

# ILLUMINA DNA PREP LIBRARY WORKFLOW

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# Introduction to sequencing


- **Sequencing:** a laboratory process that determines the precise order of the four chemical bases (adenine, guanine, cytosine, and thymine) in a nucleic acid molecule (DNA)
- **Sequencing approaches:**
  - long read sequencing (1000bp -10,000bp)
  - short read sequencing (30-500bp)

These approaches also differ in the chemistry/ technology used for sequencing

# Sequencing by Synthesis (SBS)

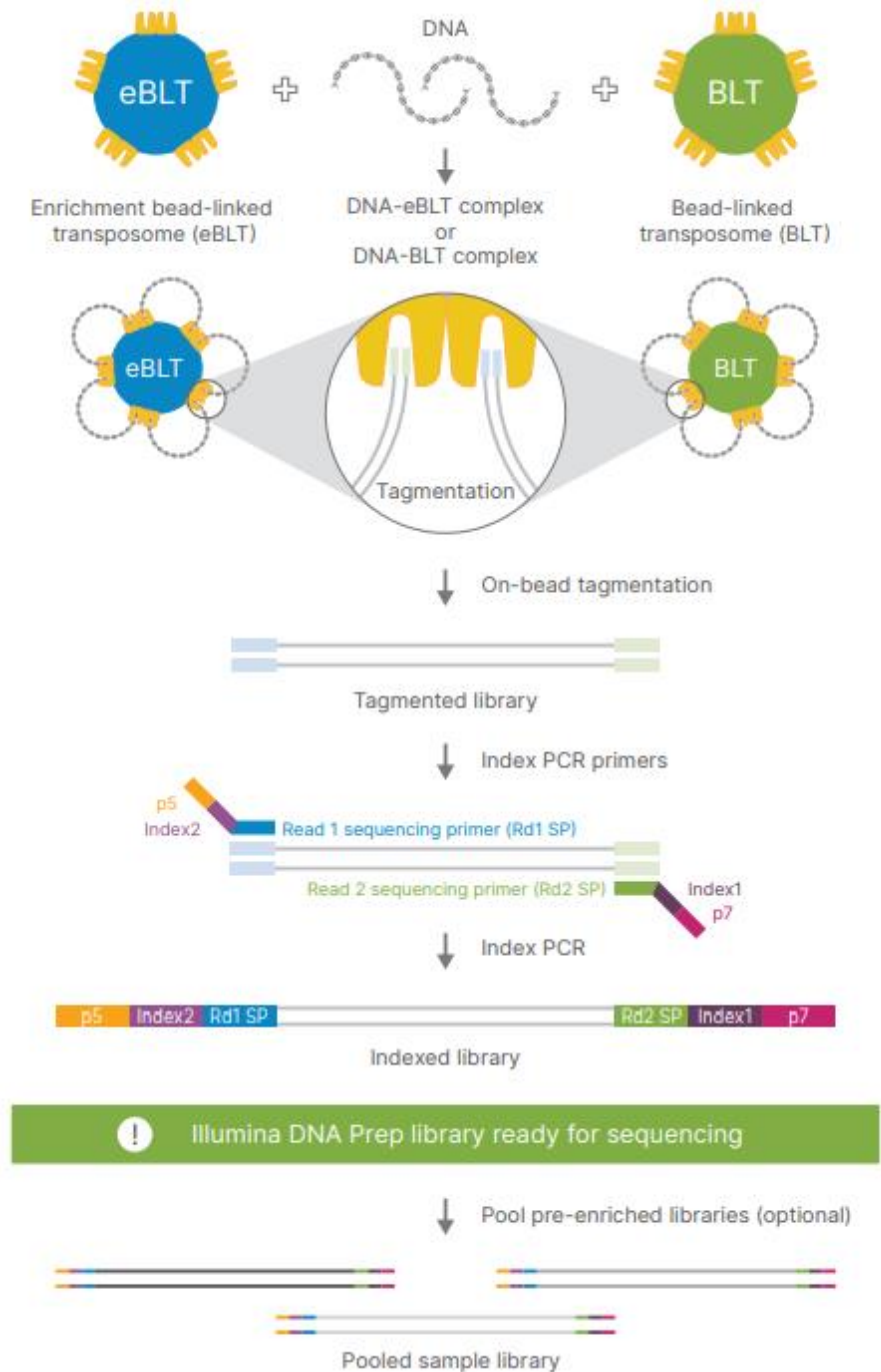
- <https://www.illumina.com/science/technology/next-generation-sequencing/beginners/ngs-workflow.html>

# NGS workflow

- **Step 1: DNA Extraction**
  - **Step 2: Library preparation**
  - **Step 3: Sequencing**
  - **Step 4: Analysis**
- 
- A teal-colored decorative curve is located in the bottom right corner of the slide, starting from the bottom edge and curving upwards and to the left.

# Library Preparation

- The library preparation process involves converting a genomic DNA sample (or cDNA sample) into a library of fragments, which can then be sequenced on an NGS instrument



# Illumina DNA Prep Workflow

## gDNA

Standard workflow: 100-299 ng ≥ 300 ng

## Whole Blood Lysis

Hands-on: 10 minutes

Total: 45 minutes

Reagents: PK1, MLB, IPB, Ethanol, RSB

## Dried Blood Spots Lysis

Hands-on: 10 minutes

Total: 50 minutes

Reagents: PK1, MLB, IPB, Ethanol, RSB

## Saliva Lysis

Hands-on: 15 minutes

Total: 30 minutes + incubation\*

Reagents: IPB, Ethanol, RSB

Safe Stopping Point

Safe Stopping Point

Safe Stopping Point

1

### Tagment Genomic DNA

Hands-on: 5 minutes

Total: 10 minutes

Reagents: RSB, BLT-PF, TB1

2

### Post Tagmentation Clean Up

Hands-on: 10 minutes

Total: 20 minutes

Reagents: ST2, TWB

3

### Ligate Indexes

Hands-on: 5 minutes

Total: 15 minutes

Reagents: ELM, IDT for Illumina DNA/RNA UD Indexes, TWB, diluted HP3

4

### Clean Up Libraries

Hands-on: 15 minutes

Total: 45 minutes

Reagents: IPB, Ethanol, RSB

Safe Stopping Point

5

### Quantify and Pool Libraries

6

### Dilute to Starting Concentration

Reagents: RSB

7

### Sequencing Set up

(Depends on NovaSeq Reagents Version)

A

### Custom Primer Workflow

(NovaSeq Reagent Kits v1.0)

B

### Standard workflow

(NovaSeq Reagent Kits v1.5 or newer)

# Illumina Sequencing Systems



iSeq™ 100



MiniSeq™



MiSeq™



MiSeq™ i100  
MiSeq™ i100 Plus



NextSeq™ 550



NextSeq™ 1000  
NextSeq™ 2000

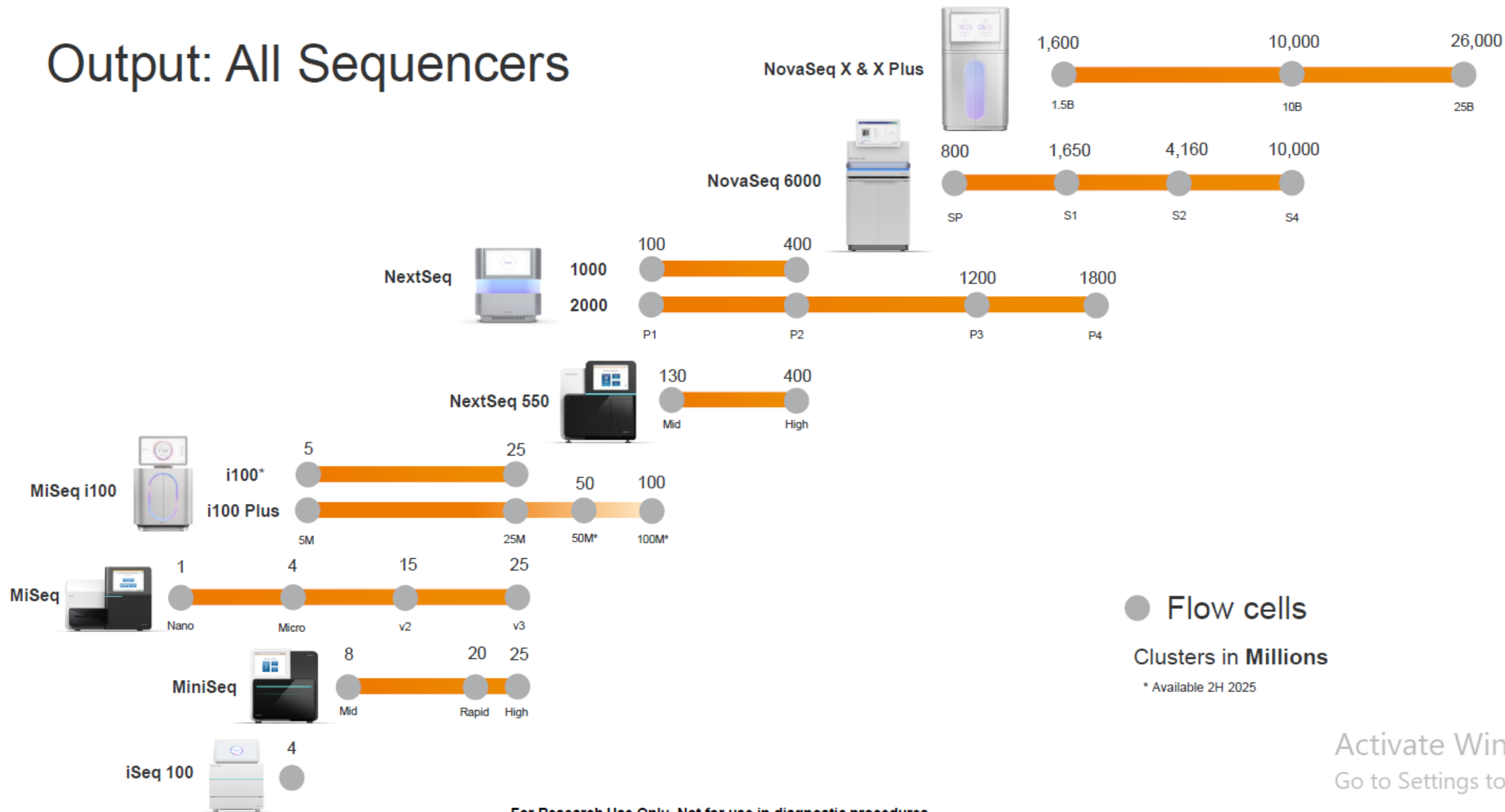


NovaSeq™ 6000



NovaSeq™ X  
NovaSeq™ X Plus

# Output: All Sequencers



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