Implementation of ISO15189 accredited Next Generation Sequencing for NSCLC on the novel Ion Torrent Genexus™ in a clinical diagnostic laboratory.

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Introduction

The proposal to establish a Next Generation Sequencing (NGS) service for non-small cell lung carcinoma (NSCLC) in this clinical diagnostic Pathology laboratory with no previous experience of NGS was due to the following main drivers:

- Targeted therapy has increased **clinical demand** for timely solid tumour sequencing[1].
- Compliance with national and international **best practice guidelines** for lung cancer diagnostics[2,3,4,5].
- **Limited tissue** availability; NGS allows multiple genes to be assessed on a single specimen[6].
- Research & **Clinical Trials** requiring investigation with comprehensive NGS panels.

Aims

1. Evaluation of **automated** NGS methodology and workflows for sample preparation, sequencing, and analysis of clinical lung tumour samples.
2. Introduction of an **accredited**, NGS service in a clinical diagnostic laboratory with no previous NGS experience, utilising the novel Ion Torrent Genexus™ sequencing platform.
3. Optimisation and verification of a targeted commercial panel (Oncomine Precision Assay) on clinical tumour samples **from sample to report**.
4. Reduce **turnaround times (TAT)** of NGS results to clinicians, in line with best practice guidelines.

Materials and Methods

A multifaceted project, over **12 months,** from initial concept to ISO15189 accreditation.

- Workflow evaluation based on:
  - **Turn Around Time.**
  - **Complexity** i.e., User expertise including bioinformatics.
  - **Tissue requirements** i.e., sample input volume.
  - **Cost per assay** i.e., penalty for small NGS run.
- Instrument selected - **Ion Torrent Genexus™** sequencer with integrated Purification Instrument (Fig. 1); a **novel** sequencing instrument that automates specimen to report workflow.

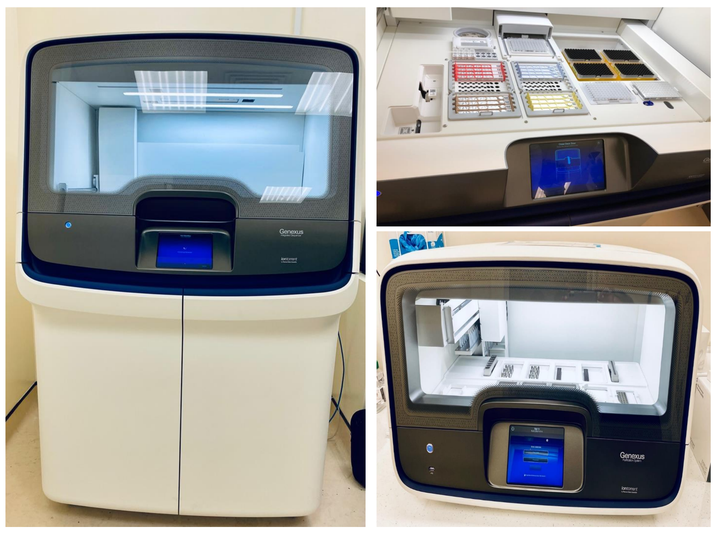

Figure 1. Ion Torrent Genexus™ sequencer and Genexus™ Purification Instrument in-situ with close-up of reagent deck.

- Assay evaluated – Targeted pan cancer panel, **Oncomine Precision Assay** (OPA) encompassing 78 variants, including mutations (45), CNVs (14), and fusion variants (19), across 50 key genes.
- 11 genes selected for NSCLC specific validation; *ALK, BRAF, EGFR, ERBB2, KRAS, MET, NTRK1, NTRK2, NTRK3, RET, ROS1.*
- Optimisation and establishment of laboratory-specific performance metrics for use with OPA on NSCLC samples, including; limits of detection (LOD), minimal depth of coverage, minimum read counts, minimum mapped reads, uniformity and variant allelic fraction (AF).
- Reporting software **Oncomine Reporter™** verified to allow for sample to report integrated workflow.

- Verification on formalin fixed paraffin embedded (FFPE) real world clinical samples including surgical resections, biopsy and cytology cell blocks, commercial reference material (FFPE), and genomic DNA/RNA (n=181) (Fig. 2).

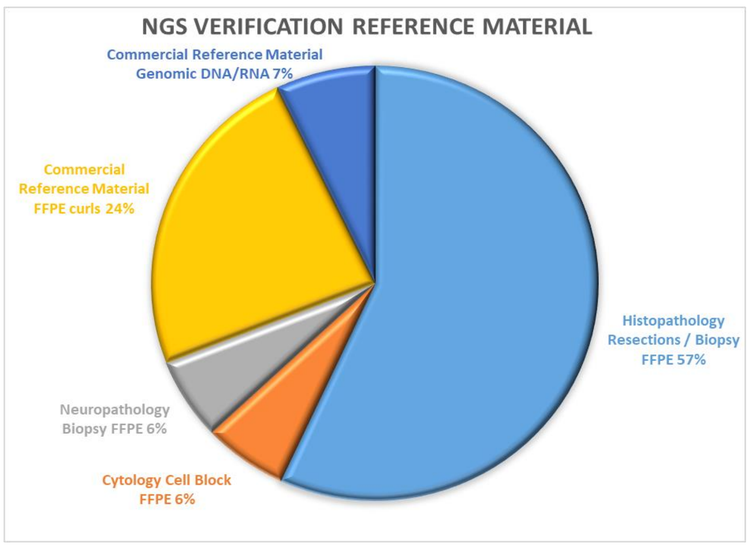

Figure 2. Range of NSG verification reference material spanning multiple specimen types.

- Assessment parameters include determining analytical sensitivity, specificity, positive percent agreement and positive predictive value (PPA/PPV), accuracy, reproducibility, Inter-lot, Inter-operator and Inter-run variability.

Results

- **Automated** Ion Torrent Genexus™ novel sequencing workflow established with OPA and Oncomine Reporter selected as optimal solution for NSCLC samples in this diagnostic laboratory (Fig. 3).

Figure 3. NGS Genexus sample to report automated workflow established at optimisation.

Sequencing Performance:

- Tissue requirements for OPA NGS; minimum input as low as 10ng/µl DNA/RNA were utilised.
- Commercial reference samples (n=56) tested on OPA assay over 20 runs. Oncospan DNA Reference Standard HD832 (Fig. 4) **concordant** with allelic fraction (AF) for each variant detected versus expected AF, all results within range 0.016 - 0.31.

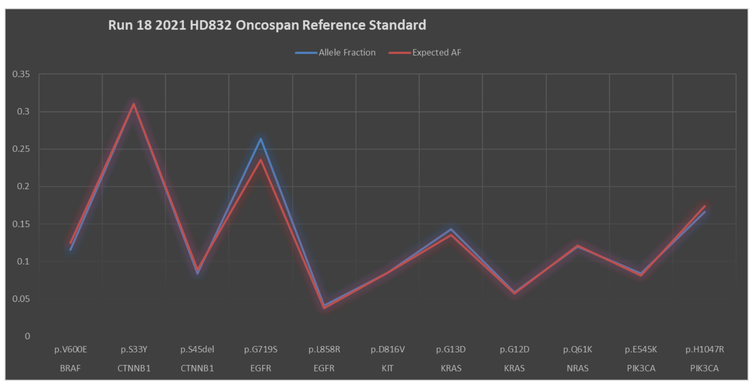

Figure 4. Run 18 HD832 Oncospan: AF for each variant detected versus expected AF.

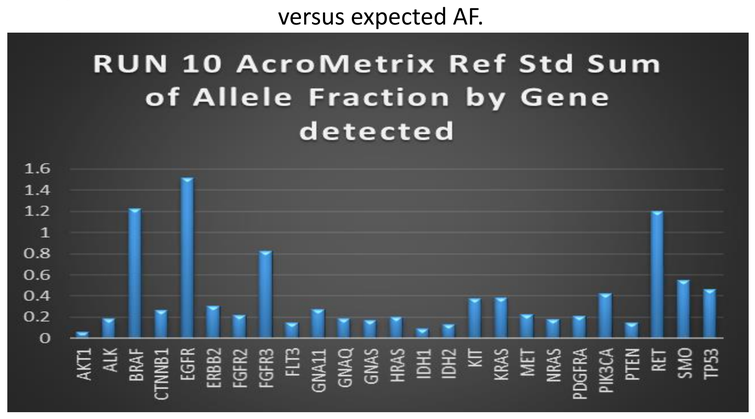

Fig. 5 Acrometrix Oncology Hotspot standard, variants detected & AF.

- AcroMetrix controls 100% specificity and 100% sensitivity across genes in OPA assay (Fig. 5).
- Extensive coverage and concordance of 11 key NSCLC variants included in validation.
- **Reproducibility** 6 runs, different days/operators; results within range (Fig. 6).

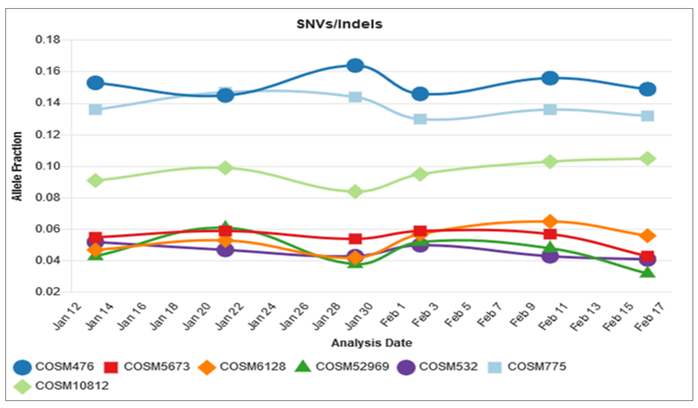

Figure 6. HD789 Horizon multiplex control reproducibility assays, AF consistent & within range.

- **Performance metrics** established over 20 optimisation runs collated from run reports and data analytics from Genexus software (Fig. 7).

GENEXUS OPA RUN PERFORMANCE METRICS	
Metric	**Target**
Final Reads	10-12M
Raw read accuracy	98-99%
% Loading	87-92%
Enrichment	99.90%
Library	99.90%
Mapped reads / DNA library	> 500K (>800 for 5% LOD)
Mapped reads /RNA library	> 100K
% Reads on Target	>90%
Base Coverage Depth	>1000(>2,500 for 5% LOD)
Uniformity	97-99% (>90% for 90%)
End to End reads	>90%
Reads / Amplicon	>500
Allelic Fraction	> 5% / 0.05
MAPD	<0.5 (0.18-0.24)
RNA detection	>5/7
Mean Read Length DNA	85-100
Mean Read Length RNA	70-100
Base Call Accuracy	97-99%

Figure 7. Performance metrics established at optimisation.

- **Irish National Accreditation Board (INAB) assessment Results :** Attained ISO15189 INAB **accreditation**; **no non-conformances**.
- **Turnaround Time (TAT)** audit at 6 months post implementation: **> 47%** reduction in **TAT of NGS** results to clinicians.

Conclusions

- Optimal performance with OPA panel on the Genexus™ using a wide range of specimen types, including NSCLC biopsies, excisions and cytology cell blocks in small **cost effective** runs.
- Successful implementation of an **accredited** NGS service utilising novel technology with a fast **TAT** currently unavailable in any other pathology laboratory in Ireland.
- Successful integration of Oncomine Reporter software to **streamline reporting** without in-house bioinformatics expertise.
- Expansion of NGS to other cancer types ongoing.

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The Ion Torrent Genexus™ was purchased through charity fundraising CUH Charity Cork University Hospital 'Saving and Changing Lives'