

## High-Sensitivity Mutation Scanning in Tumor Specimens using SURVEYOR® Nuclease and the WAVE® HS System

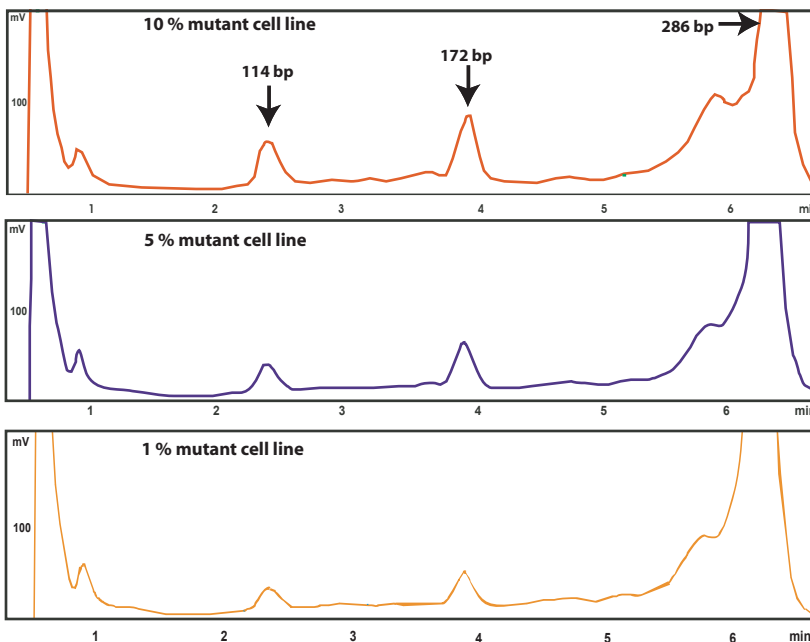
**Background** – Mutations in a number of different cancer-associated genes have been shown to correlate with disease progression, response to therapy, and/or emergence of drug resistance in a number of reports. Mutational analysis of patient tumor specimens can help relate molecular mechanisms of disease to patient outcomes.

*Sensitive and reliable mutation detection methods are critical for analysis of clinical specimens that are often heterogeneous due to the co-existence of tumor tissue, normal tissue, and/or tumor-infiltrating lymphocytes. The limited size of many biopsy specimens poses another related challenge.*

Due to the limited analytical sensitivity of current methods, preliminary microdissection of the specimen is often required to isolate a region that is comprised of 50% - 80% tumor tissue. This contributes additional time and expense to the workflow, and poses significant challenges in obtaining data from small and/or highly heterogeneous samples.

### The SURVEYOR Nuclease – WAVE HS System Solution

Transgenomic's SURVEYOR Nuclease, coupled with the WAVE HS System, has been shown to provide *superior sensitivity compared to direct DNA sequencing for the analysis of formalin-fixed paraffin-embedded (FFPE) tumor specimens*<sup>1</sup>. This method offers the potential to reduce or eliminate the need for microdissection prior to scanning for tumor-associated somatic mutations. Figure 1 shows the sensitivity of this approach in a genomic DNA dilution experiment.



**Figure 1 - Detection of Low-level EGFR Mutation in a Dilution Experiment.** DNA from a cell line containing an EGFR exon 21 mutation was diluted with EGFR wild-type genomic DNA and subjected to analysis with SURVEYOR Nuclease and the WAVE HS System. The 114 bp and 172 bp fragments are SURVEYOR cleavage products resulting from the presence of the mutation. (Analysis by Navigator™ Software).

### PROTOCOL OVERVIEW

**STEP 1: PCR Amplification**

**STEP 2: SURVEYOR Endonuclease Digestion**

**STEP 3: WAVE High Sensitivity Analysis**

Appropriate DNA size standards facilitate determination of fragment sizes and approximate location of the mutation, aiding subsequent sequence analysis.

#### **Heteroduplex Enrichment (Optional)**

For very low level mutations the samples can be rerun under partially denaturing conditions. A series of fractions can be automatically collected for re-amplification and subsequent sequence confirmation of the mutation.

### Key Advantages

- Increased sensitivity vs. direct DNA sequencing
- Unbiased scanning approach
- Decrease or eliminate need for microdissection
- More specimens suitable for analysis
  - Formalin-fixed, paraffin-embedded (FFPE) specimens
  - Small specimens obtained by non-surgical techniques

## Selected References

1. Janne, P.A., Borras, A.M., Kuang, Y., Rogers, A.M., Joshi, V.A., Liyanage, H., Lindeman, N., Lee, J.C., Halmos, B., Maher, E.A., Distel, R.J., Meyerson, M. and Johnson, B.E. (2006) A rapid and sensitive enzymatic method for epidermal growth factor receptor mutation screening. *Clin. Cancer Res.* 12, 751-758.
2. Sattler, M., Walz, C., Crowley, B.J., Lengfelder, E., Janne, P.A., Rogers, A.M., Kuang, Y., Distel, R.J., Reiter, A. and Griffin, J.D. (2006) A sensitive high-throughput method to detect activating mutations of Jak2 in peripheral-blood samples. *Blood* 107, 1237-1238.
3. Qiu, P., Shandilya, H., D'Alessio, J.M., O'Connor, K., Durocher, J. and Gerard, G.F. (2004) Mutation detection using Surveyor nuclease. *Biotechniques* 36, 702-707.

## Products

### SURVEYOR Nuclease

Description	Catalog No.	Quantity
SURVEYOR Mutation Detection Kit for WAVE and WAVE HS Systems	706030	100 reactions
SURVEYOR Mutation Detection Kit for WAVE and WAVE HS Systems	706035	25 reactions

### WAVE HS Systems

Model	Description	Catalog No.
4500HT-HS	High Sensitivity WAVE Nucleic Acid Fragment Analysis System Model 4500HT-HS	NHS-99-4500

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