand cDNA

Clean Up/Size Selection

Adenylate 3' Ends

Ligate Adpaters

Clean Up/Size Selection

PCR Amplfication

Clean Up/Size Selection

Final Library

Quality Control

Pool

Sequence

Preparing Sample for Transcriptomics Sequencing (RNAseq): 3 steps

1.) Extraction
2.) ribosomal RNA depletion
3.) Library Construction

PCR Workstation

- Vertical Laminar air flow
- Hepa filtration system
- Build in UV
- Reduce Exposure to RNases

rRNA Depletion/Fragment

Clean Up Size Selection

First Strand cDNA

Second Strand cDNA

Clean Up/Size Selection

Adenylate 3' Ends

Ligate Adpaters

Clean Up/Size Selection

PCR Amplfication

Clean Up/Size Selection

Final Library

Quality Control

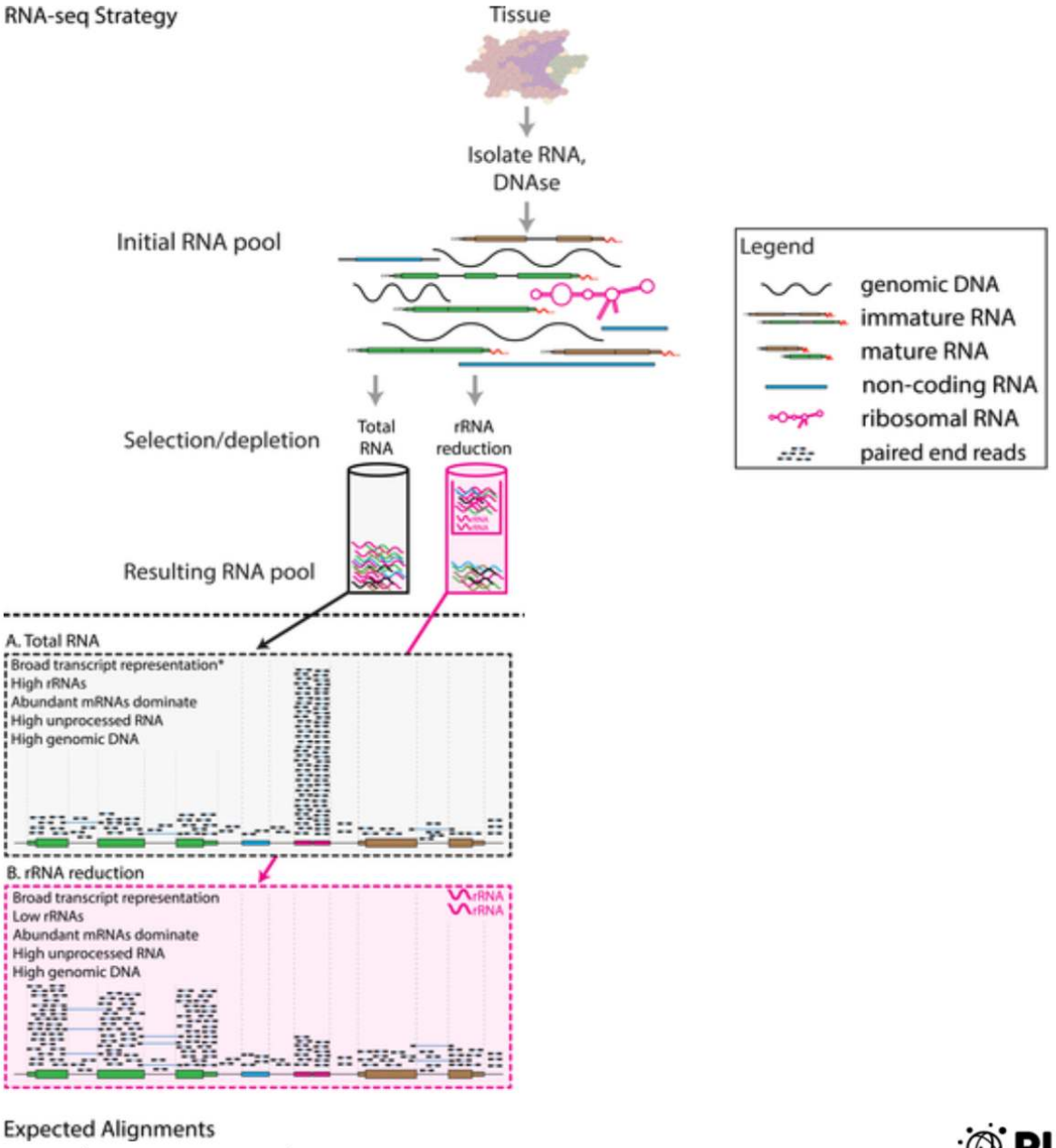
Pool

Sequence

Ribosomal RNA Depletion and Fragmenting

- Ribo Zero: iron core bead with rDNA conserved sequence hybridized to rRNA in sample
- Fragmented 150-300bp and primed for cDNA synthesis

RNA-seq library enrichment strategies that influence interpretation and analysis.



http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393

PLOS | COMPUTATIONAL BIOLOGY

Clean Up-Post Fragmentation

- Select for fragments 150-300bp
- Remove left over components from rRNA depletion

rRNA Depletion/Fragment

Clean Up Size Selection

First Strand cDNA

Second Strand cDNA

Clean Up/Size Selection

Adenylate 3’ Ends

Ligate Adpaters

Clean Up/Size Selection

PCR Amplfication

Clean Up/Size Selection

Final Library

Quality Control

Pool

Sequence

First Strand Synthesis

- Cleaved RNA fragments are copied into cDNA using RT and random primers

rRNA Depletion/Fragment

Clean Up Size Selection

First Strand cDNA

Second Strand cDNA

Clean Up/Size Selection

Adenylate 3’ Ends

Ligate Adpaters

Clean Up/Size Selection

PCR Amplfication

Clean Up/Size Selection

Final Library

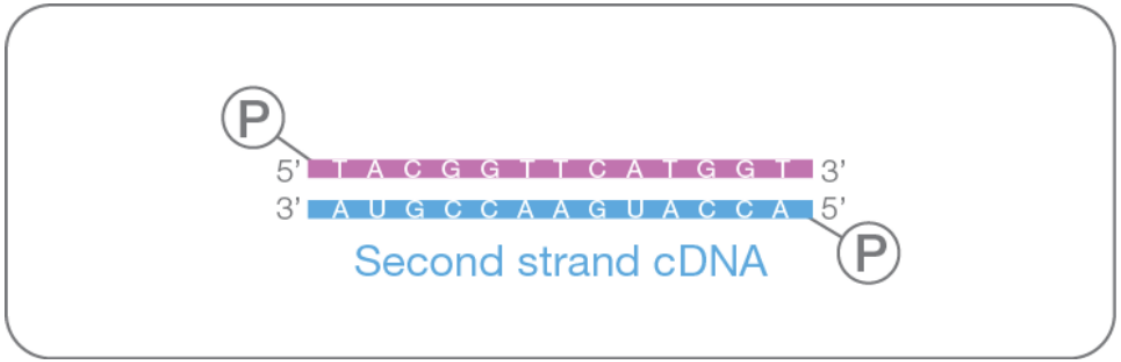
Quality Control

Pool

Sequence

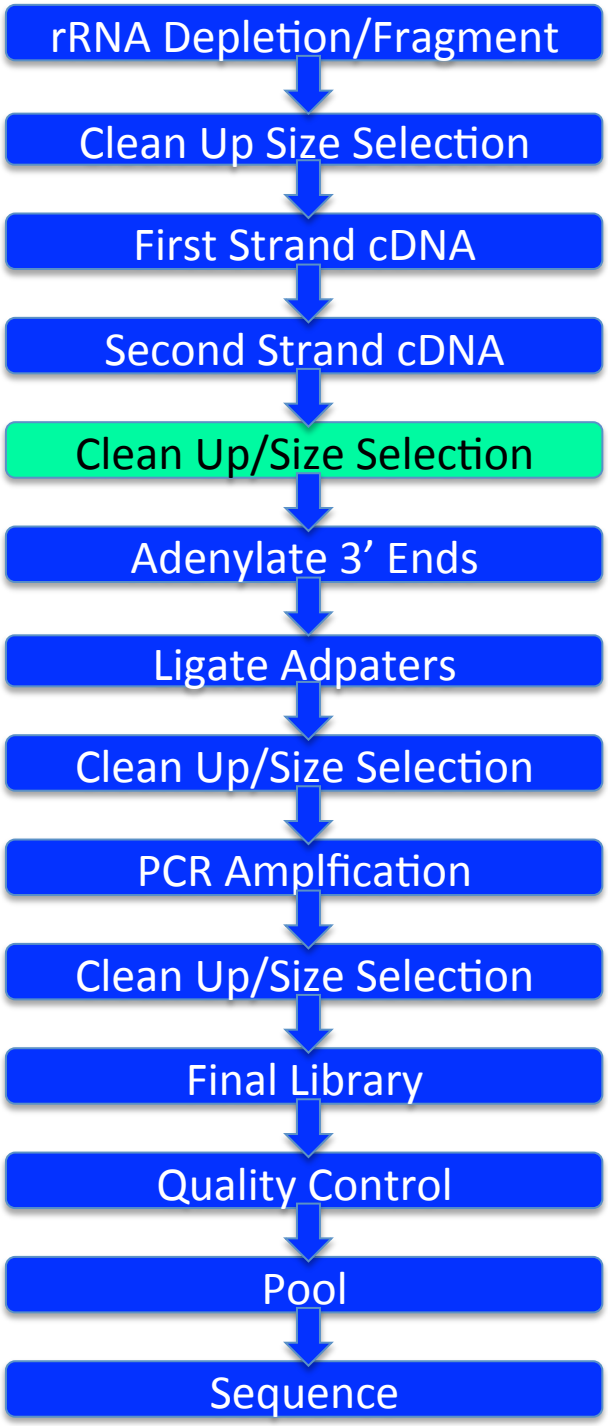
Second Strand Synthesis

- Double stranded cDNA
- Incorporation of dUTP in SSS quenches the second strand during amplification



Strand Specificity

- Important for looking at overlapping genes
- Identification of antisense expression



Clean Up- Post ds cDNA synthesis

- Select for fragments 150-300bp
- Remove left over components from cDNA synthesis

rRNA Depletion/Fragment

Clean Up Size Selection

First Strand cDNA

Second Strand cDNA

Clean Up/Size Selection

Adenylate 3’ Ends

Ligate Adpaters

Clean Up/Size Selection

PCR Amplfication

Clean Up/Size Selection

Final Library

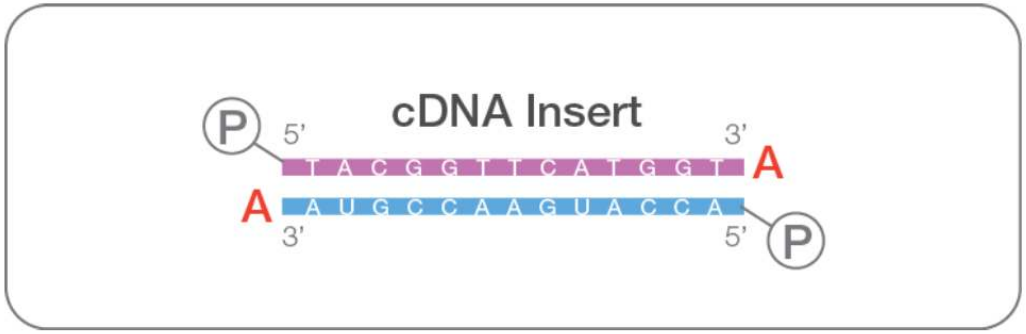
Quality Control

Pool

Sequence

A-tailing

- Single A nucleotide is added to 3’ ends of blunt fragments
- Single T nucleotide on the adapter provides complementary overhang for ligating the adapter



rRNA Depletion/Fragment

Clean Up Size Selection

First Strand cDNA

Second Strand cDNA

Clean Up/Size Selection

Adenylate 3’ Ends

Ligate Adpaters

Clean Up/Size Selection

PCR Amplfication

Clean Up/Size Selection

Final Library

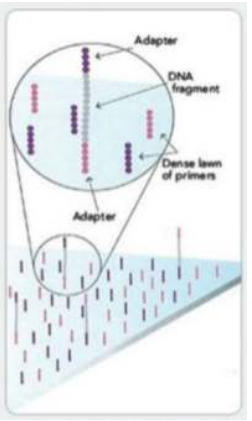
Quality Control

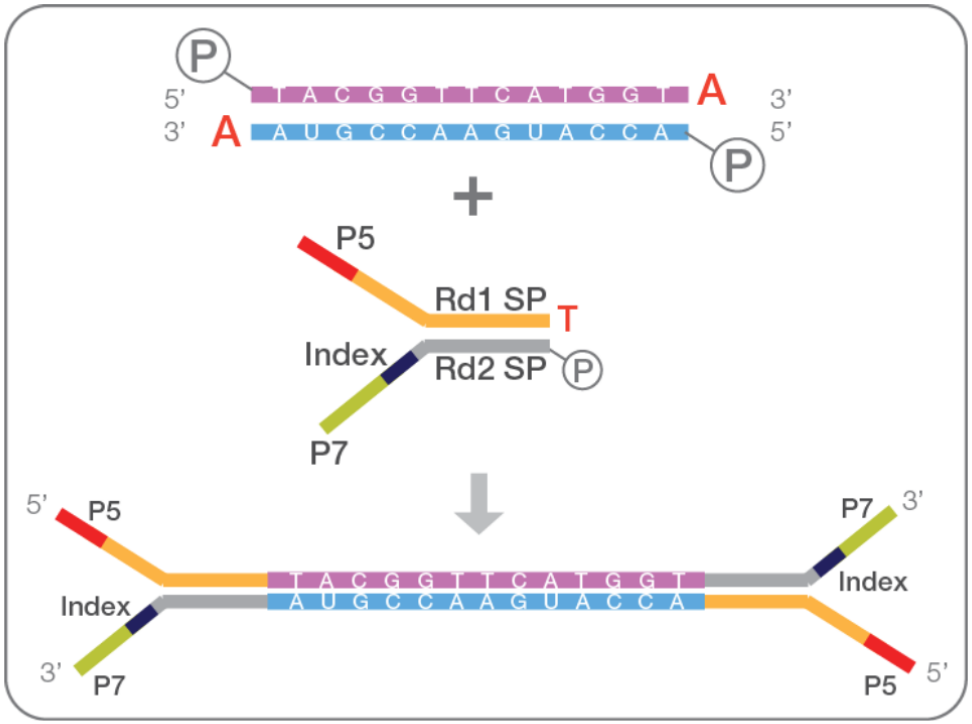
Pool

Sequence

Adapter Ligation

- P5 and P7 are used for amplification
- Barcodes are part of adapter





Clean Up- Post Adapter Ligation

- Size Selection 150-300bp
- Remove unused ligation reaction components, adapter dimers, and concatemers

rRNA Depletion/Fragment

Clean Up Size Selection

First Strand cDNA

Second Strand cDNA

Clean Up/Size Selection

Adenylate 3’ Ends

Ligate Adpaters

Clean Up/Size Selection

PCR Amplfication

Clean Up/Size Selection

Final Library

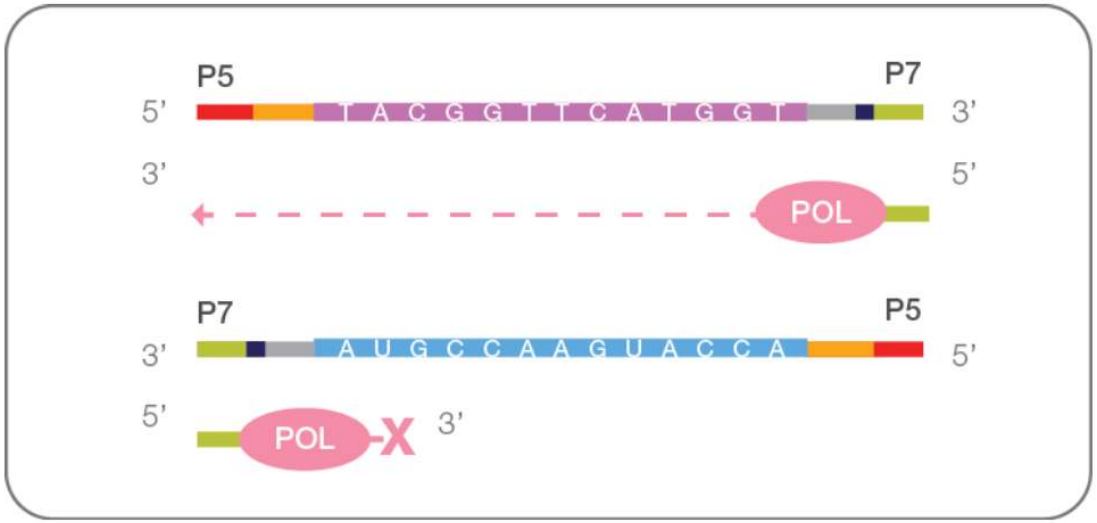
Quality Control

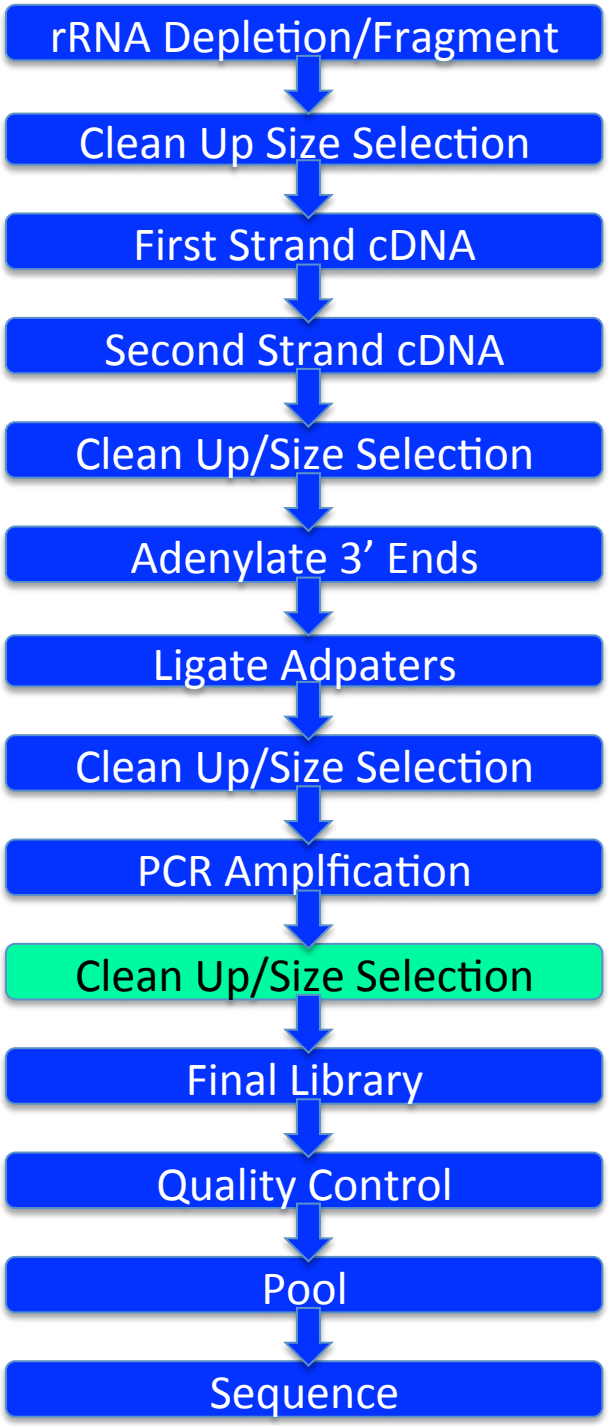
Pool

Sequence

PCR Amplification

- Polymerase does not incorporate past dUTP. Second strand is quenched during amplification.
- Products enriched with PCR amplification.





Clean Up- Post PCR Amplification

- Remove free barcodes, nucleotides
- Remove adapter dimers

rRNA Depletion/Fragment

Clean Up Size Selection

First Strand cDNA

Second Strand cDNA

Clean Up/Size Selection

Adenylate 3’ Ends

Ligate Adpaters

Clean Up/Size Selection

PCR Amplfication

Clean Up/Size Selection

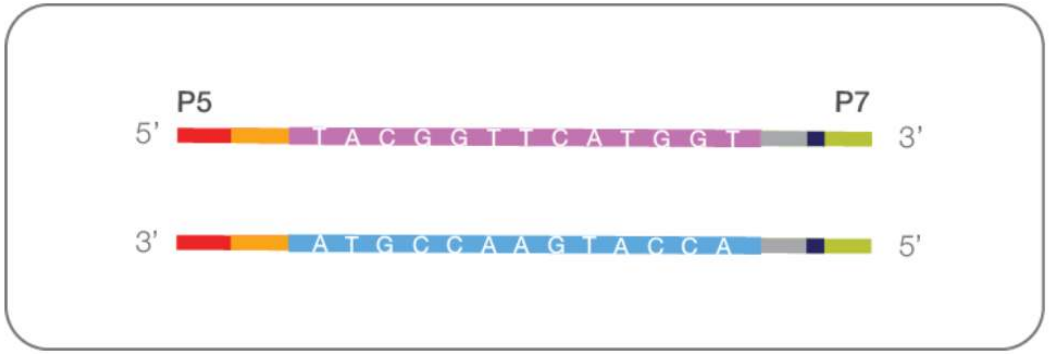
Final Library

Quality Control

Pool

Sequence

Final Library



rRNA Depletion/Fragment

Clean Up Size Selection

First Strand cDNA

Second Strand cDNA

Clean Up/Size Selection

Adenylate 3' Ends

Ligate Adpaters

Clean Up/Size Selection

PCR Amplfication

Clean Up/Size Selection

Final Library

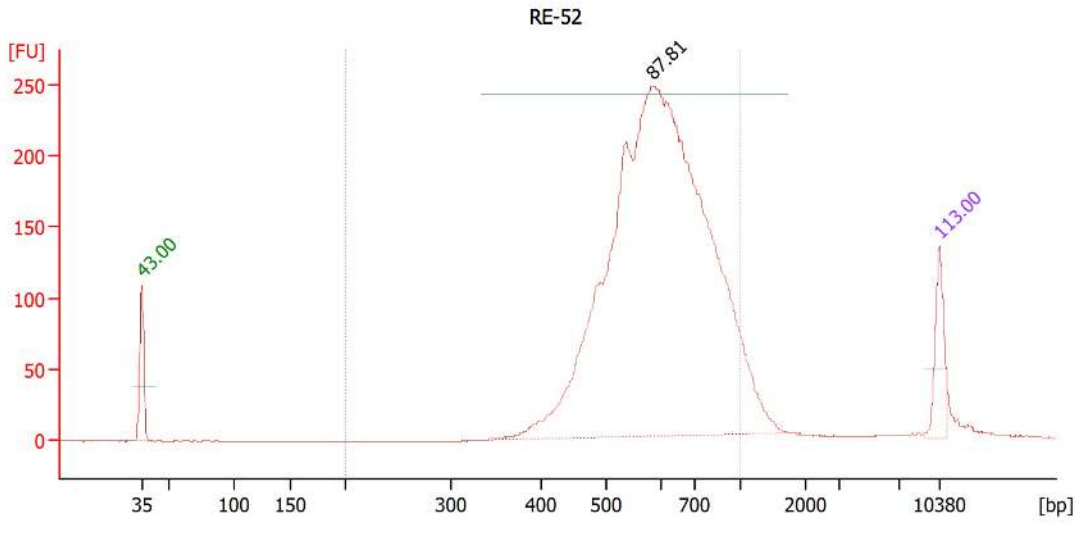
Quality Control

Pool

Sequence

Quality Control

- Agilent Bioanalzyer 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

rRNA Depletion/Fragment

Clean Up Size Selection

First Strand cDNA

Second Strand cDNA

Clean Up/Size Selection

Adenylate 3' Ends

Ligate Adpaters

Clean Up/Size Selection

PCR Amplfication

Clean Up/Size Selection

Final Library

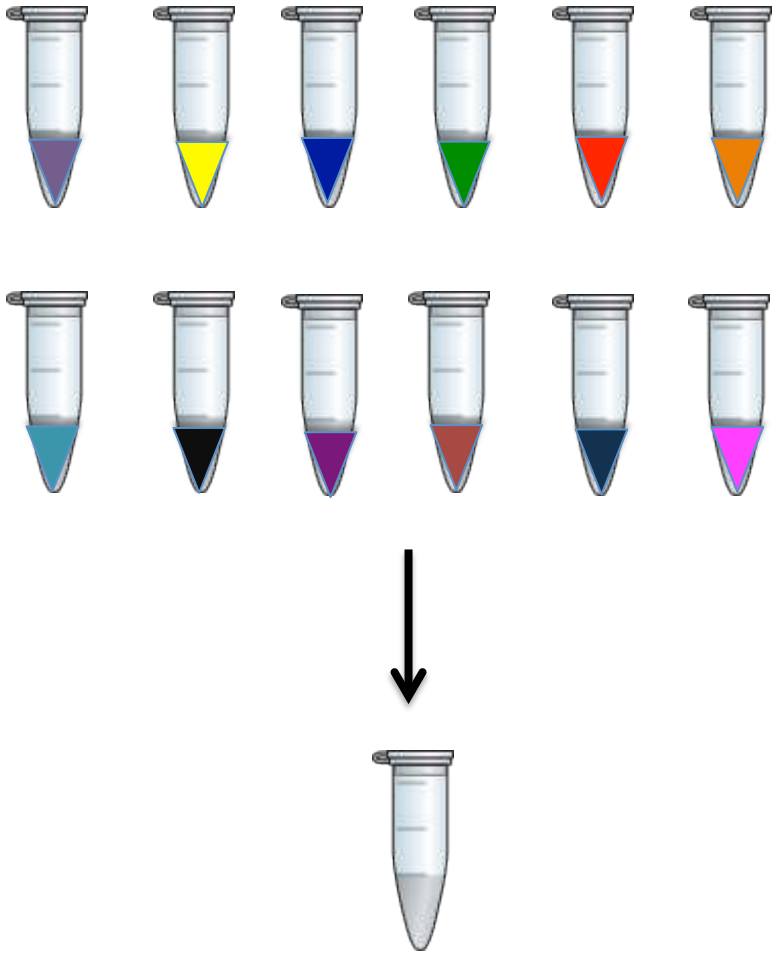
Quality Control

Pool

Sequence

Pool Final Libraries

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



rRNA Depletion/Fragment

Clean Up Size Selection

First Strand cDNA

Second Strand cDNA

Clean Up/Size Selection

Adenylate 3’ Ends

Ligate Adpaters

Clean Up/Size Selection

PCR Amplfication

Clean Up/Size Selection

Final Library

Quality Control

Pool

Sequence

Illumina HiSeq 2500 2X101

(pick your favorite)

How much will my experiment cost?

Generating RNA-Seq: *How to Choose?*

Platform	Project Firefly 2018	MiniSeq	MiSeq	Next Seq 550	HiSeq 2500 RR	Hiseq 2500 V3	HiSeq 2500 V4	HiSeq 4000	HiSeq X	Nova Seq S1 2018	Nova Seq S2	Nova Seq S4	5500 XL	318 HiQ 520	Ion 530	Ion Proton P1	PGM HiQ 540	RS P6-C4	Sequel	R&D end 2018	Smidg ION RnD	Mini ION R9.5	Grid ION X5	Prome thION RnD	Prome thION theor etical	QiaGen Gene Reader	BGI SEQ 500	BGI SEQ 50	#
Reads: (M)	4	25	25	400	600	3000	4000	5000	6000	3300	6600	20000	1400	3-5	15-20	165	60-80	5.5	38.5	--	--	--	--	--	--	400	1600	1600	--
Read length: (paired-end*)	150*	150*	300*	150*	100*	100*	125*	150*	150*	150*	150*	150*	60	200 400	200 400	200	200	15K	12K	32K	--	--	--	--	--	--	100*	50	--
Run time: (d)	0.54	1	2	1.2	1.125	11	6	3.5	3	1.66	1.66	1.66	7	0.37	0.16	--	0.16	4.3	--	--	--	2	2	2	--	--	1	0.4	--
Yield: (Gb)	1	7.5	15	120	120	600	1000	1500	1800	1000	2000	6000	180	1.5	7	10	12	12	5	150	4	8	40	2400	11000	80	200	8	--
Rate: (Gb/d)	1.85	7.5	7.5	100	106.6	55	166	400	600	600	1200	3600	30	5.5	50	--	93.75	2.8	--	--	--	4	20	1200	5500	--	200	20	--
Reagents: ($K)	0.1	1.75	1	5	6.145	23.47	29.9	--	--	--	--	--	10.5	0.6	--	1	1.2	2.4	--	1	--	0.5	1.5	--	--	0.5	--	--	--
per-Gb: ($)	100	233	66	50	51.2	39.1	31.7	20.5	7.08	18	15	5.8	58.33	--	--	100	--	200	80	6.6	--	62.5	37.5	20	4.3	--	--	--	--
hg-30x: ($)	12000	28000	8000	5000	6144	4692	3804	2460	849.6	1800	1564	700	7000	--	--	12000	--	24000	9600	1000	--	7500	4500	2400	500	--	600	--	
Machine: ($)	30K	49.5K	99K	250K	740K	690K	690K	900K	1M	999K	999K	999K	595K	50K	65K	243K	242K	695K	350K	350K	--	--	125K	75K	75K	--	200K	--	--
#Page maintained by http://twitter.com/albertvilella http://tinyurl.com/ngslytics #Editable version: http://tinyurl.com/ngsspecsshared																													
#curl "https://docs.google.com/spreadsheets/d/1GMMfhyLK0-q8Xklo3YxlWaZA5vVMuhU1kg41g4xLkXc/export?gid=4&format=csv" | grep -v '^#' | grep -v '^"' | column -t -s\, | less -S																													

*Not all shown at scale

Brian Haas, Broad

16S/ITS- $18/sample
Virome- $250/sample
Transcriptomics RNAseq- $390/sample

Thank You!

Handley Lab
Scott Handley
Chandni Desai
Barry Hykes
Alan Catalano

Holtz Lab
Lori Holtz
Cindy Rodriquez

Wang Lab
Dave Wang
Susan Vernon

Center for Genome Sciences
Jessica Hoisington-Lopez

Skip Virgin