

# Routine Detection of *BRAF* and *NRAS* mutations in FFPE tissue by Pyrosequencing

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## Summary

*BRAF* mutations are found in cutaneous melanoma and colorectal cancer, while *NRAS* mutations are common in cutaneous melanoma and myeloid leukaemias. We have developed, and are routinely using for archival (FFPE) tissue, pyrosequencing assays to detect the most common mutations of these two genes. The improved sensitivity of these assays over conventional capillary sequencing, coupled with prior macrodissection to increase tumour burden of FFPE sections, ensures that these assays are robust and reproducible.

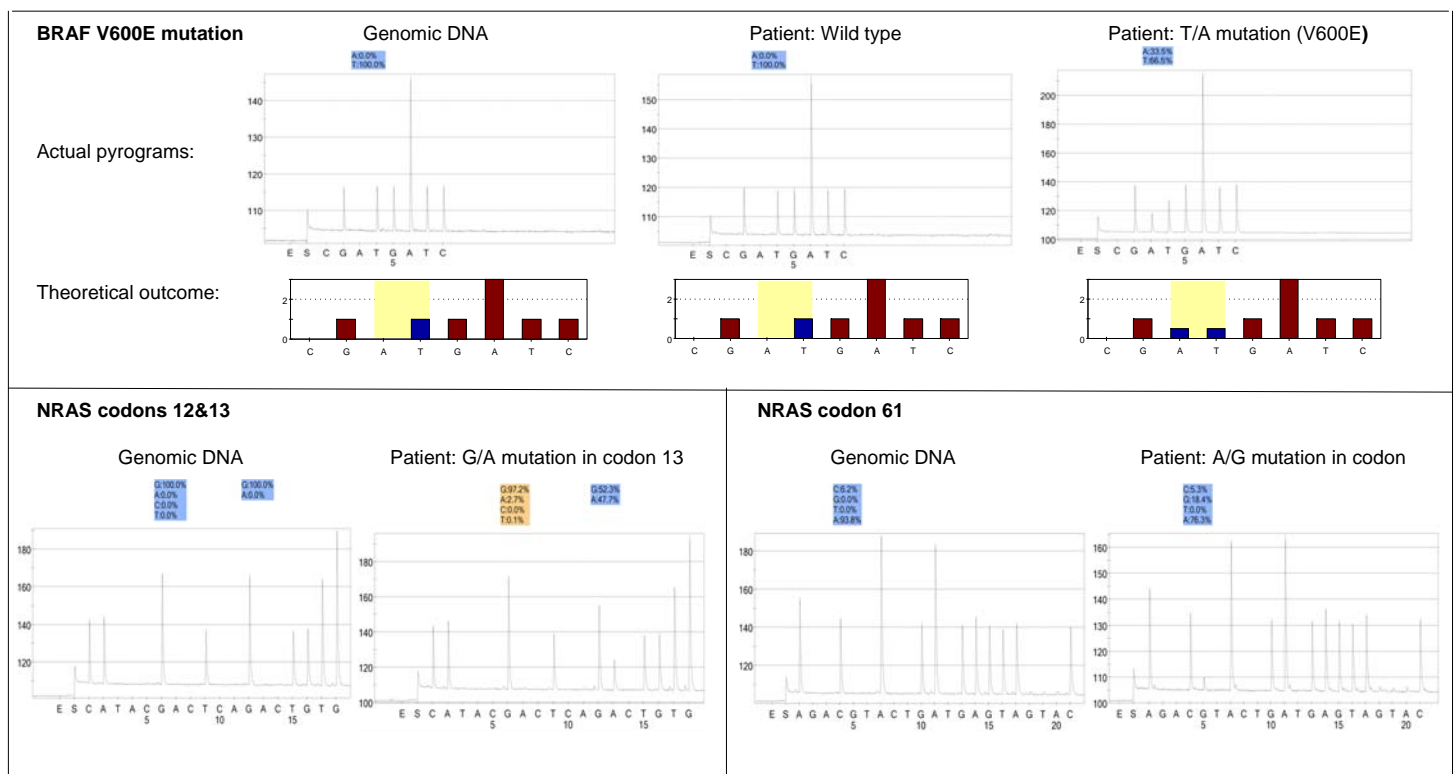
## Introduction

Acquired mutations of genes involved in proliferation, differentiation and apoptosis lead to the transformation of normal cells to cancer cells. In addition, many of these mutations are now known to contribute to resistance and, in some cases sensitivity to the new targeted therapeutics. Much of this valuable genetic information is locked away in FFPE (formalin-fixed, paraffin-embedded) tumour samples. There is a growing requirement for robust methods by which mutations can be analysed retrospectively in older samples, but which also dispense with the logistic problems of frozen samples. *BRAF* mutations are found in cutaneous melanoma (60%) and colorectal cancer (10%), while *NRAS* mutations are common in cutaneous melanoma (20%) and myeloid leukaemias (10%). Such mutations may have prognostic value, but more importantly, are now showing increasing importance as predictors of response, for a range of therapies. We have developed proprietary pyrosequencing assays for detecting the most common activating mutations of the *BRAF* and *NRAS* genes, namely the V600E mutation in *BRAF*, and mutations of codons 12, 13, and 61 in *NRAS*.

## Materials and methods

The assays, run on the PyroMark PSQ96 pyrosequencing platform, were validated using DNA extracted from melanoma cell lines with known mutations of the *NRAS* and *BRAF* genes, and from FFPE sections of appropriate tissue. The results were compared with those generated by capillary sequencing of the same samples, and (in the case of *BRAF*) the Qiagen DxS ARMS-based test for the V600E mutation. In the case of FFPE tissues, 5 x 5 micron sections are cut from blocks and, where tumour burden is below 70%, macrodissected to enrich for tumour DNA. The overall sensitivity of these assays, i.e. their ability to detect a mutant allele against a background of normal alleles, is approximately 10-15%, which compares favourably with capillary sequencing (sensitivity approx. 25-30%).

## Results



## Conclusions

These tests represent powerful and cost-effective tools for determining presence of mutations in key genes in archival material, and can contribute significantly in clinical trial and other drug development scenarios.

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