

# Evaluation of alterations in the *EGFR* gene for patient stratification

Jackie Rodgers, Graeme Denton, Tom Burr, Sam Haldenby, and Cliff Murray  
Source BioScience UK Ltd, Nottingham UK

## Summary

In non-small-cell lung cancer there is compelling evidence that response to both gefitinib (Iressa™) and erlotinib (Tarceva™) is associated with the presence of certain activating mutations of the *EGFR* gene. Source BioScience has developed a set of assays for point mutations occurring in exons 18-21 of the *EGFR* gene, as well as the CA repeat polymorphism seen in intron 1 of the *EGFR* gene. These assays, which are based on conventional capillary (Sanger) sequencing, are suitable for use on FFPE (archival) tissue, and can be carried out with as little as two 4 micron FFPE sections of tissue.

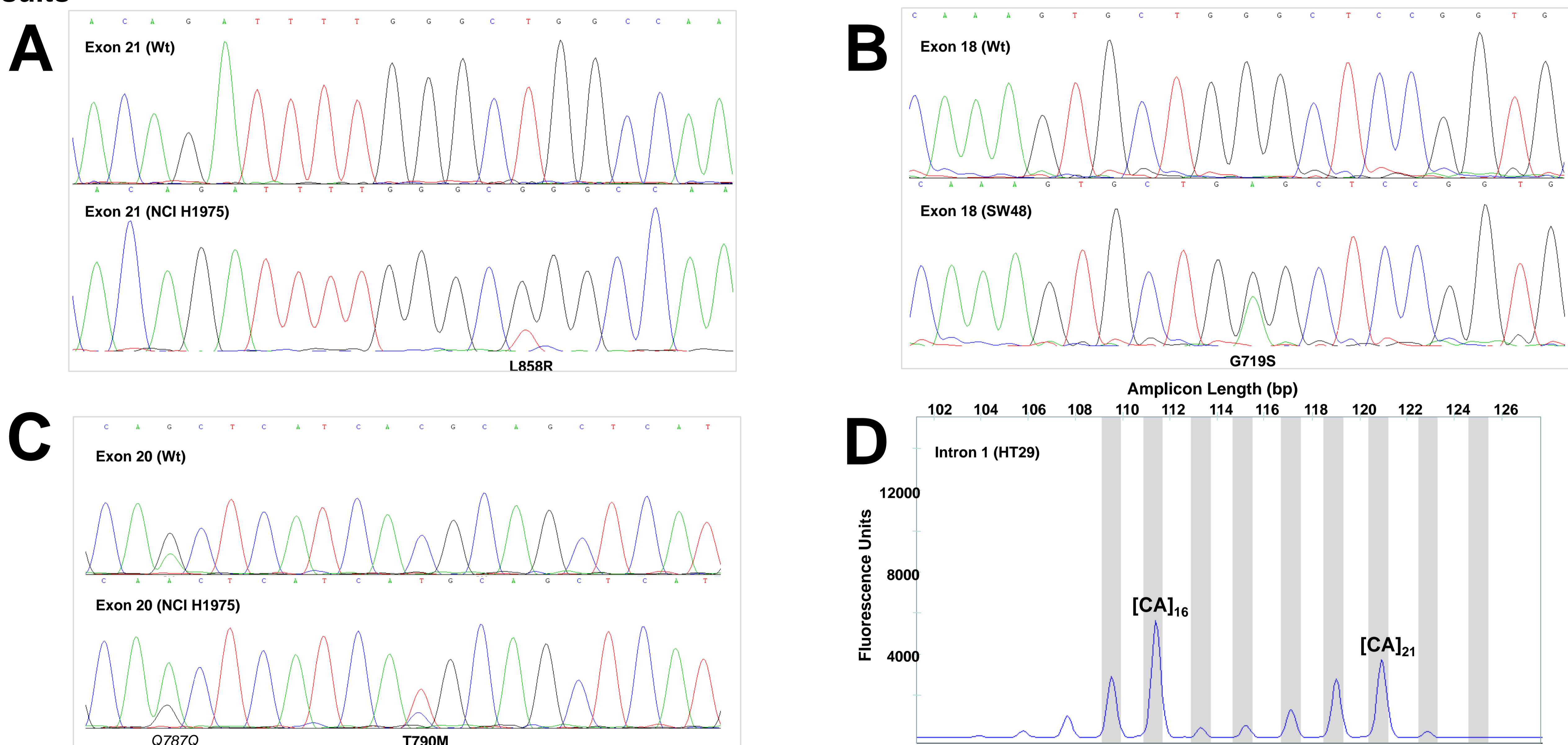
## Introduction

In different cancer types, in particular those of lung and colorectum, subsets of patients have been shown to benefit from anti-EGFR therapies. In non-small-cell lung cancer there is compelling evidence that response to both gefitinib (Iressa™) and erlotinib (Tarceva™) is associated with the presence of certain activating mutations of the *EGFR* gene, as well as with changes in *EGFR* gene copy number. The picture is less clear with respect to the expression of the receptor protein itself, and the use of immunohistochemistry to evaluate levels of expression is of questionable utility. The importance of EGFR lays in its key role as a receptor tyrosine kinase, controlling two major cellular signalling pathways, one stimulating cell proliferation and growth, and the other controlling the so-called survival pathway.

## Materials and methods

Cell-lines SW48 and NCI H1975 were obtained from LGC and cell-line HT29 was obtained from ECACC. DNA extraction was carried out directly. Primer pairs were designed for the amplification of *EGFR* exons 18, 19, 20, 21 and the CA repeat in intron 1. Exon 18, 19, 20 and 21 primers were designed to generate amplicons of 348, 271, 358 and 353 bp, respectively, and Sanger sequencing reactions were carried out on both wildtype and mutant amplicons using nested sequencing primers. The resulting traces from both wildtype and mutant samples were visually compared using Chromas-Lite (Technelysium Pty LTD). For intronic CA repeat PCRs, the forward primer was fluoroscein labelled and the products were analysed using the ABI Prism 3700 DNA Analyser and GeneMapper software (Applied Biosystems) to determine the size of amplicons and thus the CA repeat number.

## Results



**A,B,C:** Sequencing chromatograms of wildtype and mutant *EGFR* in exons 18, 20 and 21. Mutations are represented as the resulting amino acid substitutions below each chromatogram. **D:** GeneScan trace showing amplicon lengths resulting from HT29 *EGFR* intron 1 PCR. The two largest fluorescence peaks indicate a heterozygous genotype of 16/21CA repeats.

## Conclusions

Source BioScience has developed a set of assays able to detect important *EGFR* mutations. Using this assay, we were able to detect Gefitinib/Erlotinib sensitising mutations (G719S Exon 18, L858R Exon 21) and a mutation associated with drug resistance (T790M Exon 20)<sup>1</sup>. The high sensitivity afforded by Sanger sequencing of short PCR amplicons makes this methodology ideal for detecting mutations even in low tumour burden samples, and bi-directional sequences reduces the incidence of false positive/negative that may arise from sequencing errors. The ability to detect repeat polymorphisms such as [CA]<sub>n</sub> in *EGFR* intron 1 may prove invaluable as evidence exists suggesting that this polymorphism may be a predictive marker for Gefitinib sensitivity<sup>2</sup>. The small amplicon lengths also make highly fragmented FFPE tissue-derived DNA amenable to these tests. Together, these assays cover a range of known drug-response genetic markers, and enable their rapid detection at reasonable cost.

## References

- Sharma SV, Bell DW, Settleman J, Haber DA. **Epidermal growth factor receptor mutations in lung cancer.** Nat Rev Cancer. 2007 Mar;7(3): 169-81
- Tiseo M, Capelletti M, De Palma G, Franciosi V, Cavazzoni A, Mozzoni P, Alfieri R, Goldoni M, Galetti M, Bortesi B, Bozzetti C, Loprevite M, Boni L, Camisa R, Rindi G, Petronini PG, Ardizzone A **Epidermal Growth Factor Receptor Intron-1 Polymorphism Predicts Gefitinib Outcome in Advanced Non-small Cell Lung Cancer.** Journal of Thoracic Oncology. Oct 2008 3(10): 1104-1111