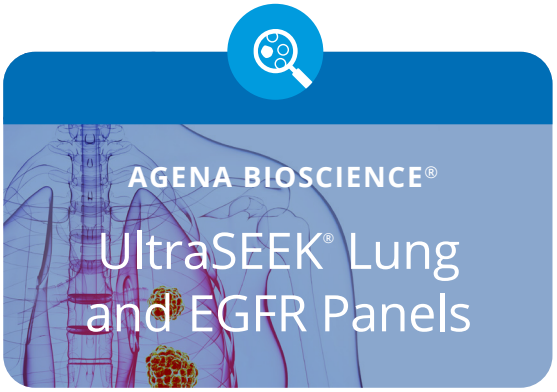
# Ultra-sensitive and accurate detection of key variants in non-small cell lung cancer liquid biopsy specimens

## INTRODUCTION




Cancer is a complex disease, with both inter- and intra- tumor heterogeneity, making it challenging to detect, treat, and manage effectively. Tissue biopsies provide only a snapshot of the disease, as they are generally limited to a single site and are rarely repeated. In addition, rare acquired secondary and tertiary variants present at very low frequencies may easily be missed when the analytical sensitivity of the detection assay is 5-10%. Liquid biopsies overcome these challenges by enabling the detection of circulating tumor DNA (ctDNA) present in blood plasma and derived from tumor sites across the body. While providing a more accurate picture of the disease, liquid biopsies have minimal risk and can easily be repeated to monitor disease and response to therapy.

The ideal technology for the detection of rare variants from cell-free DNA (cfDNA) would enable detection of multiple variants at as low as 0.1% variant allele frequency, have a quick turnaround time with simple analysis, and come at low cost. Next generation sequencing (NGS) assays are well suited for the discovery of new variants that may have clinical utility in the future. However, due to the long turnaround times and extended costs associated with the increase in read depth to achieve the required limit of detection, they are not ideally suited for rapid detection of known actionable targets and disease monitoring. Digital droplet (ddPCR) and real-time PCR (RT-PCR) assays are quick and not as expensive as NGS. However, they require much more input DNA to achieve the required sensitivity and coverage across multiple genes and variants.

In this white paper, we present the MassARRAY® System and UltraSEEK® chemistry as an ideal solution for detecting rare variants from liquid biopsies in non-small cell lung cancer (NSCLC). Using minimal DNA input of 20 ng, the majority of the variants can be detected at as low as 0.1% variant allele frequency (VAF). The MassARRAY System uses a simple, PCR-based single-day workflow with easy analysis, making it ideally suited for disease monitoring and orthogonal validation studies.





Agena® BIOSCIENCE

## ULTRASEEK® EGFR PANEL V2

The UltraSEEK EGFR Panel v2 is designed to detect 6 key activating and resistance mutations in EGFR from circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), for use in treatment monitoring studies, with a low cfDNA input of 20 ng.

**Table 1: Variants detected by the UltraSEEK EGFR Panel v2**

| Gene | Variant (Nucleotide Change) | Variant (Amino Acid Change) |
|---|---|---|
| **EFGR** | c.2235_2249del(15)GGAATTAAGAGAAGC | p.E746_A750del |
| | c.2573T>G | p.L858R |
| | c.2236_2250del(15)GAATTAAGAGAAGCA | p.E746_A750del |
| | c.2369C>T | p.T790M |
| | c.2390G>C | p.C797S |
| | c.2389T>A | p.C797S |

## ULTRASEEK® LUNG PANEL V2

The UltraSEEK Lung Panel v2 was designed to enable study of disease progression and treatment monitoring from CTCs and ctDNA. The panel detects over 70 variants across five genes associated with NSCLC, using a low cfDNA input of 20 ng extracted from a single 10 mL blood draw. In addition, the remaining PCR amplified product from the UltraSEEK EGFR Panel v2 can be used with the UltraSEEK Lung Panel v2 to detect additional variants without requiring more cfDNA sample.

**Table 2: Variants detected by the UltraSEEK Lung Panel v2**

| Gene | Coverage |
|---|---|
| **BRAF** | Exon 11 - codon 469; Exon 15 - codons 594, 600 |
| **EGFR** | Exon 19 indels; Exon 20 insertions, and substitutions across exons 18, 19, 20 and 21 |
| **ERBB2** | Exon 20 insertions |
| **KRAS** | Exon 2 – codons 12, 13; Exon 3 – codon 61 |
| **PIK3CA** | Exon 9 – Codons 542, 545; Exon 20 – codon 1047 |

## ULTRASEEK CHEMISTRY

UltraSEEK methodology uses multiplex PCR, followed by a variant-specific single base extension reaction (Figure 1). The extension reaction uses a variant-specific chain terminator labeled with a moiety for solid phase capture. After the capture, cleaning, and elution process, the extension products (analyte) are desalted, transferred to a SpectroCHIP® Array, and then loaded into the MassARRAY Analyzer and detected using time-of-flight measurements. Process and capture control assays are used to ensure the presence of DNA template in the reaction and success of the bead capture process. The Somatic Variant Report software rapidly provides an automated, easy to interpret readout of variants detected within each sample (Figure 2).

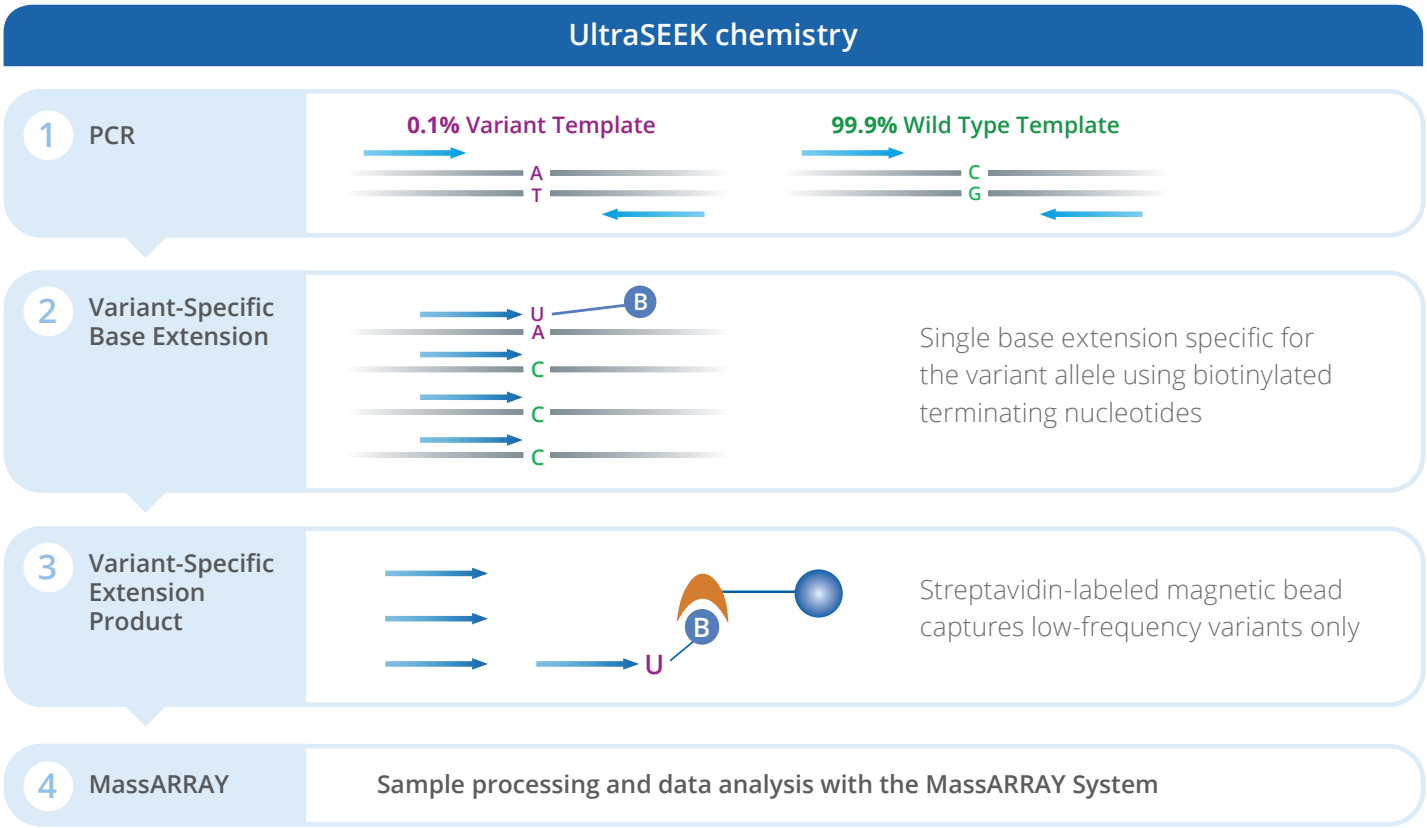



**Figure 1: Rare variant detection with UltraSEEK chemistry on the MassARRAY System**

## SOMATIC VARIANT REPORT

The Somatic Variant Report enables easy analysis of the results from the UltraSEEK EGFR and Lung Panels v2. The user can specify the z-score, peak intensity type (Area, Signal to noise ratio [SNR], height), and minimum values to be used as cutoffs for the detection of variants. Determining the z-score cutoff that gives the required sensitivity and specificity is crucial for confident calling of the data. As you decrease z-score, sensitivity increases, and specificity decreases. The opposite is true as you raise the z-score cutoff.

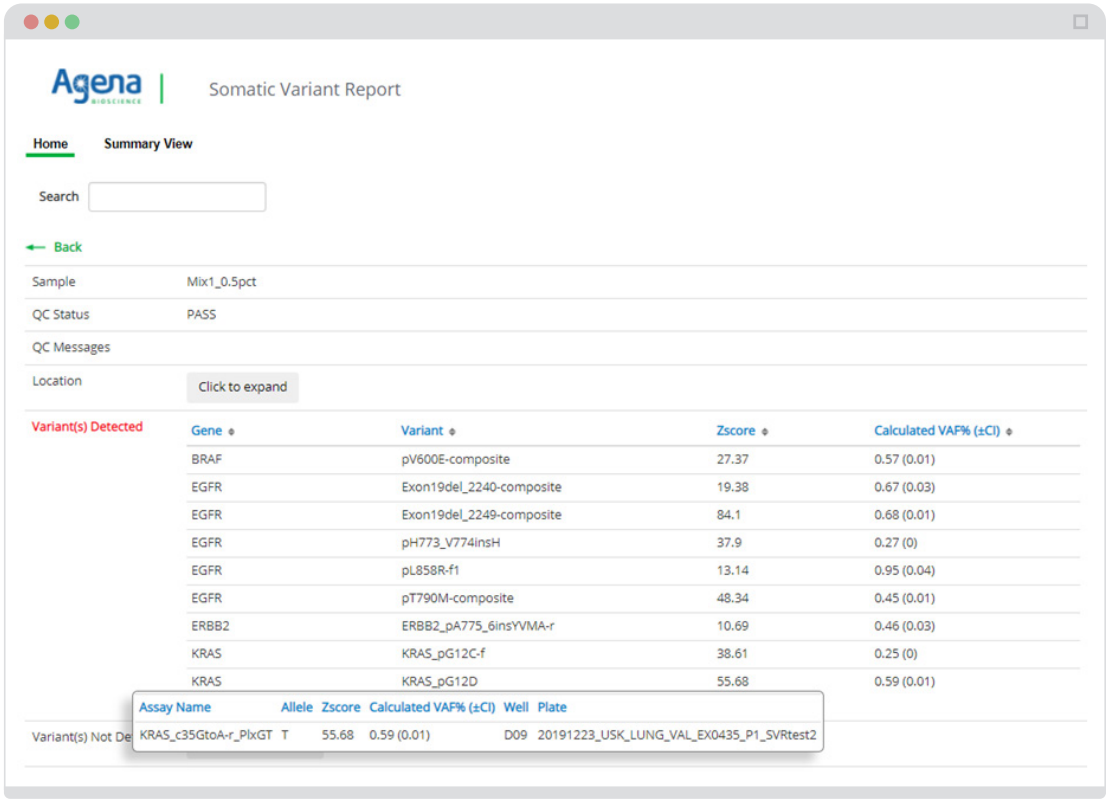



**Figure 2: Example report output for Somatic Variant Report**

## LIMIT OF DETECTION (LOD) STUDY

### Samples Tested

Limit of detection and specificity assessment were performed for every assay. A contrived model system was developed to assess the performance of each assay. The model consisted of synthetic double-stranded constructs known as gBlocks™ from Integrated DNA Technologies. Each construct contains a single variant present in the panel tested. To maximize data, up to three gBlocks were mixed into a single sample with a background of high molecular weight wild-type genomic DNA. gBlocks and genomic DNA were individually quantified for copy number by digital droplet PCR via a genomic reporter sequence. Allelic frequencies evaluated and corresponding copy numbers are listed in Table 3.

**Table 3: Number of copies of mutant and wild type DNA used to generate mutant mixes**

| % Mutant | 2 | 1 | 0.5 | 0.25 | 0.125 | 0 |
|---|---|---|---|---|---|---|
| **Mutant Copy #/Rxn** | 160 | 80 | 40 | 20 | 10 | 0 |
| **WT Copy #/Rxn** | 8000 | 8000 | 8000 | 8000 | 8000 | 8000 |

Each minor variant mix was run in quadruplicate. The assay was considered sensitive to the level tested if three out of four (75%) of the replicates were positive (i.e., had a peak intensity [area] ≥5).

### Assay Baseline

Assay baseline values were determined for each panel by processing 16 wildtype cfDNA samples on the MassARRAY System, as per the Baseline Creation Guide.[2]

## Results

Assay limit of detection and specificity were determined using a z-score of 3 and 10 on the Somatic Variant Report software. Both panels retain high specificity ≥98% and low limit of detection ≤0.5% at both z-scores.(Table 4).

**Table 4: UltraSEEK EGFR and Lung Panels v2 assay performance with commercial reference standards**

| Panel | Z-score = 3 | | | Z-score = 4 | | |
|---|---|---|---|---|---|---|
| | Sensitivity | | Specificity | Sensitivity | | Specificity |
| | Variant Allele Frequency (VAF) | % of assays detecting ≥75% of replicates | | Variant Allele Frequency (VAF) | % of assays detecting ≥75% of replicates | |
| **UltraSEEK EGFR v2** | 0.125% | 68% | 99.73% | 0.125% | 0% | 100% |
| | 0.25% | 84% | | 0.25% | 33% | |
| | 0.5% | 84% | | 0.5% | 83% | |
| | 1% | 100% | | 1% | 100% | |
| **UltraSEEK Lung v2** | 0.125% | 82% | 99.73% | 0.125% | 46% | 100% |
| | 0.25% | 97% | | 0.25% | 70% | |
| | 0.5% | 100% | | 0.5% | 90% | |
| | 1% | 100% | | 1% | 98% | |
| | 2% | 100% | | 2% | 100% | |

Sensitivity assessments were evaluated at z-scores 3-10 (Table 5 and Table 6), where sensitivity is defined as the number of assays detecting at least 3 out of 4 replicates at a given variant allele frequency. Specificity assessments were done with a minimum of 8 wild-type samples and were also evaluated using z-scores 3-10 (Table 7 and Table 8).

**Table 5: Effect of z-score on sensitivity of UltraSEEK EGFR Panel v2 assays**

| Sensitivity | % of Assays detecting ≥75% of the replicates at each VAF | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **VAF** | **z3** | **z4** | **z5** | **z6** | **z7** | **z8** | **z9** | **z10** |
| **0.125%** | 66 | 66 | 50 | 32 | 0 | 0 | 0 | 0 |
| **0.25%** | 82 | 82 | 82 | 82 | 66 | 50 | 32 | 32 |
| **0.5%** | 82 | 82 | 82 | 82 | 82 | 82 | 82 | 82 |
| **1%** | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Table 6: Effect of z-score on sensitvity of UltraSEEK Lung Panel v2 assays

| Sensitivity | % of Assays detecting ≥75% of the replicates at each VAF | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| VAF | z3 | z4 | z5 | z6 | z7 | z8 | z9 | z10 |
| 0.125% | 82 | 78 | 65 | 59 | 54 | 54 | 51 | 46 |
| 0.25% | 97 | 91 | 90 | 86 | 82 | 79 | 74 | 70 |
| 0.5% | 100 | 97 | 98 | 96 | 92 | 92 | 92 | 90 |
| 1% | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 98 |
| 2% | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Z-score can also impact the specificity of the assays (the percentage of variants detected in wild-type samples). Note that the number of variants called is less than the total number of assays, due to assay redundancy.

Table 7: Effect of z-score on specificity of UltraSEEK EGFR Panel v2 assays

| % of Total Assays at each Specificity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Specificity | z3 | z4 | z5 | z6 | z7 | z8 | z9 | z10 |
| 98% | 16 | 16 | 0 | 0 | 0 | 0 | 0 | 0 |
| 99% | 32 | 16 | 0 | 0 | 0 | 0 | 0 | 0 |
| 100% | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Table 8: Effect of z-score on specificity of UltraSEEK Lung Panel v2 assays

| % of Total Assays at each Specificity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Specificity | z3 | z4 | z5 | z6 | z7 | z8 | z9 | z10 |
| 98% | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 99% | 19 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 100% | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

The limit of detection and specificity for each assay in the UltraSEEK EGFR Panel v2 (Table 9) and the UltraSEEK Lung Panel v2 (Table 10), at a z-score of 3, are shown below.

**Table 9: UltraSEEK EGFR Panel v2 limit of detection and specificity with gBlocks using a z-score of 3 and area of 5**

| Assay | Variant | Limit of Detection | Specificity |
|---|---|---|---|
| EGFR_c2390GtoC_r | p.C797S | 0.125 | 99% |
| EGFR_c2389TtoA_ASO_f | p.C797S | 0.125 | 100% |
| EGFR_c2235_2249del15_r | p.E746_A750del | 0.5 | 98% |
| EGFR_c2236_2250del15_f | p.E746_A750del | 0.25 | 100% |
| EGFR_c2573TtoG_ASO_f | p.L858R | 0.125 | 100% |
| EGFR_c2369CtoT_f | p.T790M | 0.125 | 100% |

**Table 10: UltraSEEK Lung Panel v2 limit of detection and specificity with gBlocks using a z-score of 3 and area of 5**

| Assay | Variant | Limit of Detection | Specificity |
|---|---|---|---|
| BRAF_c1406GtoCT-f2_PlxCT | BRAF_pG469A | 0.125 | 100% |
| BRAF_c1406GtoCT-f1_PlxCT | BRAF_pG469V | 0.125 | 100% |
| BRAF_c1406GtoCT-f2_PlxCT | BRAF_pG469V | 0.125 | 100% |
| BRAF_c1799TtoA-r_PlxCT | BRAF_pV600E | 0.125 | 100% |
| BRAF_c1799TtoA-r_PlxT | BRAF_pV600E | 0.125 | 100% |
| EGFR_c2390GtoC-r_PlxGT | EGFR_pC797S | 0.125 | 98% |
| EGFR_c2309AtoG-r_aso_PlxCG | EGFR_pD770_N771insG | 0.125 | 99% |
| EGFR_c2126AtoC-r1_PlxCG | EGFR_pE709A | 0.125 | 99% |
| EGFR_c2126AtoCT-f1_PlxCT | EGFR_pE709A | 0.125 | 100% |
| EGFR_c2126AtoG-r1_PlxCG | EGFR_pE709G | 0.125 | 100% |
| EGFR_c2126AtoG-r2_PlxCG | EGFR_pE709G | 0.125 | 100% |
| EGFR_c2125GtoA-r1_PlxGT | EGFR_pE709K | 0.125 | 100% |
| EGFR_c2125GtoA-r2_PlxGT | EGFR_pE709K | 0.125 | 100% |
| EGFR_c2125GtoA-r3_PlxGT | EGFR_pE709K | 0.125 | 100% |
| EGFR_c2126AtoCT-f1_PlxCT | EGFR_pE709V | 0.125 | 100% |
| EGFR_c2249CtoA-r1_PlxCT | EGFR_pE746_A750del | 0.125 | 100% |
| EGFR_c2249CtoA-r2_PlxCT | EGFR_pE746_A750del | 0.125 | 100% |

| Assay | Variant | Limit of Detection | Specificity |
|---|---|---|---|
| EGFR_c2250AtoG-r_PlxC | EGFR_pE746_A750del | 0.125 | 100% |
| EGFR_c2245GtoA-r1_PlxGT | EGFR_pE746_E749del | 0.125 | 100% |
| EGFR_c2245GtoA-r2_PlxGT | EGFR_pE746_E749del | 0.125 | 98% |
| EGFR_c2238AtoT-f_PlxGT | EGFR_pE746_S752>D | 0.125 | 98% |
| EGFR_c2237AtoCT-f1_PlxCT | EGFR_pE746_S752toV | 0.125 | 100% |
| EGFR_c2237AtoCT-f2_PlxCT | EGFR_pE746_S752toV | 0.125 | 100% |
| EGFR_c2236GtoCT-f1_PlxCT | EGFR_pE746_T751del | 0.125 | 98% |
| EGFR_c2236GtoCT-f2_PlxCT | EGFR_pE746_T751del | 0.125 | 100% |
| EGFR_c2156GtoC-f_PlxCT | EGFR_pG719A | 0.125 | 100% |
| EGFR_c2155GtoT-f_PlxT | EGFR_pG719C | 0.125 | 100% |
| EGFR_c2308GtoC-r_PlxGT | EGFR_pH773_74insNPH | 0.125 | 100% |
| EGFR_c2320GtoC-f_PlxCT | EGFR_pH773_V774insH | 0.125 | 100% |
| EGFR_c2248GtoC-r_PlxGT | EGFR_pL747_A750toP | 0.125 | 100% |
| EGFR_c2239TtoG-f_PlxCG | EGFR_pL747_E749del | 0.125 | 99% |
| EGFR_c2240TtoC-f1_PlxCG | EGFR_pL747_P753toS | 0.125 | 100% |
| EGFR_c2251AtoCG-r_PlxCG | EGFR_pL747_T751toP | 0.125 | 100% |
| EGFR_c2582TtoAG-r_PlxCT | EGFR_pL861Q | 0.125 | 100% |
| EGFR_c2582TtoAG-r_PlxCT | EGFR_pL861R | 0.125 | 100% |
| EGFR_c2369CtoT-f1_PlxGT | EGFR_pT790M | 0.125 | 98% |
| EGFR_c2369CtoT-f2_PlxGT | EGFR_pT790M | 0.125 | 100% |
| ERBB2_c2325_2326ins12-f1_PlxCT | ERBB2_pA775_6insYVMA | 0.125 | 100% |
| ERBB2_c2325_2326ins12-f2_PlxCT | ERBB2_pA775_6insYVMA | 0.125 | 100% |
| ERBB2_c2326_2327ins3-f_PlxCT | ERBB2_pG776toVC | 0.125 | 99% |
| KRAS_c35GtoCT-f_PlxCT | KRAS_pG12A | 0.125 | 100% |
| KRAS_c34GtoCT-f_PlxCT | KRAS_pG12C | 0.125 | 99% |
| KRAS_c35GtoA-r_PlxGT | KRAS_pG12D | 0.125 | 100% |
| KRAS_c34GtoC-r_PlxGT | KRAS_pG12R | 0.125 | 100% |
| KRAS_c34GtoCT-f_PlxCT | KRAS_pG12R | 0.125 | 99% |

| Assay | Variant | Limit of Detection | Specificity |
|---|---|---|---|
| KRAS_c34GtoA-r_PlxGT | KRAS_pG12S | 0.125 | 100% |
| KRAS_c35GtoCT-f_PlxCT | KRAS_pG12V | 0.125 | 100% |
| KRAS_c37GtoT-f_PlxT | KRAS_pG13C | 0.125 | 100% |
| KRAS_c38GtoA-r_PlxT | KRAS_pG13D | 0.125 | 100% |
| KRAS_c181CtoAG-r1_PlxCT | KRAS_pQ61E | 0.125 | 100% |
| KRAS_c183AtoCT-f_PlxCT | KRAS_pQ61H_AlleleT | 0.125 | 100% |
| KRAS_c181CtoAG-r1_PlxCT | KRAS_pQ61K | 0.125 | 100% |
| KRAS_c182AtoCT-f_PlxCT | KRAS_pQ61L | 0.125 | 100% |
| KRAS_c182AtoCT-f_PlxCT | KRAS_pQ61P | 0.125 | 100% |
| PIK3CA_c1633GtoA-r_PlxGT | PIK3CA_pE545K | 0.125 | 98% |
| KRAS_c183AtoCT-f_PlxCT | KRAS_pQ61H_AlleleC | 0.125 | 100% |
| BRAF_c1406GtoCT-f1_PlxCT | BRAF_pG469A | 0.125 | 100% |
| EGFR_c2389TtoA-r_PlxCT | EGFR_pC797S | 0.125 | 100% |
| EGFR_c2247AtoC-r_PlxCG | EGFR_pK745_E749del | 0.125 | 100% |
| EGFR_c2239TtoC-f_PlxCG | EGFR_pL747_A750toP | 0.125 | 99% |
| EGFR_c2240TtoC-f2_PlxC | EGFR_pL747_P753toS | 0.125 | 100% |
| KRAS_c181CtoAG-r2_PlxCT | KRAS_pQ61E | 0.125 | 100% |
| PIK3CA_c3140AtoT-f_PlxT | PIK3CA_pH1047L | 0.125 | 100% |
| KRAS_c181CtoAG-r2_PlxCT | KRAS_pQ61K | 0.125 | 100% |
| EGFR_c2300CtoA-r_PlxT | EGFR_pD770_71insSVD | 0.125 | 100% |
| BRAF_c1781AtoG-r_PlxC | BRAF_pD594G | 0.25 | 100% |
| EGFR_c2126AtoC-r2_PlxCG | EGFR_pE709A | 0.25 | 100% |
| EGFR_c2126AtoCT-f2_PlxCT | EGFR_pE709V | 0.25 | 100% |
| EGFR_c2155GtoA-r_PlxT | EGFR_pG719S | 0.25 | 98% |
| EGFR_c2312AtoC-f_PlxCT | EGFR_pN771toTH | 0.25 | 100% |
| ERBB2_c2324_2325ins12-r_PlxCG | ERBB2_pA775_6insYVMA | 0.25 | 100% |
| EGFR_c2309AtoC-f_PlxCG | EGFR_pV769_70insASV | 0.25 | 100% |
| EGFR_c2126AtoCT-f2_PlxCT | EGFR_pE709A | 0.25 | 100% |
| EGFR_c2573TtoG-f1_PlxCG | EGFR_pL858R | 0.25 | 100% |

| Assay | Variant | Limit of Detection | Specificity |
|---|---|---|---|
| EGFR_c2573TtoG-f2_PlxCG | EGFR_pL858R | 0.25 | 100% |
| PIK3CA_c1624GtoA-r_PlxGT | PIK3CA_pE542K | 0.25 | 100% |
| EGFR_c2303GtoT-f_PlxT | EGFR_pS768I | 0.25 | 100% |
| KRAS_c182AtoG-r_PlxC | KRAS_pQ61R | 0.5 | 100% |
| PIK3CA_c3140AtoG-r_PlxC | PIK3CA_pH1047R | 0.5 | 100% |

The calibration curves derived from the LoD study display a linear response for most assays. These linear responses are used as correction coefficients to transform the normalized UltraSEEK data into allele frequency. Additionally, the observed variance of each assay is accounted for with a confidence interval around the result. The allele frequency and confidence interval are reported in the Somatic Variant Report, along with the z-score.

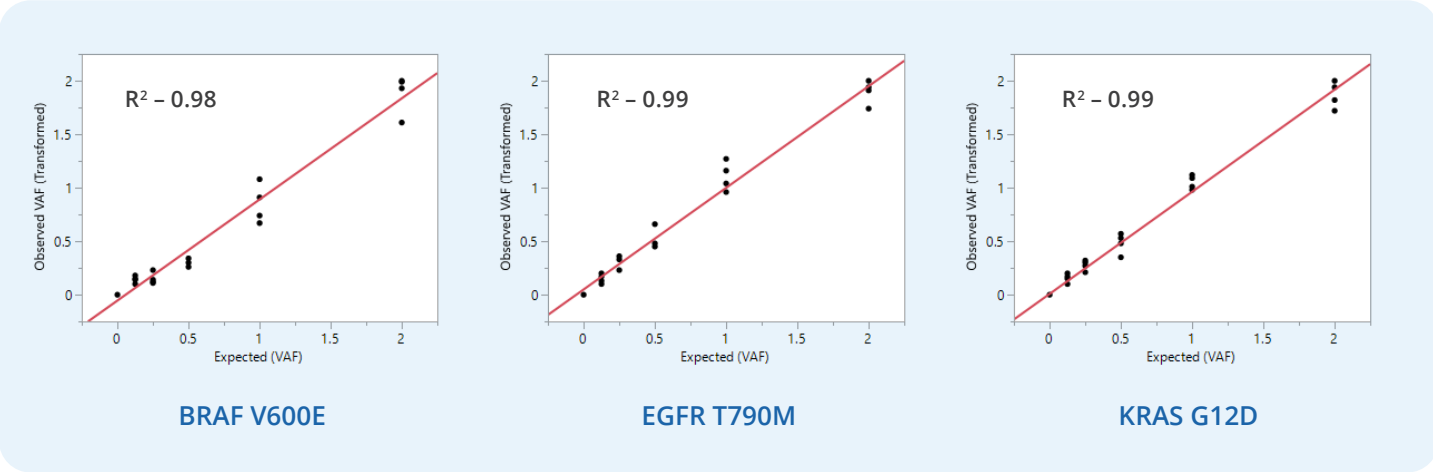



**Figure 3: Example of expected vs. observed (transformed) allelic frequency**

## ACCURACY STUDY

Assay performance in cfDNA samples was verified using the SeraCare Seraseq® ctDNA Complete™ Mutation Mix ctDNA reference standard, which contains 11 variants that can be detected using 14 assays in the UltraSEEK Lung Panel v2.

Allele frequencies of 1% (Catalog# 0710-0530), 0.5% (Catalog# 0710-0531), and 0.1% (Catalog# 0710-0532) were tested in triplicate, in addition to wild-type and non-template controls. The results for the reference standard were analyzed using a z-score of 3. Of the 14 assays characterized with this reference material, 14/14 assays (100%) achieved an LoD between 0.1% and 0.5% VAF (3/3 replicates tested).

**Table 11: UltraSEEK Lung Panel v2 assay % Positive Predictive Agreement (%PPA) with SeraCare reference standard**

| Reference Standard | %PPA |
|---|---|
| Seracare 1% | 100% (38/38)* |
| Seracare 0.5% | 100% (39/39) |
| Seracare 0.1% | 80% (31/39) |

*KRAS_c183AtoCT-f_PlxCT failed across all reference standards and was not included in the calculation. This sample was successfully detected at 0.1% upon repeat testing.*

**One replicate failed and was not counted*

The KRAS_c183AtoCT-f_PlxCT assay failed to generate a mutant signal for any of the allele frequencies and was repeated at the 0.1% allelic frequency. All three replicates were successfully detected (Table 12).

**Table 12: Repeat testing of KRAS_c183AtoCT-f_PlxCT assay (KRAS Q61H variant)**

| Variant | Assay | Allele Frequency (%) |
|---|---|---|
| KRAS_pQ61H | KRAS_c183AtoCT-f_PlxCT KRAS_pQ61H | 0.1 (3/3) |

## REPRODUCIBILITY STUDY

The reproducibility of the UltraSEEK Lung Panel v2 was determined by testing SeraCare Seraseq® ctDNA Complete Panel Mutation mix cfDNA reference standard at two independent laboratories at variant allele frequencies of 1% and 0.5%. Both sites were able to detect over 95% of the variants at 1% VAF and 90% at 0.5% VAF demonstrating the robustness of the assay across different users and instruments (Table 13).

**Table 13: Summary of reproducibility study with UltraSEEK Panel v2**

| Site | SeraCare 1% | SeraCare 0.5% |
|---|---|---|
| **Site #1** | 100% PPA (45/45) | 90% PPA (38/42*) |
| **Site #2** | 96% PPA (43/45) | 96% PPA (43/45) |

**Three failed reactions were not considered*

## EVALUATION WITH cfDNA SAMPLES

The performance of the panels was evaluated with clinical cfDNA samples which were previously tested using the cobas® EGFR Mutation Test v2 from Roche. Samples were extracted following the cobas EGFR Mutation Test v2 Instructions for Use and were not quantified. They were then interrogated with the UltraSEEK EGFR and Lung Panels v2 for orthogonal concordance and demonstration of functionality of the panel in relevant sample types. A z-score of 3 was used for data analysis.

The UltraSEEK EGFR Panel v2 showed 100% concordance with the Roche cobas® EGFR Mutation Test v2 for 6 previously characterized cfDNA samples (Table 14).

The UltraSEEK Lung Panel v2 was tested with 8 cfDNA samples previously characterized with the Roche cobas EGFR Mutation Test v2 (Table 15). The cobas EGFR Mutation Test v2 does not include mutations in several genes (BRAF, KRAS, ERBB2, and PIK3CA) that are characterized by the UltraSEEK Lung Panel v2. The stated cobas EGFR Mutation Test v2 LoD is 1.3-13.4% VAF with 50 ng of input cfDNA, which is above the LoD of the UltraSEEK Lung Panel v2 for the same EGFR mutations as tested on contrived and reference material. Discordant results in samples LB16-0622 is likely due to the lower LoD of the UltraSEEK panel.

Table 14: UltraSEEK EGFR Panel v2 results with cfDNA samples

| Sample | Variants Detected by UltraSEEK EGFR Panel v2 | VAF | Variants Detected by cobas EGFR Mutation Test v2 |
|---|---|---|---|
| LB16-0279 | EGFR p.T790M | 0.36 | EGFR T790M |
| LB16-0443 | EGFR p.E746_A750Del | >2 | Exon 19 Del & EGFR_T790M |
| | EGFR p.T790M | >2 | |
| LB16-0617 | EGFR p.E746_A750Del | >2 | Exon 19 Del & EGFR_T790M |
| | EGFR p.T790M | >2 | |
| LB17-0015 | EGFR p.E746_A750Del | >2 | Exon 19 Del & EGFR_T790M |
| | EGFR p.T790M | >2 | |
| LB17-0232 | EGFR p.E746_A750Del | >2 | Exon 19 Del & EGFR_T790M |
| | EGFR p.T790M | >2 | |
| LB17-0293 | EGFR p.T790M | >2 | EGFR_T790M |

Table 15: UltraSEEK Lung v2 Panel results with cfDNA samples

| Sample | Variants Detected by UltraSEEK Lung Panel v2 | VAF | Variants Detected by cobas EGFR Mutation Test v2 |
|---|---|---|---|
| LB16-0031 | BRAF p.V600E* | 0.88 | EGFR L858R, EGFR T790M *(BRAF V600E, KRAS G12AV not part of Cobas Assay)* |
| | EGFR p.T790M* | 0.46 | |
| | EGFR p.L858R* | 1.18 | |
| LB16-0039 | EGFR p.E746_S752toV | >2% | EGFR EX19 DEL |
| LB16-0514 | EGFR p.L747_A750toP | >2% | EGFR EX19 DEL |
| LB16-0622 | EGFR p.G719A | 0.43 | WT |
| | EGFR p.L747_A750toP | 0.27 | |
| | EGFR p.L861QR | 1.67 | |
| LB16-1039 | BRAF p.V600E* | 0.34 | WT (BRAF V600E, KRAS G12S, PIK3CA H1047R not part of Cobas Assay) |
| | KRAS p.G12S | 1.08 | |
| | PIK3CA p.H1047R | 0.33 | |
| LB17-0015 | EGFR p.E746_A750del* | >2% | EGFR EX19 DEL, EGFR T790M |
| | EGFR p.T790M* | >2% | |
| LB17-0232 | EGFR p.E746_A750del* | >2% | EGFR EX19 DEL, EGFR T790M |
| | EGFR p.T790M* | >2% | |

| Sample | Variants Detected by UltraSEEK Lung Panel v2 | VAF | Variants Detected by cobas EGFR Mutation Test v2 |
|---|---|---|---|
| LB17-0293 | EGFR p.L747_P753toS | >2% | EGFR EX19 DEL, EGFR T790M |
| | EGFR p.T790M* | >2% | |

**Result confirmed via redundant assays*

## SUMMARY

≥80% of assays in the UltraSEEK Lung and EGFR Panels have a limit of detection ≤0.5%, and 100% specificity (when z-score = 10). These UltraSEEK panels are a reliable and ultrasensitive alternative for detecting clinically relevant variants in NSCLC and can be used to detect variants at a sensitivity appropriate for liquid biopsy samples. The range of somatic mutations in each panel allows the user to maximize coverage of clinically relevant variants while minimizing DNA input. All assays across both panels were characterized for limit of detection, and calibration curves were developed for each assay. These values are implemented in the Somatic Variant Report software, to enable reporting of variant allele frequencies for all assays. The linear response at liquid biopsy-relevant allelic frequencies makes these UltraSEEK panels an ideal choice for monitoring the efficacy of treatment or residual disease in non-small cell lung cancer.

## References

1. DNA input and effect of copy number at different allele frequencies
https://blog.seracare.com/ngs/how-many-target-copies-are-present-in-your-plasma-dna-sample

2. Baseline Creation Guide - iPLEX® HS and UltraSEEK® Panels v2 and ClearSEEK™ Panels [USG-CUS-135]





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