H3ABioNet
Pan African Bioinformatics Network for H3Africa

wellcome connecting science

Next Generation Sequencing Bioinformatics Course 2021

Module 2: Session 2
Introduction to NGS
Illumina Sequencing

Illumina Sequencing

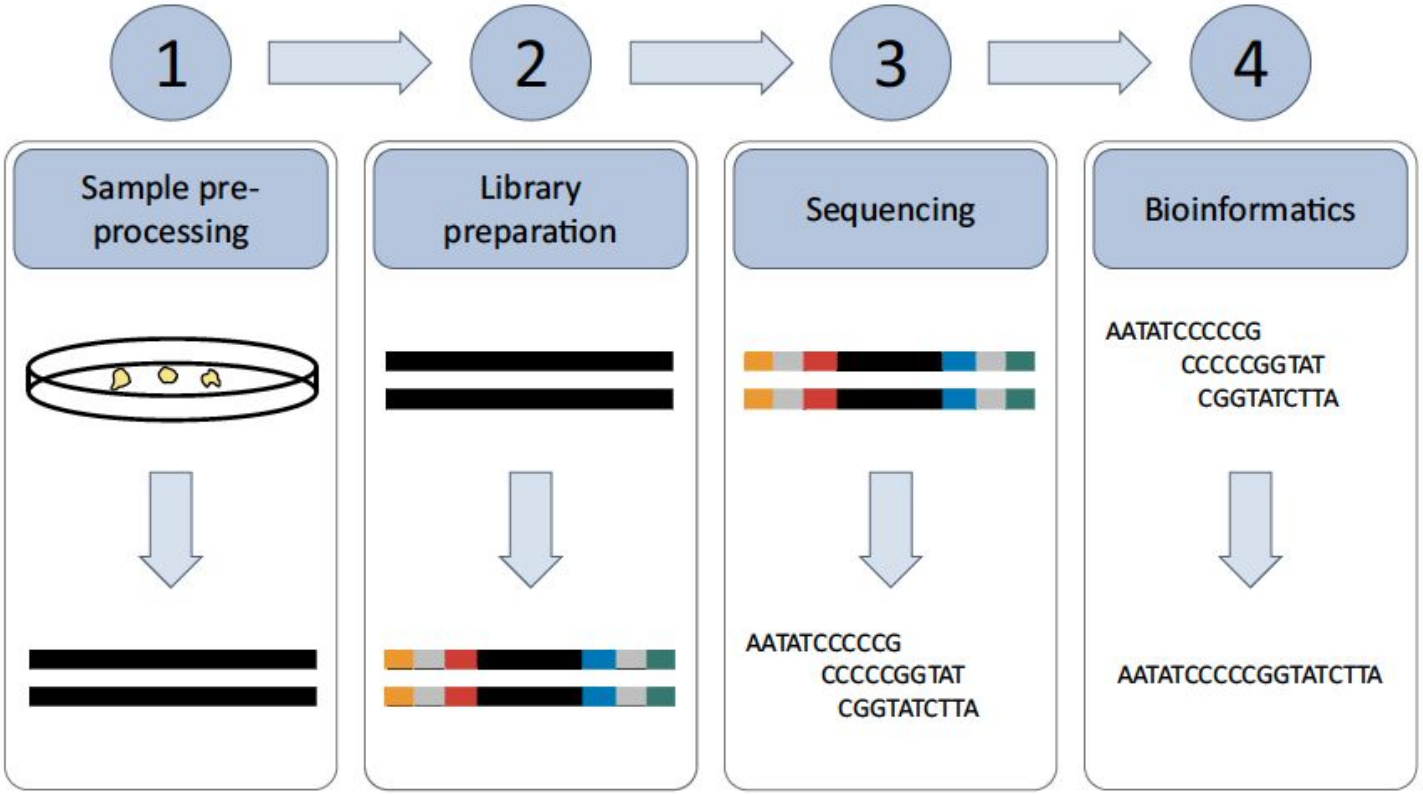
1. **Current market leader**

2. **Captures ~70% of the sequencing market**

3. **Lowest per base sequencing cost**

Platform (Run Time)	Read Length	Maximum Output (Gbp/run)	Cost/ Human Genome (US$)
iSeq 100 (10 – 19 hours)	2 X 150 bp	1.2 Gb	NA
MiniSeq (4 – 24 hours)	2 X 150 bp	7.5 Gb	NA
MiSeq (4 – 55 hours)	2 X 300 bp	15.0 Gb	NA
NextSeq (12 – 48 hours)	2 X 150 bp	120 - 330 Gb	NA
HiSeq (1-6 days)	2 X 150 bp	1500 Gb	2,500
HiSeq X (<3 days)	2 X 150 bp	1800 Gb	1,000
NovaSeq 6000 (13 – 44 hours)	2 X 250 bp	6000 Gb	800
Capillary sequencing	700-1000 bp	0.6 Gb	3,000,000,000

Illumina Sequencing: workflow

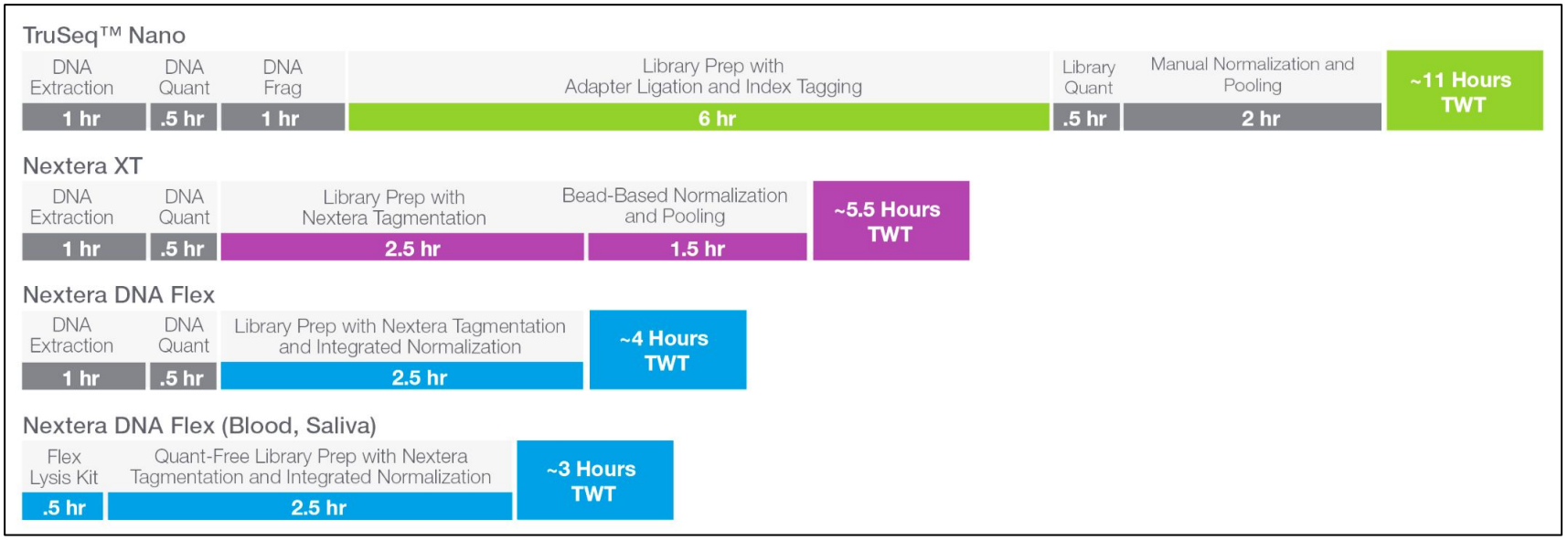


Preprocessing

1. **Genomic DNA quantity ~5-ng(MiSeq)**

2. **High quality gDNA:** RNA contamination ,Absorbance 260/280 ~1.8 (DNA)/ 2.0 (RNA), No contamination from chemicals (EDTA, phenol)

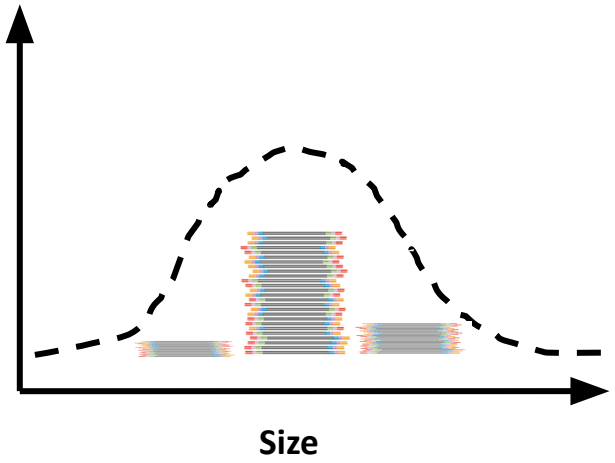
3. **Assess the yield/integrity using Qubit (PicoGreen):** detects double stranded DNA (>= 50bp)



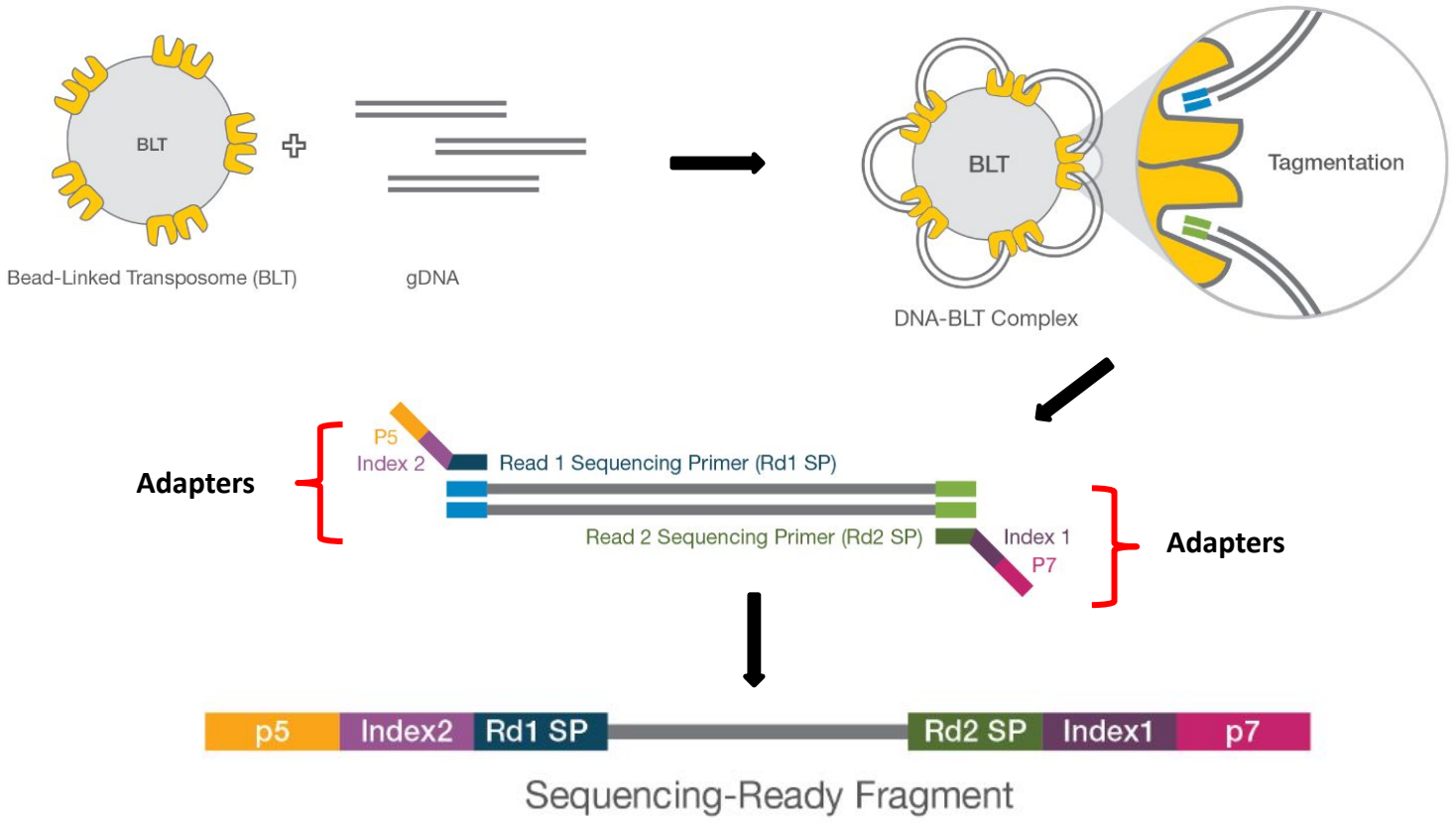
Library preparation: Fragmentation

- Separation or breaking DNA strands into pieces.
- DNA Fragmented by:

1. Enzymatic digestion
2. Shearing
3. Nebulization
4. Sonication
5. Transposon mediated fragmentation



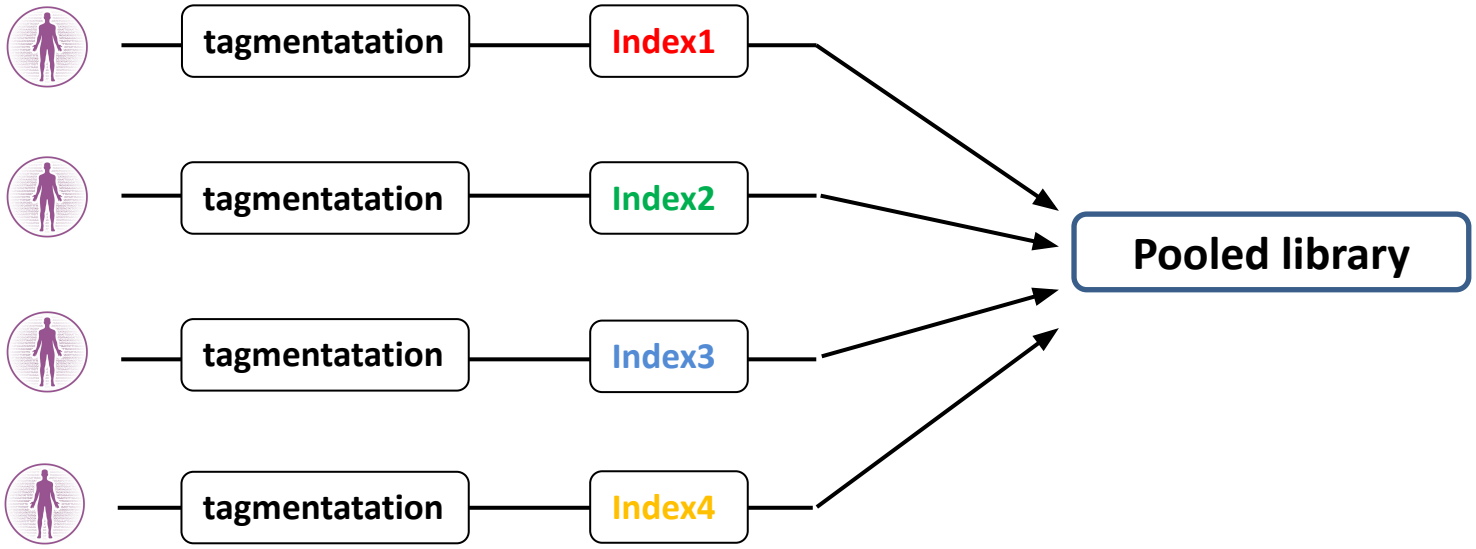
Library preparation: Tagmentation



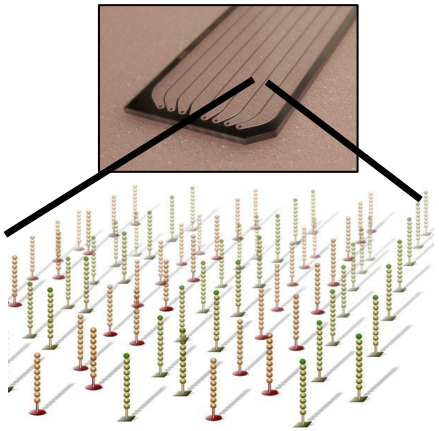
Ref: https://www.illumina.com/content/dam/illumina-marketing/documents/products/appnotes/nextera-dna-flex-human-genomes-application-note-770-2017-018.pdf

Library preparation: Multiplexing

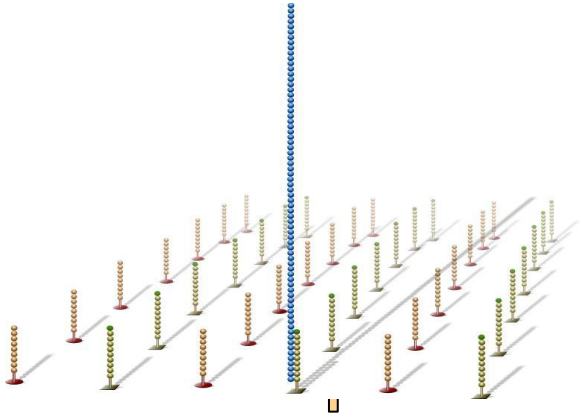
Sequencing multiple samples at the same time



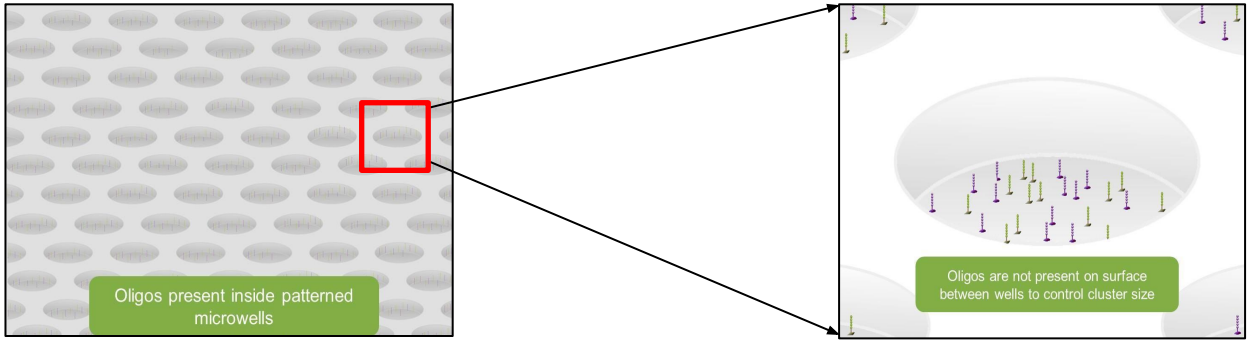
Illumina flow cells



Glass flow cell



Glass flow cell



Patterned flow cell

Please continue with slides Module2_Sessions3_Introduction to NGS-IlluminaSequencing