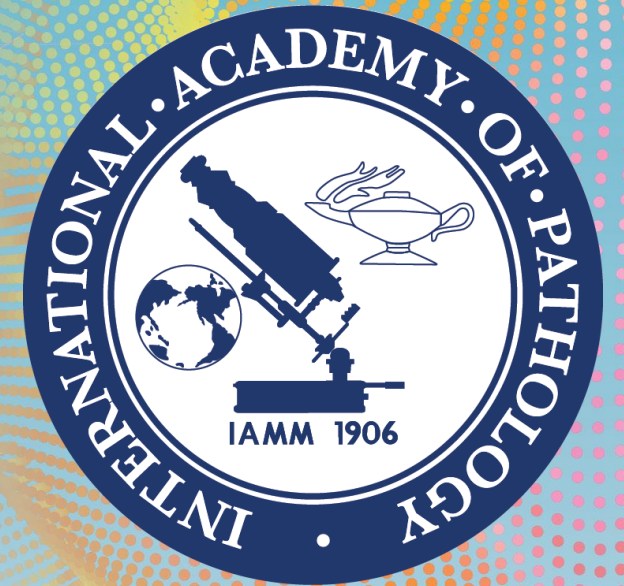


Illumina vs Genexus NGS Platforms: Selecting the Optimal Solution for Somatic Mutation Testing

Chelsee Hewitt

Molecular Pathology, Peter MacCallum Cancer Centre



Disclosure of Relevant Financial Relationships

No relevant financial relationships

Evolution of Sequencing Technology

First Generation

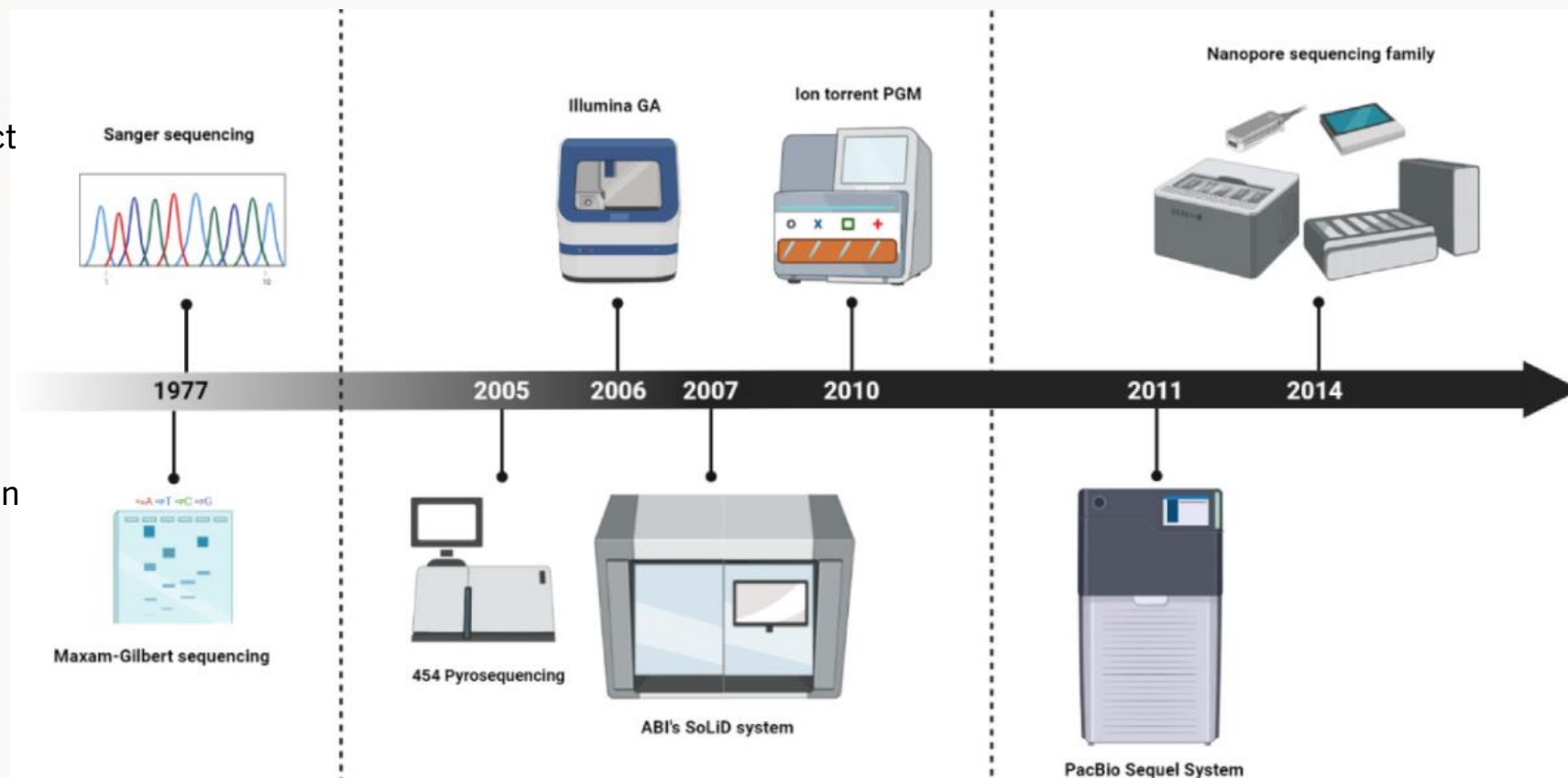
- Enabled decoding of large genomes & human genome project

Second Generation

- Revolution of massively parallel sequencing
- Short read sequencers produced tremendous amounts of data at unprecedented speed & low cost
- Become standard tool for precision molecular medicine

Third Generation

- Single-molecular sequencing in real-time
- No need for amplification



First Generation

Second Generation

Third Generation

Athanasopoulou K et al. (2021). *Third-Generation Sequencing: The Spearhead towards the Radical Transformation of Modern Genomics*. *Life (Basel)* 12 (1).

Illumina vs Genexus NGS Platforms: Selecting the Optimal Solution for Somatic Mutation Testing

Predominant Short Read Sequence Technologies

Illumina NovaSeq 6000



ThermoFisher Genexus



Characteristics of Illumina and Ion Torrent (by ThermoFisher) Sequencing

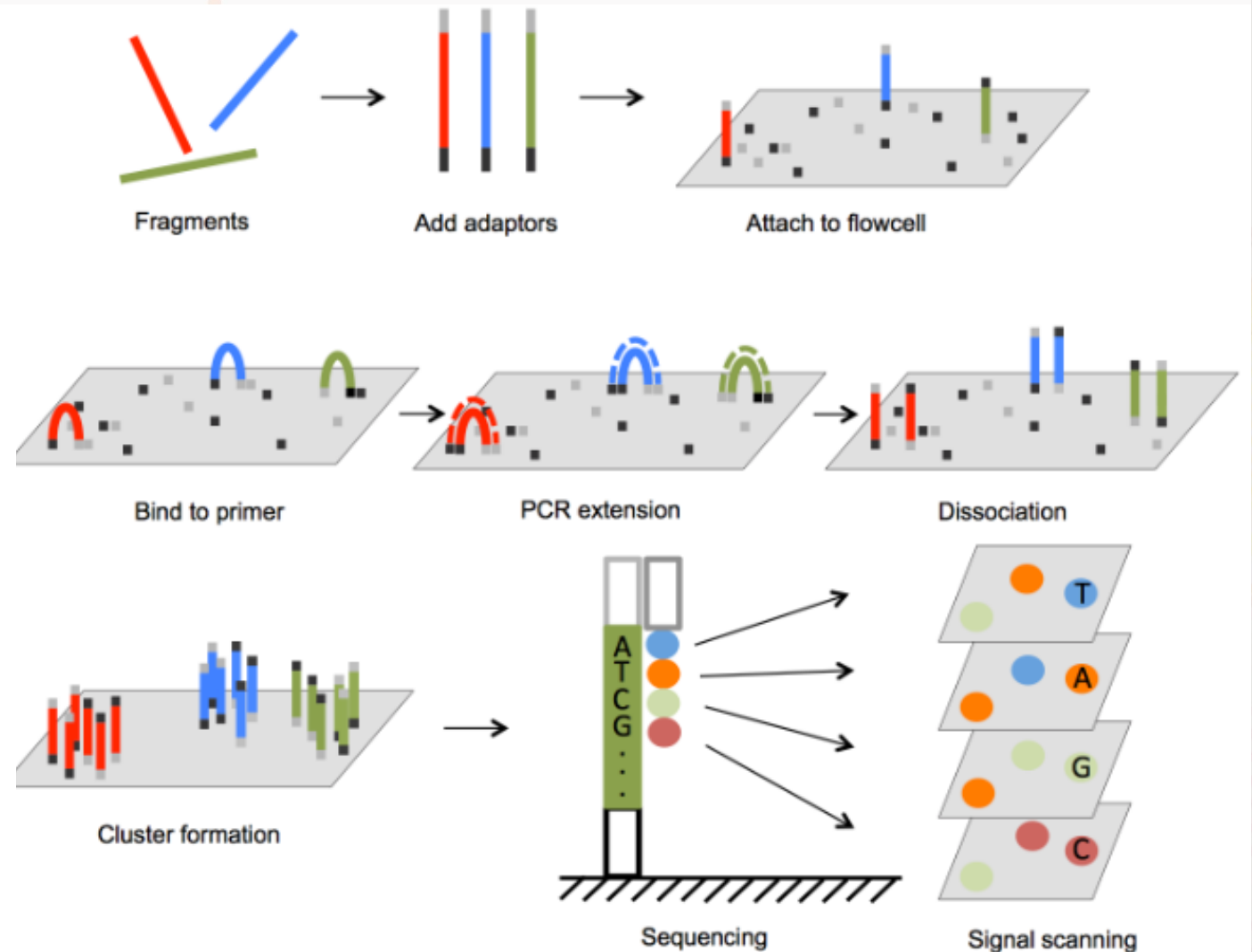
Company	Illumina								Ion Torrent by ThermoFisher		
Library Prep & Sequencing	PCR or hybridisation based-capture library prep. Sequence by Synthesis.								PCR library prep. Sequence by Synthesis.		
Detection Method	Fluorescent								Ion		
Platform	<u>iSeq</u>	<u>MiniSeq</u>	<u>MiSeq</u>	NextSeq550	<u>NextSeq 1000&2000</u>	<u>NovaSeq 6000</u>	<u>MiSeqDx</u>	NextSeq550 Dx	<u>GeneStudio SS</u>	<u>Genexus</u>	Ion PGM-Dx
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Weight (Kg)	15.9	45	57.2	83	141	481	54.4	84.4	63.5	204.1	30
Sequencing Applications	Small WGS, targeted, small RNA		Small WGS, targeted, <u>ChiP-Seq</u> , Small RNA	Small WGS, targeted, exome & transcriptome		WGS, targeted, WES, epigenome & transcriptome	Small WGS, targeted	Targeted, exome & transcriptome	Targeted, epigenetic, exome & transcriptome	Targeted	Targeted
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Automation	Perform sequencing only									Turnkey NGS solution (GPI & Sequencer)	
Bioinformatics	Large choice of analysis software options available, but more bioinformatics support required								Torrent Suite software		

* Dependant on flow cell / chip

Adapted from Hu T et al. (2021). Next-generation sequencing technologies: An overview. Hum Immunol 82 (11), pp. 801-811.

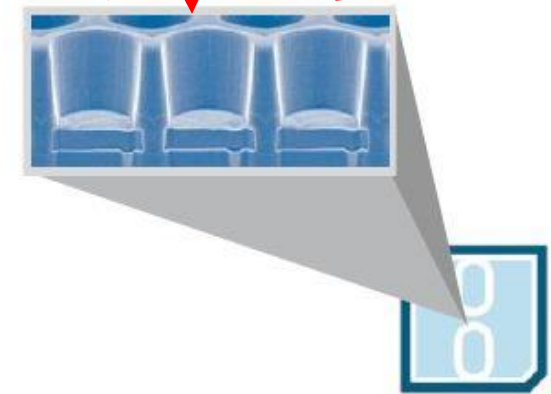
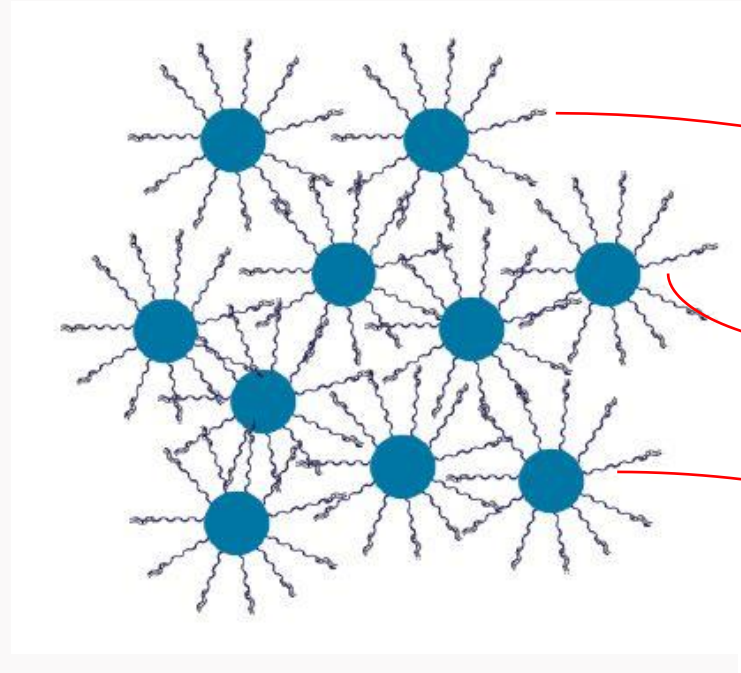
Illumina Sequencing

- PCR or hyb capture library preparation
- Adaptors added, allow binding to primer-loaded flow cell
- Bridge PCR for amplification – clusters of fragments
- Fragments denatured
- Each sequencing cycle a fluorescently-labelled nucleotide is incorporated into each growing strand
- Laser excites the fluorophores & signals collected, dNTPs identified and turned into DNA sequence
- Fluorescent labels enzymatically cleaved prior to next sequence cycle



Lu, Y et al., (2016) Next Generation Sequencing in Aquatic Models, Next Generation Sequencing – Advances, Applications and Challenges doi: 10.5772/61657

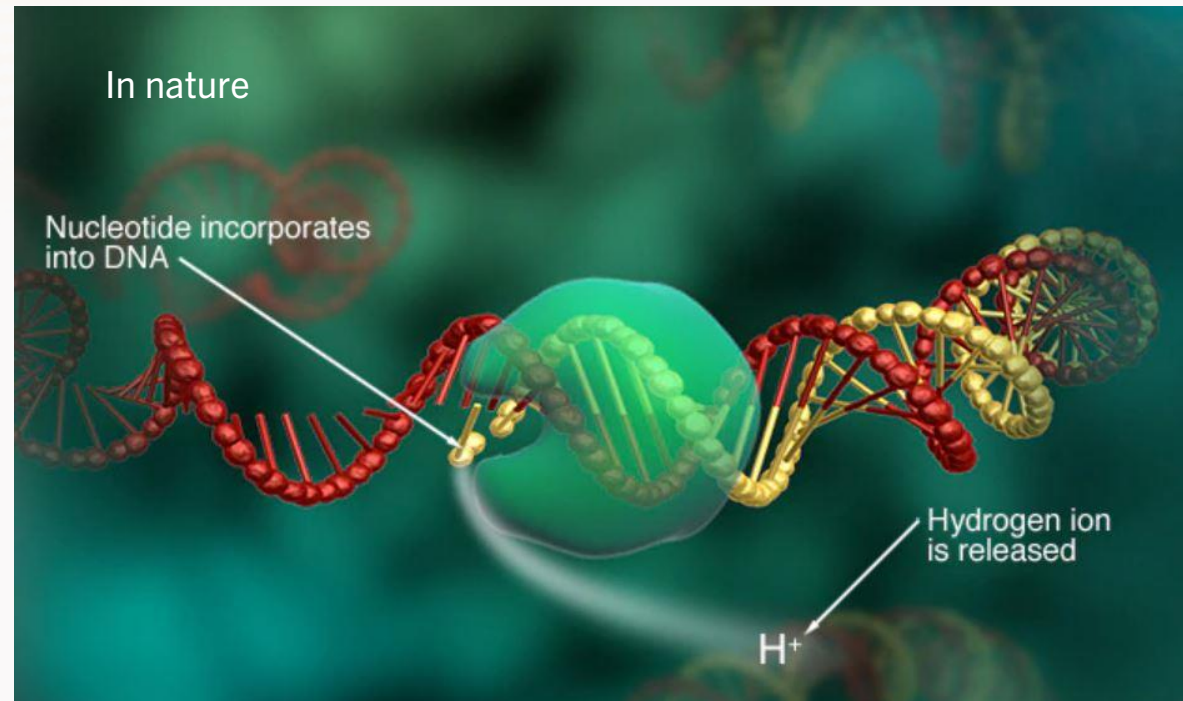
Ion Torrent Sequencing



- PCR based library preparation
- Fragments attached to single beads (Ion Sphere Particles - ISPs)
- Fragments copied to cover ISP
- Enriched ISPs primed for sequencing by annealing of sequence primer
- Beads loaded singly into wells of ion chip

Image: Altered from ThermoFisher Scientific

Ion Torrent Sequencing

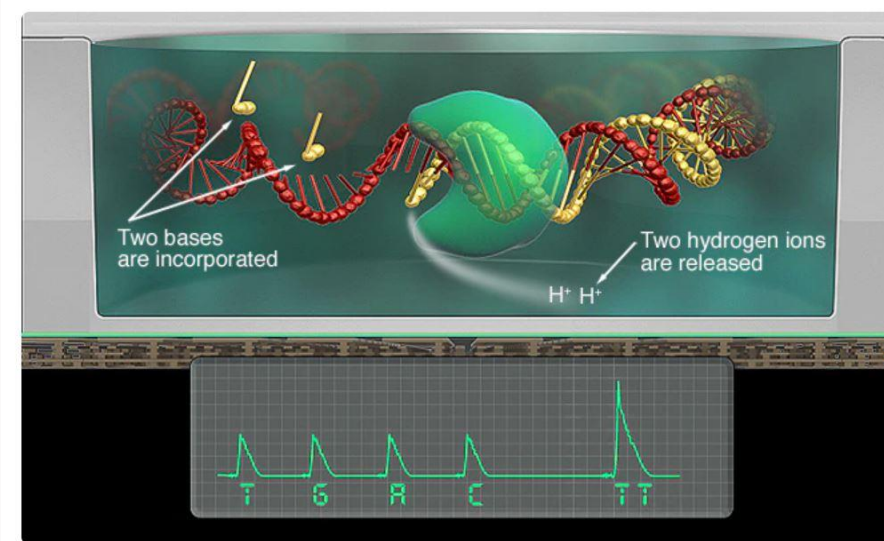
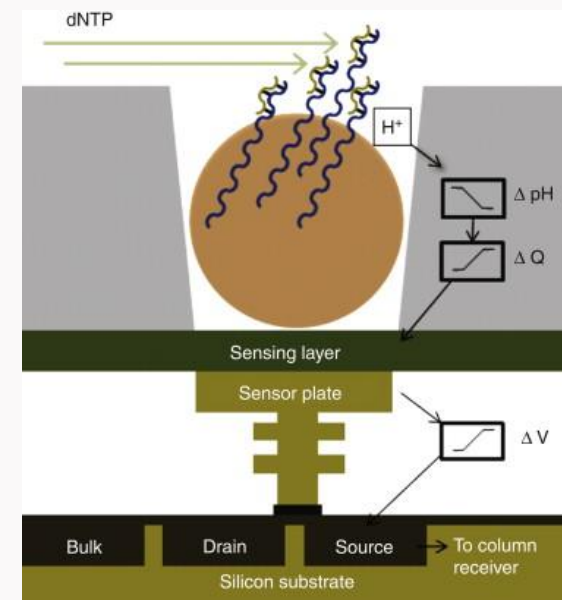


<https://www.thermofisher.com/au/en/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-technology.html>

Ion Torrent Sequencing

- Chip flooded with unmodified dNTPs in specific sequence
- Complementary dNTPs incorporated & H ion released
- pH of solution in well altered induces change in surface potential of ion-sensitive layer of base of well & source terminal of sensor under each well creating an output voltage
- Series of electrical pulses turned into DNA sequence
- Quantitative e.g., 2 identical bases in a row doubles output voltage, or no match – no voltage change
- Direct detection = very fast

<https://www.thermofisher.com/au/en/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-technology.html>



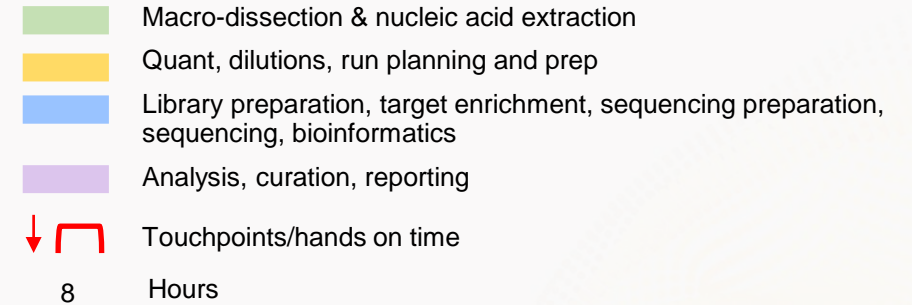
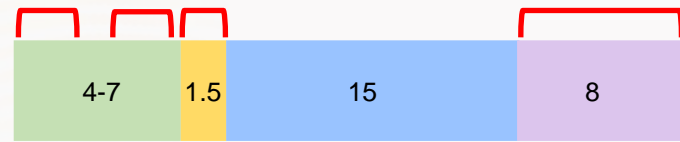
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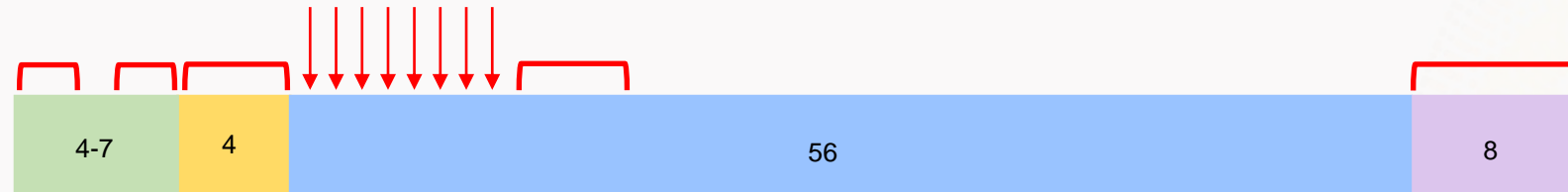
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Turn Around and Hands on Time

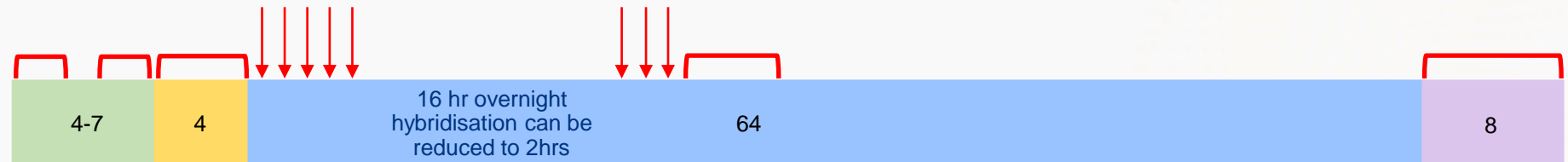
OPA on Genexus



Amplicon Panel on NextSeq



Hyb Capture Panel on NextSeq



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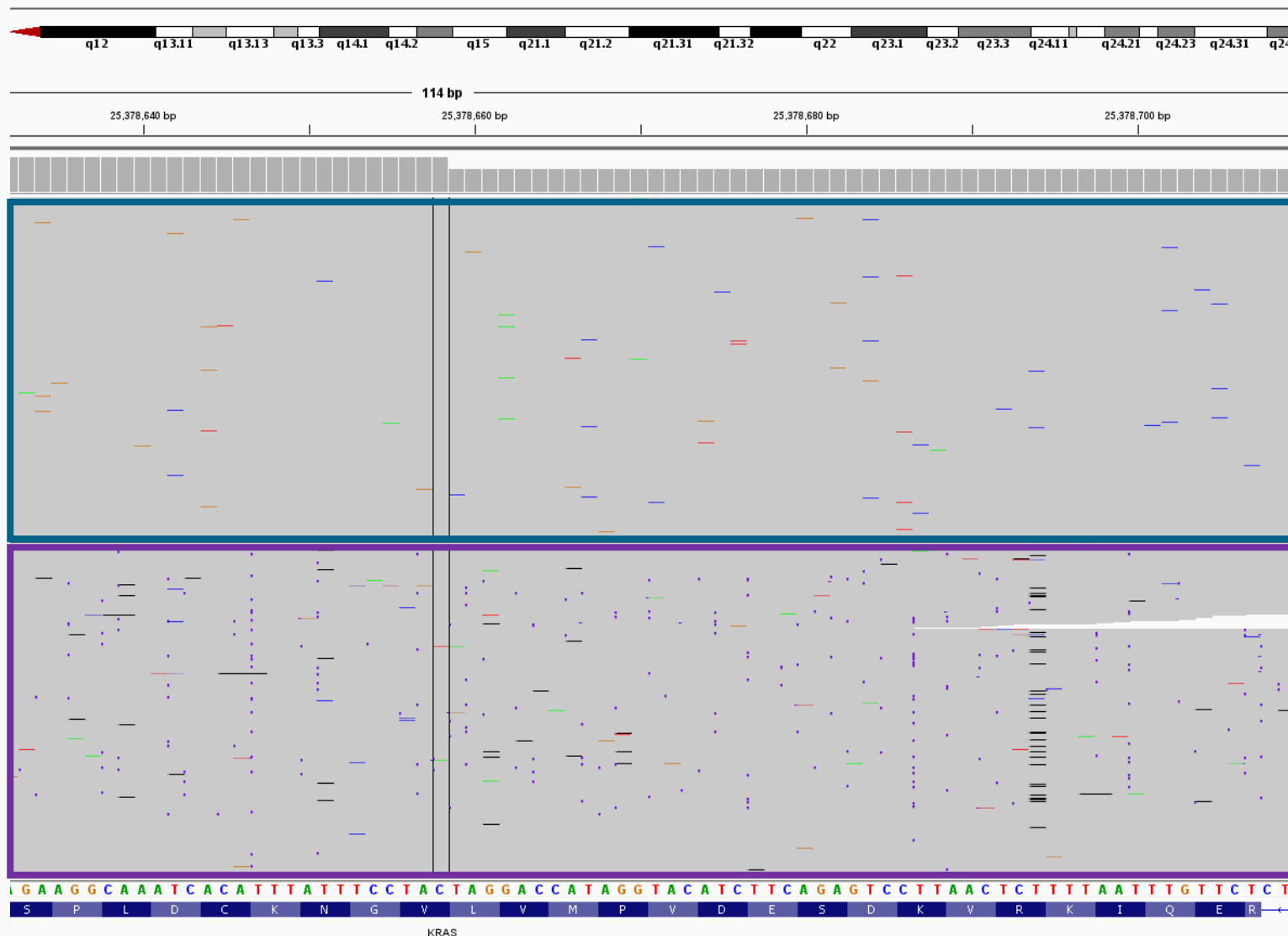
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Sequence Error Rates

Illumina sequencing of in-house amplicon targeted panel

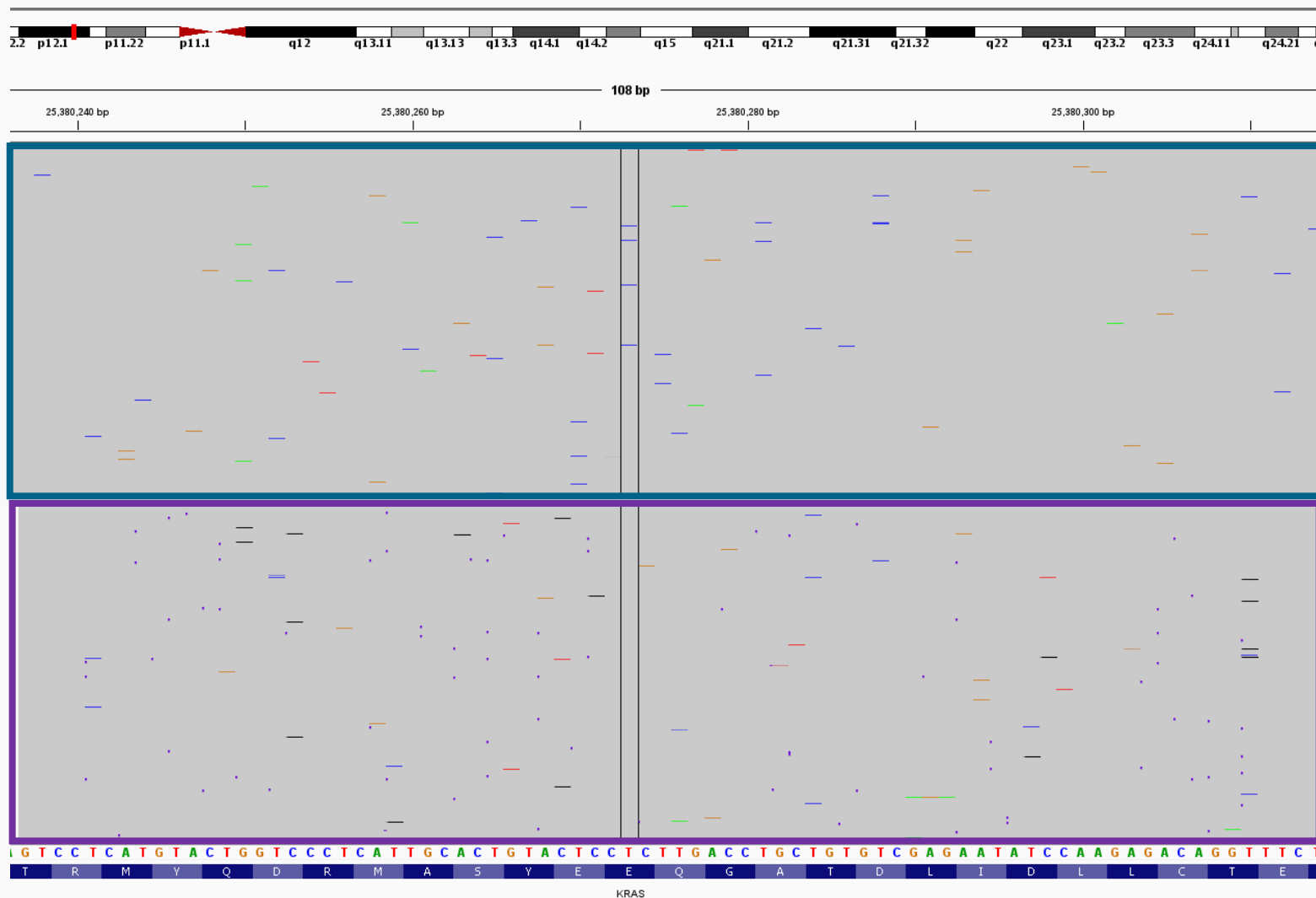
Genexus sequencing of Oncomine Precision Assay (OPA)



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NGS Panel Availability

ThermoFisher For Genexus



- **Ion Torrent OncoPrint Tumour Specific Panels:** Small (15-30 gene) dynamic manufactured on demand eg HRR pathway, melanoma, gastric and oesophageal, CRC and pancreatic
- **OncoPrint Precision Assay:** Clinically relevant small DNA variants in 45 genes, 14 copy number alterations (CNA) genes and 17 gene fusions and splice variants
- **Ion Torrent OncoPrint Comprehensive Assay v3:** Small DNA variants in 87 hotspot genes, 43 gene amplifications, 48 full CDS for del variants, 51 gene fusions
- **Ion Torrent OncoPrint Comprehensive Assay Plus:** Small DNA variants in >366 genes, 49 gene fusions and splice variants, CNA, MSI, TMB & Genomic Instability Metric (GIM - HRD solution)

Illumina



- **TruSight Tumor 15:** Small DNA variants in 15 genes
- **TruSight Tumor 170:** Small DNA variants in 148 genes, 55 gene fusions and splice variants, gene amplifications
- **TruSight Oncology 500:** Small DNA variants in 523 genes, 55 gene fusions and splice variants, gene amplifications, TMB & MSI (HRD add on)
- **TruSight RNA Fusion:** 507 fusion associated genes to detect known and novel fusion gene partners
- **TruSight RNA Pan-Cancer:** 1385 oncology genes for gene expression, variant and fusion detection

Off the shelf panels from other companies E.g., Qiagen, Twist Bioscience, IDT Archer, SOPHiA

NGS Custom Panels

ThermoFisher For Genexus

Custom panels restricted to ThermoFisher AmpliSeq and AmpliSeqHD library preparation chemistry

Illumina

Custom panels using many library preparations eg:

- Illumina
- Twist Bioscience
- Qiagen
- IDT Archer

Summary

Genexus Platform

- Turnkey NGS Solution
- Small/flexible sample batching, fast turnaround (similar to IHC results) with low hands-on time

Illumina NGS Platforms

- Higher sample throughput
- Flexible NGS library preparation and analysis options (may require bioinformatic support)

Acknowledgements

Peter Mac Pathology Department

- Molecular Oncology Team
- Laboratory Operations Team
- Pathologists

