

# A comparison of KRAS mutation detection techniques

Zoe Allen, Stewart Payne.

Northwest Thames Regional Genetics Service (Kennedy Galton Centre)

## INTRODUCTION

KRAS mutations are present in a number of cancers. Approximately 35-45% of metastatic colorectal cancer tumours have an activating KRAS or BRAF mutation which makes them less likely to respond to anti-EGFR therapies.

Testing for KRAS mutations in metastatic colorectal cancer will:

- Enable patients without a KRAS mutation to receive effective treatment
- Stop patients with mutant KRAS being exposed to toxic effects of anti-EGFR therapies
- Give an overall cost saving to the NHS

## KRAS TESTING

KRAS testing can be challenging as the quality of tumour specimens received can vary. Fragmented, low quality DNA extracted from FFPE tissues, and the percentage of wild type cells present in the sample can compromise mutation detection.

This issue has been addressed by biotechnology companies over the last year, and several products are now being marketed, claiming to detect mutations down to a 1% level in a wild type background.

A comparison of three techniques has been performed:

- A shifted termination assay (Life Technologies)
- Fluorescent ARMS (Elucigene)
- Sanger sequencing

## COMPARISON OF METHODS

	Shifted termination assay	Fluorescent ARMS	Sequencing
Mutations detected	KRAS codons 12 and 13	KRAS codons 12 and 13, and BRAF V600E	All
Marketed sensitivity	1-5%	1%	5-10%
Equipment required	ABI 3130 or similar	ABI 3130 or similar	ABI 3730 or similar
Hands on time	~3 hours	~1 hour	~3 hours
Cost per sample (reagents only)	~£60	~£80	~£40

## SEQUENCING

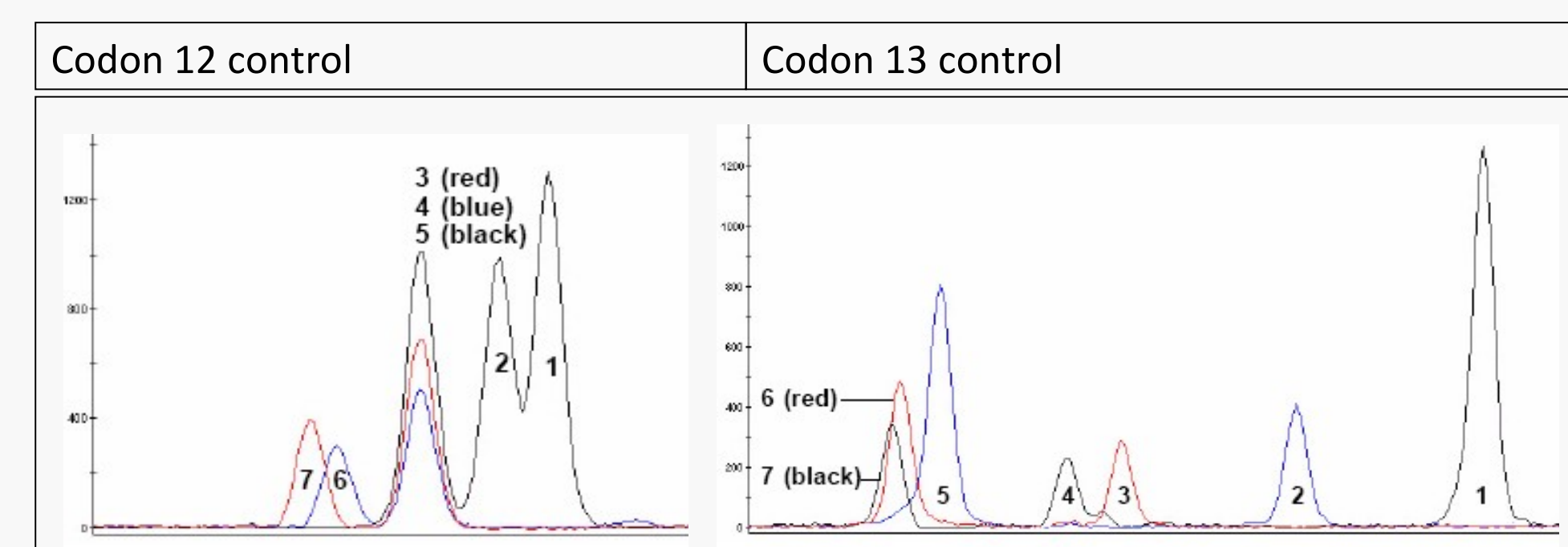
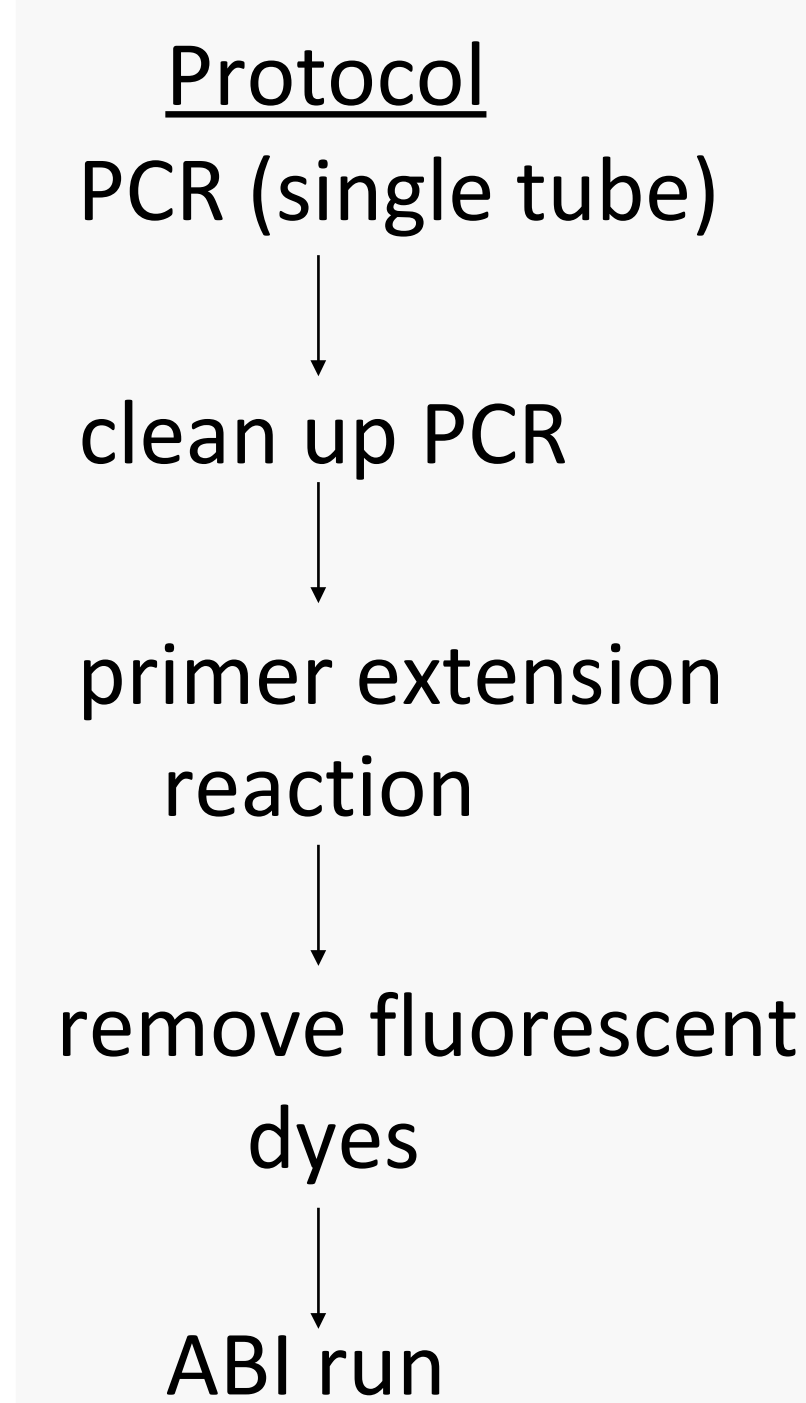
PCR using Megamix Royal and sequencing using BigDye v1.1 was able to reliably detect mutations at approximately 10% mutation in a wild type background (results not shown).

## CONCLUSIONS

Both methods are superior to Sanger sequencing and give comparably sensitive results, detecting down to 5% mutant in a wild type background.

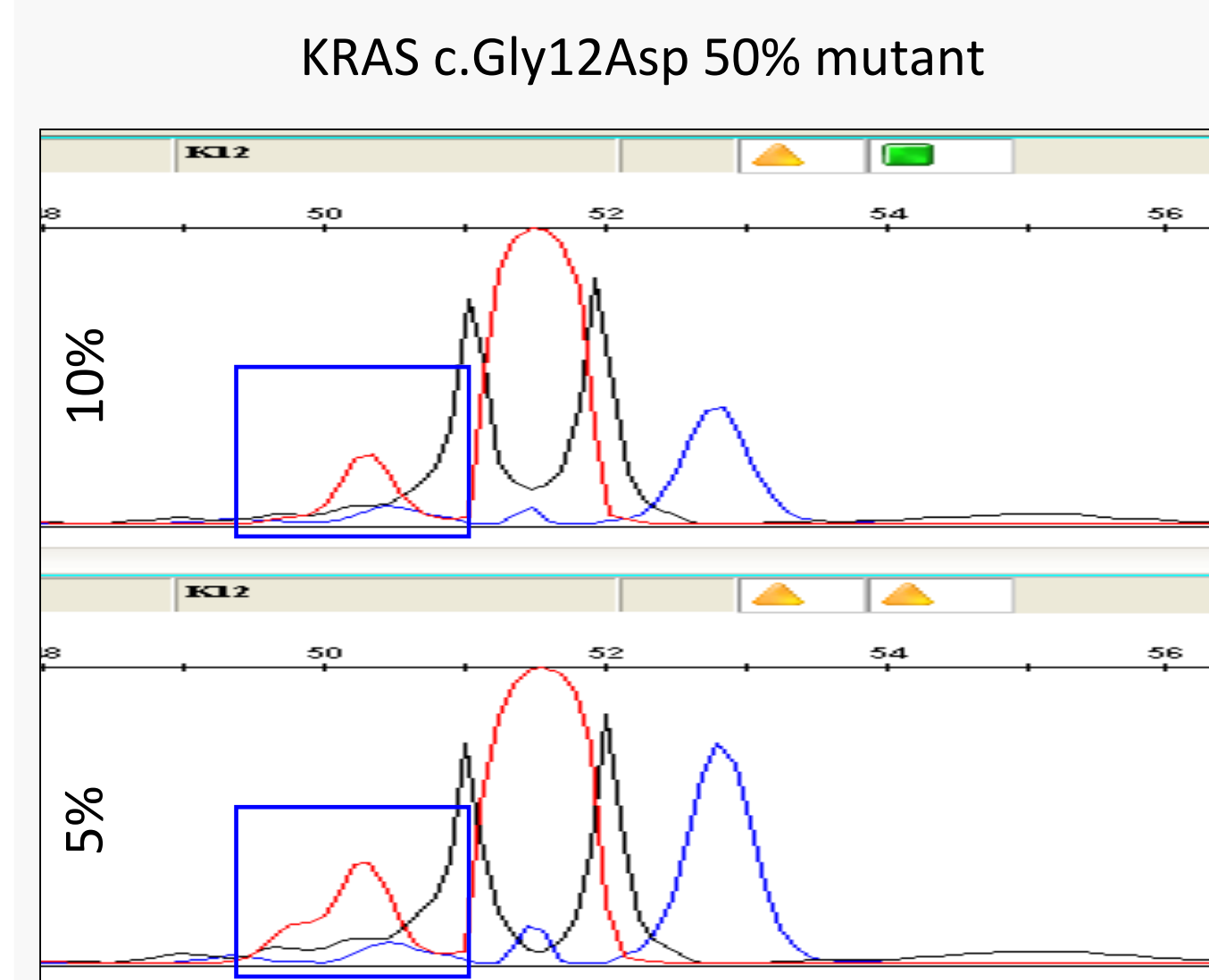
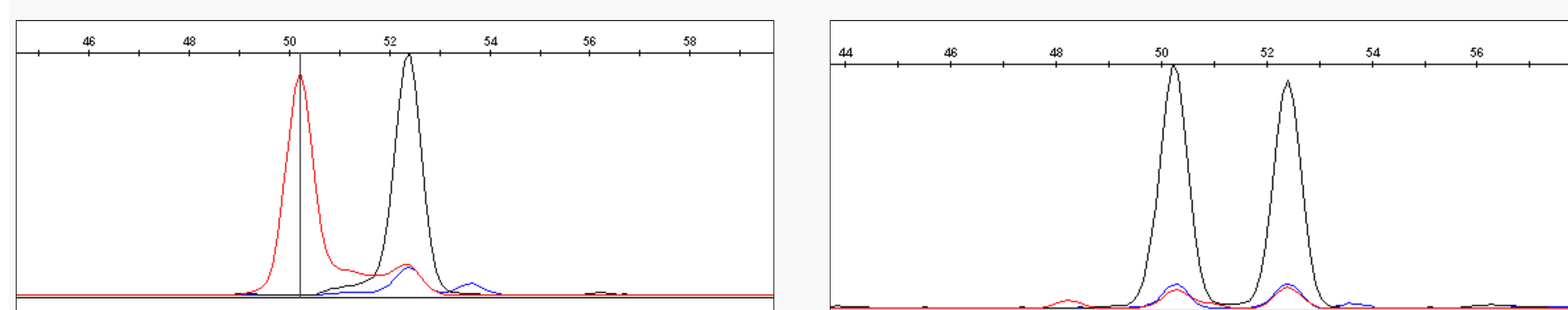
In an era where detection of genetic variants in tumours is being used for personalized medicine, the advances in sensitivity by Life Technologies and Elucigene will allow laboratories to provide a rapid and reliable test and enable patients to benefit from targeted therapy.

## SHIFTED TERMINATION ASSAY



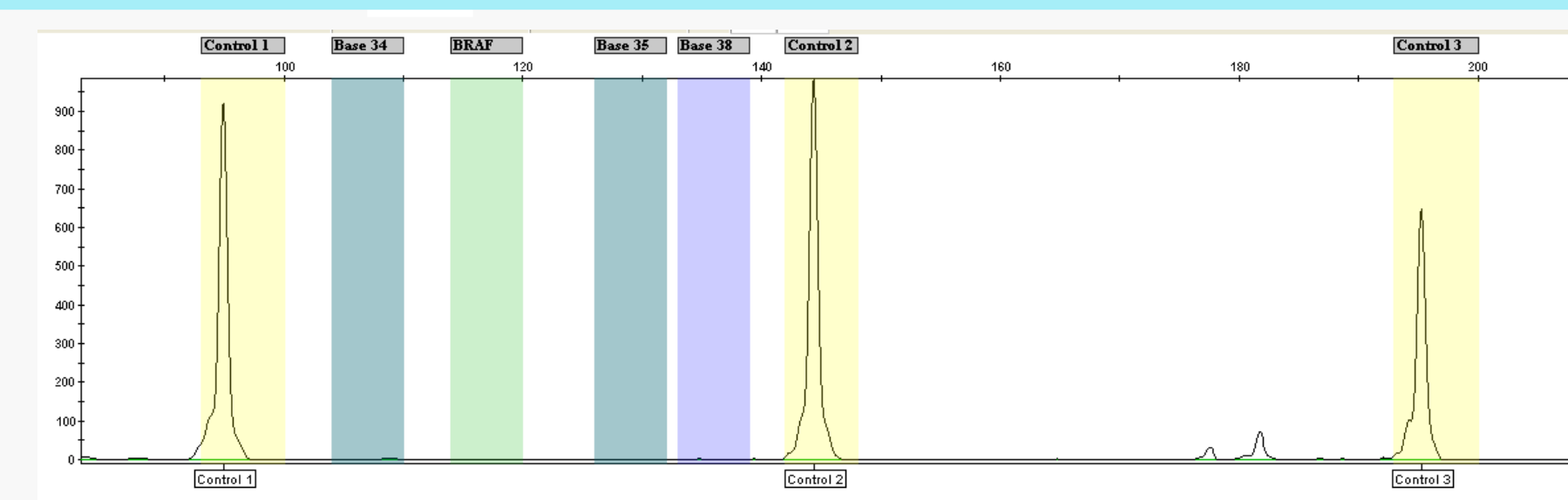
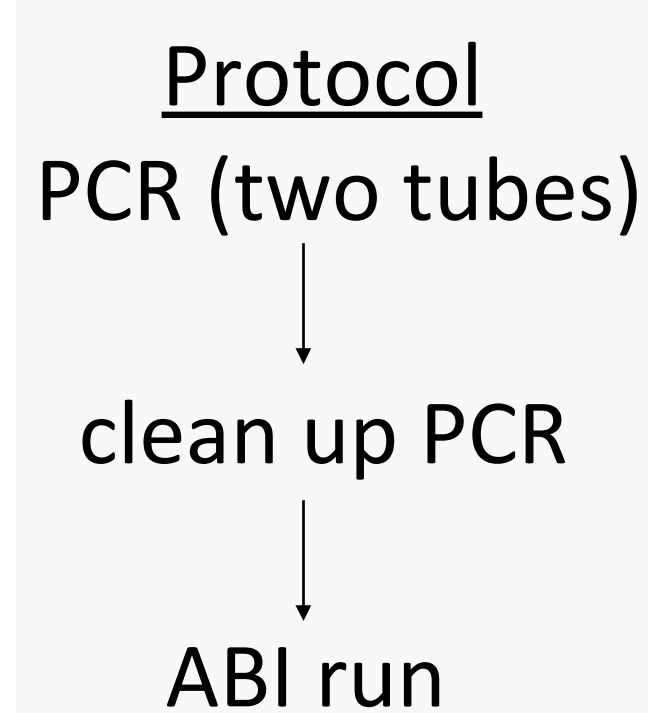
Peak number	Colour	Sequence	Peak number	Colour	Sequence
1	Black	Wild type	1	Black	Wild type
2	Black	GGT>GTT	2	Blue	GGC>CGC
3	Red	GGT>GAT	3	Red	GGC>AGC
4	Blue	GGT>GCT	4	Black	GGC>TGC
5	Black	GGT>TGT	5	Blue	GGC>GCC
6	Blue	GGT>CGT	6	Red	GGC>GAC
7	Red	GGT>AGT	7	Black	GGC>GTC

Summary of results using a control containing all possible variants



The top panel shows a c.Gly12Asp 10% mutant, and the bottom a 5% mutant. Although the data shown is very overloaded (with split peaks), the mutation has been clearly detected.

## ARMS ASSAY



3 internal control peaks are used to assess the sample quality. These can be used as an indicator of how well the ARMS reaction will perform for that sample. Equal peak height = good quality sample. Decrease in peak height = poor sample

