Molecular Pathology: The Pakistani Perspective

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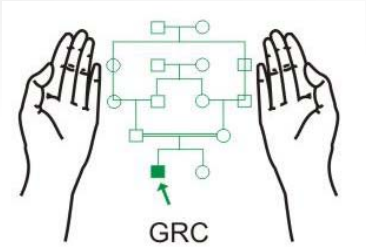
Genetics Resource Centre (GRC)
Rawalpindi



www.grcpk.com

Sickle Cell Anemia: a Molecular Disease.
Pauling & Italiano. Science November 1949

Watson & Crick: 1953

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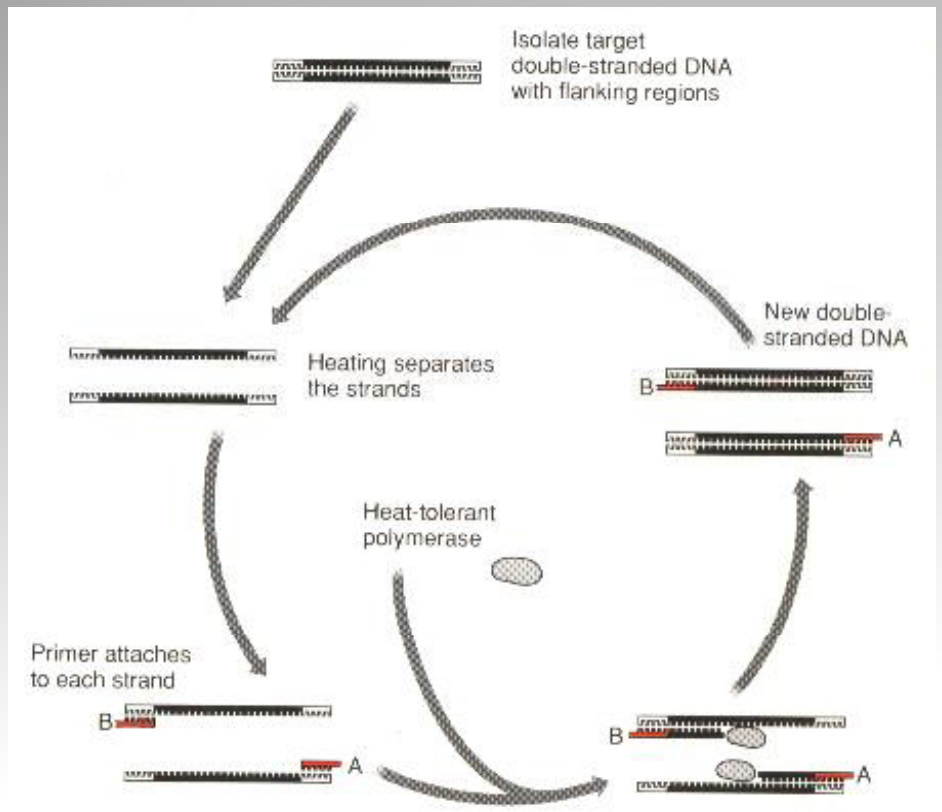
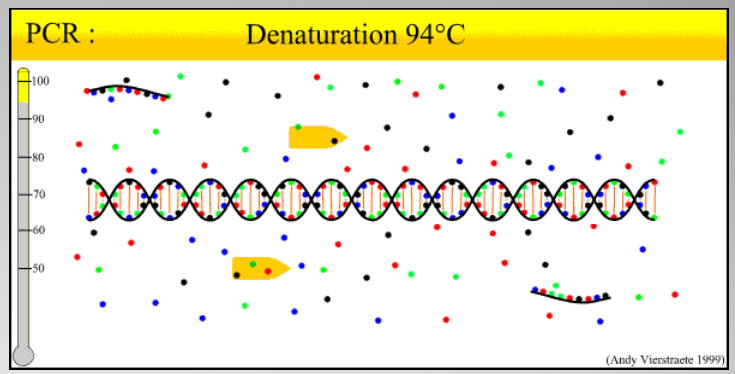
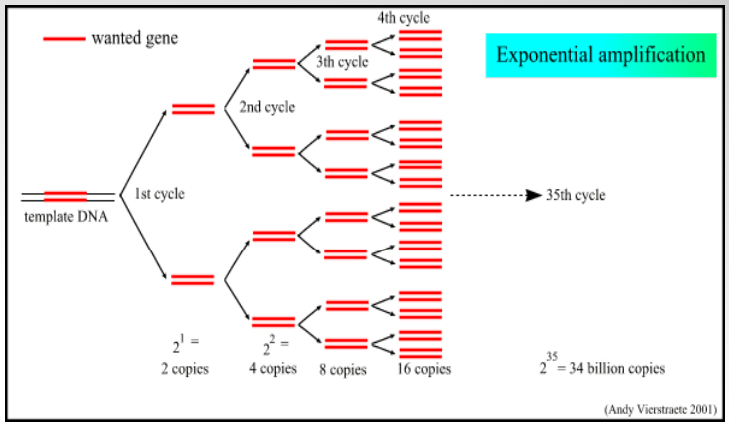
Polymerase Chain Reaction (PCR)







(Kary Mullis 1985)

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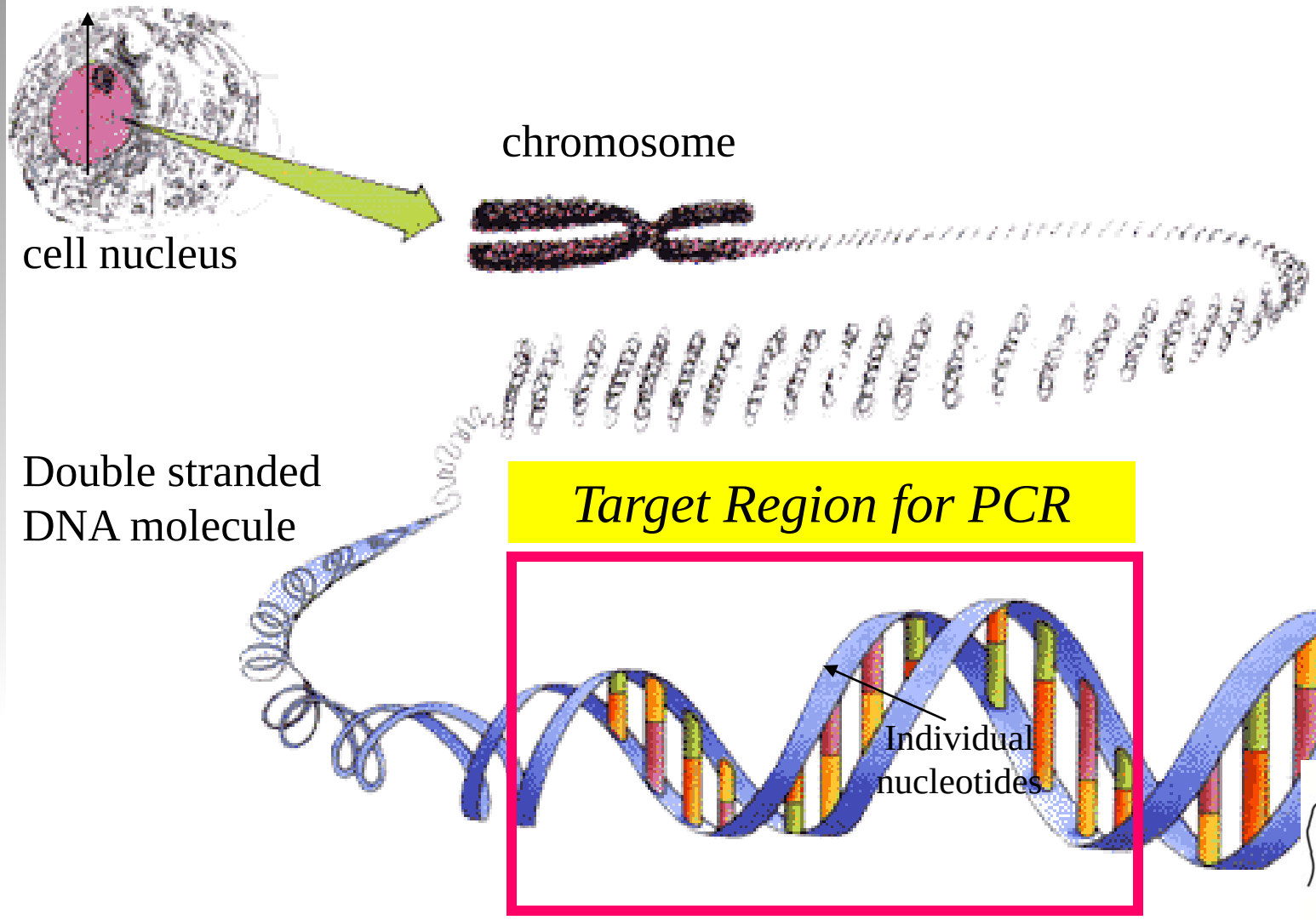
DNA


chromosome
cell nucleus
Double stranded
DNA molecule
Target Region for PCR
Individual
nucleotides
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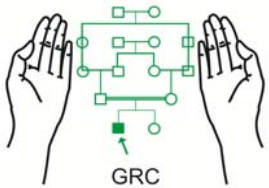
Molecular Pathology: Applications

- 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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Mutation	Punjabi	Pathan	Sindhi	Baluchi	Mohajir	All
Common mutations						
IVSI-5 (G-C)	107 (27·2%)	27 (12·9%)	114 (43·9%)	131 (76·2%)	75 (41·4%)	454 (37·3%)
Fr 8–9 (+G)	146 (37·2%)	103 (49·1%)	29 (11·2%)	14 (8·1%)	23 (12·7%)	315 (25·9%)
Del 619 bp	14 (3·6%)	4 (1·9%)	36 (13·9%)	2 (1·2%)	29 (16·0%)	85 (7·0%)
Fr 41–42 (−TTCT)	36 (9·2%)	18 (8·6%)	16 (6·2%)	1 (0·6%)	11 (6·1%)	82 (6·7%)
IVSI-1 (G-T)	19 (4·8%)	4 (1·9%)	33 (12·7%)	2 (1·2%)	7 (3·9%)	65 (5.4%)
Uncommon mutations						
Cd 15 (G-A)	14 (3·6%)	13 (6·2%)	5 (1·9%)	9 (5·2%)	8 (4·4%)	49 (4·0%)
Cd 30 (G-C)	15 (3·8%)	1 (0·5%)	19 (7·3%)	3 (1·7%)	4 (2·2%)	42 (3·5%)
Cd 5 (−CT)	11 (2·8%)	16 (7·6%)	0 (0·0%)	1 (0·6%)	2 (1·1%)	30 (2·5%)
Fr 16 (−C)	6 (1·5%)	8 (3·8%)	6 (2·3%)	6 (3·5%)	3 (1·7%)	29 (2·4%)
Cap+1 (A-C)	9 (2·3%)	8 (3·8%)	0 (0·0%)	0 (0·0%)	3 (1·7%)	20 (1·6%)
Hb-E	3 (0·8%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	10 (5·5%)	13 (1·1%)
Cd 30 (G-A)	3 (0·8%)	2 (1·0%)	0 (0·0%)	2 (1·2%)	4 (2·2%)	11 (0·9%)
IVSII-1 (G-A)	6 (1·5%)	1 (0·5%)	0 (0·0%)	1 (0·6%)	2 (1·1%)	10 (0·8%)
Rare mutations						
−88 (C-T)	1 (0·3%)	2 (1·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	3 (0·3%)
IVSI-1 (G-A)	1 (0·3%)	0 (0·0%)	1 (0·4%)	0 (0·0%)	0 (0·0%)	2 (0·2%)
Fr 47–48 (+ATCT)	2 (0·5%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	2 (0·2%)
Fr 126–131 (−17 bp)	0 (0·0%)	2 (1·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	2 (0·2%)
Cd 39 (C-T)	0 (0·0%)	1 (0·5%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	1 (0·1%)
IVSI minus 25	0 (0·0%)	0 (0·0%)	1 (0·4%)	0 (0·0%)	0 (0·0%)	1 (0·1%)
Total	393 (100%)	210 (100%)	260 (100%)	172 (100%)	181 (100%)	1216 (100%)

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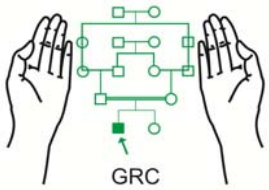

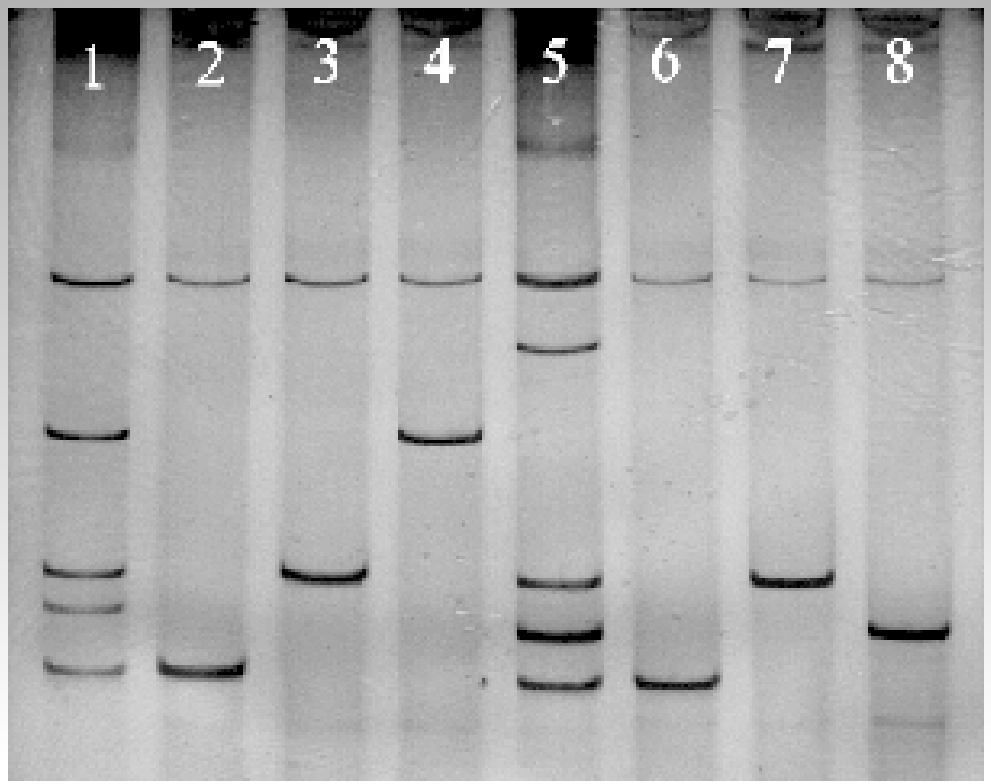

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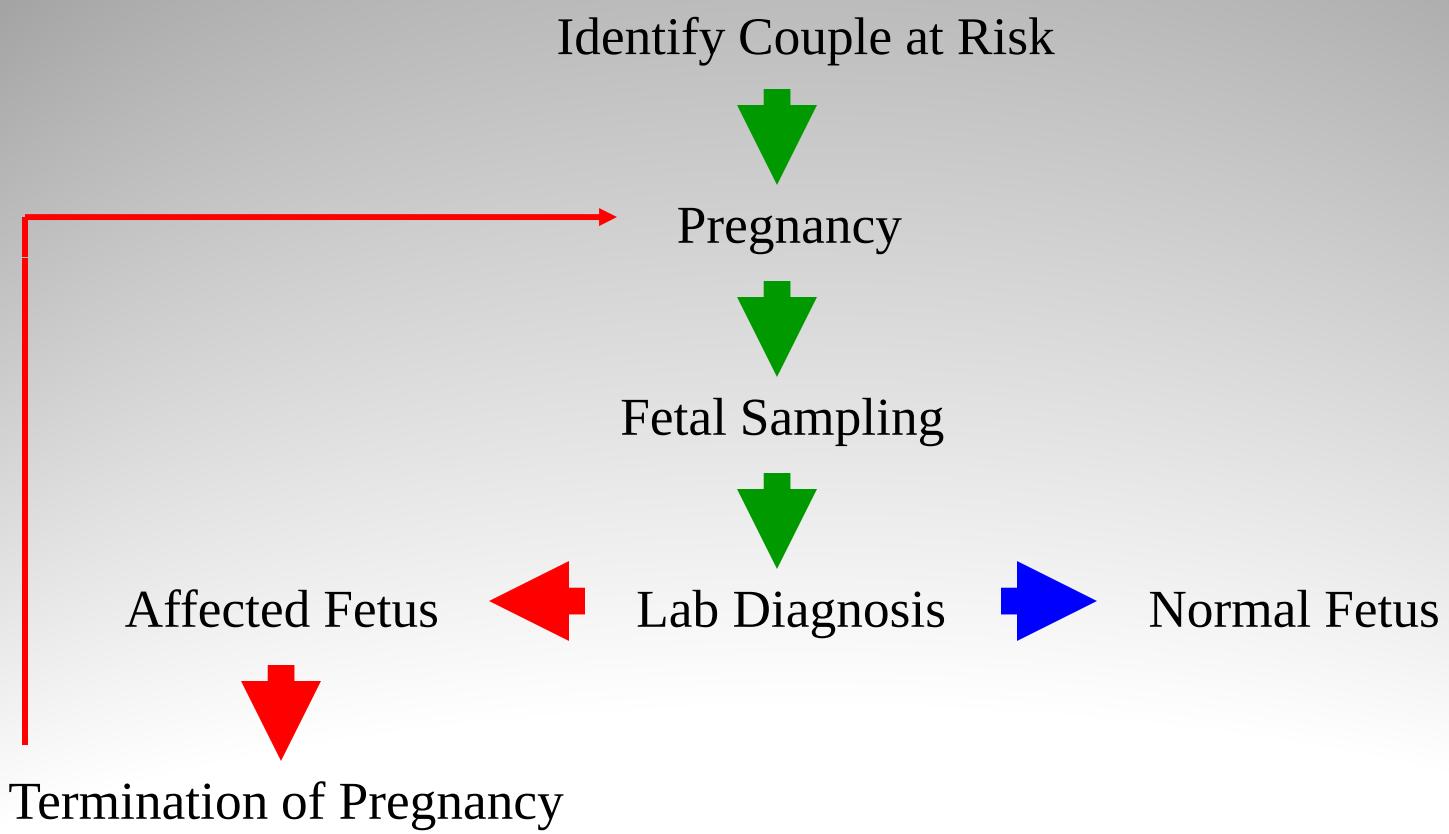
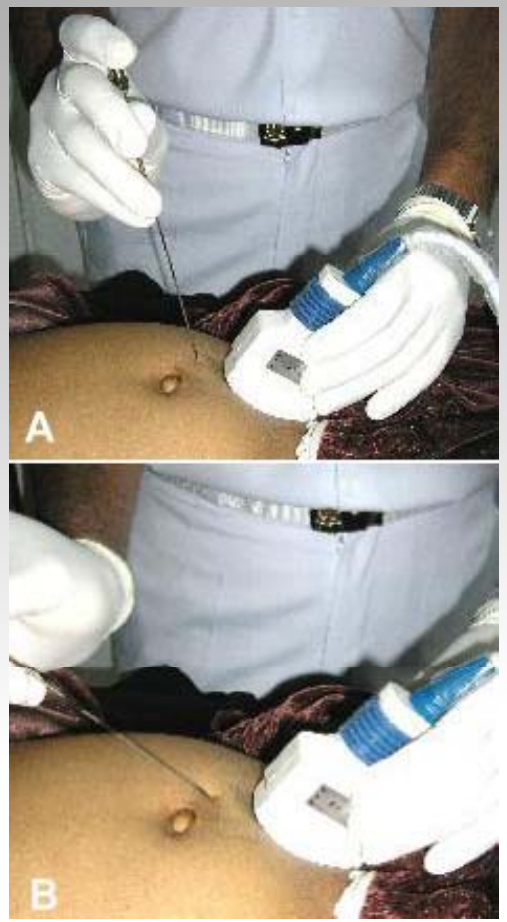
Prenatal Diagnosis



Chorionic Villus Sampling (CVS)



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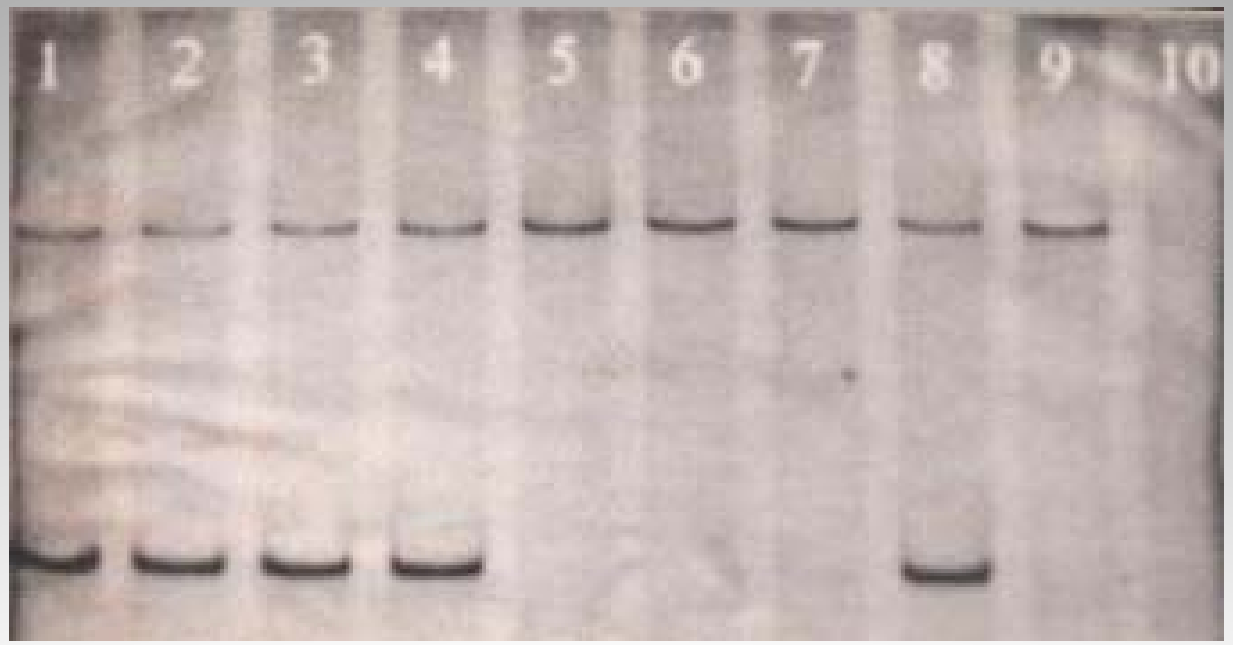

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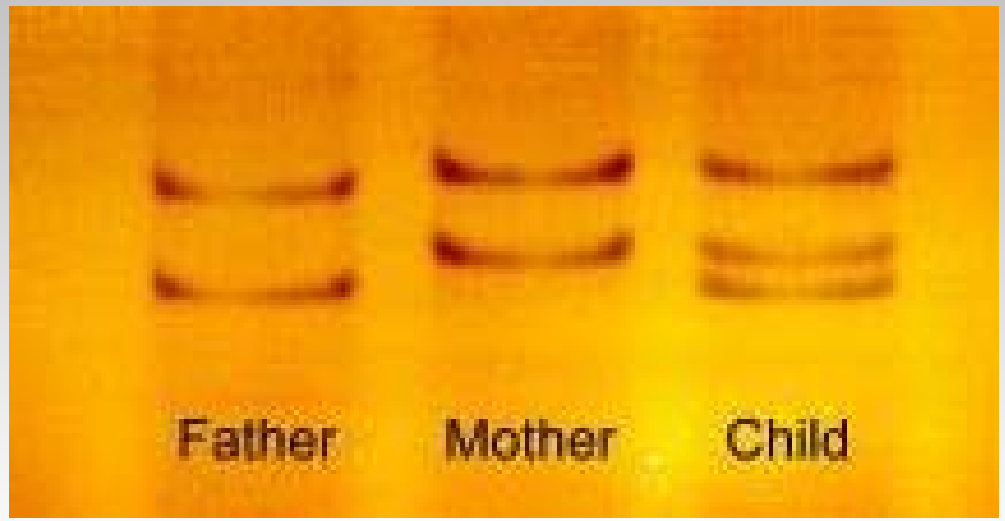

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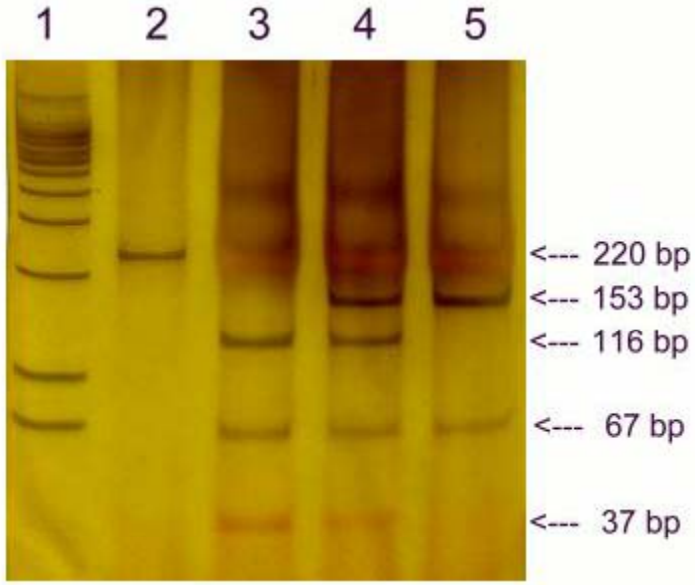
PCR based detection of Factor V Leiden



Lane 1: 100bp ladder
Lane 2: Uncut 220 bp amplified fragment
Lane 3: Wild type (normal) DNA
Lane 4: Heterozygous Factor V Leiden
Lane 5: Homozygous Factor V Leiden

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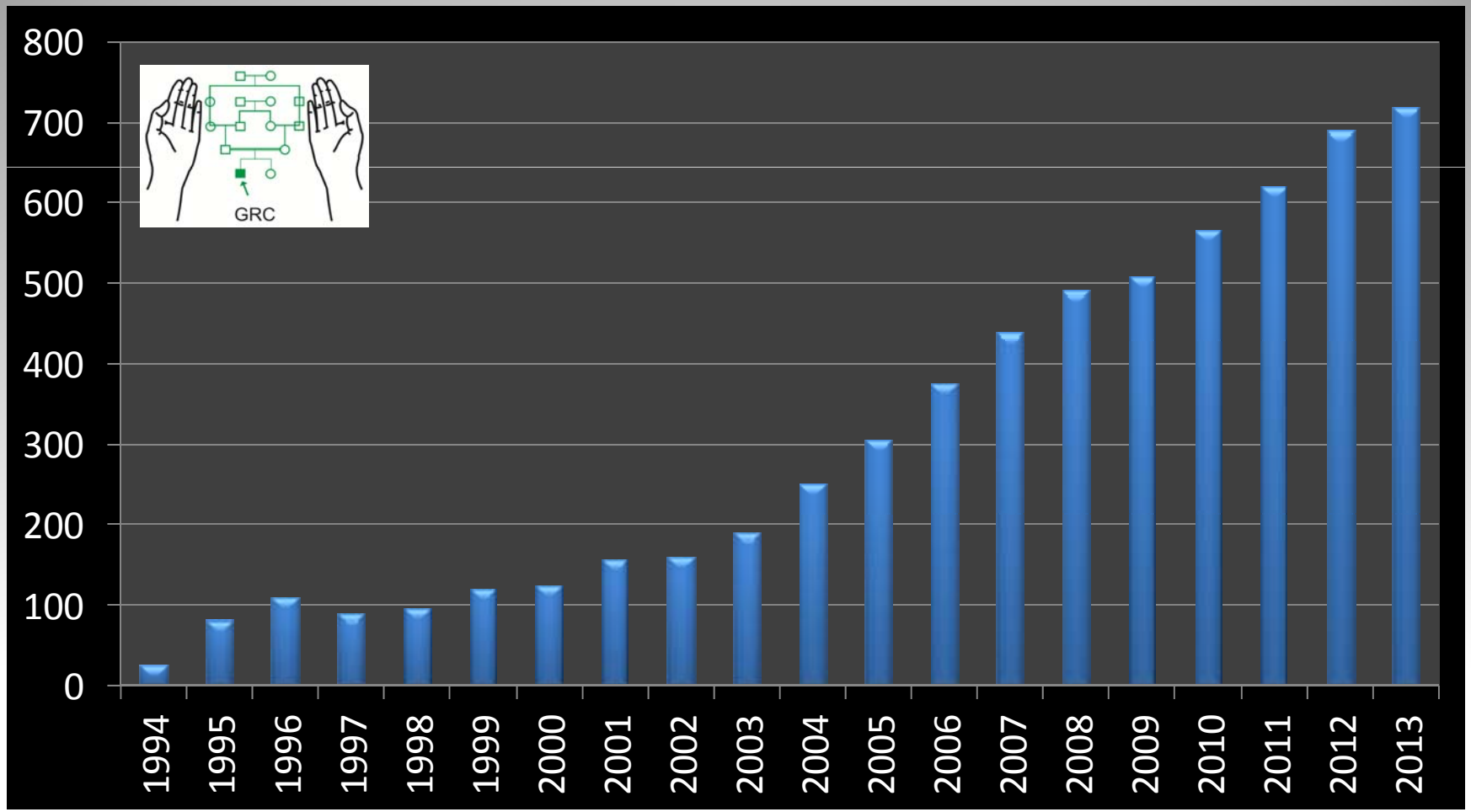
Prenatal Diagnosis of Thalassaemia



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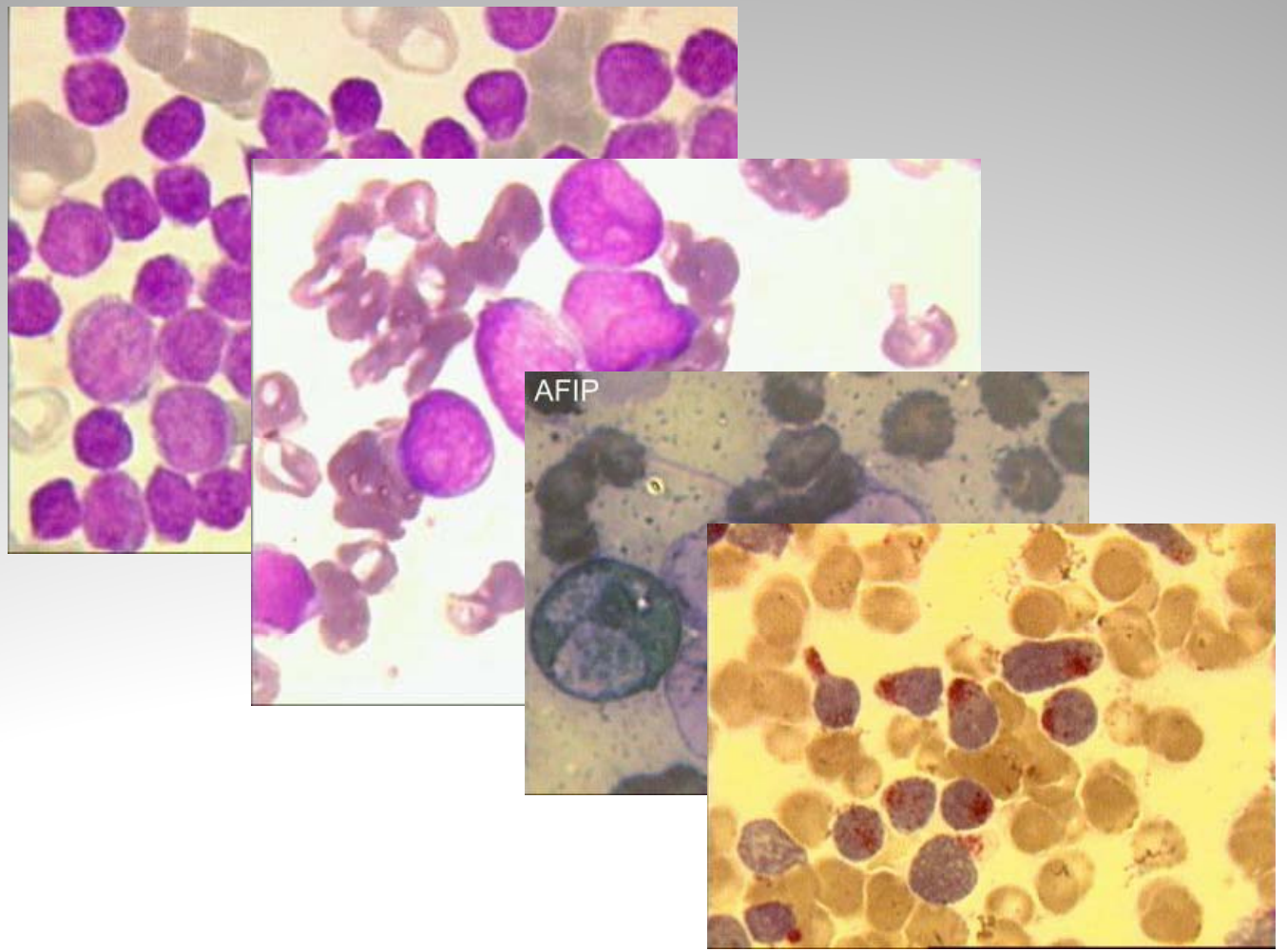
Molecular Diagnosis of Cancer



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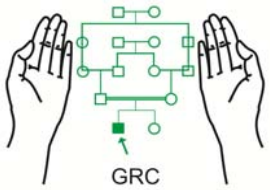
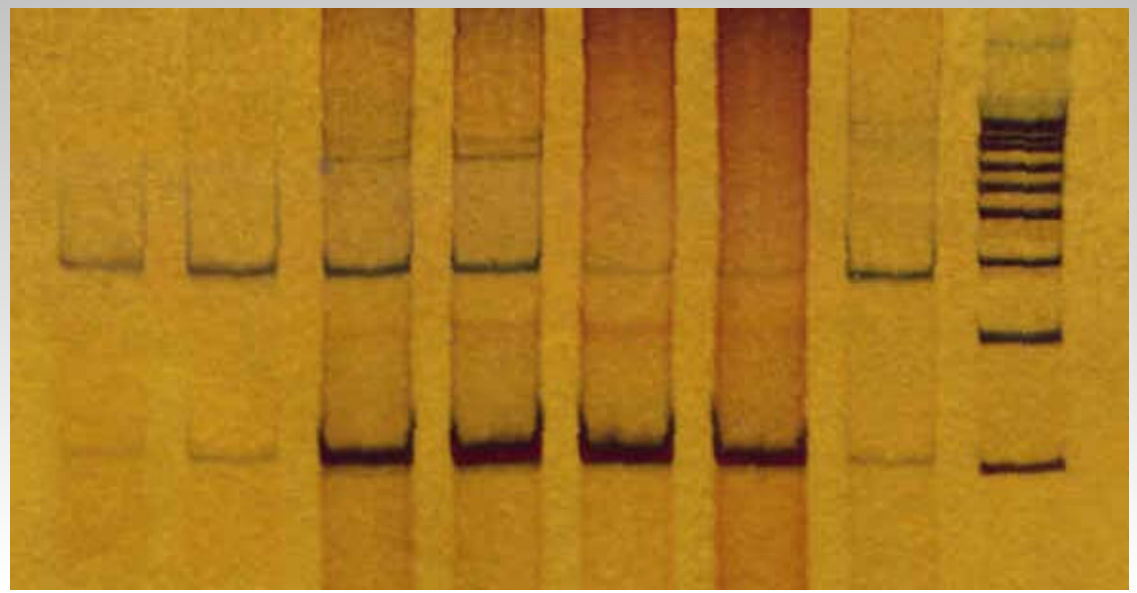
Molecular Markers

- 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



JAK2 Mutation



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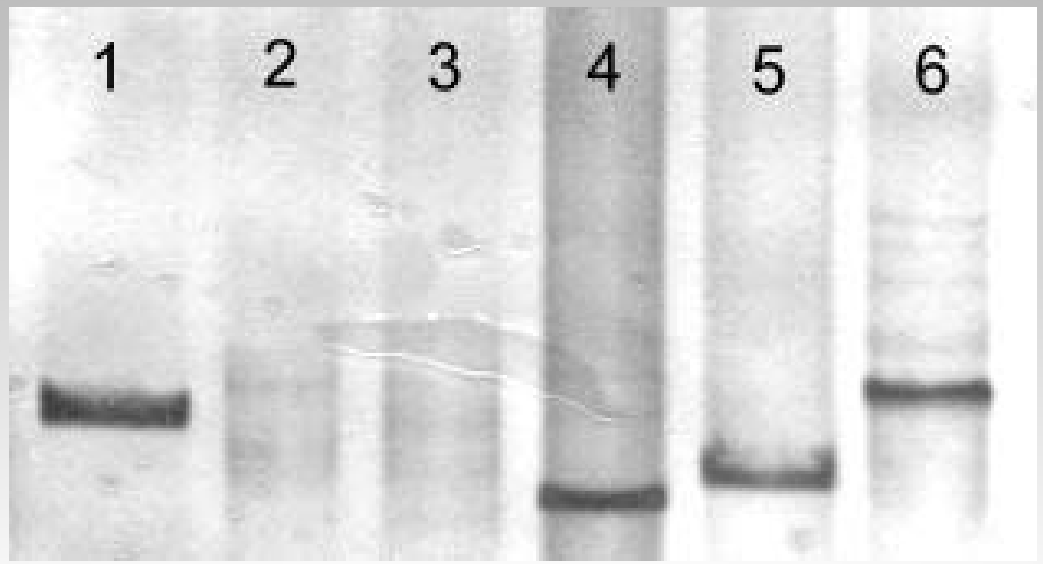

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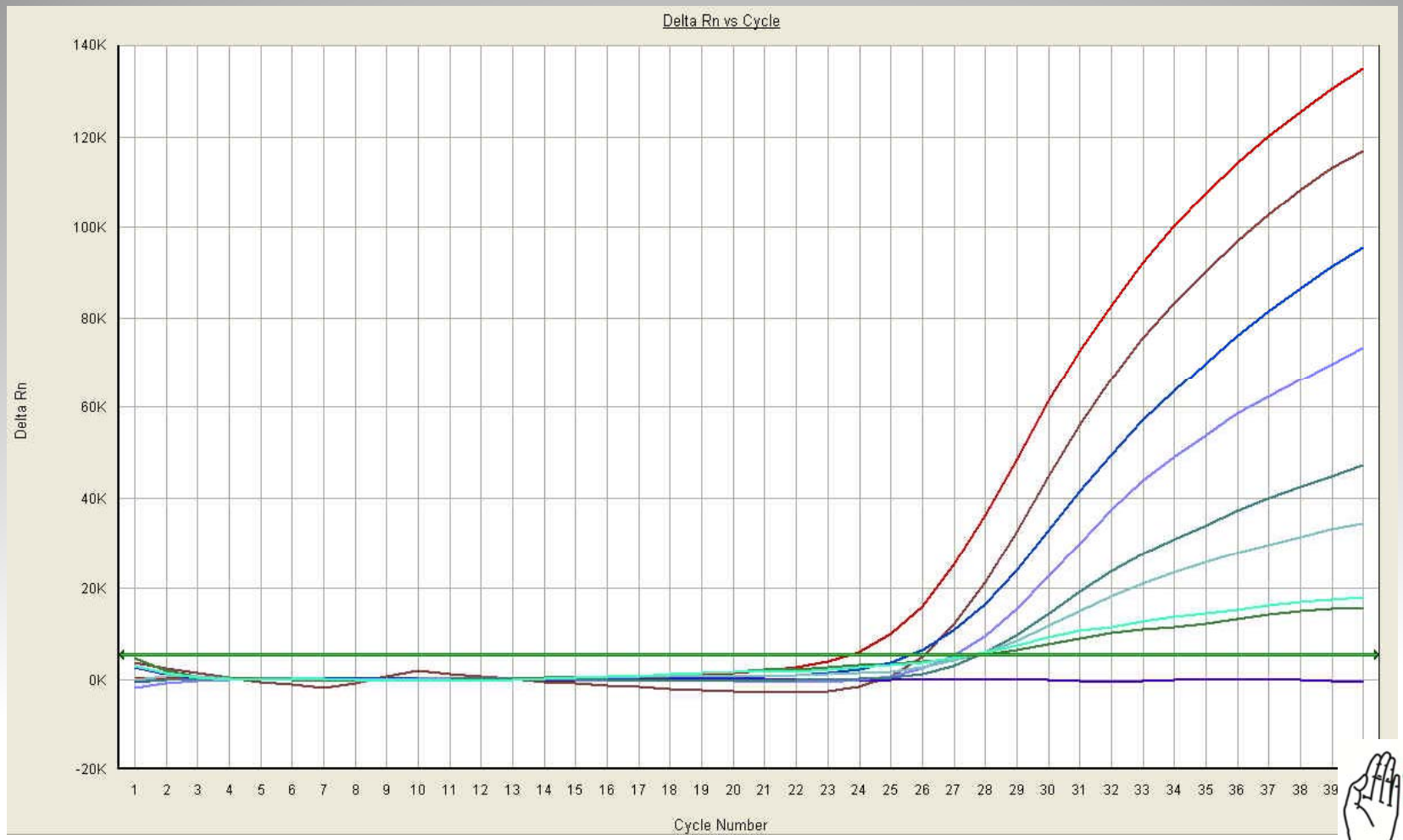
Real Time PCR for *Bcr-abl*



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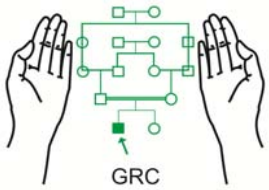
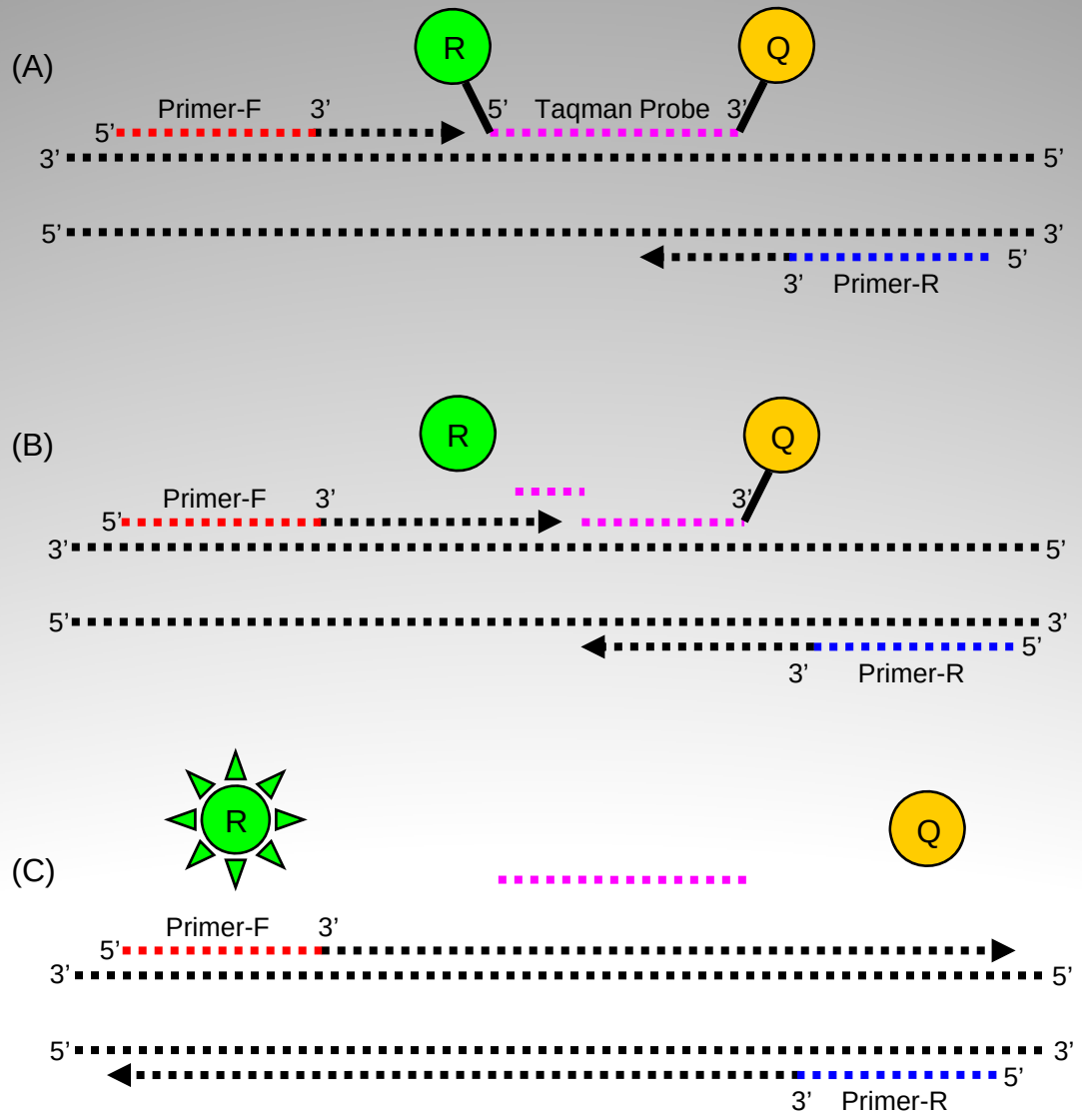
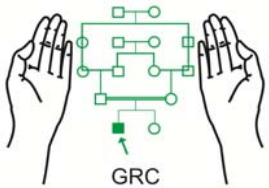
Infectious Diseases

- Viral
  - HCV, HBV, HIV, CMV, HPV, Dengue etc.
- Bacterial
  - Tuberculosis
  - Antibiotic resistance
- Parasitic
  - Leishmania, Malaria
- Others




(A)
R
Q
Primer-F
3'
5'
Taqman Probe
3'
5'
3'
5'
5'
3'
5'
3'
Primer-R
(B)
R
Q
Primer-F
3'
5'
3'
5'
3'
5'
5'
3'
5'
3'
Primer-R
(C)
R
Q
Primer-F
3'
5'
3'
5'
5'
3'
5'
3'
Primer-R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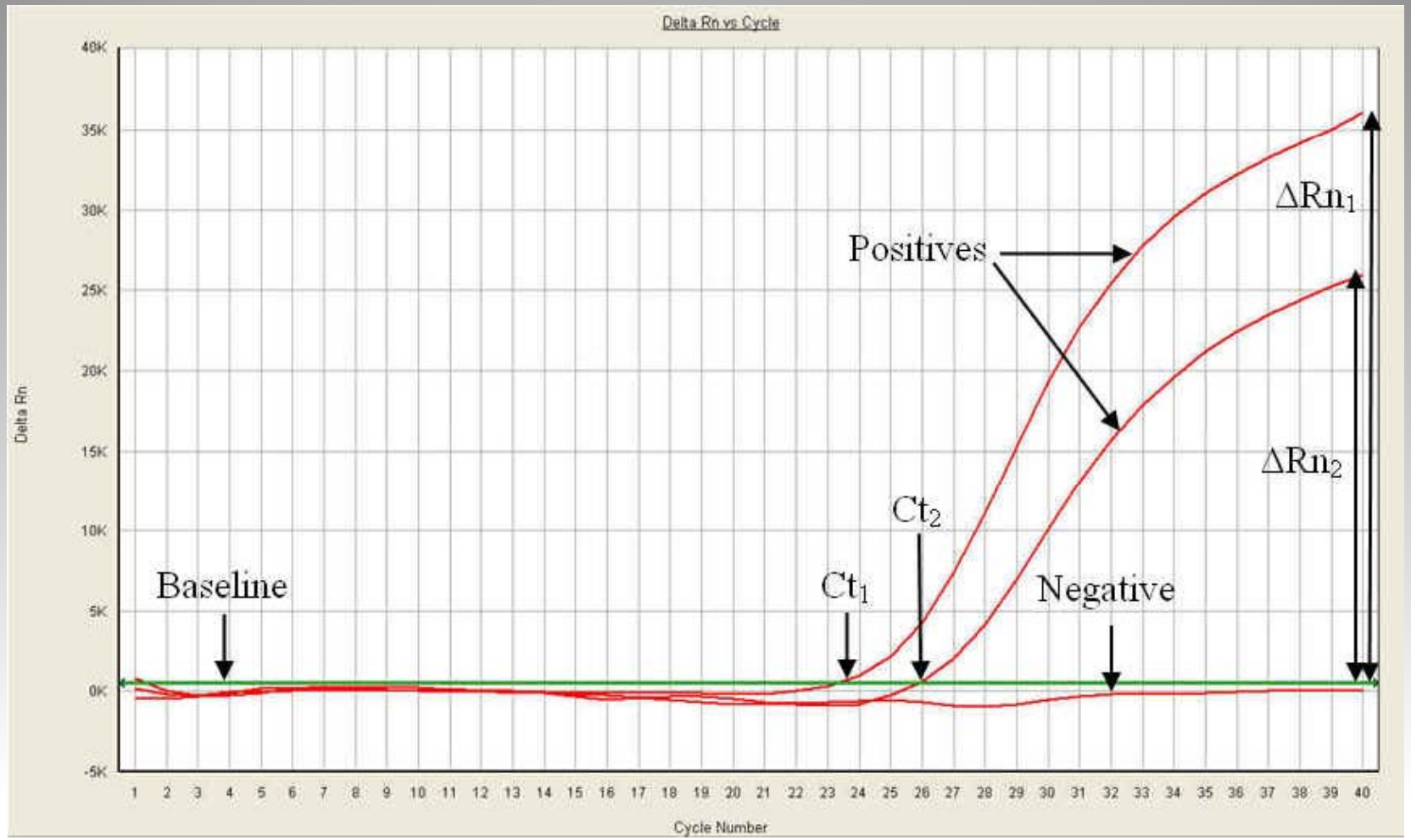
Delta Rn vs Cycle
Delta Rn
Cycle Number
40K
35K
30K
25K
20K
15K
10K
5K
0K
-5K
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
Baseline
Ct1
Ct2
Negative
Positives
ΔRn1
ΔRn2

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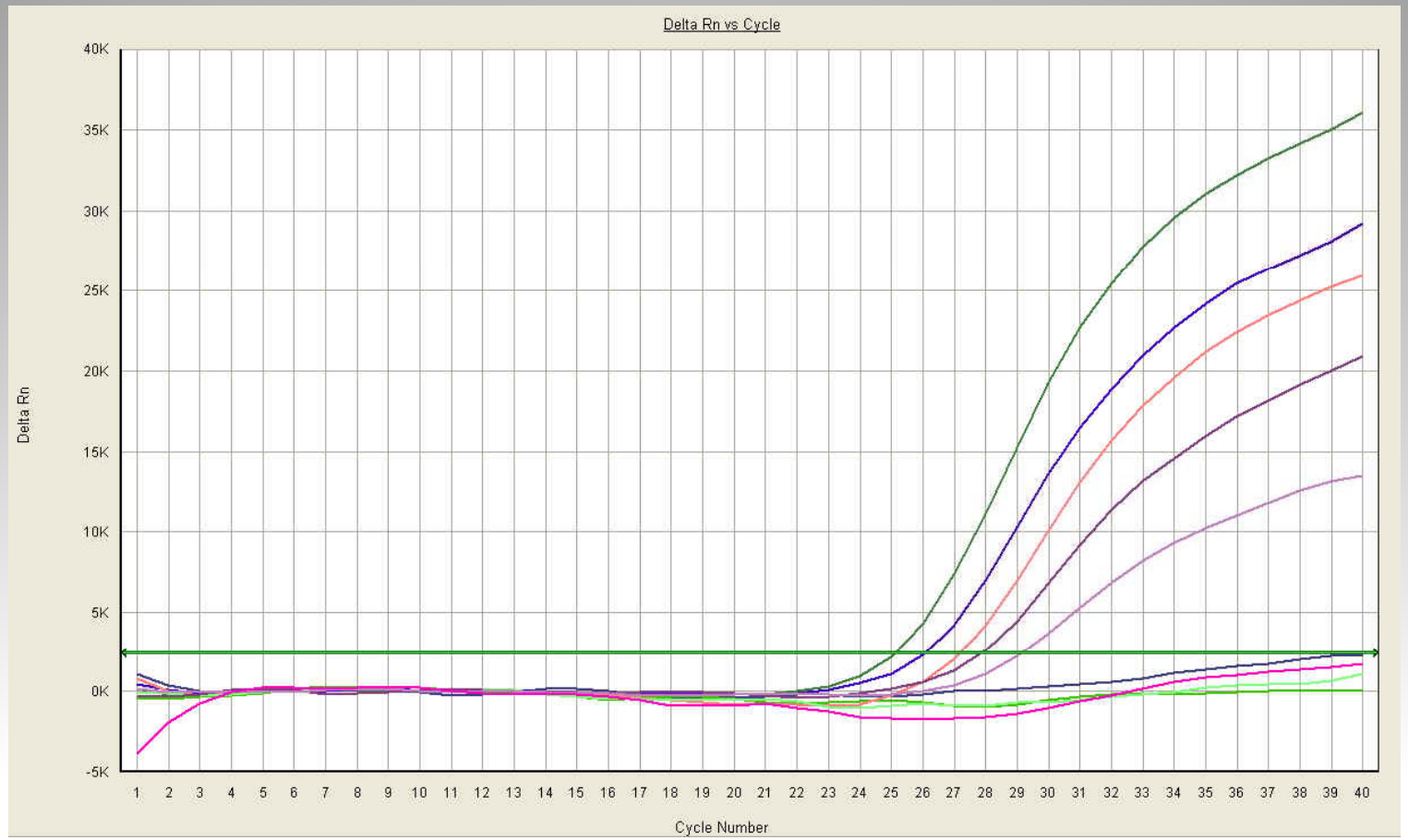
Real Time PCR for HCV



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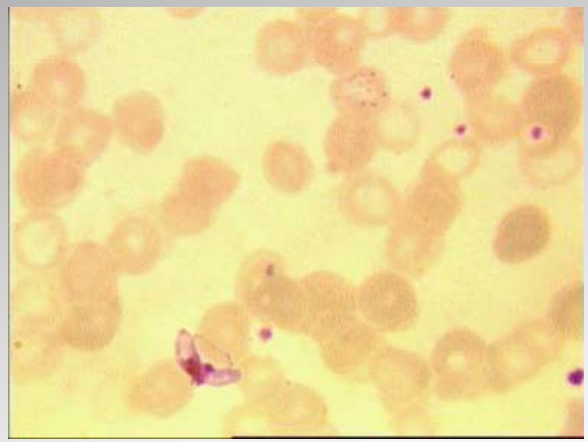
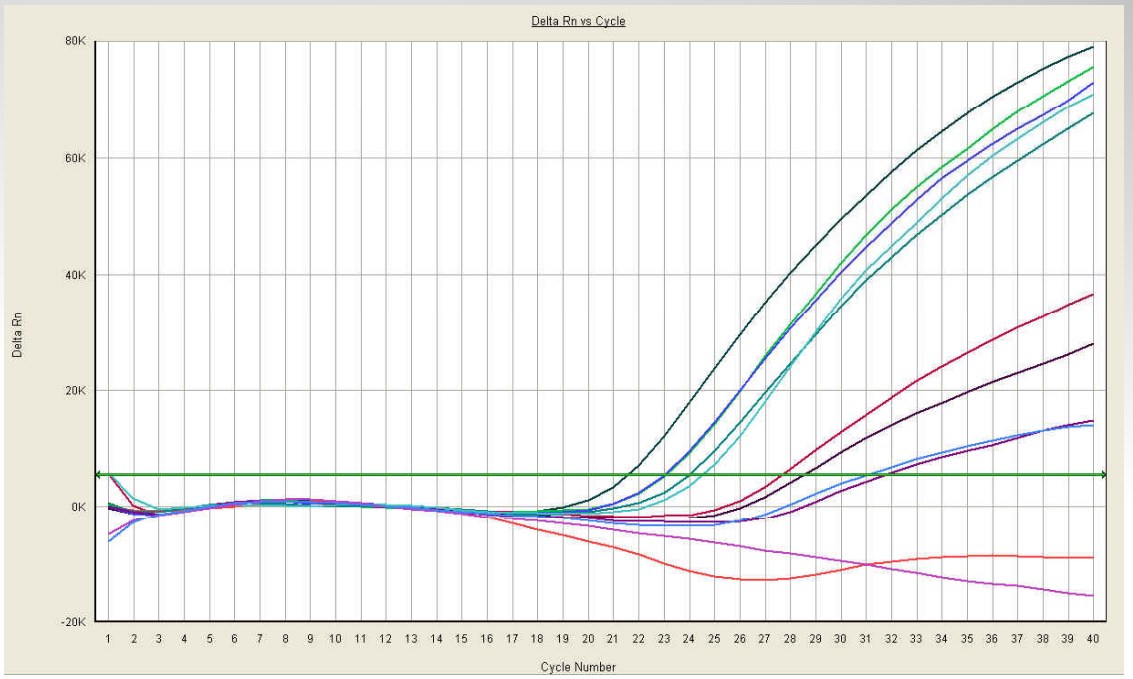
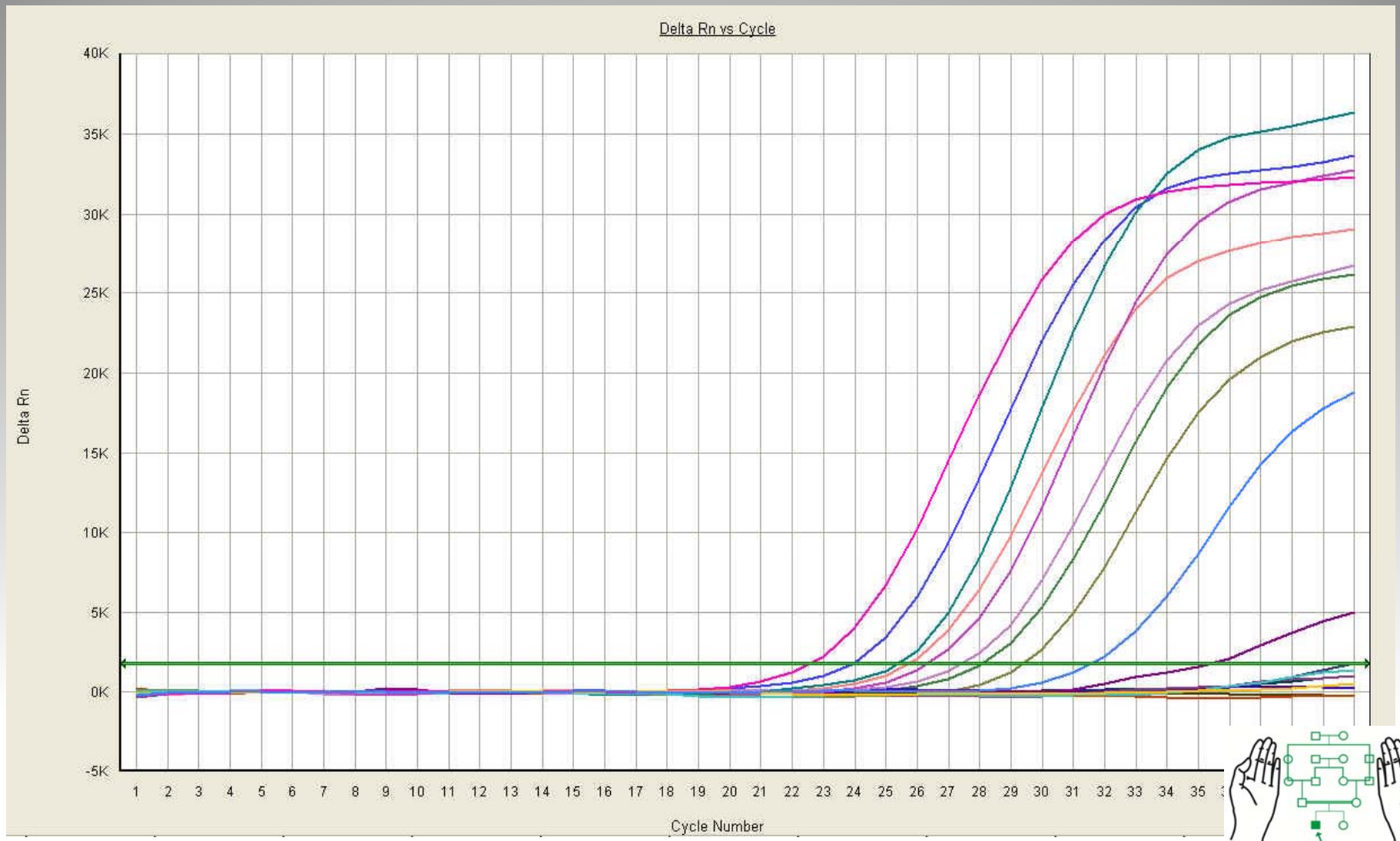
Malaria




Delta Rn vs Cycle
80K
60K
40K
20K
0K
-20K
Delta Rn
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

GRC


Delta Rn vs Cycle
Delta Rn
40K
35K
30K
25K
20K
15K
10K
5K
0K
-5K
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
Cycle Number

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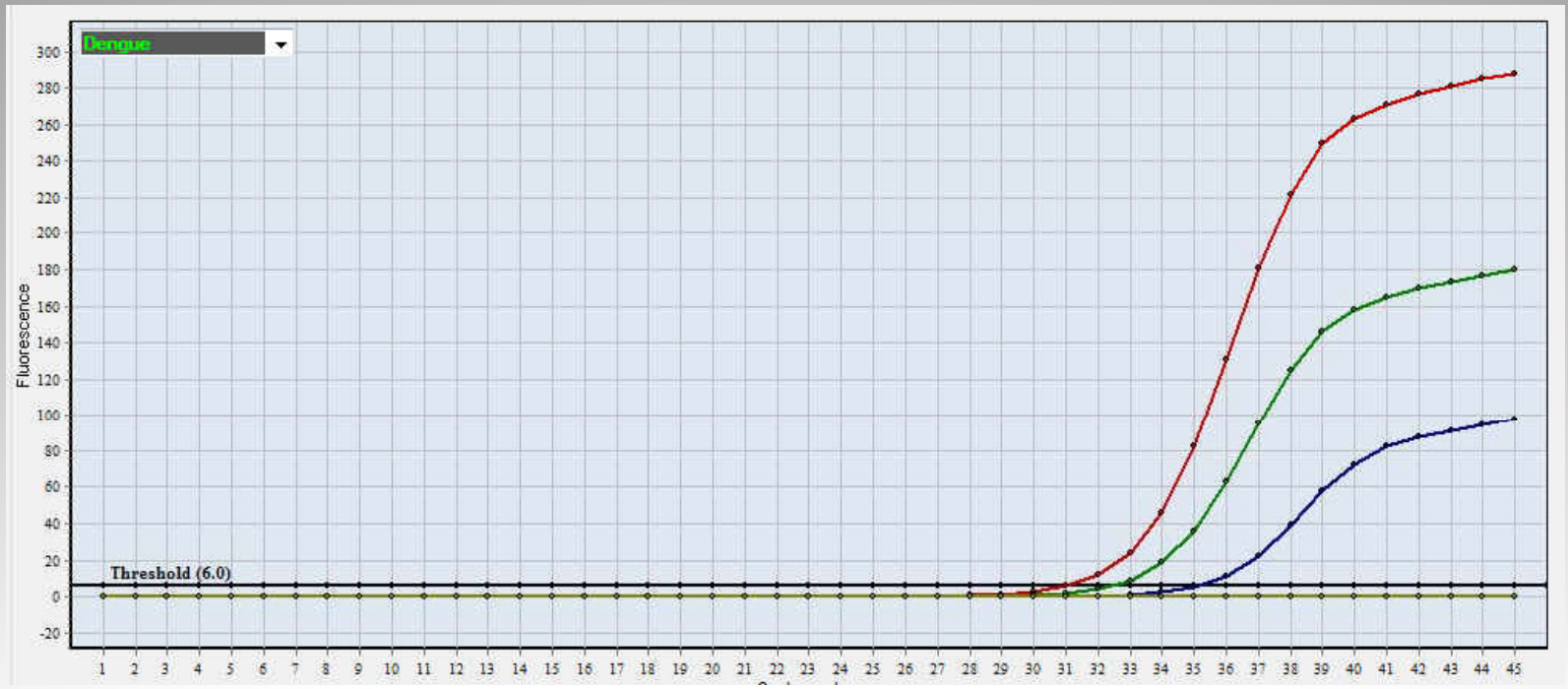
Dengue
Fluorescence
Threshold (6.0)
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45

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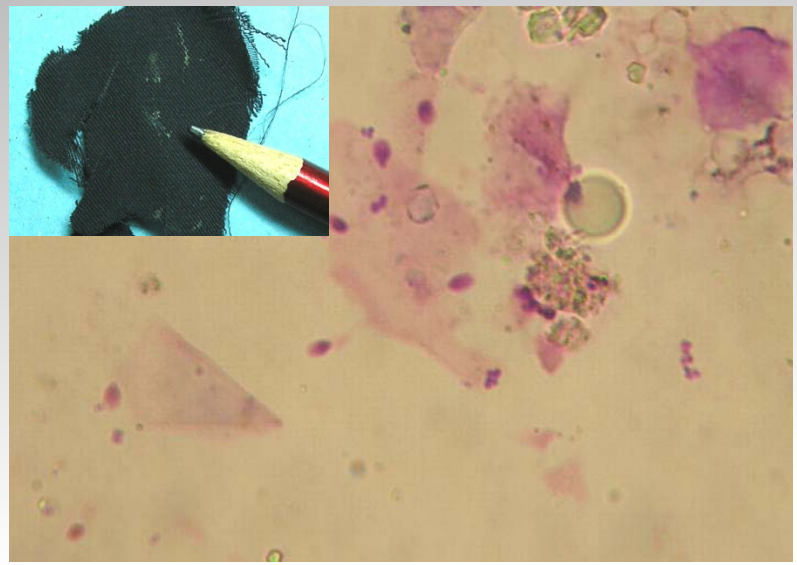
Human Identification (Forensic DNA Testing)



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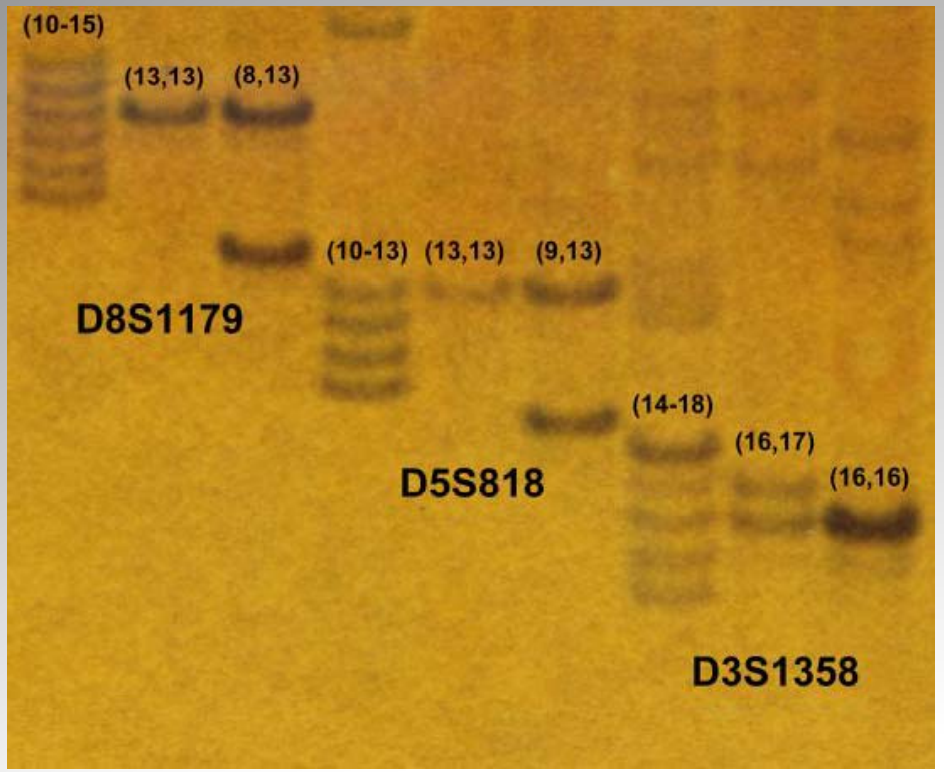
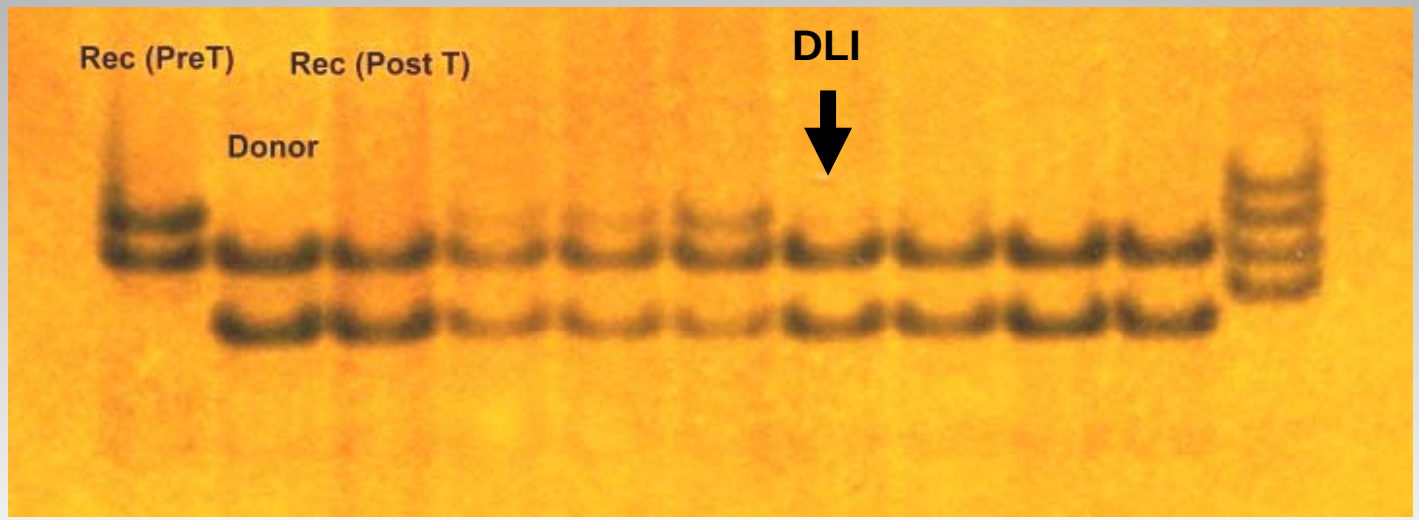
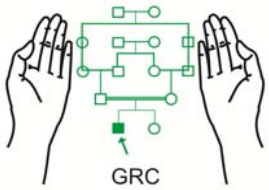

(10-15)
(13,13)
(8,13)
(10-13)
(13,13)
(9,13)
D8S1179
(14-18)
(16,17)
(16,16)
D5S818
D3S1358

GRC

Donor Chimerism



A Low Cost DNA Lab: "Made in Pakistan"



A Mobile DNA Lab

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GTI-16 DNA Thermal Cycler

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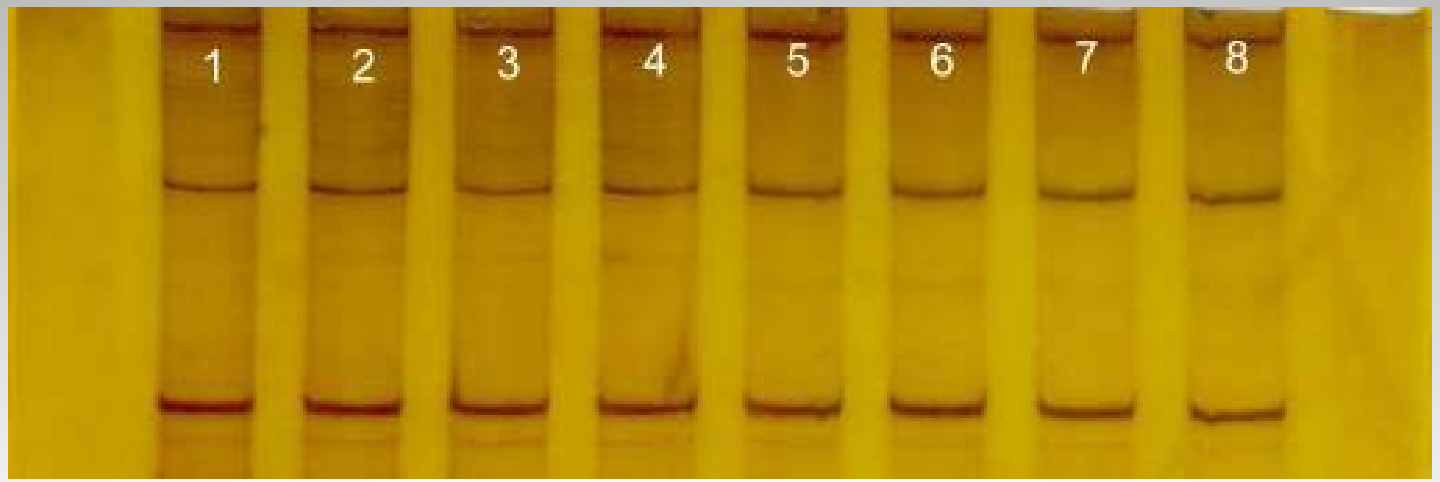
GTI-16 ABI-9700 (USA)



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GTI-PCR Reader-G

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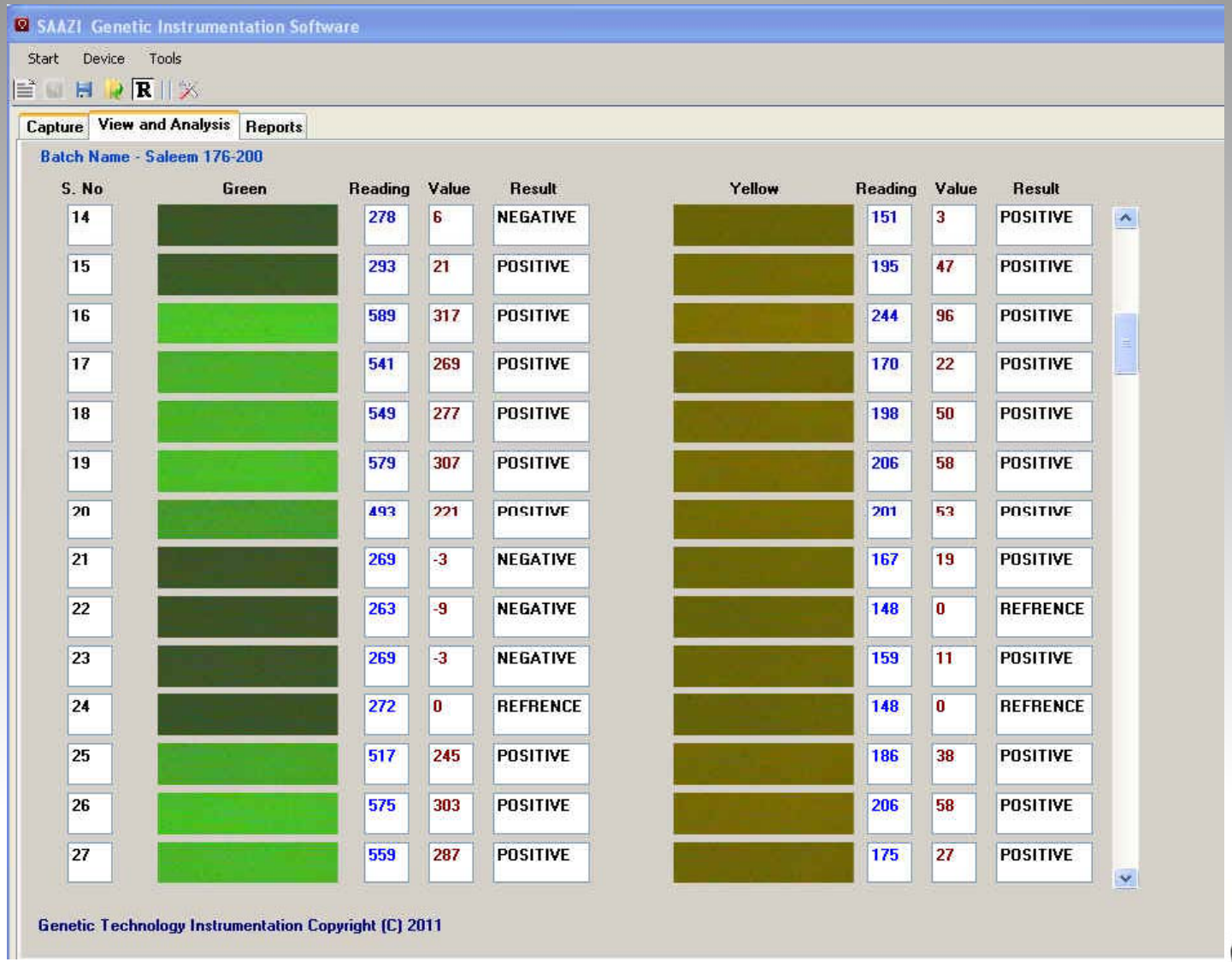
SAAZI Genetic Instrumentation Software
Start
Device
Tools
Capture
View and Analysis
Reports
Batch Name - Saleem 176-200
S. No
Green
Reading
Value
Result
Yellow
Reading
Value
Result
14 278 6 NEGATIVE 151 3 POSITIVE
15 293 21 POSITIVE 195 47 POSITIVE
16 589 317 POSITIVE 244 96 POSITIVE
17 541 269 POSITIVE 170 22 POSITIVE
18 549 277 POSITIVE 198 50 POSITIVE
19 579 307 POSITIVE 206 58 POSITIVE
20 493 221 POSITIVE 201 53 POSITIVE
21 269 -3 NEGATIVE 167 19 POSITIVE
22 263 -9 NEGATIVE 148 0 REFRENCE
23 269 -3 NEGATIVE 159 11 POSITIVE
24 272 0 REFRENCE 148 0 REFRENCE
25 517 245 POSITIVE 186 38 POSITIVE
26 575 303 POSITIVE 206 58 POSITIVE
27 559 287 POSITIVE 175 27 POSITIVE
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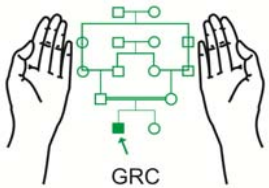
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GTI-Microcentrifuge & Dry Bath

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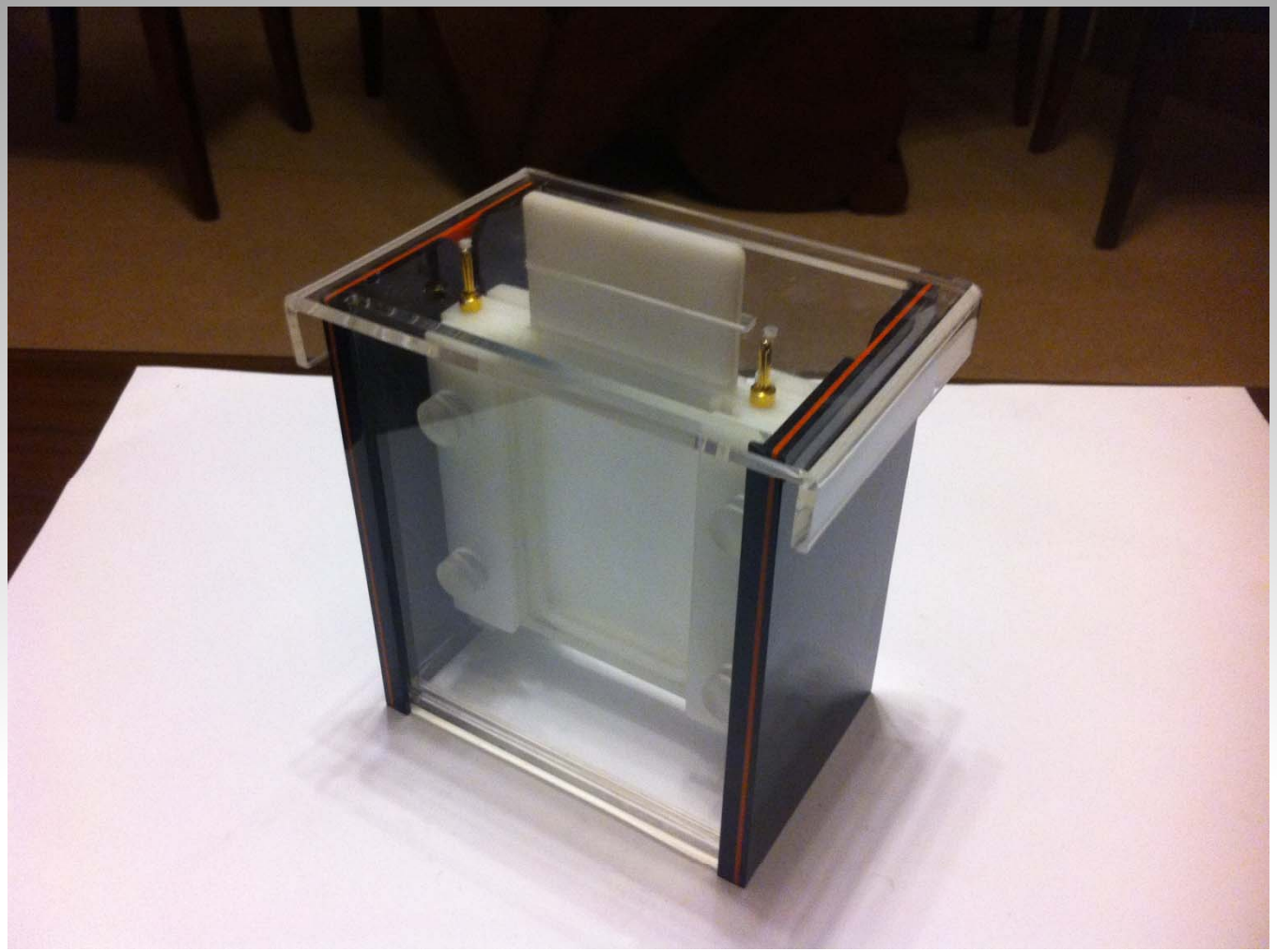
GTI-DC Power Supply

GRC

GTI-PAGE Apparatus



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