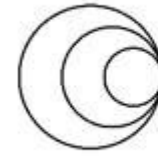




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science**

Next Generation Sequencing Bioinformatics Course 2021

Module 2: **Session 2** Introduction to NGS Illumina Sequencing



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NGS Bioinformatics Course Africa 2021
Narender Kumar

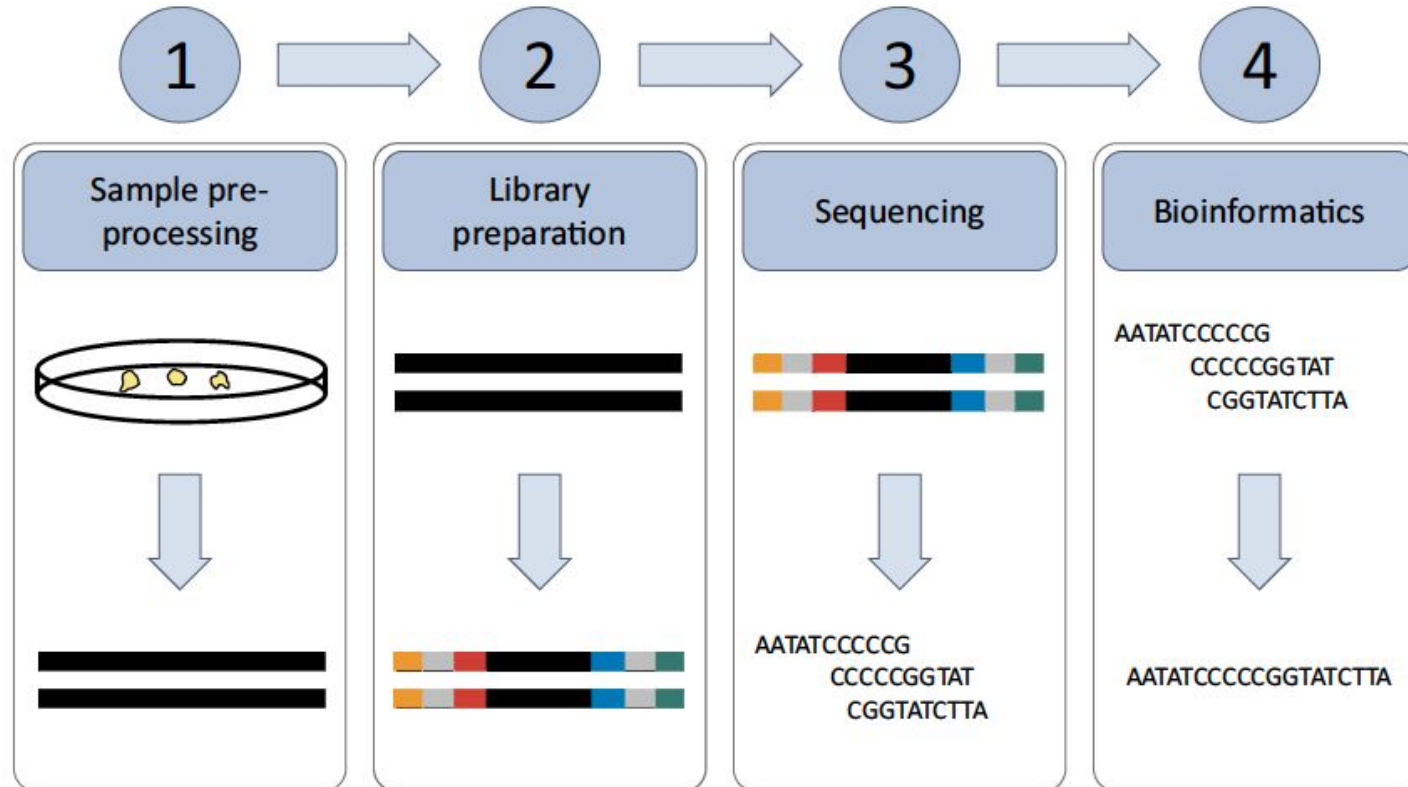
Illumina Sequencing

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



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Next Generation Sequencing Bioinformatics
Trainer Name: Narender Kumar

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)



TruSeq™ Nano

DNA Extraction	DNA Quant	DNA Frag	Library Prep with Adapter Ligation and Index Tagging	Library Quant	Manual Normalization and Pooling	~11 Hours TWT
1 hr	.5 hr	1 hr	6 hr	.5 hr	2 hr	

Nextera XT

DNA Extraction	DNA Quant	Library Prep with Nextera Tagmentation	Bead-Based Normalization and Pooling	~5.5 Hours TWT
1 hr	.5 hr	2.5 hr	1.5 hr	

Nextera DNA Flex

DNA Extraction	DNA Quant	Library Prep with Nextera Tagmentation and Integrated Normalization	~4 Hours TWT
1 hr	.5 hr	2.5 hr	

Nextera DNA Flex (Blood, Saliva)

Flex Lysis Kit	Quant-Free Library Prep with Nextera Tagmentation and Integrated Normalization	~3 Hours TWT
.5 hr	2.5 hr	



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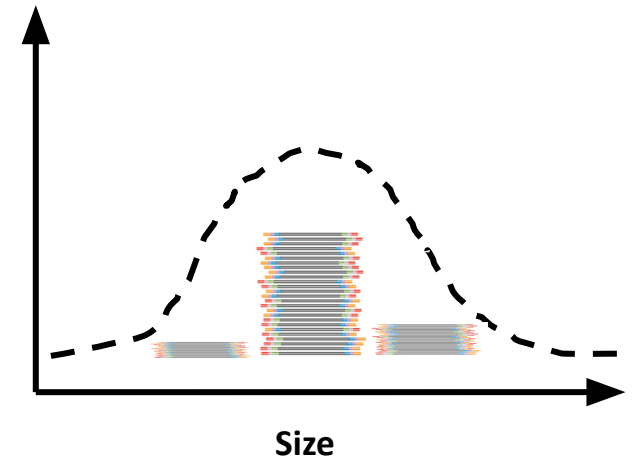
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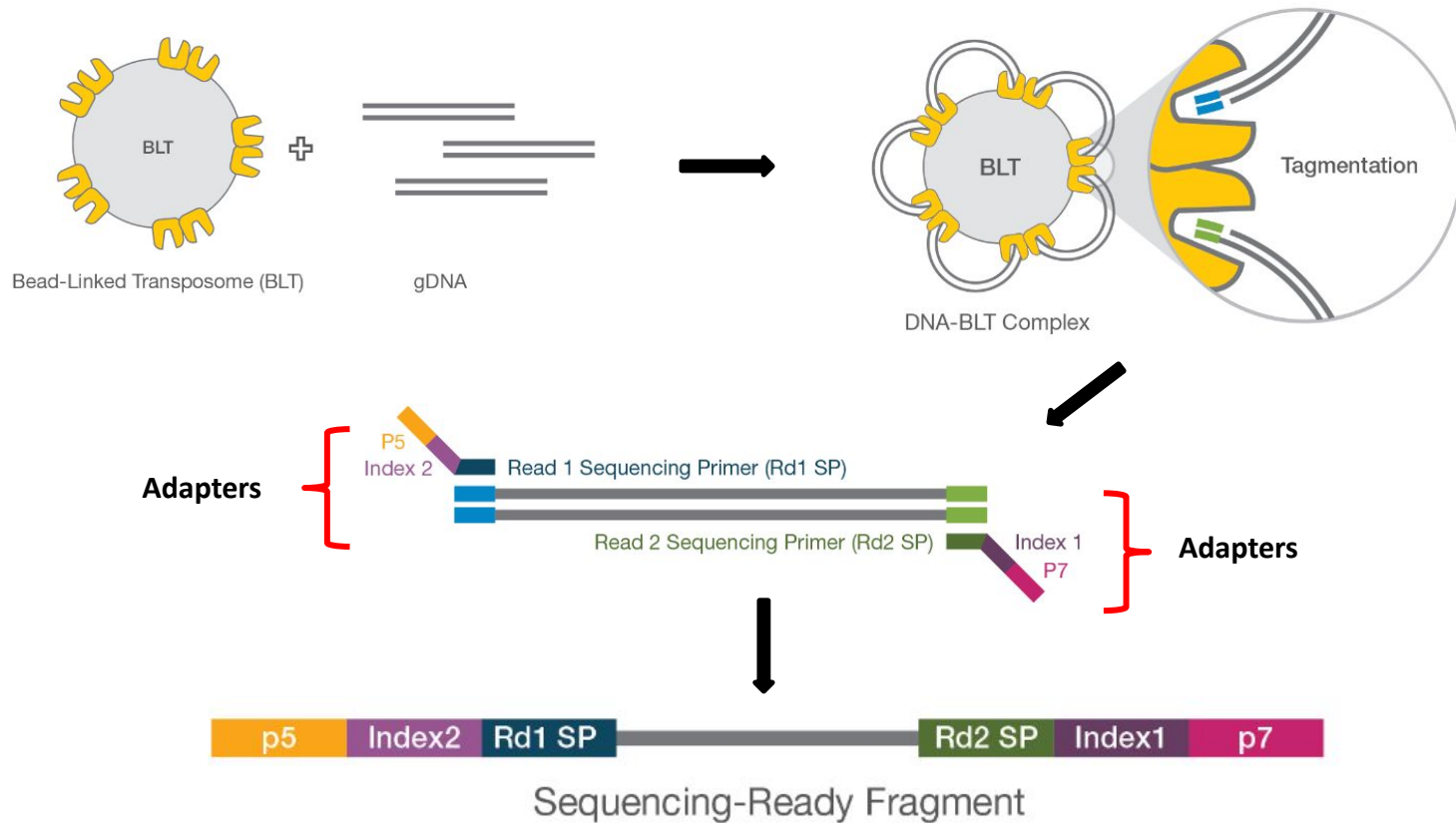
Next Generation Sequencing Bioinformatics
Trainer Name: Narender Kumar

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>



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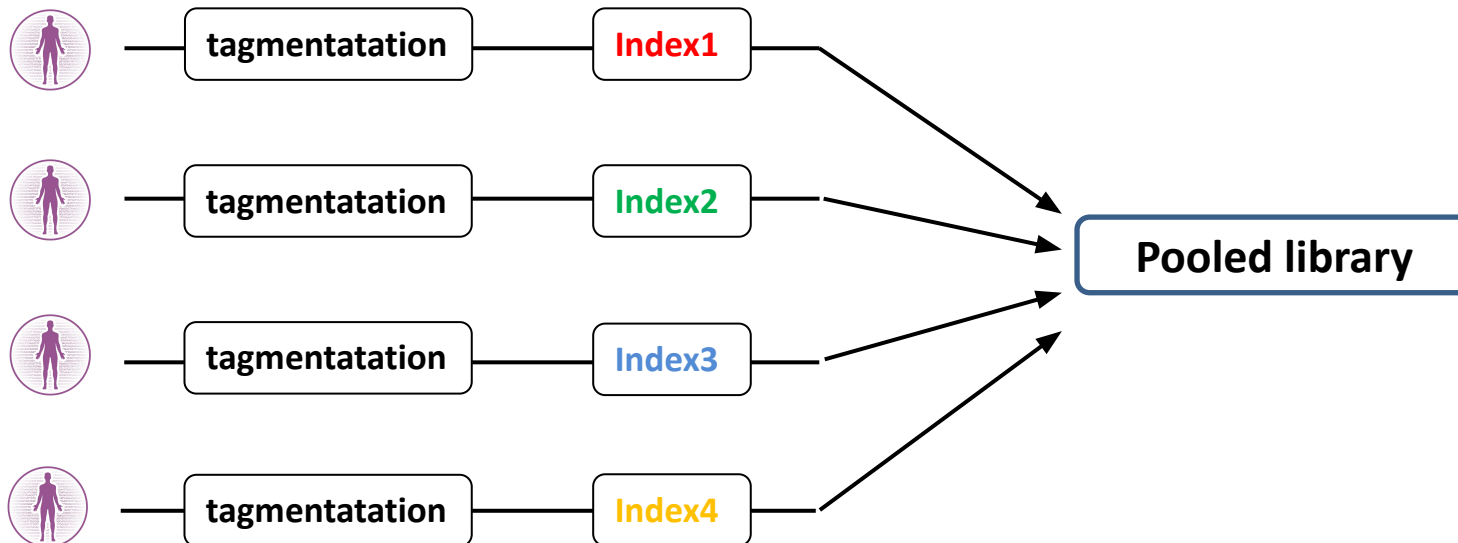
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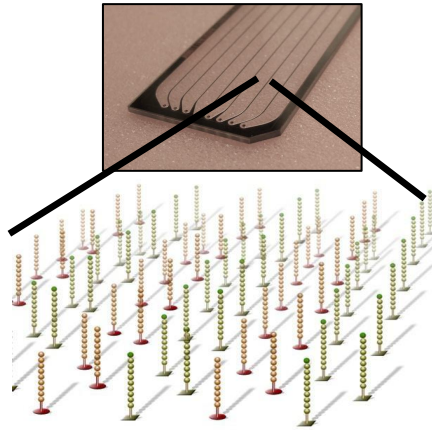
Next Generation Sequencing Bioinformatics
Trainer Name: Narender Kumar

Library preparation: Multiplexing

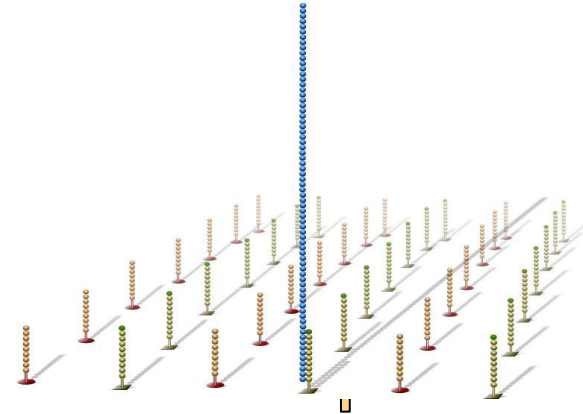
Sequencing multiple samples at the same time



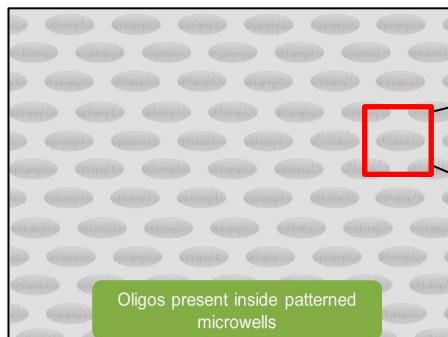
Illumina flow cells



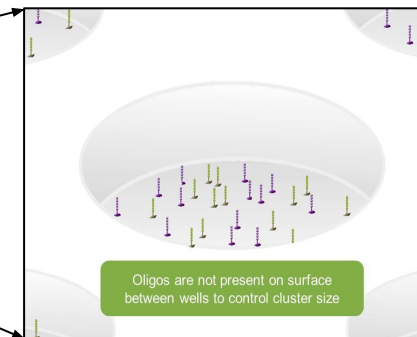
Glass flow cell



Glass flow cell



Patterned flow cell



Please continue with slides Module2_**Sessions3**_Introduction
to NGS-IlluminaSequencing



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