The CRMGEN Project

Certified Reference Materials for Molecular Genetic Testing

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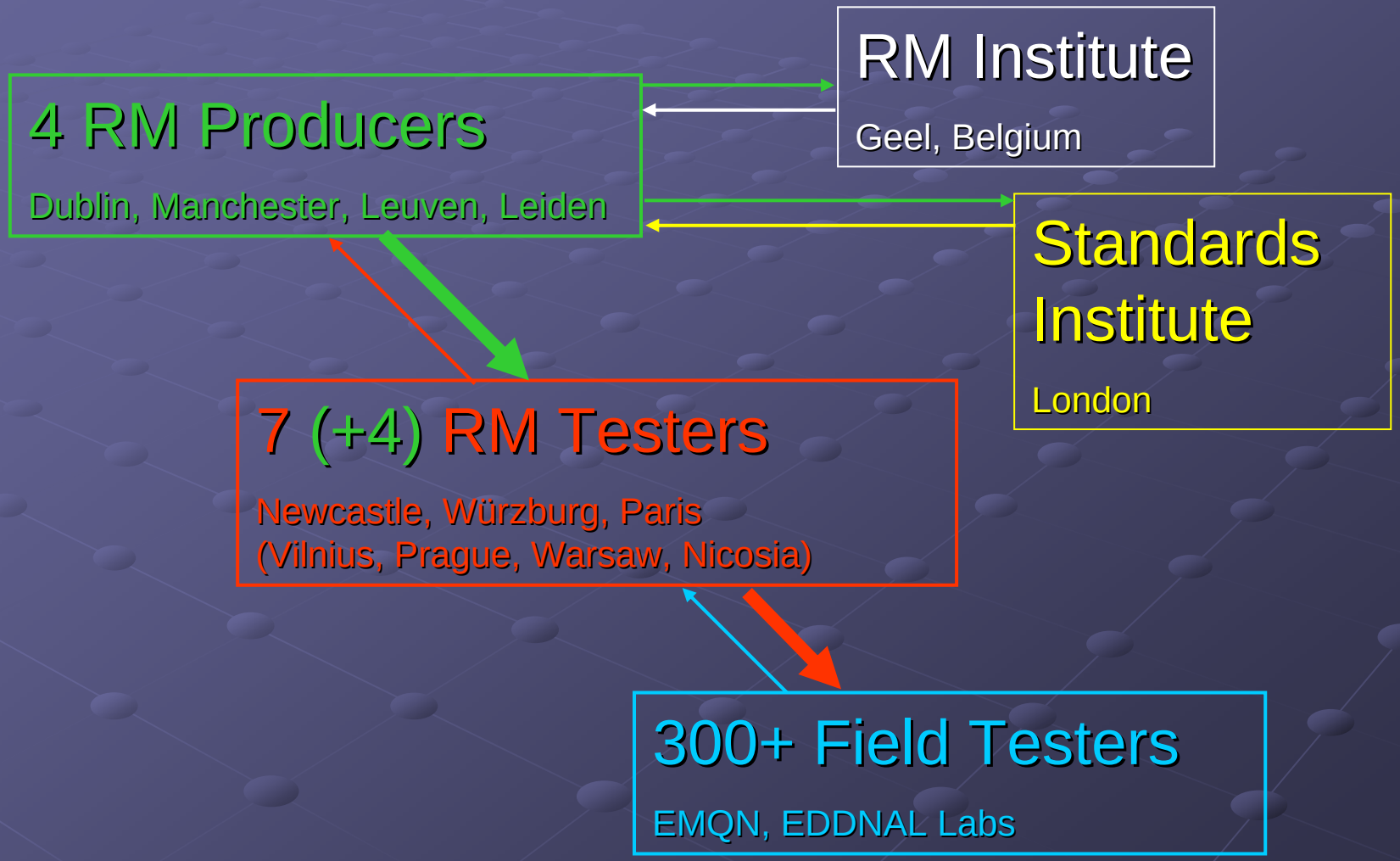
The CRMGEN project - History

- Idea generated by EMQN Management Group
- Expression of interest submitted Dec 1999
- Published as call for proposals April 2000
- Proposal submitted September 2000
- Contract negotiation started Jan 2001
- Contracts signed October 2001
- Project started November 2001
- Four NAS partners added November 2002
- Project funding expired October 2005

The CRMGEN project - People

- Co-ordinator
  - David Barton, Dublin
- Management Group
  - Jean-Jacques Cassiman, Leuven
  - Bert Bakker, Leiden
  - Rob Elles, Manchester
  - Clemens Muller, Würzburg
  - Glyn Stacey, London
  - Christoph Klein, Geel
- Partners
  - Su Stenhouse, Glasgow
  - Michel Goossens, Paris
  - Christine Mannhalter, Vienna
  - Milan Macek Jr, Prague
  - Jerzy Bal, Warsaw
  - Roula Christodoulou, Nicosia
  - Vaidutis Kuchinskas, Vilnius

CRMGEN Consortium Design



The CRMGEN project - Plan

- Develop prototype RMs in four formats
  - PCR products - Dublin
  - Cell lines - Leuven
  - Genomic DNA - Manchester
  - Recombinant DNA - Leiden
- Send prototype RMs to testing labs
- Study stability and presentation issues
- Field-test prototype RMs
- Develop guidelines for future RM production
- Develop plan for future RM production

The CRMGEN project - RMs

Disorder	RMs to be developed	RM Type*
Cystic Fibrosis	ΔF508, G542X, G551D, N1303K	CL
Haemochromatosis	C282Y, H63D	PCR
Fragile X syndrome	Normal, premutation, expansion	CL, gDNA
Sickle cell anaemia	HbS	PCR
Beta thalassaemia	Codon 39 (C->T), IVSI- 110 (G->A)	PCR
Factor V Defect	R506Q, Factor V “Leiden"	rDNA
HNPCC	Representative nonsense mutations	gDNA
DMD	Deletions, duplications	rDNA

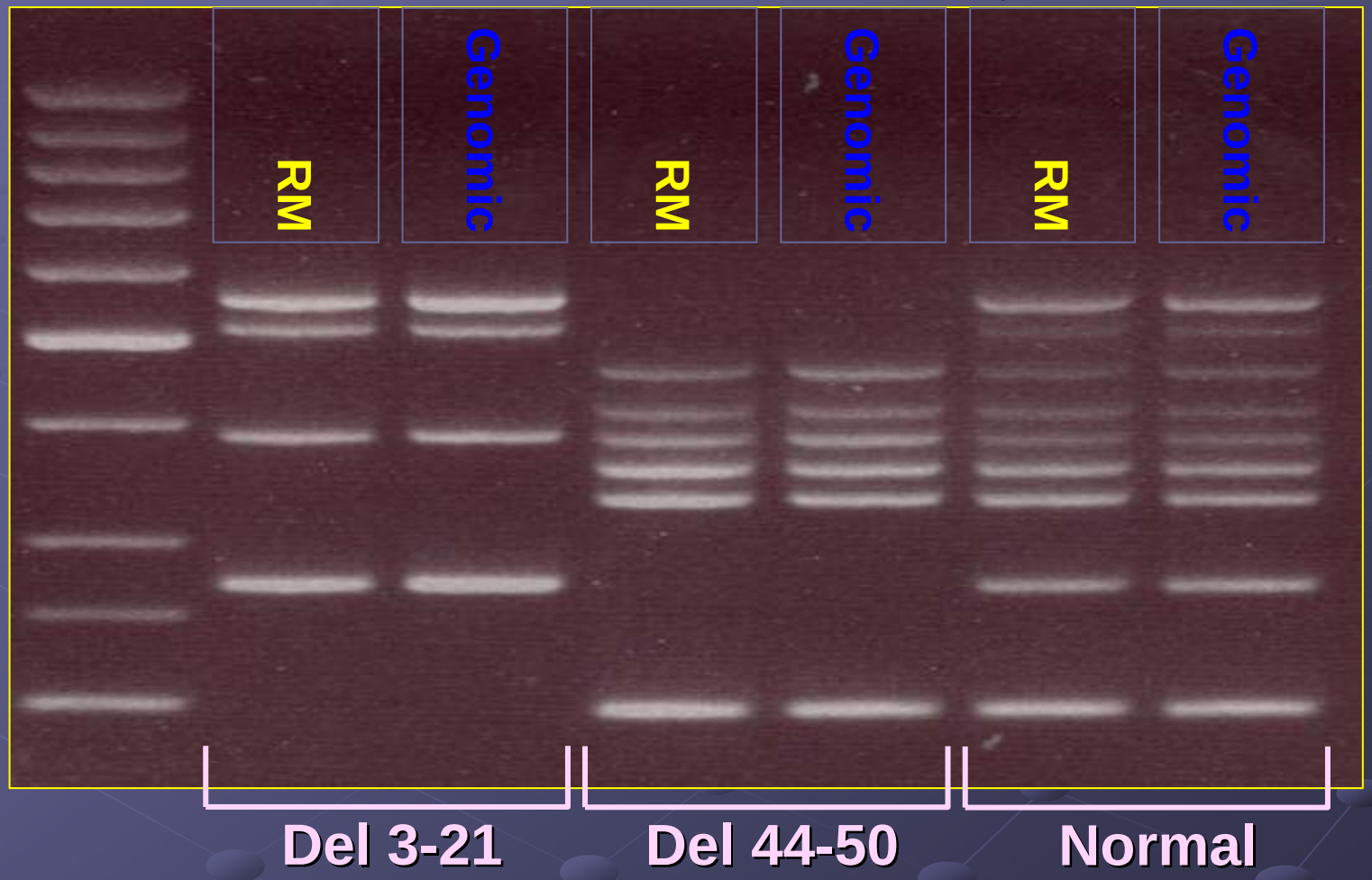
*KEY: CL, cell line; PCR, polymerase chain reaction product; gDNA, genomic DNA; rDNA, recombinant DNA

Issues around RM format

Type	Similar to usual samples	Versatile	Stable	Economical to produce	Storage cost	Ethical issues
Cell Line	+++	+++	-	+	-	-
Genomic DNA	++	++	+	++	++	-
Recombinant DNA	+	++	++	+++	++	++
PCR Product	+	++	++	+++	++	++
Synthetic DNA	-	-	++	++	++	+++

Plasmid-based RM for DMD

Francesco Russo & Bert Bakker, Leiden



Chamberlain multiplex; 50fg each RM / 200 ng genomic DNA

Plasmid-based RM for DMD

Francesco Russo & Bert Bakker, Leiden

Sample:	CM + PL 3-7													
Control:	Neg C 1	Neg C 3												
Opmerking:														
EXONS	SAMPLE	CONTROL	2	3	4	5	6	8	9	12	13	17	19	30
Pm	7953	31678	0.99	2.26	2.11	1.83	2.16	1.16	1.08	1.06	1.06	1.02	1.09	1.00
2	10610	42808		2.29	2.14	1.86	2.19	1.17	1.10	1.08	1.07	1.03	1.11	1.01
3	12580	22199			0.94	0.81	0.96	0.51	0.48	0.47	0.47	0.45	0.48	0.44
4	15625	29444				0.87	1.02	0.55	0.51	0.50	0.50	0.48	0.52	0.47
5	8228	17871					1.18	0.63	0.59	0.58	0.58	0.55	0.60	0.54
6	15010	27695						0.54	0.50	0.49	0.49	0.47	0.51	0.46
8	15182	52267							0.93	0.92	0.91	0.88	0.95	0.86
9	14994	55246								0.98	0.98	0.94	1.01	0.92
12	11690	43788									1.00	0.96	1.03	0.94
13	4704	17701										0.96	1.03	0.94
17	9163	35874											1.08	0.98
19	15431	56170												0.91
30	8660	34666												

Duplication exons 3-7

MLPA analysis; 100fg each RM / 100 ng genomic DNA

Prototype Genomic DNA RMs

Sample No.	Disease	Affection Status	Gender	Cell Line Established?
CRM4/03/0001	FraX	Full Mutation	Female	Yes
CRM4/03/0003	FraX	Full Mutation	Male	Yes
CRM4/03/0004	FraX	Normal Transmitting Male	Male	Yes
CRM4/03/0005	FraX	Premutation (Unstable mosaic)	Female	Yes
CRM4/03/0013	FraX	Normal	Female	Yes
CRM4/03/0006	HNPCC	MSH2 (c.2182_2199del)	Female	Yes
CRM4/03/0014	HNPCC	MSH2 (c.1165C>T)	Female	Yes
CRM4/03/0015	HNPCC	MSH2 (c.2131C>T)	Male	Yes
CRM4/03/0017	HNPCC	MLH1 (c.901C>T)	Male	Yes
CRM4/03/0018	HNPCC	MLH1 (c.350C>T & c.1852-1853AA>GC)	Female	Yes
CRM4/03/0019	HNPCC	MLH1 (c.677G>T)	Male	Yes

Prototype RM Characterisation

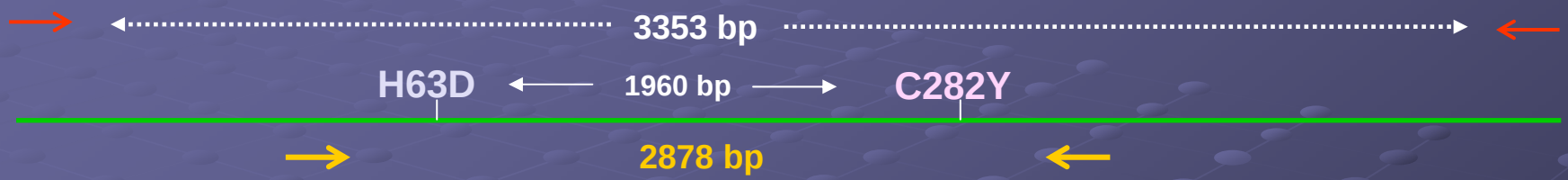
- All characterisation complete:
  - Allele size and methlyation status determined (FraX)
  - Exons plus boundaries of hMLH1 and hMSH2 sequenced (HNPCC) by us and MRC Geneservice
- Summary reports produced for each prototype:
  - Sample Numbers.
  - DNA concentration and extraction method.
  - Gender.
  - Gene.
  - Mutation and polymorphism details.
  - Methylation status (FraX only).
  - Relevant worksheet numbers.

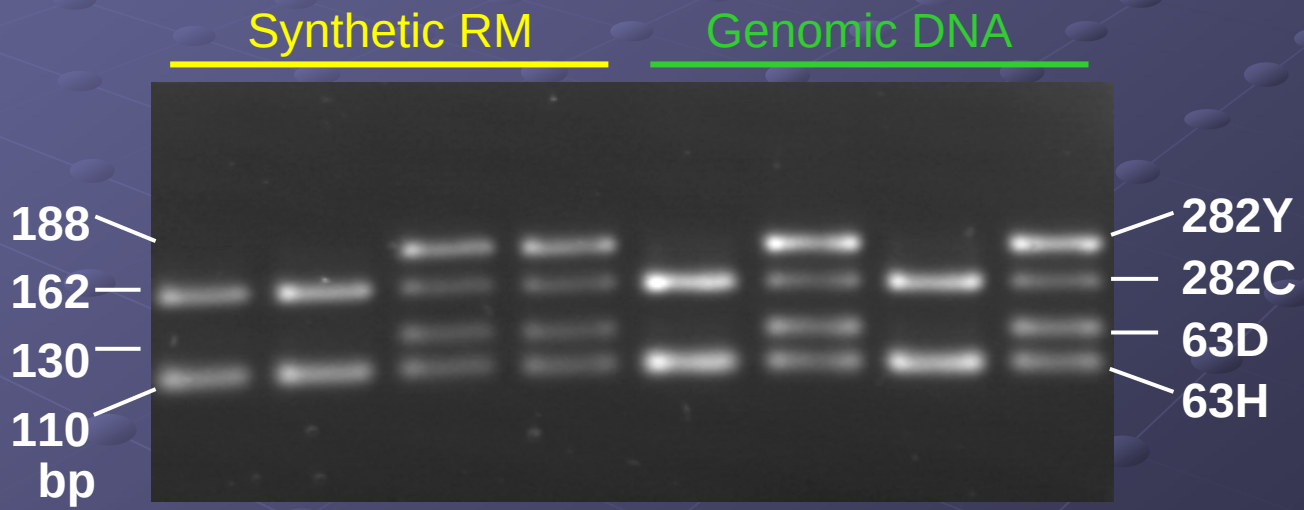
Summary – Genomic DNA

- 4 FraX X and 6 HNPCC prototype RMs have been developed:
  - Fully characterised.
  - Field tested.
  - Short term stability studies completed.
- Genomic DNA is a suitable format for producing RMs:
  - × Long production process (informed consent, bleed patient, grow cell line, extract DNA).
  - ✓ Characterisation fairly quick.
  - ✓ Field trial performance good.

PCR product-based RM for HH

Liz Donohoe & David Barton, Dublin





BbrPI digest (Stott et al, 1999)

Cell line-based RMs

GOALS:

- Development of RMs for cystic fibrosis testing
- Four common CFTR mutations
- Uncommon CFTR mutations (disease causing or polymorphisms) that might interfere with common CFTR mutations in diagnostic CFTR tests.
- 6 cell lines

Cell line-based RMs

STRATEGY

- Obtain patient bloods, create cell lines
  - EBV transformation
  - Fingerprint ID
  - Mycoplasma test
  - Karyotype
- Genetic Analysis
  - Develop CF gene sequencing protocol
  - 61 sequencing reactions, both strands
  - complete coding region, and exon/intron junctions
  - Develop “minisequencing” protocol to test all mutations/variants

Cross-cutting Activities

- Stability & homogeneity studies
- Presentation and packaging issues
  - Liquid/freeze-dried, additives, etc.
- Decontamination protocols
- Carrier DNA
  - Salmon sperm, yeast, hexamers, UV cross-linking
- Cell line banking and validation
- Field trial design
- Market surveys
- Study of patent implications

The CRMGEN Project - Results

- Prototype RMs produced as
  - Genomic DNA – FRAX, HNPCC, Cystic Fibrosis
  - Plasmid Mixes – Duchenne MD, FV Leiden
  - PCR products – Haemochromatosis, Hb, Cystic Fibrosis
  - Cell lines – CF, FRAX, HNPCC
- Know-how developed
  - Design
  - Production
  - Homogenization
  - Stabilization
  - Validation
- Guidelines drafted
- Fragile X RMs going forward for WHO certification

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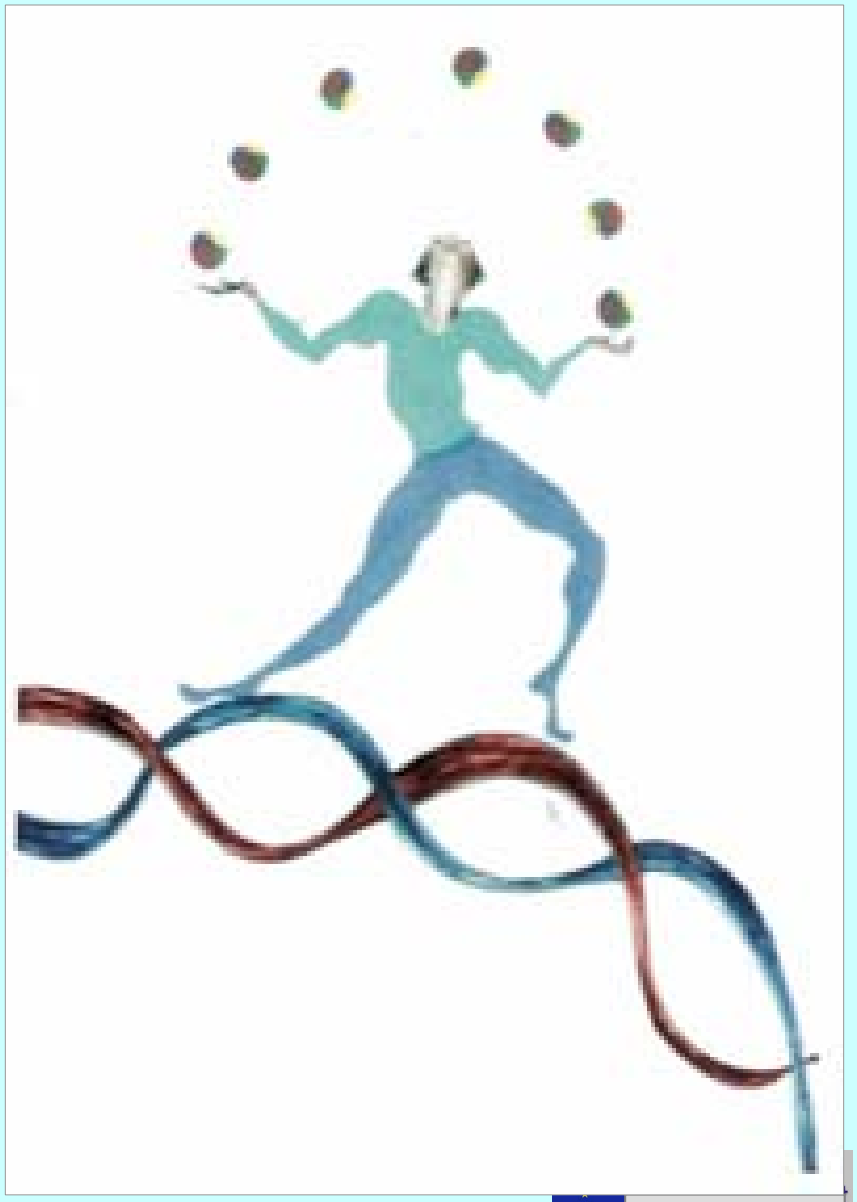
EuroGentest

Genetic testing in Europe:

A Network for test development, harmonization, validation and standardization

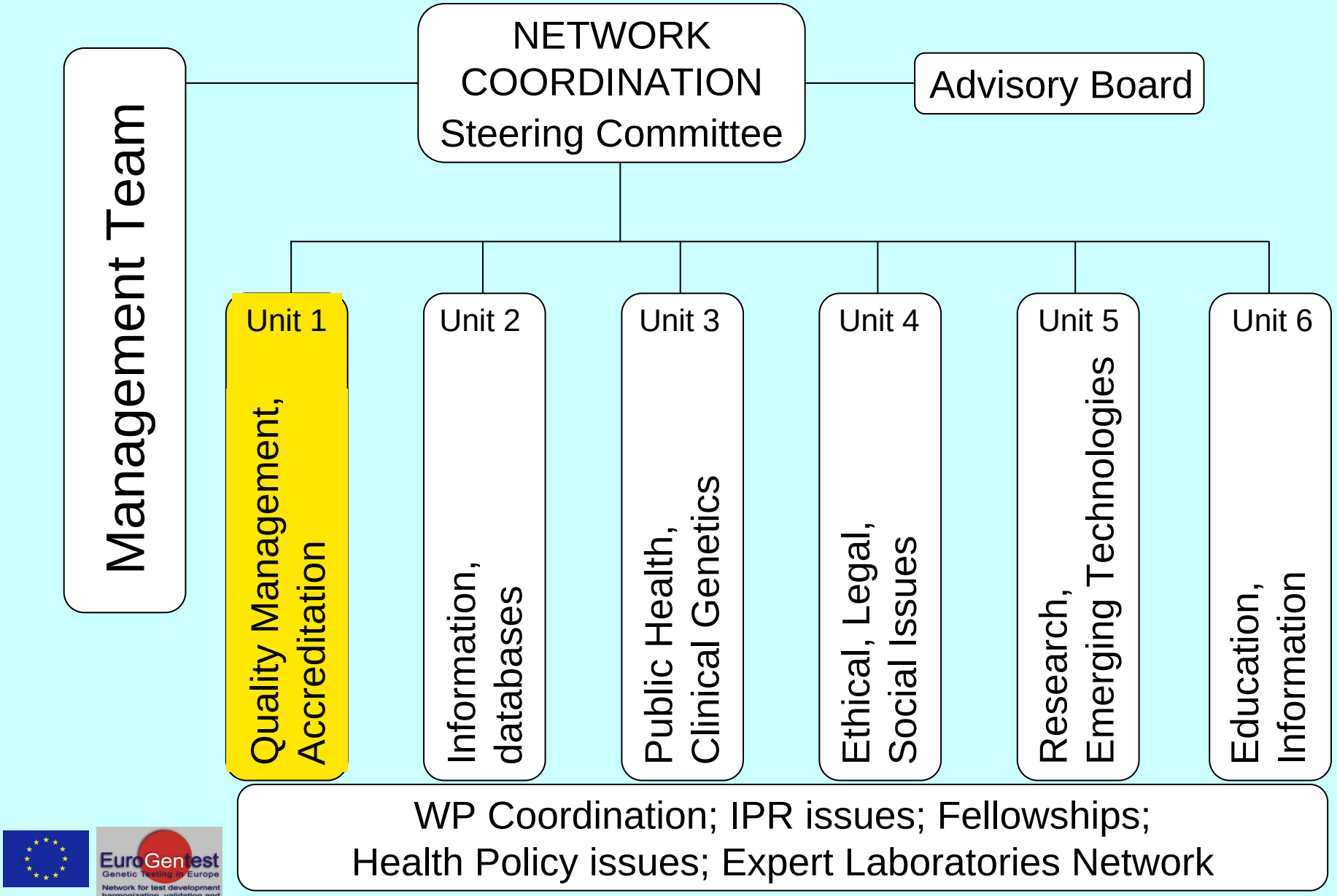


By Rebecca Kent

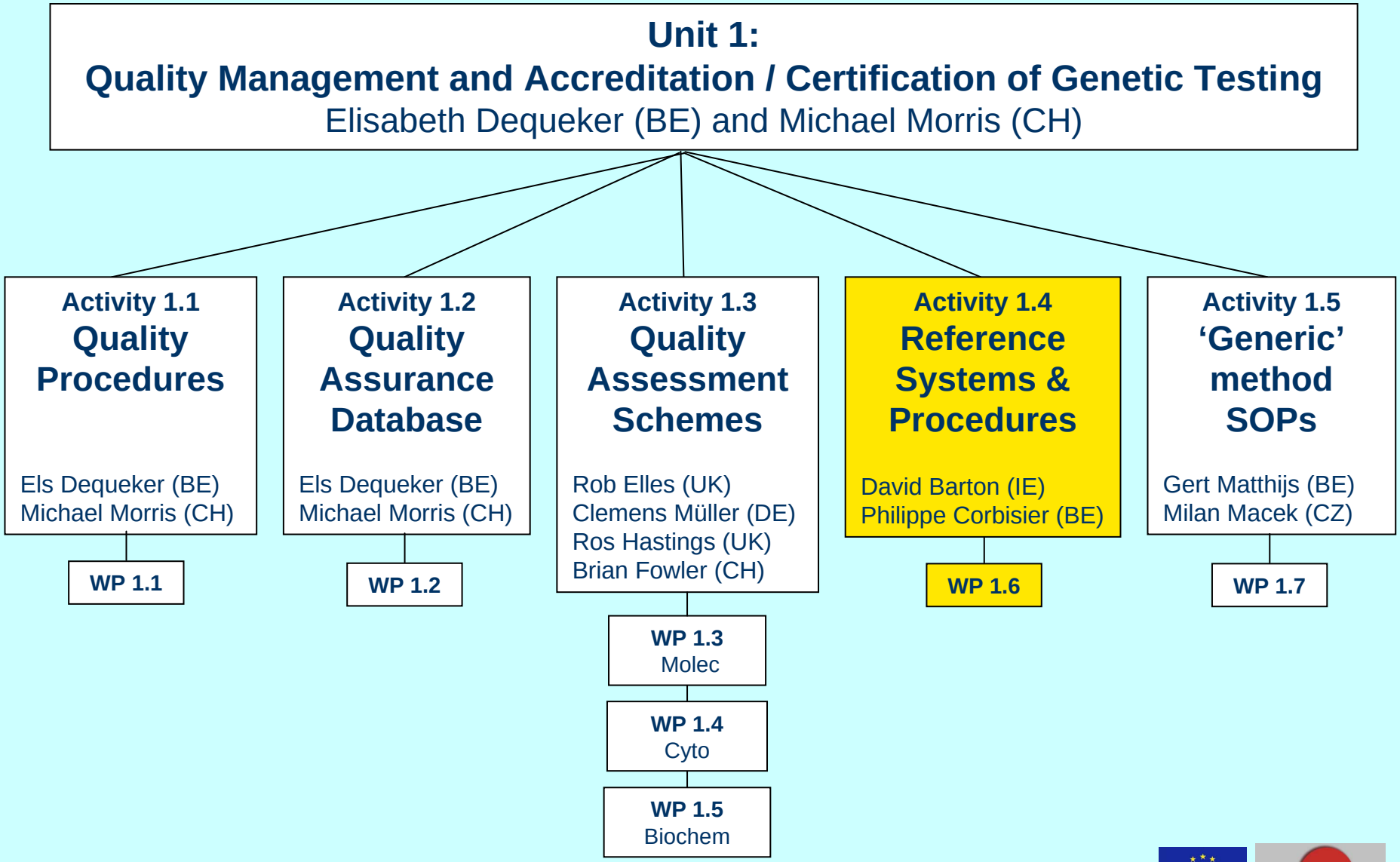
Co-ordinator: Jean-Jacques Cassiman
Katholieke Universiteit
Leuven, Belgium

JRC-IRMM, November 2005

EuroGentest Network Structure



Relation between the units, activities and workpackages



JRC-IRMM, November 2005

WP 1.6 Reference Measurement Systems

- Identify the present and future needs for Reference Materials (RM) and Reference Measurement Procedures (RMP) for genetic testing.
- Set priorities for the development of new RMs
- Support implementation of traceability for routine diagnostic methods and IVDs to Certified Reference Materials
- Support improvement of quality and harmonization of genetic testing by definition of guidelines for the development of Reference Materials in this field.
- Build an enduring network, involving all the key stakeholders in Reference Measurement Systems development.
- Study Regulation and patenting issues in genetic testing in Europe
- Participate in targetd RM development projects


EuroGentest
Genetic Testing in Europe
Network for test development harmonization, validation and standardization of services

Generic RMs for Mutation Detection

Helen White & Nick Cross, NGRL Wessex

- Generic set of reference reagents to assess mutation detection techniques.
- 48 plasmids produced which can be used to determine the sensitivity and specificity of these techniques by analysing
  - Type of base substitution
  - GC content of the amplicon
  - Location of the mutation in the fragment.
- Currently using these reagents to evaluate CSCE and dHPLC
- Running a more extensive field trial in 2005.