PCR in Diagnostic Pathology

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Genetics Resource Centre (GRC)

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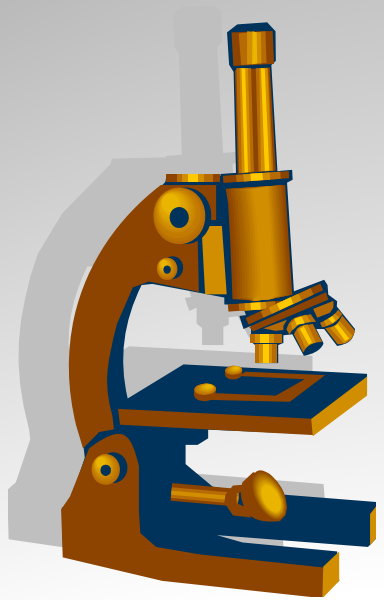
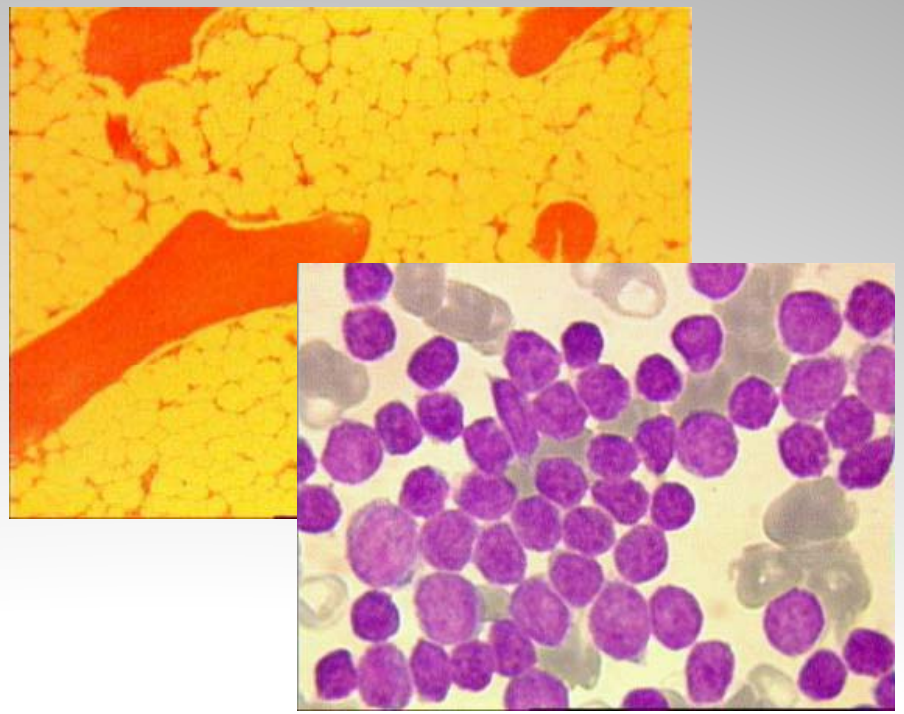
www.grcpk.com

Pathology: A descriptive science





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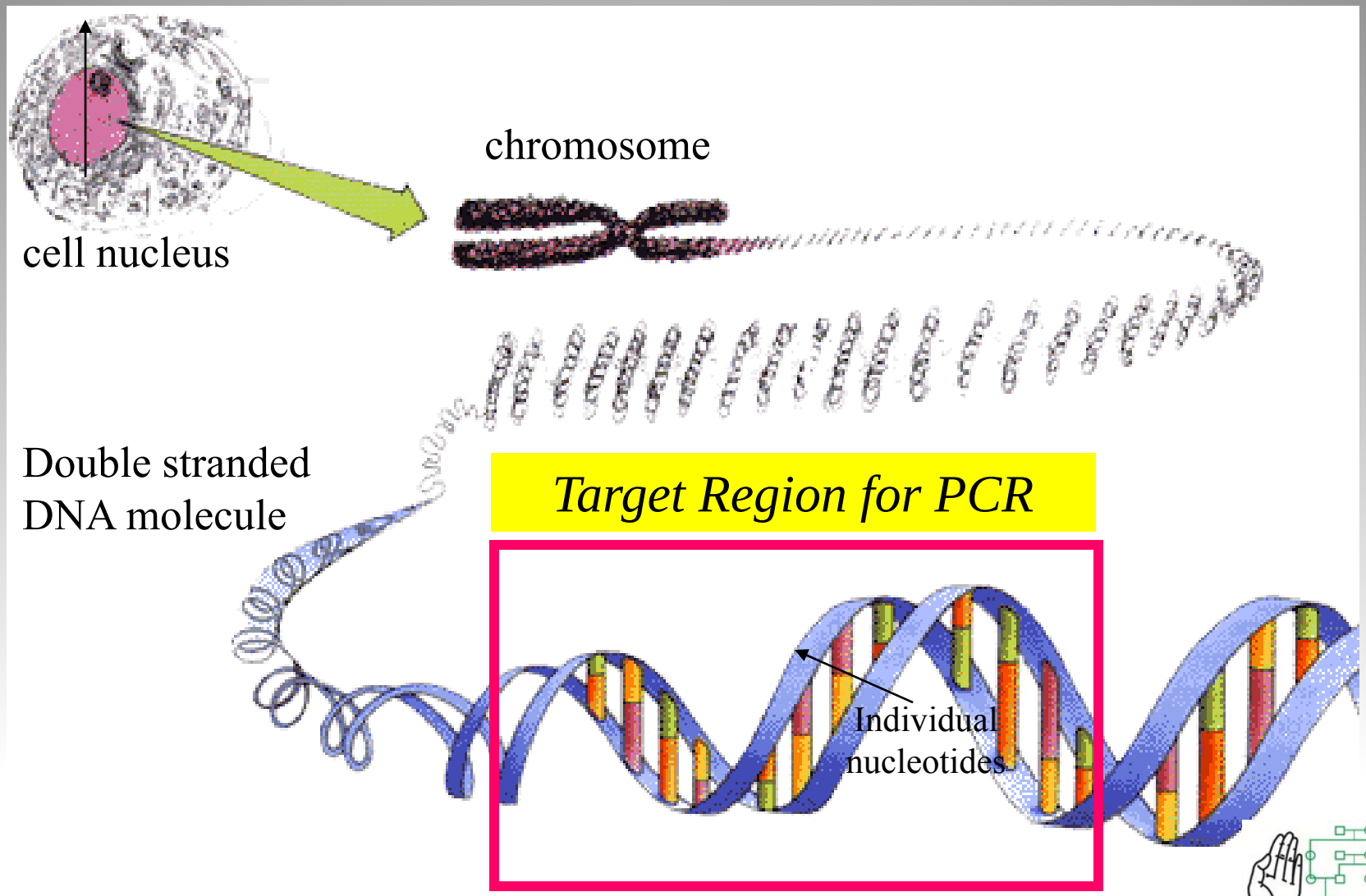
chromosome
cell nucleus
Double stranded
DNA molecule
Target Region for PCR
Individual
nucleotides

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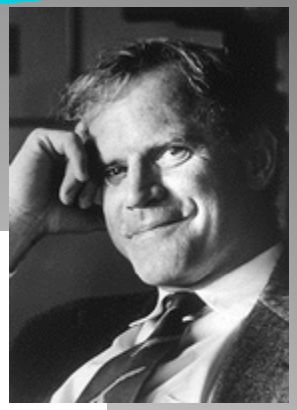
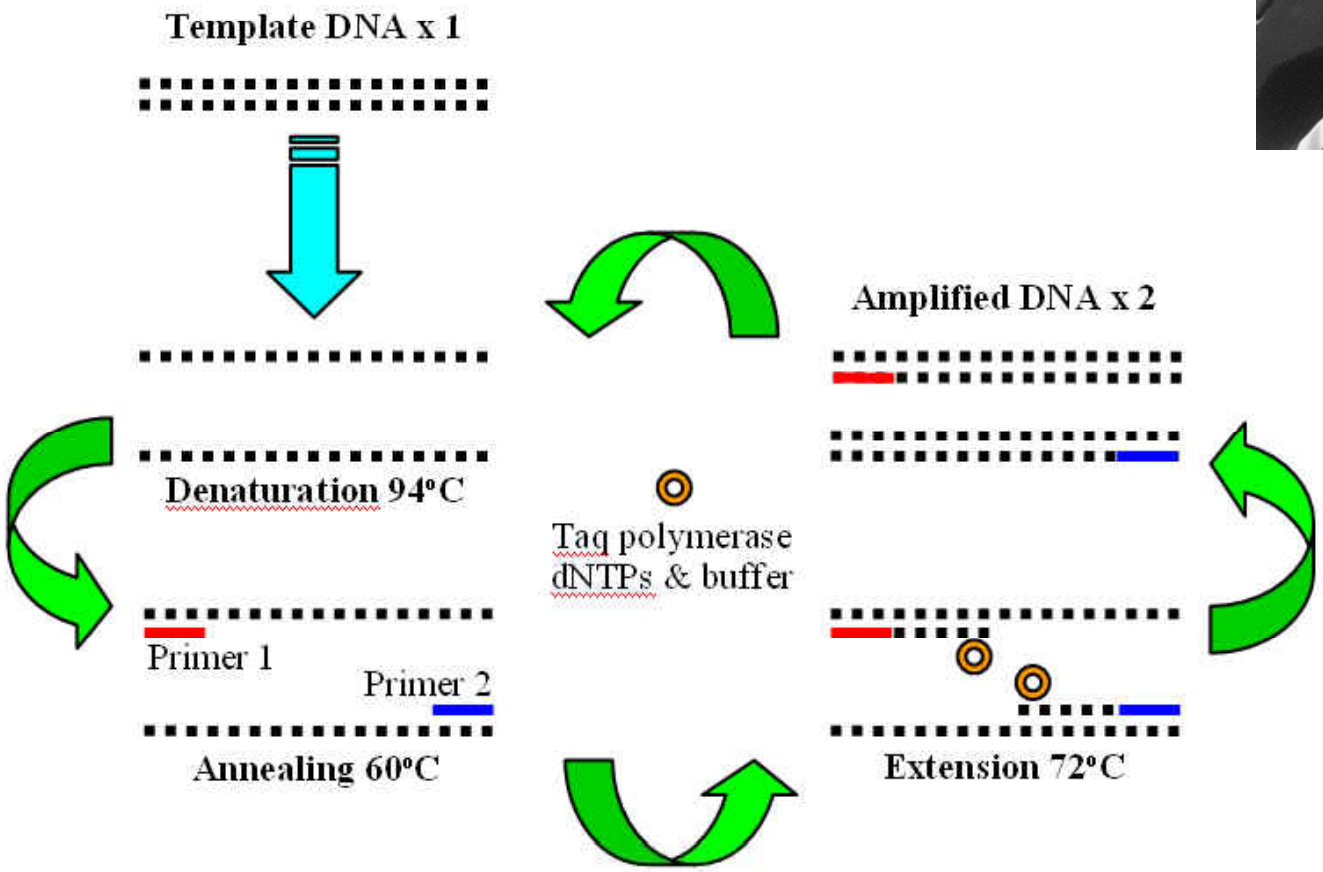
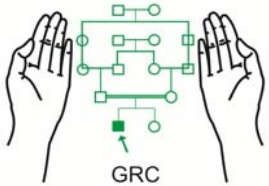



Template DNA x 1
Denaturation 94°C
Primer 1
Primer 2
Annealing 60°C
Tag polymerase
dNTPs & buffer
Amplified DNA x 2
Extension 72°C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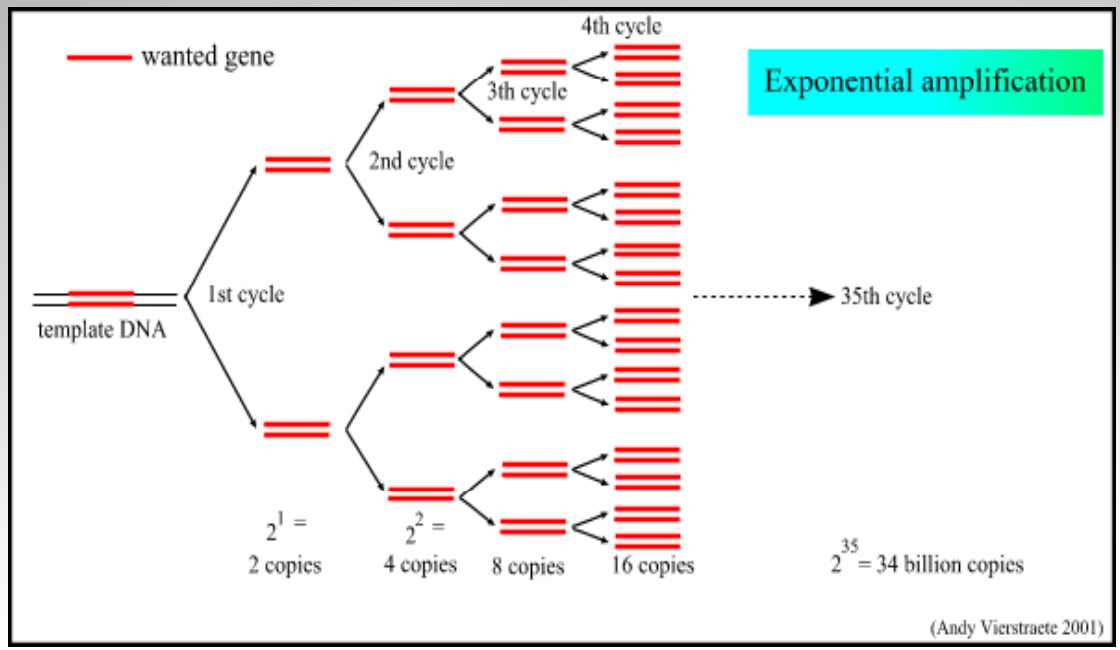
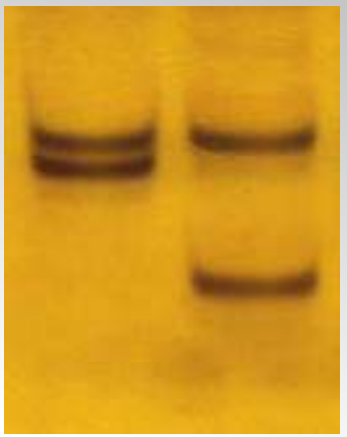
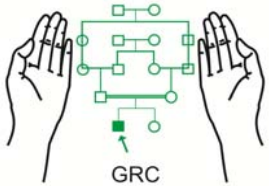

wanted gene
Exponential amplification
4th cycle
3th cycle
2nd cycle
1st cycle
template DNA
35th cycle
2^1 =
2 copies
2^2 =
4 copies
8 copies
16 copies
2^{35} = 34 billion copies
(Andy Vierstraete 2001)

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Real Time PCR

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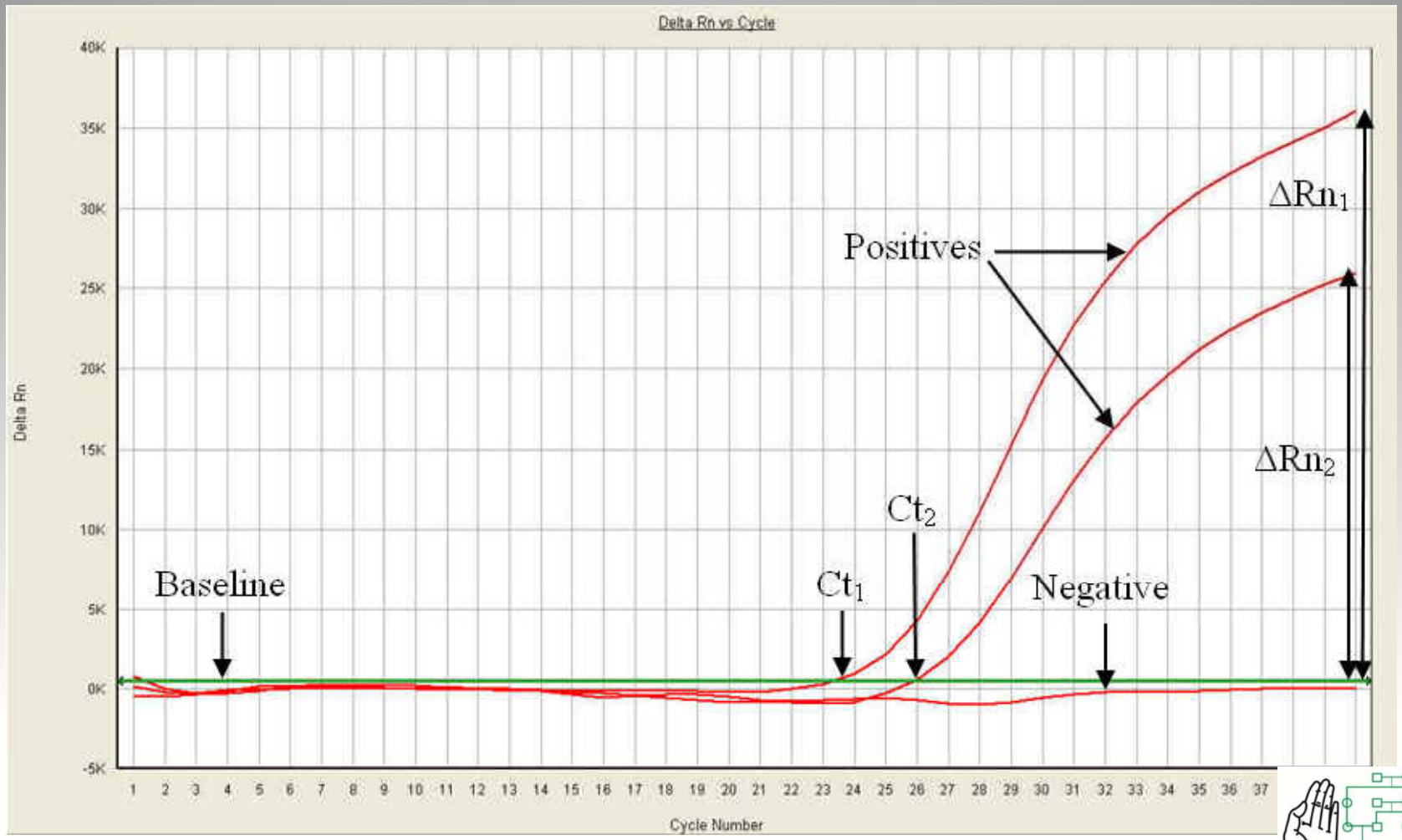
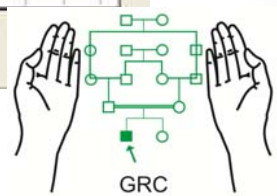
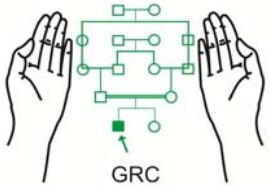
Real Time PCR





PCR Applications

- Inherited Disorders
- Cancer
- Infectious Diseases
- Human Identification
- Others



Of the 5000 known Genetic Disorders
2500 can be diagnosed by Genetic Analysis

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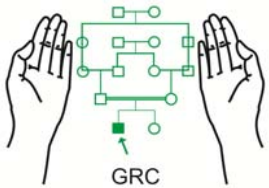
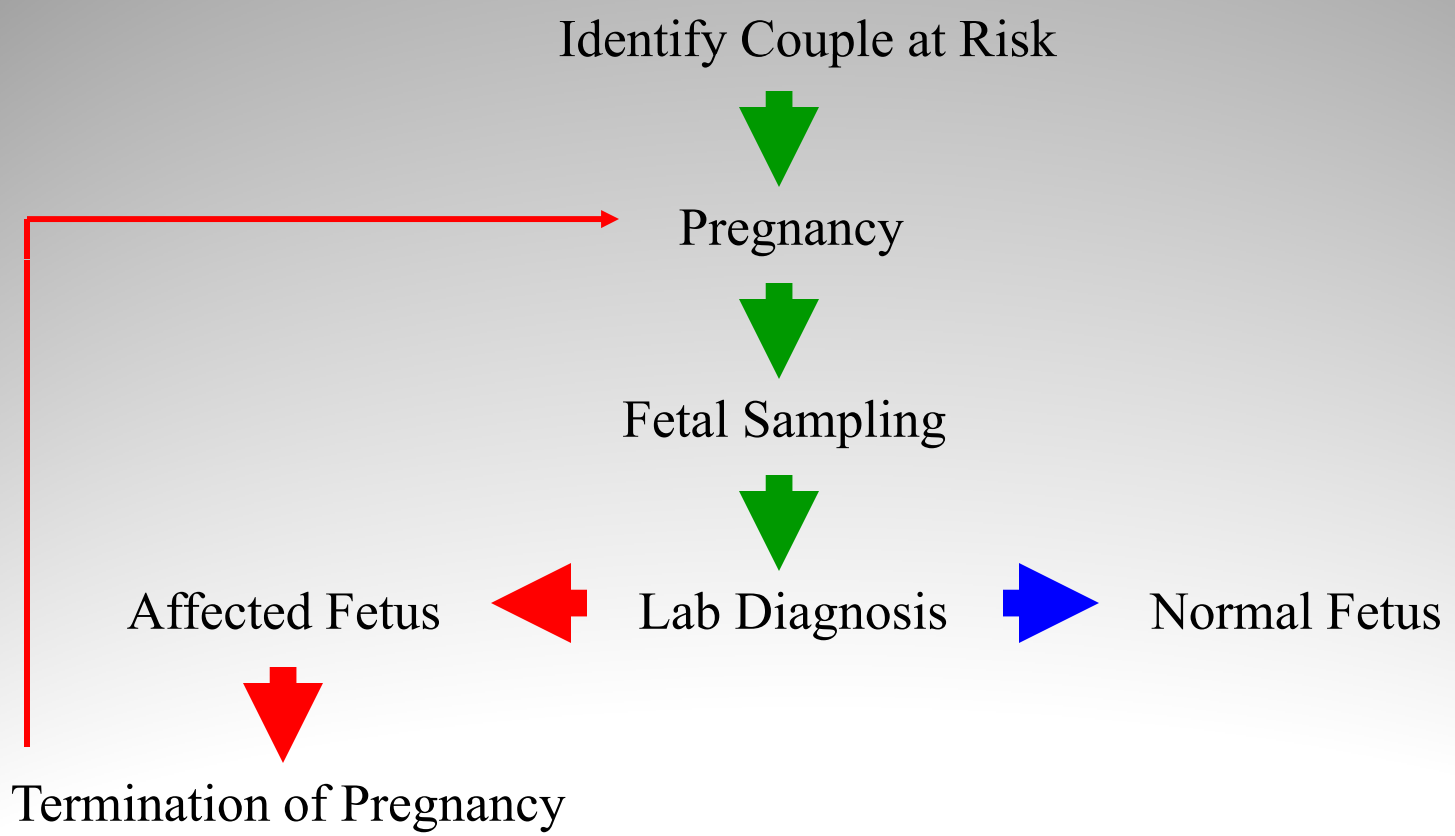
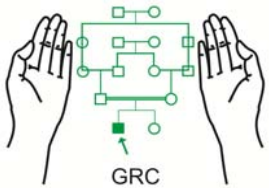
PCR in Thalassaemia

- Prenatal Diagnosis
- Diagnosis in previously transfused patients
- Silent thalassaemia alleles
- Distinction between structural variants
- Thalassaemia intermedia
- α-thalassaemia
- β-Thalassaemia carriers in certain situations
- Rare thalassaemias



Prenatal Diagnosis





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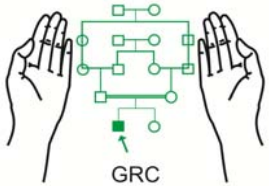
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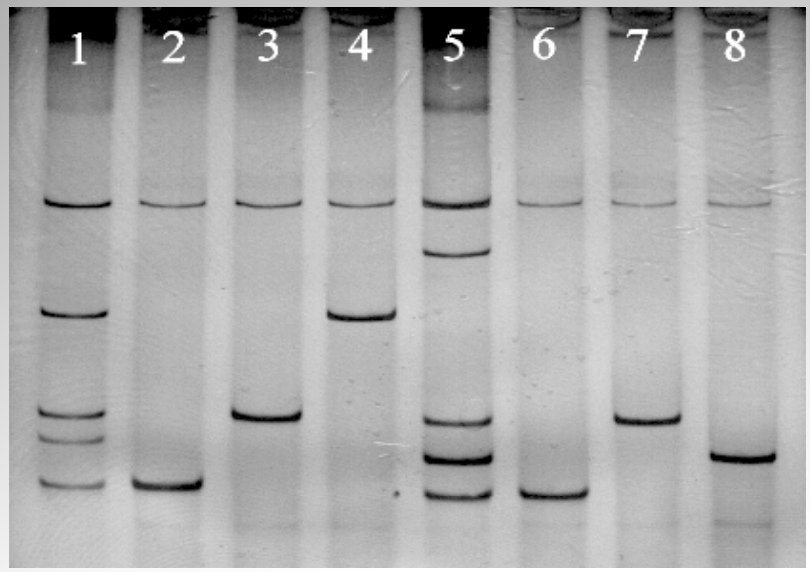
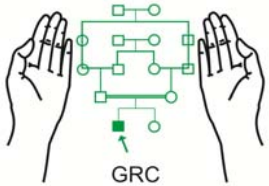
ARMS PCR for Thalassaemia

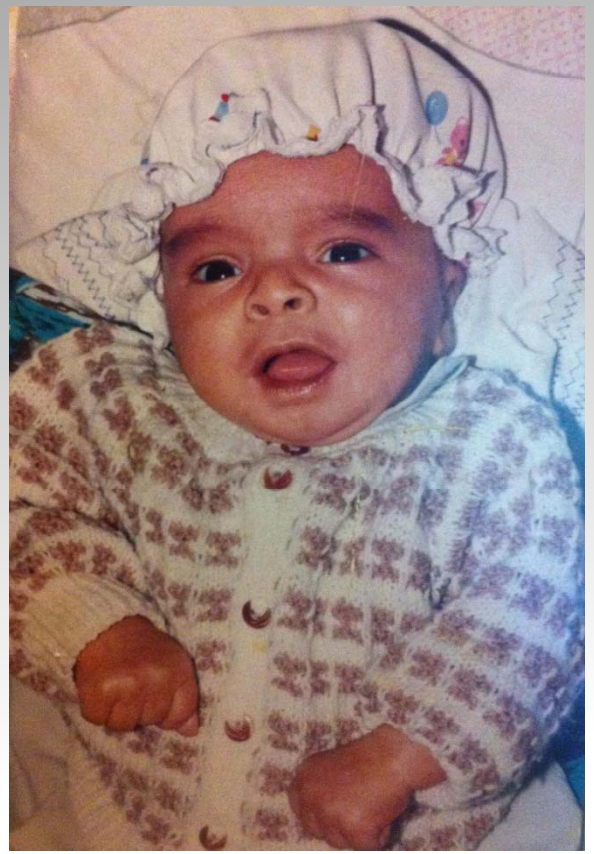


Primer ID:	Mutations Pooled:	Amplified Product size:
AD-1	Fr 8-9 (+G) IVSI-5 (G-C) Fr 41-42 (-TTCT) IVSI-1 (G-T) Del 619bp	215 bp 285 bp 439 bp 280 bp 242 bp
AD-2	Cd 5 (-CT) Fr 16 (-C) IVSI-1 (G-T) Cd 30 (G-C) Cd 30 (G-A) IVSII-1 (G-A)	205 bp 238 bp 280 bp 280 bp 280 bp 634 bp
AD-3	Cd 15 (G-A) Cap+1 (A-C)	500 bp 567 bp

(Ahmed et al, Prenatal Diagnosis 2000)



PND in Pakistan (1st May 1994)



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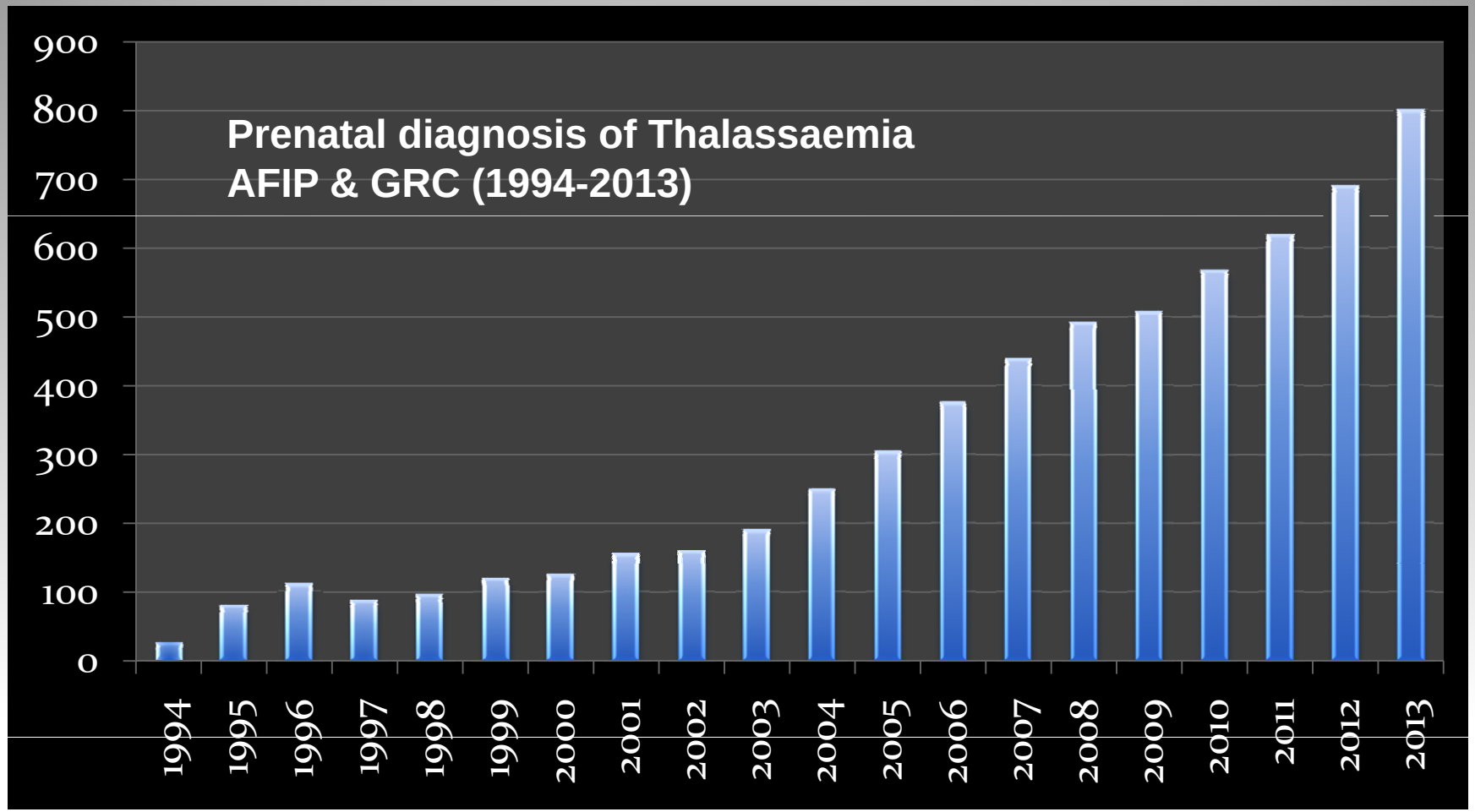
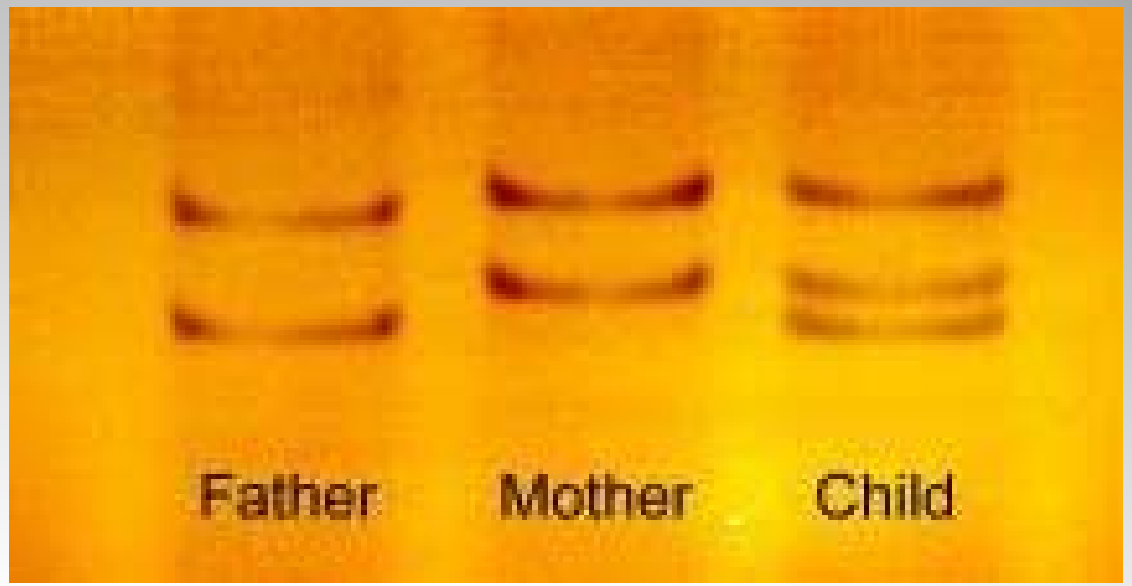
PND for Thalassaemia in Pakistan (AFIP & GRC)





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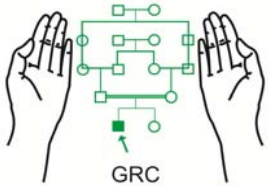
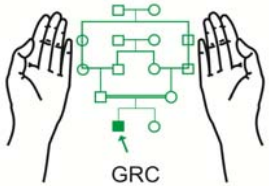
Prenatal Diagnosis at GRC

- Thalassaemias
- Trisomies 13, 18, 21
- Cystic fibrosis (Δ508 mutation)
- DMD
- Fetal Rh-D Typing

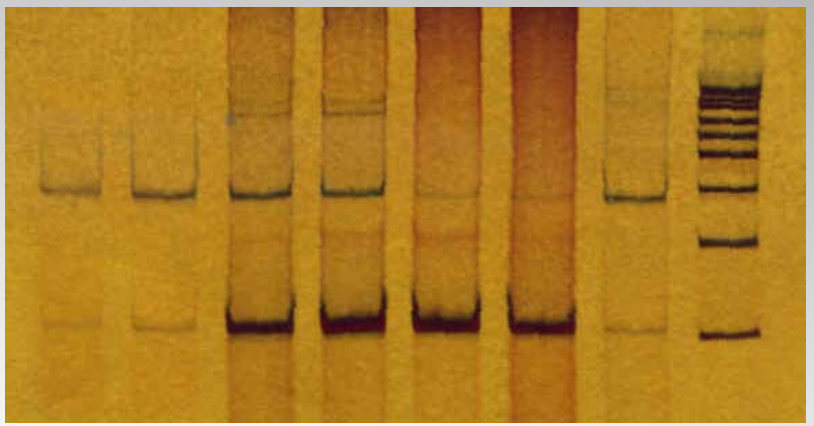


PCR Applications in Cancer



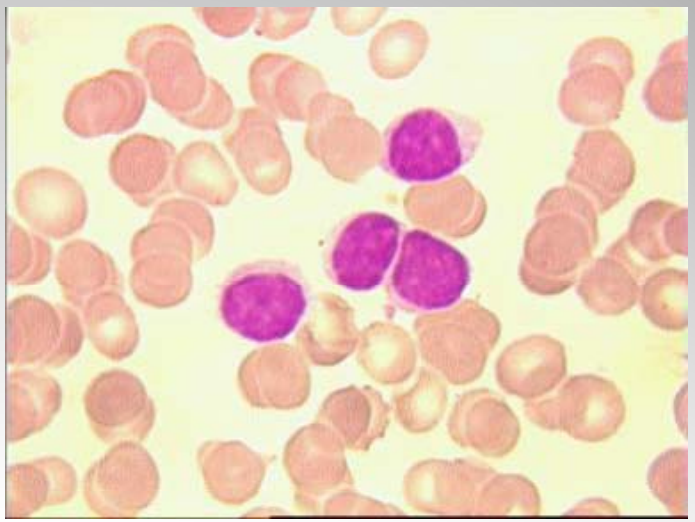
55 years old man

- TLC: 11.0×10^9/L
- Hb: 18.5 g/dl
- PCV 0.54 L/L
- TRBC: 6.1×10^{12}/L
- MCV: 76.1 fl
- MCH: 29.2 pg
- Platelets: 370×10^9/L
- Retics: 1.5%
- ESR: 4 mm in 1st hour



JAK-2 mutation

- 60 yrs old man incidently found to have:
- Hb: 13.5 g/dl
- TLC: 15 X 10^9/L
- Lymphocytes: 9 X 10^9/L
- Platelets: 235 X 10^9/L



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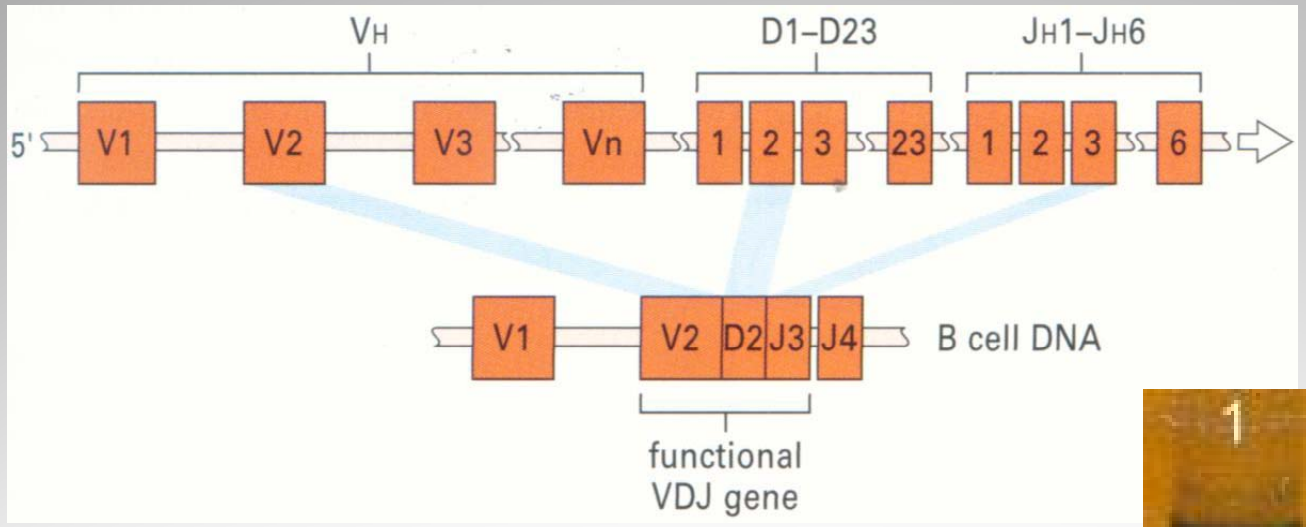
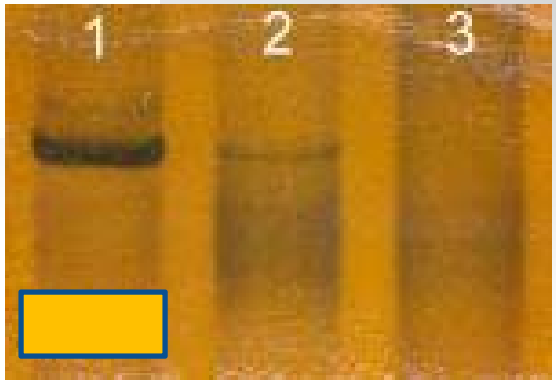
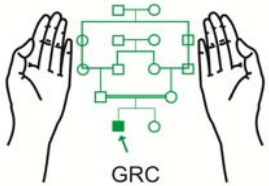
Clonal Ig Gene Rearrangement

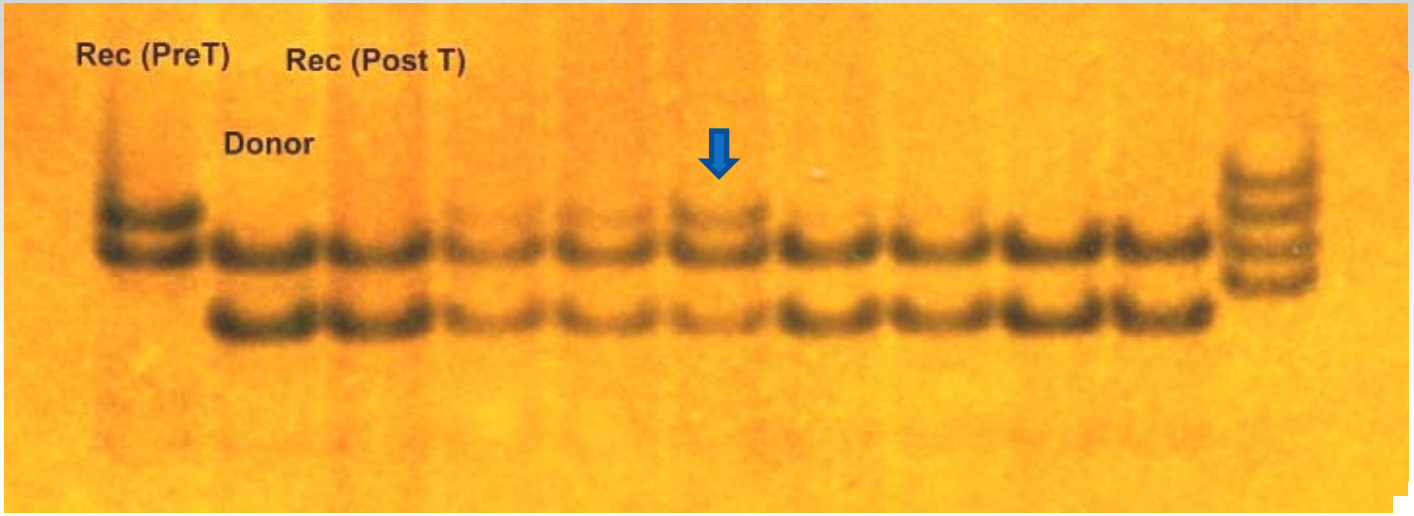


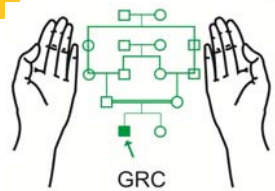




Donor Chimerism

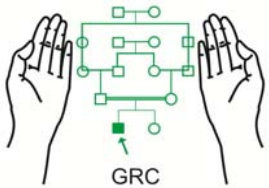
33 yrs old male with relapsed NHL
Allo-BMT done after reduced intensity conditioning
Atypical cells in peripheral blood



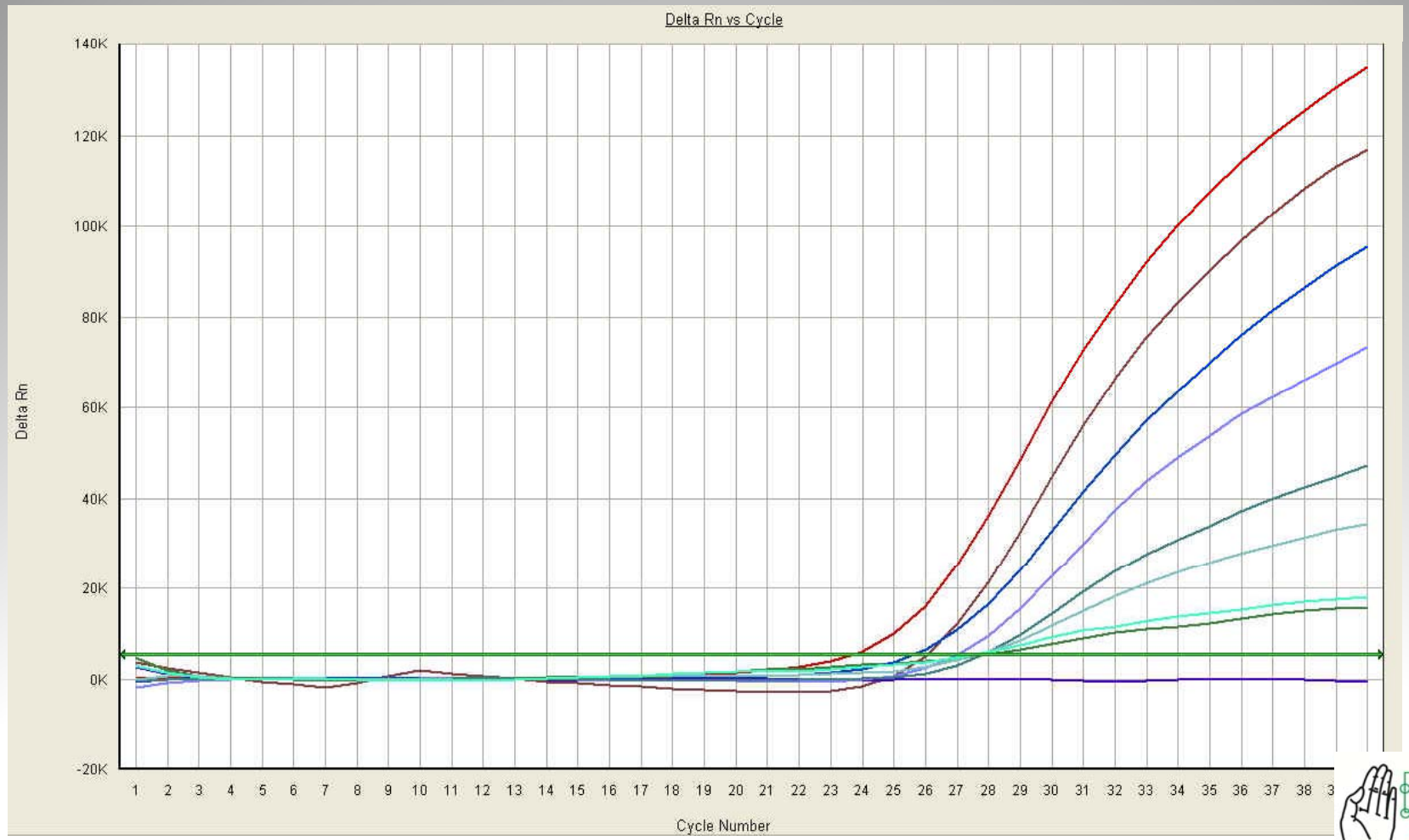


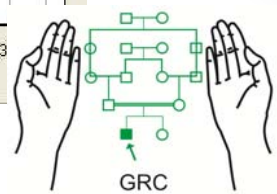
Molecular Markers in Haematological Malignancies

- CML bcr-abl
- ALL bcr-abl, Ig-gene, TCR-gene
- AML M2 t(8;21)
- AML M3 t(15;17)
- AML M4Eo inv(16)
- NHL bcl-II, Ig-gene, TCR-gene
- MPD JAK2 mutation

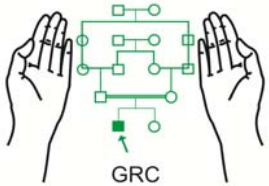


Minimal Residual Disease (MRD)





PCR Applications in Infectious Diseases



Viral

HCV, HBV, HIV, CMV
HPV, Dengue etc.

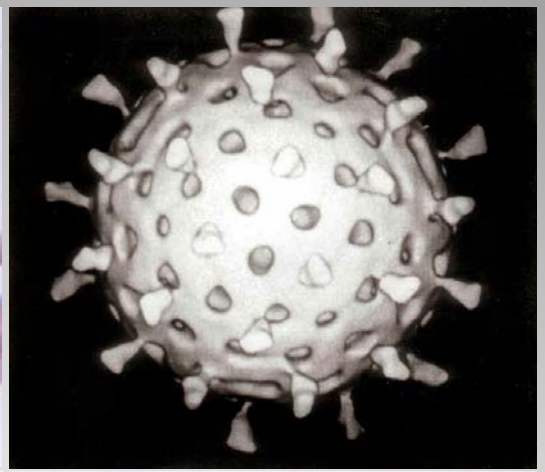
Bacterial

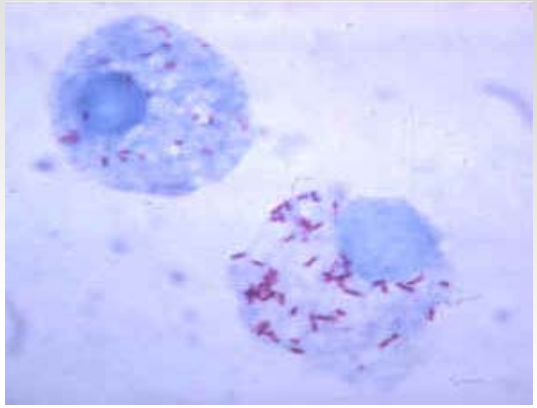
Tuberculosis
Antibiotic resistance

Parasitic

Malaria, Leishmania

Others





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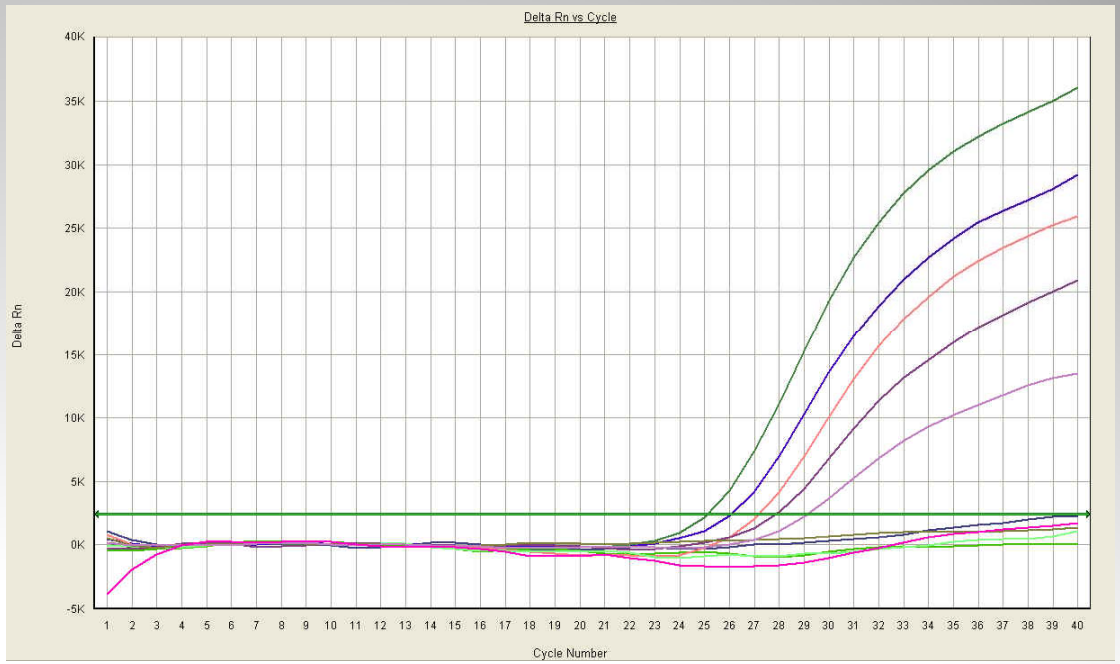
Viral Hepatitis

- Qualitative PCR
- Quantitative PCR
- Genotyping



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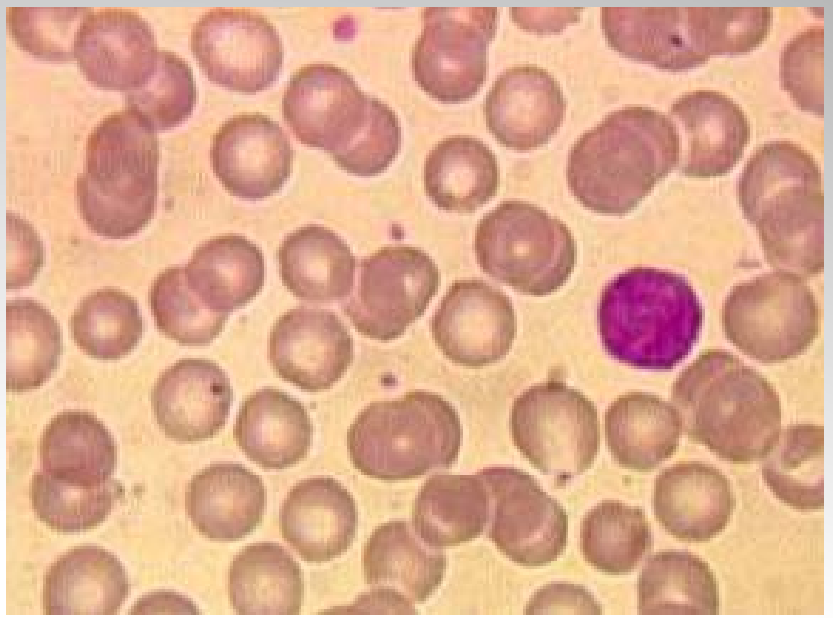
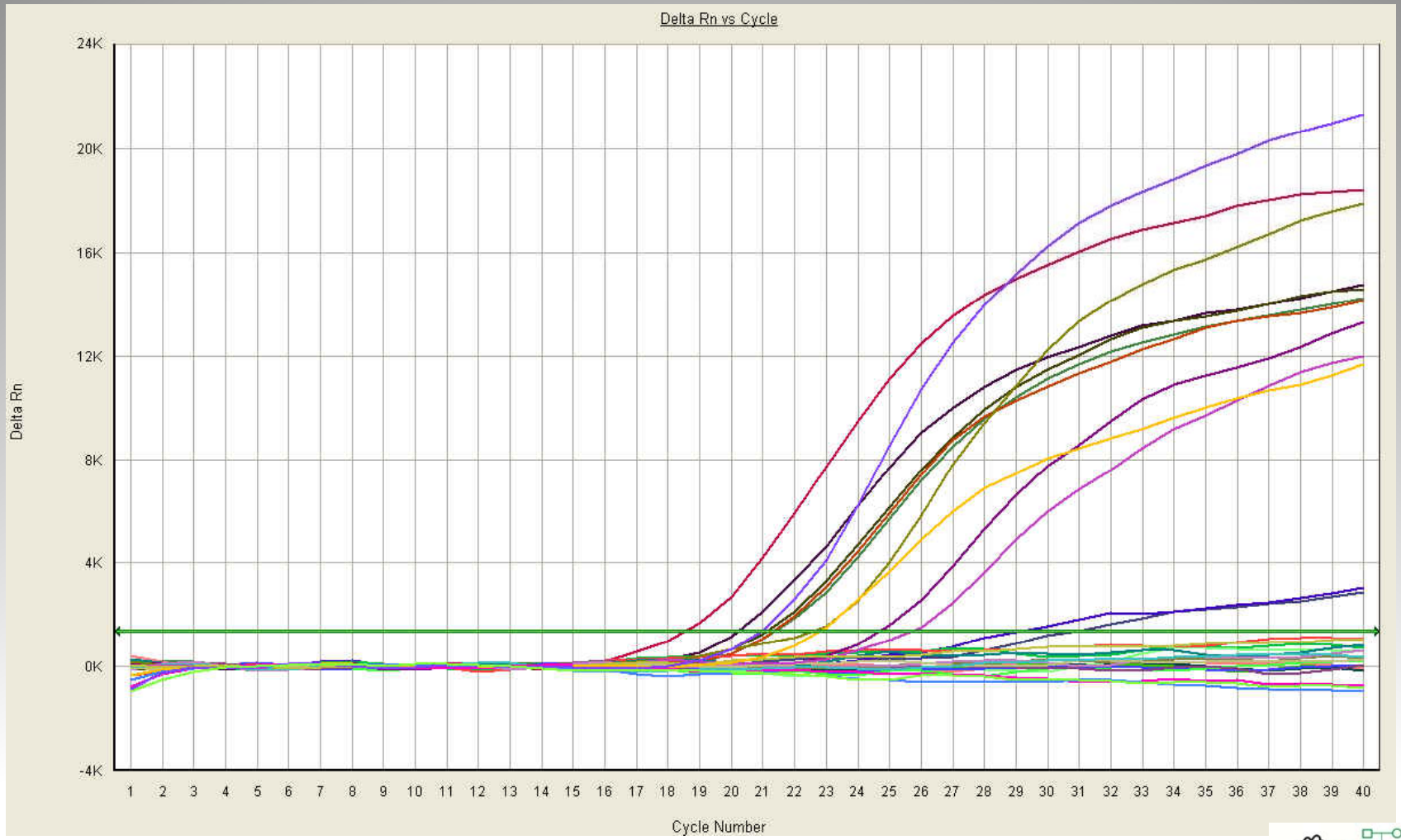
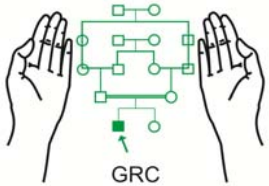
A young man with three days history of high grade fever with chills, headache and vomiting

- WBC: 3.2×10^9/L
- RBCs: 5.12×10^{12}/L
- Hb: 18.2 g/dl
- MCV: 83.5 fl
- MCV: 28.2 pg
- Platelets: 63




Delta Rn vs Cycle
Delta Rn
24K
20K
16K
12K
8K
4K
0K
-4K
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
Cycle Number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Microscopy PCR & ICT in Malaria

Patients (%)	Microscopy	ICT Malaria (Optimal)	PCR
300 (100%) BT: 83% MT: 13% Mixed: 4%	106 (+4) (35.3%)	100 (33.3%)	121 (40.3%)

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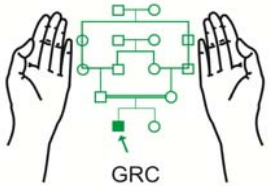
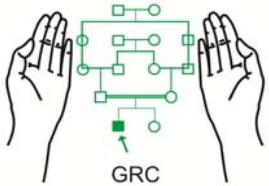
PCR in Infectious Diseases

- Diagnosis
- Monitoring the progress of disease
- Screening of blood donors
- Epidemiology

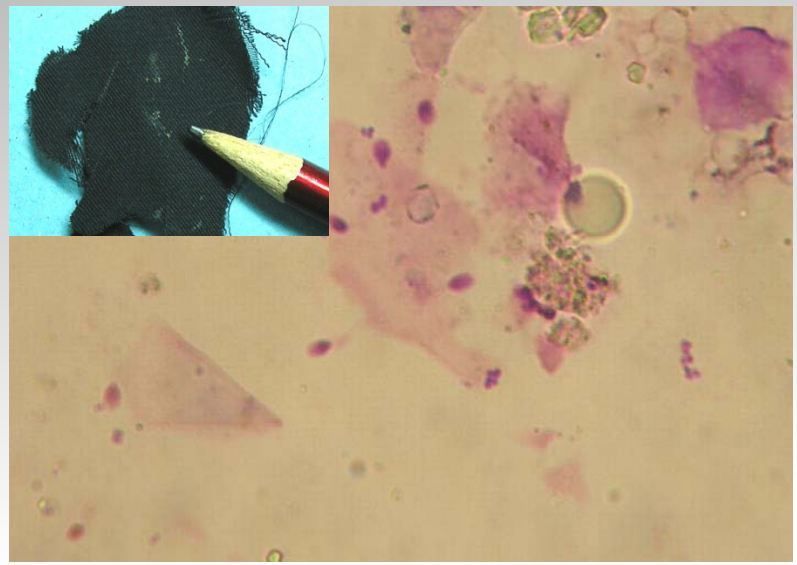


Limitations

- DNA Polymorphism in the infectious agent
- Cross homology
- Is the organism dead or alive?
- False positive & negative results



Forensic DNA Testing



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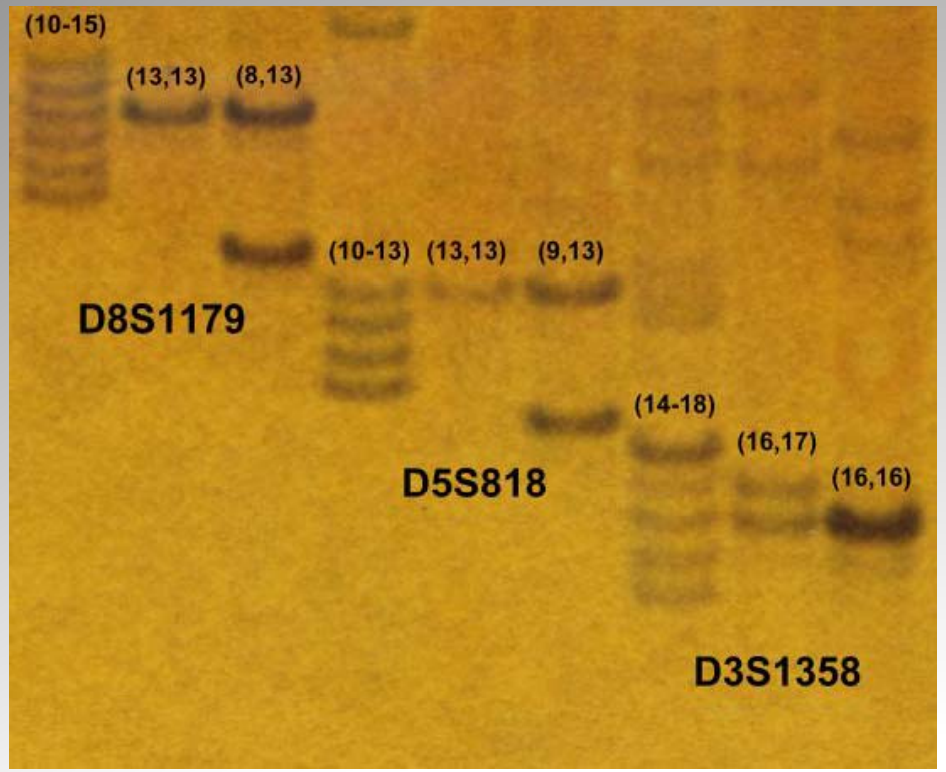
(10-15)
(13,13)
(8,13)
(10-13)
(13,13)
(9,13)
D8S1179
(14-18)
(16,17)
D5S818
(16,16)
D3S1358

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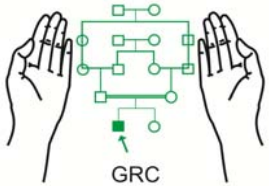
Setting up PCR Lab



DNA Thermal Cycler

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PCR Reader

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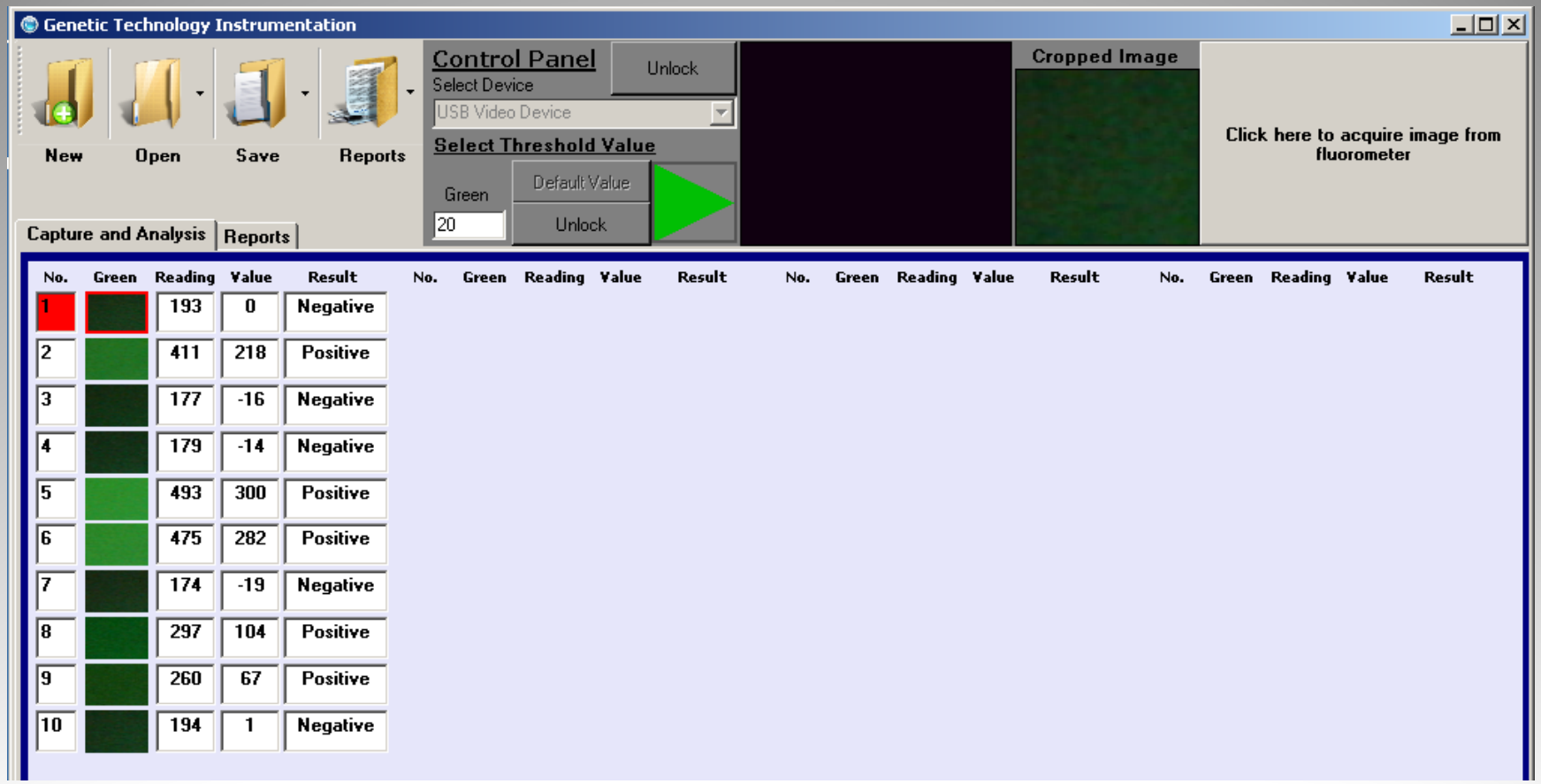
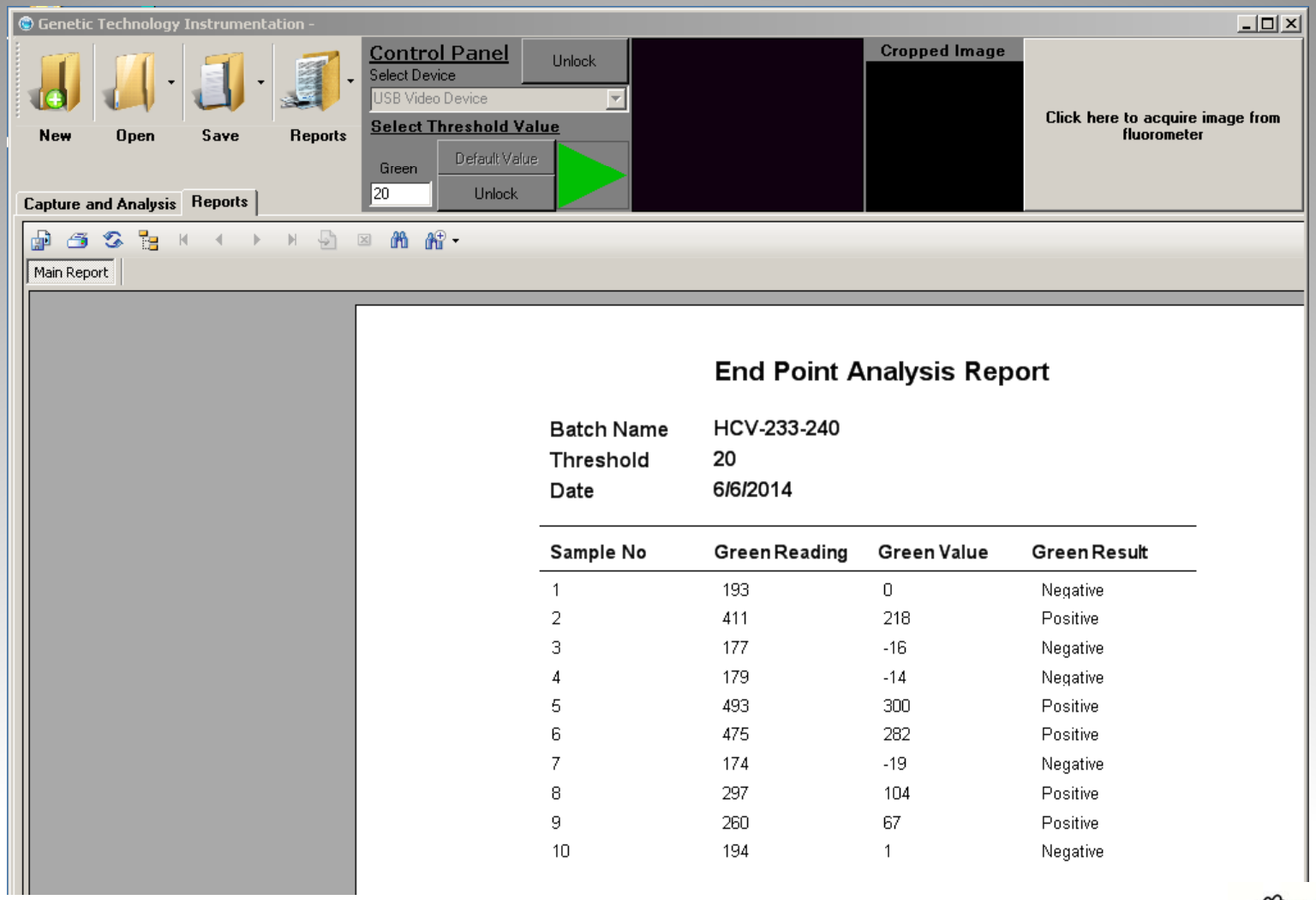

Genetic Technology Instrumentation
New
Open
Save
Reports
Control Panel
Unlock
Select Device
USB Video Device
Select Threshold Value
Green
Default Value
20
Unlock
Cropped Image
Click here to acquire image from fluorometer
Capture and Analysis
Reports
No. Green Reading Value Result
1 193 0 Negative
2 411 218 Positive
3 177 -16 Negative
4 179 -14 Negative
5 493 300 Positive
6 475 282 Positive
7 174 -19 Negative
8 297 104 Positive
9 260 67 Positive
10 194 1 Negative

GRC



End Point Analysis Report

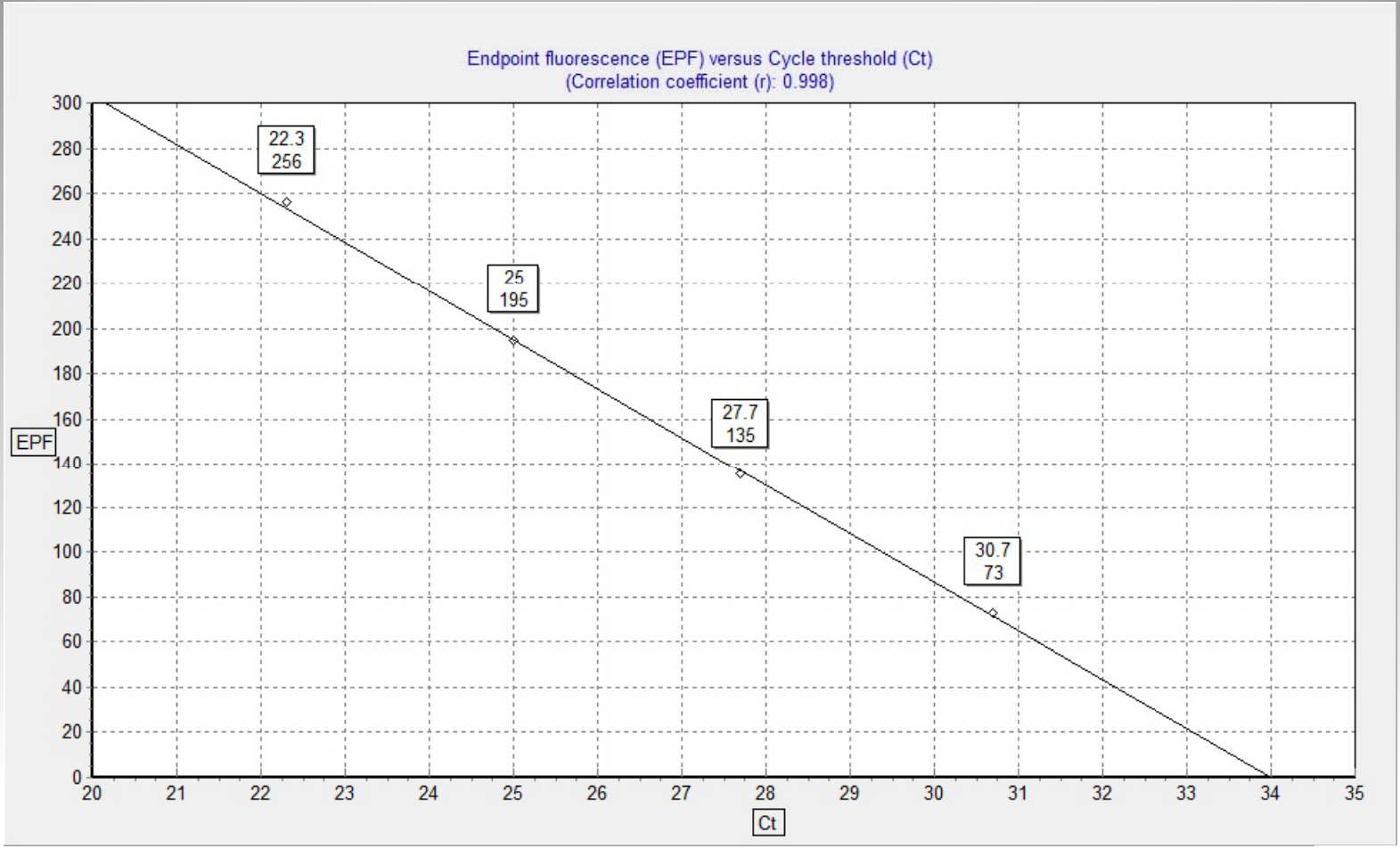
Batch Name	HCV-233-240
Threshold	20
Date	6/6/2014

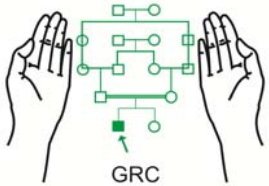
Sample No	Green Reading	Green Value	Green Result
1	193	0	Negative
2	411	218	Positive
3	177	-16	Negative
4	179	-14	Negative
5	493	300	Positive
6	475	282	Positive
7	174	-19	Negative
8	297	104	Positive
9	260	67	Positive
10	194	1	Negative

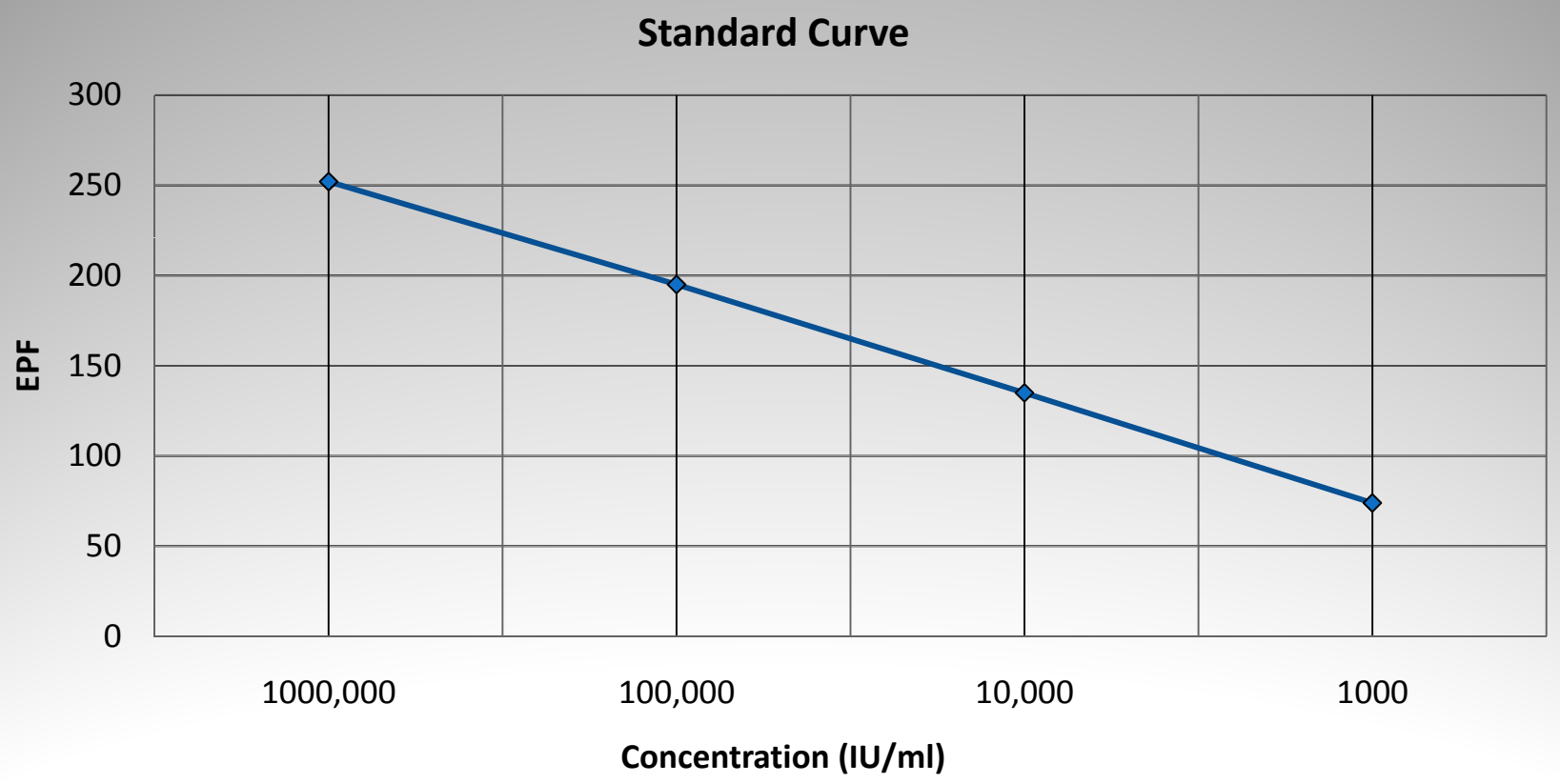
GRC

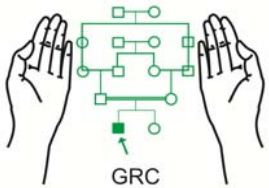
Dil	1/1		1/10		1/100		1/1000		Neg	
Conc IU/ml	1000,000 IU/ml		100,000 IU/ml		10,000 IU/ml		1000 IU/ml		0 IU/ml	
Repeat	Ct	Fluor	Ct	Fluor	Ct	Fluor	Ct	Fluor	Ct	Fluor
1	22.3	247	25.1	200	28.1	138	31.4	63	0	4
2	22.1	248	25.3	182	28.1	134	30.9	68	0	8
3	22.1	252	25.2	190	28.1	138	30.7	74	0	1
4	22.7	252	24.8	205	27.8	128	31	79	0	1
5	22.1	261	25	196	27.4	140	31	76	0	5
6	22.3	248	24.8	197	27.5	134	30.1	74	0	1
7	22.1	247	24.8	196	27.3	138	30.1	80	0	2
8	22.2	254	24.9	186	27.6	132	29.9	79	0	4
9	22.5	258	24.7	198	27	133	31.7	61	0	2
10	22.2	249	25	203	27.6	137	30.6	84	0	1
Mean	22.25	251.6	24.96	195.3	27.65	135.2	30.74	73.8	0	2.9
SD	0.21	4.84	0.20	7.29	0.37	3.65	0.58	7.57	0.00	2.33
CV	0.95	1.92	0.78	3.73	1.36	2.70	1.90	10.26		80.38

GRC

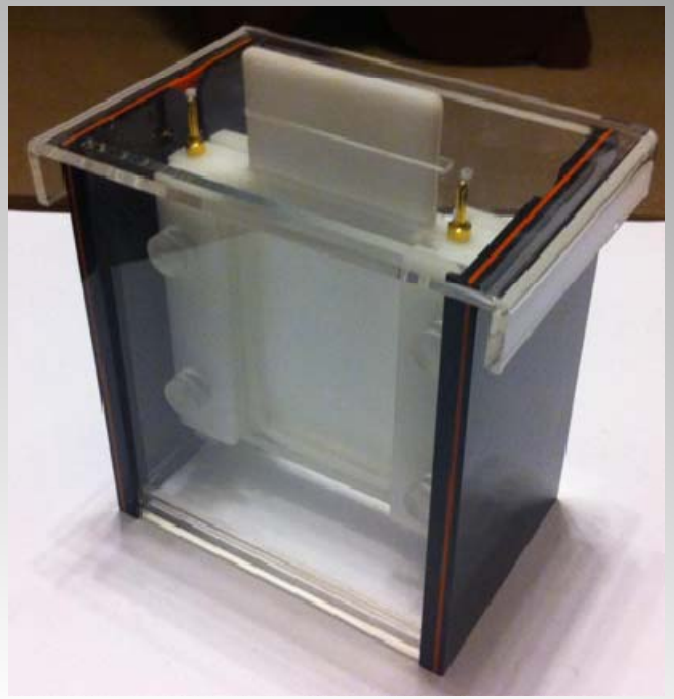

Endpoint fluorescence (EPF) versus Cycle threshold (Ct)
(Correlation coefficient (r): 0.998)
EPF
Ct
22.3
256
25
195
27.7
135
30.7
73
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

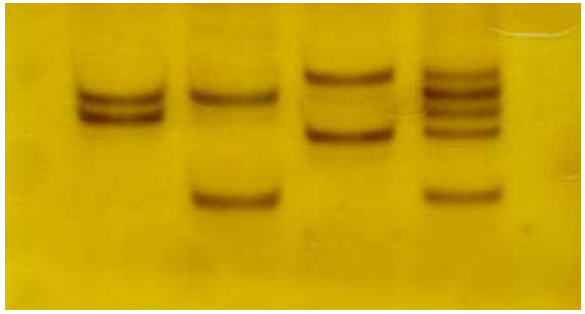

GRC

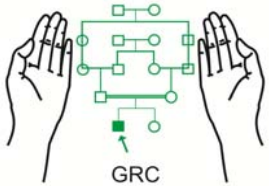

Standard Curve
300
250
200
150
100
50
0
EPF
1000,000
100,000
10,000
1000
Concentration (IU/ml)


GRC

DC Volts
Voltage
Minutes
Start
Power
Stop

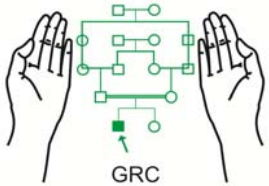





GRC

GRC

PERFORMANCE GUARANTEED
PCR


GRC