Current Quality Control Materials for "Genetic" Tests: Guidelines, Oversight And Availability

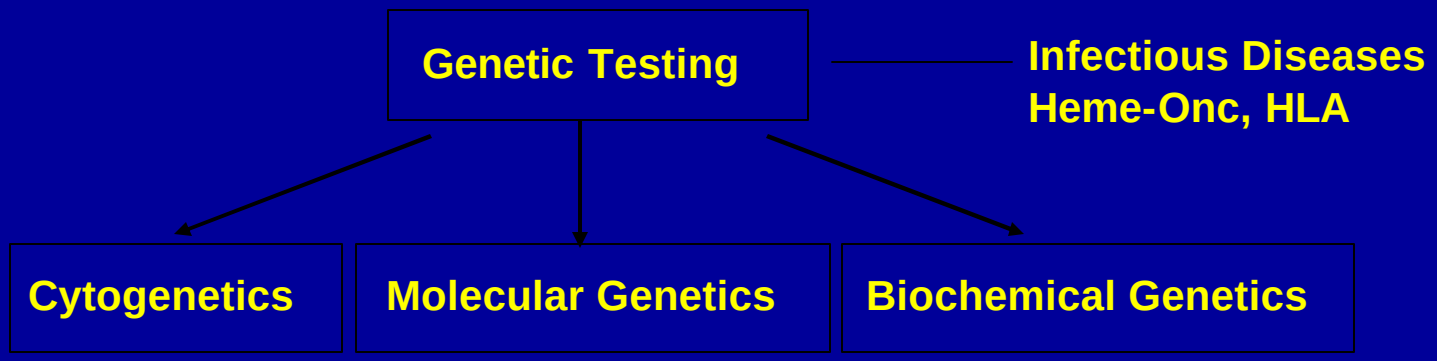
Lawrence M. Silverman, PhD.
September 15, 2003

Developing QC Materials For GENETIC TESTING

PROGRAM

- Stabilized Synthetic Nucleic Acids
- Cell Transformation
- Genetic Engineering
- European QC Materials
- Dried Blood Spot Materials
- Multiplex CF Control Materials
- Innovative QC
- Future Needs for QC Materials
- Panel Discussions

Quality Control Issues



Quality Assurance
Method Validation
Proficiency Testing

Quality Control Issues (continued)

- Technology Driven
- Rapidly Changing Target
  - Qualitative
  - Quantitative
- Issues Regarding Patient Samples for QC/QA
- Matrix Effect
- RNA/ RT-PCR

Quality Control Issues (continued) – Infectious Diseases

- HSV (Herpes Simplex Virus)
  - Method Dependent
  - Type 1 Versus Type 2
  - Control Material
- Entereovirus
  - Method Dependent
  - “Mixed” Control Material
- Neisseria Gonorrhea
  - Method Dependent
  - Non-specific Interference

Quality Control Issues (continued) - Cytogenetics

- FISH Analyses
  Control Material/Patient Samples
- AneuVision
  Commercial Controls Available for
  Trisomies 13, 18, and 21

Quality Control Issues (continued) – Heme/Onc

- Bcr/abl
  - Breakpoint Dependent
  - Method Dependent (housekeeping gene)
  - Control Material
- Her2/neu
  - Method Dependent
  - Control Material

Quality Control Issues (continued) - HLA

- Control Material for Rare Alleles
- Method Dependent

Quality Control Issues (continued) – Sequence-based Diagnostics

- Limited Sequencing
  - BRCA1/2
  - HIV/HCV Genotyping
  - HLA Genotyping
  - Gleevac Resistance

Genetic Testing for Cystic Fibrosis (NIH Consensus Conference)

"…recommended that testing for gene mutations that cause cystic fibrosis be offered as an option to all pregnant couples and those planning pregnancy."

Heterogeneity in Molecular Genetic Disease

- **Clinical heterogeneity – variability in disease severity and organ involvement within a single molecular disorder**
- **Allelic heterogeneity – multiple mutations within a single gene that each give rise to an abnormal phenotype**
- **Locus heterogeneity – mutations in more than one gene can give rise to very similar phenotypes**

Mutations Classified by Severity of Disease

Severe (Lung dis, PI)	Mild (Lung dis, PS)	Lung dis, PS Brdl/nl sweat Cl	No lung dis, CBAVD
ΔF508	R117H (5T)	R117H (7T)	R117H (7T)
G542X	3849+10kb C>T	3849+10kb C>T	D1152H
G551D	2789+5G>A	G551S	D1270N
R553X	R334W	D1152H	P67L
W1282X	G85E	A455E	5T
N1303K	G91R		
3905insT	R347P		
1078delT	R347H		
621+1G>T	R347L		
1717-1G>A	D1152H		
ΔI507	A455E		
R560T	Y563N		
S549N	P574H		
3659delC	S945L		
G480C	L1065P		
	F1286S		

(Yankaskas, Knowles, 1999)

Recommended Core Mutation Panel for General Population CF Carrier Screening

DF508	DI507	G542X	G551D	W1282X
N1303K	R553X	621+1G>T	R117H	1717-1G>A
A455E	R560T	R1162X	G85E	R334W
R347P	711+1G>T	1898+1G>A	2184delA	1078delT
3849+10kbC>T	2789+5G>A	3659delC	3120+1G>A	I148T

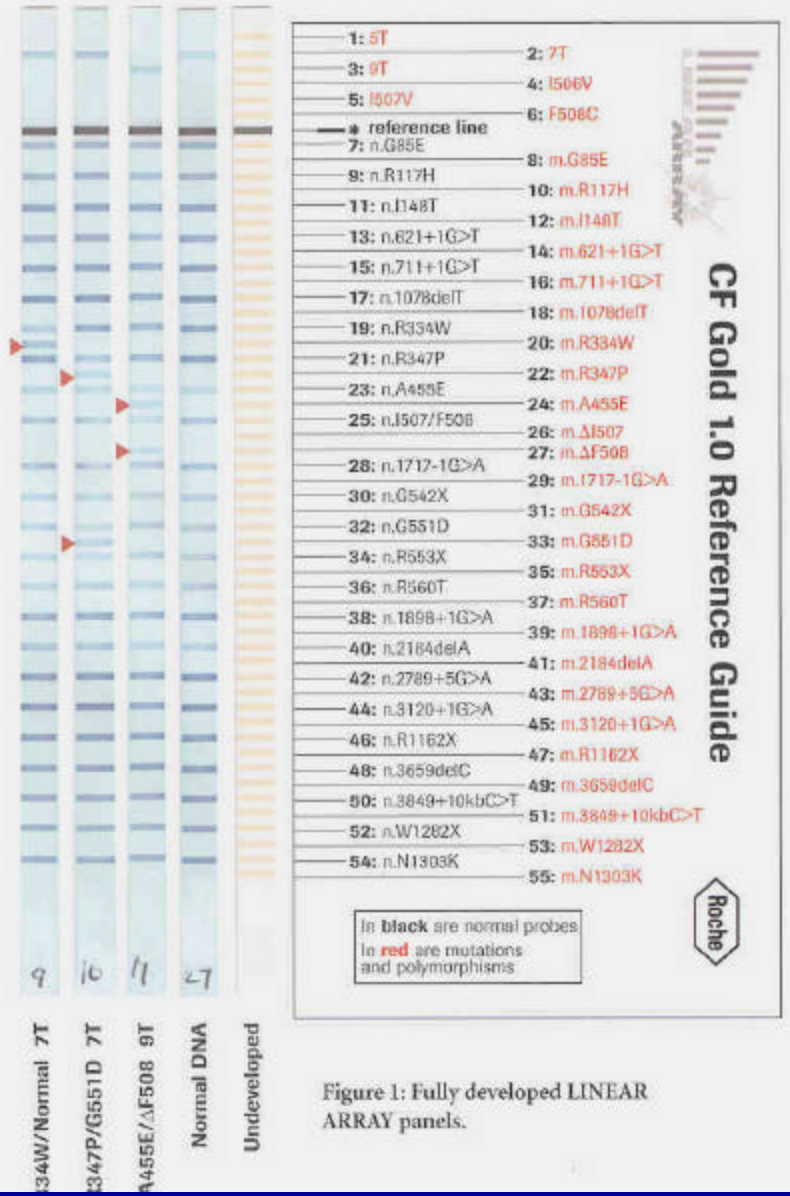


Figure 1: Fully developed LINEAR ARRAY panels.

I148T occurs on Multiple Haplotypes and Confers A Variable Phenotype (con't)

- I148T-9T, observed in all unaffected and 1 affecte

- I148T-9T-3199del6, observed in 4/5 affecteds (not seen in 386 non-CF chromosomes)

- Thus, I148T occurs on at least two backgrounds, and only with the 3199del6 appears to be disease-causing.

Clinical Utility of Poly-T

- Determination of Poly-T status in individuals positive for R117H is useful for predicting phenotype.
- The odds of being affected with CF is significantly greater when R117H is in cis to 5T rather than 7T
- In males, the odds being affected with CAVD is significantly greater when R117H is in cis to 7T rather than 5T

Amended Core Mutation Panel for General Population CF Carrier Screening

DF508	DI507	G542X	G551D	W1282X
N1303K	R553X	621+1G>T	R117H	1717-1G>A
A455E	R560T	R1162X	G85E	R334W
R347P	711+1G>T	1898+1G>A	2184delA	1078delT
3849+10kbC>T	2789+5G>A	3659delC	3120+1G>A	I148T
D1152H				

Table I. Proportion of Exon9− CFTR Transcripts in Cultured Nasal Epithelial Cells

Genotype	Haplotype	Sample no.	Proportion exon 9− transcripts	*n*	Proportion exon 9− transcripts/ haplotype
I	D: TG10/T9/M470	1	0.03±0.01	19	
	B: TG10/T7/M470				
II	D: TG10/T9/M470	2	0.11±0.01	13	D: 0.02–A: 0.22
III	B: TG10/T7/M470	4	0.05±0.01	9	
	B: TG10/T7/M470				
IV	B: TG10/T7/M470	5	0.08±0.01	15	B: 0.04–A: 0.14
	A: TG11/T7/V470	6	0.09±0.01	17	B: 0.06–A: 0.12
		7	0.10±0.01	15	B: 0.07–A: 0.13
		8	0.10±0.01	12	B: 0.06–A: 0.15
V	A: TG11/T7/V470	9	0.12±0.01	14	
	A: TG11/T7/V470	10	0.13±0.01	11	
		11	0.14±0.02	9	
		12	0.16±0.01	13	
		13	0.17±0.01	10	
		14	0.12±0.01	13	
		15	0.15±0.01	15	
		16	0.12±0.01	10	
		17	0.16±0.01	12	
		18	0.14±0.01	15	
		19	0.12±0.01	12	
		20	0.13±0.01	13	
		21	0.16±0.01	10	
		22	0.12±0.01	8	
VI	A: TG11/T7/V470	23	0.21±0.01	15	C: 0.29–A: 0.08
	C: TG12/T7/M470	24	0.20±0.01	14	C: 0.30–A: 0.05
		25	0.23±0.02	13	C: 0.32–A: 0.03
VII	B: TG10/T7/M470	26	0.46±0.01	9	B: 0.10–E: 0.72
	E: TG12/T5/V470				

A: TG11/T7/V470

Fewer T's: less normal splicing
More TG's: less normal splicing

Perhaps $(TG)_m$ tract explains penetrance of the 5Tallele, i.e, more TGs, less normal splicing, higher penetrance or worse clinical outcome.

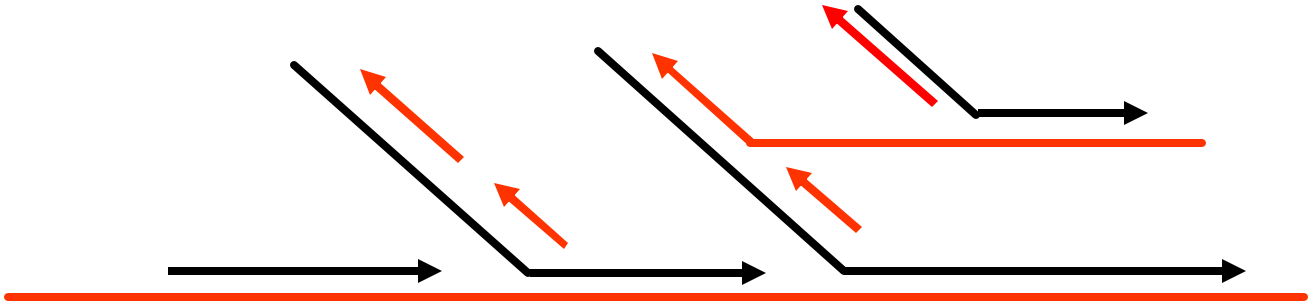
Haplotypes associated with less normal splicing and reduced Cl^- conductance correlate with worse clinical outcome

Table III. T5 Haplotypes

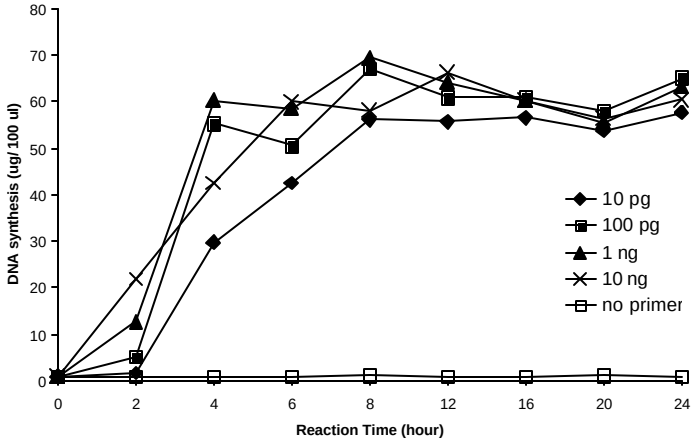
Haplotypes	CBAVD	Parents	Random	CF
	n = 18	*n = 13*	*n = 5*	*n = 1*
F: TG11/T5/M470	1	13	4	0
E: TG12/T5/V470	15	0	1	0
G: TG13/T5/M470	2	0	0	0
H: TG13/T5/V470	0	0	0	1

REPLI-g WGA
Isothermal gDNA Amplification

Multiple
Displacement
Amplification



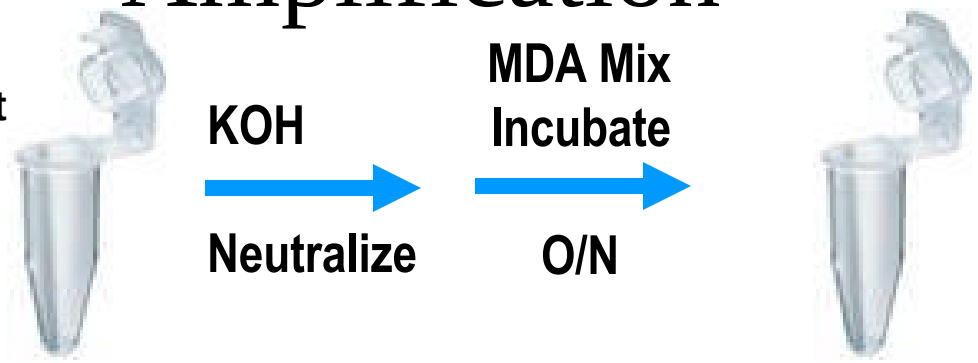
Genomic DNA



- Random Hexamers
- Phi29 Polymerase
  - Strand Displacing
  - Synthesis Rate (55-200 nt/s)
  - Highly processive
  - High fidelity
- Isothermal Reaction (30°C)

Single-Tube Whole Genome Amplification

- 10+ ng gDNA
- 0.5 mL Blood, Buffy Coat
- single buccal swab
- 3 mm blood spot
- 2 mm tissue section



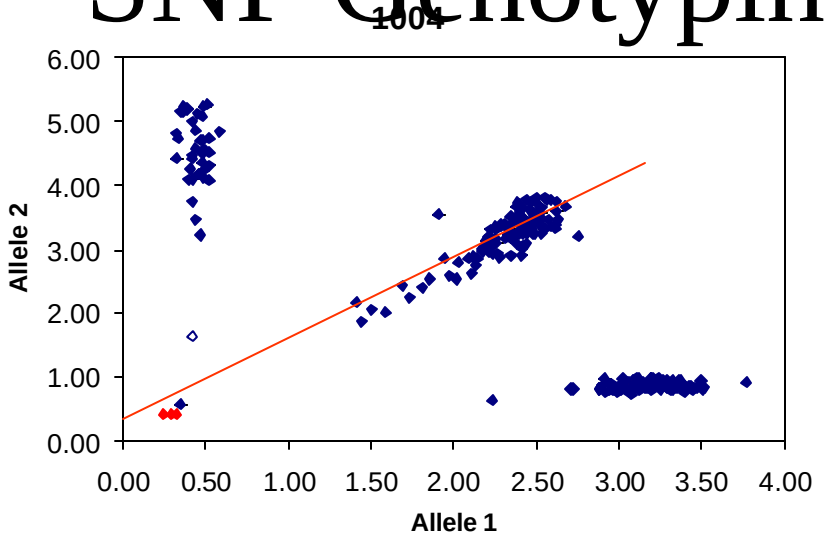
100+mg DNA ready for use

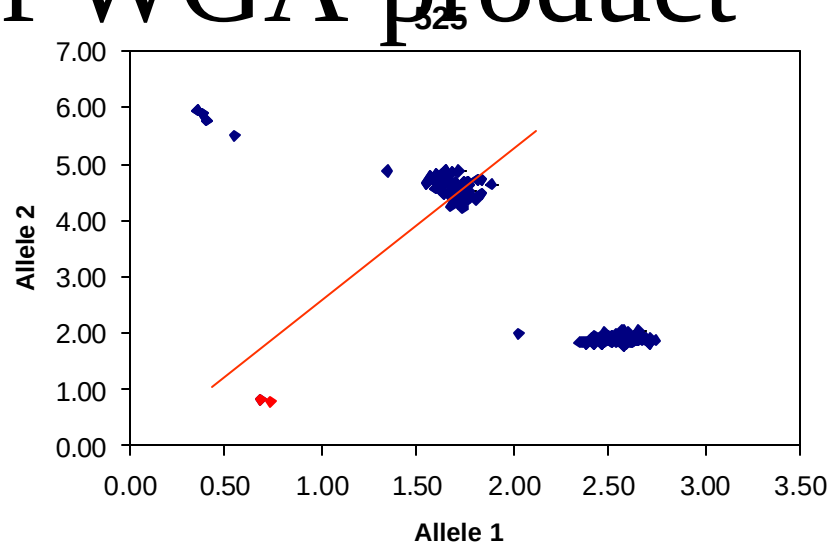
- **Scalable up to 10 mL reaction (8-10 mg of DNA!)**

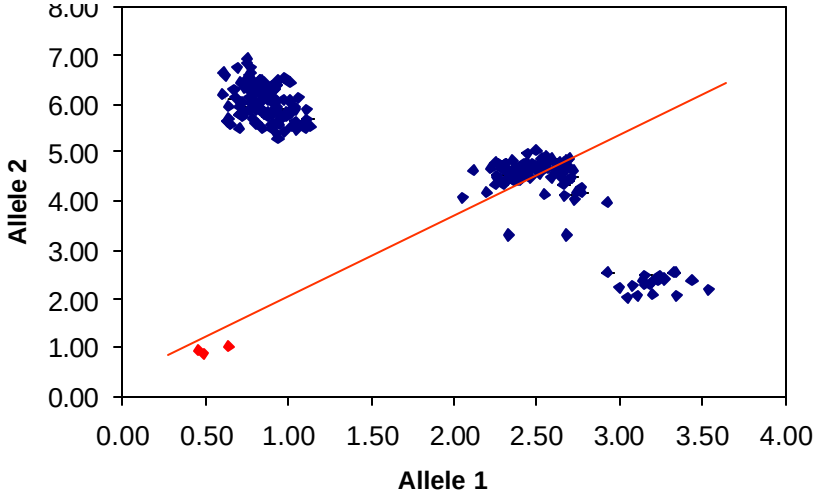
Dean et al. *Proc. Natl. Acad. Sci.,USA* 2002 (99) 5261

Hosono et al. *Genome Research*, 2003 (13) 954

SNP Genotyping of WGA product

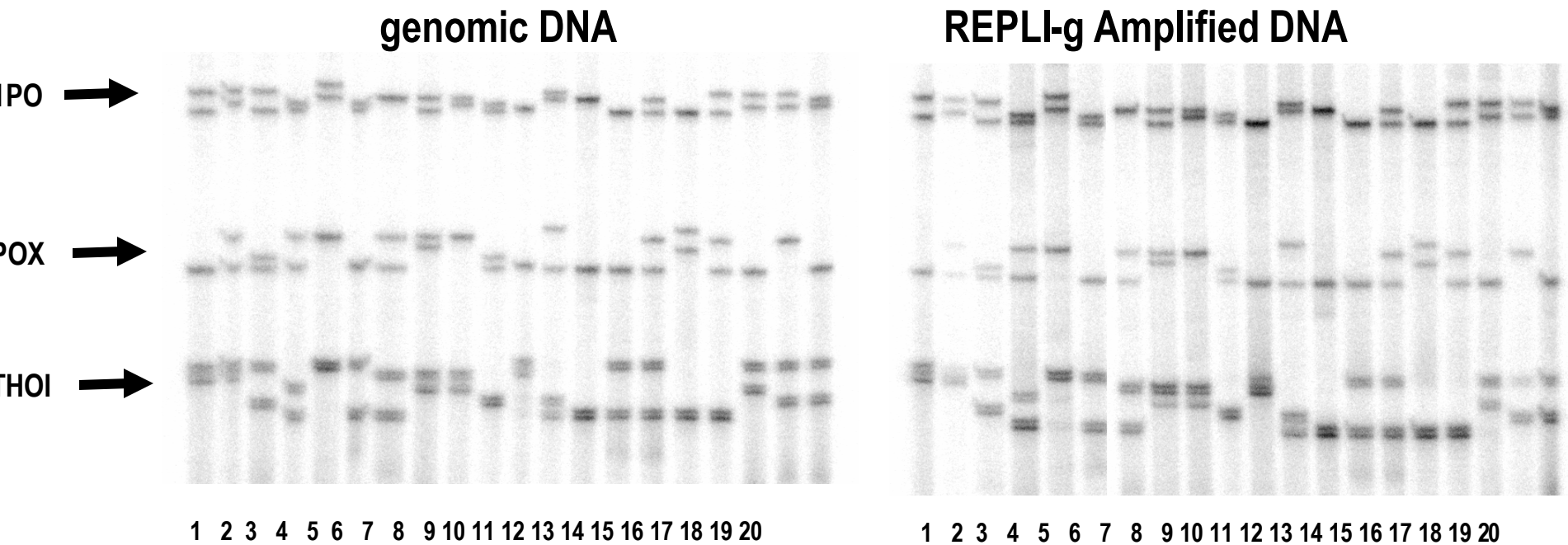






- **252 different genomic DNA**
- **Genotyped with 3 SNP TaqMan Assay**

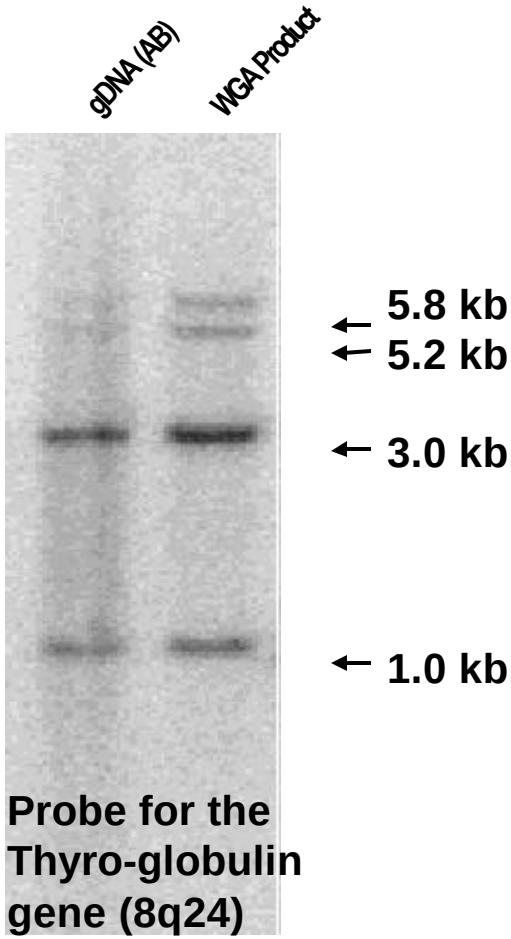
STR Genotyping of WGA product

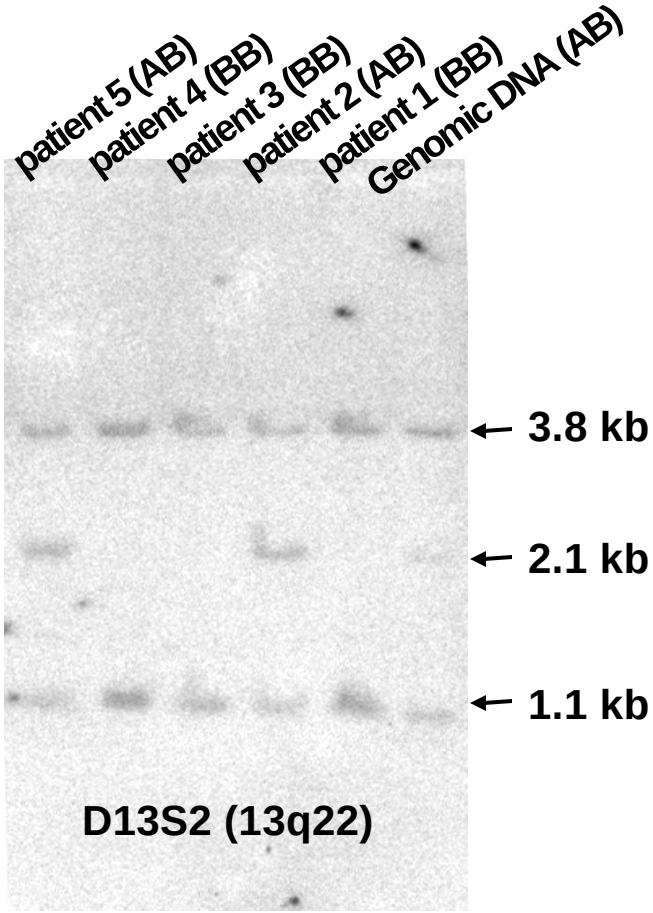


- 3 STR loci Multiplex PCR
  GenePrint â STR from PROMEGA

- CSF1PO (chr 5q33.3-34) $(agat)^2$
- TPOX (chr 2p25.1-pter) $(aatg)^2$
- THO1 (chr 11p15.5) $(aatg)^2$

Quality and Length of WGA Product RFLP Southern Analysis





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