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DNA extraction, 16S rRNA amplification and sequencing

Dr. Sérgio Rocha

27.06.2024

General approach

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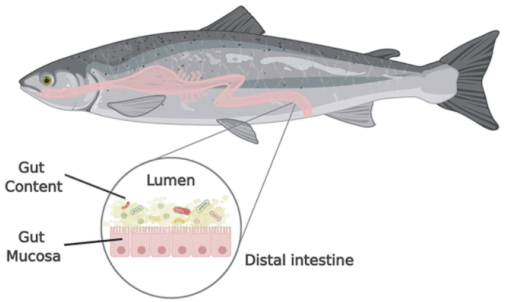
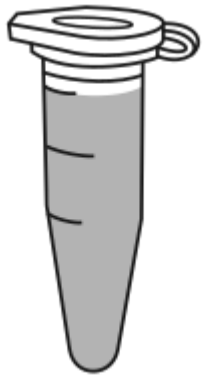


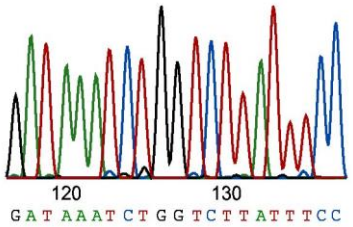


Digesta sample

16S rRNA gene amplification

16S rRNA

16S rRNA gene sequencing



DADA2 Amplicon Sequencing. Exactly. Version 1.14

silva high quality ribosomal RNA databases

Total DNA extraction → 16S amplificantion and library preparation → Sequecing → Data processing

Our M&M in Atlantic salmon

Norwegian University of Life Sciences

Weththasinghe *et al. Animal Microbiome* (2022) 4:9
https://doi.org/10.1186/s42523-021-00161-w

Animal Microbiome

RESEARCH ARTICLE Open Access

Modulation of Atlantic salmon (*Salmo salar*) gut microbiota composition and predicted metabolic capacity by feeding diets with processed black soldier fly (*Hermetia illucens*) larvae meals and fractions

Pabodha Weththasinghe[1*], Sérgio D. C. Rocha[1], Ove Øyås[1,2], Leidy Lagos[1], Jon Ø. Hansen[1], Liv T. Mydland[1] and Margareth Øverland[1*]

Agboola *et al. Animal Microbiome* (2023) 5:21
https://doi.org/10.1186/s42523-023-00242-y

Animal Microbiome

RESEARCH Open Access

Effect of yeast species and processing on intestinal microbiota of Atlantic salmon (*Salmo salar*) fed soybean meal-based diets in seawater

Jeleel O. Agboola[1*], Sérgio D. C. Rocha[1], Dominic D. Mensah[1], Jon Ø. Hansen[1], Ove Øyås[1,2], David Lapeña[2], Liv T. Mydland[1], Magnus Ø. Arntzen[2], Svein J. Horn[2] and Margareth Øverland[1*]

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Norway spruce extracts (NSEs) as bioactive compounds in novel feeds: Effect on intestinal immune-related biomarkers, morphometry and microbiota in Atlantic salmon pre-smolts

Sérgio D.C. Rocha[a], Byron Morales-Lange[a,*], Ruth Montero[a], Dawit Teklay Okbayohanese[a], Purushothaman Kathiresan[b], Charles McLean Press[b], Liv Torunn Mydland[a], Margareth Øverland[a,*]

frontiers | Frontiers in Immunology

From a cell model to a fish trial: Immunomodulatory effects of heat-killed Lactiplantibacillus *plantarum* as a functional ingredient in aquafeeds for salmonids

Sérgio Domingos Cardoso Rocha, Peng Lei, Byron Morales-Lange*, Liv Torunn Mydland and Margareth Øverland*

Department of Animal and Aquacultural Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, Ås, Norway

GitHub and GitLab

Total DNA extraction

DNA isolation from digesta samples

Norwegian University of Life Sciences

QIAamp® Fast DNA Stool Mini Handbook

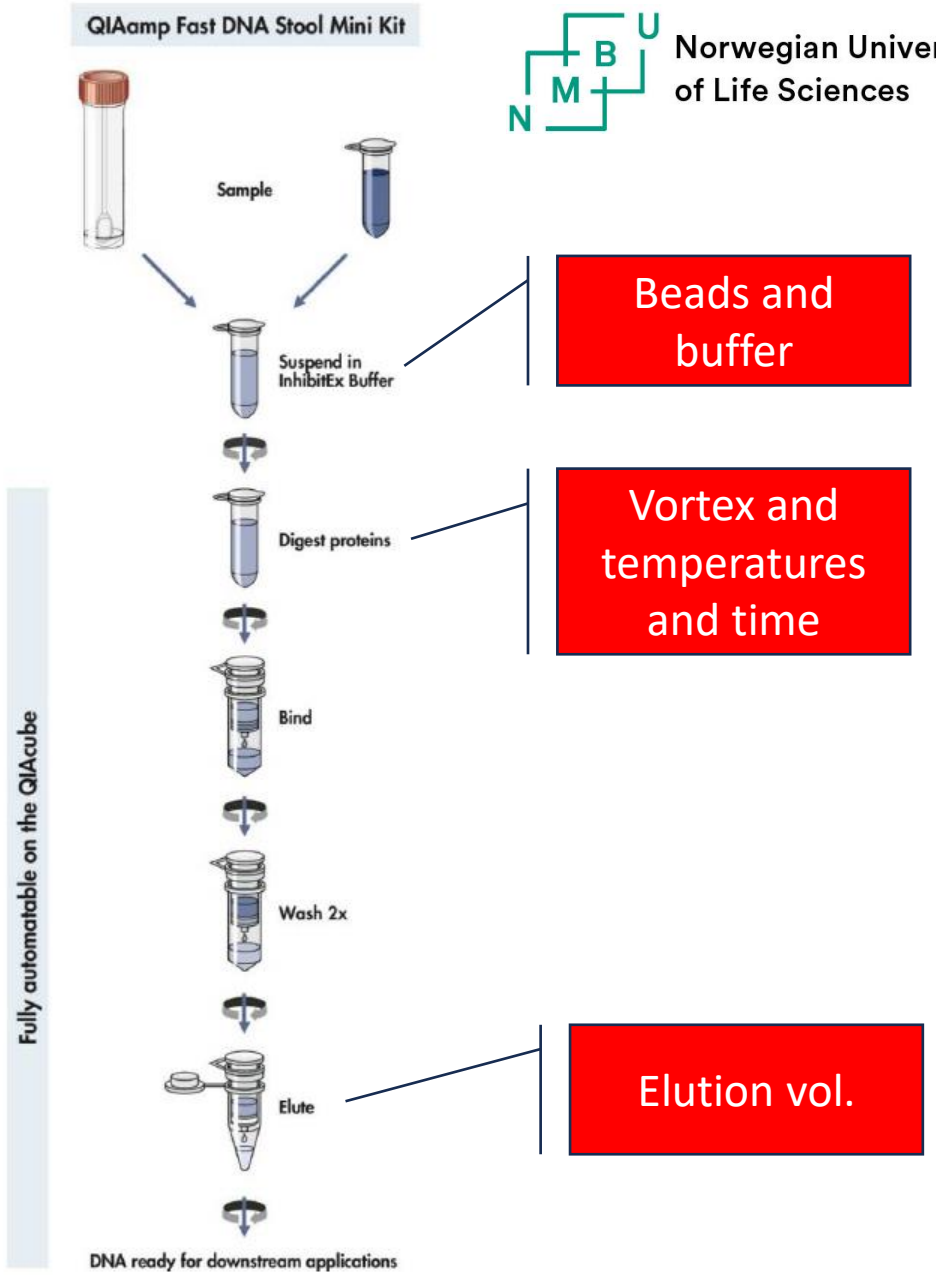
For fast purification of genomic DNA from stool samples

QIAGEN

Protocol: Isolation of DNA from Stool for Pathogen Detection

Lysis conditions in this protocol are optimized to increase the ratio of nonhuman DNA to human DNA. Human DNA is not excluded by this protocol.

→ Column method



Beads and buffer

Vortex and temperatures and time

Elution vol.

DNA isolation from other samples

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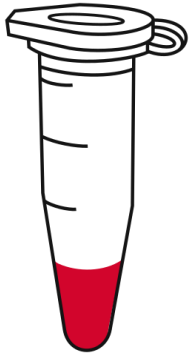
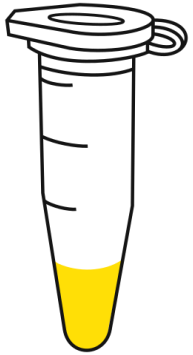
Feed



Grinded
frozen
pellets

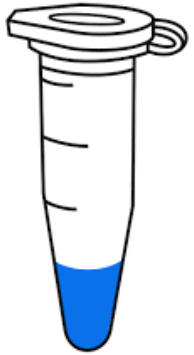
100mg feed
+
500uL ASL

Mock



75uL
+
200uL ASL

Water

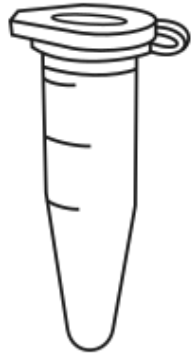


Filter paper from 500mL water
+
600uL ASL

Filter paper

Filter paper
+
600uL ASL

Blank



Mock sample

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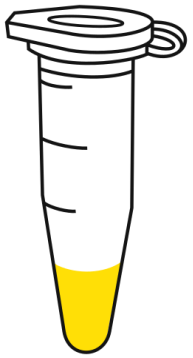
Mock

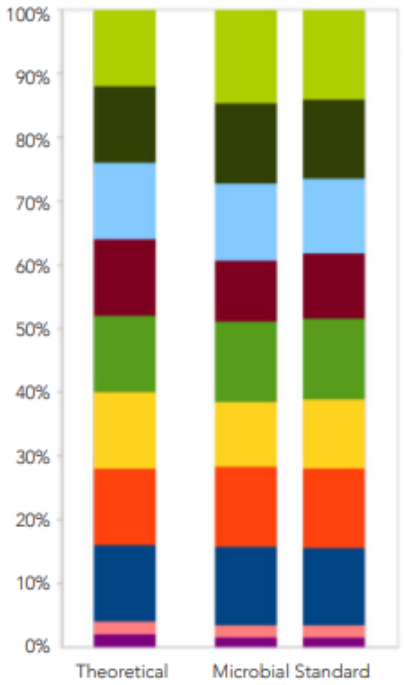


75uL

+

200uL ASL

Defined Microbial Community



Species	Avg. GC (%)	Gram Stain	gDNA Abun. (%)
Pseudomonas aeruginosa	66.2	-	12
Escherichia coli	56.8	-	12
Salmonella enterica	52.2	-	12
Lactobacillus fermentum	52.8	+	12
Enterococcus faecalis	37.5	+	12
Staphylococcus aureus	32.7	+	12
Listeria monocytogenes	38.0	+	12
Bacillus subtilis	43.8	+	12
Saccharomyces cerevisiae	38.4	Yeast	2
Cryptococcus neoformans	48.2	Yeast	2

The ZymoBIOMICS® Microbial Community Standard contains three easy-to-lyse bacteria, five tough-to-lyse bacteria, and two tough-to-lyse yeasts.

Zymo- BIOMICS™, Zymo Research, California, USA; catalog no., D6300

Mock sample

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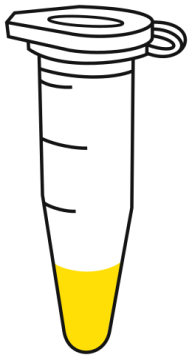
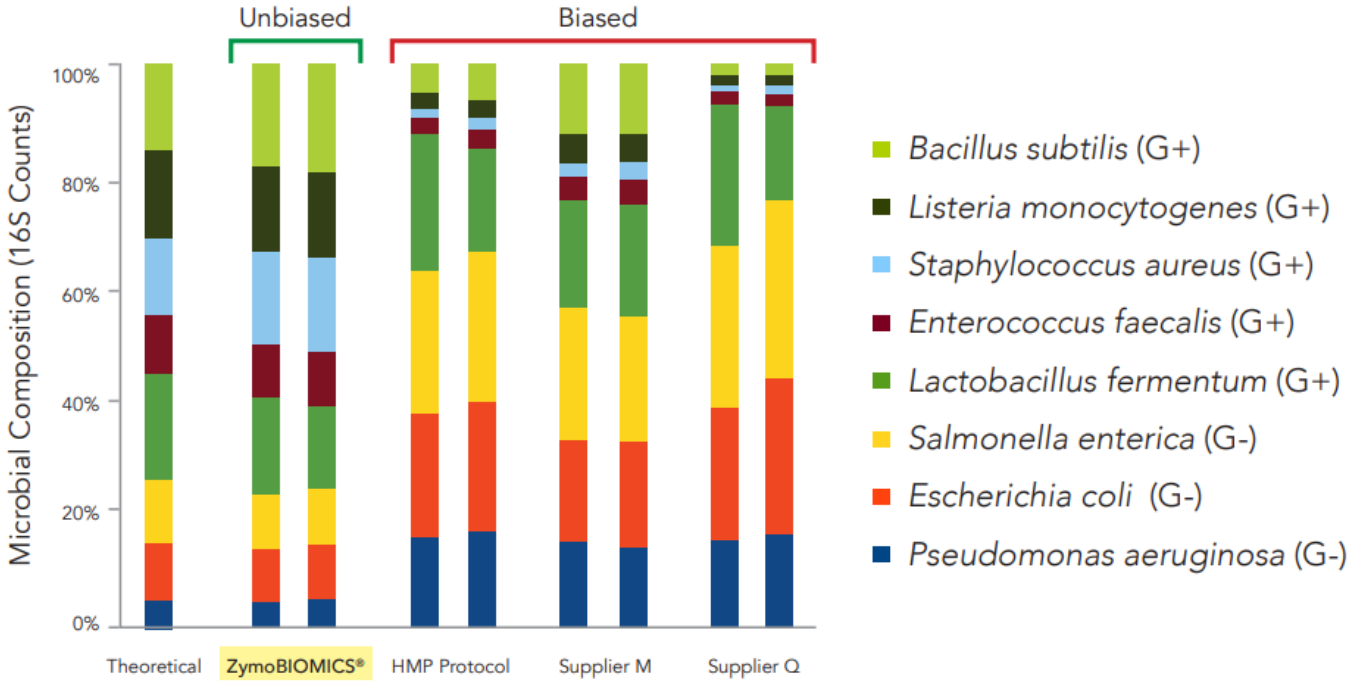
Mock



75uL

+

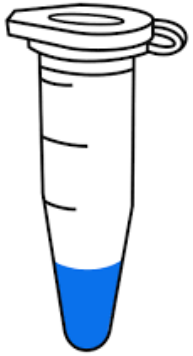
200uL ASL



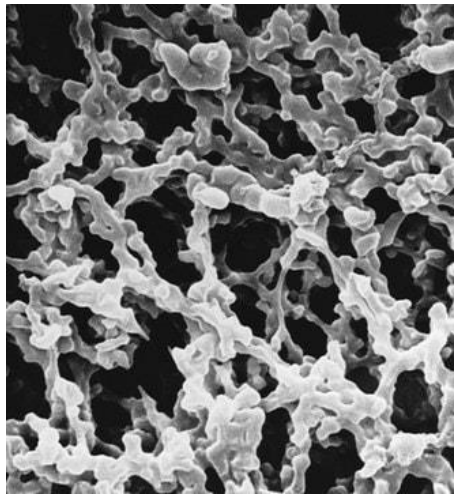
The ZymoBIOMICS® Microbial Community Standard was used to compare different DNA extraction protocols. DNA samples were profiled by 16S rRNA gene targeted sequencing.

Water

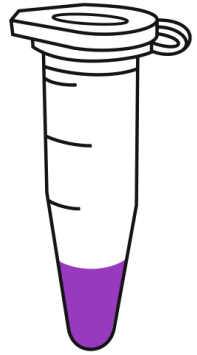
Water



(half) **Filter paper from 500mL water**
+
600uL ASL



Filter paper



Filter paper (wet with PCR water)
+
600uL ASL

16S rRNA amplification and library preparation

Amplification of 16S gene

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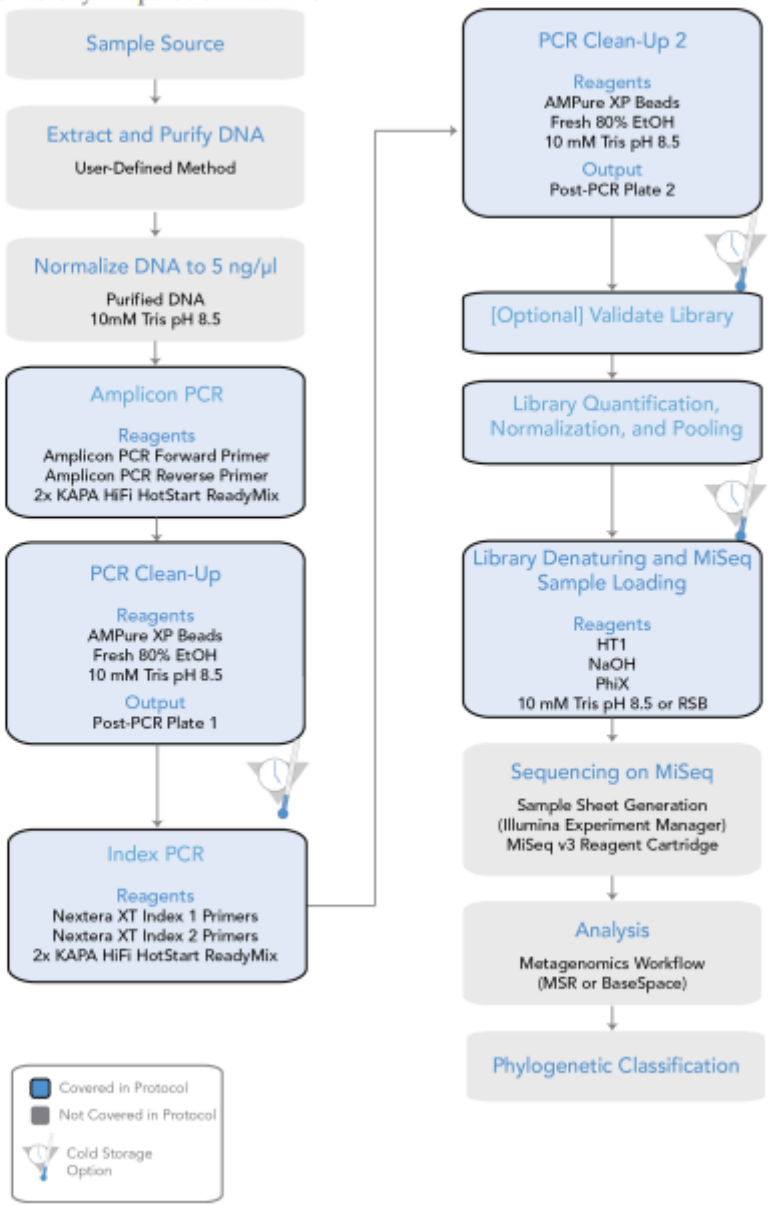
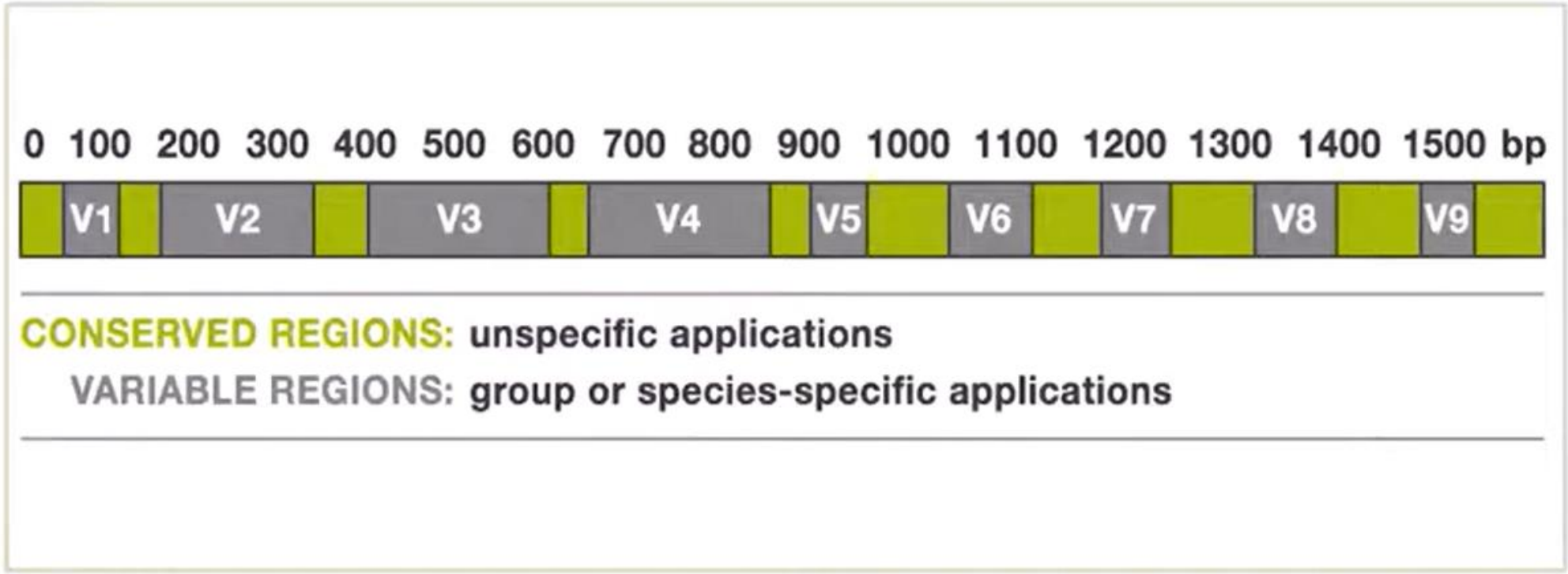
16S Metagenomic Sequencing Library Preparation

Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System

Figure 2 16S Library Preparation Workflow



Amplification of 16S gene



Amplification of 16S gene

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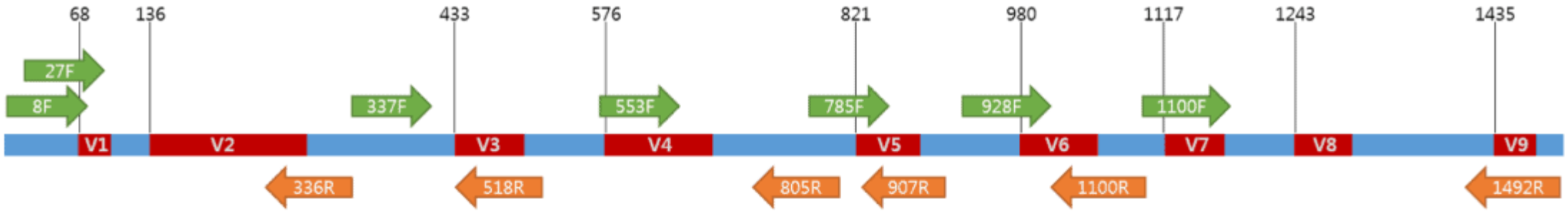


V1-V3 ~510 bp for Roche 454

V3-V4 ~428 bp for MiSeq PE

V3-V5 ~548 bp for Roche 454

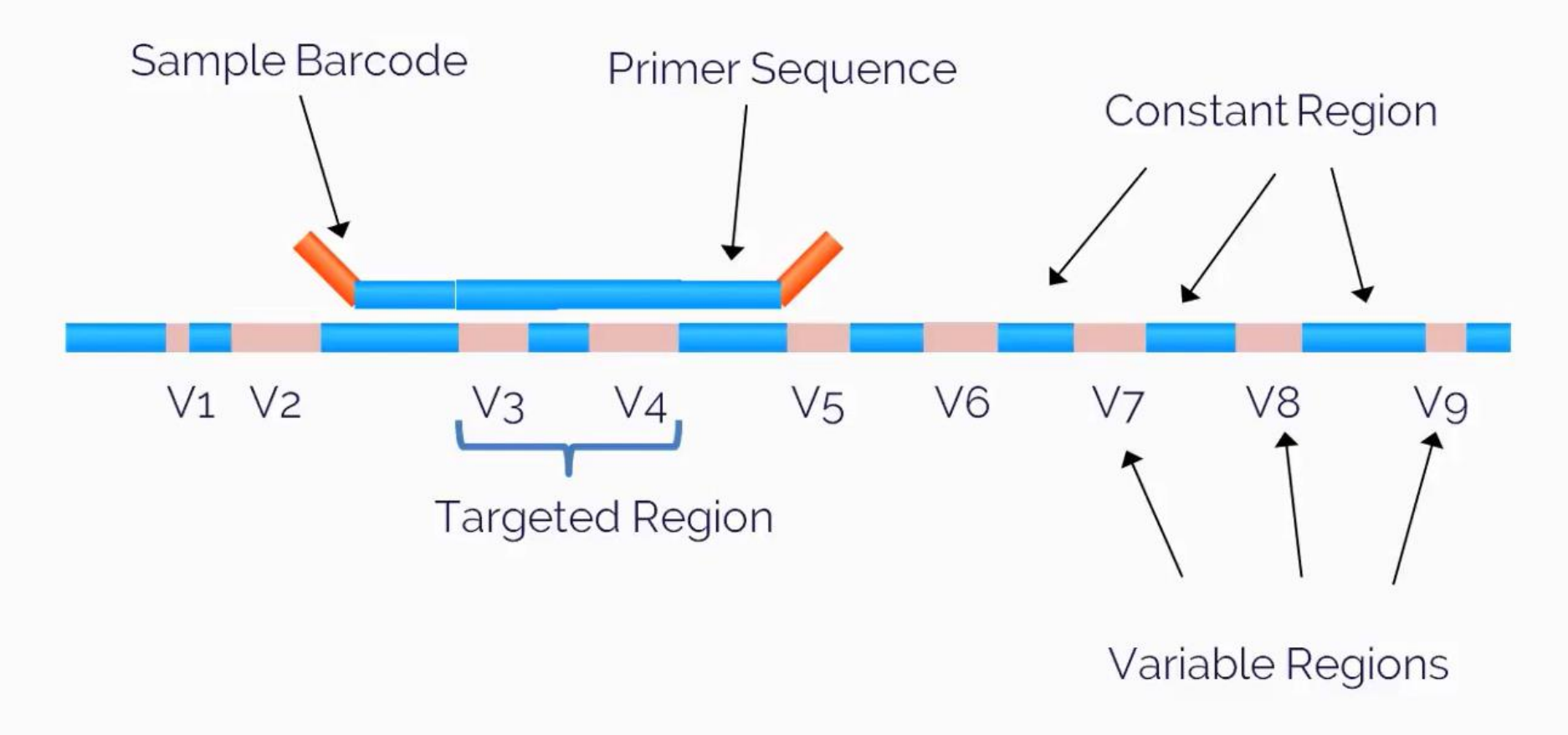
V4 ~252 bp for HiSeq

~562 bp for Roche 454 V6-V9

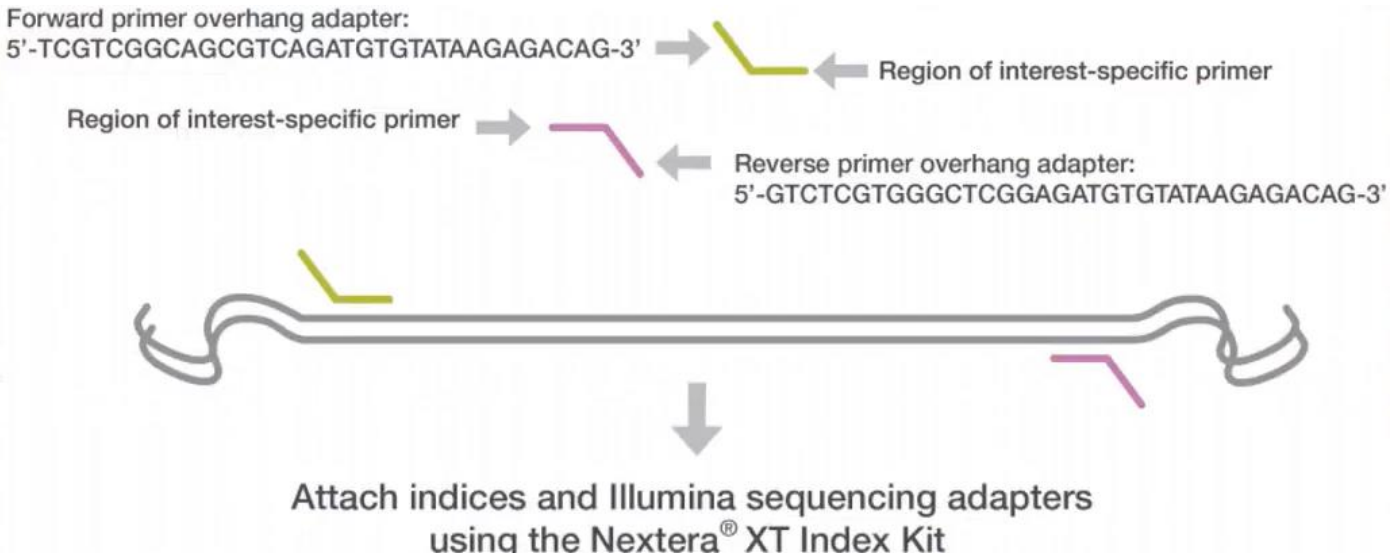
V1-V9 (Full-length)

Pacific Biosciences

Amplification of 16S gene



Amplification of 16S gene



Forward Primer:

5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG

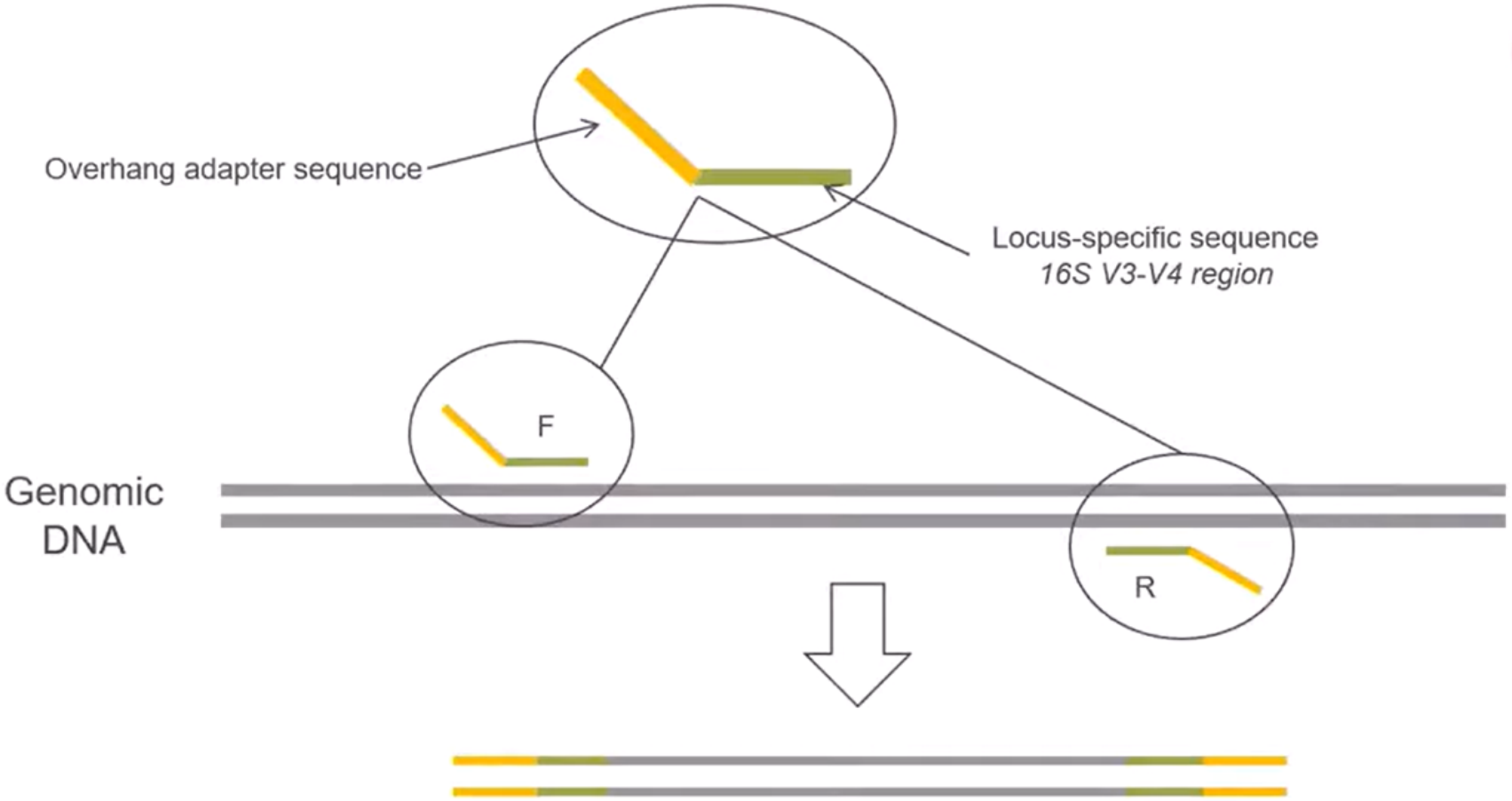
Overhang Adapter Sequence — Locus-Specific Sequence *16S V3-V4*

Reverse Primer:

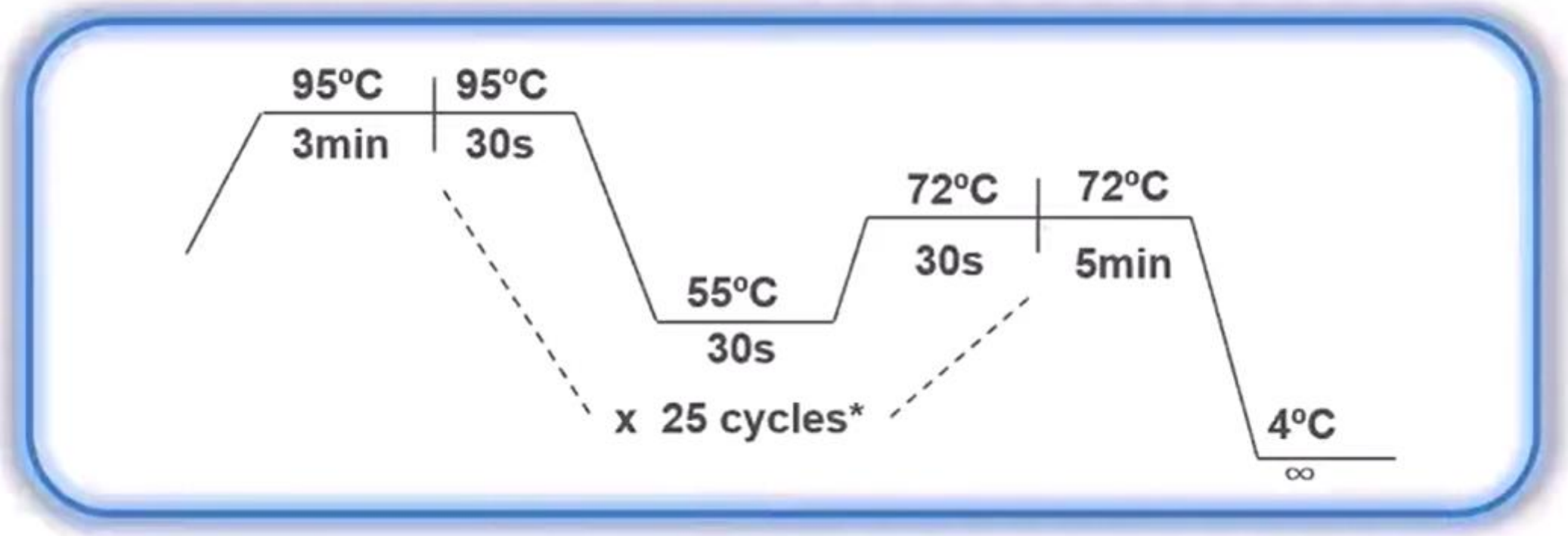
5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC

Overhang Adapter Sequence — Locus-Specific Sequence *16S V3-V4*

Amplification of 16S gene



Amplification of 16S gene



Clean up with Ampure XP beads

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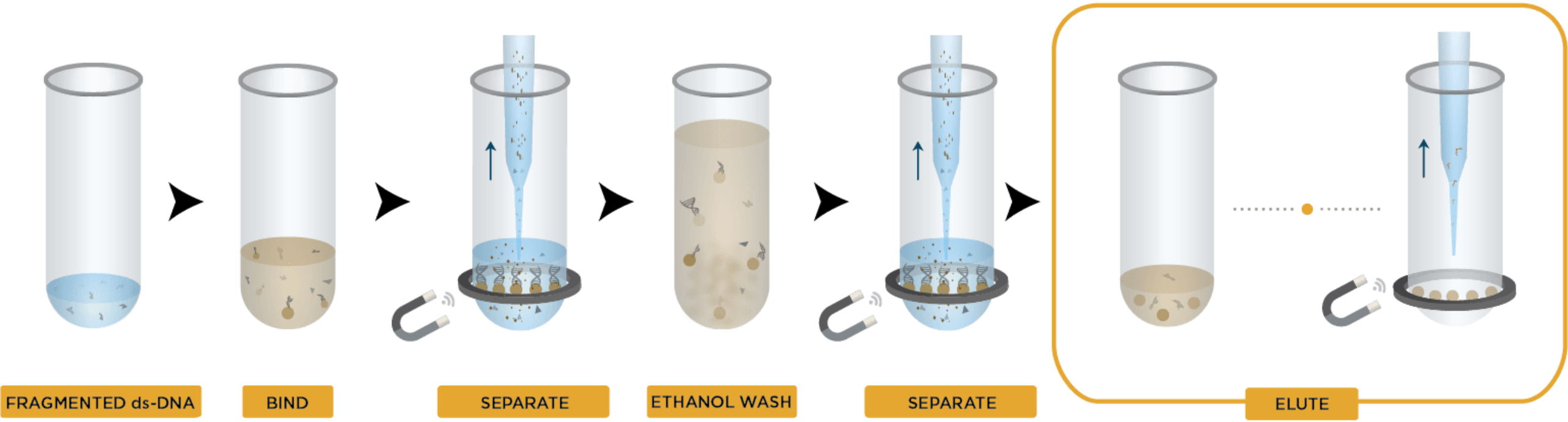
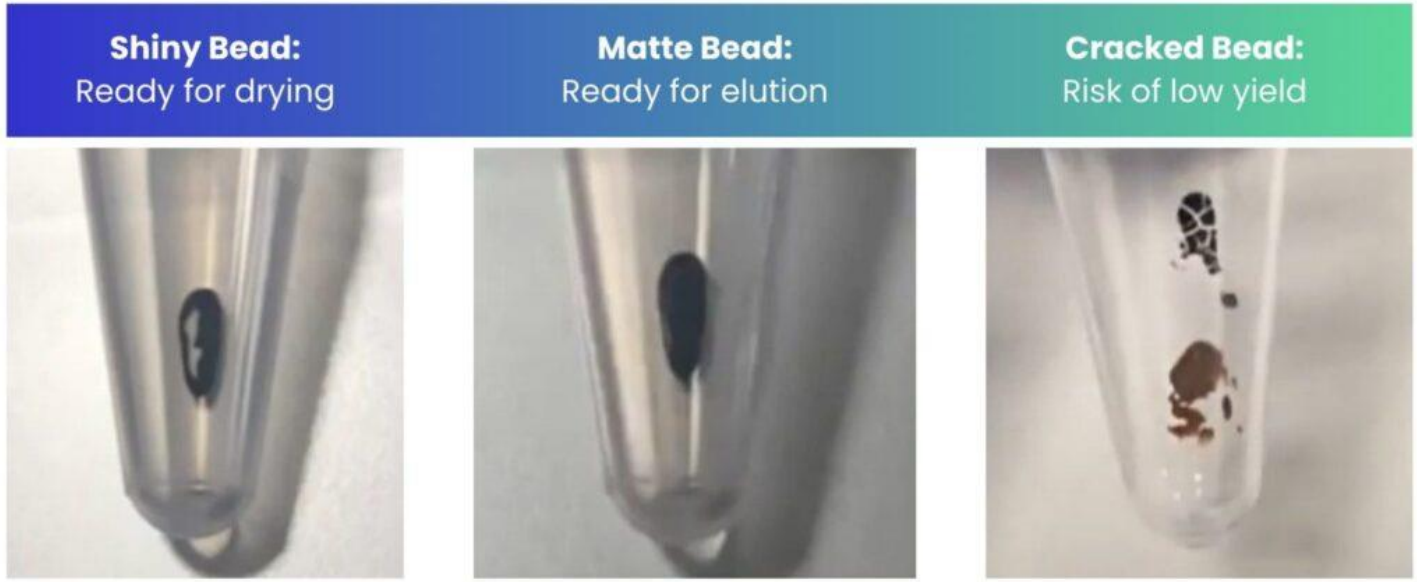
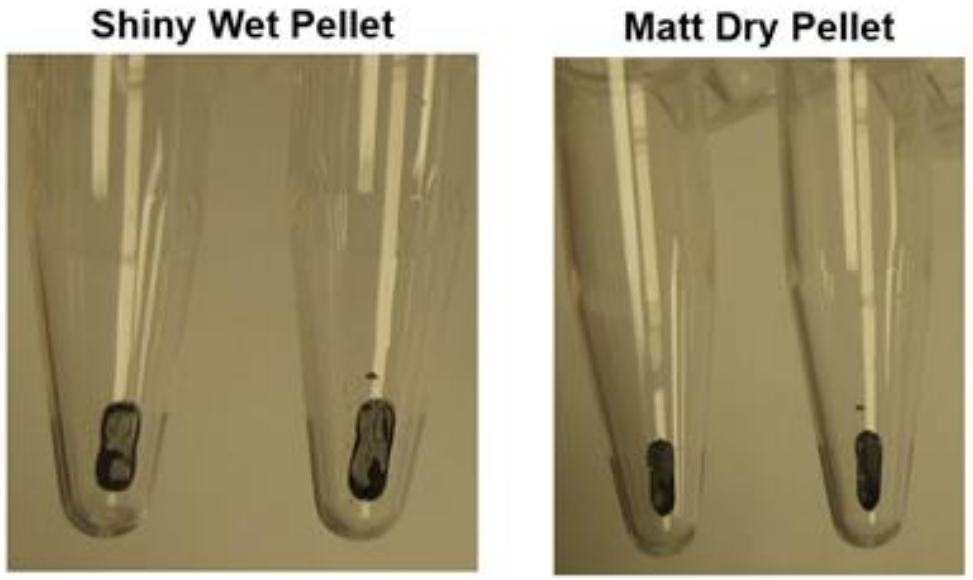
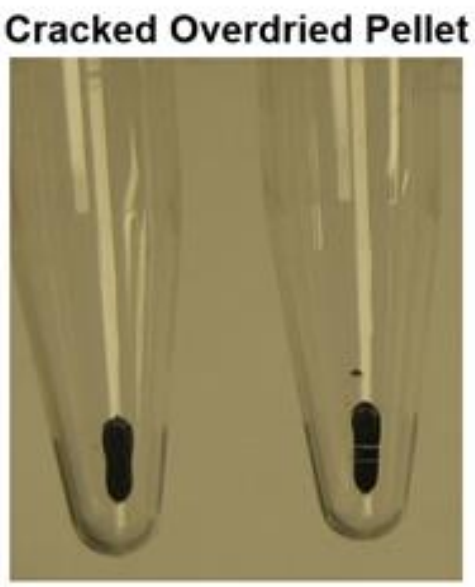


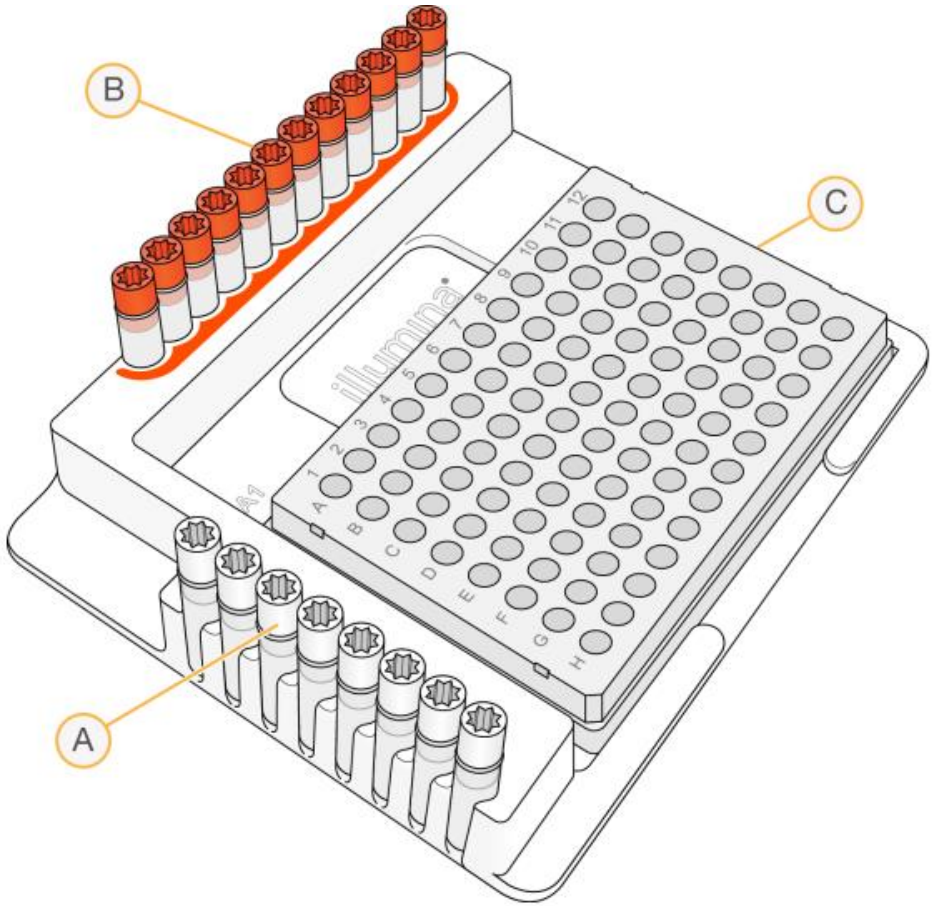
Clean up with Ampure XP beads







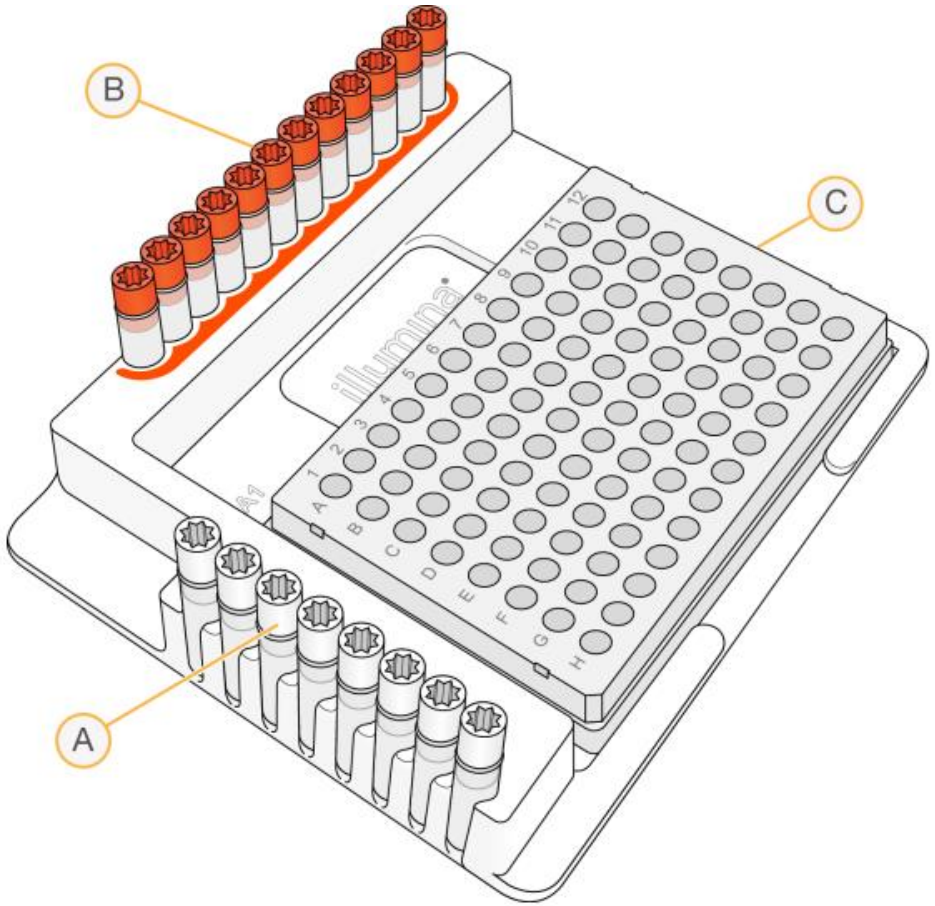
Library construction



A Index 2 primers (white caps)
B Index 1 primers (orange caps)
C 96-well plate

Index 1 (i7)	Sequence	Index 2 (i5)	Sequence
N701	TAAGGCGA	S501	TAGATCGC
N702	CGTACTAG	S502	CTCTCTAT
N703	AGGCAGAA	S503	TATCCTCT
N704	TCCTGAGC	S504	AGAGTAGA
N705	GGACTCCT	S505	GTAAGGAG
N706	TAGGCATG	S506	ACTGCATA
N707	CTCTCTAC	S507	AAGGAGTA
N708	CAGAGAGG	S508	CTAAGCCT
N709	GCTACGCT		
N710	CGAGGCTG		
N711	AAGAGGCA		
N712	GTAGAGGA		

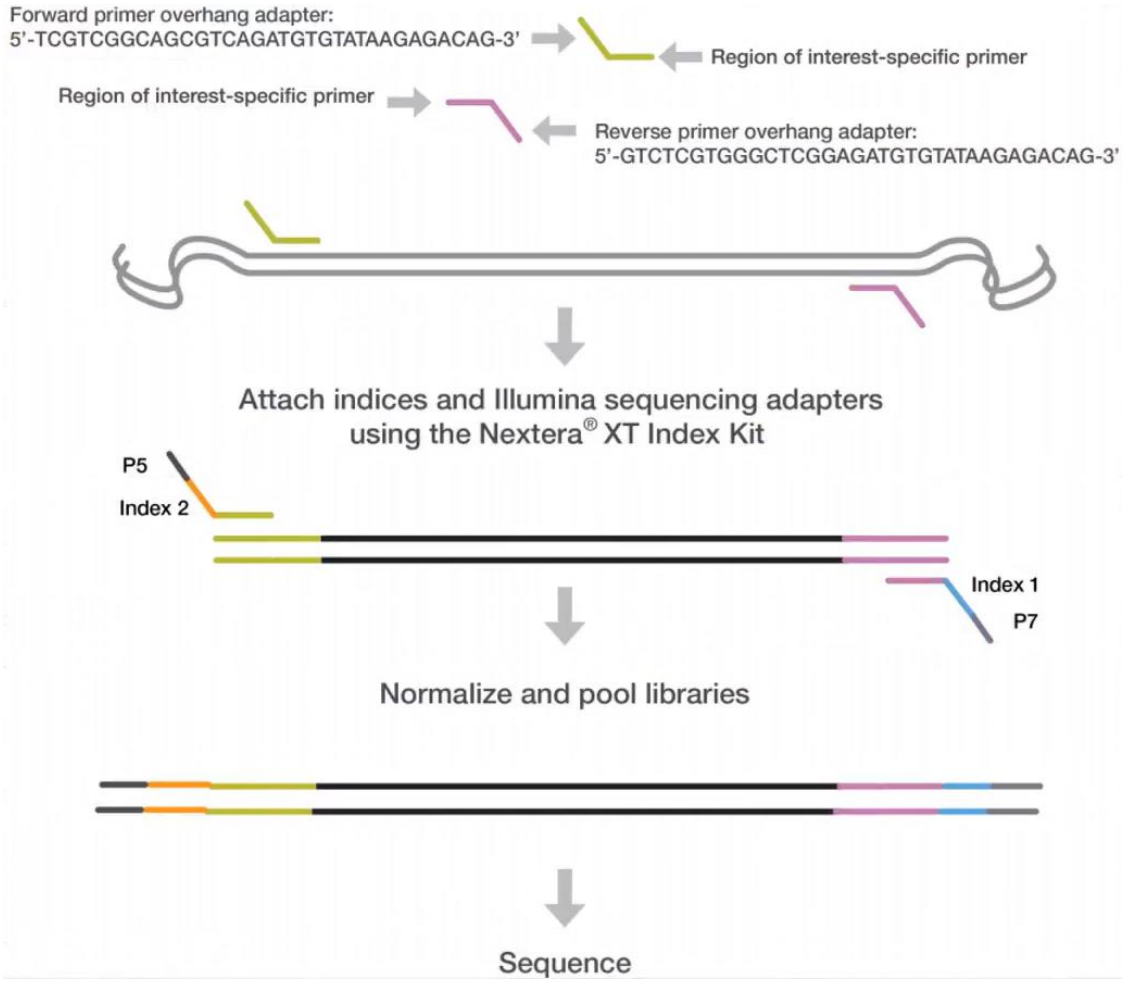
Library construction



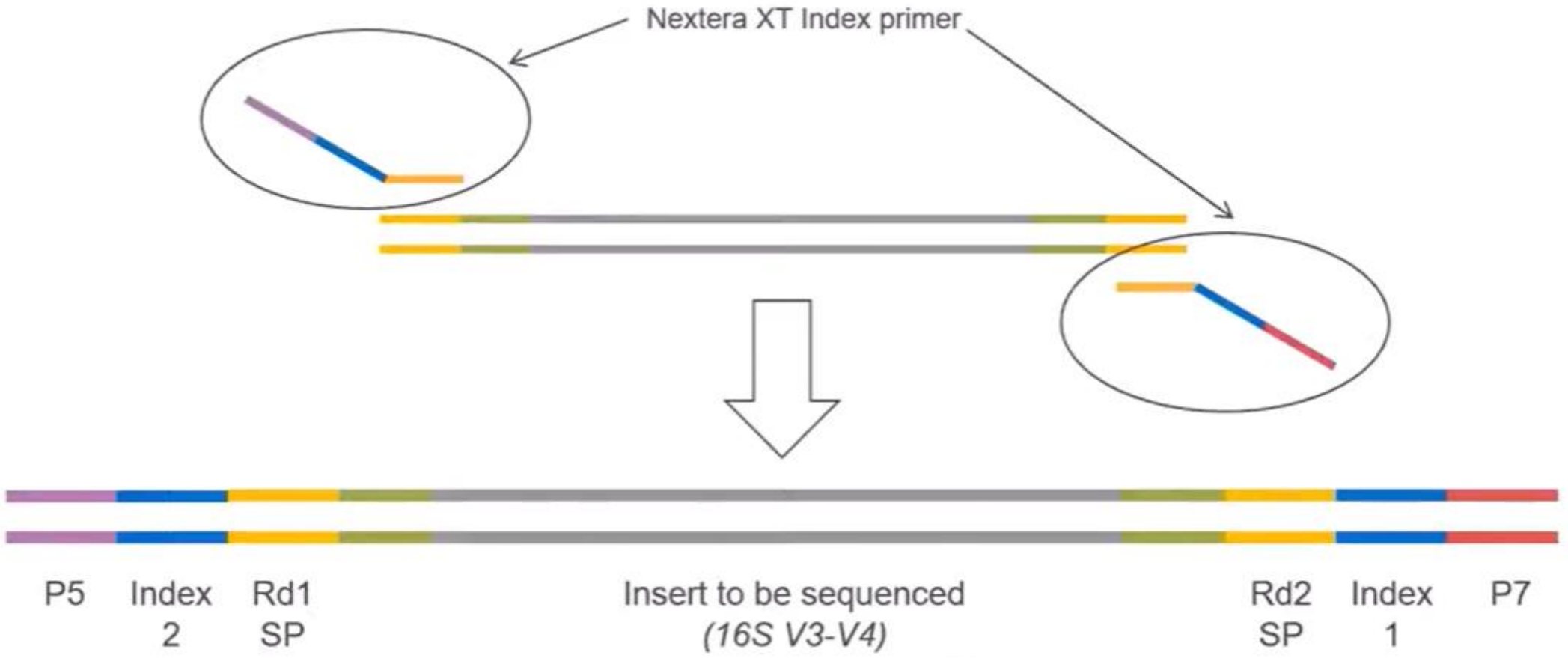
A Index 2 primers (white caps)
B Index 1 primers (orange caps)
C 96-well plate

	A	B	C	D	E
	N701	N702	N703	N704	N705
1	S501					
2	S502					
3	S503					
4	S504					
5	S505					

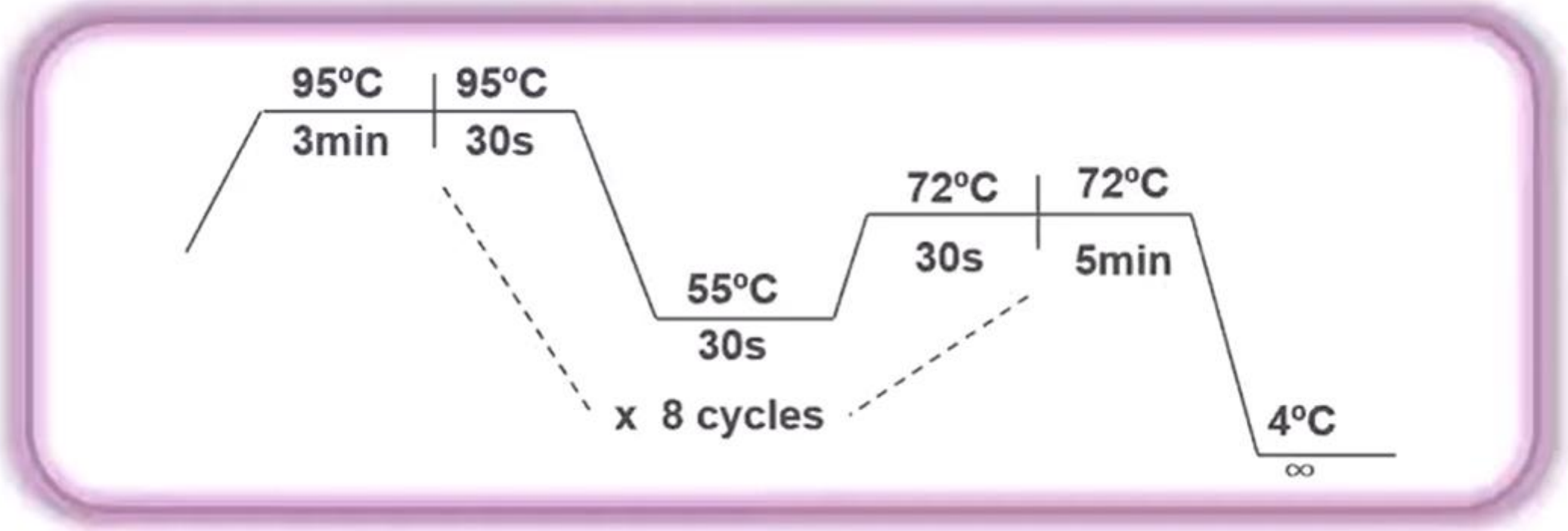
Index PCR


Forward primer overhang adapter:
5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'
Region of interest-specific primer
Region of interest-specific primer
Reverse primer overhang adapter:
5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'
Attach indices and Illumina sequencing adapters
using the Nextera® XT Index Kit
P5
Index 2
Index 1
P7
Normalize and pool libraries
Sequence

Index PCR


Nextera XT Index primer
P5
Index 2
Rd1 SP
Insert to be sequenced
(16S V3-V4)
Rd2 SP
Index 1
P7

Index PCR



Quality control

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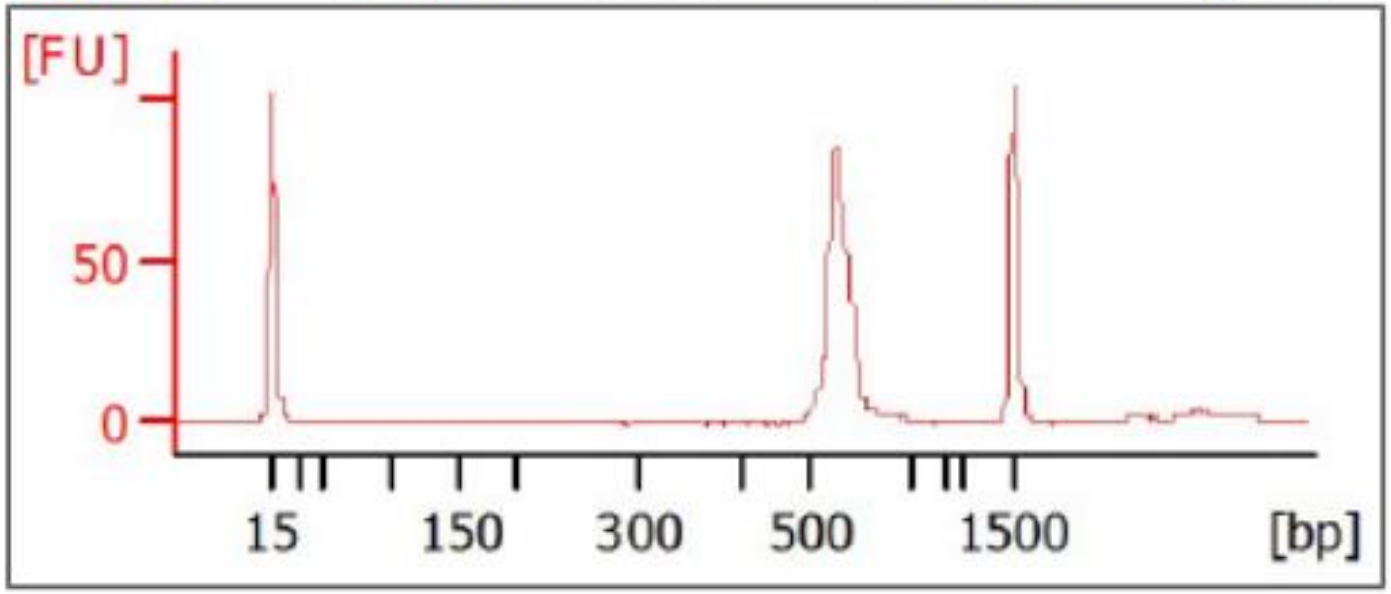
Figure 3 Example Bioanalyzer Trace after Amplicon PCR Step



Sequencing

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Sequencing

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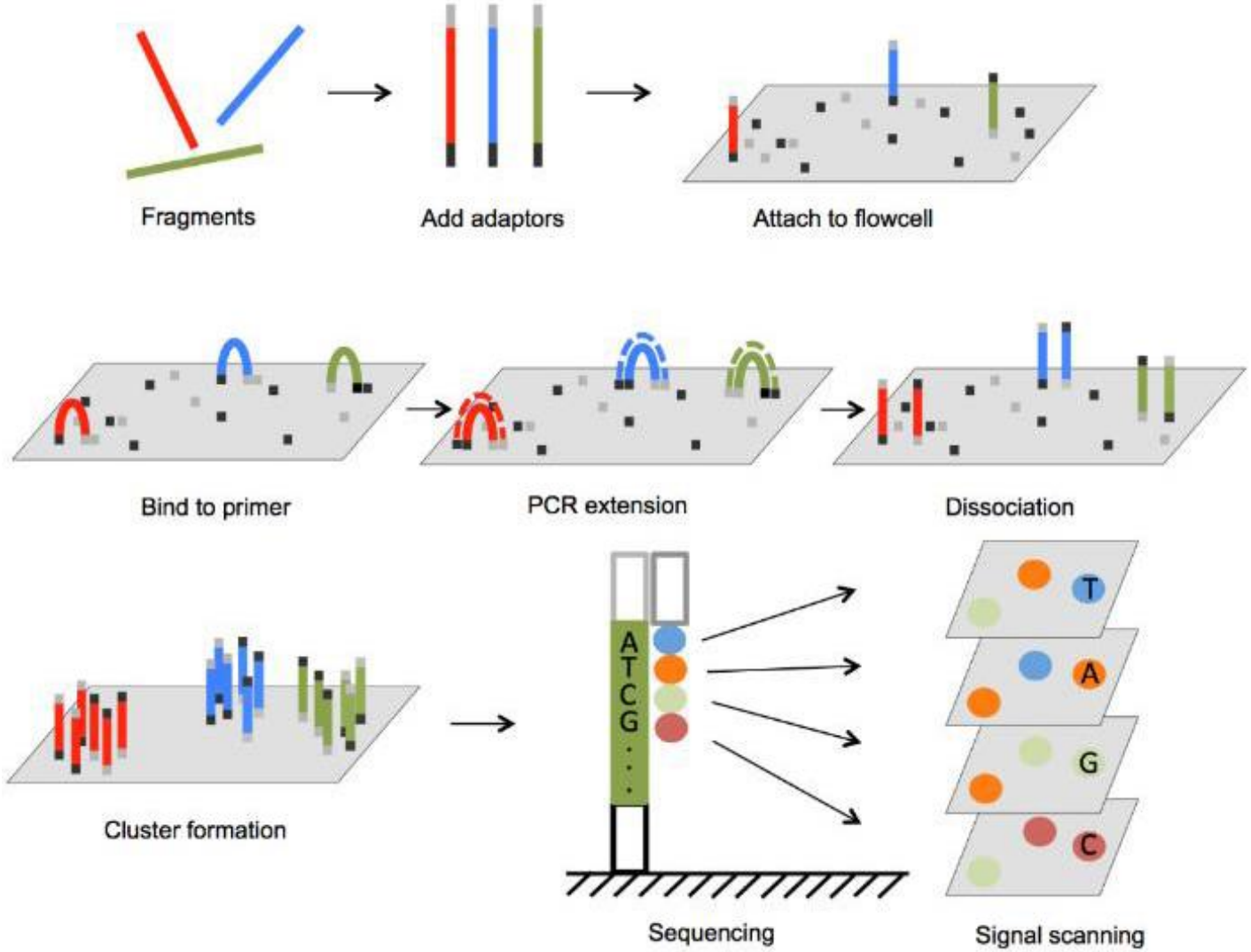
Library Pooling Considerations

- Pool sample libraries into MiSeq run
- Total MiSeq v3 run output is > 20 million paired-end reads
- This will generate >100,000 reads per sample which is commonly recognized to be sufficient for metagenomic surveys

Sequencing Considerations

- Optimal raw cluster density is 800-1000k/mm^2
- Cluster densities below 500k/mm^2 and above 1000k/mm^2 is not recommended
- Spike-in minimum 5% PhiX library to serve as an internal control for low-diversity library

Sequencing


Fragments
Add adaptors
Attach to flowcell
Bind to primer
PCR extension
Dissociation
Cluster formation
ATCG...
Sequencing
T
A
G
C
Signal scanning