

**Comparing the sensitivity of methods  
routinely used for the detection of acquired  
*EGFR* mutations in Non-Small Cell Lung  
Cancer**

**A CMGS Scientific Sub-Committee study**

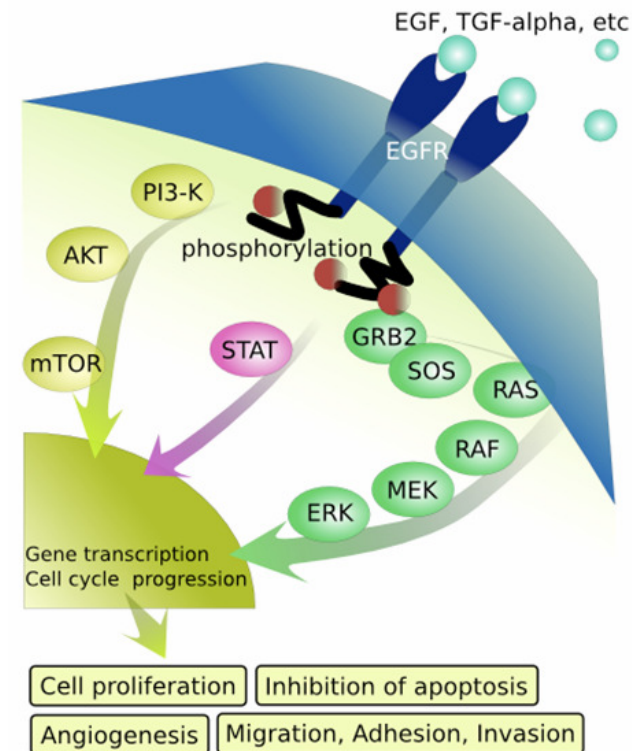
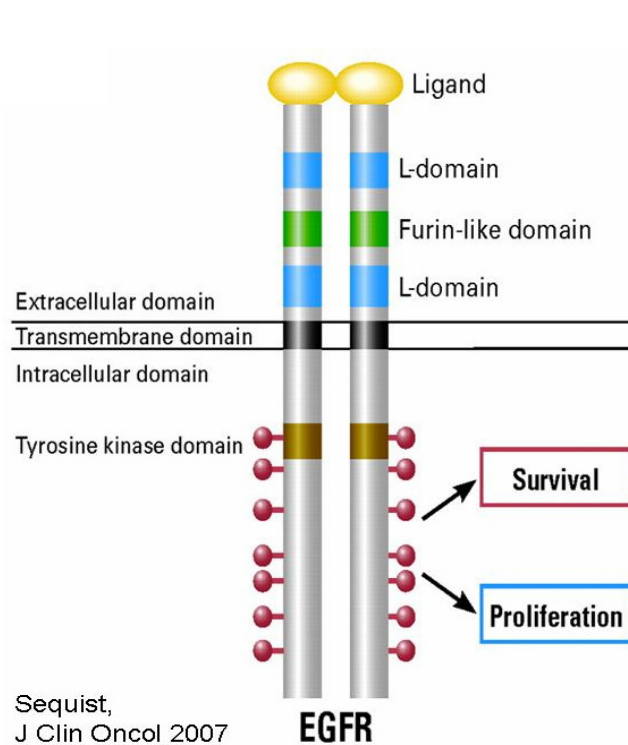
Martina Owens  
Molecular Genetics  
Royal Devon & Exeter Hospital, Exeter

# Non-Small Cell Lung Cancer (NSCLC)

- Accounts for 80% of lung cancer
- Any type of epithelial lung cancer other than small cell lung carcinoma
- 31,000 new cases each year in England and Wales
- 30,000 deaths in England and Wales in 2007
- Biologically aggressive - survival rate from diagnosis:  
25% >1 year, 7% >5 years
- Relatively insensitive to chemotherapy
- Small-molecule inhibitors of the Epidermal Growth Factor Receptor (EGFR) for the treatment of NSCLC were approved in 2003

# Epidermal Growth Factor Receptor (EGFR)

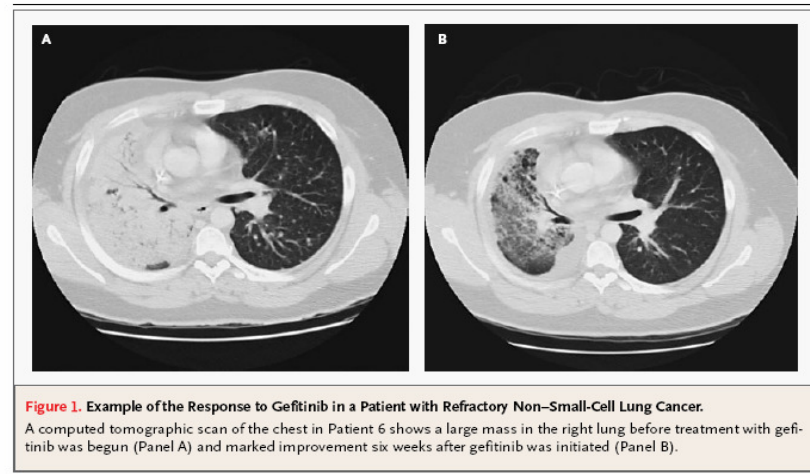
- Transmembrane tyrosine kinase receptor for members of EGF family ligands (EGF and TGF $\alpha$ )



- Activating mutations in *EGFR* result in constitutive activation of EGFR

# Patients with NSCLC can show a significant clinical response to TKIs

~10% of patients have a rapid and often dramatic clinical response to the tyrosine kinase inhibitors (TKIs), gefitinib (Iressa) and erlotinib (Tarceva).



Response is dependent on the mutational status of *EGFR*

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Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non-Small-Cell Lung Cancer to Gefitinib

Thomas J. Lynch, M.D., Daphne W. Bell, Ph.D., Raffaella Sordella, Ph.D., Sarada Gurubhagavatula, M.D., Ross A. Okimoto, B.S., Brian W. Brannigan, B.A., Patricia L. Harris, M.S., Sara M. Haserlat, B.A., Jeffrey G. Supko, Ph.D., Frank G. Haluska, M.D., Ph.D., David N. Louis, M.D., David C. Christiani, M.D., Jeff Settleman, Ph.D., and Daniel A. Haber, M.D., Ph.D.

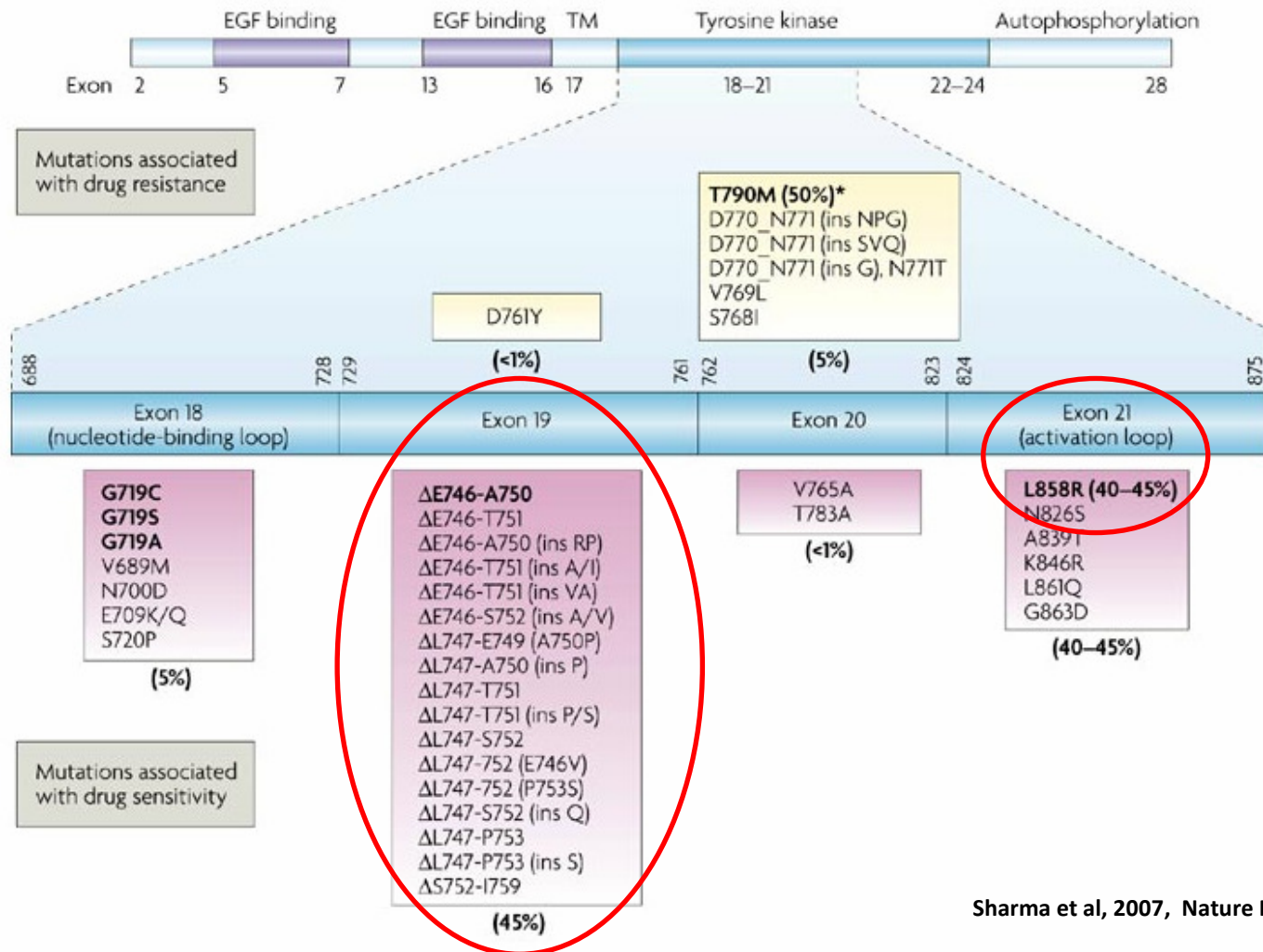
EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib

William Pao<sup>\*\*\*</sup>, Vincent Miller<sup>15</sup>, Maureen Zakowski<sup>1</sup>, Jennifer Doherty<sup>\*</sup>, Katerina Politi<sup>\*</sup>, Inderpal Sarkaria<sup>1</sup>, Bhuvanesh Singh<sup>1</sup>, Robert Heelan<sup>\*\*</sup>, Valerie Ruschl, Lucinda Fulton<sup>11</sup>, Elaine Mardis<sup>11</sup>, Doris Kupfer<sup>11</sup>, Richard Wilson<sup>11</sup>, Mark Kris<sup>15</sup>, and Harold Varmus<sup>\*</sup>

<sup>\*</sup>Program in Cancer Biology and Genetics and Departments of <sup>1</sup>Medicine, <sup>15</sup>Surgery, <sup>3</sup>Pathology, and <sup>\*\*</sup>Radiology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; and <sup>11</sup>Genome Sequencing Center, Washington University School of Medicine, 4444 Forest Park Boulevard, St. Louis, MO 63108

Contributed by Harold Varmus, July 19, 2004

# EGFR mutation spectrum



Sharma et al, 2007, Nature Reviews Cancer

Exon 19 deletions and p.Leu858Arg account for ~90% of patients with activating mutations

# Testing Methodology: Scanning v Targeted

## Gene mutation screening technologies

### ***Advantages***

- All mutations, including novel mutations may be detected
- Technology is available in many molecular genetics labs

### ***Disadvantages***

- Sensitivity tends to be lower than targeted methods
- Experienced operators needed
- Tends to be more labour intensive

## Gene Mutation targeted technologies

### ***Advantages***

- Only mutations assayed for may be detected – therefore less time consuming
- Sensitivity tends to be higher than screening technologies

### ***Disadvantages***

- Mutations not assayed may be missed
- Reagents may be more expensive

## Aim of Study

- Different methodologies employed by labs
- Variation in reported sensitivity of methods employed
- CMGS Scientific Sub-Committee - study **to compare the sensitivity of different methods for the detection of acquired *EGFR* mutations**
- Labs invited to participate in study at National meeting

# 15 labs agreed to participate in the study



- Newcastle (New Gene)
- Sheffield
- Manchester
- Birmingham
- Oxford
- Cambridge
- King's College Hospital
- Royal Marsden
- Barts
- Royal Surrey County Hospital
- Cancer Laboratory & Translational Oncology Research Centre, Portsmouth
- Salisbury
- Bristol
- Cardiff
- Exeter

# Preparation of mutant samples

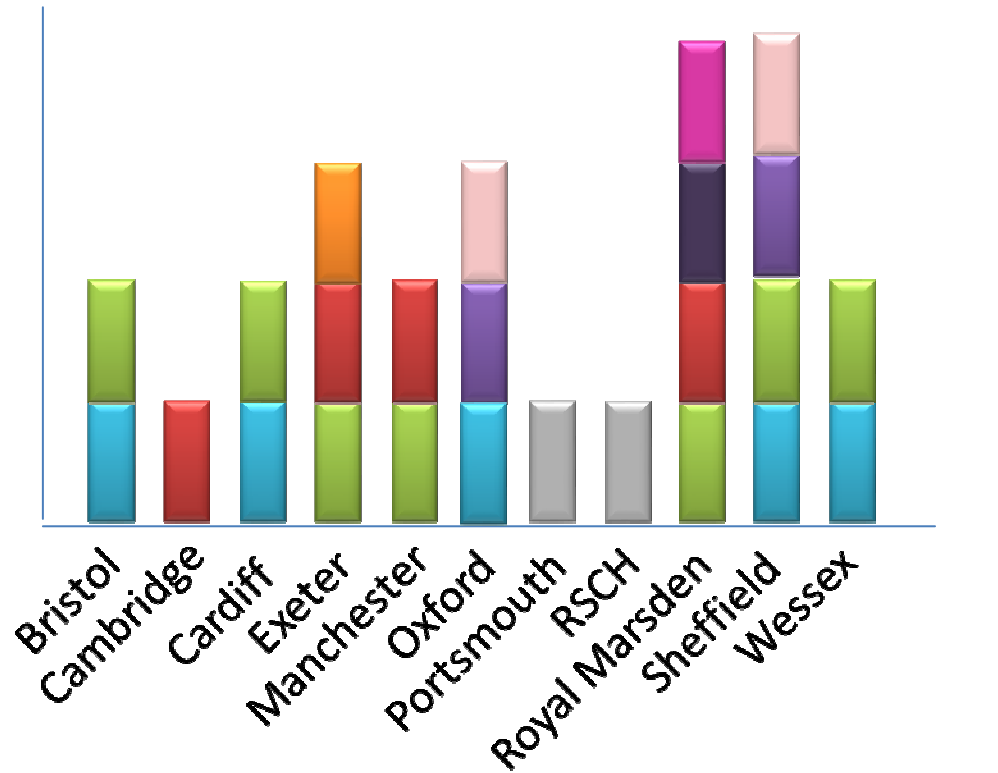
- ***EGFR* p.Leu858Arg (exon 21)**
  - commercial cell line (H1975) from ATCC
  - grown in RPMI media until confluent
  - the number of cells in the flask was calculated
  - mixed with 100% wild type (HeLa cell line) with same number of cells/ml to create mutant mixes
  - DNA was extracted from cell pellet
- ***EGFR* c.2236del15 (exon 19)**
  - c.2236del15 DNA supplied by Cardiff laboratory
  - quantified and mixed with HeLa cell line with the same concentration to give the required mutation level

## 16 blind samples sent to each lab

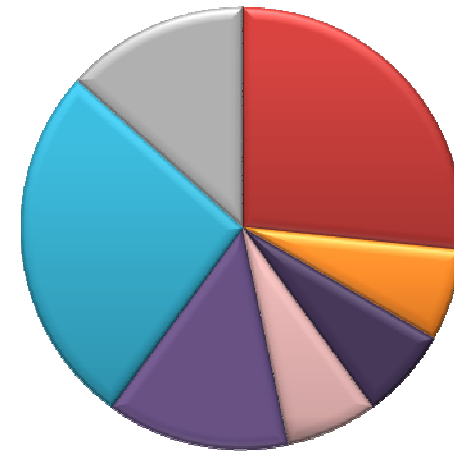
- Mixes containing 0-15% mutant (wild type, 1, 2, 5, 7.5, 10, 15%)
- Duplicate samples were included for the 10% mutation
- 8 samples for each mutation and questionnaire distributed to 15 participating labs
- Questionnaire:
  - Method used
  - Mutation detected (would it be reported as positive)
  - Mutation description (if applicable)
  - Estimated level of mutation
  - Do you routinely report mutation load?

# Results – methodology

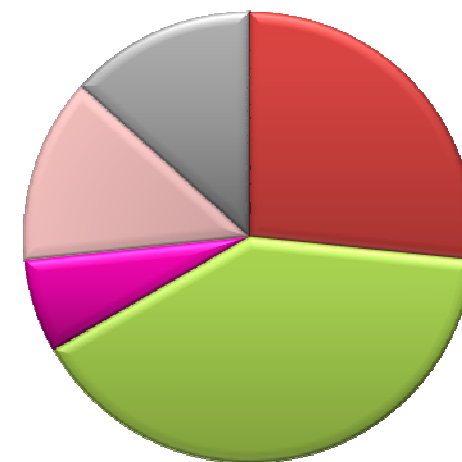
Results obtained for 11 labs



p.Leu858Arg

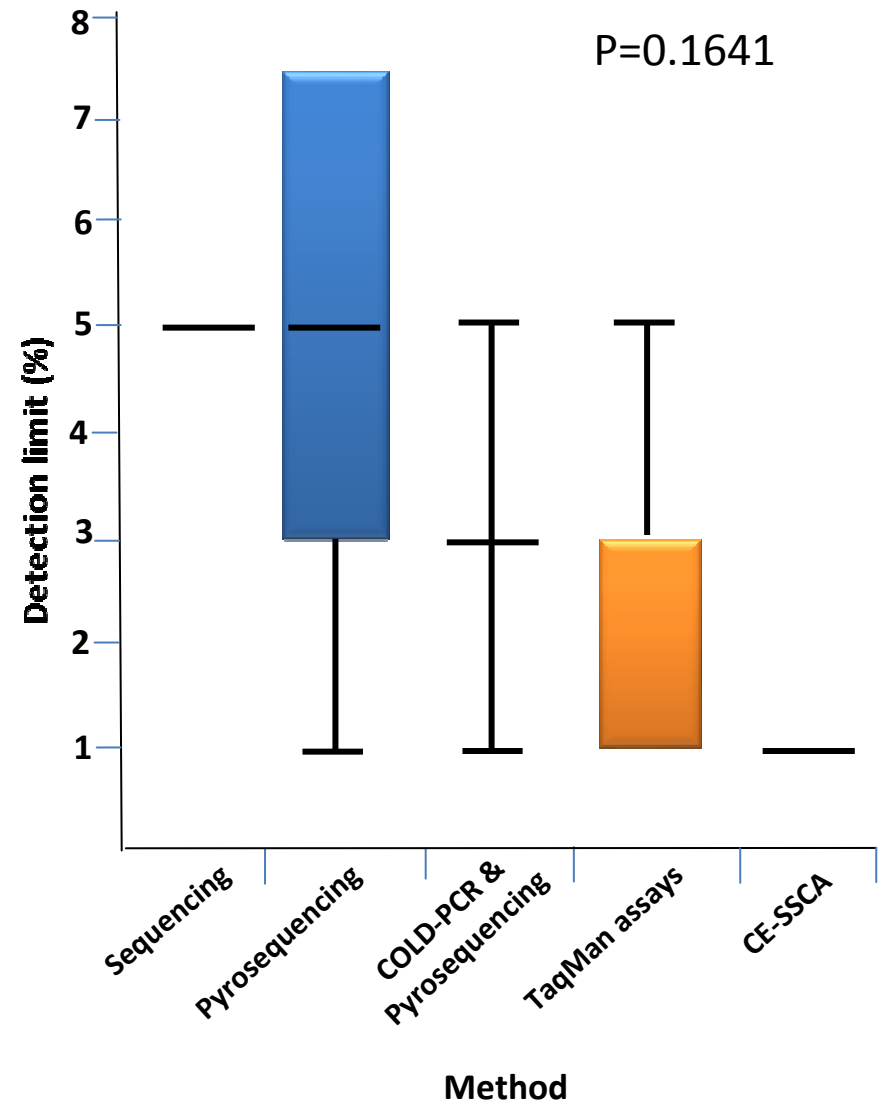
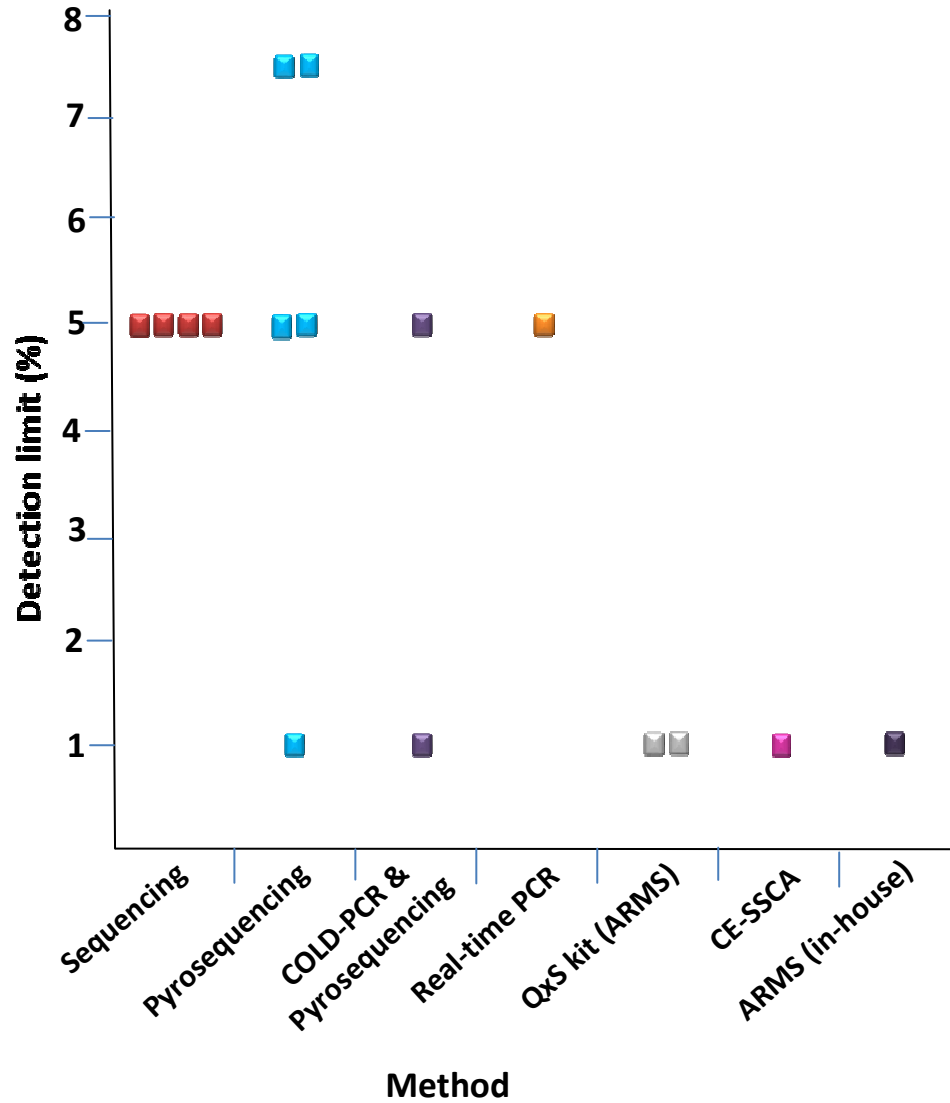


Exon 19 deletion

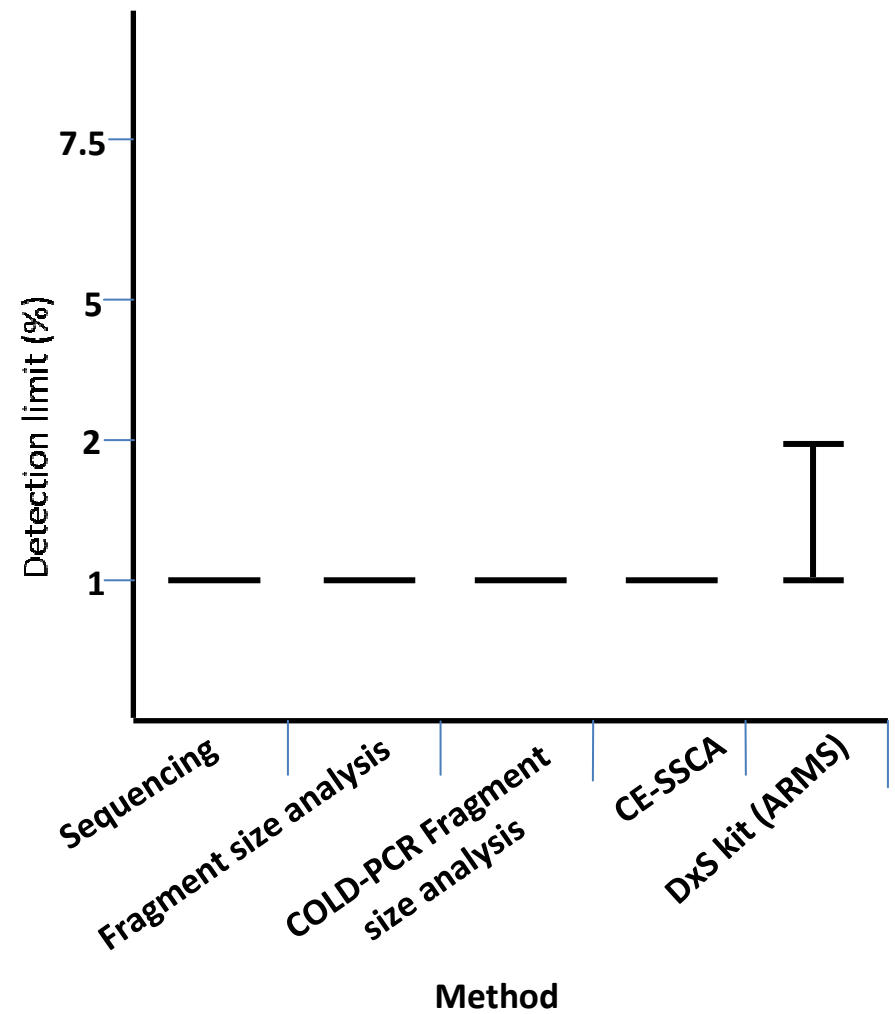
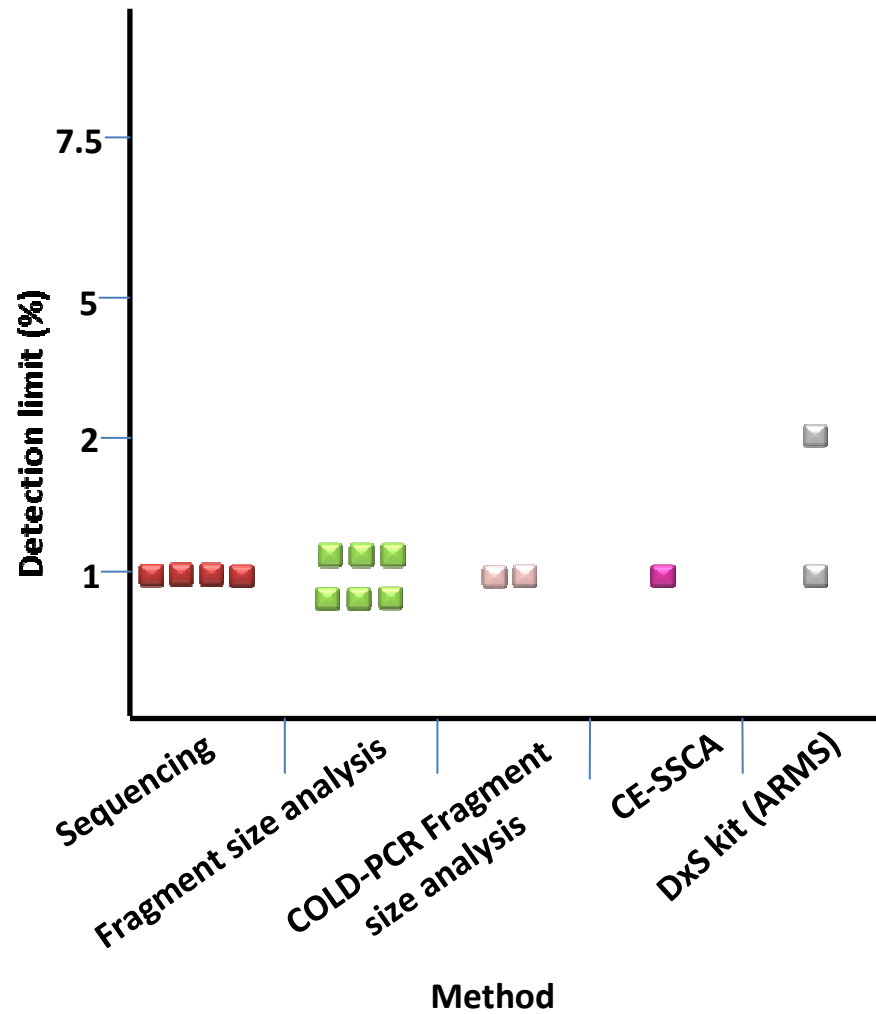


- Pyrosequencing
- Fragment size analysis
- Sequencing
- Real-time PCR
- CE-SSCA
- COLD-PCR & Pyroseq
- COLD-PCR & Frag
- QxS kit (QIAGEN)
- ARMS (In-house)

# Results - sensitivity (p.Leu858Arg)

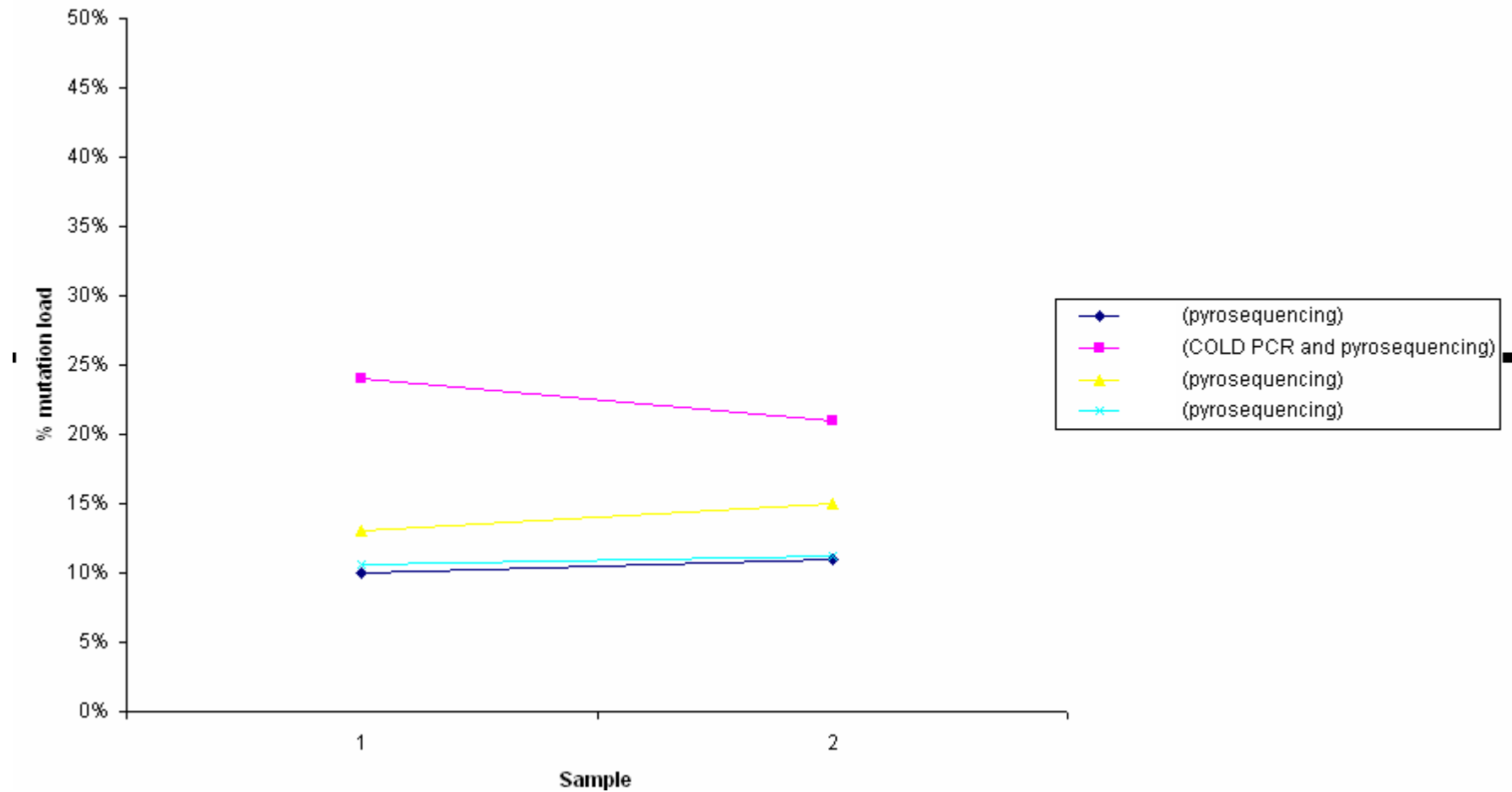


# Results - sensitivity (Exon 19 deletion)



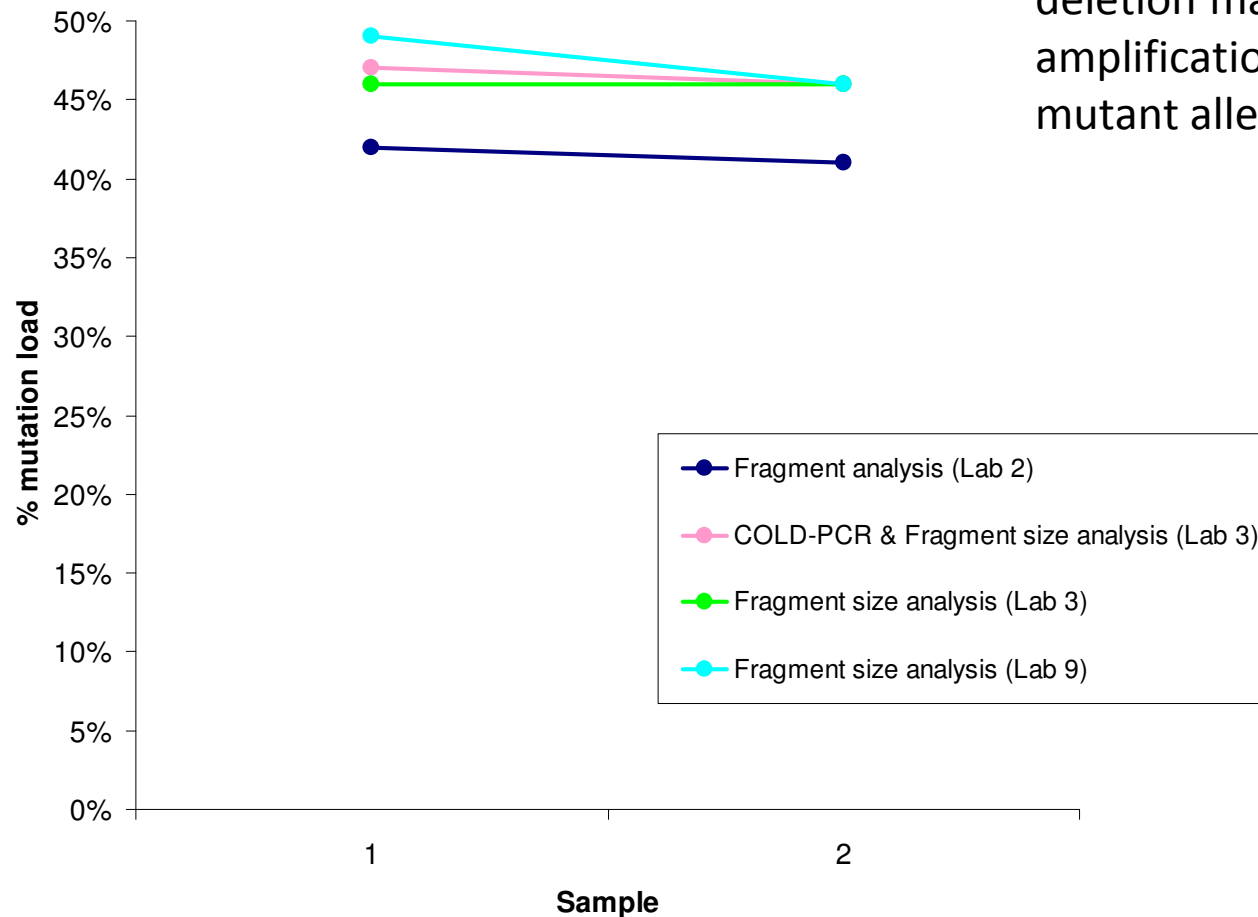
# Results – p.Leu858Arg quantification reproducibility

Samples 3 and 6 – 10% mutation



# Results – Exon 19 del quantification reproducibility

## Samples 11 and 14 – 10% mutation



High sensitivity for this 15bp deletion may reflect preferential amplification of the smaller mutant allele

# Summary of findings

- Six different methodologies in use by the laboratories who participated in the study
- No individual assay demonstrated increased sensitivity compared to the others
- Sequencing and Pyrosequencing are the most common methods used to identify the p.L858R mutation
- Sequencing and Fragment size analysis are the most common methods used to identify deletions in exon 19
- None of the labs who participated routinely reported mutation load
- Choice of method dependent on technologies already in use in the lab, cost and analysis time

# Acknowledgements

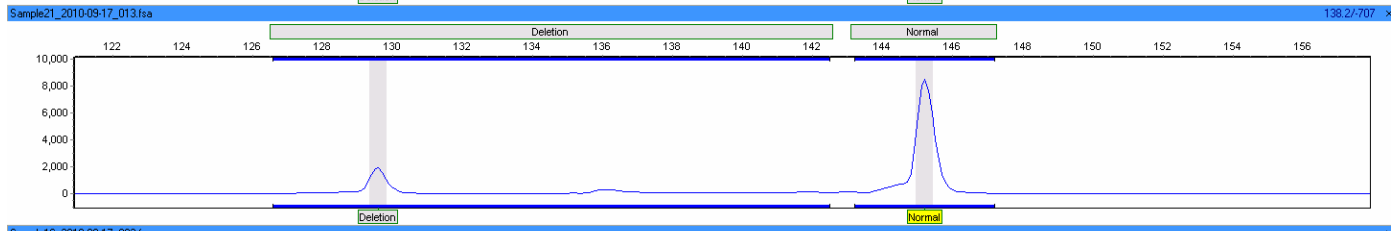
- **Exeter**  
S Ellard, E Young, M Day, N Goodman
- **Bristol**  
C Crosby, M Williams
- **Cambridge**  
J Knight, Y Huang, H Liu
- **Cardiff**  
I Abebiyi, L Lazarou, H Muglaasi
- **Oxford**  
T Bedenham, T Cranston
- **Wessex**  
J Callaway, A Skinner
- **Manchester**  
A Wallace
- **Portsmouth**  
I Cree
- **Royal Marsden**  
G de Castro
- **RSCH, Surrey**  
L Lavender
- **Sheffield**  
A Milano

# Results – Exeter Fragment Size Analysis

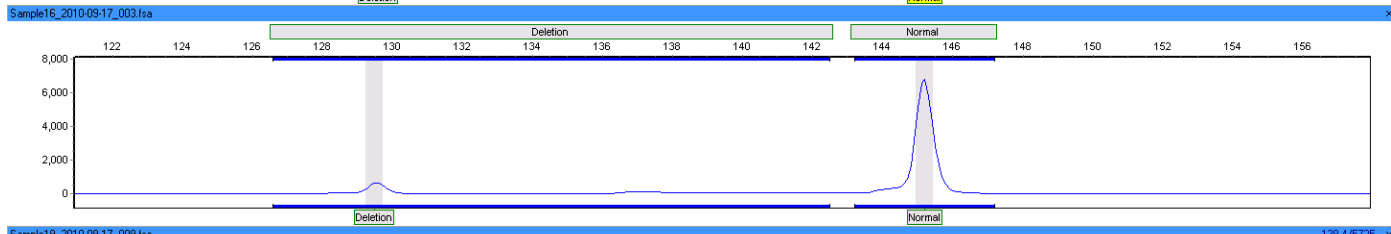
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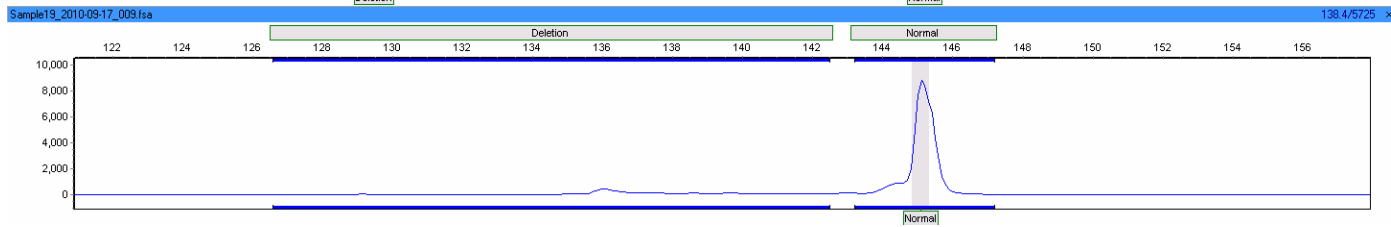
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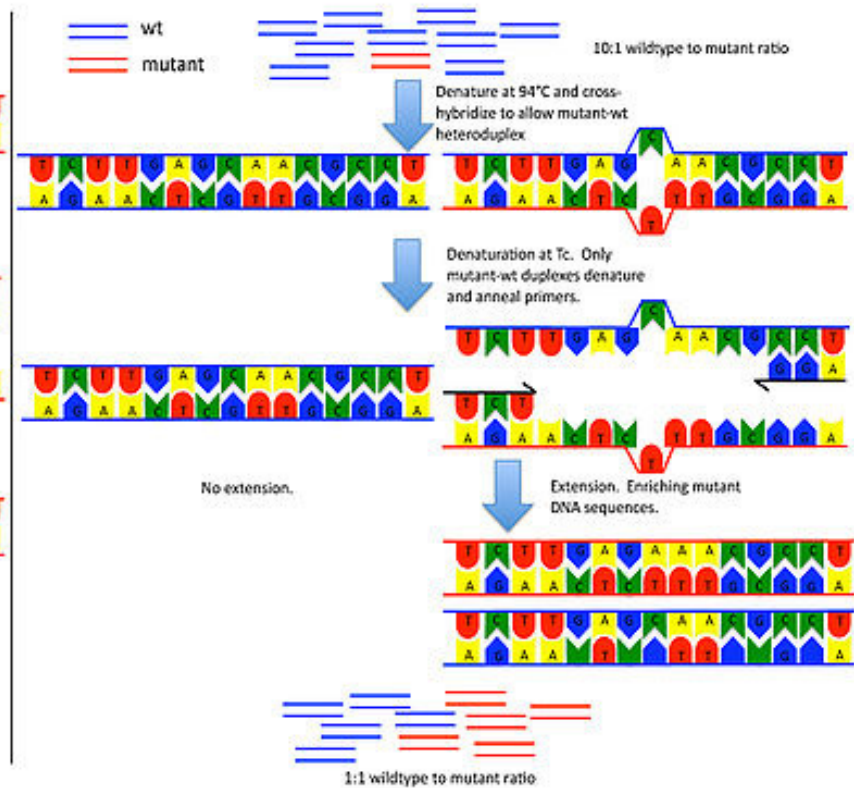
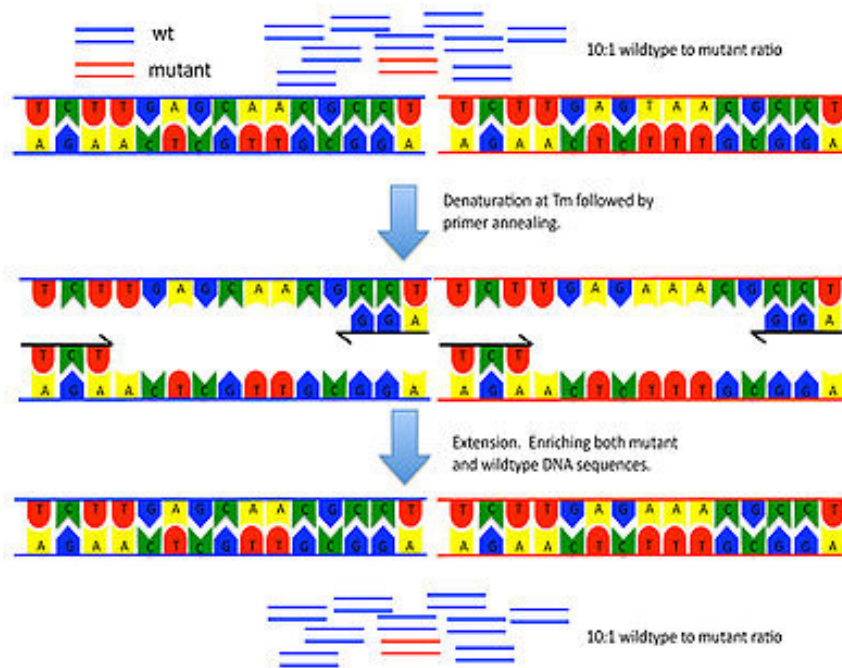


1%



0%





Conventional PCR

COLD-PCR

# Results - sensitivity (p.Leu858Arg - all methods)

