FM-CSCE
A unified screening method for identification of deletions, duplications and point mutations

Emma Ashton
DNA Laboratory
Guy's Hospital, London

White paper targets

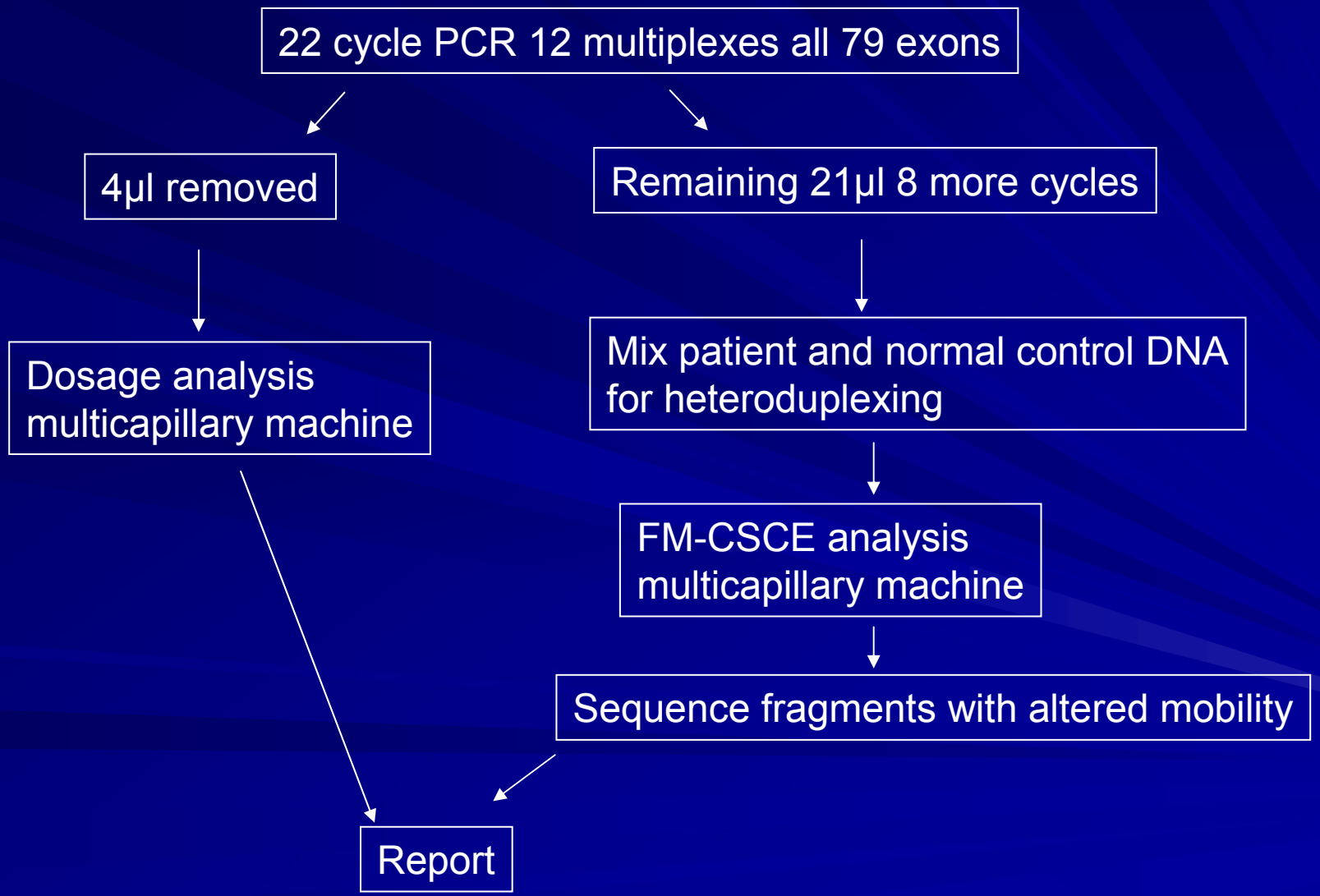
- Screen for mutations in large genes in 8 weeks
- Current screening methods screen for one mutation type only

- Developed FM-CSCE as a radical approach to screen for nearly all mutation types simultaneously
- More efficient than separate screens
- Full gene screen for each sample is faster

Improved screening strategy

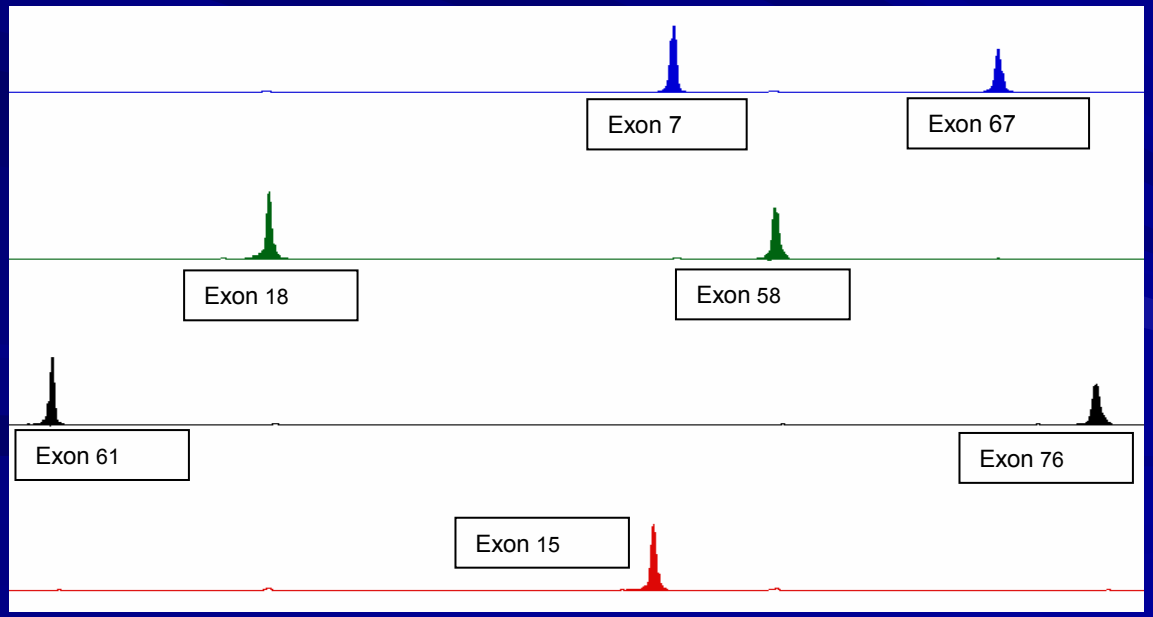
- Single screen for large deletions, duplications and point mutations
- Performed rapidly using genomic DNA
- Applicable to female carriers
- Combination of fluorescent dosage analysis with FM-CSCE – rapid screen of large gene for nearly all mutation types

Dystrophin assay: streamlined single approach for detection of all mutation types



Design of multiplexes

- Primers designed at least 50 bp into intron
- Products 200-500 bp
- 84 fragments split into 12 multiplexes (96-well plate)



Temperature

- Panel of females with known point mutations
- FM-CSCE at 18-30°C in 3 degree increments
- Heteroduplexes clearest at °C
- 1 mutation only picked up at °C (3 only at °C and °C)

Capillary length

- Original testing with cm capillary
- capillary improved pick-up rate (83% compared to 96%)

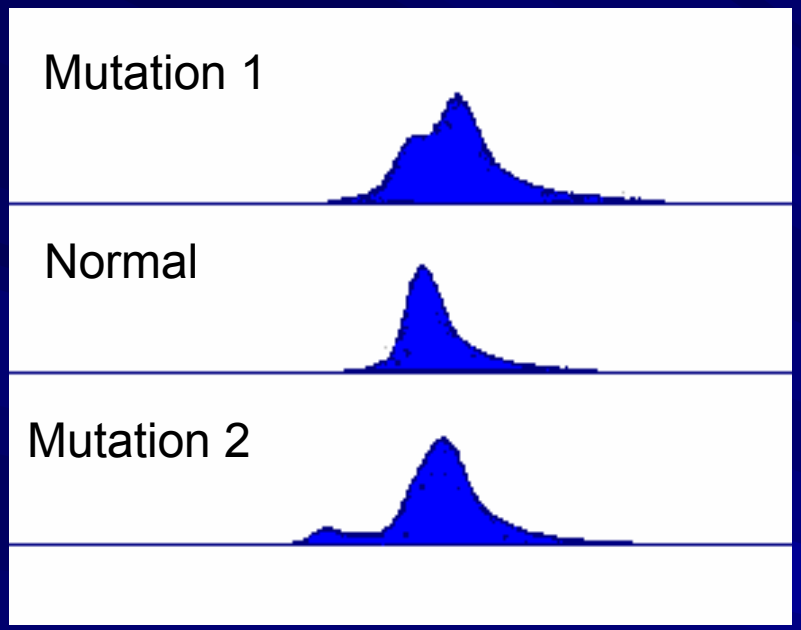
Run voltage

- 3100 low-voltage modules from ABI
- Tested voltages 10kV-15kV
- 1 mutation only picked up at kV
- 1 mutation only picked up at kV and kV
- Other mutations detected at all voltages

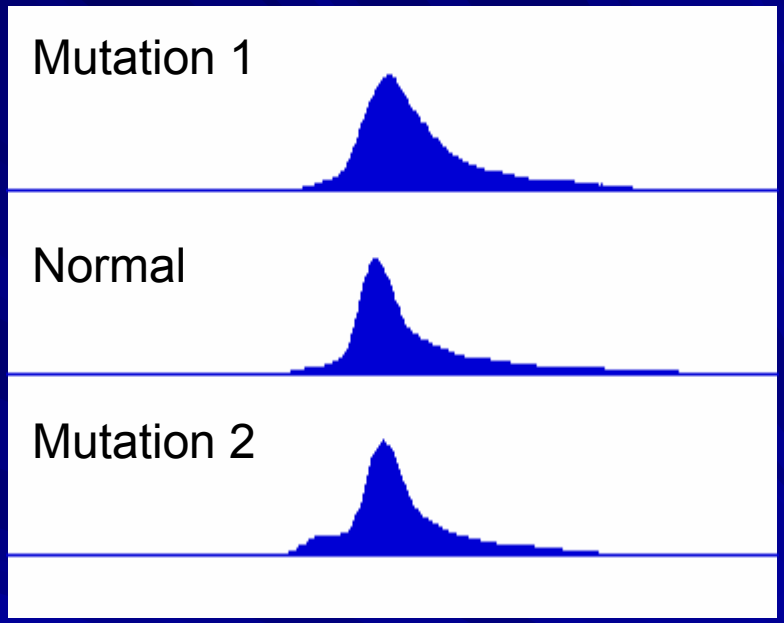
Polymer

- 7% non-denaturing polymer (ABI) plus:
  - Various additives
- Tested with a panel of known mutations
- Best results obtained with

Polymer comparison



Polymer 1



Polymer 2

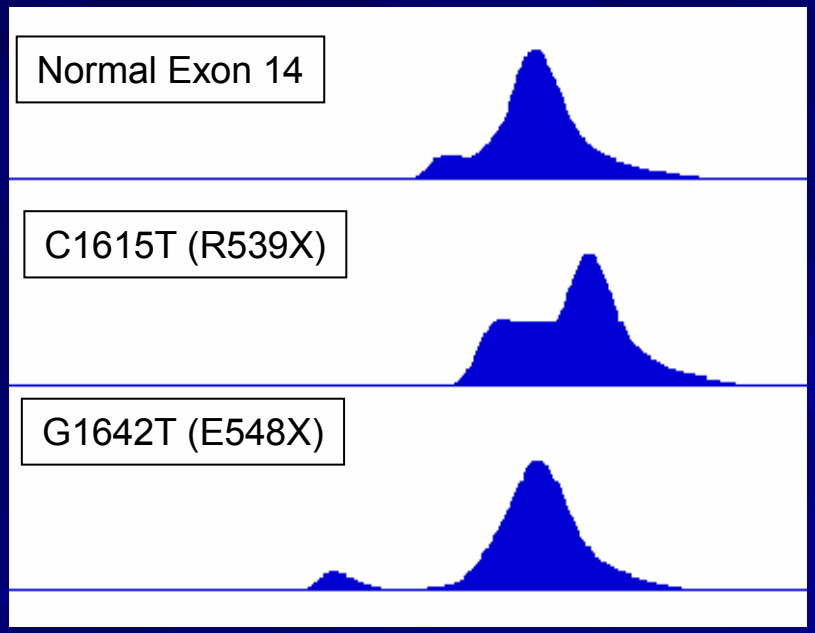
Cycle number

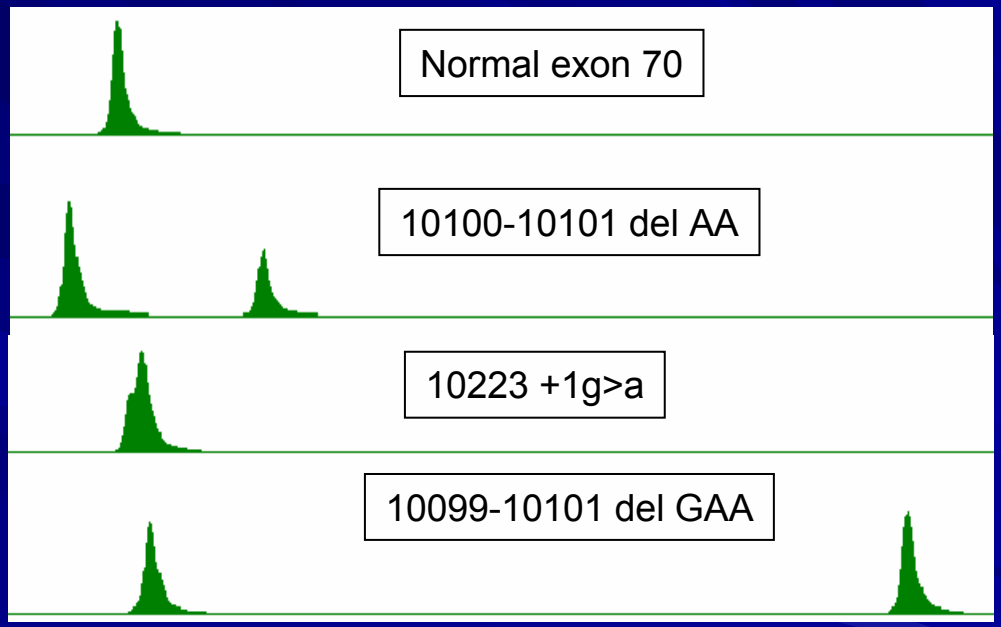
- Originally aimed for one cycle number for dosage and CSCE
- But…. better dosage stats at lower cycle numbers, clearer CSCE changes at higher cycle numbers
- Tested CSCE at 21-30 cycles PCR

Assessment of FM-CSCE sensitivity

- Initial test of CSCE conditions using 48 samples from affected males and carrier females with known substitution mutations (total of 31 different mutations)
- 47/48 samples (98%) showed a pattern clearly different from normal controls during CSCE
- 1 mutation detected in affected male, only a slight difference seen in his mother

Examples of sequence variants detected by FM-CSCE





Blind trial

- 50 male and 50 female samples tested

Mutation type	Number in blind trial	Number detected
Large deletions	29	29
Large duplications	27	27
Small ins/dels (1-67 bp)	15	15
Nonsense mutations	11	12
Splice site mutations	3	3
Normal controls	16	15
TOTAL	100	100

Blind trial II

- 100% sensitivity and specificity
- Denaturing polymer clearly picks up and characterises small in/dels
- Important that peaks are strong. Heteroduplexes often smaller than main peak

Current screening

- Screen 50 DMD + 50 BMD males with definite diagnosis no del/dup to determine pick up rate
- 11 patients with large del/dups of exons not in standard multiplexes
- 46 patients with point mutations
- 2 pathogenic missense mutations identified
- 39 samples screening on-going
- 13 samples no mutation detected

How long does this take?

Currently using 1 x 3100

- To screen 1 batch of 27 patients plus controls
  - 24 dosage runs 45 hrs 15 min
  - 24 CSCE runs 38 hrs 50 min
  - TOTAL ~ 85 hours

- Requested 2 x 48 capillary machines (white paper)
  - 8 dosage runs ~ 8 hrs
  - 8 CSCE runs ~ 5 hrs
  - Total ~ 13 hrs

Alternatives

- MLPA developed since we started project
- Advantage of screening large number of exons simultaneously
- Two-tier screening again – does not detect point mutations
- Caution- case recently where MLPA suggested a deletion, actually a small insertion

Future applications

- Currently developing assay for Alports disease
- Future development for BRCA genes and congenital muscular dystrophies
- If gene with only point mutations, CSCE only
- Apply to smaller genes for simultaneous detection of deletions, duplications and point mutations e.g. HNPCC, FAP

Acknowledgements

Stephen Abbs
Zandra Deans
Michael Yau

Guy's and St Thomas' Charitable Foundation