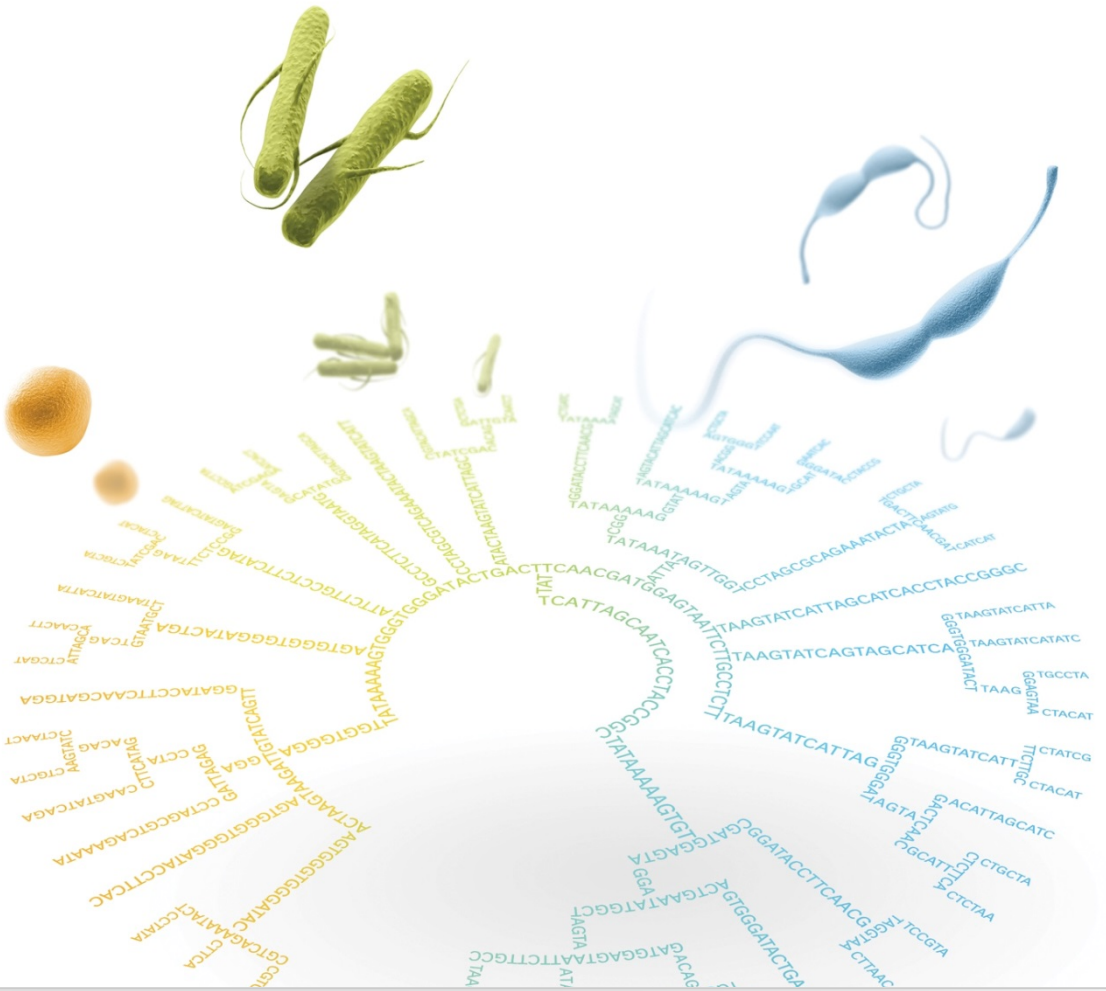
16S Metagenomics Sequencing Workflow



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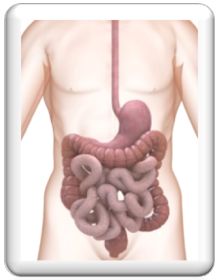
Metagenomics and Microbial Diversity

Diverse Applications

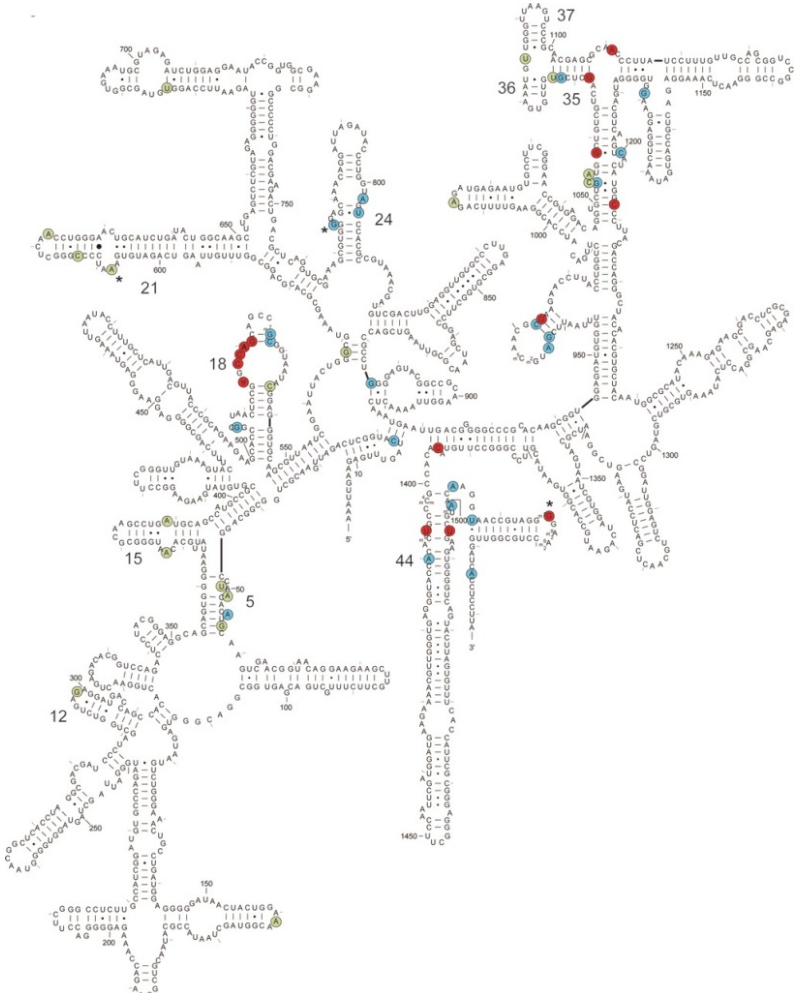
Metagenomic studies can give insight into:

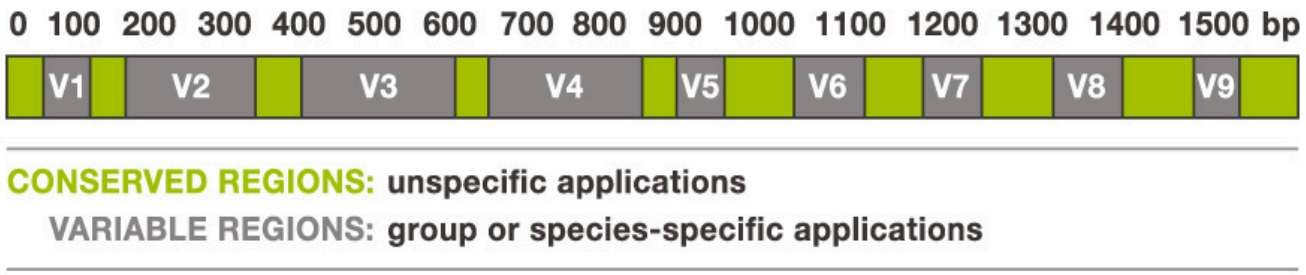
- Microbial diversity in an environmental habitat
- Abundance of microbial species
- Gene content in a sample and the discovery of novel genes
- Meta-transcriptomics: analysis of the expressed genome in a sample
- Pathogen detection
- Signature profile of an environment or disease state



16S rRNA Gene Sequencing

- 16S rRNA is a part of the **ribosomal RNA** of prokaryotic cells which is about 1,542 nucleotides long.
- It has been observed that this molecule contains regions which are highly conserved (not altered much due to mutation) among species.
- Thus if these molecules are sequenced and the sequences of various species are compared the microbes can be phylogenetically classified.
- **This is a powerful tool used for classification and genome analysis.**





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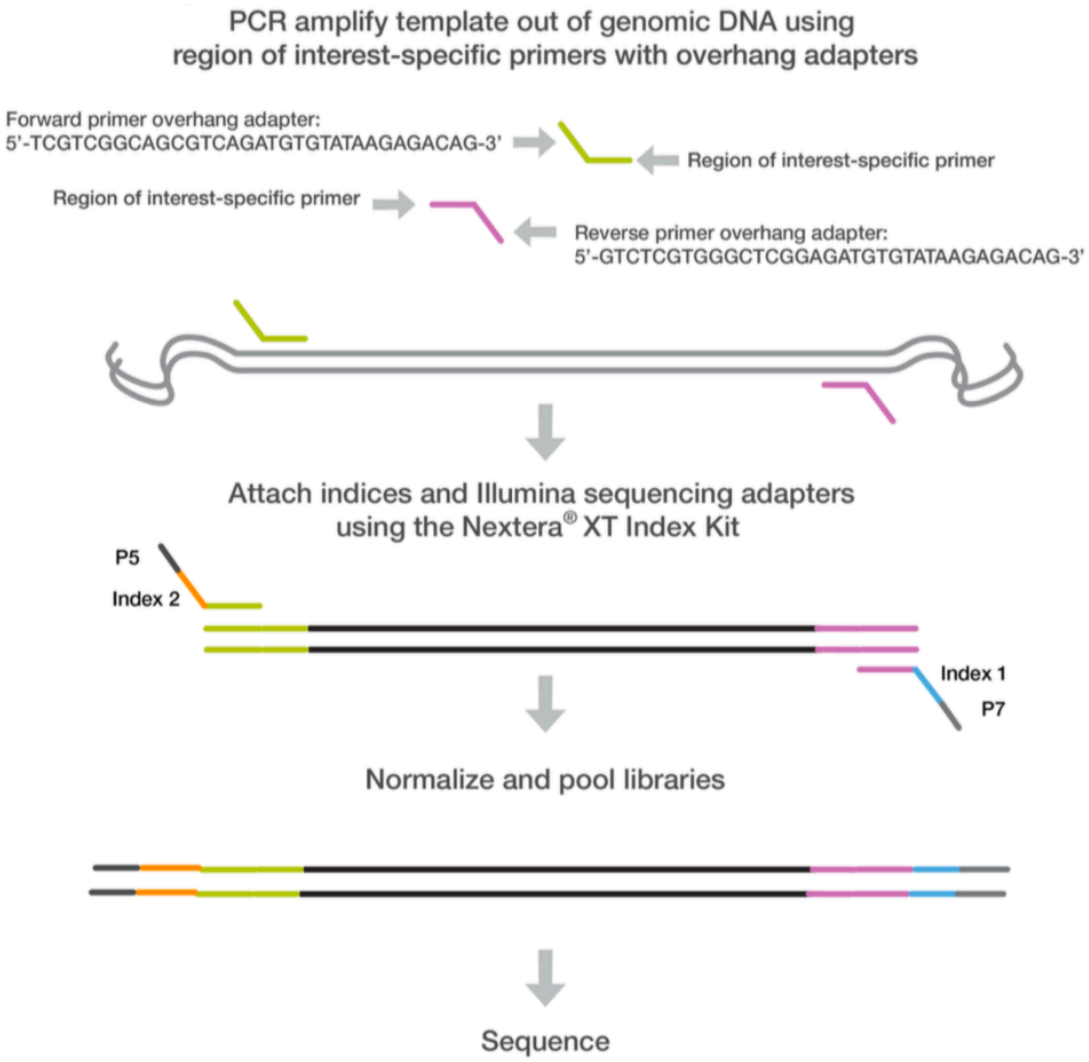
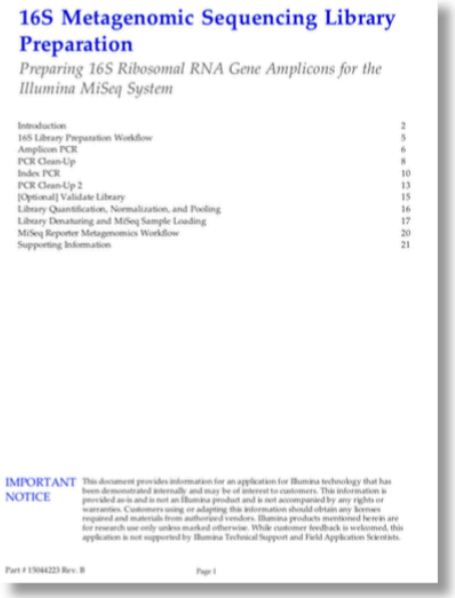
16S Metagenomics Library Preparation Workflow

Illumina-demonstrated protocol





16S Metagenomic Sequencing Library Preparation

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

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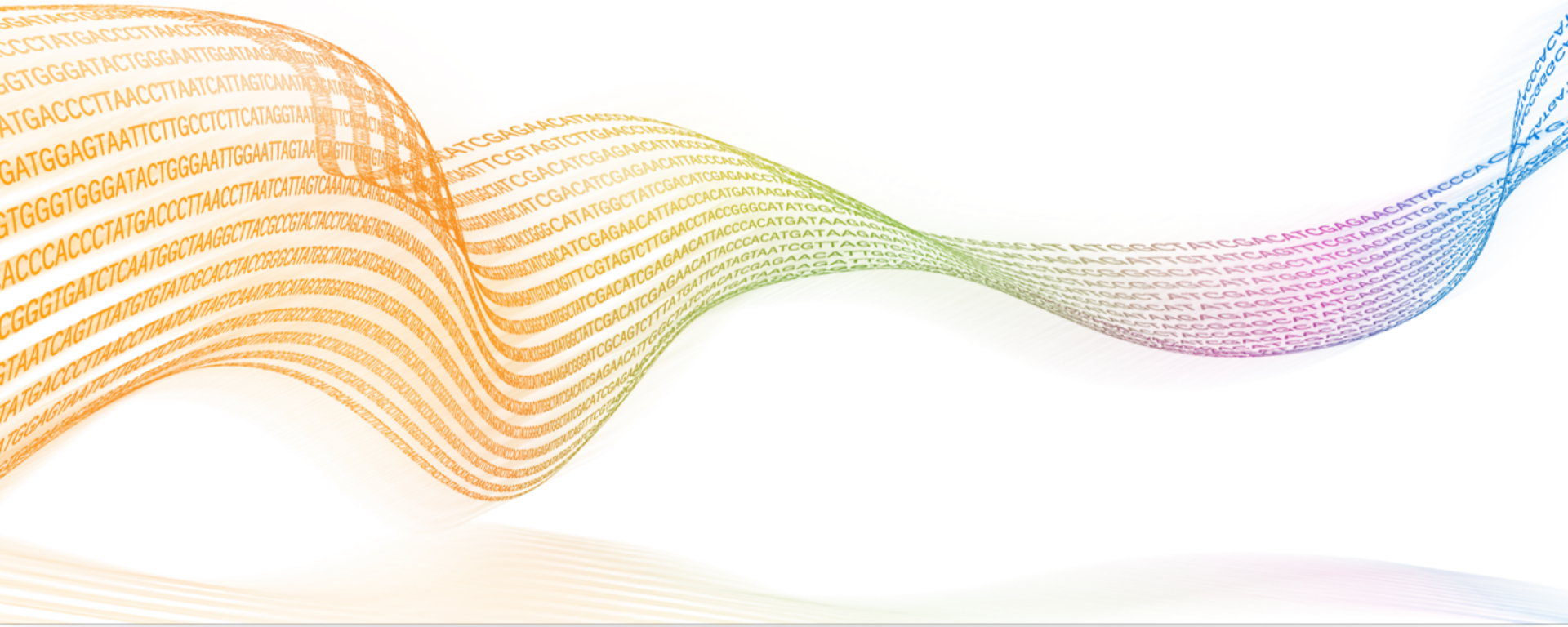
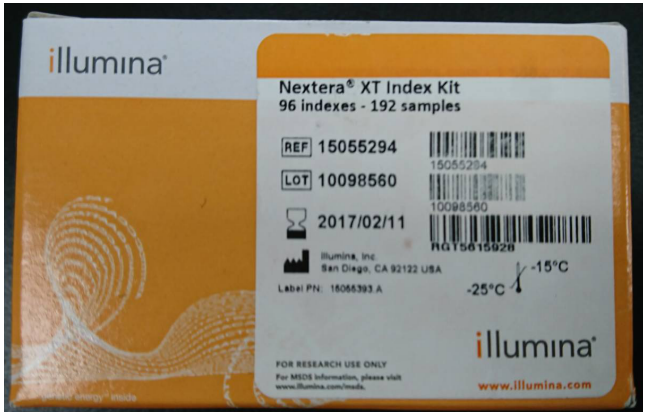
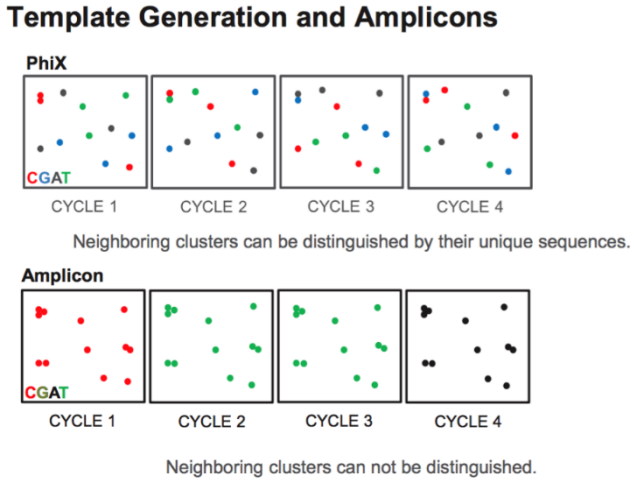
Required Reagents and Equipment



What do I need for 16S Metagenomics workflow?

Items to Order from Illumina





Catalog Number	Product Description
FC-131-2001~2004 20027213	**Nextera XT Index Kit v2 Set A~D, 384 samples (96 indices)** **IDT for Illumina Nextera DNA UD index Set A, 96 samples (96 indices)**
FC-110-3001	**PhiX Control v3**
(2019 Q2) or MS-102-3003	**iSeq 100 i1 Reagent (500cycle Single Kit) or** **MiSeq Reagent Kit v3**
FC-130-1005 (Optional)	**TruSeq Index Plate Fixture and Collar Kit (2 Each)**

What do I need for 16S Metagenomics workflow?

User-supplied equipment and consumables

Equipment for library prep
Plate centrifuge
Magnetic stand (96-samples)
Thermocycler

Reagents for library prep
Forward & Reverse oligo primers
AMPure XP Beads
KAPA HiFi HotStart Ready Mix

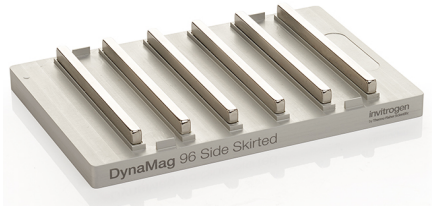
Equipment for input & library QC
Fluorometer (Qubit preferred)
Bioanalyzer 2100

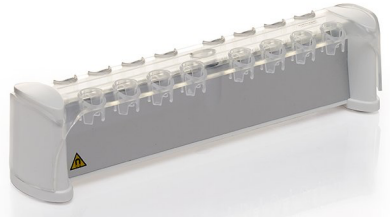
Reagents for input & library QC
Fluorometric DNA Quant kit (Qubit preferred)
Bioanalyzer DNA 1000 Kit

KAPA HiFi HotStart Ready Mix

Ethyl alcohol

Magnetic stand





Thermal Cycler

Beckman Coulter AMPURE XP 60ML

encompass

Manufacturer: Beckman Coulter A63881

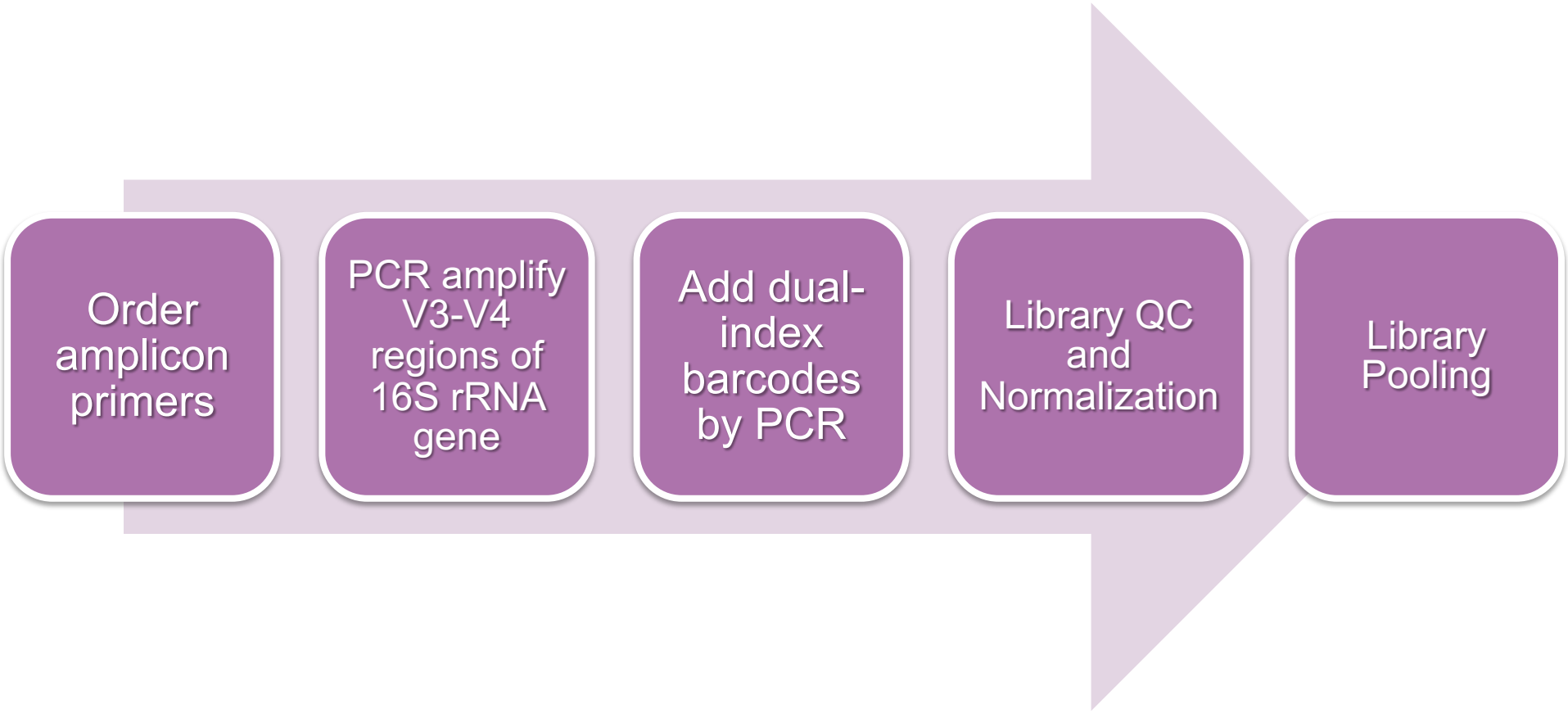
Agencourt AMPure XP 60 mL Kit: size 1,666/3,333 preps with kit components Agencourt AMPure XP Reagent The Agencourt AMPure XP system is a highly efficient, easily automated PCR purification system that delivers superior quality DNA with no salt carryover. Requiring no centrifugation or filtration, Agencourt AMPure XP can be easily used in manual and automated 96- or 384-well formats

Qubit

Bioanalyzer

16S Metagenomics Library Preparation Workflow

Illumina-demonstrated protocol



Step 1: Order Amplicon Primers

Primers target 16S rRNA gene V3-V4 region

- A single amplicon of approximately **460 bp** is created
- Primers **include overhang** adaptor sequences
- Illumina recommends using **standard desalting purification** when ordering oligo primer sets

Forward Primer:

5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTACGGGNGGCWGCAG

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

Reverse Primer:

5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GACTACHVGGGTATCTAATCC

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

DNA Isolation Kits

Epicentre DNA isolation kits are optimized to isolate bacterial DNA obtained from various environments

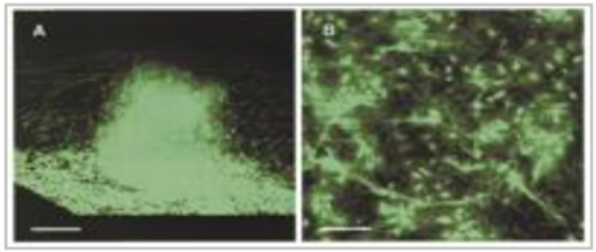
Sample Type	Epicentre Isolation Kit
Water	**Metagenomic DNA Isolation Kit for Water**
Soil	**SoilMaster DNA Extraction Kit**
Fecal matter	**ExtractMaster Fecal DNA Extraction Kit**
Difficult-to-culture species present in environmental water, soil, or compost	**Meta-G-Nome DNA Isolation Kit**

DNA Quantitative Analysis

DNA input of only 12.5ng of microbial genomic DNA
- 260/280 = 1.8 – 2.0

Quantify genomic DNA use fluorescence-based quantification method
- Qubit
- PicoGreen

PicoGreen®



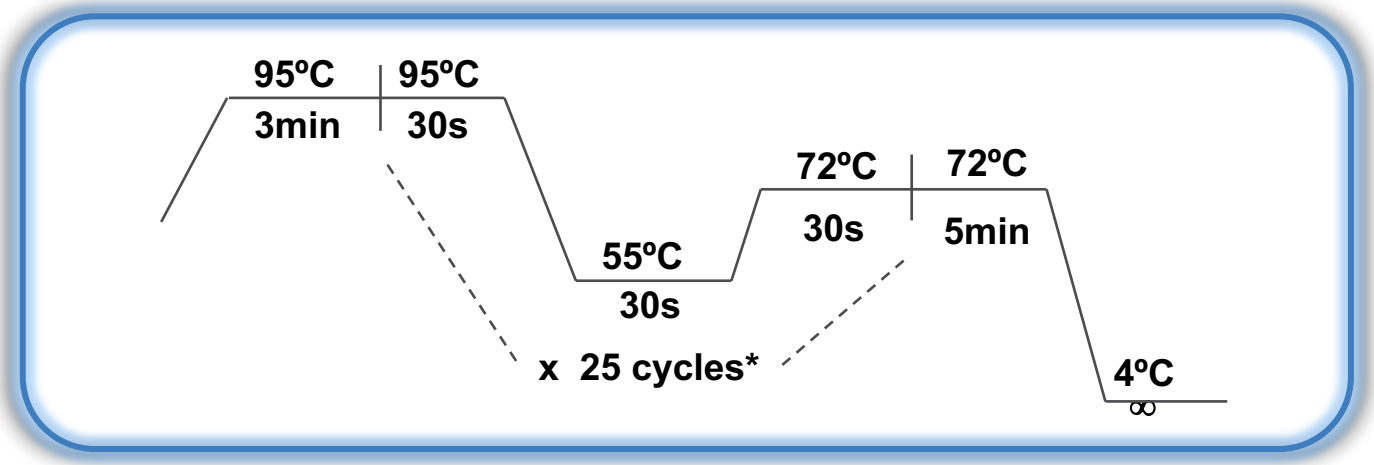
- Easier for high-throughput
- Fluorometer plate reader available

Qubit™

- Very easy-to-use
- Good for smaller number of samples

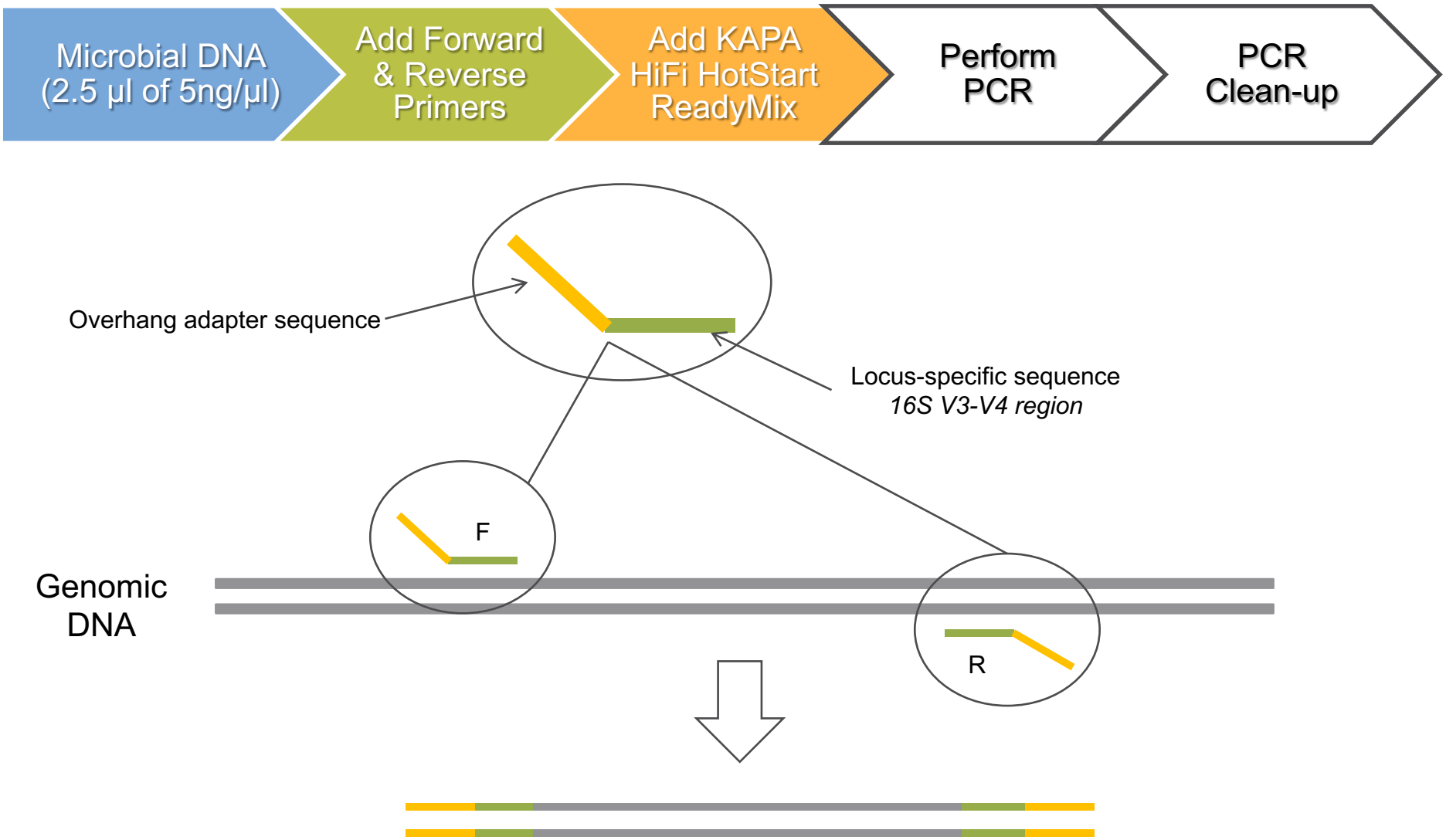
Step 2: PCR Amplify V3-V4 regions of 16S rRNA gene

Microbial DNA (2.5 µl of 5ng/µl) → Add Forward & Reverse Primers → Add KAPA HiFi HotStart ReadyMix → Perform PCR → PCR Clean-up



* Number of PCR cycles may have to be optimized for different samples

Step 2: PCR Amplify V3-V4 regions of 16S rRNA gene



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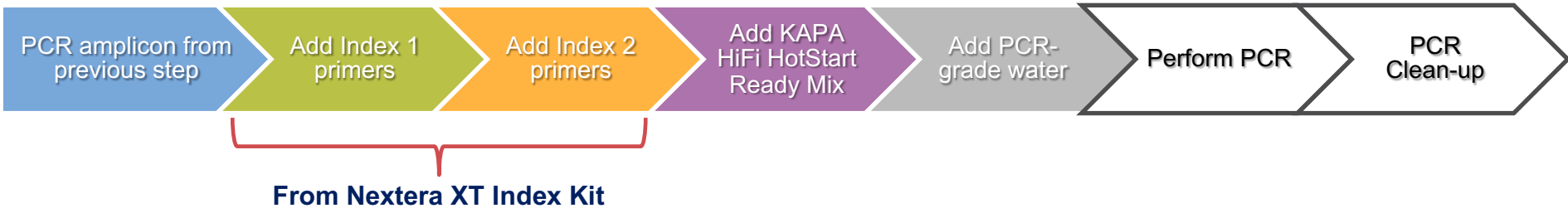
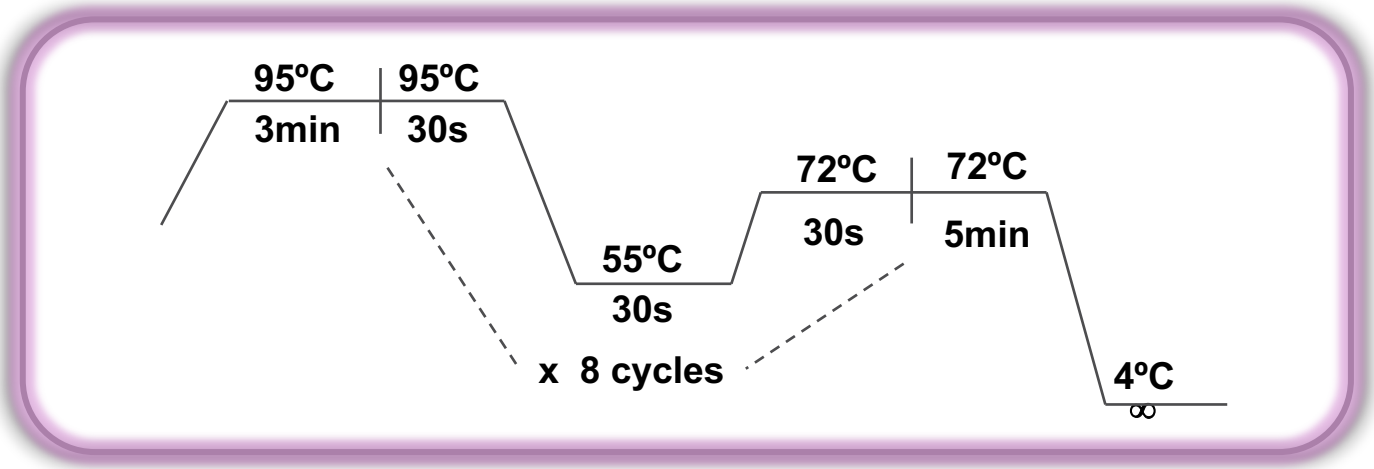
Step 3: 2nd PCR to Add Indices and Adaptors





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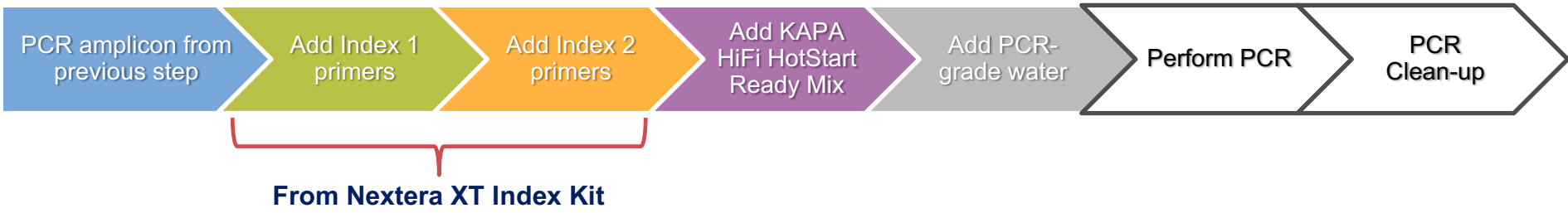
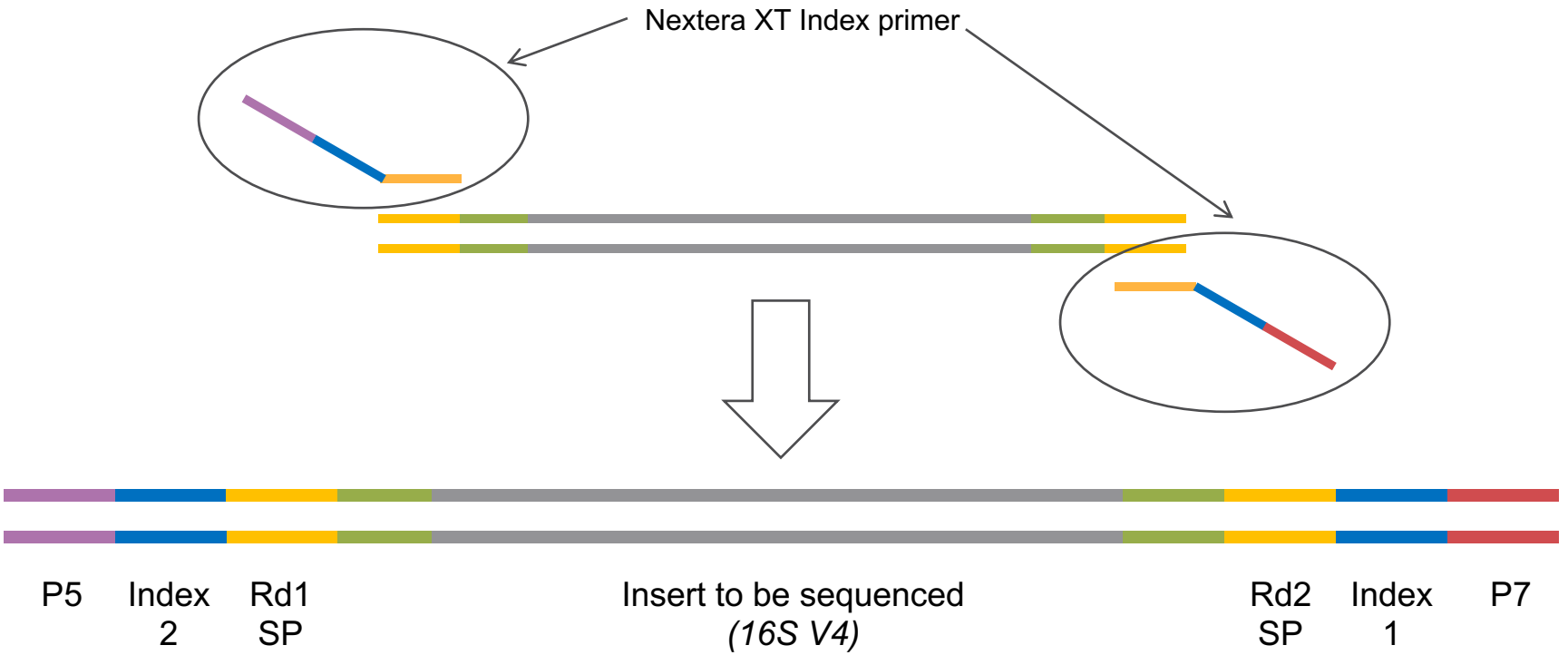
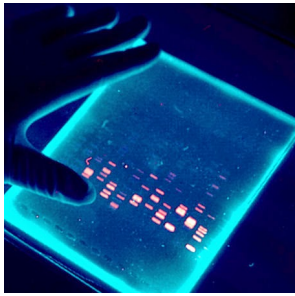


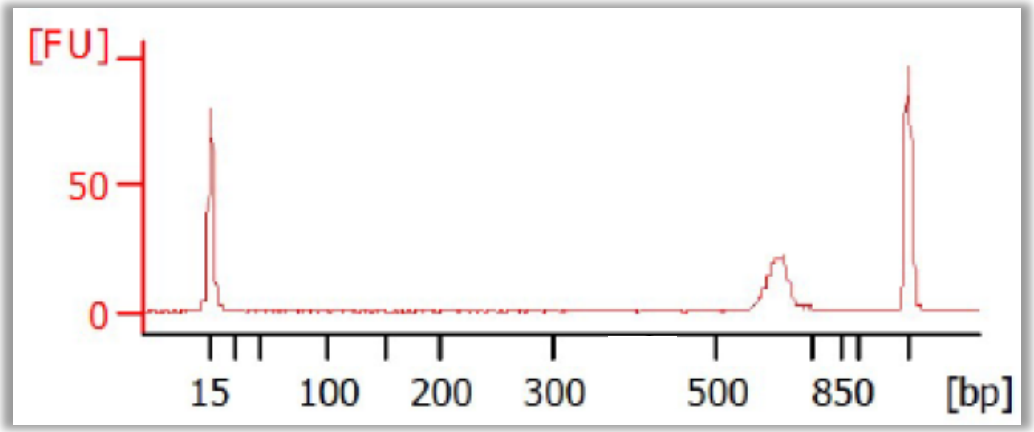


Step 4: Library QC



Quantity

- Use dsDNA-specific fluorescent dye (Qubit or PicoGreen)



Example Bioanalyzer Trace of Final Library

Quality

- Run on Agilent Bioanalyzer DNA 1000 chip
- Expected library size ≈ 630bp

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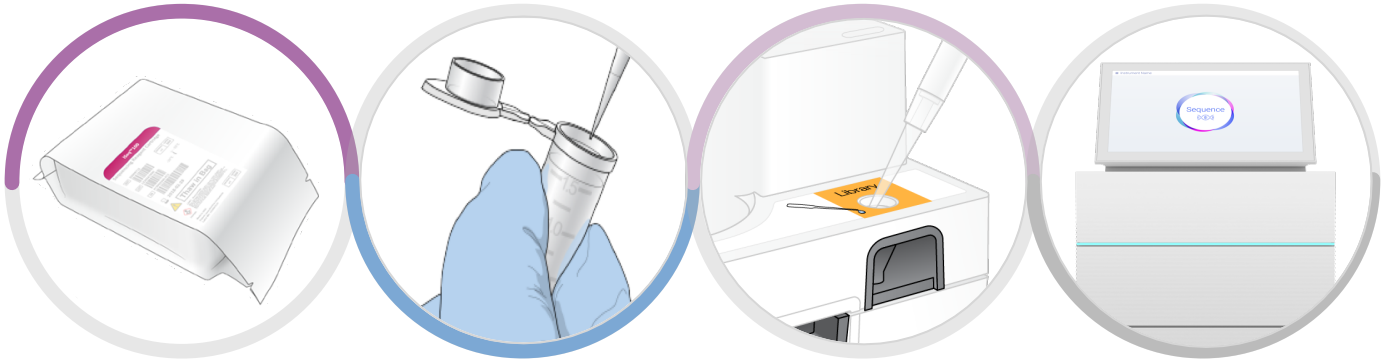
Set Up an iSeq 100 Run

iSeq 100 Workflow



Thaw

Thaw cartridge IN the foil bag according to recommended methods

Prepare

Prepare sequence-ready libraries from input DNA or RNA

Load

Insert diluted libraries into the prepared sequencing consumables

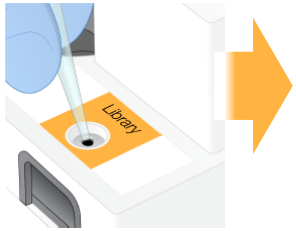
Start Run

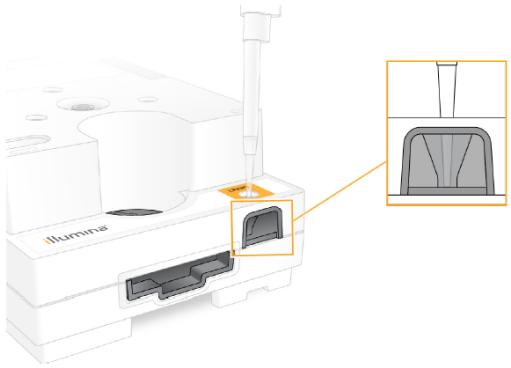
Initiate a run using instrument control software

Load Consumables Into the Cartridge

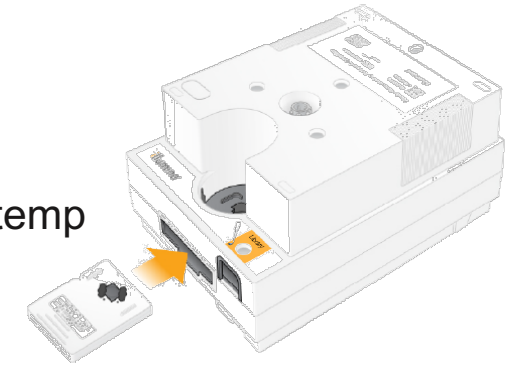
- **Tear open package**

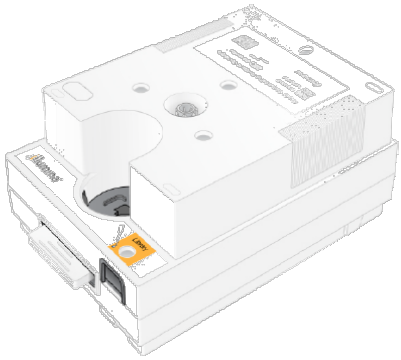
- **Load library**
  - Use a pipette tip to pierce the foil of the Library reservoir
  - Add 20 µl to the bottom of the reservoir





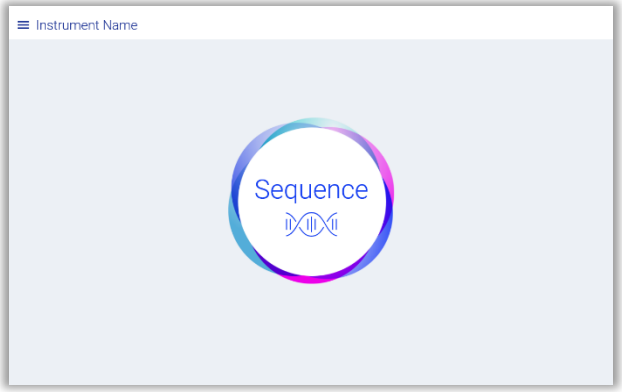
- **Load flow cell**
  - Tip! Allow the flow cell to sit at room temp for 10-15 minutes to prevent forming condensation



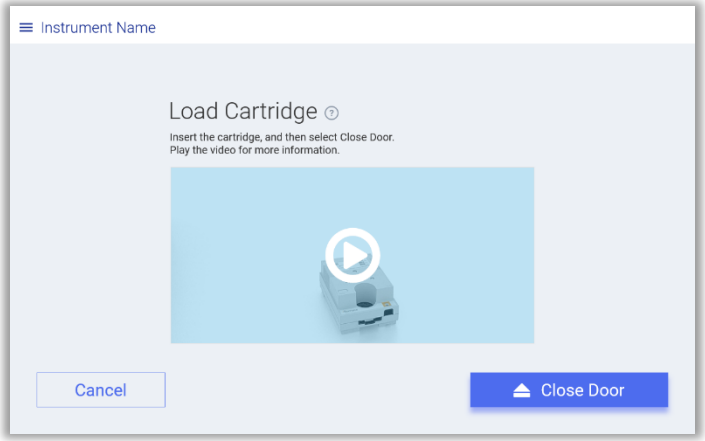


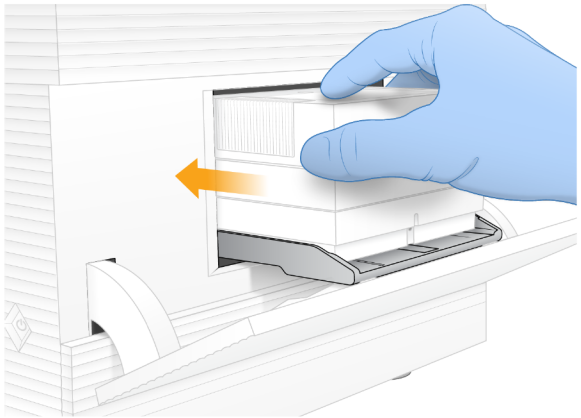
Set Up a Run Sequencing Run

- **Select *Sequence***

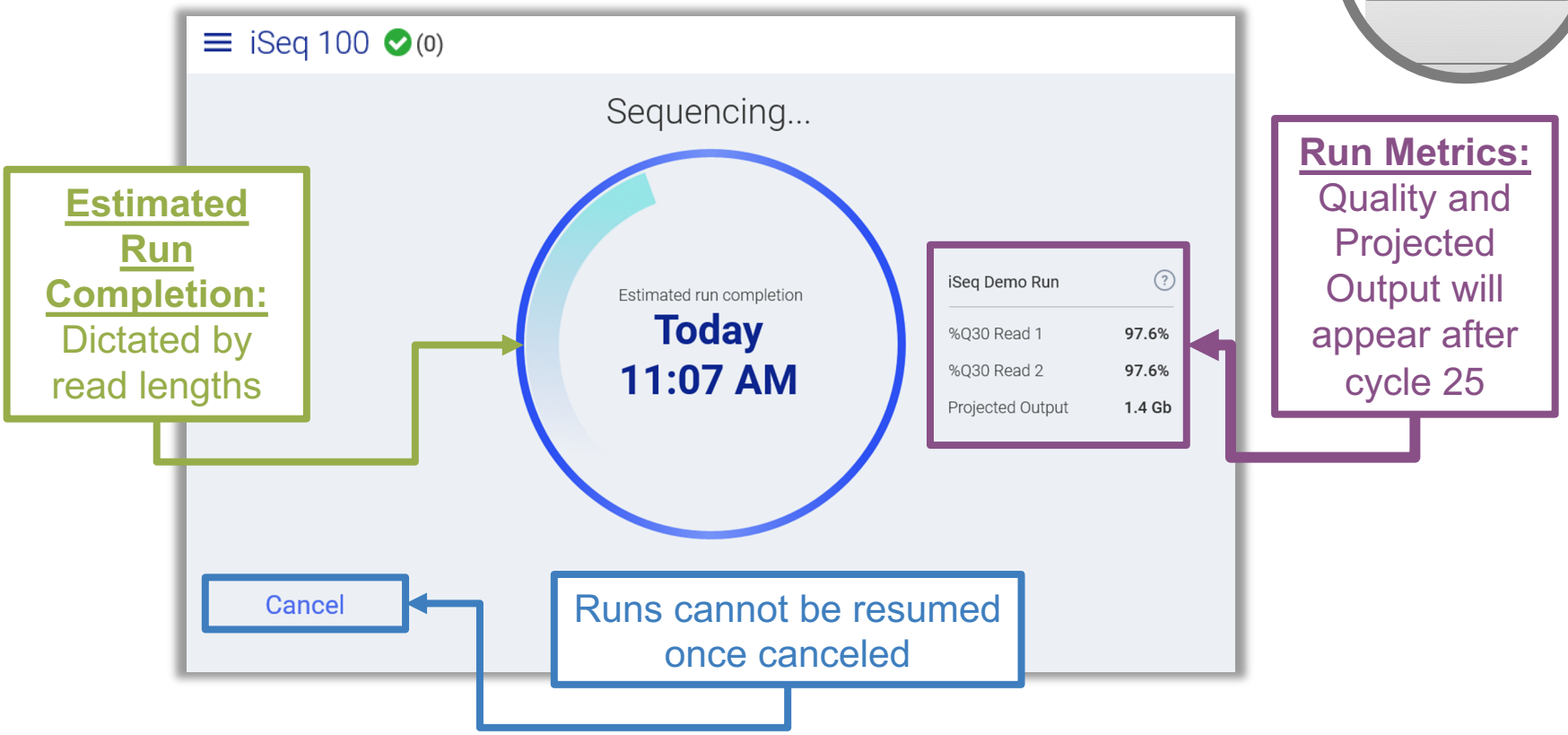


- **Load Consumables**



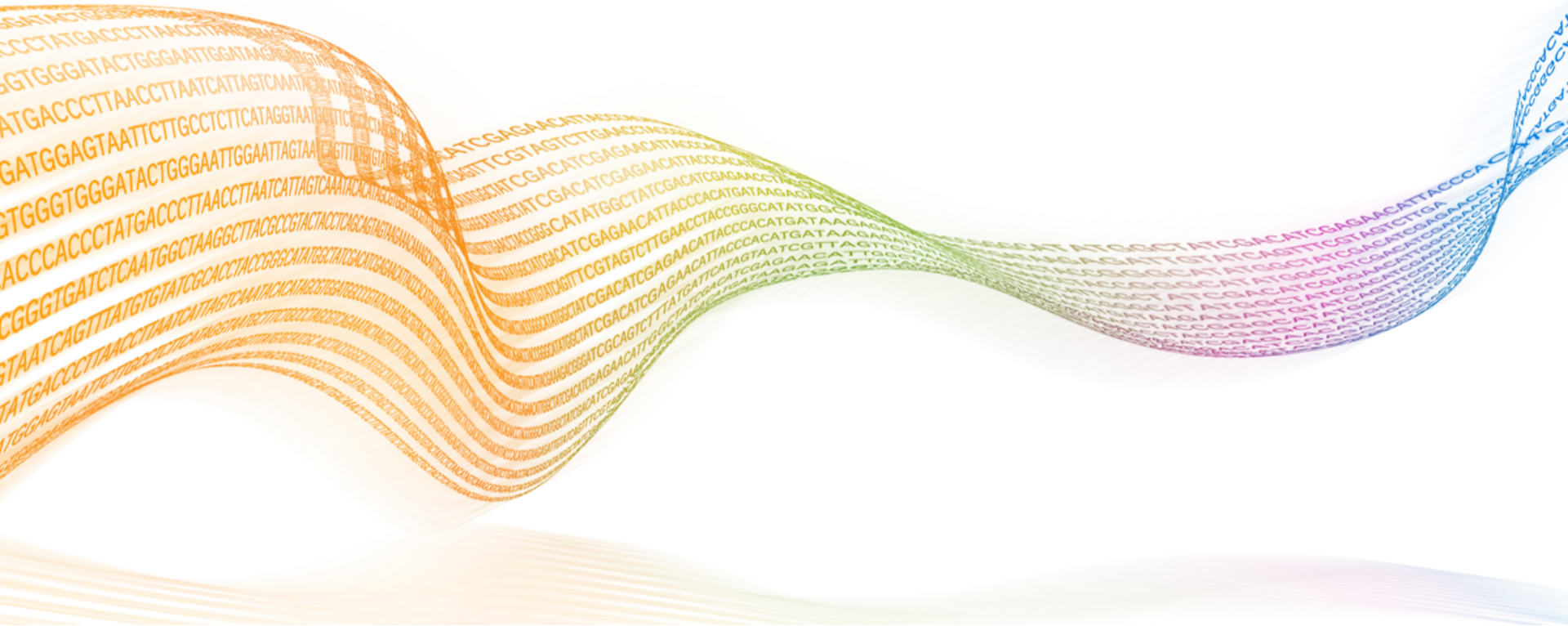


Run Monitoring



- **Paired-end 2X151 plus dual index 8+8 run time is approximately 18.5 hours**

Questions?



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