# Principles of Genetic Testing (DMD)

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# Duchenne / Becker Muscular Dystrophy

- Highly elevated CK in young children
- X-linked – Dystrophin Gene
  - 2.4Mb - 79 Exons
- Females are carriers, Boys are affected
- Vast mutation spectrum

# DMD/ BMD

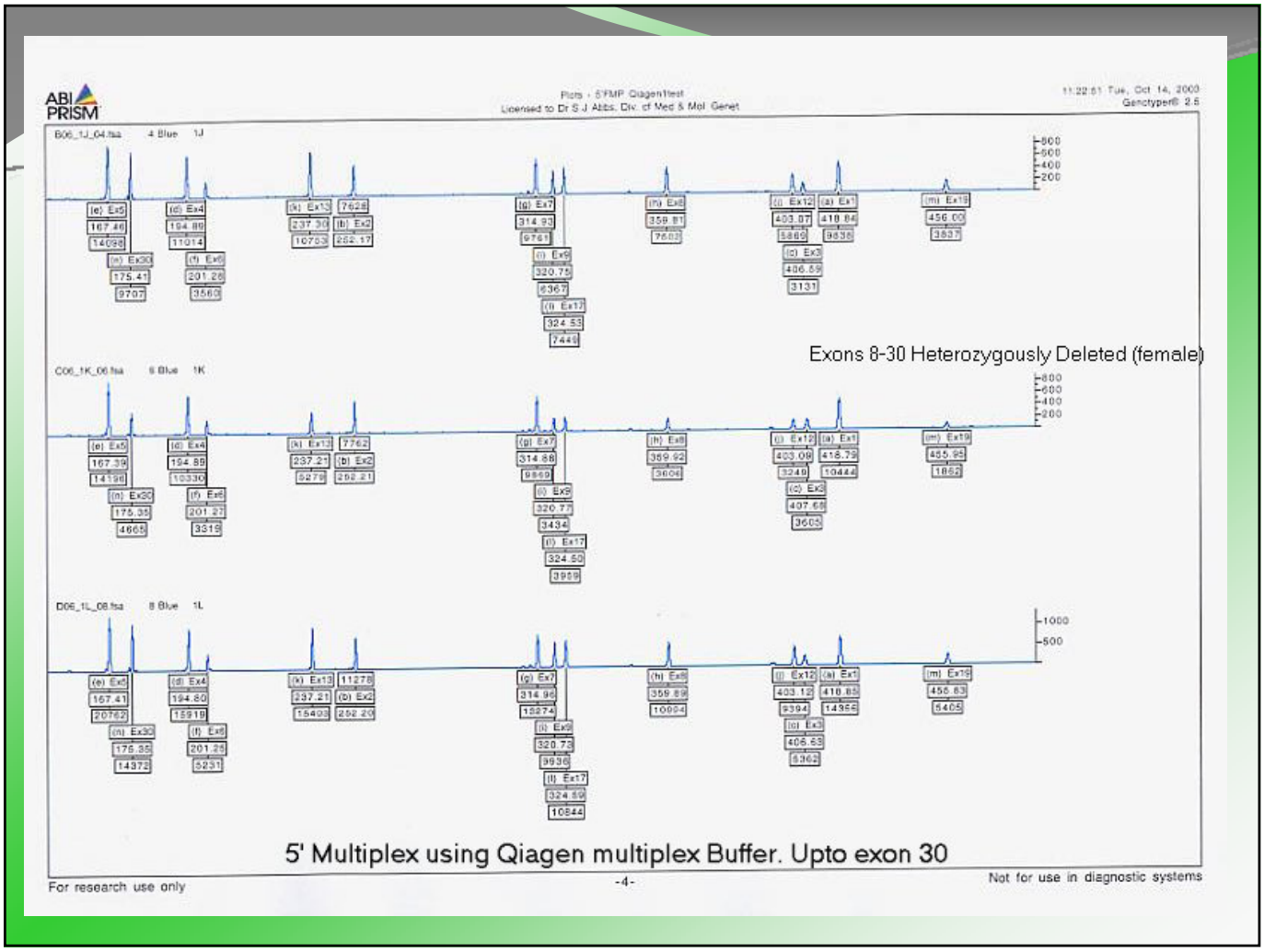
- Mutations
  - Point mutations
  - Whole exon deletions/duplications
  - Small insertions/deletion
  - Non sense, frameshift (truncating protein)
- Upto 10% Mosaicism
  - No mutation detected in Mother of boys with known mutations

# Screening – QF PCR

- DNA Quality Test
- 2 Multiplexes of14 Exons
- ABI 3100 Genetic Analyser
- Analysed on Software and Excel sheet
- Detect female carrier status, duplicated males/ females

➢ Detects 99% del, 96% Dup & >1bp ins/del

➢ However, only accounts for ~ 65-70% dystrophin mutations


ABI PRISM
Exons 8-30 Heterozygously Deleted (female)
5' Multiplex using Qiagen multiplex Buffer. Upto exon 30
For research use only
-4-
Not for use in diagnostic systems

5' FMP QIAGEN2 STATS

| NAME: | 2K 40109 F | | RESULT | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CONTROL | SAMPLE | EXONS | | | | | | | | | | | | | |
| EXONS | | | Pm | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 12 | 13 | 17 | 19 | 30 | MEAN |
| Pm | 13332 | 12495 | - | 1.12 | 1.03 | 1.19 | 1.13 | 1.22 | 1.07 | 2.33 | 2.1 | 2 | 2.24 | 2 | 2.02 | 2.26 | 1.67 |
| 2 | 10520 | 8818 | 0.89 | - | 0.92 | 1.06 | 1.01 | 1.09 | 0.96 | 2.09 | 1.88 | 1.79 | 2.01 | 1.79 | 1.81 | 2.03 | 1.49 |
| 3 | 5174.5 | 4725 | 0.97 | 1.09 | - | 1.16 | 1.1 | 1.19 | 1.04 | 2.27 | 2.05 | 1.95 | 2.18 | 1.95 | 1.97 | 2.21 | 1.62 |
| 4 | 16270 | 12838 | 0.84 | 0.94 | 0.86 | - | 0.95 | 1.02 | 0.9 | 1.96 | 1.77 | 1.68 | 1.89 | 1.68 | 1.7 | 1.91 | 1.39 |
| 5 | 18532.5 | 15403 | 0.89 | 0.99 | 0.91 | 1.05 | - | 1.08 | 0.95 | 2.07 | 1.86 | 1.77 | 1.99 | 1.77 | 1.79 | 2.01 | 1.47 |
| 6 | 5381 | 4145 | 0.82 | 0.92 | 0.84 | 0.98 | 0.93 | - | 0.88 | 1.92 | 1.73 | 1.64 | 1.84 | 1.64 | 1.66 | 1.86 | 1.36 |
| 7 | 12427.5 | 10872 | 0.93 | 1.04 | 0.96 | 1.11 | 1.05 | 1.14 | - | 2.18 | 1.96 | 1.87 | 2.09 | 1.87 | 1.88 | 2.11 | 1.55 |
| 8 | 10267.5 | 4125 | 0.43 | 0.48 | 0.44 | 0.51 | 0.48 | 0.52 | 0.46 | - | 0.9 | 0.86 | 0.96 | 0.86 | 0.87 | 0.97 | 0.67 |
| 9 | 9109 | 4061 | 0.48 | 0.53 | 0.49 | 0.57 | 0.54 | 0.58 | 0.51 | 1.11 | - | 0.95 | 1.07 | 0.95 | 0.96 | 1.08 | 0.75 |
| 12 | 8271 | 3879 | 0.5 | 0.56 | 0.51 | 0.59 | 0.56 | 0.61 | 0.54 | 1.17 | 1.05 | - | 1.12 | 1 | 1.01 | 1.13 | 0.80 |
| 13 | 15860 | 6630 | 0.45 | 0.5 | 0.46 | 0.53 | 0.5 | 0.54 | 0.48 | 1.04 | 0.94 | 0.89 | - | 0.89 | 0.9 | 1.01 | 0.70 |
| 17 | 9678 | 4538 | 0.5 | 0.56 | 0.51 | 0.59 | 0.56 | 0.61 | 0.54 | 1.17 | 1.05 | 1 | 1.12 | - | 1.01 | 1.13 | 0.80 |
| 19 | 5497.5 | 2552 | 0.5 | 0.55 | 0.51 | 0.59 | 0.56 | 0.6 | 0.53 | 1.16 | 1.04 | 0.99 | 1.11 | 0.99 | - | 1.12 | 0.79 |
| 30 | 14348 | 5939 | 0.44 | 0.49 | 0.45 | 0.52 | 0.5 | 0.54 | 0.47 | 1.03 | 0.93 | 0.88 | 0.99 | 0.88 | 0.89 | - | 0.69 |
| | | MEAN | 0.66 | 0.75 | 0.68 | 0.8 | 0.76 | 0.83 | 0.72 | 1.65 | 1.48 | 1.41 | 1.59 | 1.41 | 1.42 | 1.6 | |

Deletion of exons 8-30 in a female patient. 5' Multiplex PCR

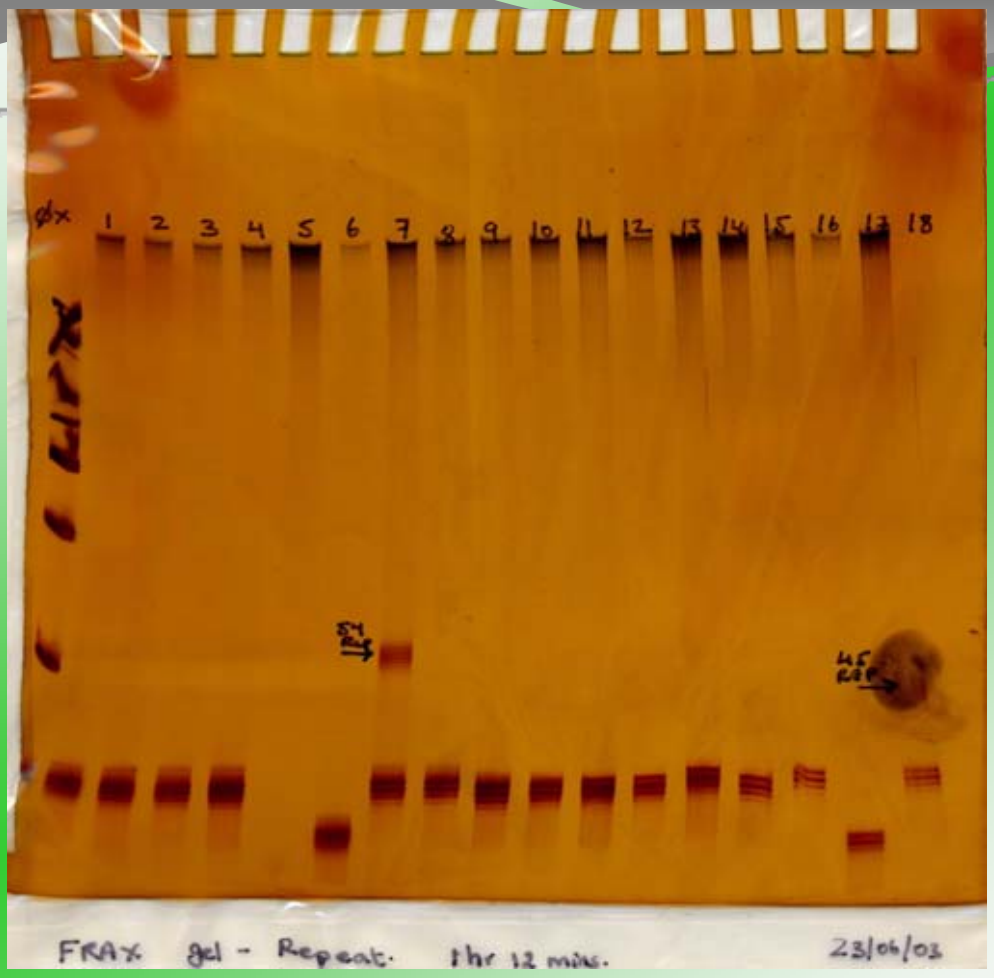

1 kb
A
B
C
D
E
← BRCA ex 11
← DMD ex 1
Confirming ex 1 del in patient A
1·5% Agarose for patient with DMD ex 1 del 90V - 40min

# Full Screen

- Full Screen
  - Full DMD gene screening (12 multiplexes, 7 exons each)
  - Heteroduplex analysis
  - QF PCR all exons
  - Both done on ABI 3100 Genetic Analyser
- Gene Sequencing
- PTT (Muscle biopsy - mosaicism)

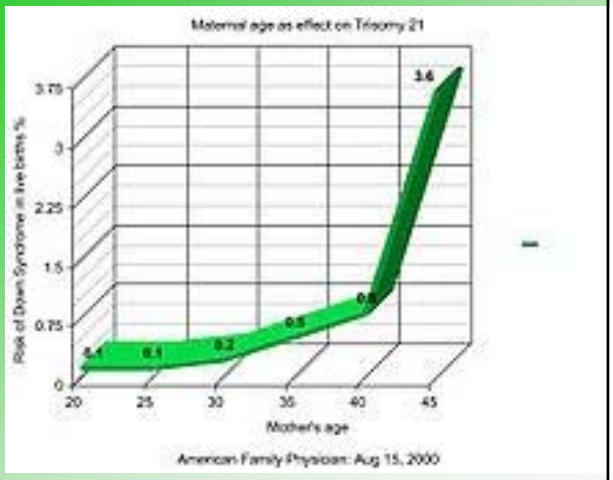
# Fragile X Syndrome

- Mutation on FMR1
  - CGG Repeat > 200
- Elongated face, large or protruding ears, flat feet, MR, speech problems
- Affects 1 in 3600 males and 1 in 4000-6000 Females
- PCR for up to 200 repeats and Southern Blot


ØX 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
FRAX gel - Repeat. 1hr 12 mins. 23/06/03

# Down's Syndrome

- 1 in 800 to 1000 births worldwide
- Trisomy 21 (full or part)
  - Meiotic Non-dysjunction
  - Full Trisomy 21 – 95% of Down's
  - 88% - maternal
- Maternal Age Risk
- Amniocentesis, CVS
  - Karyotyping, FISH
  - QF-PCR
- Triple Test
  - bHCG AFP, E2



# PCR –GOLD STANDARD BY WHO AND CDC

- Rapid, Ultra Sensitive and Specific
- CHIK, H1N1, MTB, Chlamydia etc.,
- Detects DNA unique to pathogen
- Accurately pick-up virus even in mild infections
- Used for Viral Loads (Hep B, C, HIV)
- Human Papilloma Virus- cervical cancer
- Septicaemia


Thank you