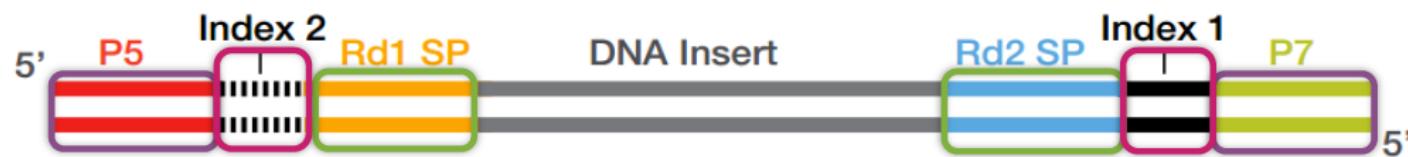
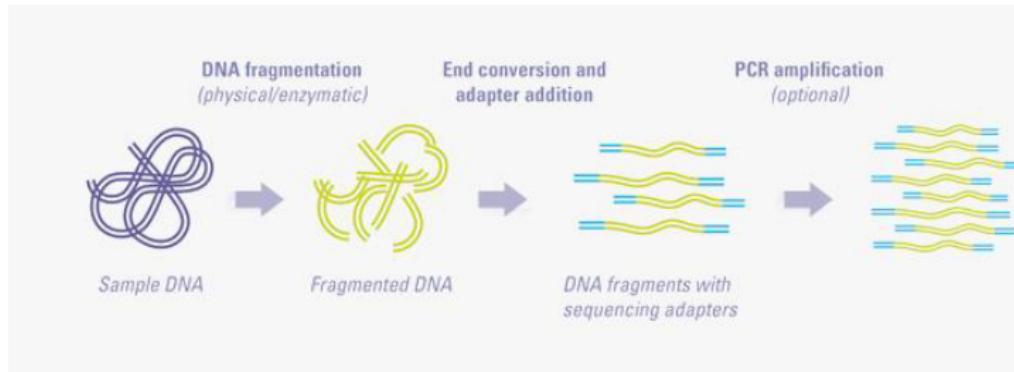


Library Prep is Critical for Successful Sequencing



For clustering:
Libraries must have P5 and P7 binding regions on either end of a library

For sequencing:
Libraries must have sequencing primer binding regions

For mixing samples:
Libraries must have a unique index or barcodes sequence

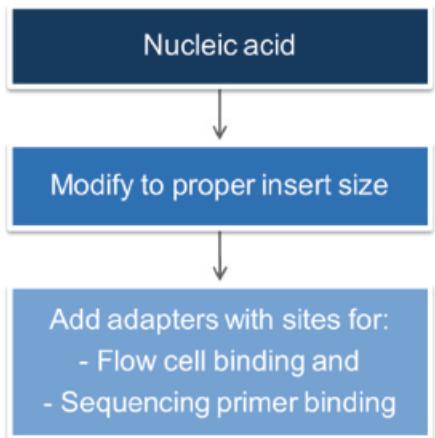
illumina®

Illumina Library Prep Training Course:

- ✓ **Nextera XT Sample Preparation Course**
- ✓ **Truseq DNA PCR-Free Sample Preparation Course**
- ✓ **Truseq Amplicon Sample Preparation Course**
- ✓ **Truseq Stranded RNA Sample Preparation Course
(Includes Total RNA or mRNA)**

✓ Truseq Stranded RNA Sample Preparation Course (Includes Total RNA or mRNA)

Library Prep Overview



Same general template architecture regardless of application

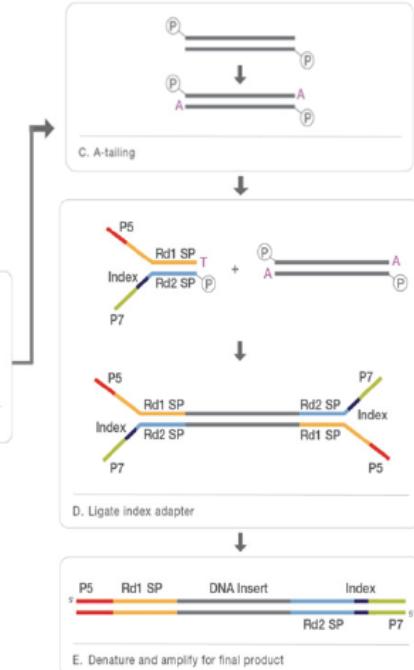
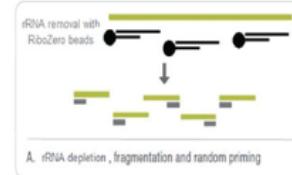
FOR RESEARCH USE ONLY

TruSeq Stranded RNA Workflows

TruSeq Stranded mRNA



TruSeq Stranded Total RNA

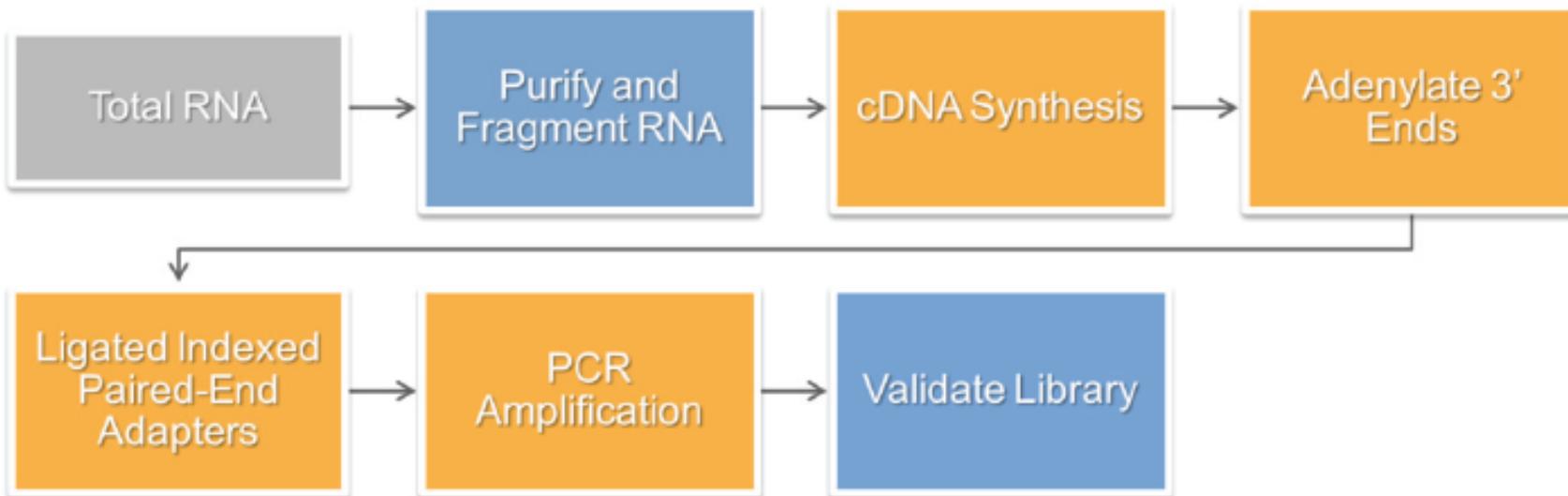


FOR RESEARCH USE ONLY

Key Steps: TruSeq Stranded RNA

- ✓ Check RNA quality on Bioanalyzer

- ✓ Use RNA best practices to avoid degradation



- ✓ Quantify by qPCR
- ✓ Quality & Size on Bioanalyzer

Recommended RNA quality check and quantification methods:



Qubit



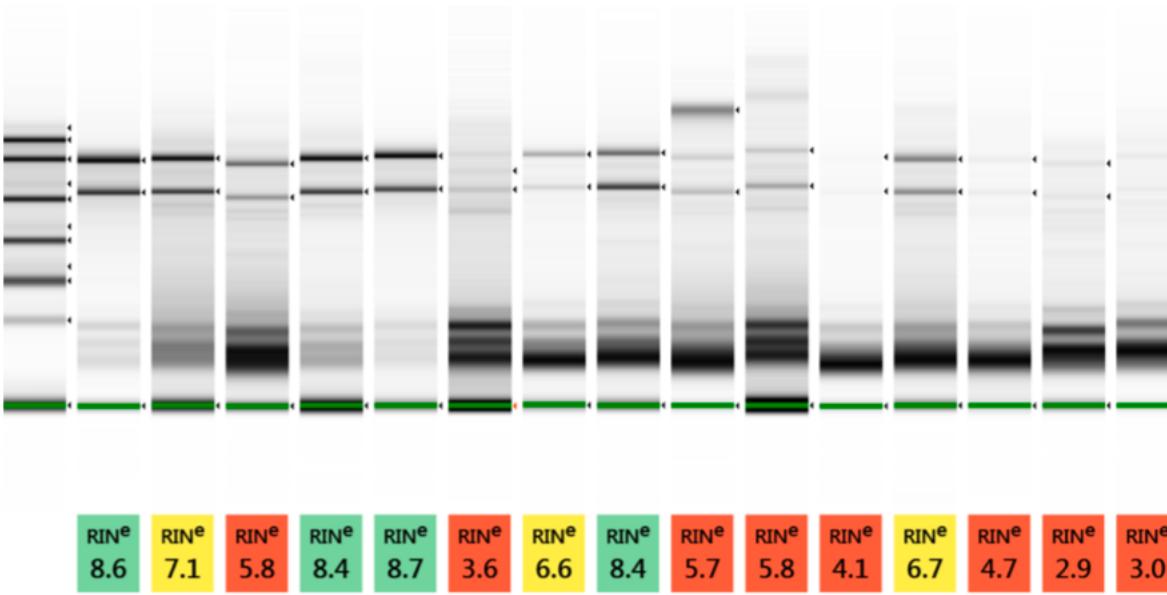
Bioanalyzer / TapeStation

gDNA -1 ug

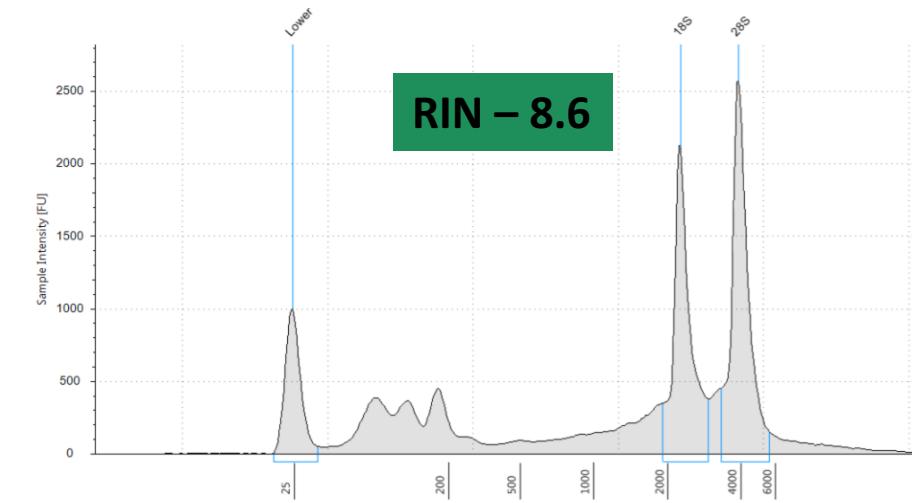
RNA – 0.5-1.0 ug

Sample Prep Method	Input Recommendations	Quality Requirements
TruSeq Stranded mRNA	0.1 - 4 μ g total RNA	High quality; RIN ≥ 8
TruSeq Stranded Total RNA	0.1 - 1 μ g total RNA	Degraded RNA can be used
TruSeq RNA v2	0.1 - 4 μ g total RNA	High quality; RIN ≥ 8

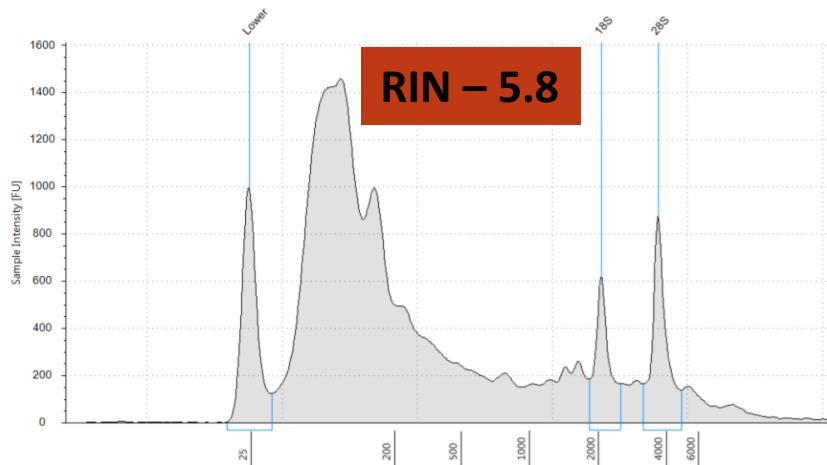
Check RNA quality on Bioanalyzer / TapeStation



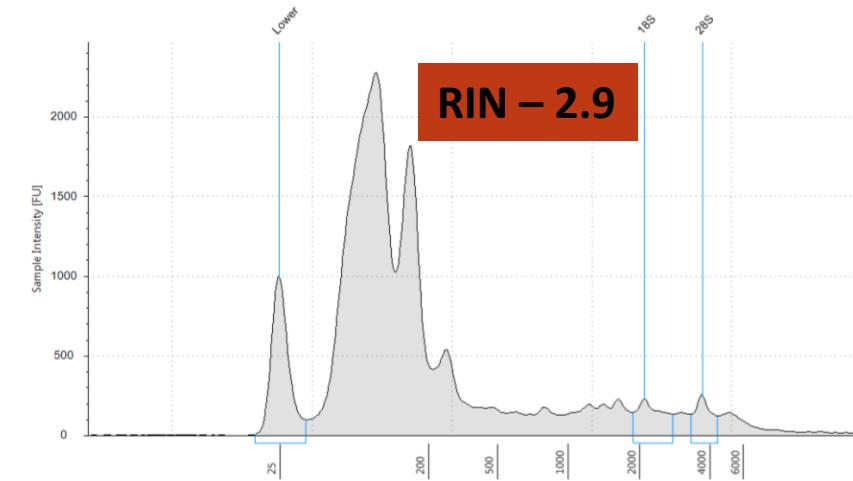
Control- Heat shock RNA sample



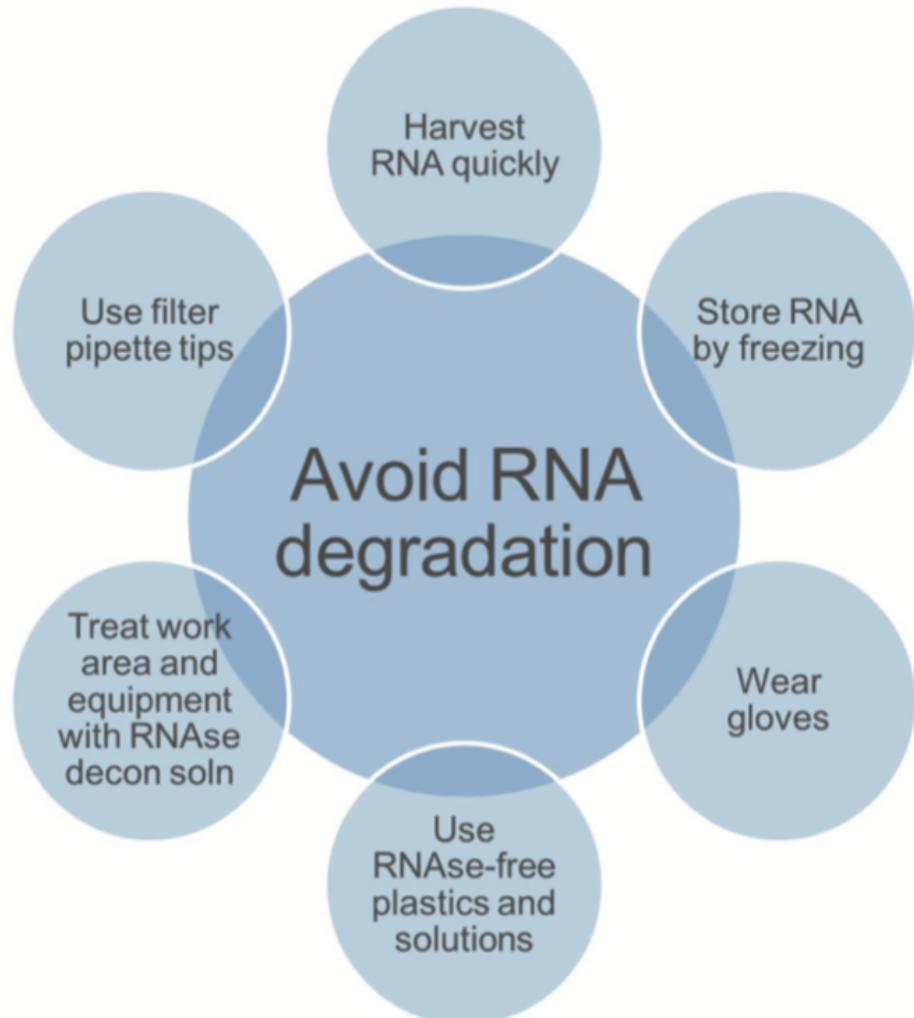
D1: NF54 Rep 1 T24



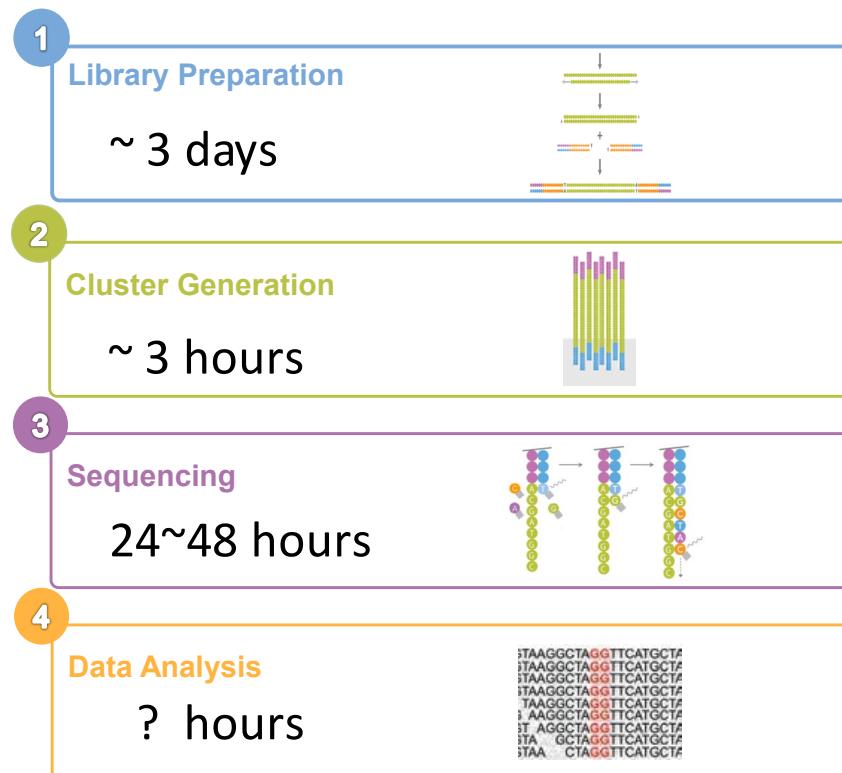
G2: PB52 Rep 1 T36_1



RNA Handling Best Practices

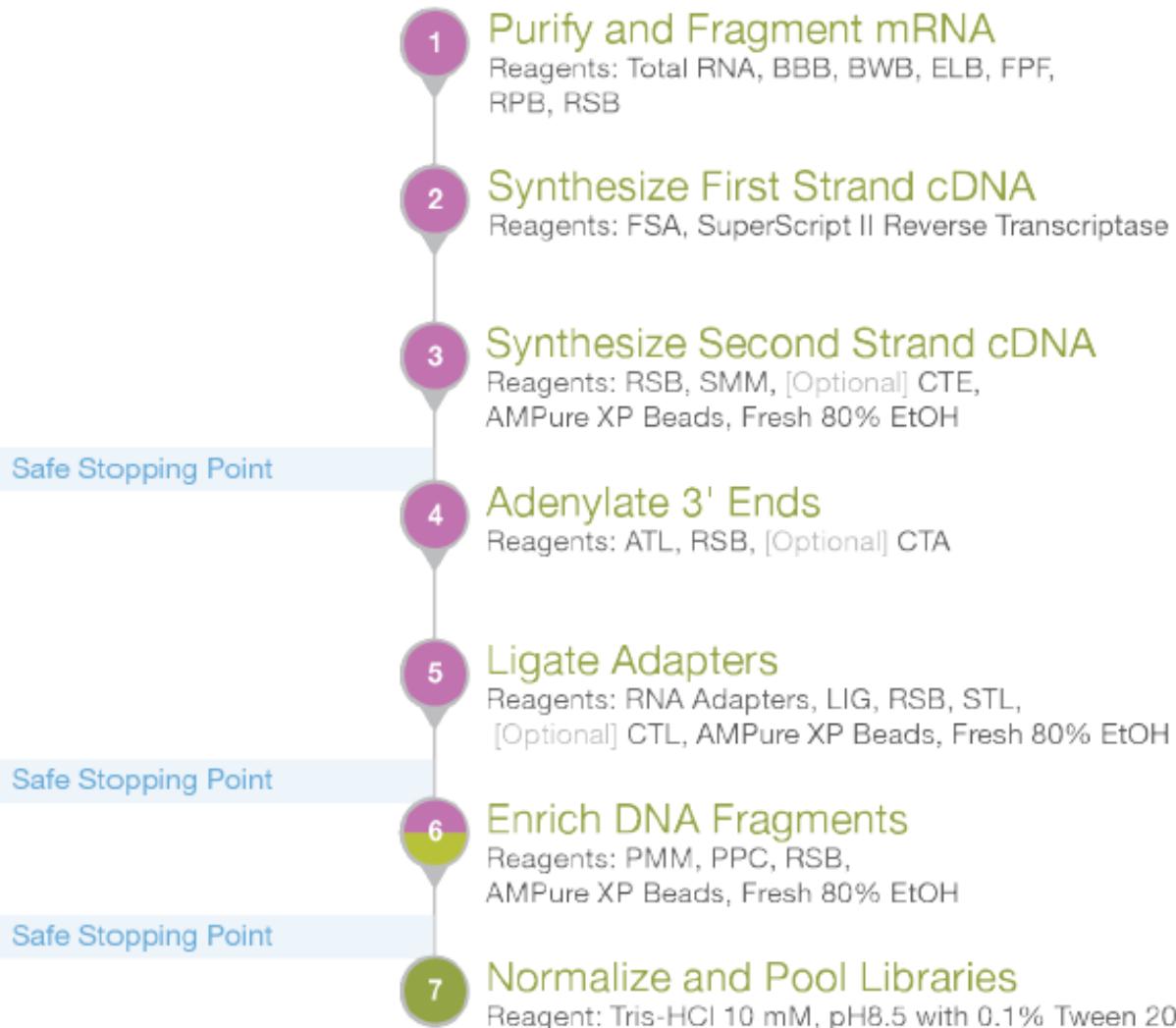


Illumina Sequencing Workflow



Library Prep Workflow

Figure 11 TruSeq Stranded mRNA Library Prep Workflow



(1) Library Construction for Next-Gen Sequencing

Day 1 & Day 2

- A. Poly-A selection, fragmentation and random priming
- B. First and second strand synthesis
- C. A-tailing
- D. Ligate index adapter
- E. Denature and amplify for final product

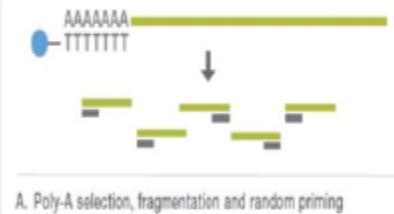
(2) Library quantification, normalization and pooling

Day 3

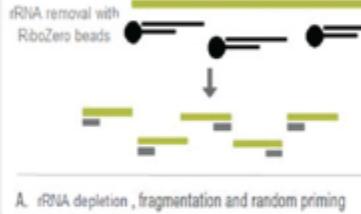
- A. Library quantification
- B. Normalization
- C. Pooling

TruSeq Stranded RNA Workflows

TruSeq Stranded mRNA



TruSeq Stranded Total RNA



FOR RESEARCH USE ONLY

(1) Library Construction for Next-Gen Sequencing

Day 1 Library Prep

- Poly-A selection, fragmentation and random priming
- First and second strand synthesis

Day 2 Library Prep

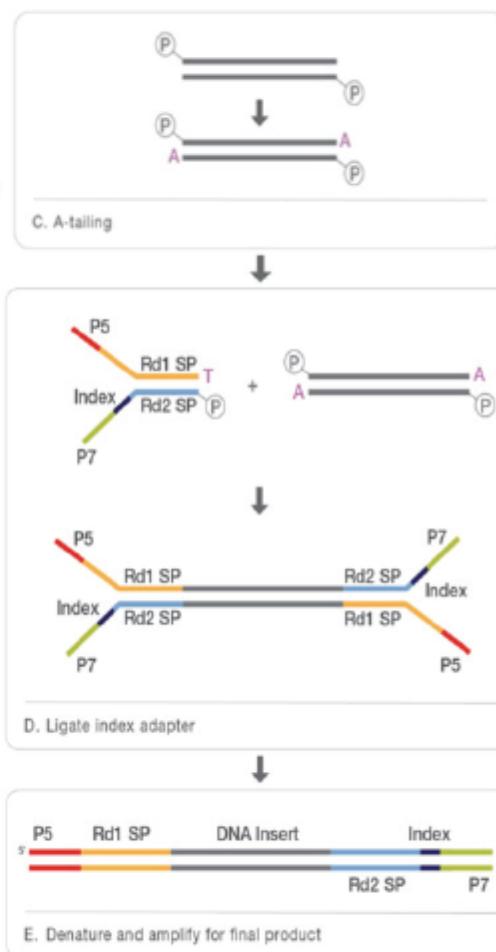
- A-tailing
- Ligate index adapter
- Denature and amplify for final product

Day 3 Library Validation

- Library quantification
- Normalization
- Pooling

Day 4 Sequencing

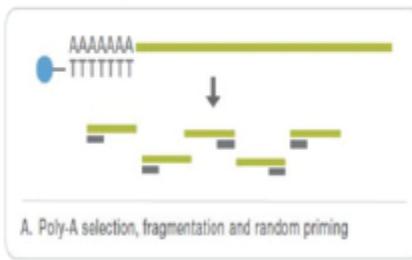
- Denature and dilute pooled library
- Create sample sheet
- Sequencing set up



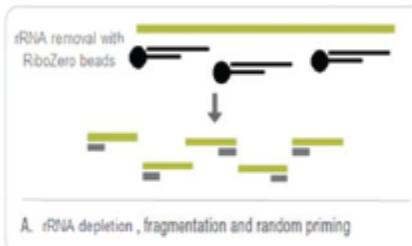
TruSeq Stranded RNA Workflows

Day 1 Library Prep Workflow

TruSeq Stranded mRNA

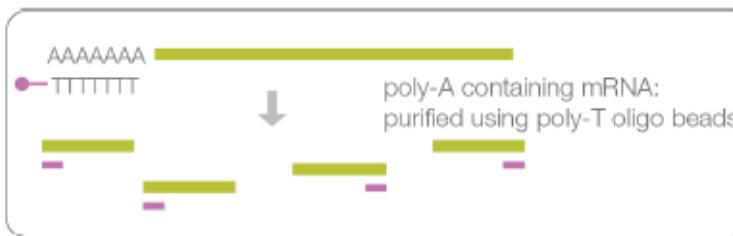


TruSeq Stranded Total RNA



FOR RESEARCH USE ONLY

Figure 1 Purifying and Fragmenting mRNA



Purify and Fragment mRNA

The Poly-A containing mRNA molecules are purified using poly-T oligo attached magnetic beads. Following purification, the mRNA is fragmented into small pieces using divalent cations under elevated temperature.

Figure 2 Synthesizing First Strand cDNA



Synthesize First Strand cDNA

Cleaved RNA fragments are copied into first strand cDNA using reverse transcriptase and random primers. Adding Actinomycin D to FSA (First Stand Synthesis Act D mix) prevents spurious DNA-dependent synthesis while allowing RNA-dependent synthesis, improving strand specificity.

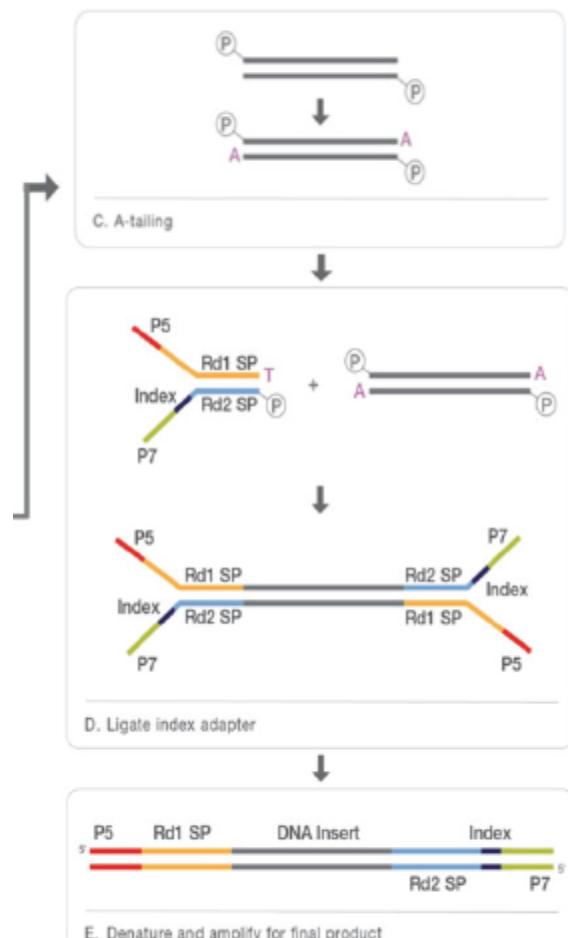
Figure 3 Synthesizing Second Strand cDNA



Synthesize Second Strand cDNA

Strand specificity is achieved by replacing dTTP with dUTP in the SMM (Second Strand Marking Mix), followed by second strand cDNA synthesis using DNA Polymerase I and RNase H. The incorporation of dUTP in second strand synthesis quenches the second strand during amplification.

Day 2 Library Prep Workflow



Adenylate 3' Ends

A single 'A' nucleotide is added to the 3' ends of the blunt fragments to prevent them from ligating to each other during the adapter ligation reaction. A corresponding single 'T' nucleotide on the 3' end of the adapter provides a complementary overhang for ligating the adapter to the fragment. This strategy ensures a low rate of chimaera (concatenated template) formation.

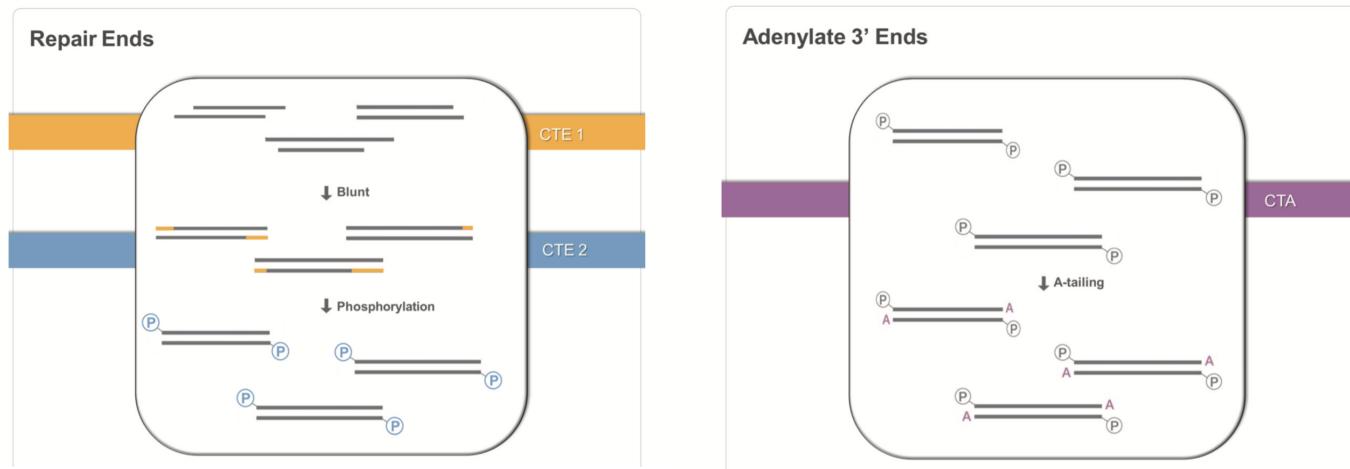
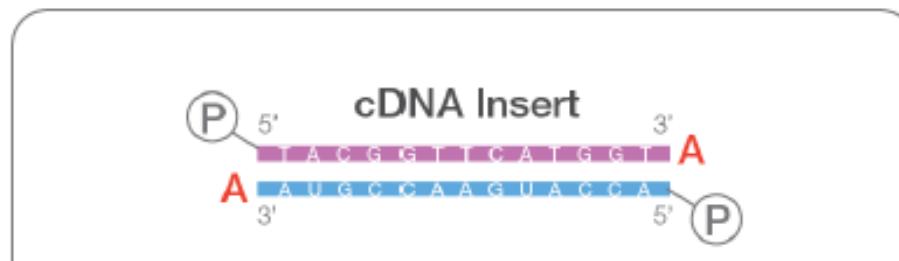
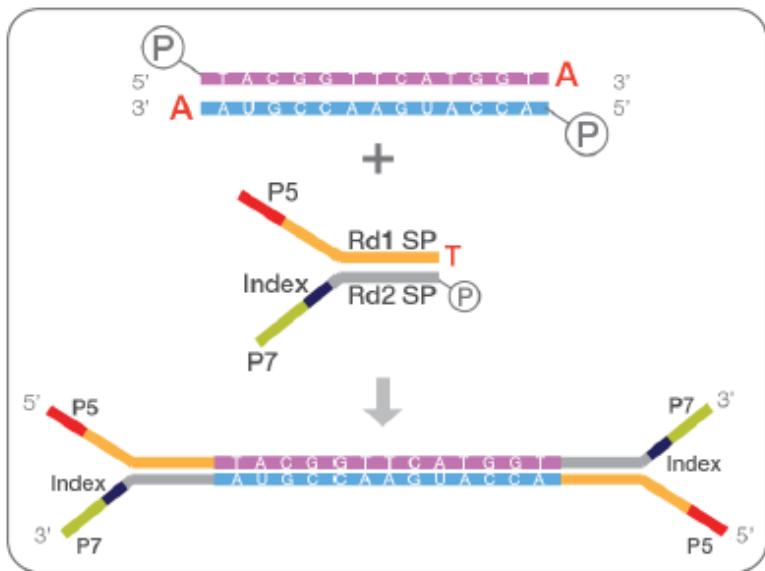


Figure 4 Adenylating 3' Ends

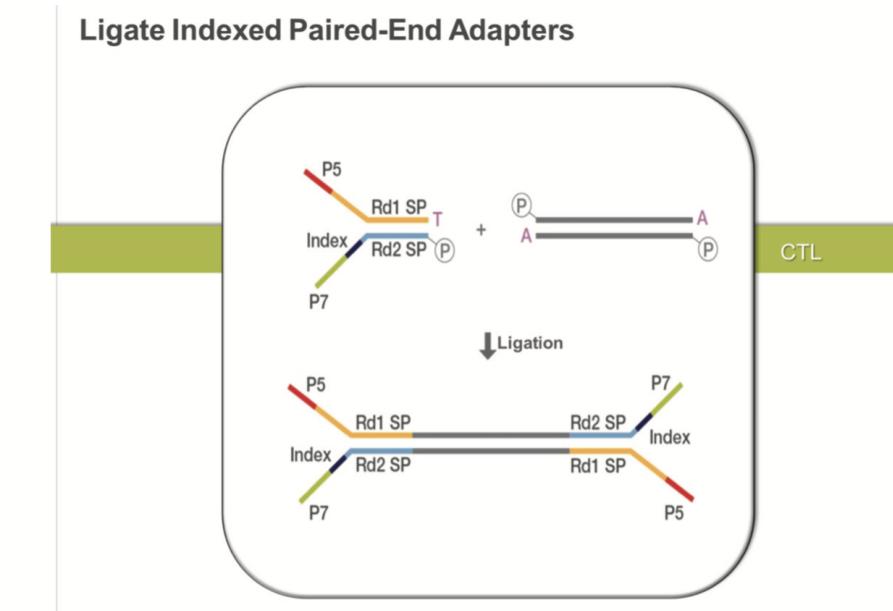


Day 2 Library Prep Workflow

Figure 5 Ligating Adapters



Ligate Indexed Paired-End Adapters

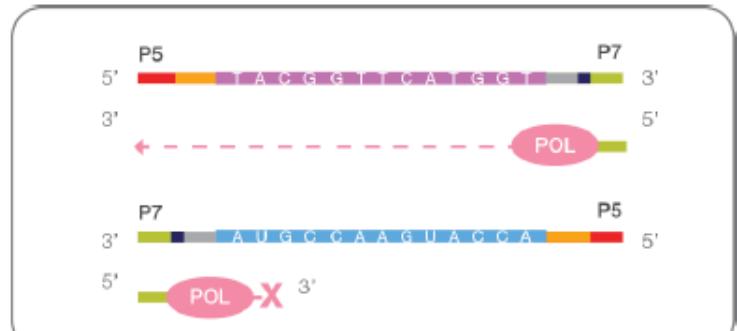


Ligate Adapters

The single-index adapter is shown in this workflow. The dual-index adapter option is not shown in this workflow. Adapter ligation prepares the ds cDNA for hybridization onto a flow cell.

Day 2 Library Prep Workflow

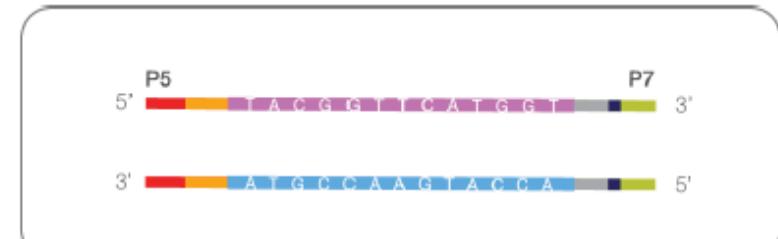
Figure 6 Enriching DNA Fragments



Enrich DNA Fragments

Polymerase used in the assay does not incorporate past dUTP. Therefore, the second strand is effectively quenched during amplification. The products are enriched with PCR and purified to create the final cDNA library.

Figure 7 LS Final Library

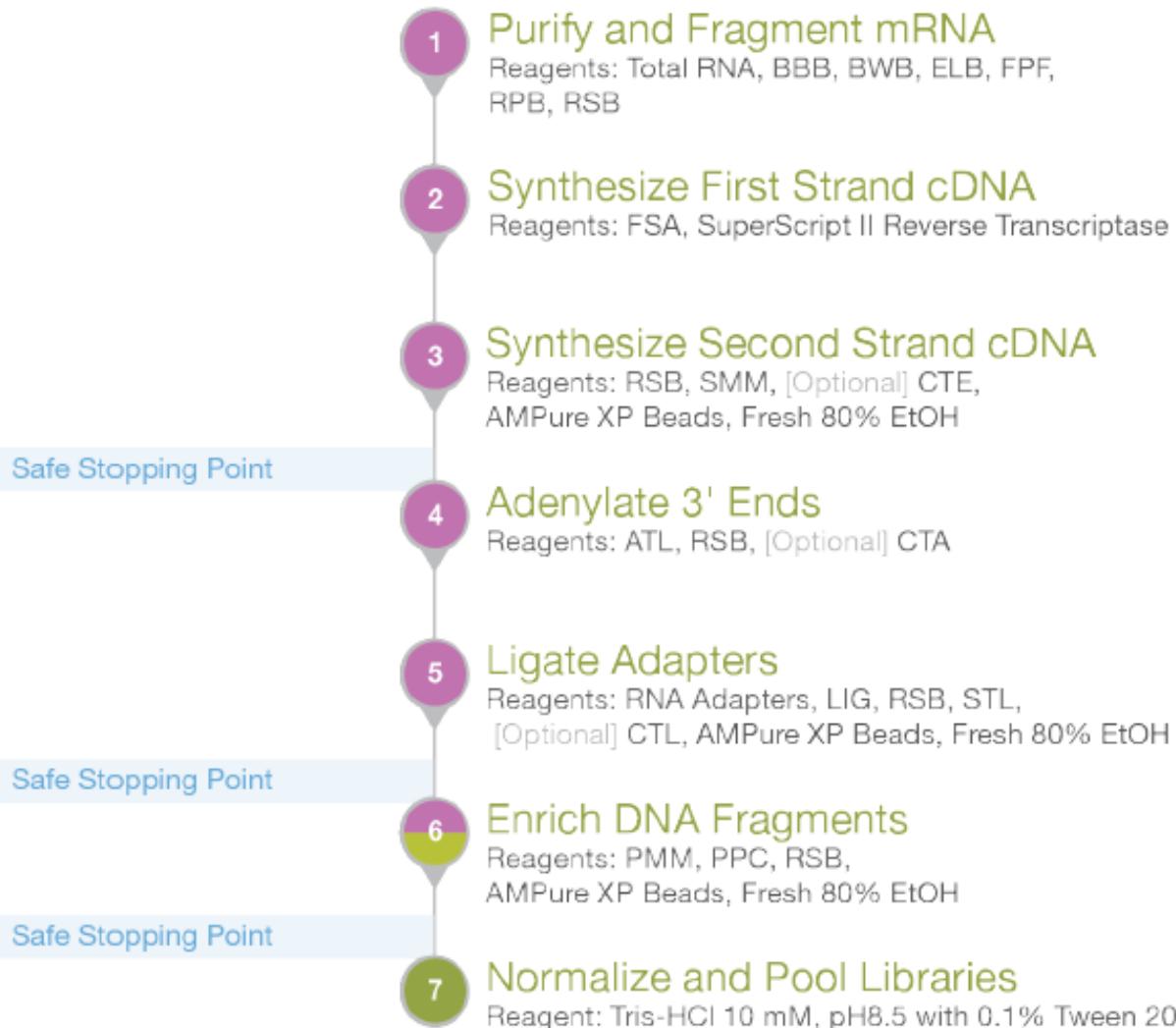


Final Library

The LS library features a single-index adapter, as shown in this workflow. The HS library features a dual-index adapter, which contains a unique index at each end. The HS library dual-index adapter is not shown in this workflow.

Library Prep Workflow

Figure 11 TruSeq Stranded mRNA Library Prep Workflow



(1) Library Construction for Next-Gen Sequencing

Day 1 & Day 2

- A. Poly-A selection, fragmentation and random priming
- B. First and second strand synthesis
- C. A-tailing
- D. Ligate index adapter
- E. Denature and amplify for final product

(2) Library quantification, normalization and pooling

Day 3

- A. Library quantification
- B. Normalization
- C. Pooling

Accurate quantification
is critical for maximizing
high-quality data output

Library quantification is
especially critical when
pooling indexed libraries

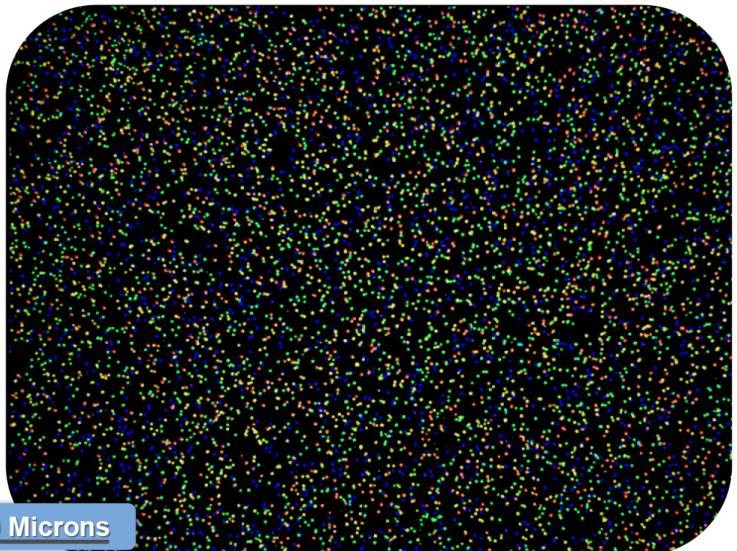
Library Validation

Use recommended
method to quantify final
libraries before
sequencing

Check library quality
using a Bioanalyzer
2100

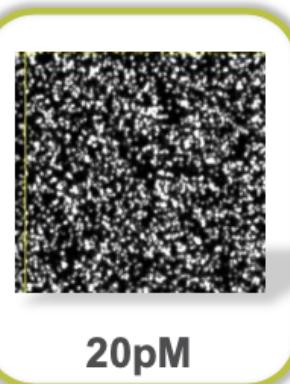
Maximize Data Quality and Quantity

Clusters

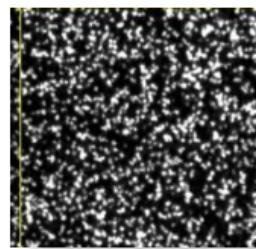


100 Microns

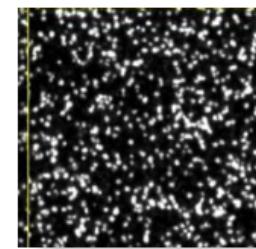
Optimized flow cell clustering determines data quality and overall data yield



20pM



10pM



5pM



1pM

Overclustering can result in:

- Loss of data quality and data output
- Loss of focus
- Reduced base calls and Q30 scores
- Complete run failure

Underclustering can result in:

- Loss of time and money
- Loss of focus
- Complete run failure

This is an actual cluster pattern from a flow cell in a 4-color overlay. Images are normally only one color.

Accurate Quantification Is Critical When Multiplexing

Calculated concentration is 10X higher for one library in pool

Sample	Expected Output	Actual Output
1	16%	66%
2	16%	6%
3	16%	6%
4	16%	6%
5	16%	6%
6	16%	6%

Sample	Expected Output	Actual Output
1	16%	20%
2	16%	20%
3	16%	20%
4	16%	20%
5	16%	20%
6	16%	2%

Calculated concentration is 10X lower for one library in pool

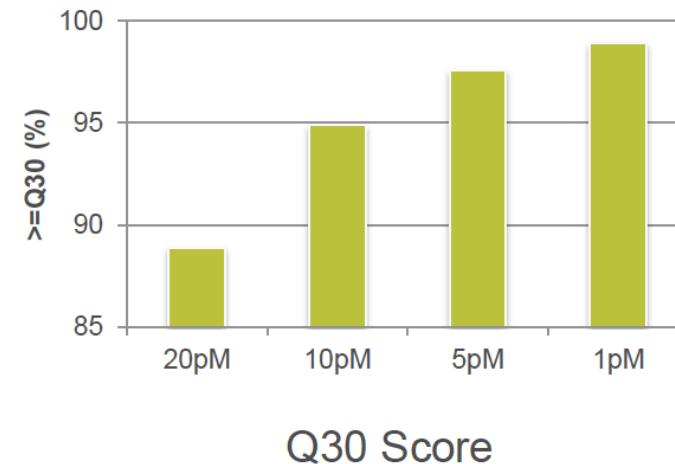
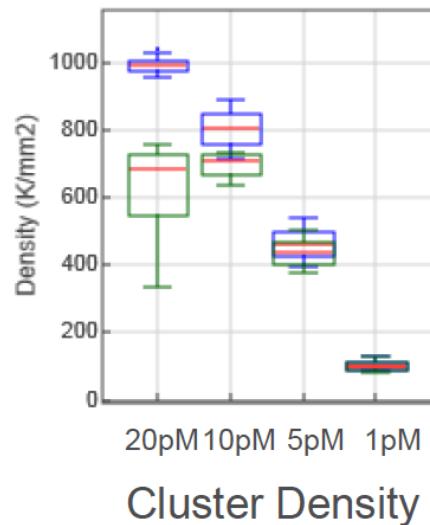
Flow Cell Titration

Flow Cell Titration

Correlate library concentration with cluster number

Uses three to four lanes of a flow cell

Data are still usable

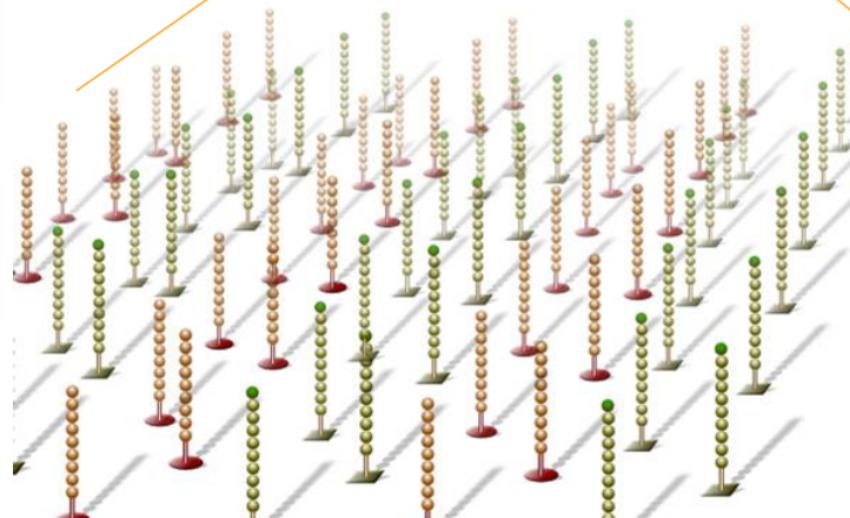
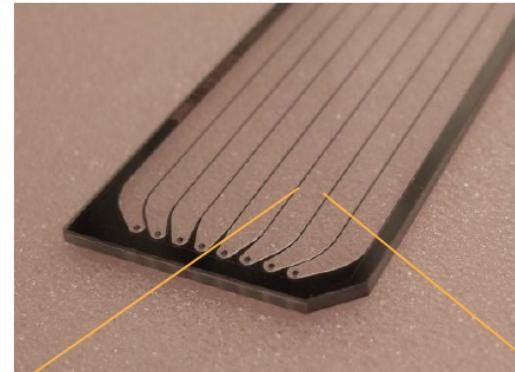


What is a Flow Cell?

Cluster generation occurs on a flow cell

A flow cell is a thick glass slide with channels or lanes

Each lane is randomly coated with a lawn of oligos that are complementary to library adapters

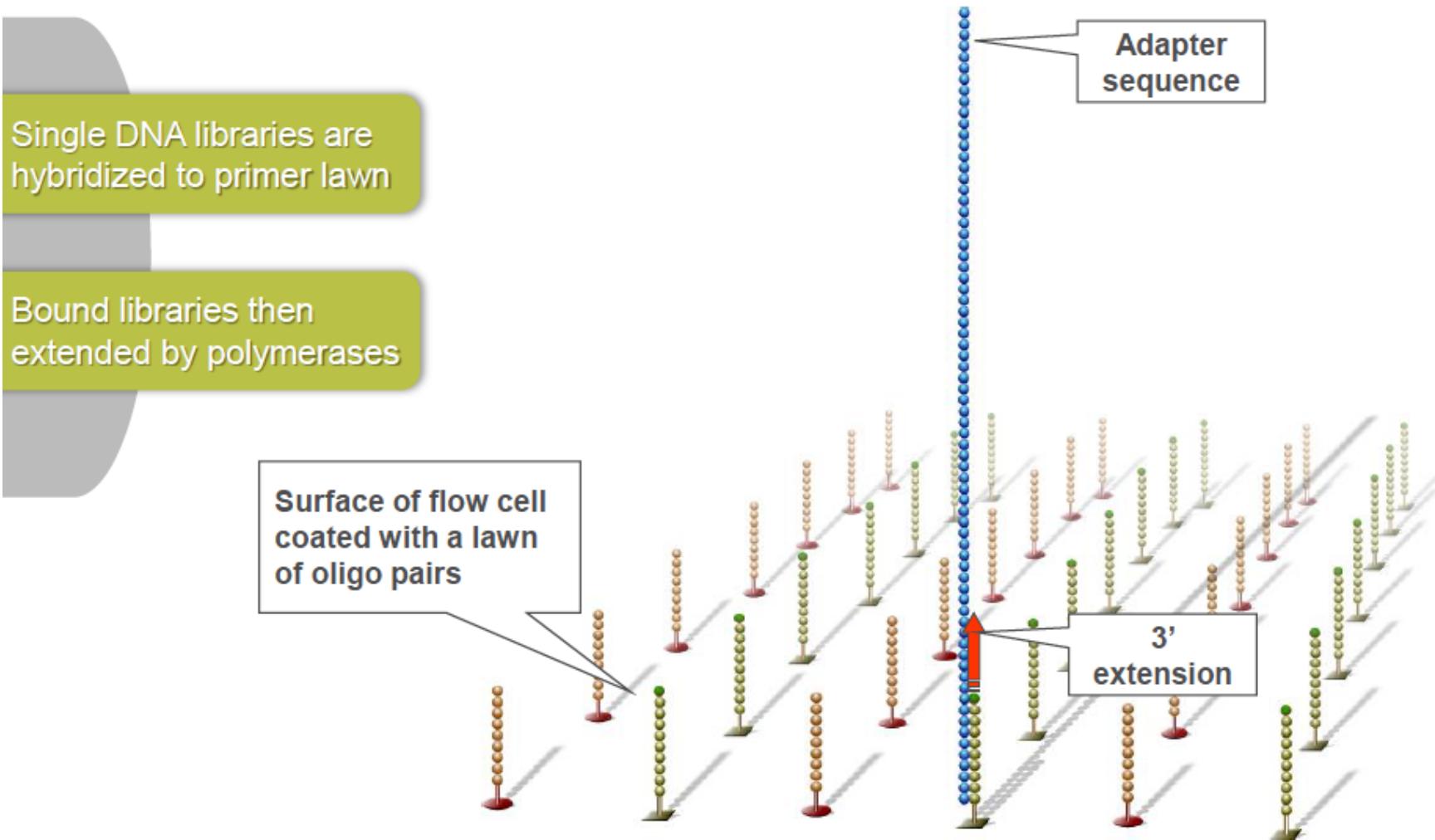


Flow cell: A glass slide with 1,2, or 8 (depending on instrument platform) physically separated lanes. Each lane is coated with a lawn of surface bound, adapter-complimentary oligos. A single sample or pool of up to 96 multiplexed samples can be run per lane depending on application parameters.

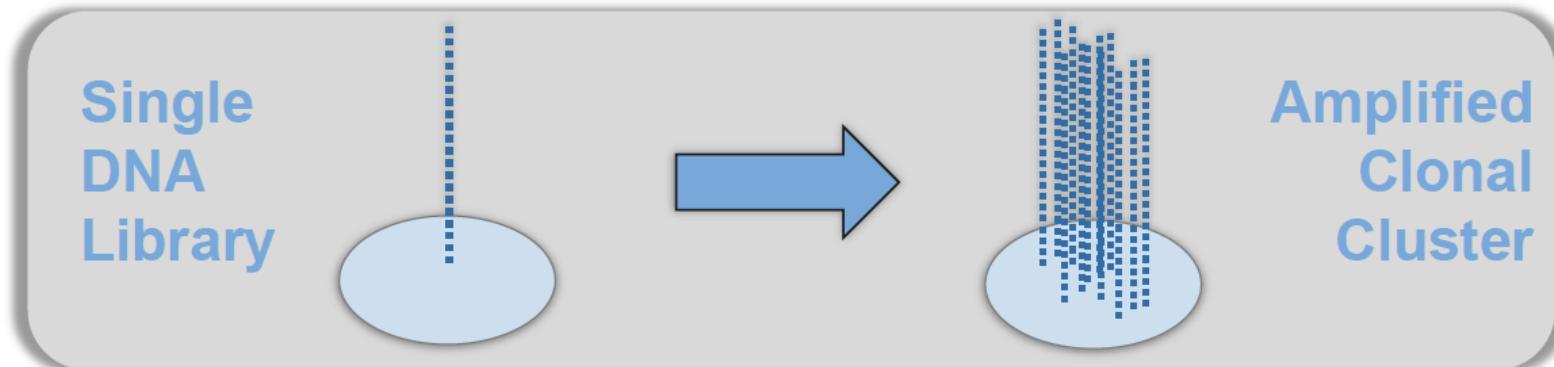
A GA IIx and HiSeq flow cell contains 8 separate lanes and is clustered on a cBot.

A MiSeq flow cell has a single lane and is clustered directly on the MiSeq in conjunction with a sequencing run.

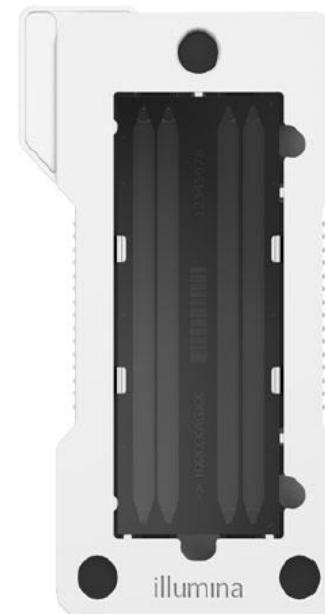
Hybridize Fragment & Extend



Cluster Generation



- Library pool loaded into reagent cartridge flows through all 4 lanes of the flow cell
- Hybridization and cluster generation are automated on the NextSeq system
- Approximately 5,000 molecules are included in a cluster



Recommended Library Quantification Methods

qPCR

- TruSeq DNA PCR-Free
- TruSeq Nano DNA
- TruSeq Stranded RNA
- TruSeq ChIP
- TruSeq Synthetic Long-Read DNA
- Nextera Mate Pair

Fluorometric dsDNA assay

- Nextera Rapid Capture
- Nextera DNA
- TruSight Rapid Capture
- TruSight One

Bioanalyzer

- TruSeq Small RNA
- TruSight Tumor
- TruSeq Targeted RNA Expression

Bead-based normalization

- Nextera XT
- TruSeq Amplicon
- TruSight DNA Amplicon

Quantification Methods of Sequencing Libraries

- Accuracy highly dependent on dilution and sample handling
- Recommended for *quality control* only, unless indicated

Bioanalyzer
2100



- Specifically detects double-stranded DNA
- Does not discriminate incomplete TruSeq libraries

Fluorometric
dsDNA assay



- Specifically measures full-length libraries
- Detection very sensitive

qPCR

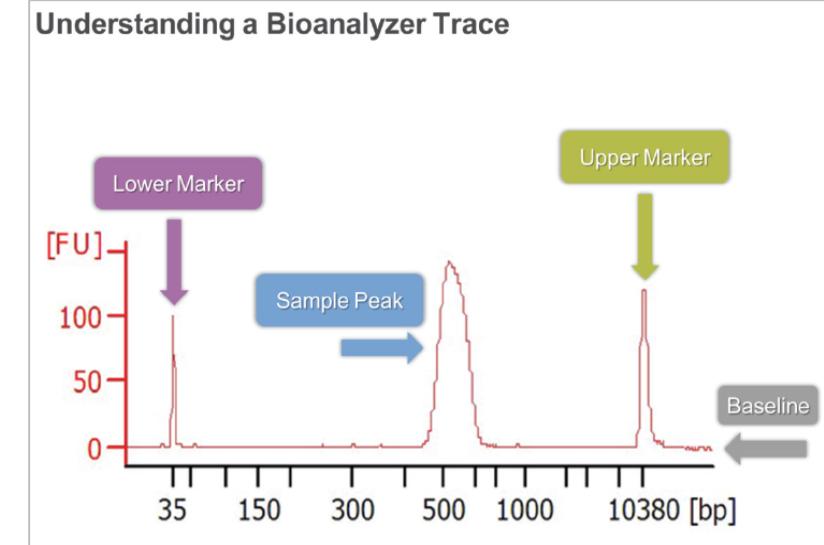
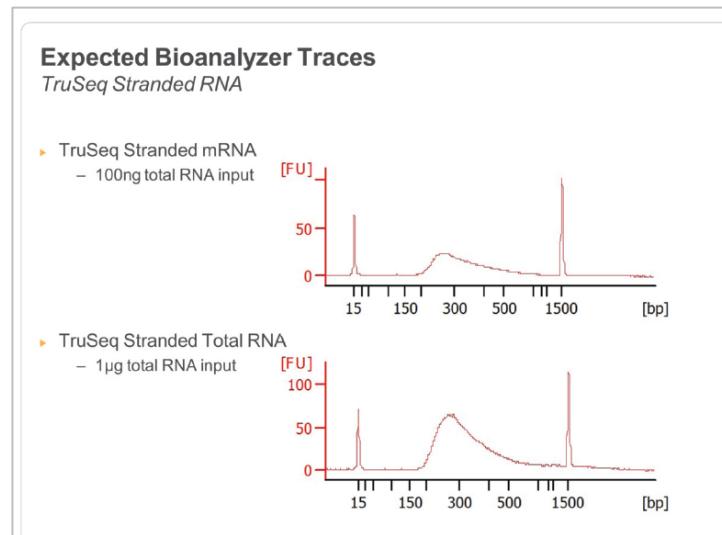


- Detects nucleic acids nonspecifically
- Contaminants elevate values
- Should not be used for input or library quantification

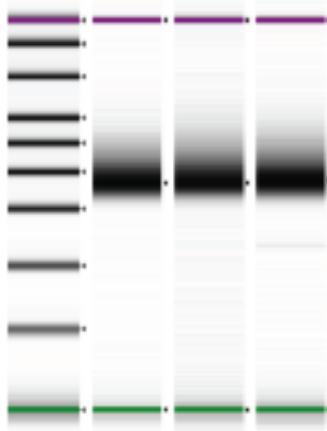
UV
spectrophotometer



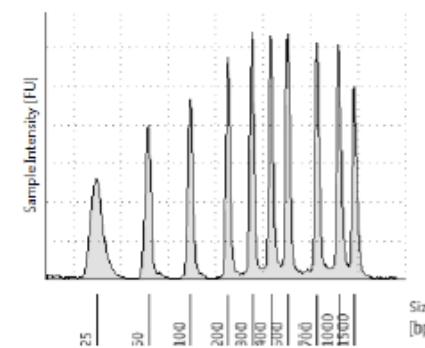
Check Library Quality (Bioanalyzer / TapeStation)



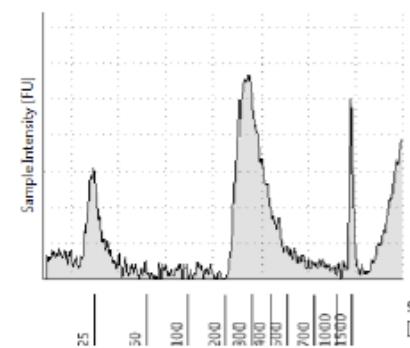
DNA library ~ 300 bp



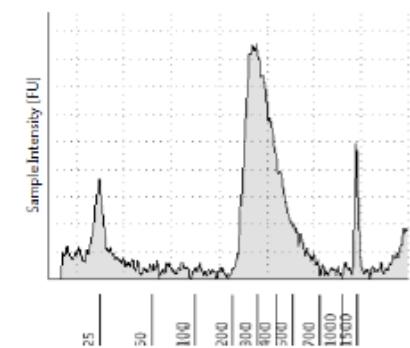
A0: Electronic Ladder



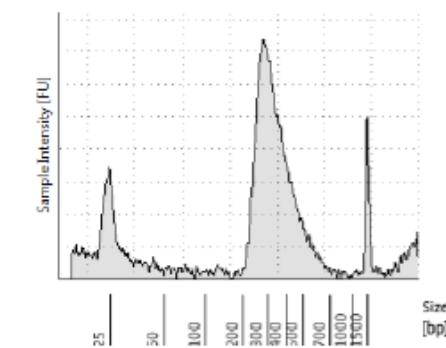
A2: cDNA ctrl



B2: 20-NF54-24hrs-JO



C2: 21-NF54-36hrs-JO



Check Libraries

Quantify Libraries

To achieve the highest-quality data on Illumina sequencing platforms, it is important to create optimum cluster densities across every lane of the flow cell. Optimizing cluster densities requires accurate quantification of DNA libraries.

- 1 Quantify the libraries using qPCR according to the *Illumina Sequencing Library qPCR Quantification Guide* (document # 11322363).

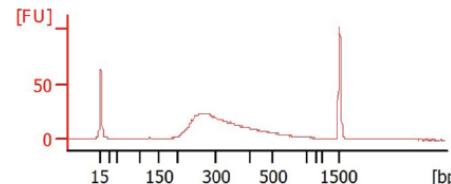
Check Library Quality

- 1 If using a Standard Sensitivity NGS Fragment Analysis Kit on an Advanced Analytical Fragment Analyzer:
 - a Dilute the DNA library 1:1 with RSB.
 - b Run 1 μ l diluted DNA library.
- 2 If using a DNA 1000 chip on an Agilent Technologies 2100 Bioanalyzer, run 1 μ l undiluted DNA library.

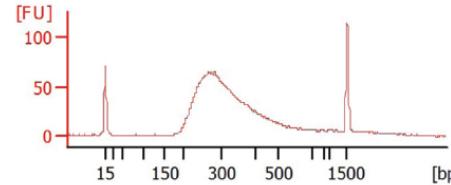
Expected Bioanalyzer Traces

TruSeq Stranded RNA

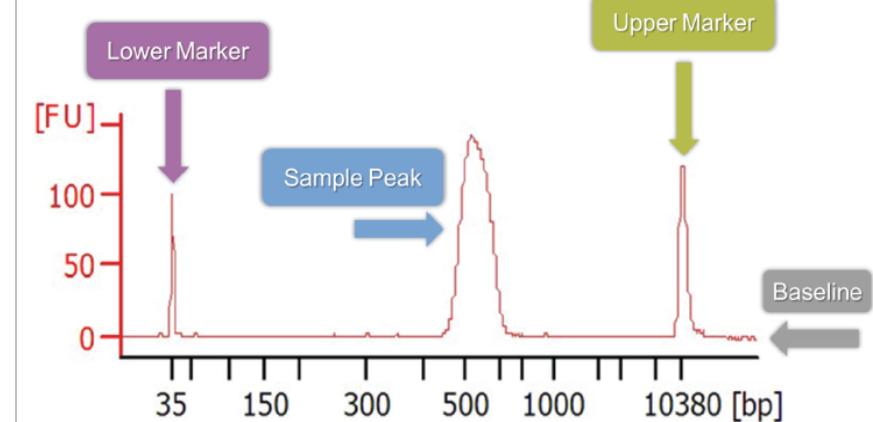
- TruSeq Stranded mRNA
– 100ng total RNA input



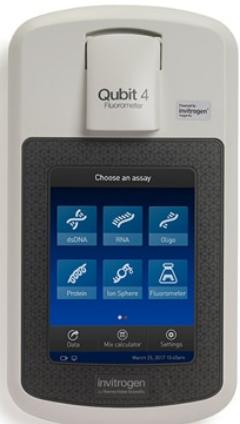
- TruSeq Stranded Total RNA
– 1 μ g total RNA input



Understanding a Bioanalyzer Trace



Library Quantification, Normalization, and Pooling



Illumina recommends quantifying your libraries using a fluorometric quantification method that uses dsDNA binding dyes.

Calculate DNA concentration in nM, based on the size of DNA amplicons as determined by an Agilent Technologies 2100 Bioanalyzer trace:

$$\frac{\text{(concentration in ng/μl)}}{(\text{660 g/mol} \times \text{average library size})} \times 10^6 = \text{concentration in nM}$$

Qubit® 4 Fluorometer

For example:

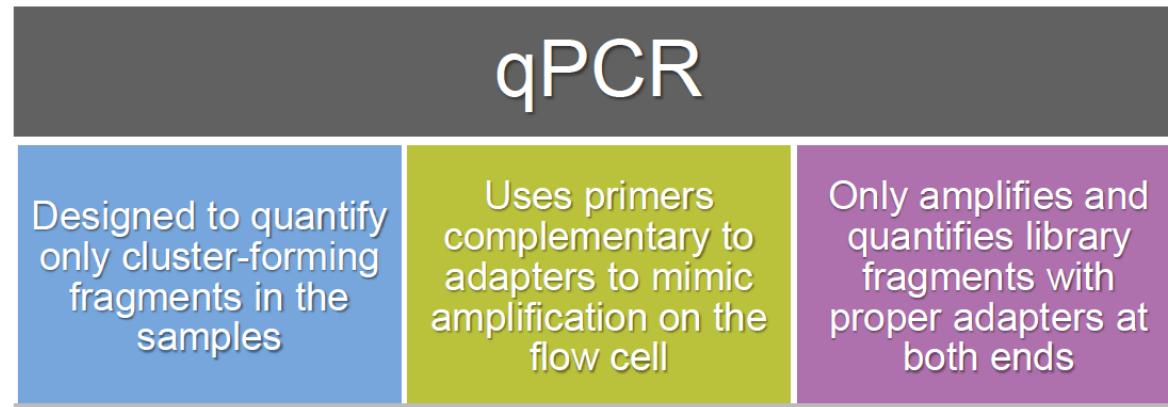
$$\frac{15 \text{ ng/μl}}{(\text{660 g/mol} \times 500)} \times 10^6 = 45 \text{ nM}$$

Dilute concentrated final library using Resuspension Buffer (RSB) or 10 mM Tris pH 8.5 to 4 nM. Aliquot 5 μl of diluted DNA from each library and mix aliquots for pooling libraries with unique indices. Depending on coverage needs, up to 96 libraries can be pooled for one MiSeq run.

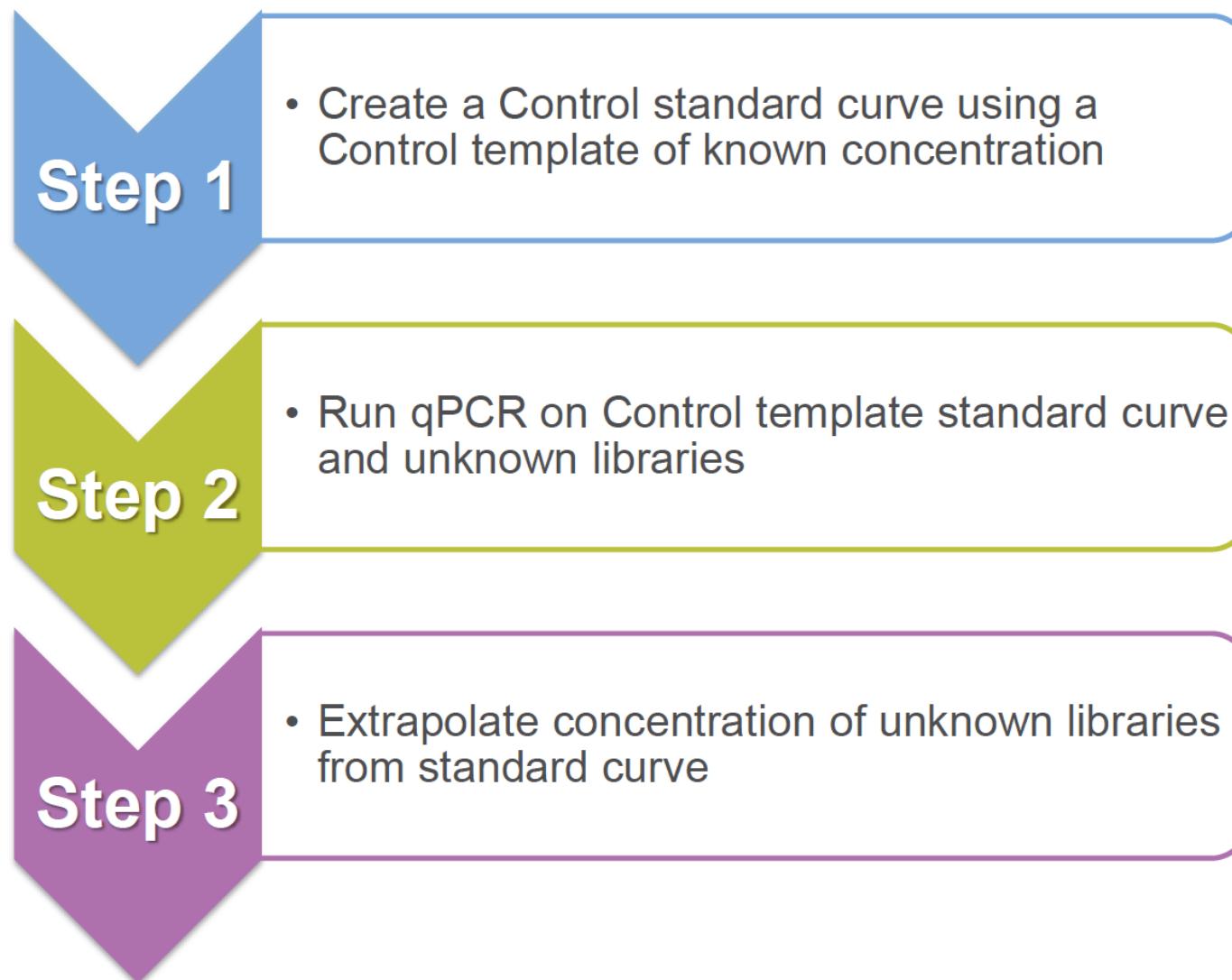
For metagenomics samples, >100,000 reads per sample is sufficient to fully survey the bacterial composition. This number of reads allows for sample pooling to the maximum level of 96 libraries, given the MiSeq output of > 20 million reads.

Calculate Library Concentrations using qPCR

Library qPCR Overview



Steps for Quantifying Libraries with qPCR



Library Quantification:

Resource – KAPA Biosystems Library Quantification Kit

Kit Components	Comments
6 DNA Standards	No dilutions needed! Caveat: Included DNA standards may not closely mimic Libraries to be quantified
Primers	Work with Illumina Universal Adapters

- *Designed specifically for libraries prepared with Illumina sample prep assays*
- *Reliable, accurate standard curve*

Best Practice:

Use control library that has an insert size and GC content similar to your sample library

Check Libraries

Quantify Libraries using qPCR

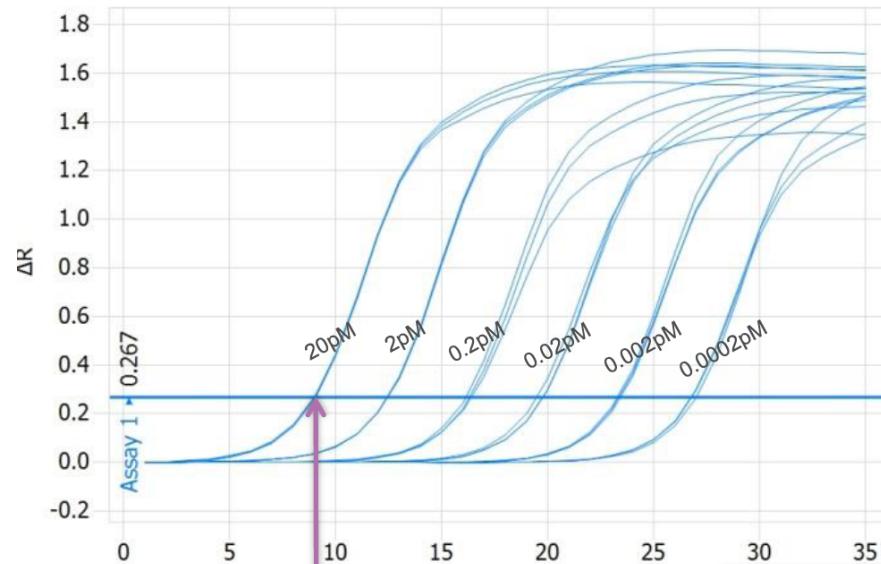
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- 1 Quantify the libraries using qPCR according to the *Illumina Sequencing Library qPCR Quantification Guide* (document # 11322363).



Roche LightCycler 96 System

Illumina Sequencing Library qPCR Quantification:



KAPA Library Quantification Kits
For Illumina sequencing platforms

Sample name ¹	dsDNA concentration (pM) ^{2,3}
Std 1	20
Std 2	2
Std 3	0.2
Std 4	0.02
Std 5	0.002
Std 6	0.0002

Export Data

Experiment Name: Sample Prep Training

Standard Curve Data

Slope	R ²	Efficiency %
-3.59	0.9997	90.04

Well	Sample Name	Assay Role	Cq	Cq Mean	Std. Dev.	Quantity	Quantity	Mean Qty.	Std. Dev. Qty.
E1	Std 1	Standard	8.06	8.04	0.03	20	pM	20	0
F1	Std 1	Standard	8.01	8.04	0.03	20	pM	20	0
E2	Std 2	Standard	11.77	11.71	0.09	2	pM	2	0
F2	Std 2	Standard	11.65	11.71	0.09	2	pM	2	0
E3	Std 3	Standard	15.35	15.24	0.15	0.2	pM	0.2	0
F3	Std 3	Standard	15.14	15.24	0.15	0.2	pM	0.2	0
E4	Std 4	Standard	18.86	18.81	0.06	0.02	pM	0.02	0
F4	Std 4	Standard	18.77	18.81	0.06	0.02	pM	0.02	0

Well	Sample Name	Assay Role	Cq	Cq Mean	Std. Dev.	Cq	Quantity	Quantity	Mean Qty.	Std. Dev. Qty.
C5	Libr C1 1:10000	Unknown	11.61	11.58	0.04	2.07	pM	2.10	0.05	
D5	Libr C1 1:10000	Unknown	11.55	11.58	0.04	2.14	pM	2.10	0.05	
A5	Libr C1 1:5000	Unknown	10.40	10.40	0.00	4.48	pM	4.48	0.00	
B5	Libr C1 1:5000	Unknown	10.40	10.40	0.00	4.48	pM	4.48	0.00	

Calculate Library Concentrations

Library name	Conc. in pM calculated by qPCR instrument (triplicate data points)			Avg. conc. (pM)	Size adjusted concentration (pM)	Conc. of undiluted library stock (pM)
Library 1:1000	A1	A2	A3	A	$A \times \frac{452}{\text{Avg. fragment length}} = W$	$W \times 1000$
Library 1:2000	B1	B2	B3	B	$B \times \frac{452}{\text{Avg. fragment length}} = X$	$X \times 2000$
Library 1:4000	C1	C2	C3	C	$C \times \frac{452}{\text{Avg. fragment length}} = Y$	$Y \times 4000$
Library 1:8000	D1	D2	D3	D	$D \times \frac{452}{\text{Avg. fragment length}} = Z$	$Z \times 8000$

1

pM taken from Eco results table
(only 2 values if run in duplicate)

2

Library size taken from Bioanalyzer data

3

Dilution value

Calculate Library Concentrations

Example

Concentration Calculation Table

A	B			C	D	E	F
Library Name and Dilution	Conc. calculated by qPCR instrument (pM) [from Excel data chart]			Avg. Conc. (pM)	Size adjusted Conc (pM) [bp from Bioanalyzer chart]	Conc. Of undiluted library stock (pM)	Library stock (nM)
Library 1:10000	X1	X2	X3	X	$X \times (452\text{bp}/\text{Avg fragment length}) = W$	$W \times 10000$	pM/1000
Library C1 1:10000	2.07	2.14	2.09	2.1	$2.1 \times (452\text{bp}/585\text{bp}) = 1.6$	$1.6 \times 10000 = 16000$	16
Library C1 1:5000	4.48	4.61	4.42	4.5	$4.5 \times (452\text{bp}/585\text{bp}) = 3.5$	$3.5 \times 5000 = 17500$	17.5

Library C1

16.7 nM

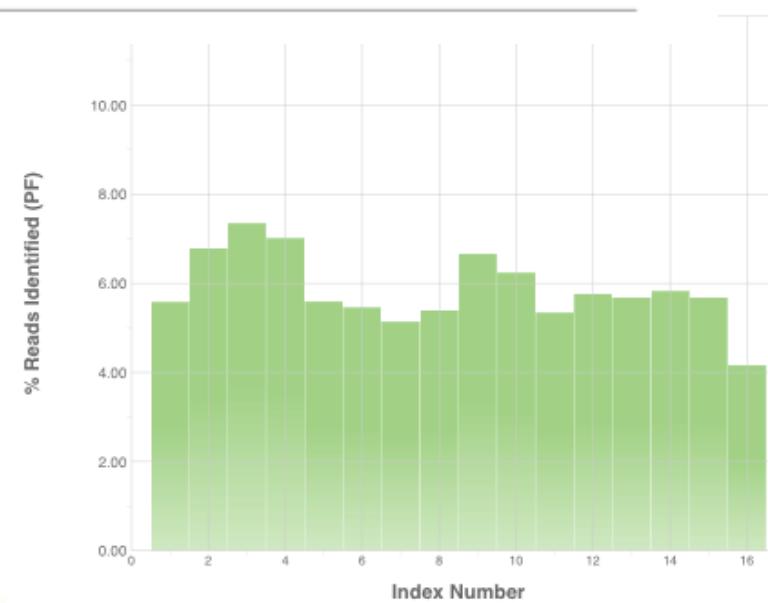
Protocol A: Standard Normalization Method

Use protocol A to denature and dilute libraries that have been normalized using standard library quantification and quality control procedures recommended in the library prep documentation.

Follow the steps most appropriate for your library and the version of MiSeq reagent kit you are using.

Chemistry	Compatible Denature and Dilute Steps
MiSeq Reagent Kit v3	4 nM library —Results in a 6–20 pM loading concentration.
MiSeq Reagent Kit v2	4 nM library —Results in a 6–20 pM loading concentration. 2 nM library —Results in a 6–10 pM loading concentration.

- Quantify Library (Qubit and qPCR)
- Check Library Quality (Bioanalyzer)
- **Normalize and Pool Libraries**
- Denature and Dilute Libraries
- Combine Library and PhiX Control
- Load Libraries onto the reagent cartridge
- Set up the Sequencing Run



Denature a 4 nM Library

- 1 Combine the following volumes in a microcentrifuge tube.
 - ▶ 4 nM library (5 µl)
 - ▶ 0.2 N NaOH (5 µl)
- 2 Vortex briefly and then centrifuge at $280 \times g$ for 1 minute.
- 3 Incubate for 5 minutes at room temperature.
- 4 Add 990 µl prechilled HT1 to the tube containing denatured library.
The result is 1 ml of a 20 pM denatured library.

Dilute Denatured 20 pM Library

- 1 Dilute to the desired concentration using the following volumes.

Concentration	6 pM	8 pM	10 pM	12 pM	15 pM	20 pM
20 pM library	180 µl	240 µl	300 µl	360 µl	450 µl	600 µl
Prechilled HT1	420 µl	360 µl	300 µl	240 µl	150 µl	0 µl

Denature and Dilute PhiX Control

Use the following procedure to denature and dilute a PhiX library for use as a sequencing control.

Follow the steps appropriate for the version of MiSeq reagent kit you are using.

Chemistry	Final PhiX Concentration
MiSeq Reagent Kit v3	Dilute the denatured PhiX control to 20 pM, which produces an optimal cluster density using v3 reagents.
MiSeq Reagent Kit v2	Dilute the denatured PhiX control to 12.5 pM, which produces an optimal cluster density using v2 reagents.

Dilute PhiX to 4 nM

- 1 Combine the following volumes in a microcentrifuge tube.
 - ▶ 10 nM PhiX library (2 μ l)
 - ▶ 10 mM Tris-Cl, pH 8.5 with 0.1% Tween 20 (3 μ l)
- 2 If not prepared within the last **12 hours**, prepare a fresh dilution of 0.2 N NaOH.

Combine Library and PhiX Control

For most libraries, use a low-concentration PhiX control spike-in of 1% as a sequencing control. For low diversity libraries, increase the PhiX control spike-in to at least 5%.

- 1 Combine the following volumes of denatured PhiX control and denatured library.

	Most Libraries (1% Spike-In)	Low-Diversity Libraries (≥ 5% Spike-In)
Denatured and diluted PhiX	6 µl	30 µl
Denatured and diluted library (from protocol A or protocol B)	594 µl	570 µl

- 2 Set aside on ice until you are ready to load it onto the reagent cartridge.



NOTE

Actual PhiX percentage varies depending upon the quality and quantity of the library pool.

Dilute Library to Loading Concentration

- 1 Combine the following volumes of pooled libraries and prechilled HT1 in a microcentrifuge tube.

Amplicon Library Pool	Prechilled HT1
6 µl	594 µl
7 µl	593 µl
8 µl	592 µl
9 µl	591 µl
10 µl	590 µl

Nextera XT Library Pool	Prechilled HT1
24 µl	576 µl

The total volume is 600 µl.

- 2 Vortex briefly and then centrifuge at $280 \times g$ for 1 minute.

Denature Diluted Library

- 1 Place the tube on the preheated incubator for 2 minutes.
- 2 Immediately cool on ice.
- 3 Leave on ice for 5 minutes.
- 4 To add a PhiX control, proceed to *Denature and Dilute PhiX Control* on page 8. Otherwise, see *Next Steps* on page 10.

Best Practices

- ▶ Always prepare freshly diluted NaOH for denaturing libraries for cluster generation. This step is essential to the denaturation process.
- ▶ To prevent small pipetting errors from affecting the final NaOH concentration, prepare at least 1 ml of freshly diluted NaOH.
- ▶ For best results, begin thawing the reagent cartridge before denaturing and diluting libraries. For instructions, see the *MiSeq System User Guide* (part # 15027617).

About Low Diversity Libraries

Low diversity libraries are libraries where a significant number of the reads have the same sequence. This lack of variation shifts the base composition because the reads are no longer random.

Low diversity can occur with some expression studies with > 25% one type of transcript, low-plexity amplicon pools, adapter dimer, or bisulfite sequencing, for example. A higher concentration spike-in of PhiX helps balance the overall lack of sequence diversity.

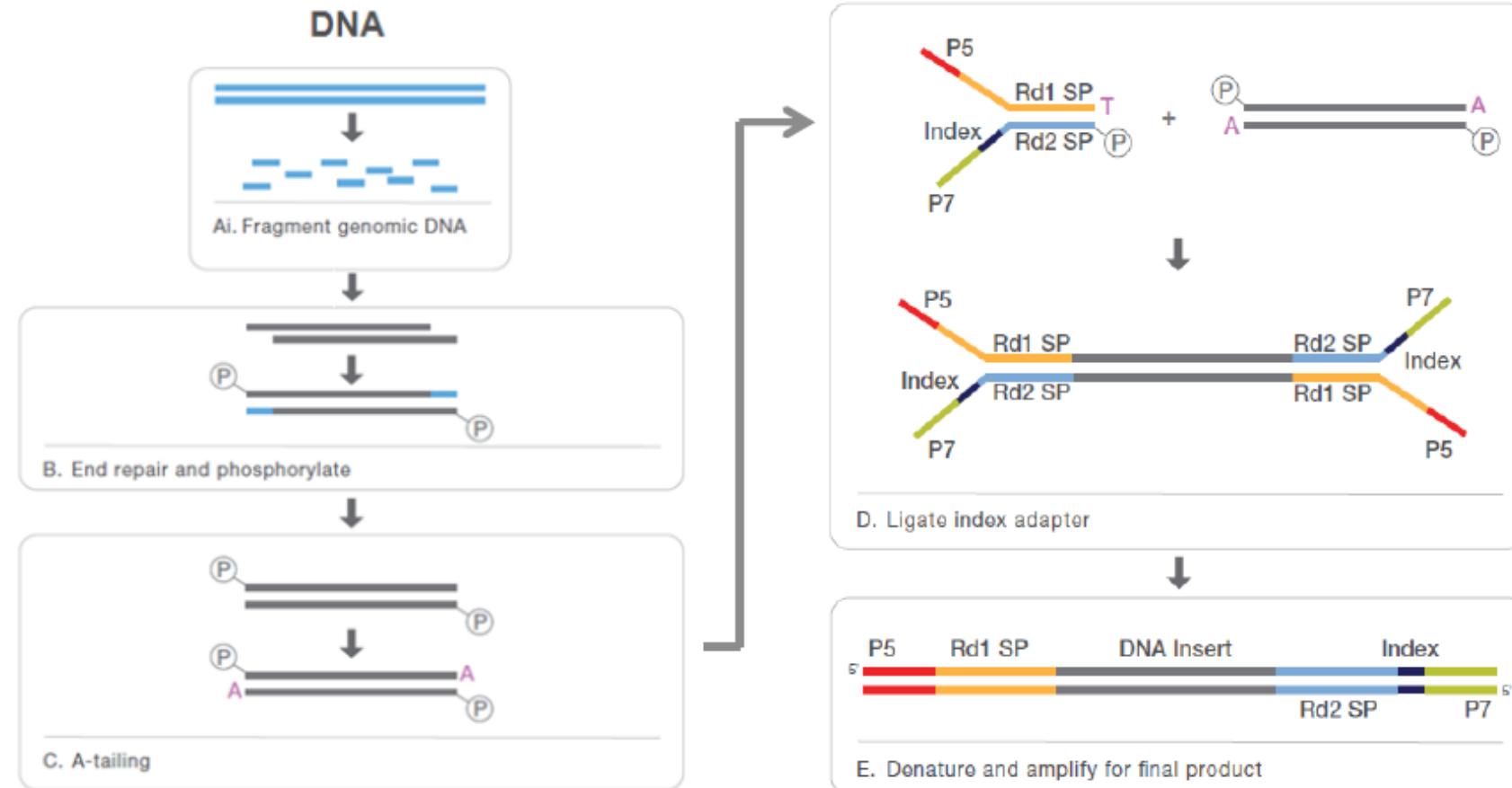


NOTE

For low diversity libraries, dilute your PhiX control library to the same concentration as your denatured library.

Library Preparation

TruSeq DNA Library Preparation Kit



Sequencing Kits We Recommend to Use:

Illumina Library-Prep Kits and Indexes Would be Recommended

Sequencing Data QC,
Index Distribution QC,
FASTQ Data Generation



Illumina BaseSpace
Sequence HUB



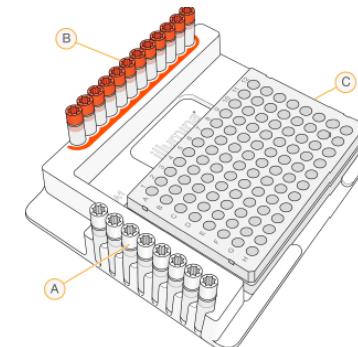
❖ RNA Sequencing: TruSeq® Stranded mRNA Library Prep
(48 Samples) 20020594

❖ 16S rRNA Sequencing:

Index PCR: Nextera XT Index kit (2 Primers N7xx, S5xx) from the Index Kit

Amplicon PCR: 16S Amplicon PCR Primers (Forward, Reverse)

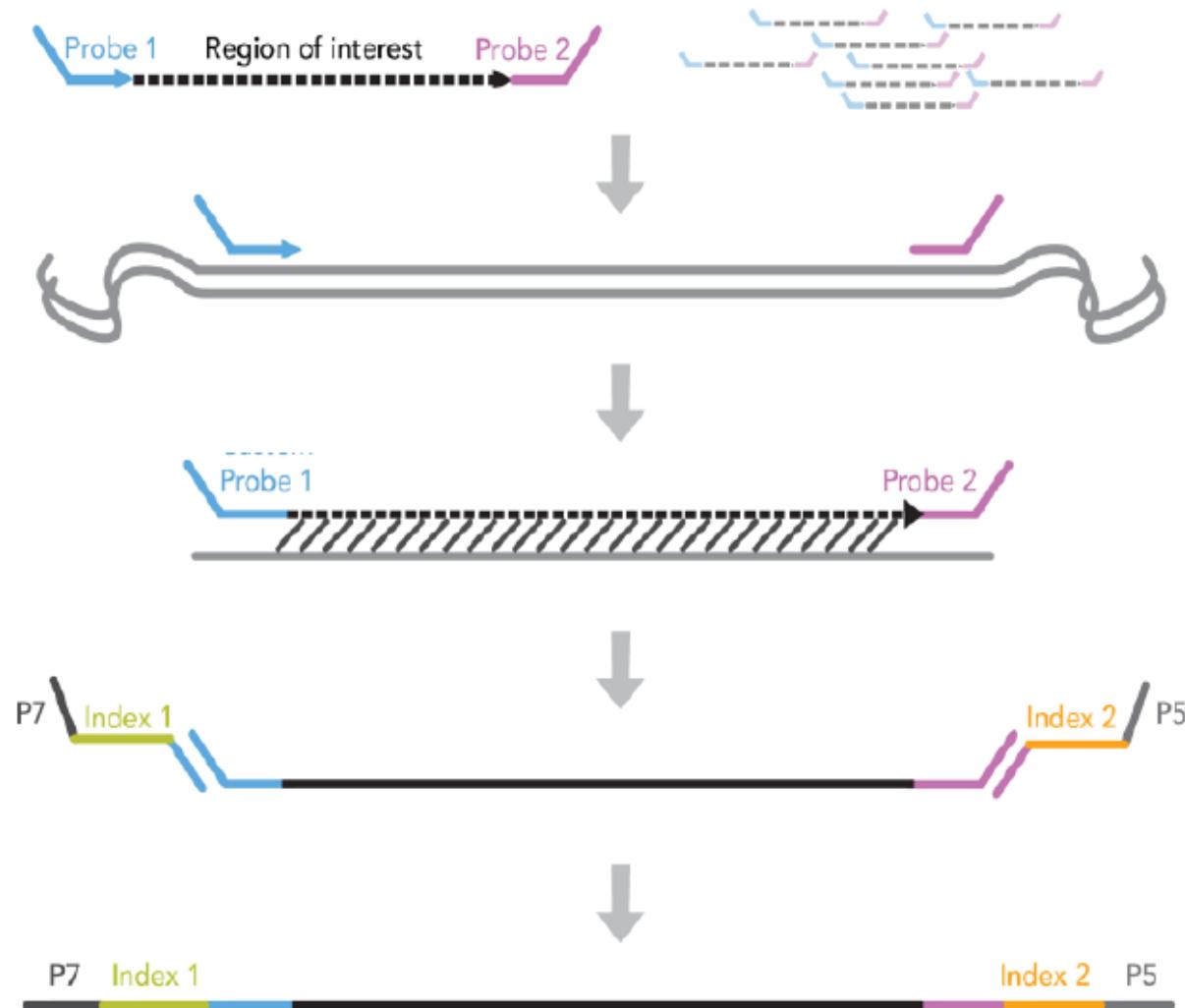
2x KAPA HiFi HotStart ReadyMix



❖ Metagenomics Sequencing

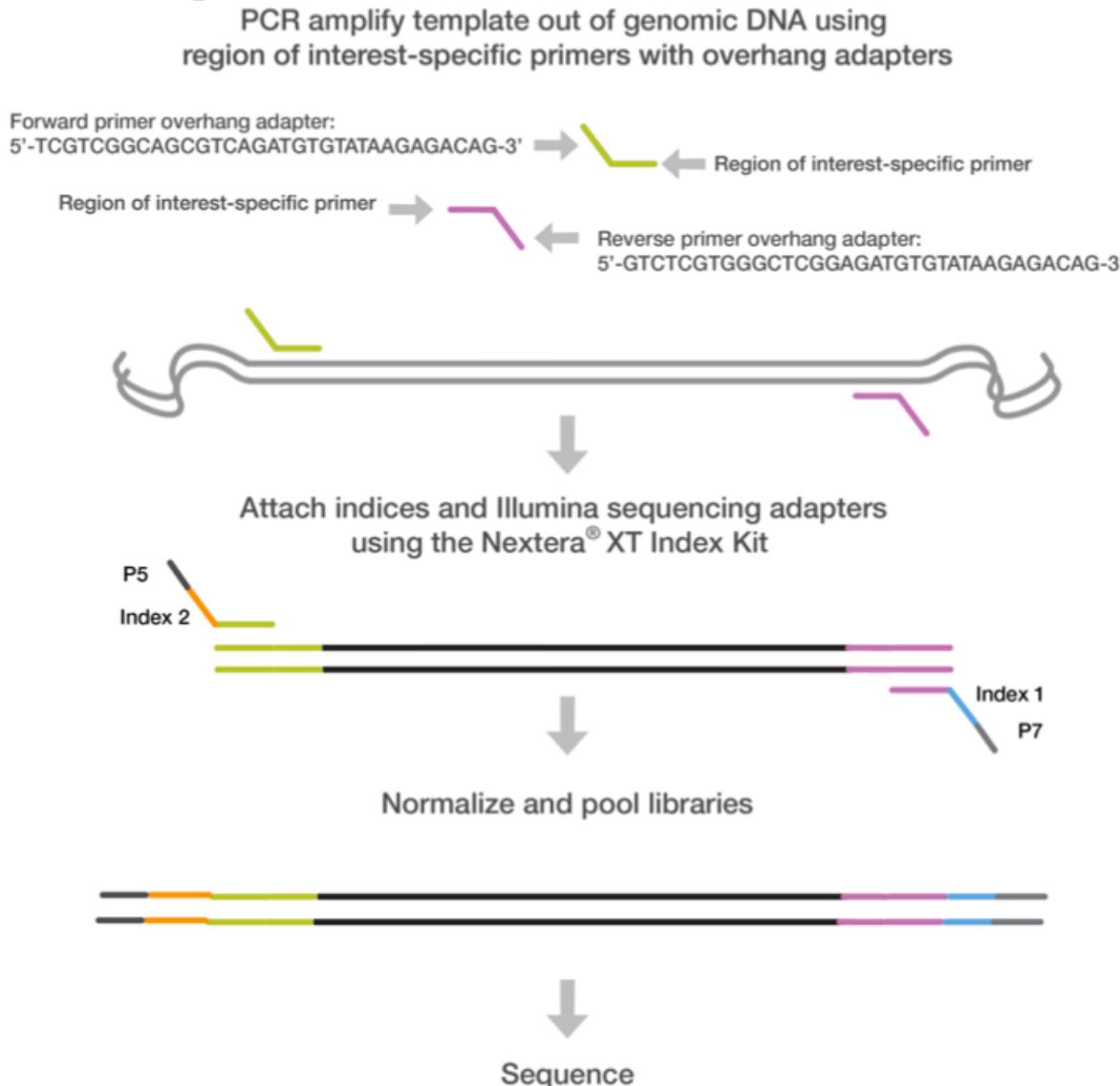
Nextera DNA Flex Library Prep Kit, Nextera DNA CD Indexes (24 Indexes, 24 Samples)

TruSeq Amplicon Workflow

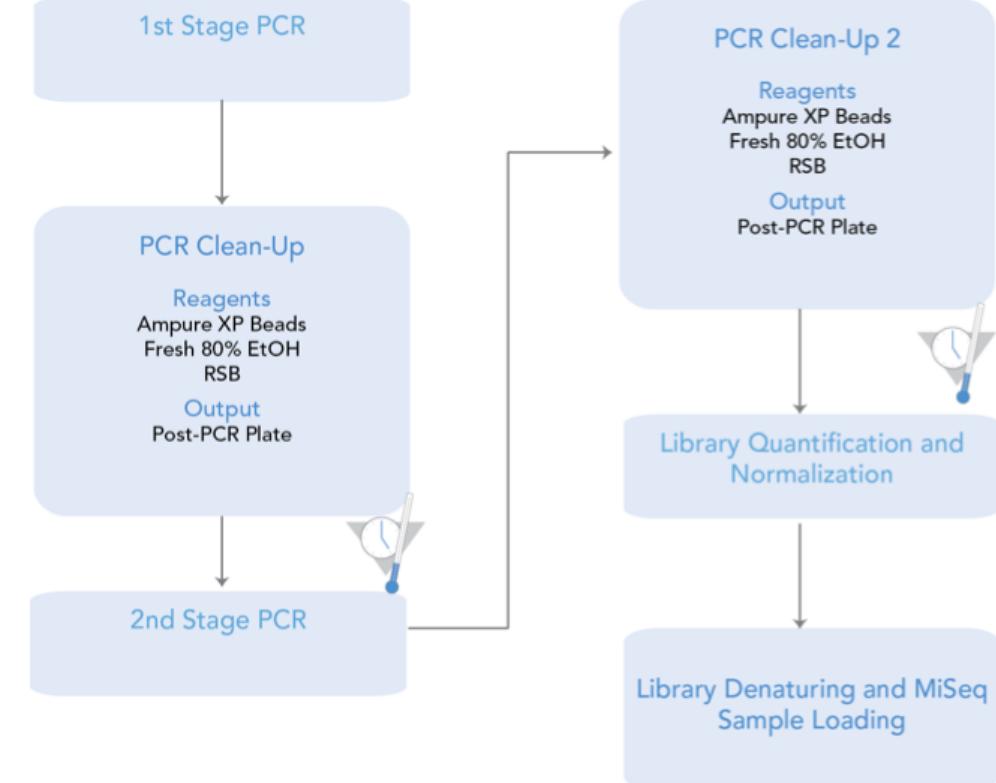


16S Metagenomic Sequencing Library

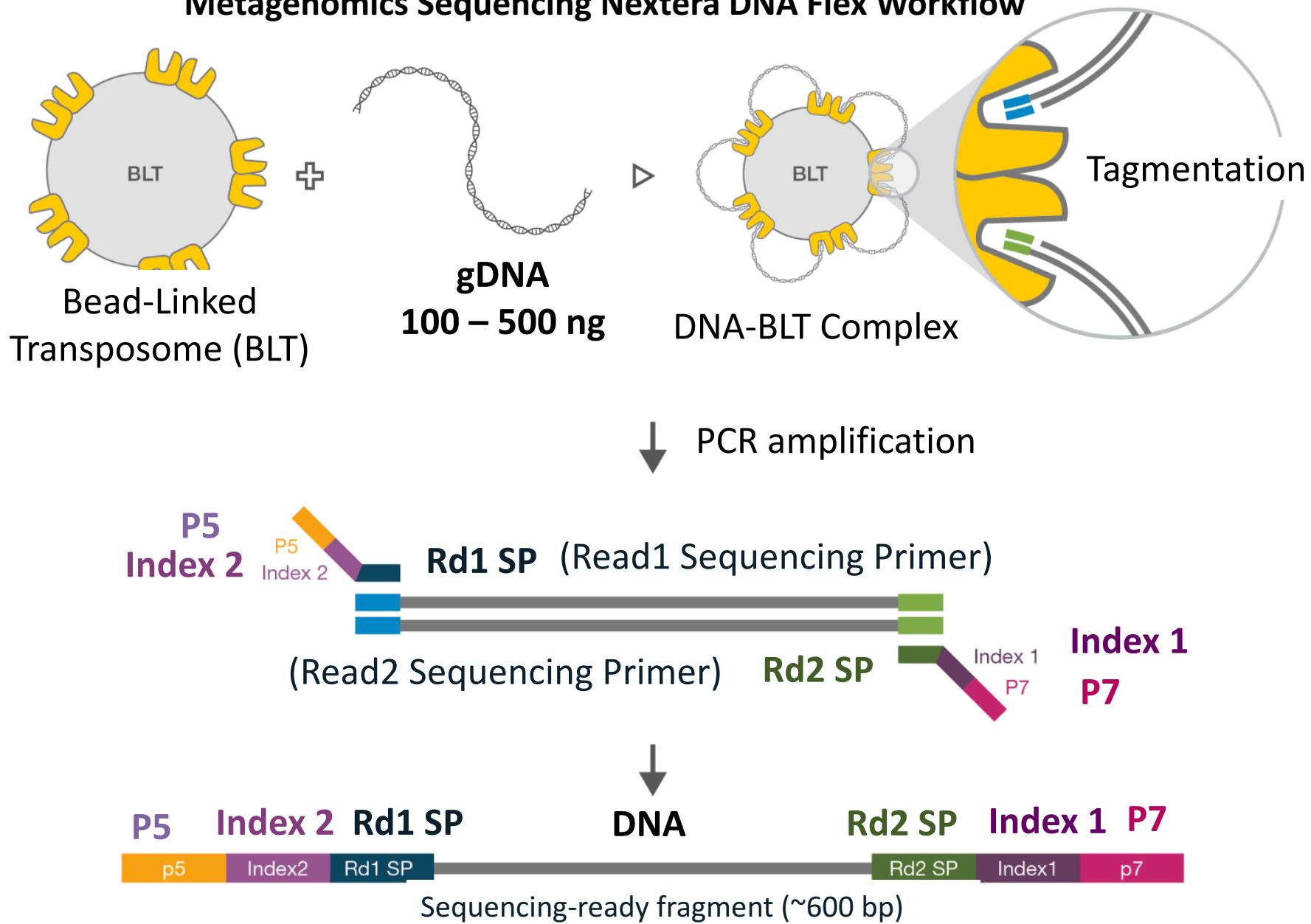
16S V3 and V4 Amplicon Workflow



16S Library Preparation Workflow



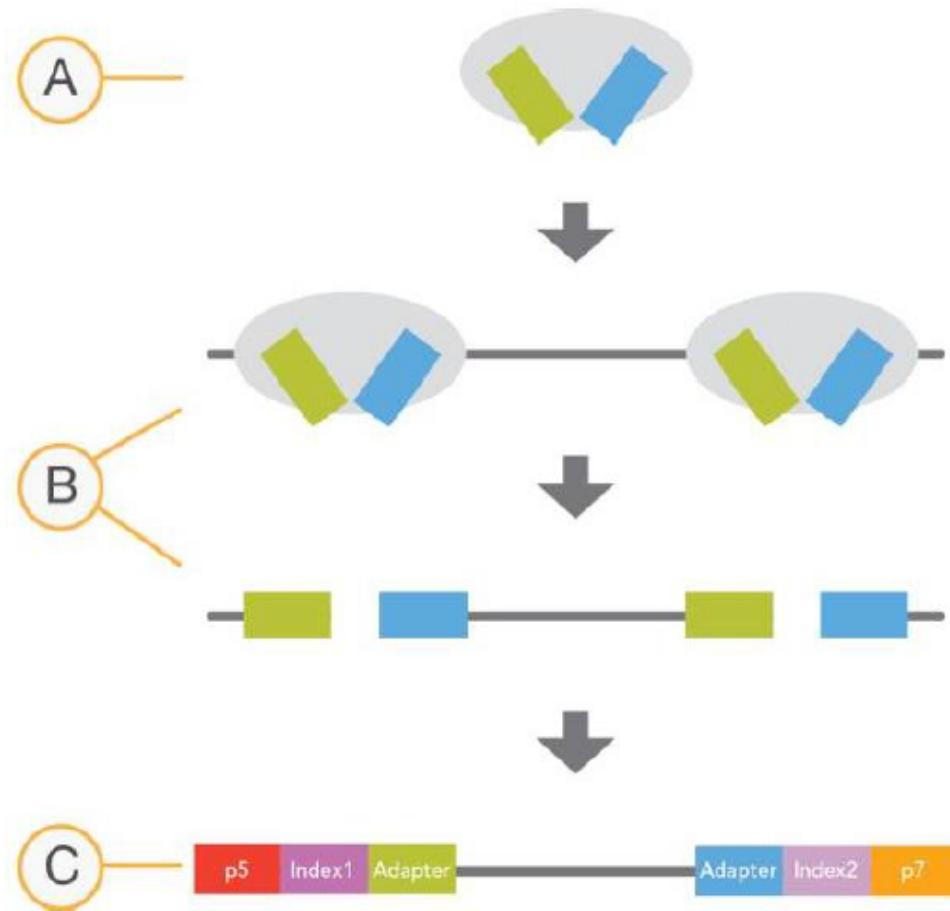
Metagenomics Sequencing Nextera DNA Flex Workflow



Sequencing with the NextSeq 500/550 High Output Kit v2.5 (300 cycles)

Library Preparation

Illumina Nextera DNA Library Preparation Kit



Separate fragmentation not required

Tag with enzyme mix

PCR polishes fragment ends and incorporates optional indexes

- A Nextera Transposome with Adaptors
- B Fragmentation to Fragment and Add Adaptors
- C Limited Cycle PCR to Add Sequencing Primer Sequences and Indices

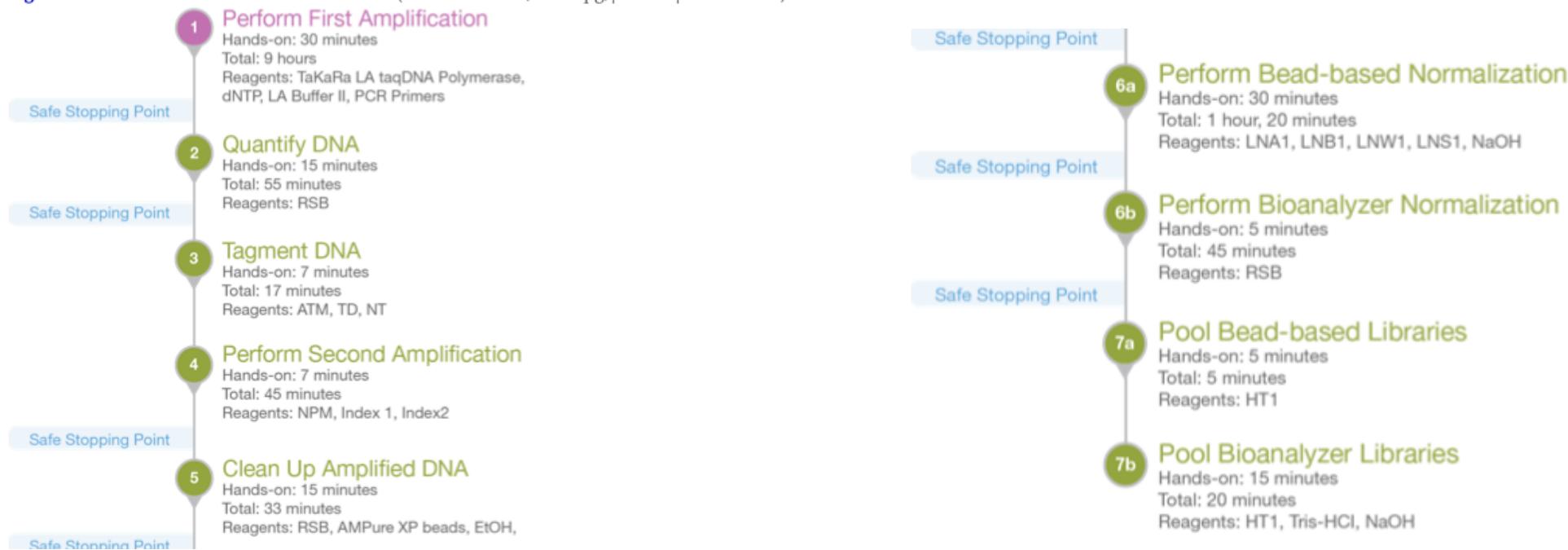
Human Mitochondria DNA Genome Sequence

Introduction

Illumina Nextera XT DNA Library Prep Kit

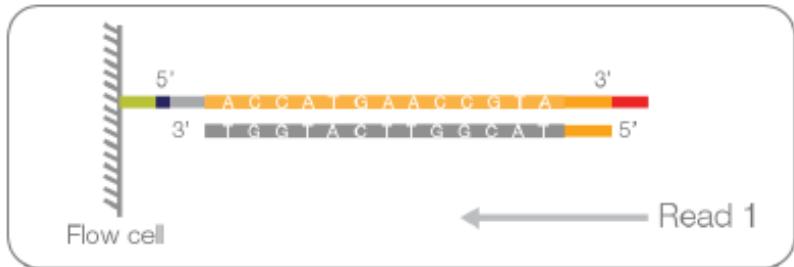
This protocol explains how to prepare, sequence, and analyze the entire human mitochondrial DNA (mtDNA) genome from clean, intact DNA samples. During sample preparation, the mtDNA genome is amplified in two PCRs to generate two long fragments spanning the entire human mitochondrial genome (16,569 bp). The amplicons are quantified and pooled before library preparation. Subsequent library sequencing on the MiSeq is followed by data analysis.

Figure 1 Human mtDNA Genome Workflow (for 12 reactions, 1-200 pg/ μ l in 10 μ l total DNA)



Cluster Generation for Read 1 and Read 2

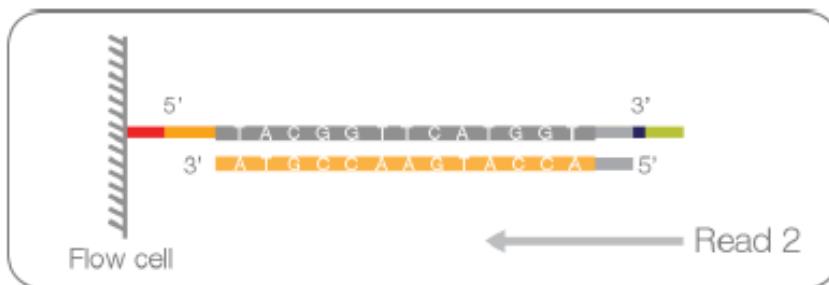
Figure 8 Cluster Generation and Read 1 Sequencing



Cluster Generation and Read 1 Sequencing

In Read 1, sequencing reads map to the antisense strand.

Figure 9 Paired-end Turnaround and Read 2 Sequencing



Paired-end Turnaround and Read 2 Sequencing

In Read 2, sequencing reads map to the sense strand.

Genomics Equipment Core Sequencing Services

NGS Services:

- ❖ Whole-Genome Sequencing
- ❖ RNA Sequencing
- ❖ Gene Expression Profiling
- ❖ 16S rRNA Sequencing
- ❖ Metagenomics Sequencing
- ❖ Targeted Gene Sequencing
- ❖ Targeted Gene Expression Profiling
- ❖ miRNA & Small RNA Analysis
- ❖ Single-Cell Sequencing
- ❖ DNA, RNA Quantification

USF Genomics Equipment Core Workflow

1. Complete 'Initial Request'

User – Complete the RNAseq Laboratory Training and register in the iLab as certified user.
User - Upload the '**PI signed the Policies and Procedures**' to the iLab.
Paige - DocuSign for the core, upload fully signed Policies and Procedures to the iLab.

2. Submit 'Service Request'

User - Upload the '**Billing Form**' and '**Sample Sheet**', submit the work request.
Judy – '**Financial Approval**', confirm with the core that Billing INFO is correct/ready to start.
Core staff – Generate the '**quote**', then submit the quote to User/PI for approval.
User - Upload the '**PI signed quote**', then clicks '**Agree**' to accept the work

3. Submit 'Reservations'

User - Submit the work '**Reservation**' through the iLab.
Core staff – Save and **approve 'Reservations'**, Click '**Begin**' when work starts.

4. Post work 'Completed'

Core staff – '**Reporting**', submit the report summary to PI and note the work completed.
Core staff – Create the '**Invoice**', and confirm with Judy '**Billing initiated**'.
Judy – Submit the '**Billing**' request to PI and financial officer, confirm with the core when payment completed.
Core staff – Click '**Finish Work**' once the work and payment are completed.

2. Submit 'Service Request'

User - Upload the 'Billing Form' and '**Sample Sheet**', submit the work request.

Sample Sheet

PROJECT INFORMATION

Project Title: **Study of Plasmodium Falciparum ART-resistant mutants**

Type of Samples: **Total RNA**

Number of Sample: **36**

Species name of organism for reference: **Plasmodium Falciparum3D7**

Size of Genome in Mbp: **23 Mbp**

Are there Reference Samples for Data Comparison? WT/Mutant; untreated/treated (yes/no): **Yes**

SEQUENCING INFORMATION (Miseq Output = ~15M reads; Nextseq Mid Output = ~120M reads; Nextseq High Output = ~350M reads; *reads number +/- 15% in each run)

Library Type (RNA-seq, gDNA-WGS, small RNA-seq, 16S rRNA-seq, Metagenomics Sequencing, Single-cell RNA-seq, etc.): **RNA-seq**

Sequence Read Length required (75, 150, 300 bp): **150 bp (300 cycle kit)**

Sequence Read Type required (Single read or Paired-end read): **Paired**

Sequence Depth required (millions of reads per sample: 2M, 5M, 10M, 20M, 40M): **5M**

Do you need consulting service for bioinformatic analysis of your sequencing data (sequence alignment, gene expression, metabolomics, proteomics, and other data analysis): **No**

Sample Sheet

CRITERIA

Please Read prior to entering sample information - All Fields Required

Sample Tubes: Please provide samples in 1.5 mL Eppendorf tubes

Clear Labels: Each sample tube should have a clearly labeled sample identifier. It should be less than 8 characters long

Amount of Material Required: Please provide no less than 2 ug of total RNA per sample for RNA-seq, 1 ug of gDNA for WGS

Concentration: All samples minimum concentration required 10 ng/ μ L (for RNA, or DNA)

Storage Solution: Purified nucleic acid storage solution should be RNase free aqueous solution such as DEPC treated water (for RNA), and nuclease free water or EB buffer for DNA)

SAMPLE INFORMATION - **All Fields Required**

Tube #	Sample Name	Amount of Material provided (ng)	NanoDrop Conc. (ng/ μ L)	RNA or DNA QC		Qubit Conc. (ng/ μ L)	Volume of Sample (μ l)	DNA/RNA sample prep kit	Notes : sample groups for data comparison, or any special instructions
				260/280	260/230				
1	001_pf_JA	2000				20	100	TRIzol Reagent (invitrogen)	
2	002_pf_JA	2000				20	100	TRIzol Reagent (invitrogen)	
3	003_pf_JA	2000				20	100	TRIzol Reagent (invitrogen)	
4	004_pf_JA	2000				20	100	TRIzol Reagent (invitrogen)	
5	005_pf_JA	2000				20	100	TRIzol Reagent (invitrogen)	
6	006_pf_JA	2000				20	100	TRIzol Reagent (invitrogen)	



Questions?

