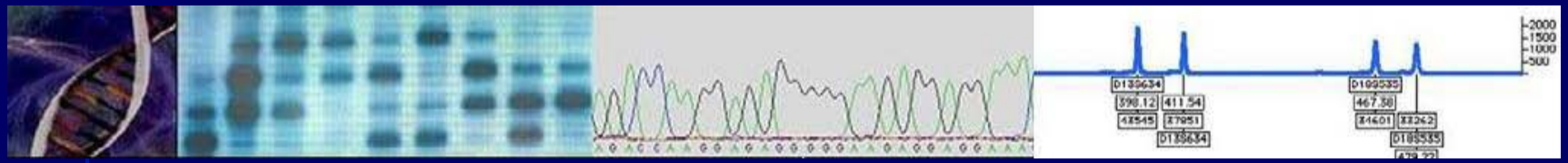


UK NEQAS FOR MOLECULAR GENETICS

External Quality Assurance
PGD for monogenic disorders

Dr Sandi Deans
Scheme Organiser

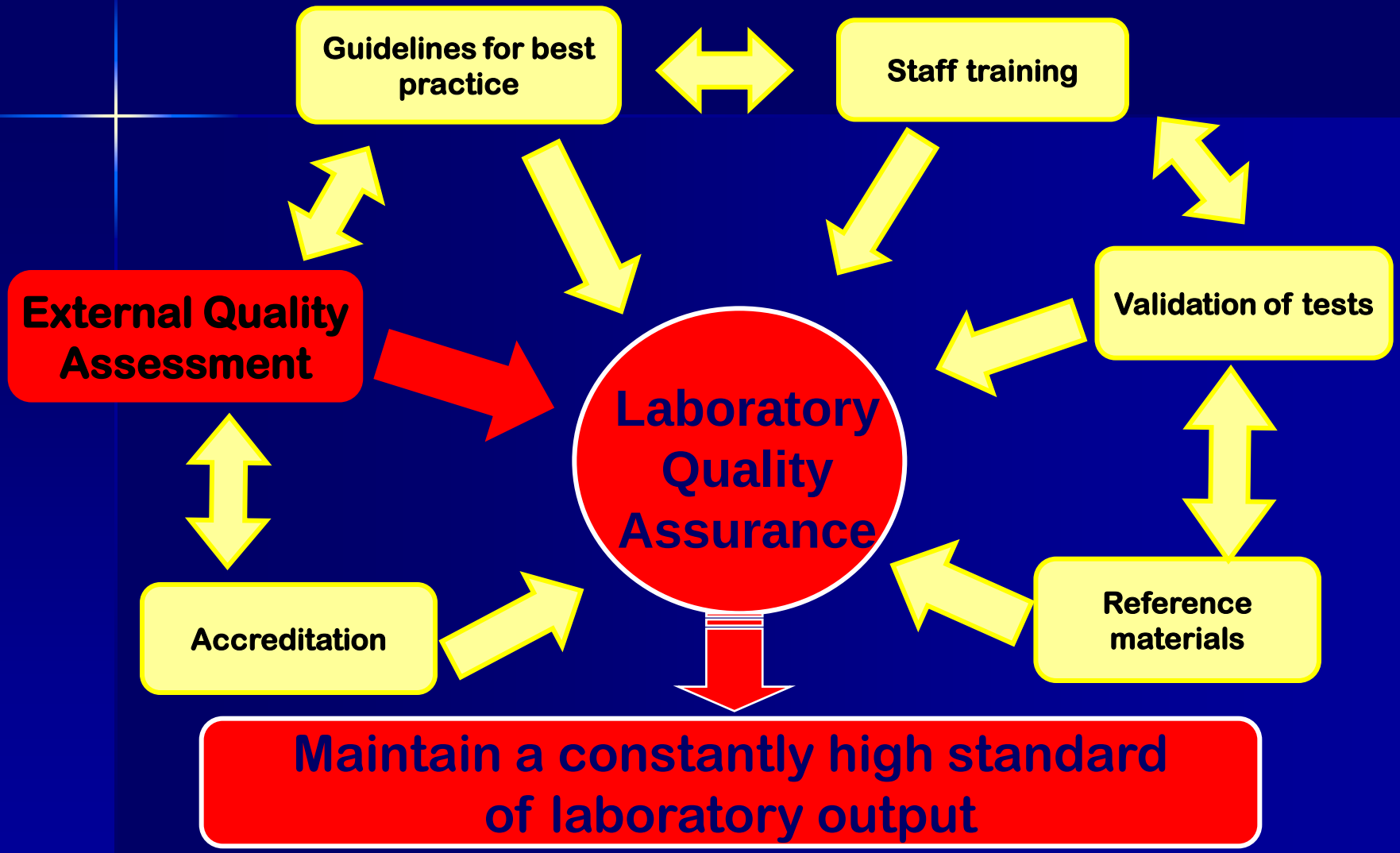
Institute of Human Genetics,
International Centre for Life,
Newcastle upon Tyne, UK

CPA

Accredited EQA Scheme
Reference No: 051

Ensuring Quality

network of issues



UK NEQAS

What is External Quality Assessment?

- EQA = proficiency testing
- Procedure for assessing and maintaining the quality and standards of output from a laboratory
- Measures the error rate of the laboratory and helps to identify any underlying problems.
- The end result is improved performance and better quality control

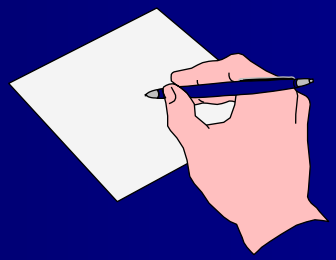
What is the purpose of EQA?

- Mechanism to quantify the quality of output of a laboratory
  - the analytical service
  - the interpretation of result
- Measure for the providers and users of the service
- Raise and harmonise standards
- Education
- Ensures that patients and clinicians receive the best possible service

Molecular Genetic Testing

Genotyping

Interpretation



Clinical context

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- **UK National External Quality Assessment Services (UKNEQAS)**
  "helping to ensure clinical laboratory test results are accurate, reliable and comparable wherever they are produced"

- **UK NEQAS for Molecular Genetics**
  - Provide EQA for Molecular Genetic testing laboratories
  - Self-funding, not-for-profit organisation
  - Full CPA Accreditation since 2001
  - Assess – Genotyping / Interpretation / Clerical Accuracy

- **Web-based system:**
  - Each laboratory has own website account
  - EQA scheme registration
  - Report return submission
  - Scheme marking
  - Publication of scores and reports
  - Record of participation and performance
  - Invoicing

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Pilot EQA for PGD monogenic disorders

Initial Stages

- Identified need for EQA
- CPA (UK) funding for pilot scheme 2008/09 (1 year)
- Collaboration with ESHRE
- Formed PGD Molecular EQA Working Group

Aims of Pilot EQA

- Assess all stages of the process
  - many aspects to providing a good service
  - PGD is not just testing embryo cells
  - gather information on differing laboratory practice
  - review reporting strategies

- Stage 1 - Ability to perform a feasibility work-up for PGD
  - Genotyping & Interpretation of results

- Stage 2 -Technical ability to perform single cell PCR
  - Genotyping, Interpretation & Reporting of results

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Pilot EQA for Cystic fibrosis

- 12 PGD labs performing CF participated
- Commercially sourced samples from CF families
  - Independently validated samples
  - DNA samples for feasibility work-up
  - Cell lines for single cell PCR
- No participation fee
- Use UK NEQAS for Molecular Genetics website

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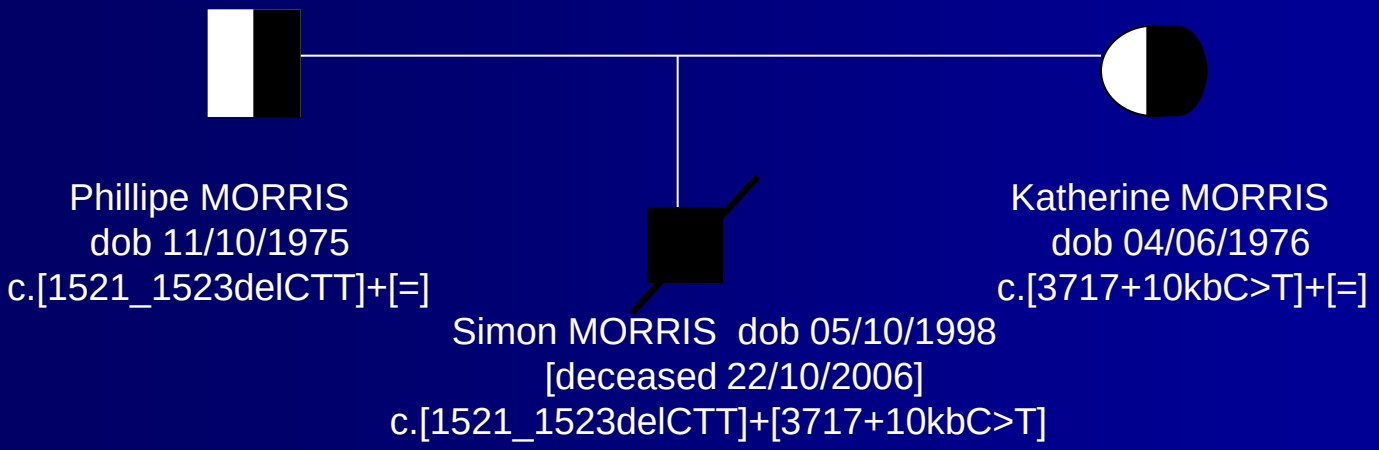
Stage 1 - December 2008

★ **Distribution of DNA samples for Feasibility Study**



★ **6 weeks given for testing (over holiday)**
★ **Results submitted using usual reporting format**
★ **Requested participants complete *proforma* to gather information**

Mutation nomenclature is given using NM_000492.3 with numbering starting at the A of the ATG initiation codon according to HGVS guidelines.

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Stage 1 - Results

- **100% result return rate with high standard of genotyping**
- **11/12 labs offered PGD** (1 lab – no optimised protocol for required markers)
- **Reports scored for Genotyping, Interpretation & Clerical Accuracy** (max. 2.00)
- **Scheme mean scores:**

Genotyping	***1.93***
Interpretation	***1.78***
Clerical Accuracy	***1.95***

Stage 1 – Marking

★ **Genotyping – *0.1 mark deducted for each incorrect locus***

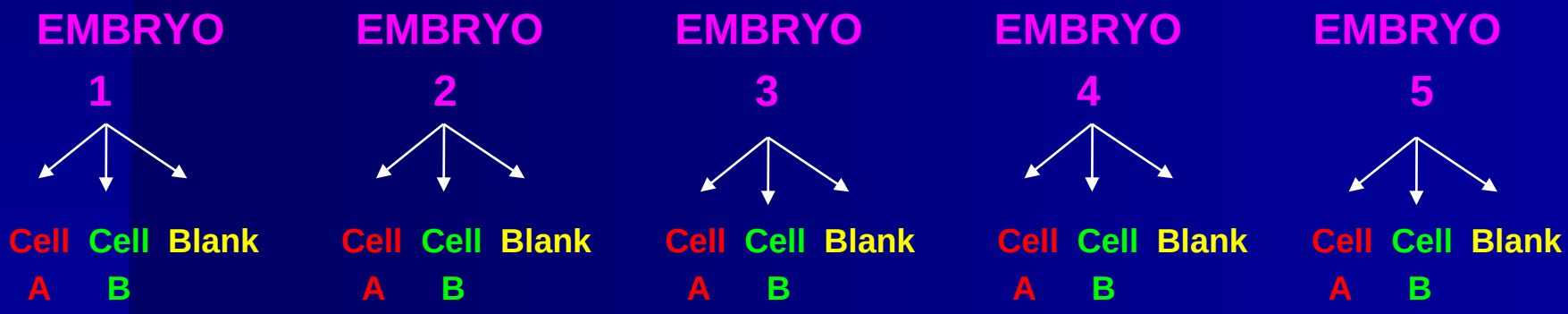
★ **Interpretation – Reports should include (*0.1 mark deducted for each omission*)**
- **Disease being tested**
- **Methods performed**
- **Markers to be used for PGD case**
- **Error rates clearly stated**
- **Use of Human Genome Variation Society (HGVS) mutation nomenclature**
- **Stating reference sequence when using mutation nomenclature**

★ **Clerical Accuracy - Reports should include (*0.1 mark deducted for each omission*)**
- **Two identifiers per patient**
- **Stating which samples were tested**
- **Indication of name/signature of authoriser**
- **Stating issue date of report**

Stage 2 - February 2009

★ Validation and distribution of testing single cells
- ★7 validation laboratories
- ★range of PCR assays performed
- ★range of distribution times

★ Distribution of single lymphocytes for PGD case



★ 4 weeks given for testing and results submitted using usual reporting format

Stage 2 – Results

	Genotype	Interpretation
Embryo 1	c.[1521_1523delCTT]+[3717+10kbC>T] *delta F508 / 3849+10kbC>T compound heterozygote*	Affected embryo Transfer not recommended
Embryo 2	c.[1521_1523delCTT]+[=] *delta F508 carrier*	Unaffected embryo Transfer recommended
Embryo 3	c.[1521_1523delCTT]+[3717+10kbC>T] *delta F508 / 3849+10kbC>T compound heterozygote*	Affected embryo Transfer not recommended
Embryo 4	c.[3717+10kbC>T]+[=] *849+10kbC>T carrier*	Unaffected embryo Transfer recommended
Embryo 5	c.[1521_1523delCTT]+[=] *delta F508 carrier*	Unaffected embryo Transfer recommended

Mutation nomenclature is given using NM_000492.3 with numbering starting at the A of the ATG initiation codon according to HGVS guidelines

Stage 2 – Marking

- **Genotyping –** ***0.1 mark deducted for each incorrect locus for each embryo***

- **Interpretation – Marks were given for the correct interpretation of results ie. whether the embryo should be transferred or not**

- **Clerical Accuracy - Reports should include (*0.1 mark deducted for each omission*)**
  - **Report date or egg collection date**
  - **Samples being tested**
  - **Disease being tested**
  - **Methods performed**
  - **Error rates clearly stated**
  - **Use of Human Genome Variation Society (HGVS) mutation nomenclature**
  - **Stating reference sequence when using mutation nomenclature**
  - **Indication of name/signature of authoriser**

Stage 2 – Results

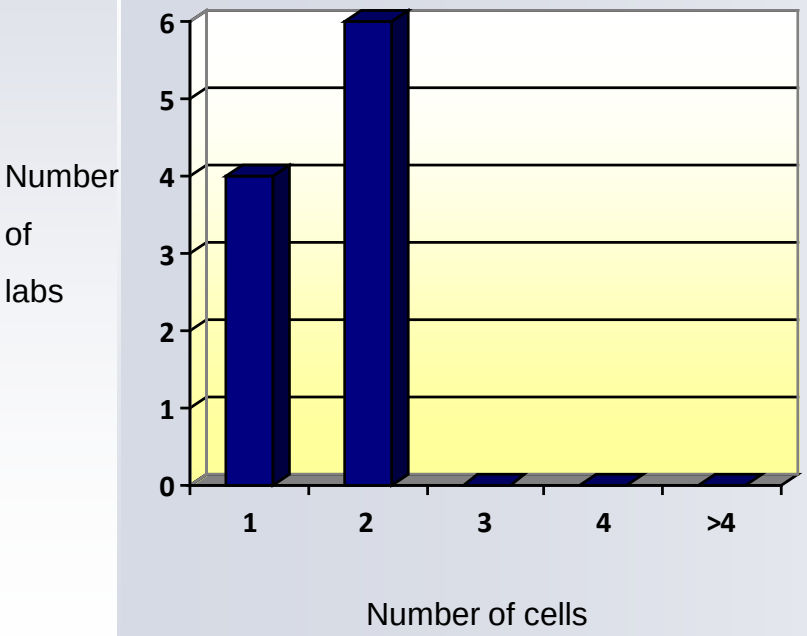
- Scheme mean scores:

Genotyping	***1.99***
Interpretation	***2.00***
Clerical Accuracy	***1.77***

- Stage 2 participant returns
  - 6/11 labs obtained results for all 5 embryos
  - 2/11 labs obtained results for 4 embryos
  - 2/11 labs = no results but sent data to interpret/report
  - 1/11 lab obtained results for 2 embryos

Strategy Survey 1

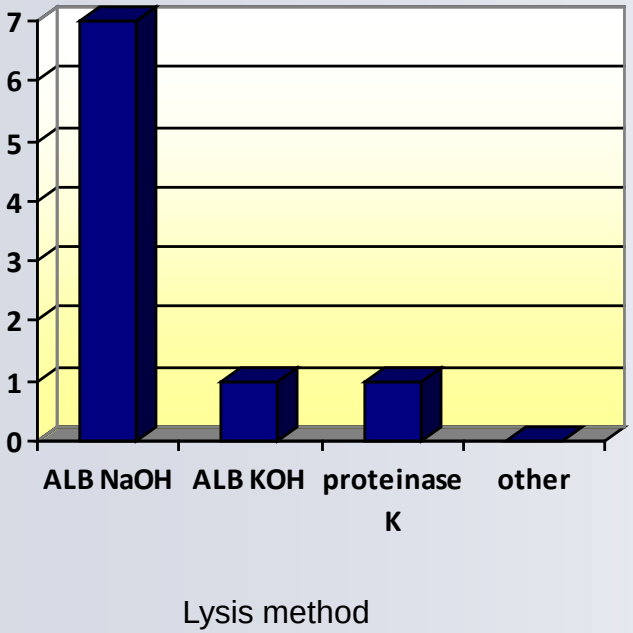
How many embryo cells do you routinely test?



- ★ 40% of labs test 1 cell
- ★ 60% of labs test 2 cells
- ★ No labs tested >2 cells

Strategy Survey 2

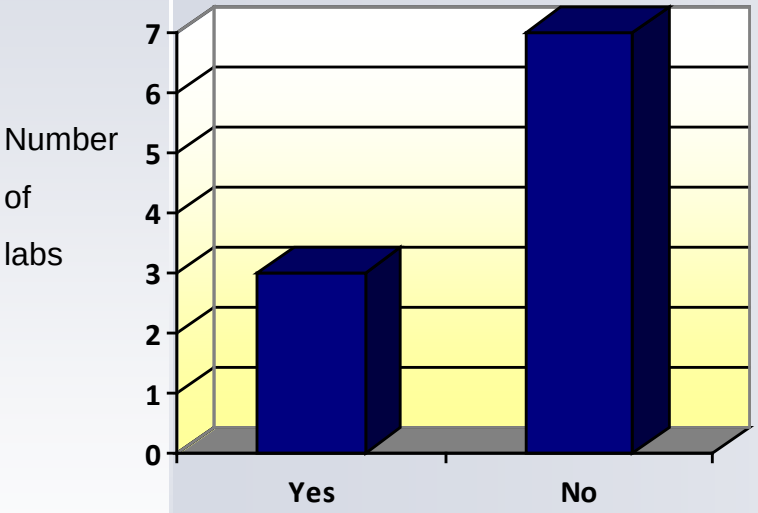
Which lysis method do your routinely use?



- ★ 3 methods stated
- ★ 70% of labs use NaOH alkaline lysis buffer

Strategy Survey 3

Do you perform whole genome amplification?



- ★ 30% labs routinely perform whole genome amplification
- ★ They all used NaOH alkaline lysis buffer
- ★ 2 of them test 1 cell per embryo (the other tests 2 cells)

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Pilot Scheme Summary

- Pilot EQA scheme looked at a PGD case for cystic fibrosis:
  Feasibility Study and Single Cell testing
- Participants given a measure of against an external source of validated material and against other PGD laboratories
- No critical genotyping or interpretation errors detected
- Highlighted variability of reporting formats and local procedures
- Single lymphocytes successfully used test embryo cell testing

Future

★**Second year of pilot EQA scheme - 2010**

Format

- Opened to all interested parties
- 15 participants
- Fees charged

Timetable

- Feasibility Study – January/February 2010
- Single Cell Testing – March/April 2010
- Scheme Marking – May/June 2010
- Scheme Scores and Report published – June 2010

Modifications to scheme

- *Proforma* provided for Feasibility Study results
- Improved instructions for processing single lymphocytes
- Streamline marking process to improve turnaround time

Acknowledgements

★ **Molecular PGD EQA Working Group**

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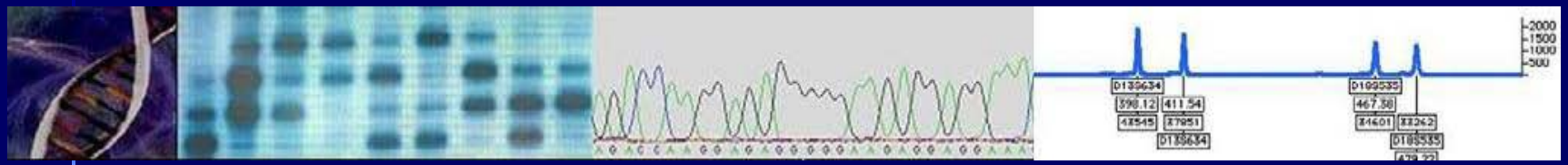
★ **Coriell Institute for Medical Research**
★ **Dr Francesco Fiorentino and his team at Genoma, Italy**
★ **ESHRE**
★ **UK NEQAS for Molecular Genetics Steering Committee**



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