# Utility of a New Targeted Next Generation Sequencing Test for Liquid Biopsy Samples from Patients with NSCLC

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Abstract #7440

## Background

Blood-based methods for profiling tumor-sourced nucleic acids have become increasingly important in the diagnostic workup for cancer patients, particularly in non-small cell lung cancer (NSCLC). Blood-based assays address several limitations of tissue-based testing approaches such as inaccessible lesions, limited tissue, extended time to results, and invasive specimen collection procedures. Broad-profiling next-generation sequencing (NGS) methods are used to detect rare variants in blood samples from cell-free nucleic acid (cfNA). Here we describe the utility of a newly developed GeneStrat52 (GS52) NGS test, which uses the GeneStudio™ S5 Prime platform. We report on performance verification of the assay using contrived clinical specimens. We also present a case study which demonstrates that assay's use in during monitoring. These studies add to the growing body of knowledge supporting the usefulness of molecular detection of actionable and emerging biomarkers in liquid biopsy.

## Materials and Methods

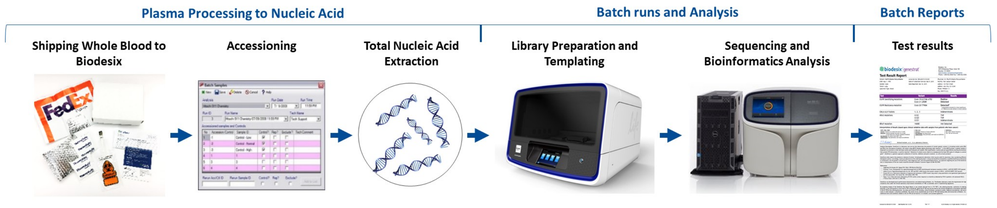


**Figure 1. GeneStrat52 Next Generation Sequencing Test Workflow.** The test process begins when whole blood arrives at the Biodesix laboratory. Test samples are accessioned and processed to isolate circulating nucleic acids, both cfDNA and cfRNA. Following nucleic acid extraction and cDNA synthesis, 20ng input is entered into library preparation using the Oncomine™ Pan-Cancer Cell-Free Total Nucleic Acid Assay according to the manufacturer's guidelines. Templating occurs overnight on the Ion Chef™ System. Sequencing is then performed on the Ion GeneStudio™ S5 Prime, and analysis is completed with the Torrent Suite™/Ion Reporter™ bioinformatics workflow to make variant calls.

## Precision Analyses

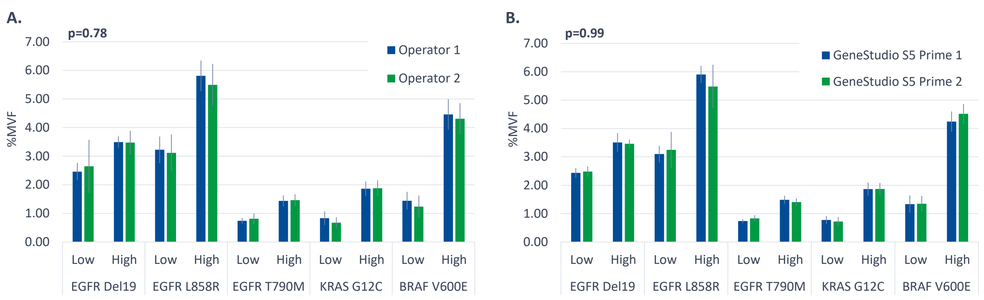


**Figure 2. Performance Comparison for Operators and Instruments.** One high- and one low-concentration specimen were tested for each variant over 18 replicate observations on nine processing days. (A) All data generated by each operator, regardless of day or instrument, was averaged for each variant level. (B) All data generated using each instrument, regardless of operator or day, was averaged for each variant level. A student's t-test was used to determine the p-value for each graph with no significant difference observed between operators or instruments. Error bars indicate ± standard deviation.

## Acknowledgements

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## Clinical Case Study

**Emergence of EGFR C797S[2]:**

- Female, never-smoker presented with new-onset seizures
  - Diagnosed with extensive brain and bony metastatic positive lung adenocarcinoma, EGFR exon 19 deletion (Del19) was identified
- Initial treatment: whole-brain radiation therapy and EGFR receptor tyrosine kinase inhibitor, erlotinib (TARCEVA®) with an excellent initial response
- Acquired resistance using ddPCR and GeneStrat52 NGS: EGFR T790M emerged and treatment was switched to osimertinib (TAGRISSO®)
- Patient discontinued therapy due to resistance and disease progression

**Key Takeaway from clinical case study:**

- This case study further demonstrates clinical utility of NGS for longitudinal monitoring over 540 days for clearance followed by re-emergence of EGFR positive cfNA harboring EGFR C797S resistance mutation
  - EGFR C797S resistance typically presents approximately 10 months following treatment in 40% of NSCLC cases[3]

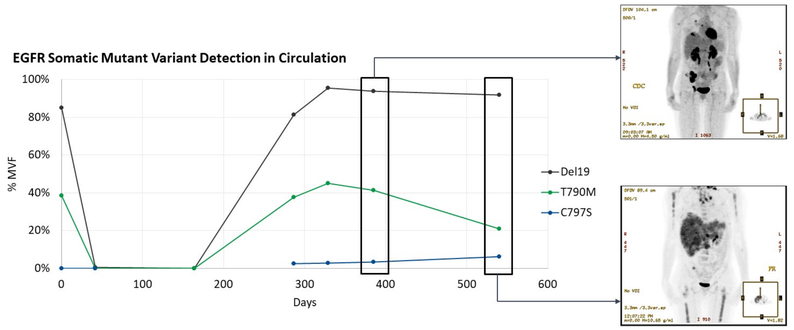
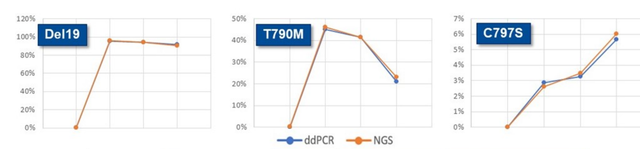



**Figure 3.** Detection of EGFR Del19 driver and T790M erlotinib resistance mutations compared to EGFR C797S osimertinib resistance mutation (%MVF) over 540-day osimertinib treatment cycle.




| Patient Blood Draw | EGFR Del19 | | EGFR T790M | | EGFR C797S | |
|---|---|---|---|---|---|---|
| | %MVF ddPCR | %MVF NGS | %MVF ddPCR | %MVF NGS | %MVF ddPCR | %MVF NGS |
| Day 42 | 0.57% | 0.43% | 0.00% | 0.19% | 0.00% | 0.00% |
| Day 287 | 95.64% | 96.16% | 45.18% | 46.07% | 2.86% | 2.62% |
| Day 385 | 93.96% | 94.23% | 41.41% | 41.62% | 3.26% | 3.50% |
| Day 540 | 91.97% | 90.32% | 21.10% | 23.03% | 5.68% | 6.04% |

**Figure 4.** Comparison of the %MVF achieved with ddPCR and GeneStrat52 NGS of EGFR Del19, T790M, and C797S mutations over 540-days of osimertinib treatment.

| Concordance | | NGS Result | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| ddPCR Result | Positive | 77 | 7 | 84 |
| | Negative | 0 | 37 | 37 |
| | Total | 77 | 44 | 121 |
| Sensitivity: 91.7% | | Specificity: 100% | | |

**Table 1. Concordance between ddPCR and NGS at the variant level.** The %MVF for the GeneStrat® Test[1] ddPCR reference result for each variant were compared to the %MVF results using the GeneStrat52 NGS test on the Ion Torrent GeneStudio S5 Prime.

## Accuracy Analyses

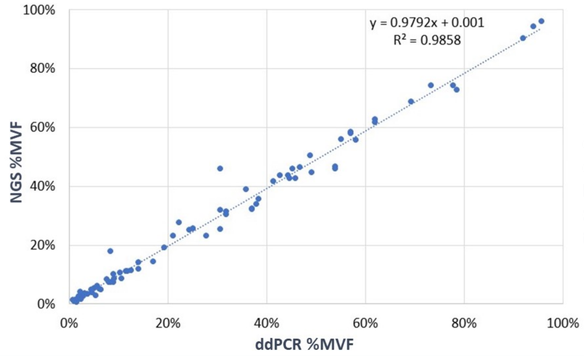



**Figure 5.** Graphical representation of the range of allele frequencies detected with ddPCR (x-axis) and NGS (y-axis). High concordance ($R^2$=0.98) was observed between the two methods.

## Conclusions

- 101 cfNA (cell free nucleic acid) specimens were evaluated from donors previously diagnosed with NSCLC.
- 84 variants for EGFR (E746-A750del, T790M, L858R, G719X, C797S), BRAF V600E or KRAS G12X were detected with allele frequencies ranging from 0.85-96.16% using the GeneStrat52 NGS test. An additional 37 negative specimens were tested.
- Results were highly concordant between the GeneStrat® ddPCR Test and GeneStrat52 NGS ($R^2$=0.986) with 91.7% sensitivity and 100% specificity.
- NGS and ddPCR were successfully utilized for longitudinal monitoring of disease resistance and progression.

## References

[1]Mellert et al. JMD. 2017. v.19 (3) 404 – 416. https://doi.org/10.1016/j.jmoldx.2016.11.004

[2]Bowling et al. Cancer Drug Resist 2019 Aug 27. http://dx.doi.org/10.20517/cdr.2019.53

[3]Thress et al. Nat Med. 2015 Jun;21(6):560-2. doi: 10.1038/nm.3854.

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