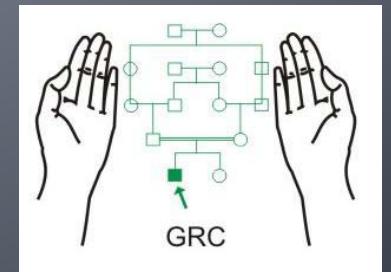


Molecular Diagnosis of Malaria and Dengue

Maj Gen (R) Suhail Ahmed, HI (M)
MBBS; MCPS; FCPS; PhD (London)

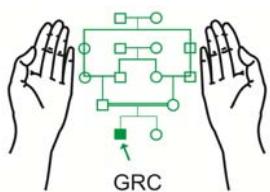
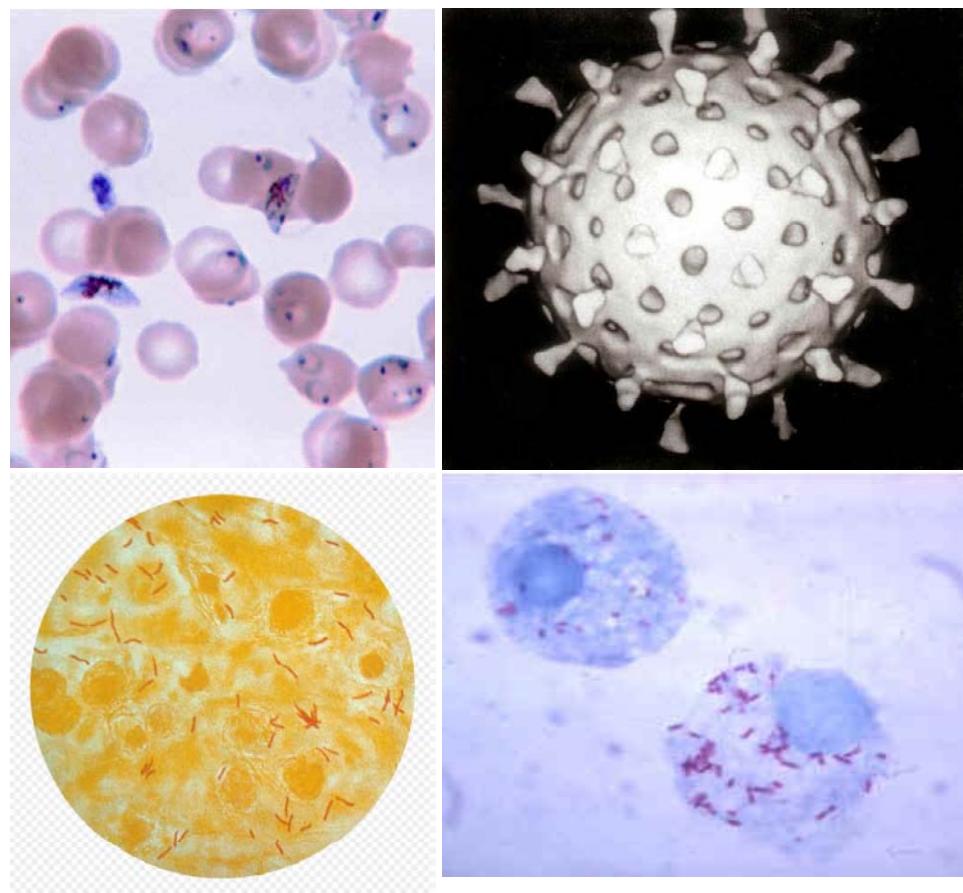
Genetics Resource Centre (GRC)

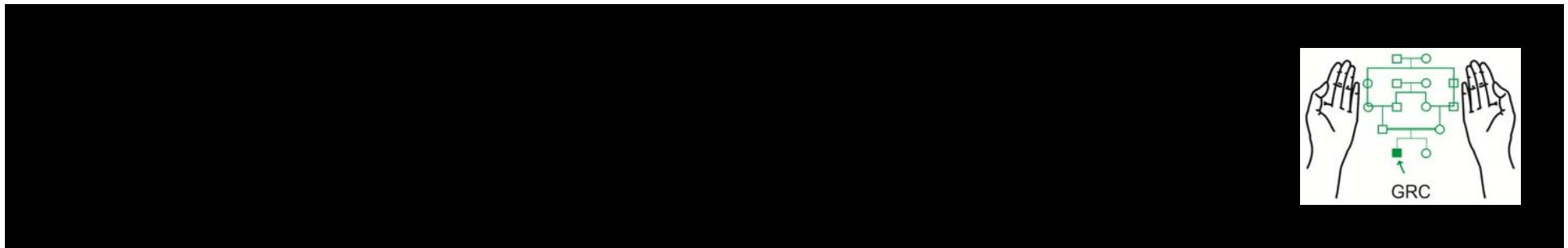


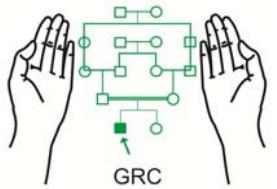
www.grcpk.com

Molecular Diagnosis of Infectious Disorders

- Microbial
- Parasitic
- Viral
- Fungal
- Rickettsial

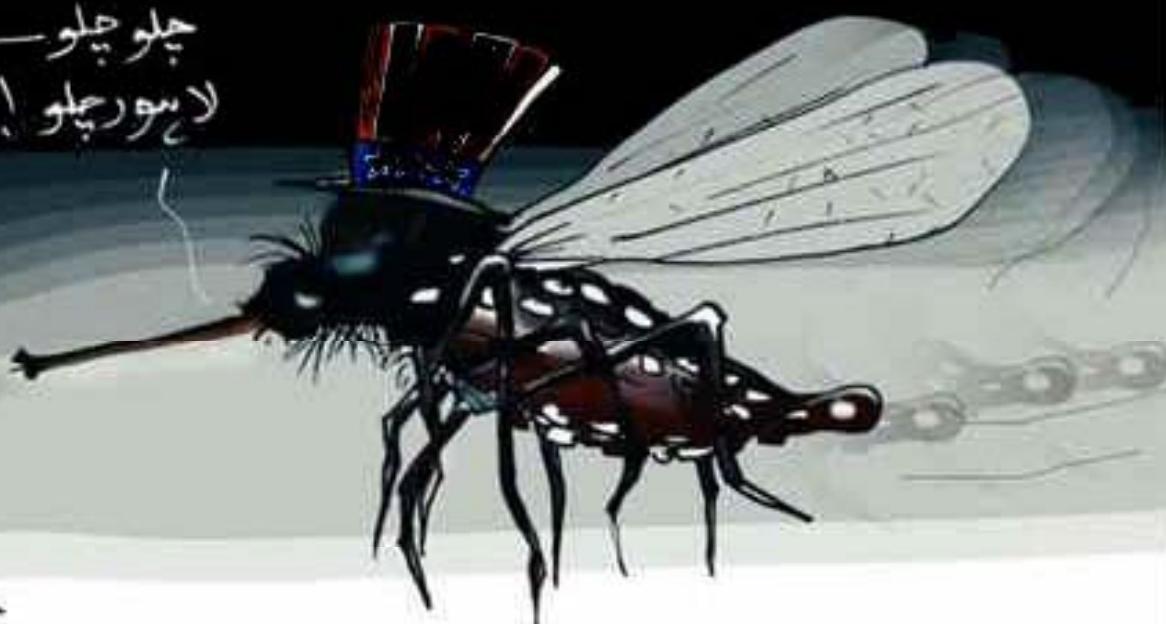






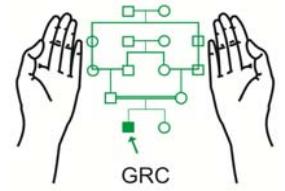
کراچی کے بعد لاہور میں بھی ڈینگی کا پھیلاؤ ۔

چلو چلو ۔
لاہور چلو ।



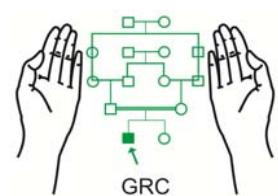
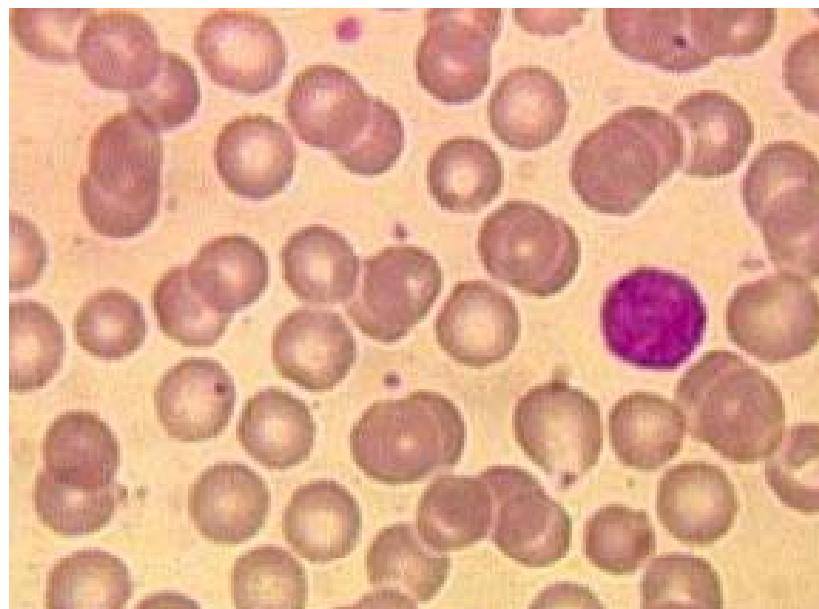
خواجہ

18-10-2010

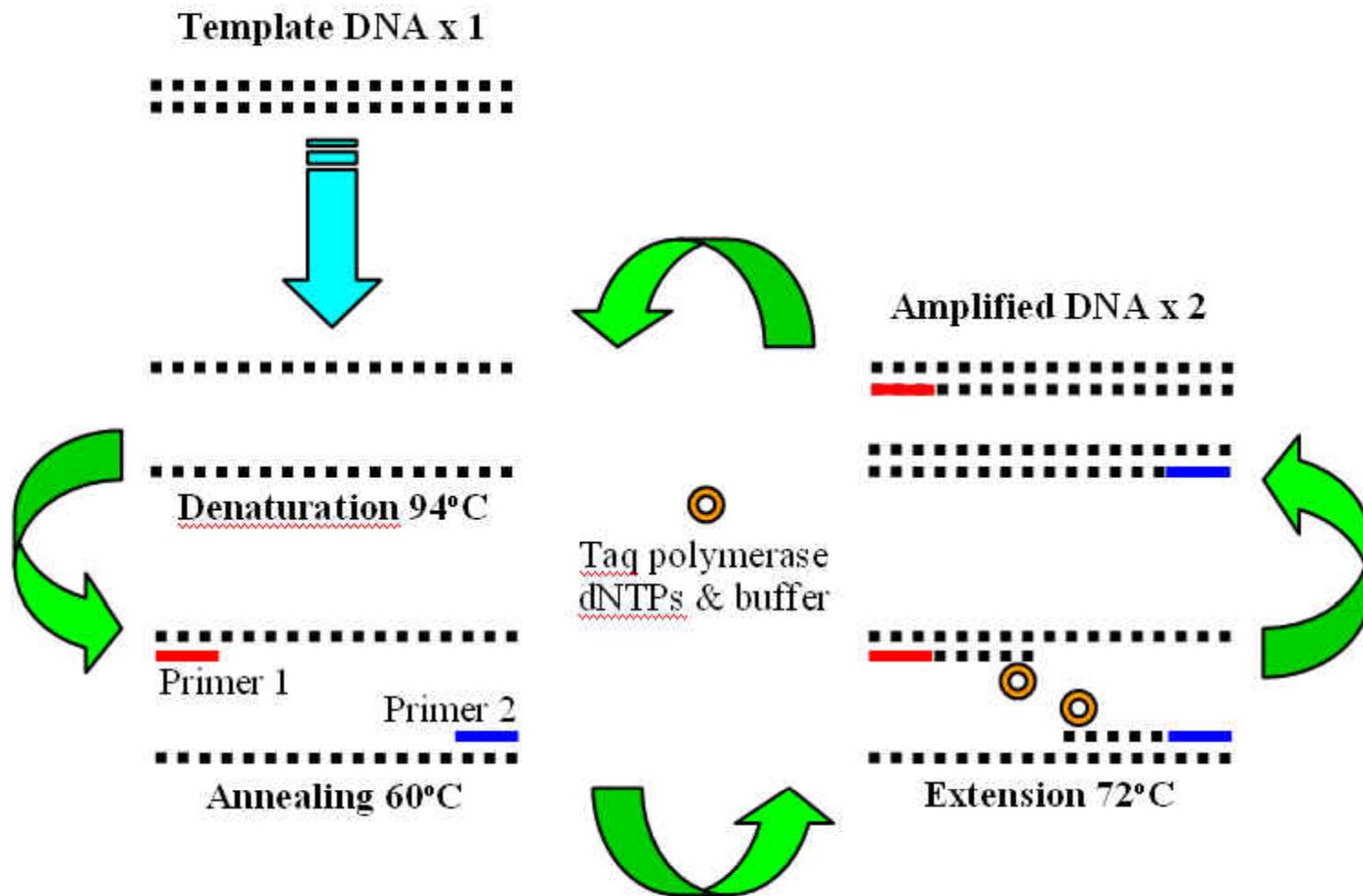
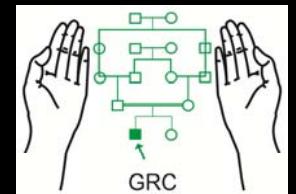


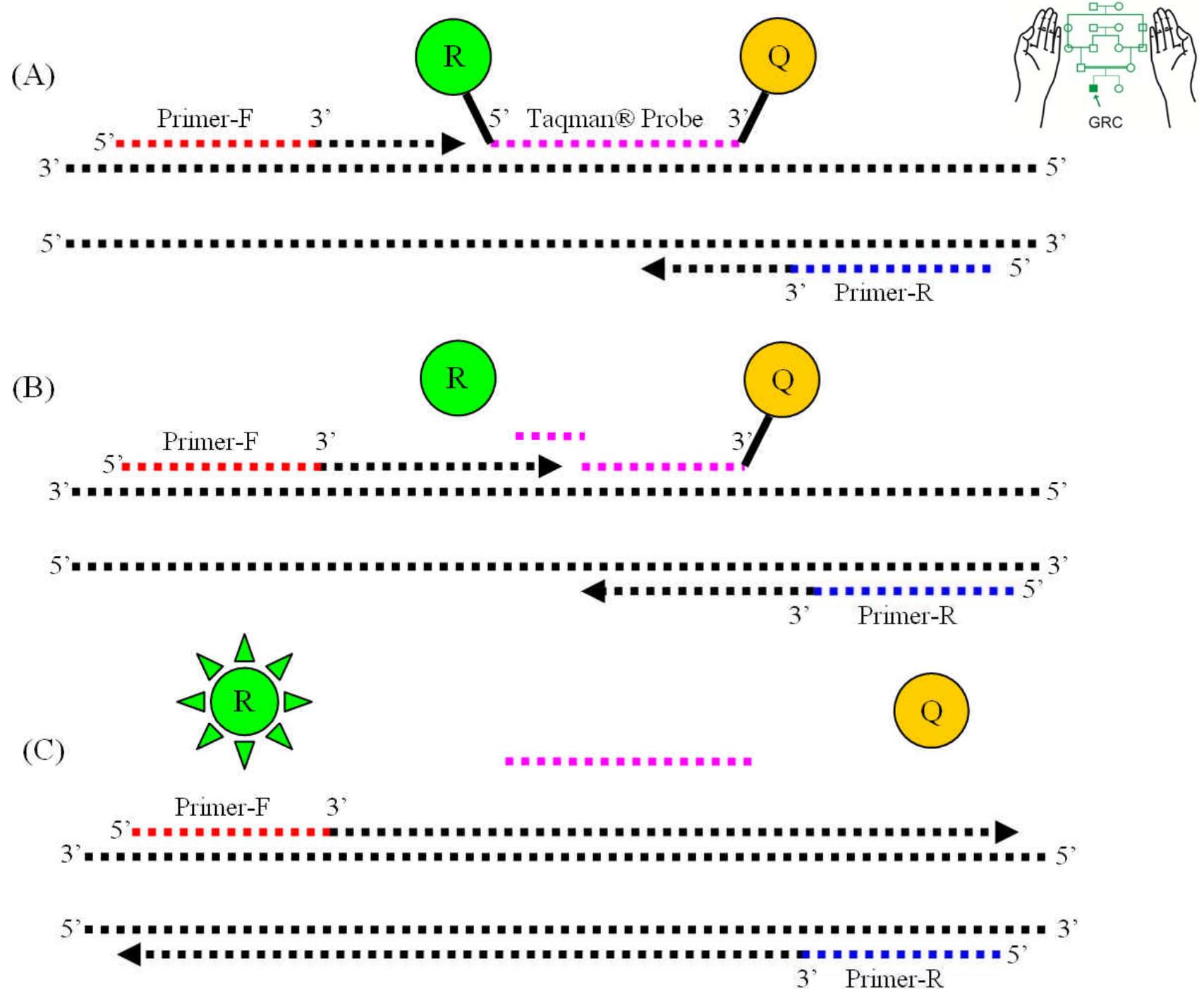
A young man with three days history of high grade fever with chills, headache and vomiting

- WBC: $4.3 \times 10^9/L$
 - RBCs: $5.12 \times 10^{12}/L$
 - Hb: 18.2 g/dl
 - MCV: 83.5 fl
 - MCV: 28.2 pg
 - Platelets: 63
-
- Anti Dengue IgM: Negative

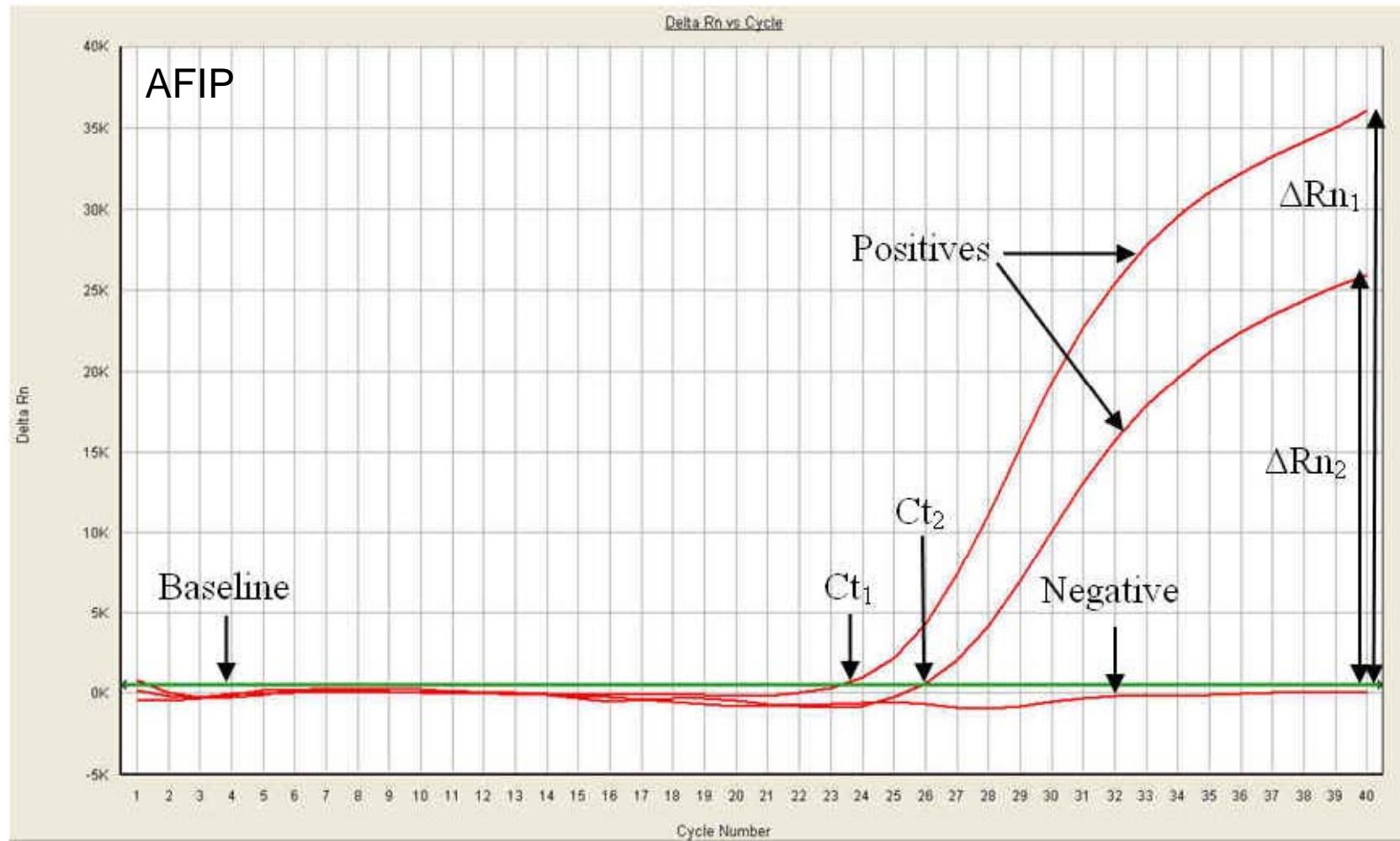
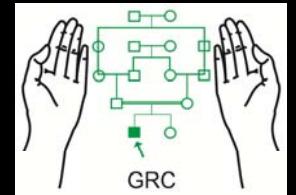


PCR

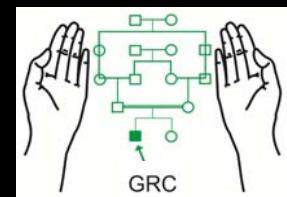




Real Time PCR



SSU rRNA genes



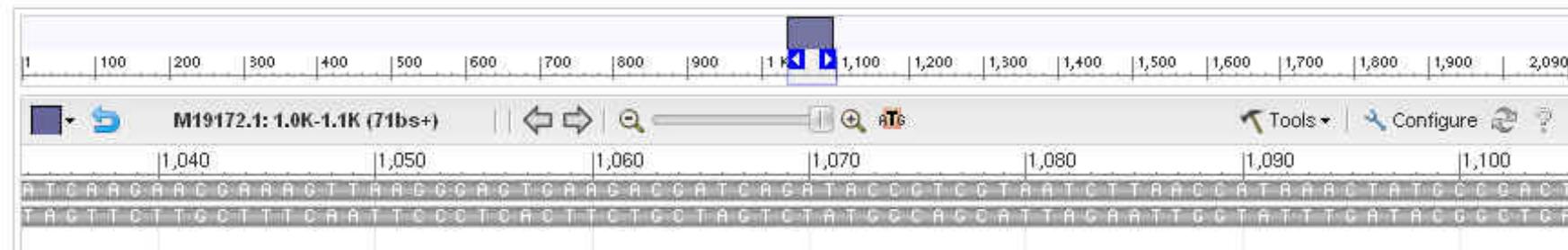
- *Plasmodium falciparum* (M19172)
- *Plasmodium vivax* (X13926)
- *Plasmodium malariae* (M54897)
- *Plasmodium ovale* (L48987)

P.falciparum 18S ribosomal RNA in asexual parasites

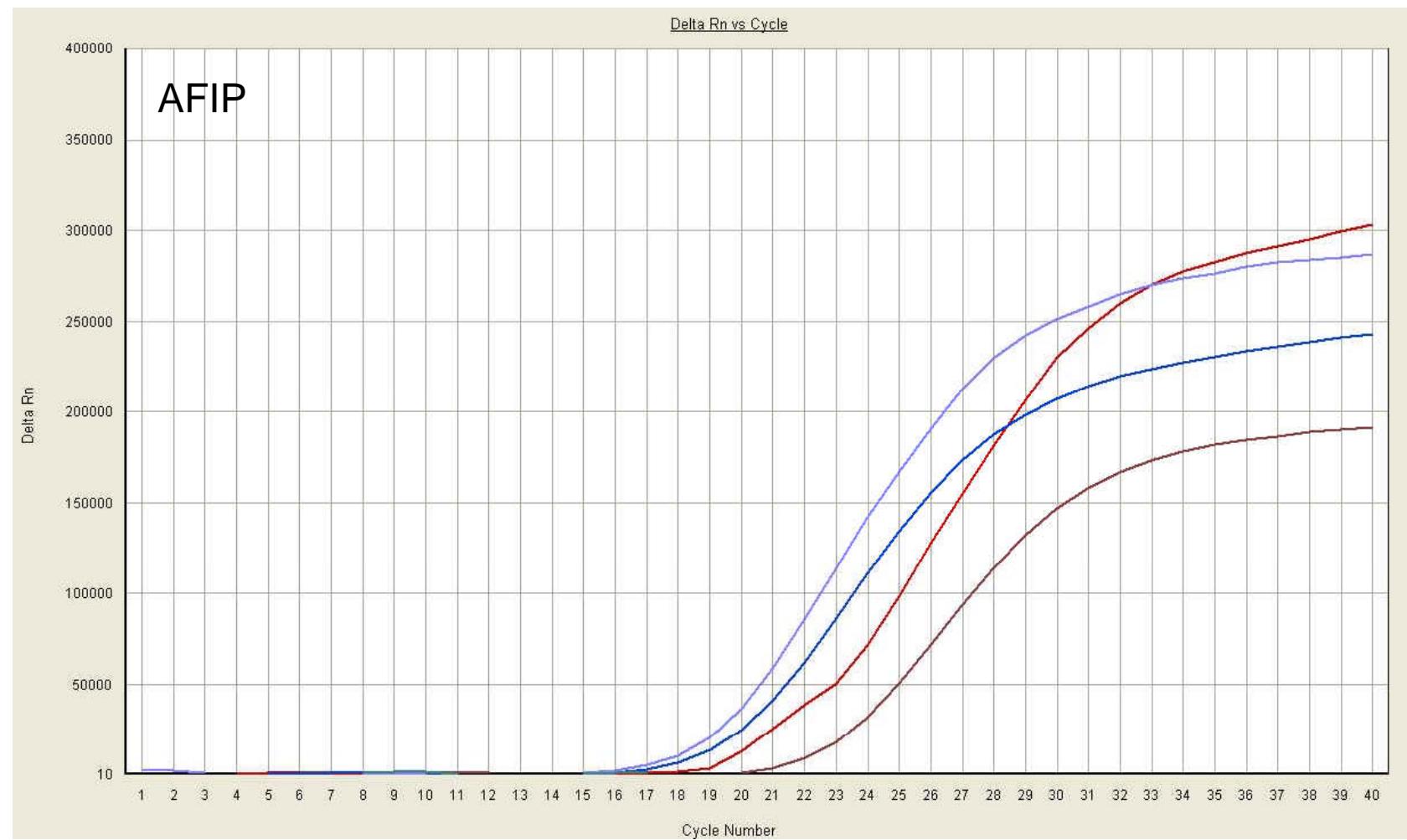
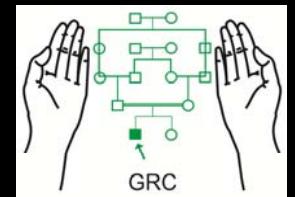
GenBank: M19172.1

[GenBank](#) [FASTA](#)

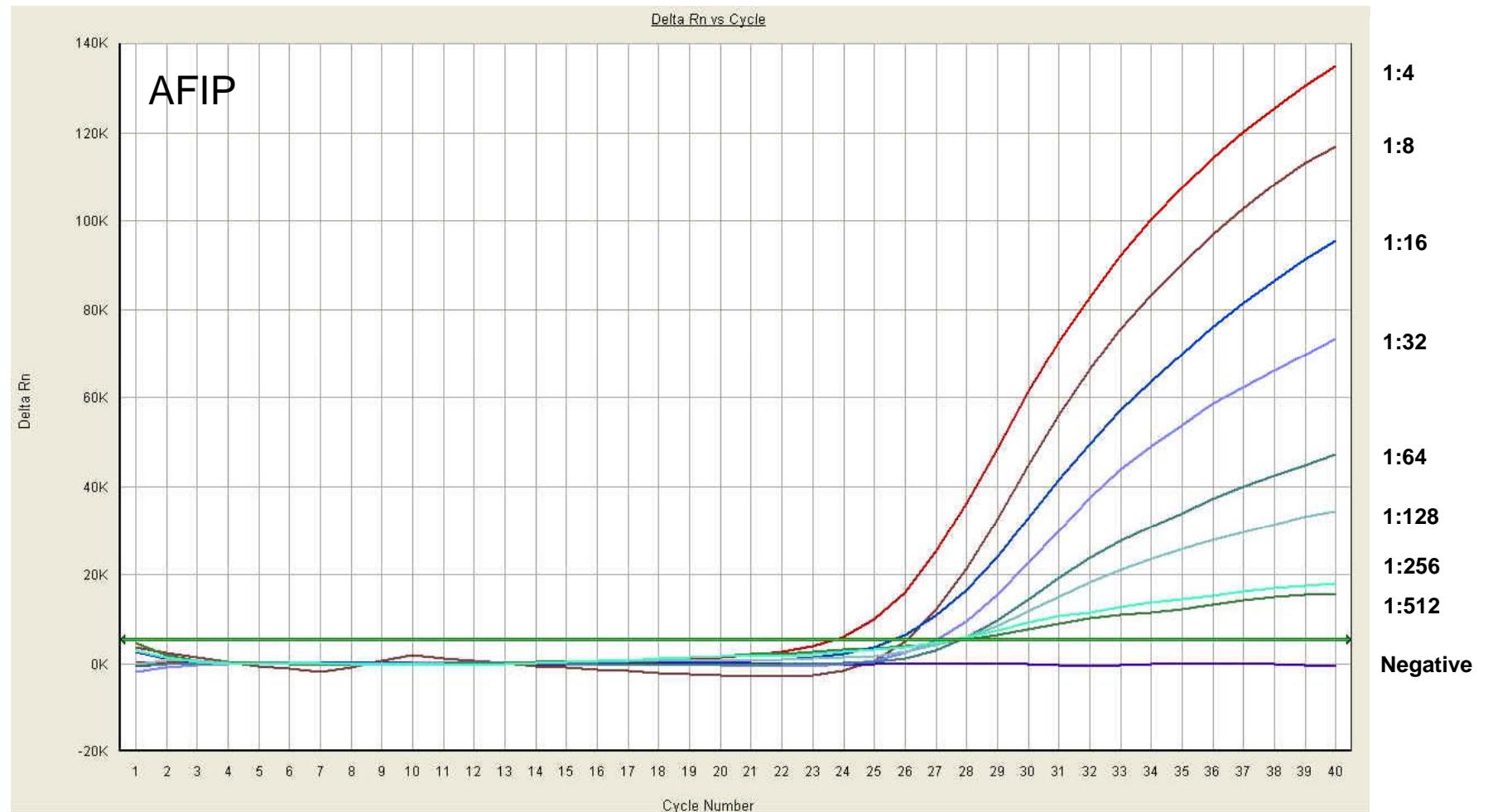
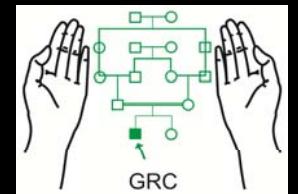
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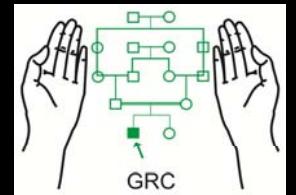
Real Time PCR for Malaria



PCR vs Microscopy in Malaria

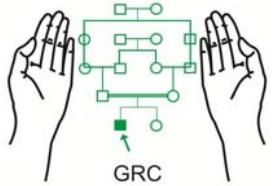


Microscopy PCR & ICT in Malaria



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

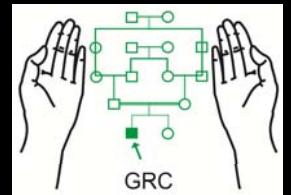
(Saleem et al, 2013)



<u>Sample No</u>	<u>ABI 7500 (CT)</u>	<u>Fluorometer</u>	<u>Microscopy</u>	<u>ICT Malaria</u>
15	28.71	59	Negative	Negative
17	25.2	220	Negative	Negative
35	27.22	120	Negative	Weak Positive
47	25.26	198	Negative	Negative
49	28.14	131	Negative	Weak Positive
63	26.19	177	Negative	Negative
65	30.46	80	Negative	Negative
109	29.33	60	Negative	Negative
123	30.3	47	Negative	Negative
124	27.34	110	Negative	Negative
132	28.29	109	Negative	Negative
141	26.08	105	Negative	Negative
163	28.53	90	Negative	Negative
186	26.54	204	Negative	Negative
238	32.46	29	Negative	Negative
284	28.95	100	Negative	Negative

(Ahmed & Saleem 2011, unpublished data)

Dengue Virus



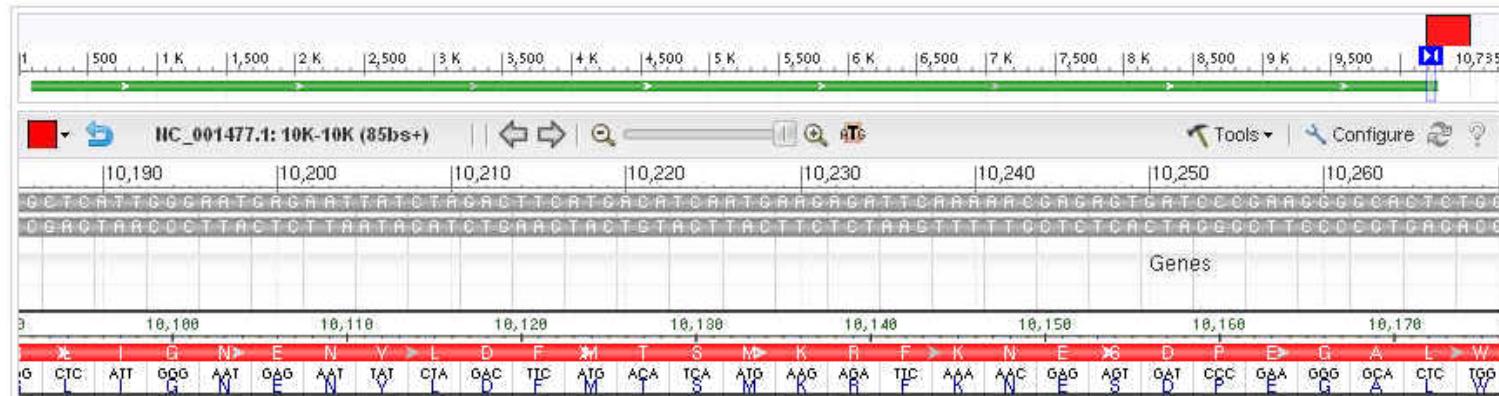
- *Dengue Virus Type-1 (NC_001477.1)*
- *Dengue Virus Type-2 (AF038403.1)*
- *Dengue Virus Type-3 (NC_001475.2)*
- *Dengue Virus Type-4 (NC_002640.1)*

Dengue virus 1, complete genome

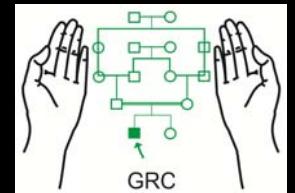
NCBI Reference Sequence: NC_001477.1

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3'-UTR of Dengue Virus



Dengue Virus Type-1 (NC_001477.1)

- **ACAACAA**CAAACAGCATATTGACGCTGGGA**G**AGACCAGAGATCCTGCTGTCTACAG
CATCATTCCAGGCACAGAACGCCA**AAAA**ATGGAATGGTGCTGTTGAATCACACAGGTTCT

Dengue Virus Type-2 (AF038403.1)

- **AAAACAA**AAACAGCATATTGACGCTGGGA**A**AGACCAGAGATCCTGCTGTCTCAG
CATCATTCCAGGCACAGAACGCCA**GAAA**ATGGAATGGTGCTGTTGAATCACACAGGTTCT

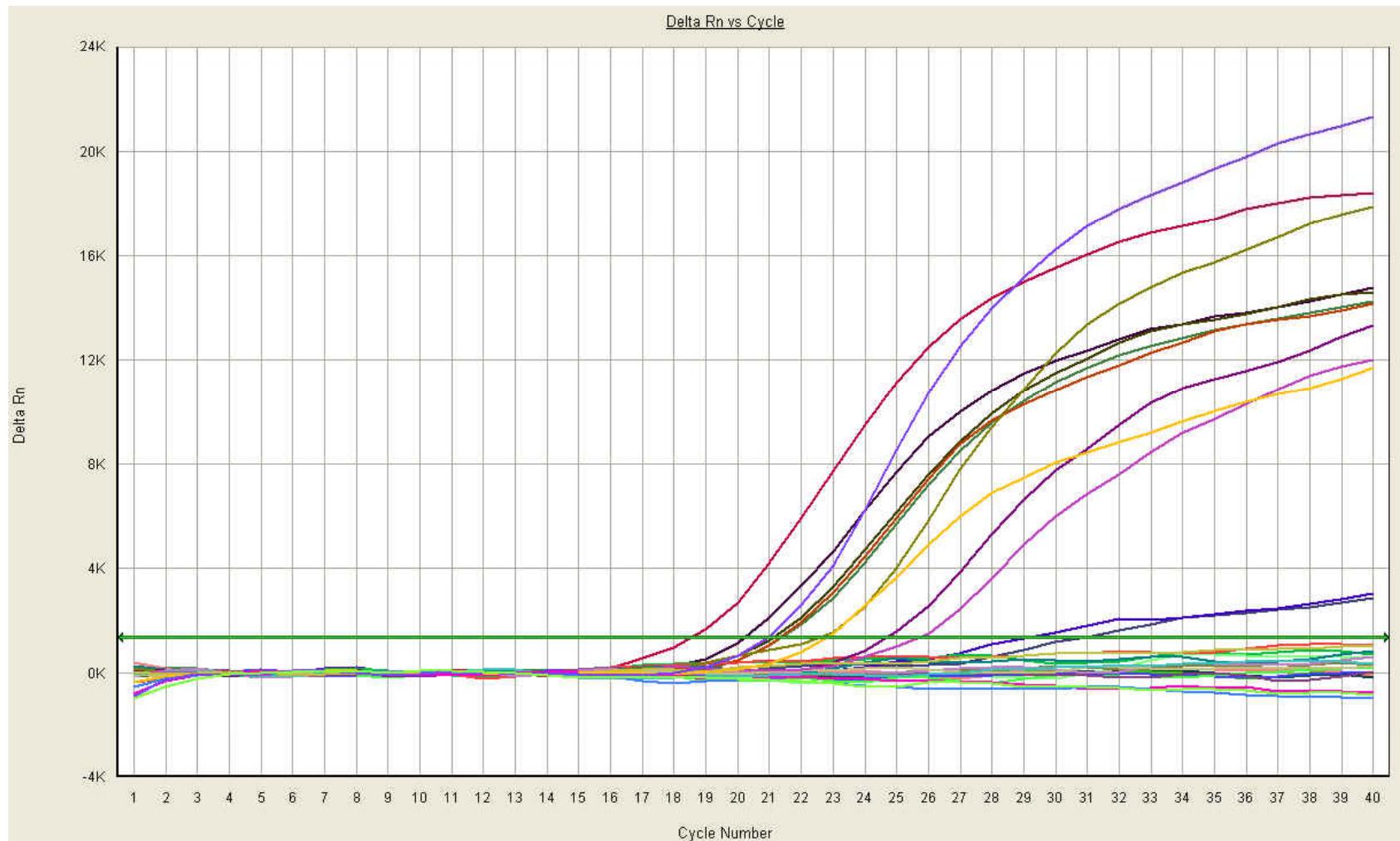
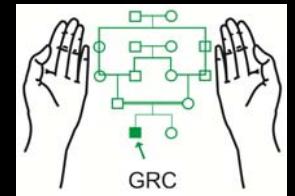
Dengue Virus Type-3 (NC_001475.2)

- **GCAAATA**AAACAGCATATTGACGCTGGGA**G**AGACCAGAGATCCTGCTGTCTCAG
CATCATTCCAGGCACAGAACGCCA**GAAA**ATGGAATGGTGCTGTTGAATCACACAGGTTCT

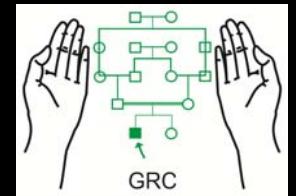
Dengue Virus Type-4 (NC_002640.1)

- **CCAACACA**AAACAGCATATTGACGCTGGGA**A**AGACCAGAGATCCTGCTGTCTGCA**A**
CATCA**A**TCCAGGCACAGA**GCGCCG**CAAGATGGA**TTGGTGT**TTGTTGA**T**CCAACACAGGTTCT

Real time PCR for Dengue



PCR for Malaria & Dengue



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

Cost of Consumables for Real Time PCR

■ Malaria:

- DNA extraction (Chelix method) Rs. 60
- Real time PCR (in house by Taqman probe): Rs. 40
- Total Rs. 110

■ Dengue:

- RNA extraction (commercial microcolumn) Rs. 225
- cDNA synthesis (in house) Rs. 80
- PCR (in house) Rs. 40
- Total Rs. 345

