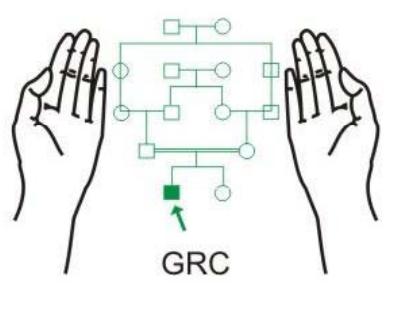


Quantitative Polymerase Chain Reaction (Q-PCR)

Maj Gen Suhaib Ahmed, HI (M)
MBBS; MCPS; FCPS (Pak); PhD (London)

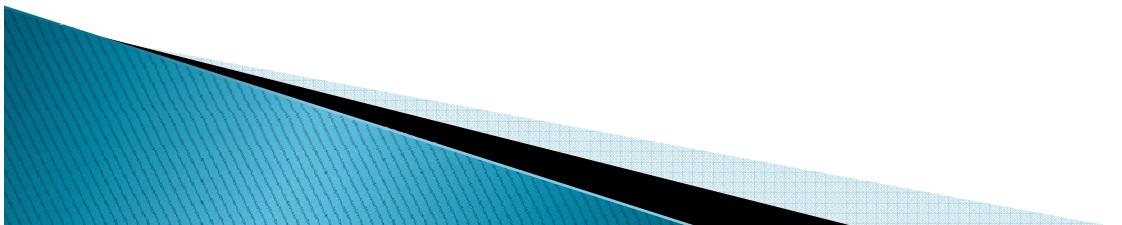
Genetics Resource Centre (GRC)



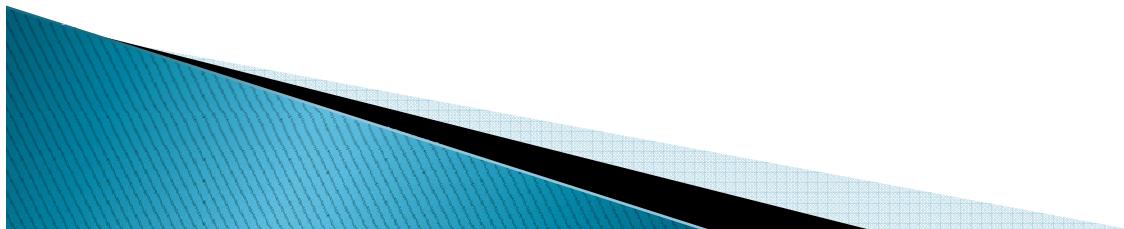
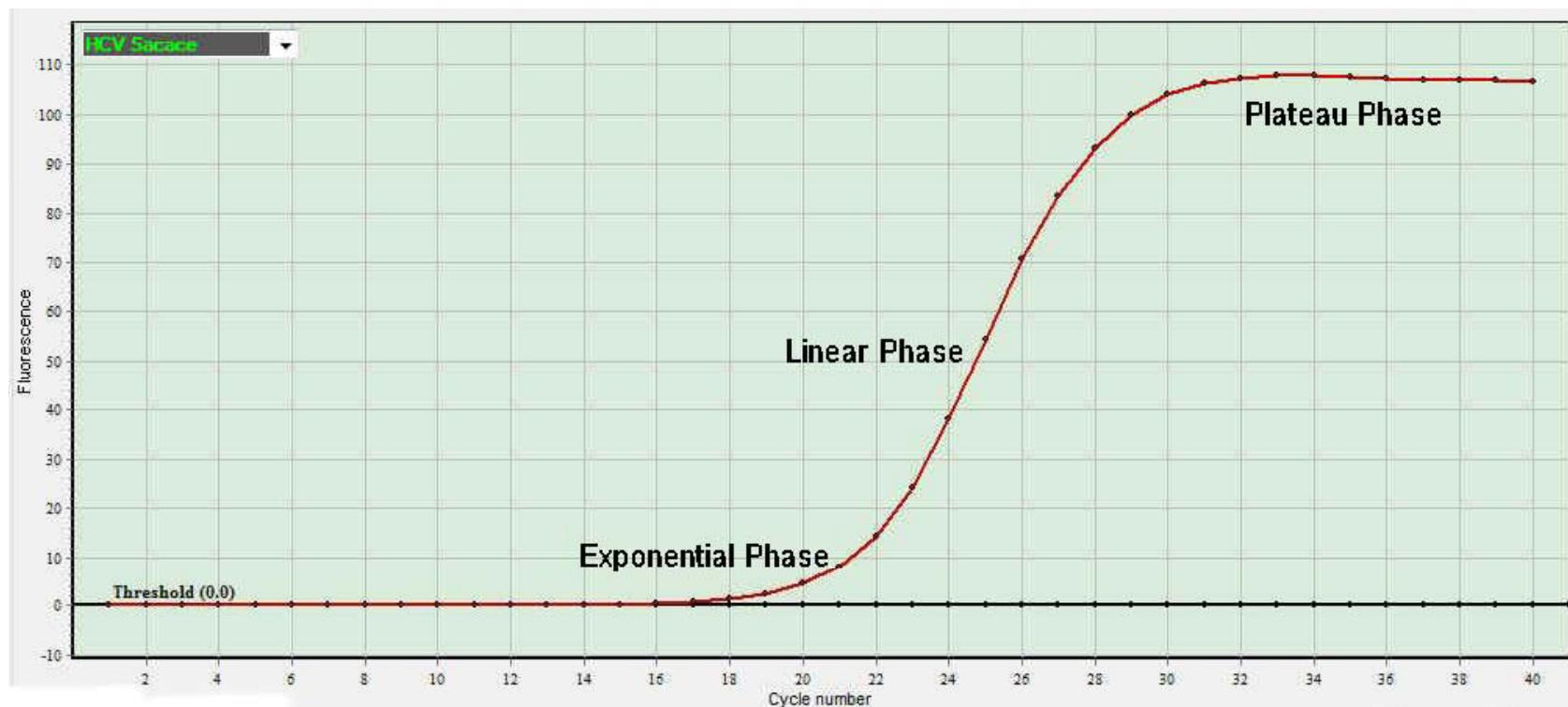
www.grcpk.com

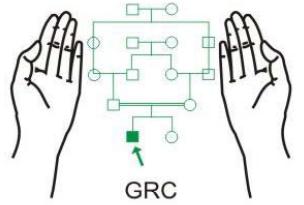
Phases of PCR

- ▶ Exponential: a short lived phase in which the DNA molecules double with each cycle. This is the most efficient phase of reaction.
- ▶ Linear: as the reaction components are consumed the doubling of DNA is progressively slowed down and it may take more than one cycle for the DNA molecules to double.
- ▶ Plateau: when the reaction components are consumed to a large extent the doubling of DNA molecules also comes to a halt. If the reaction is allowed to proceed indefinitely the number of amplified DNA may actually decrease due to their degradation. This is the most inefficient phase of the reaction.

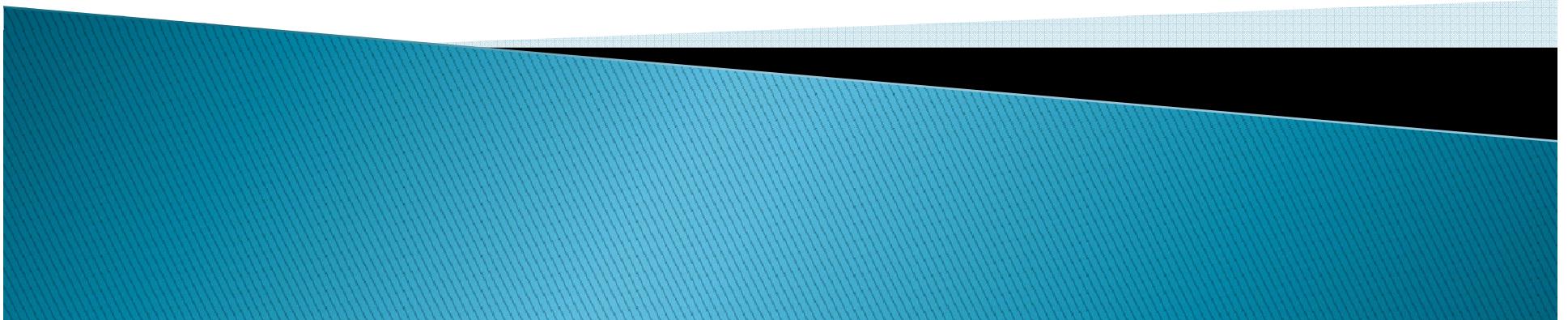


Phases of PCR

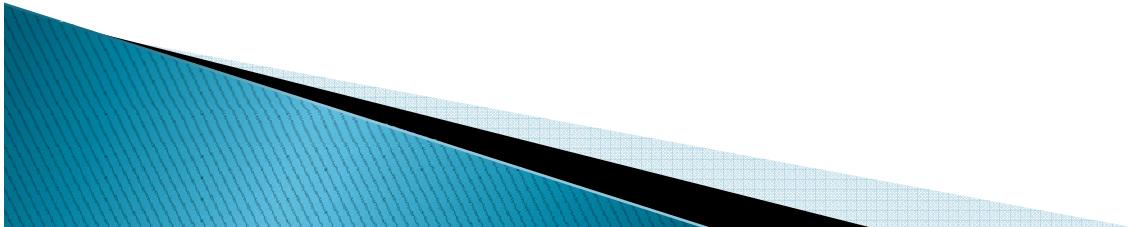
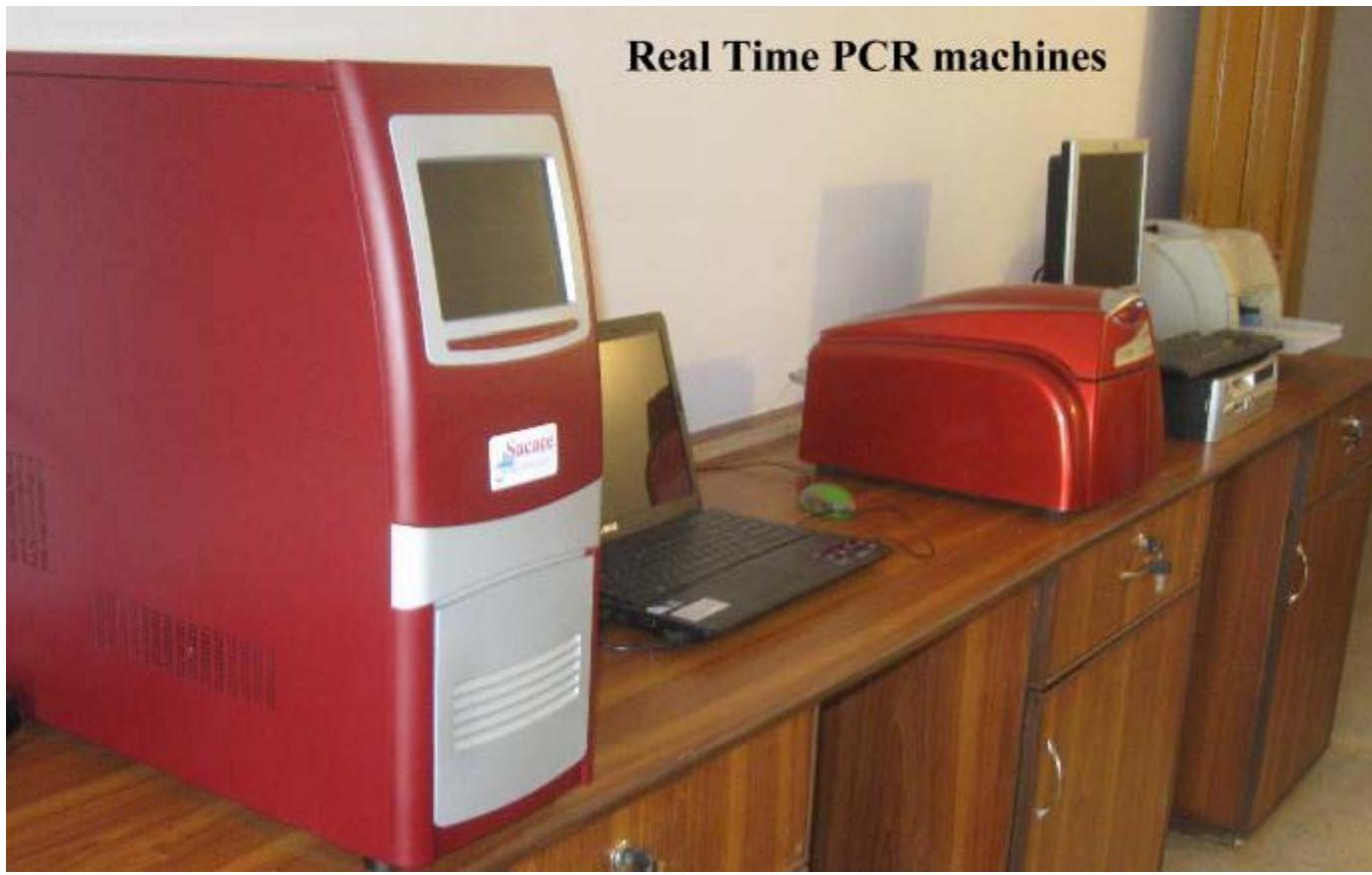


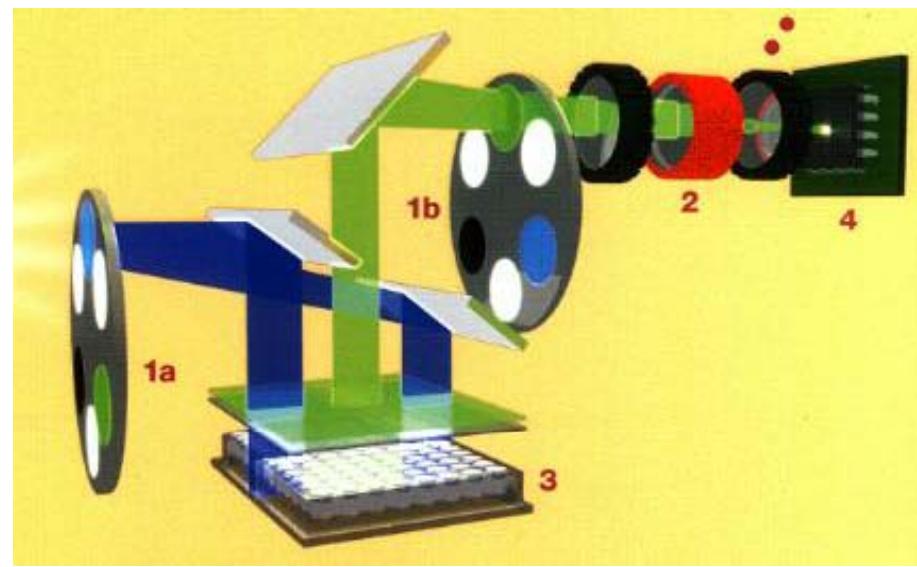


Real Time PCR

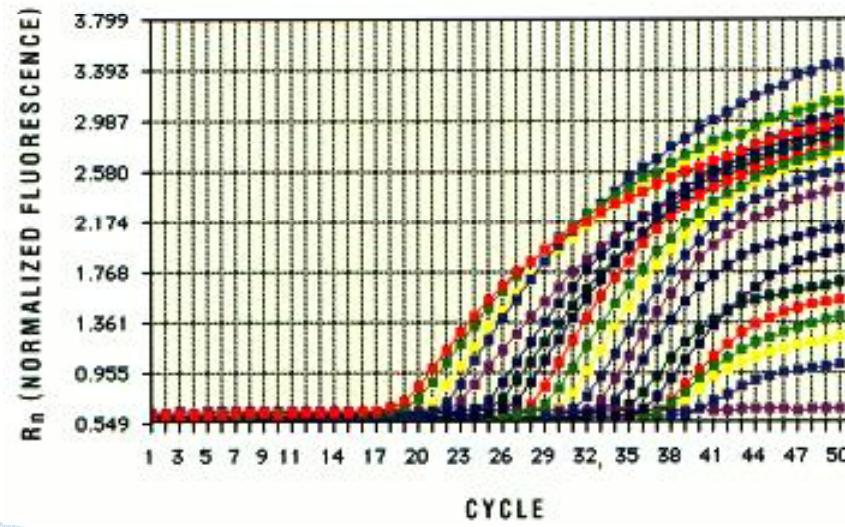


Real Time PCR machines

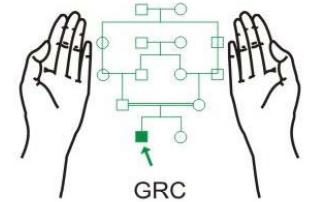




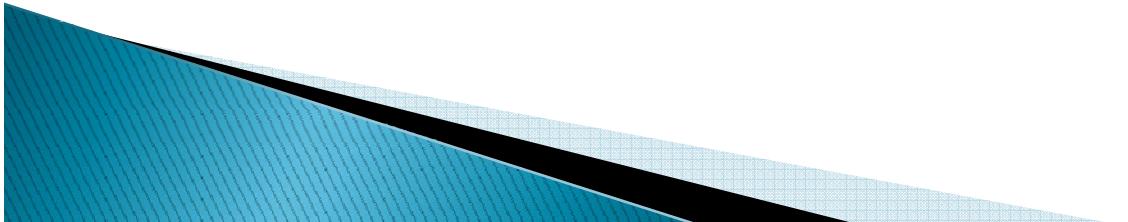
AMPLIFICATION PLOT



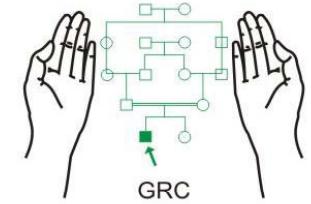
Monitoring of PCR



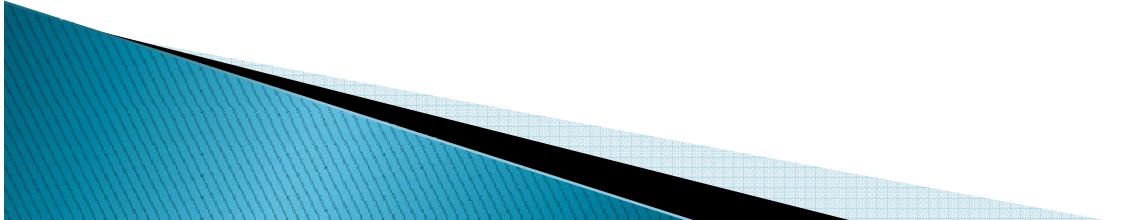
- ▶ SYBR Green Method
- ▶ Molecular Probe Method
- ▶ TaqMan Probe Method
- ▶ Molecular Beacon Method
- ▶ FRET Probe Method



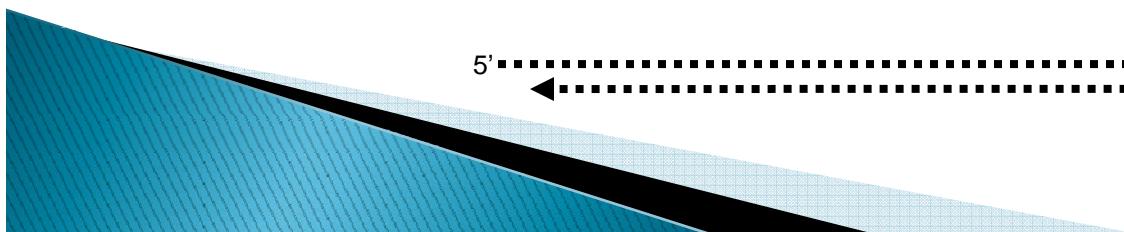
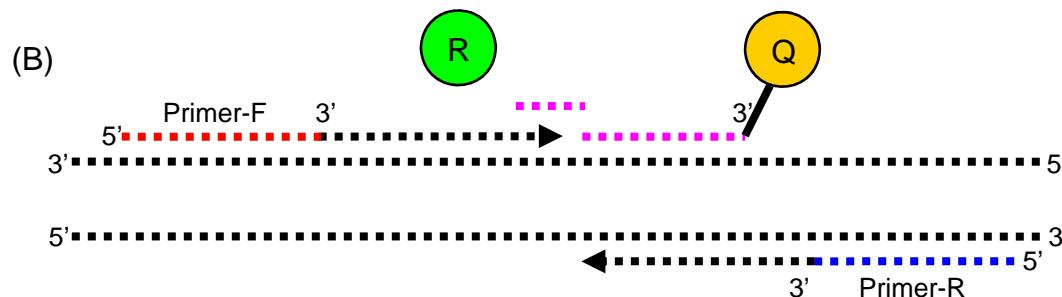
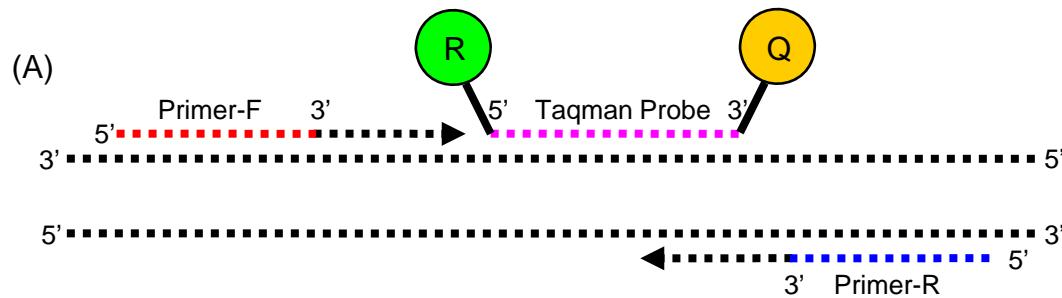
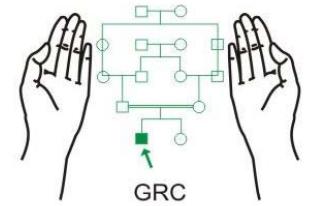
TaqMan Probe



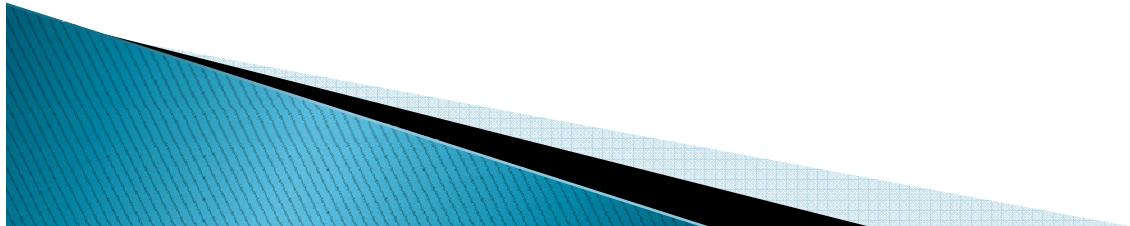
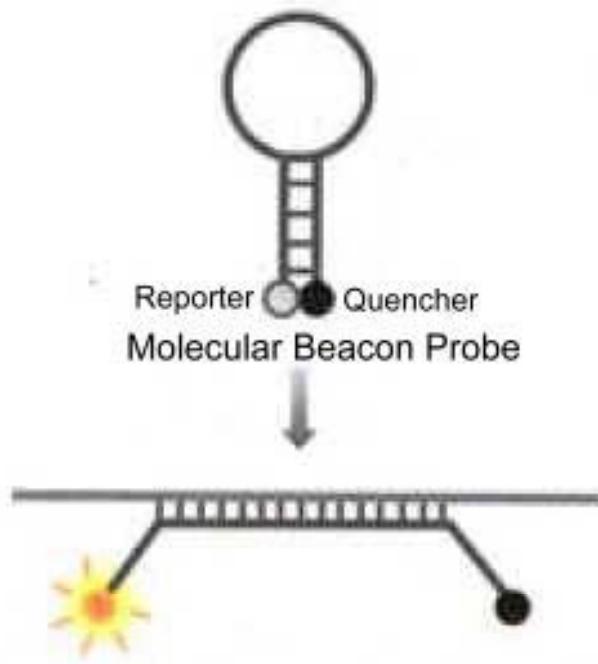
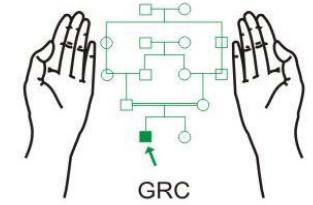
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GTGGCCAGATGCACCGTCGAACGGCTGATGACCAAACTCGGCCTGTCCGGGA  
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TGGGTAGCAGAC
```

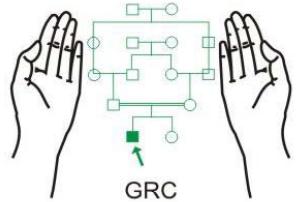


TaqMan Probe

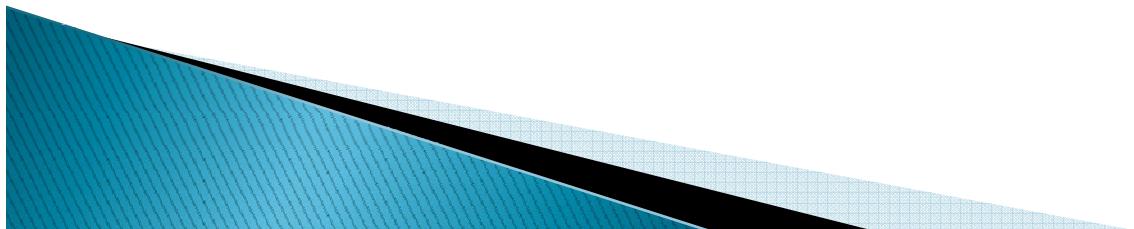


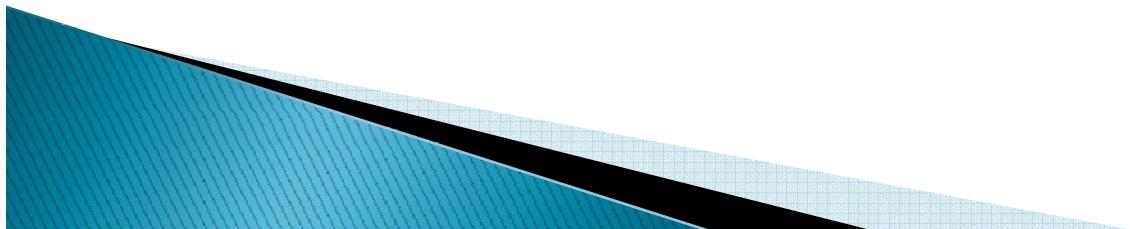
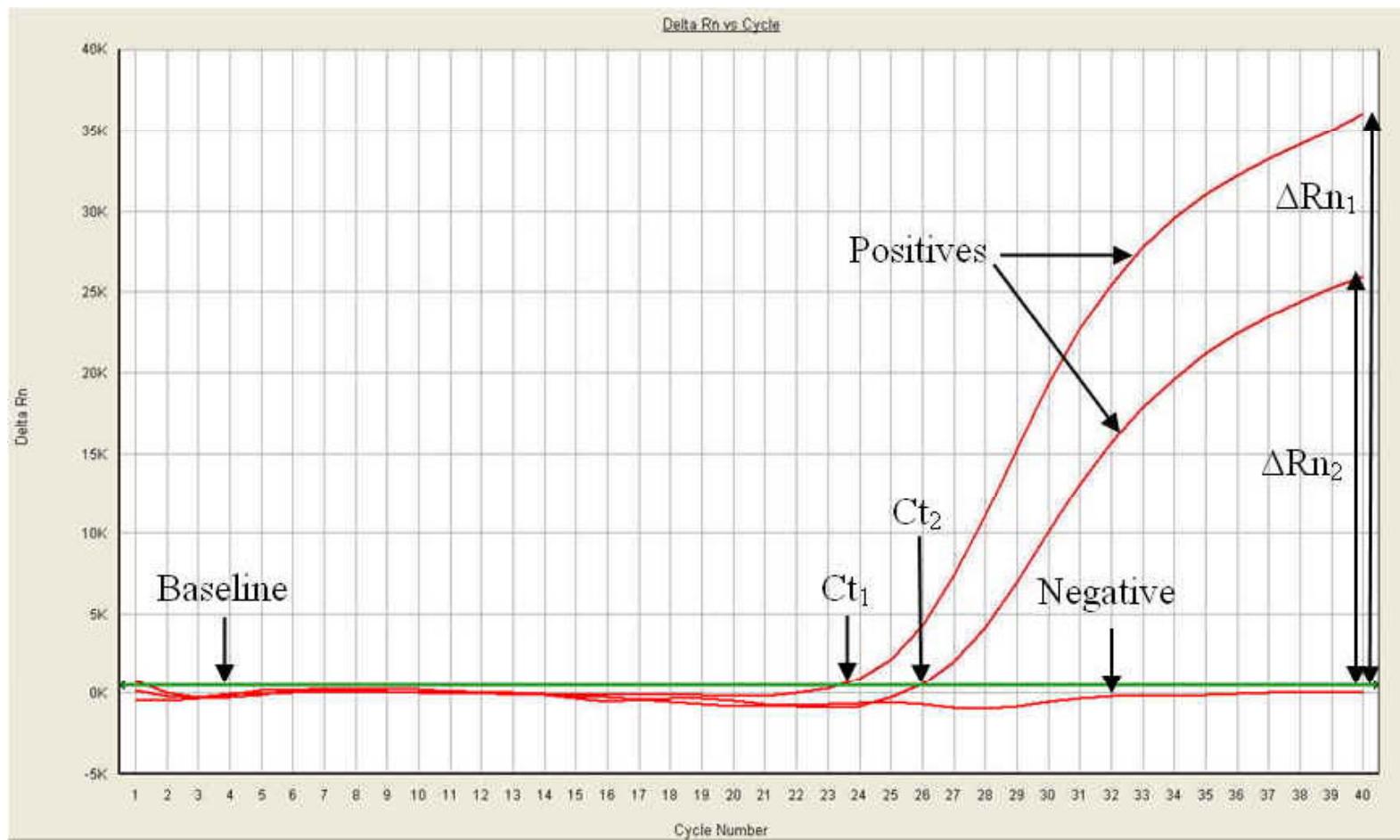
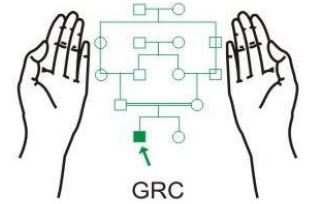
Molecular Beacon

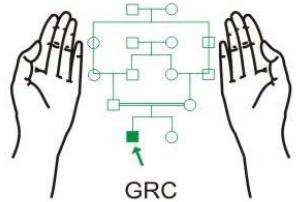




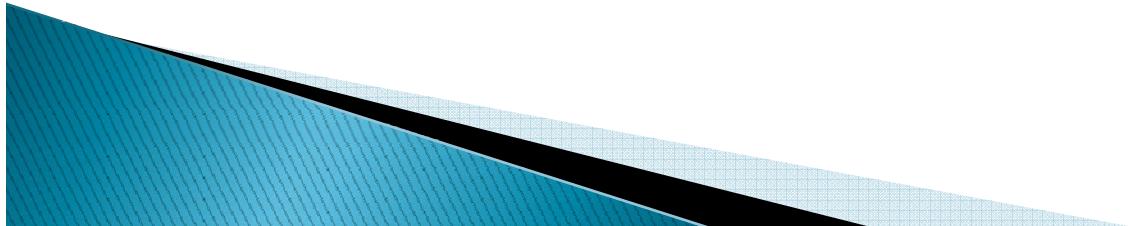
Reporter dye	Incident light wavelength	Emergent light wavelength	Compatible quenchers
FAM/SYBR Green	492	516	TAMRA, DABCYL, BHQ1
TET	517	538	TAMRA, DABCYL, BHQ1
HEX/JOE/VIC	535	555	TAMRA, DABCYL, BHQ1
Cy3	545	568	BHQ2
TAMRA	556	580	BHQ2
ROX/Texas Red	585	610	BHQ2
Cy5	635	665	BHQ3



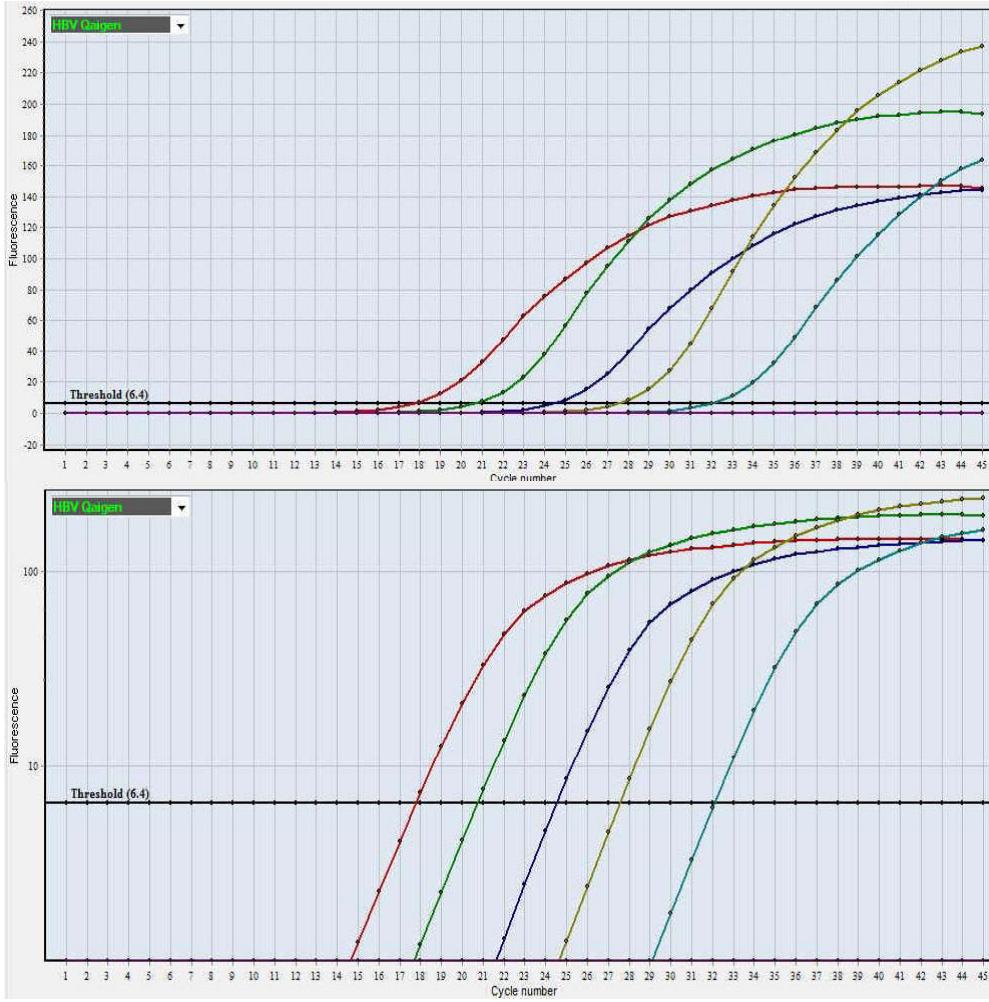




Cycle No.	DNA Molecules	Cycle No.	DNA Molecules	Cycle No.	DNA Molecules
1	2	11	2048	21	2,097,152
2	4	12	4096	22	4,194,304
3	8	13	8192	23	8,388,608
4	16	14	16,384	24	16,777,216
5	32	15	32,768	25	33,554,432
6	64	16	65,532	26	67,108,864
7	128	17	131,072	27	134,217,728
8	256	18	262,144	28	268,435,456
9	512	19	524,288	29	536,870,912
10	1024	20	1,048,576	30	1,073,741,824

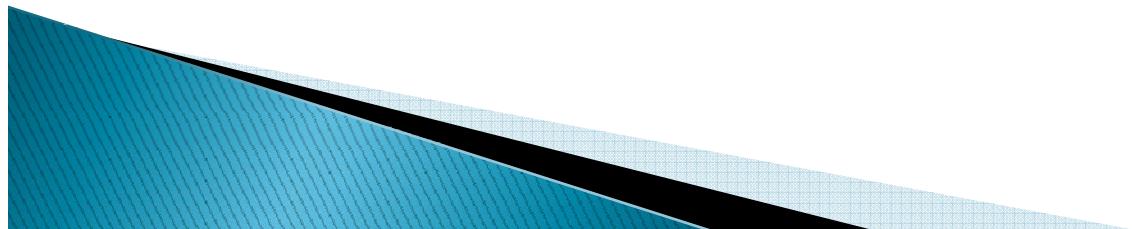


Linear Vs Log Recording

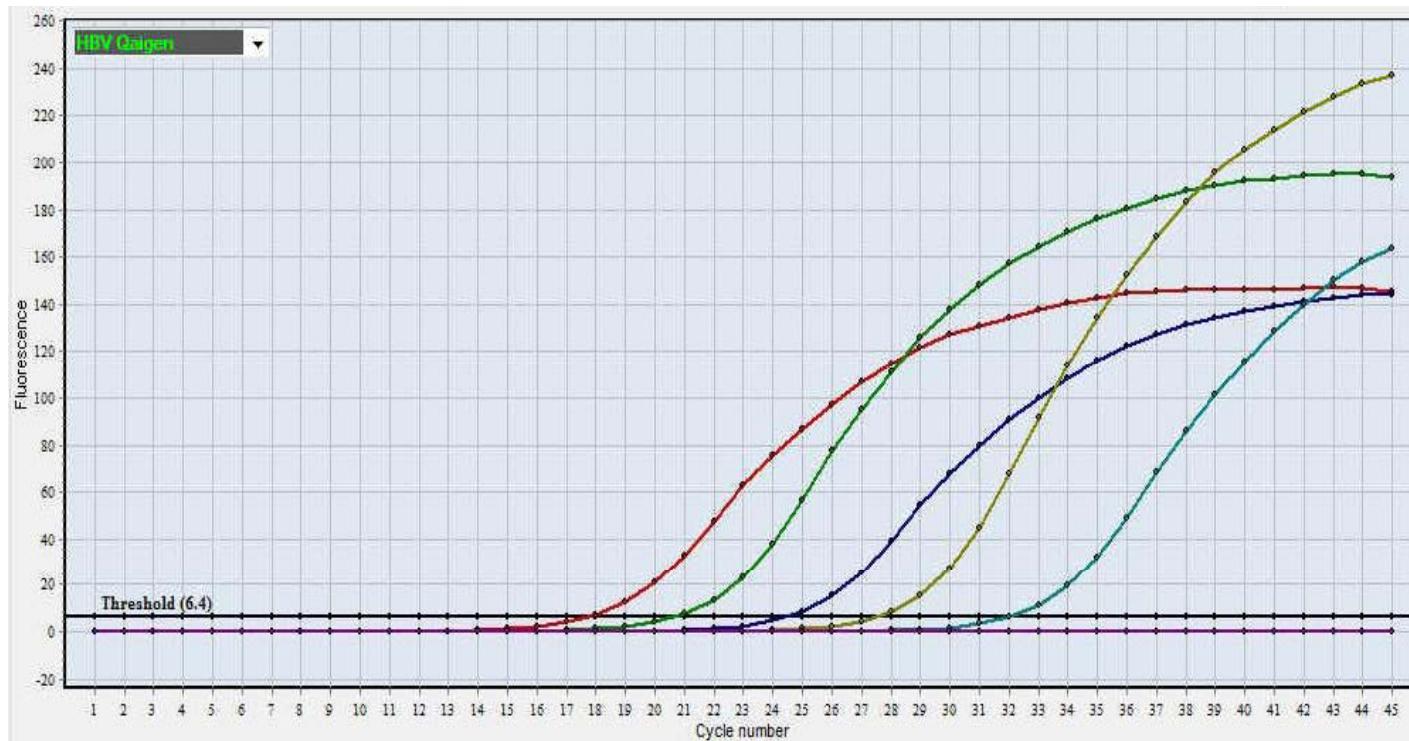


Q-PCR

- ▶ Absolute Quantification
- ▶ Relative Quantification



Absolute Quantification



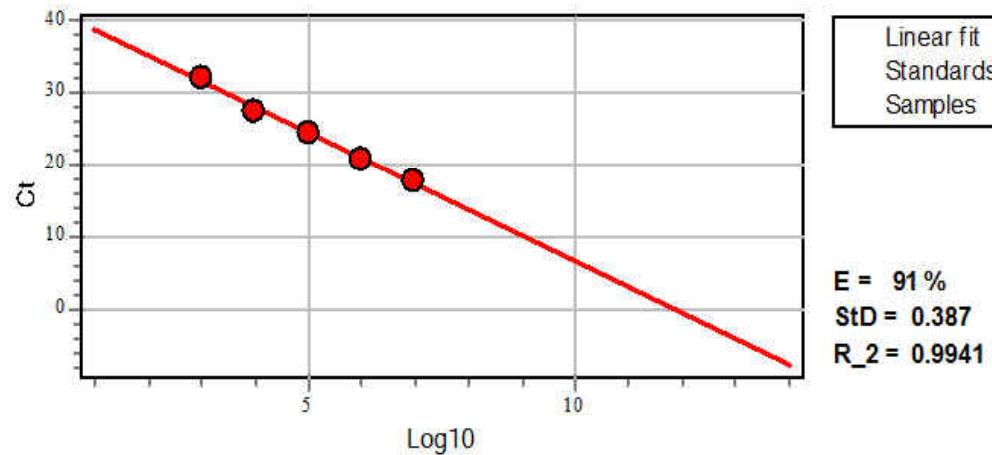
Standard Curve

Quantitative analysis

Number of the hole	Identifier of the tube	Ct, Fam	Ct, Hex	Concentration, copies/ml
A1	QS-1 (HBV Qaigen)	17.7		1.0E+07
A2	QS-2 (HBV Qaigen)	20.7		1.0E+06
A3	QS-3 (HBV Qaigen)	24.4		100,000
A4	QS-4 (HBV Qaigen)	27.5		10,000
A5	QS-5 (HBV Qaigen)	32.1		1,000
A6	C_- (HBV Qaigen)			

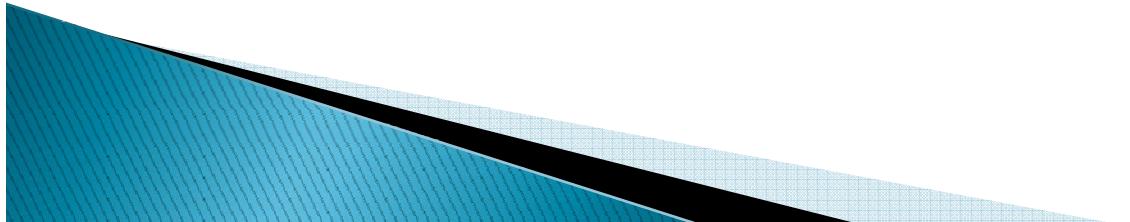
* Manual(Threshold) analysis method (B.F) Threshold_FAM = 6.4 Threshold_HEX = 0.0

$$Y = 42.211 - 3.549 \cdot X$$

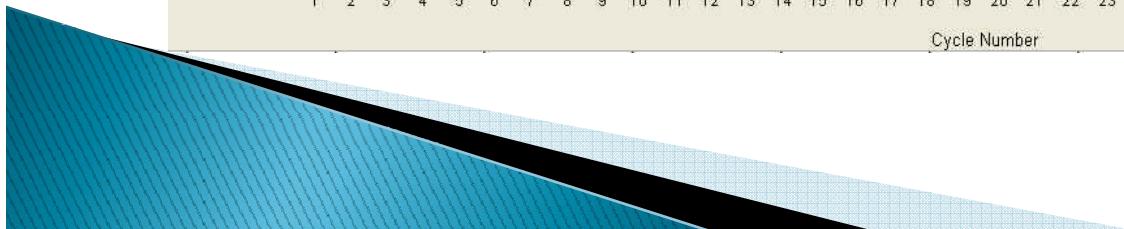
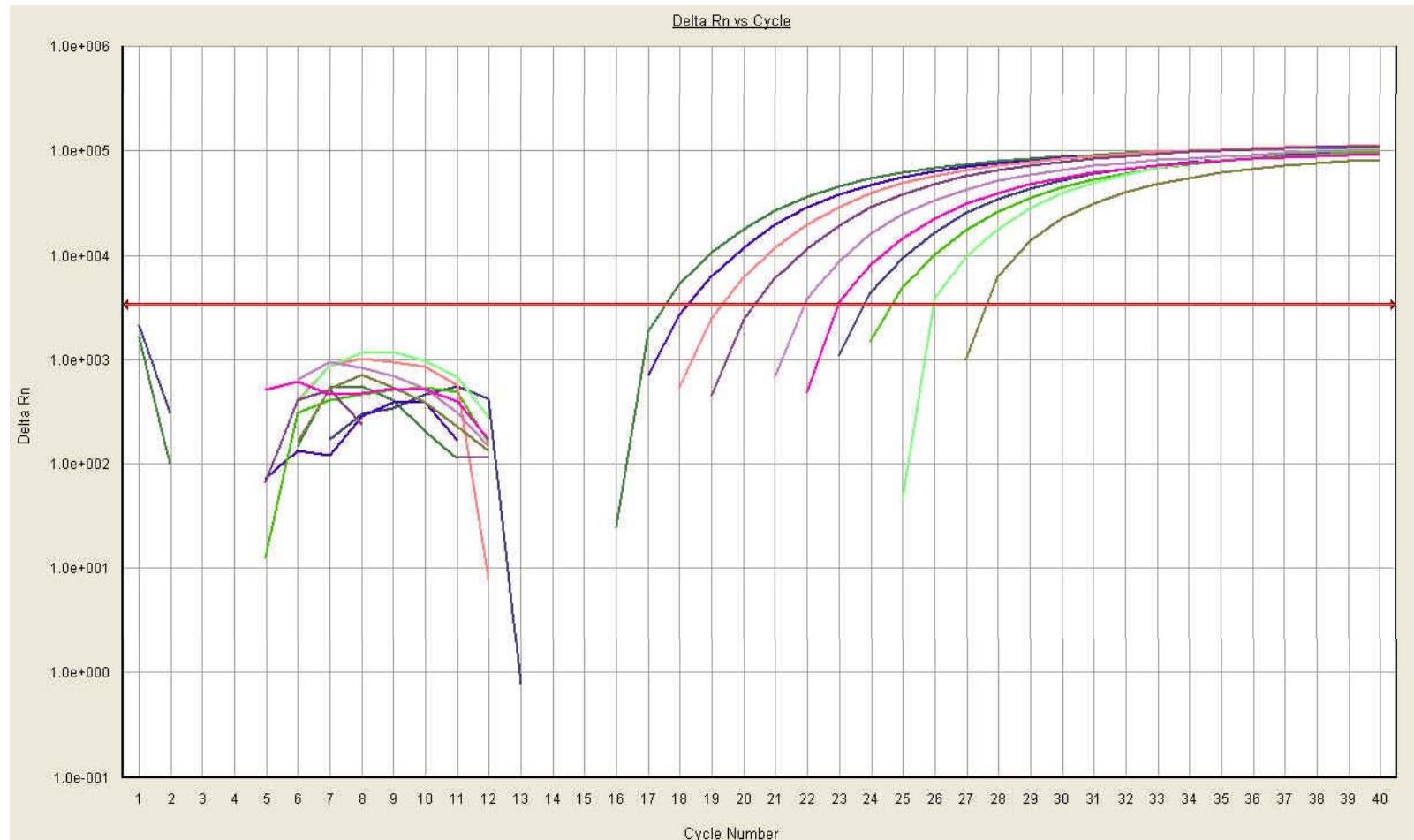


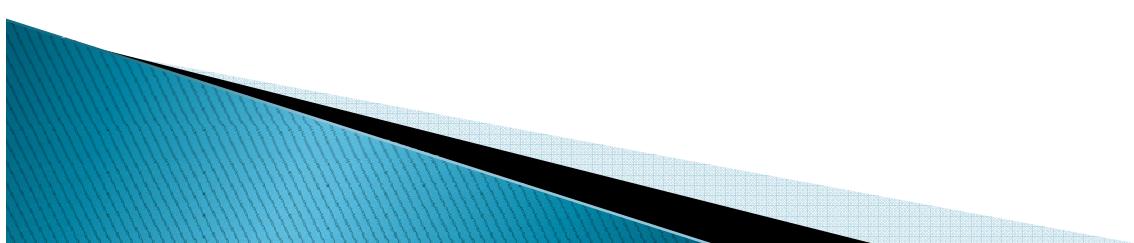
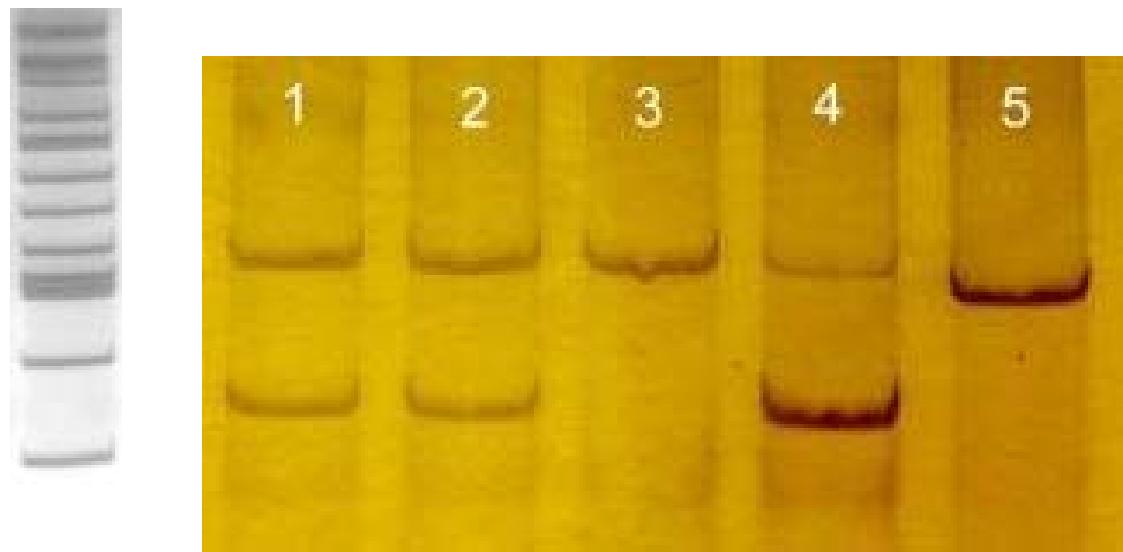
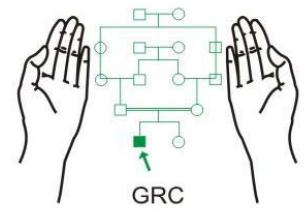
Relative Quantification

- ▶ $\Delta Ct = Ct \text{ target} - Ct \text{ reference (standard)}$
- ▶ The amount of target: $2^{-\Delta Ct}$
- ▶ Example-1:
- ▶ $\Delta Ct = +2.0$
- ▶ $2^{2.0} = 4.0$
- ▶ Or the sample has four times (fold) DNA or RNA as compared to that present in the reference (standard).
- ▶ Example-2:
- ▶ $\Delta Ct = -2.0$
- ▶ $2^{-2.0} = 0.25$
- ▶ Or the sample has 0.25 (1/4) fold DNA or RNA as compared to that present in the reference (standard).

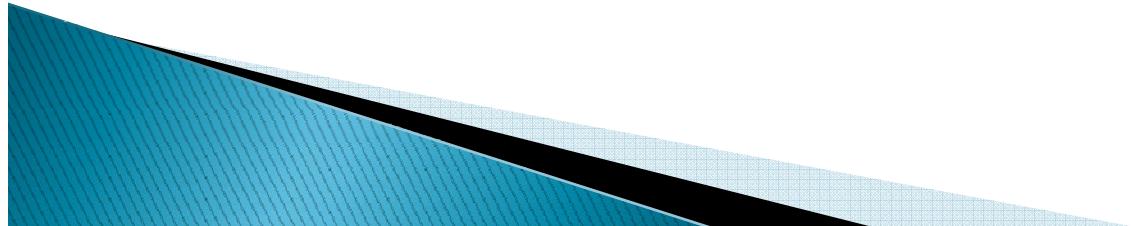


Q-PCR by Gel Electrophoresis

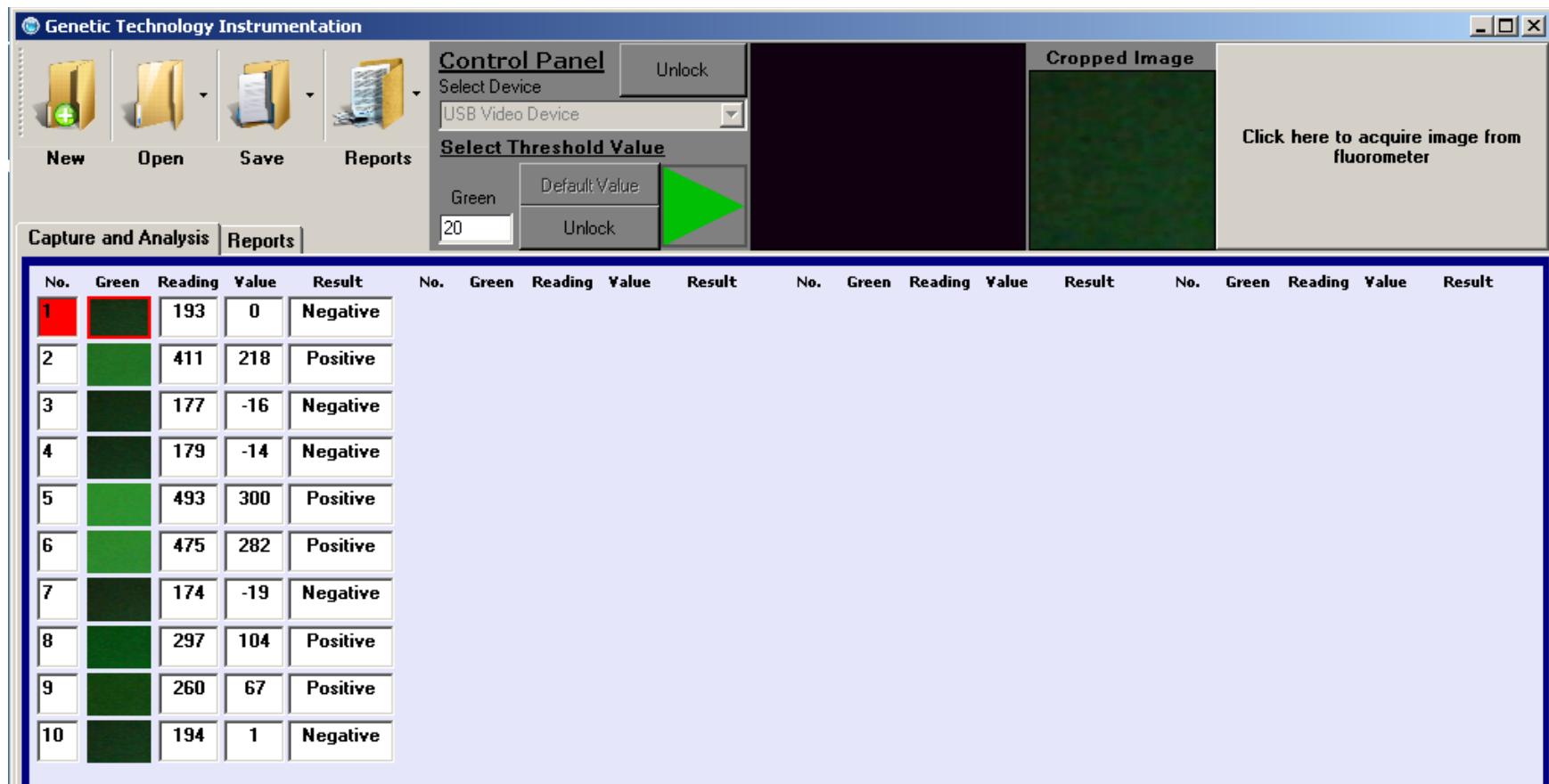


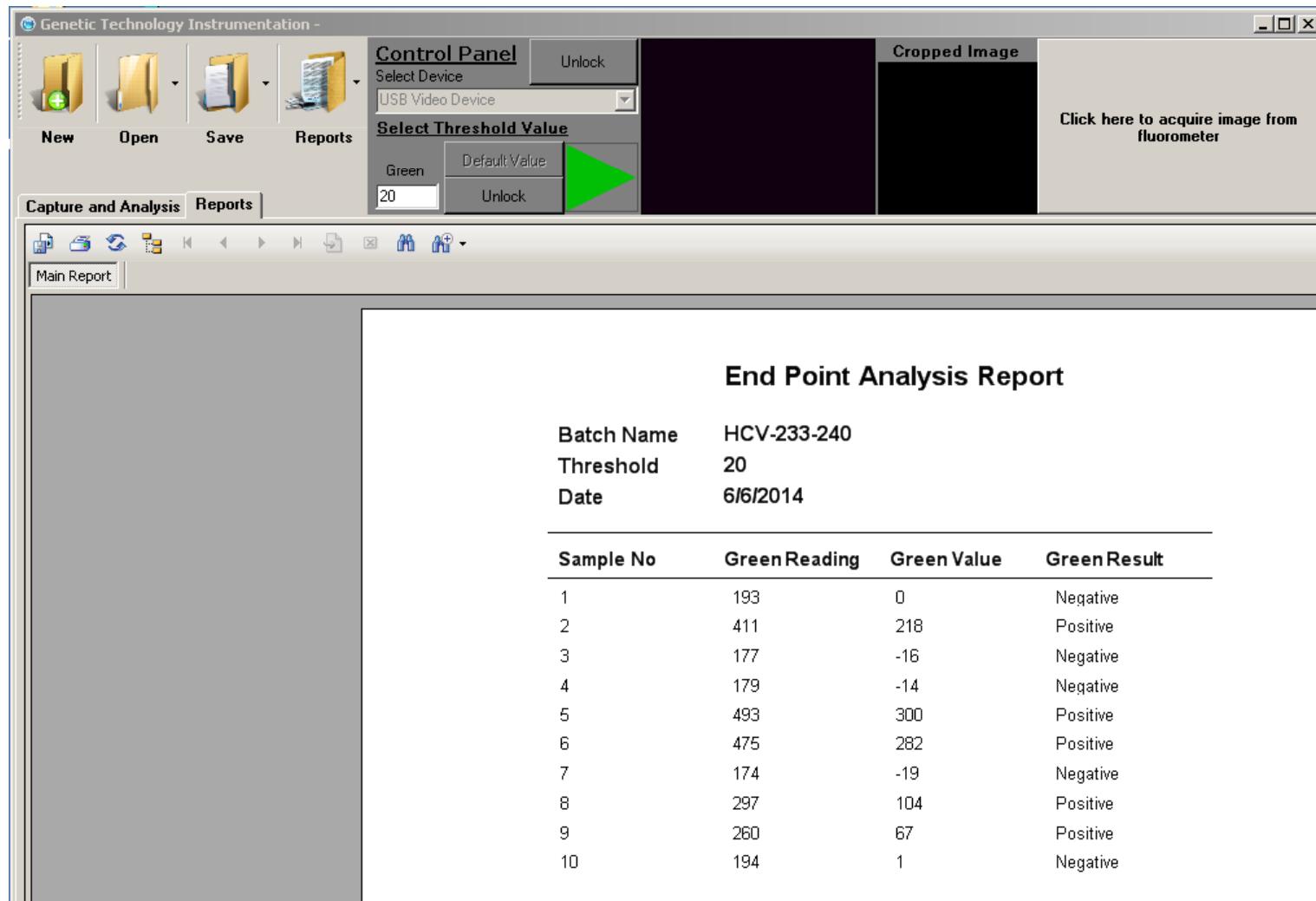


Q-PCR by Endpoint Fluorescence



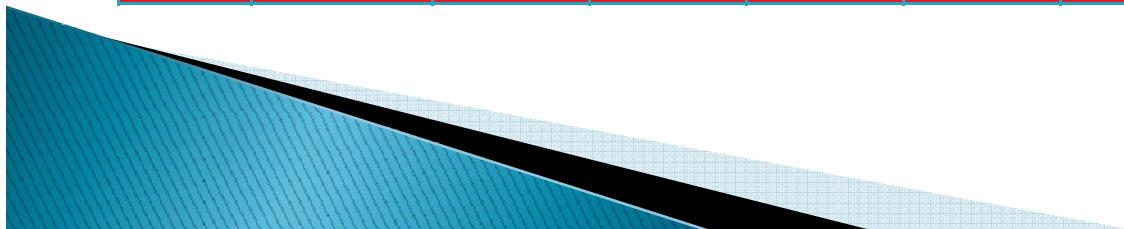
Q-PCR by Endpoint Fluorescence





Q-PCR by Endpoint Fluorescence

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



Q-PCR by Endpoint Fluorescence

