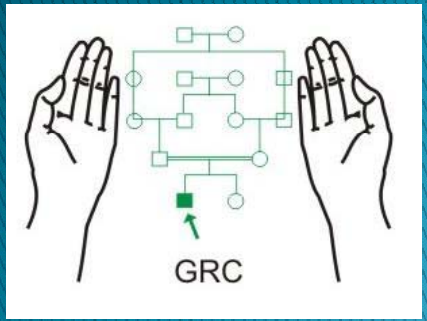
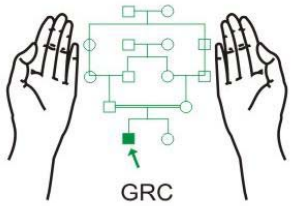
Polymerase Chain Reaction (PCR-III)

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

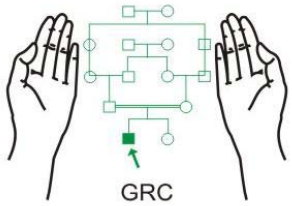
Genetics Resource Centre (GRC)



www.grcpk.com

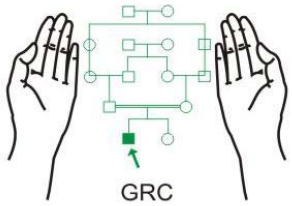


Short Tandem Repeat (STR) Analysis



Human Genome

- Nuclear DNA (3000 Mb)
  - Genes (20%)
  - Extra-genic DNA (80%)
    - Unique DNA (75%)
    - Repetitive DNA (25%)
      - Non Tandem Repeats
      - Tandem Repeats
- Mitochondrial DNA (16.6 Kb)



GATCCTAGAGTTACGTACGTAGTCATTCAAGCTAGGTAACGCCACTAGAC
CGTACGTAGTCATTCAAGCTAGGTAACGCCACTACGTACGTAGTCATTGG
CAAGCTAGGTAATTACGTACGTAGTCATTCAAGCTCGCCACTACGTACGC
GTAGTCATTCAAGCTAGGTAACGGATCCTAGAGTTACGTACGTCCACTAT
AGATCCTAGAGTTACGTCCGT CTGG CTGG CTGG CTGG CTGG CTGG
CTGG CTGG CTGG CTGG CTGG CTGG CTGG CTGG CTGG CTGG CTGG
CTGG CTGG CTGG CTGG GTCATTCAAGCTAGGTAACGCCACTACAGTCG
GTAGAATCG GATCCTAGAGTTACGTACGTAGTCATTCAAGCTAGGTAACG
CCACTAGACCGTACGTAGTCATTCAAGCTAGGTAACGCCACTACGTACGTA
GTCATTGGCAAGCTAGGTAATTACGTACGTAGTCATTCAAGCTCGCCACTA

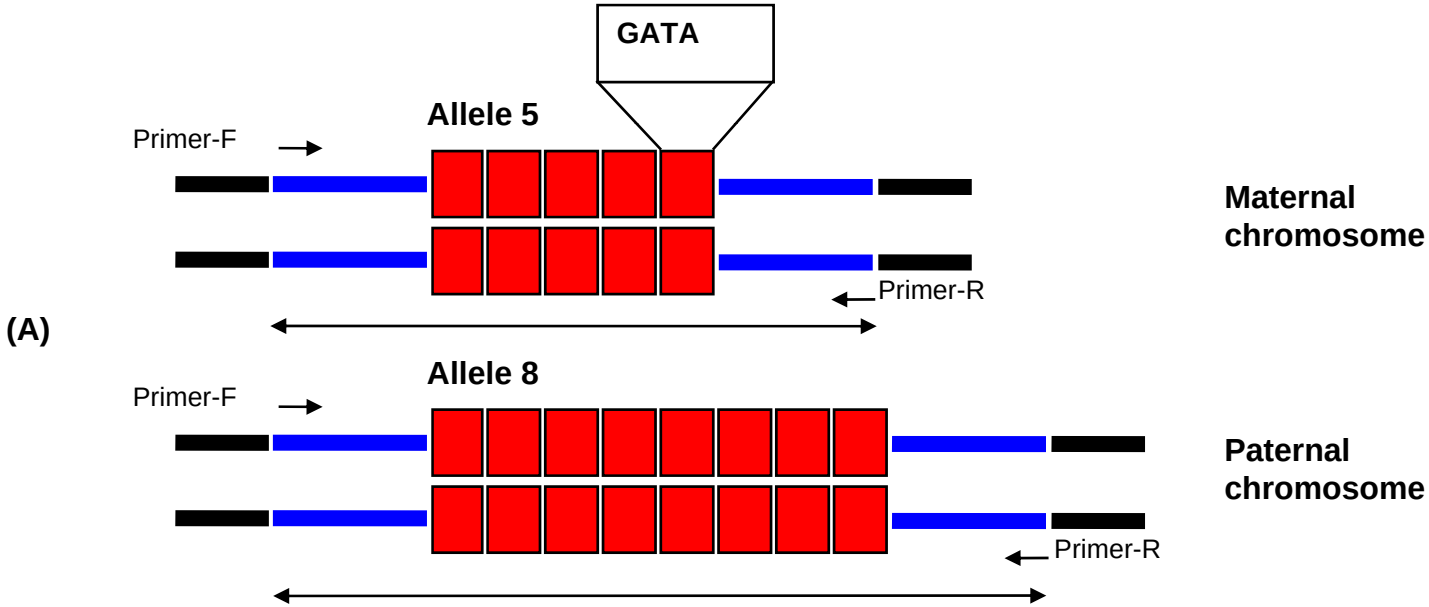
CTGG X 18, 19, 20, 21, 22, 23, 24....

Short Tandem Repeats (STR)

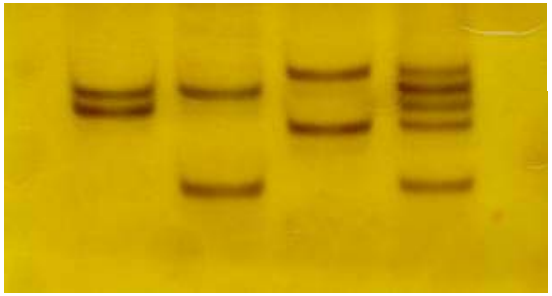
D5S818 (GenBank: G08446.1) The sequence GATA is repeated 11 times.

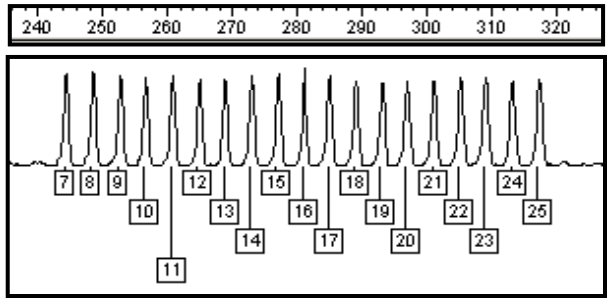
ATATTTTAATAGCAAGTATGTGAC*AAGGGTGATTTTCCTCT*
*TTGG*TATCCTTATGTAATATTTTGAA**GATAGATAGATAGAT**
AGATAGATAGATAGATAGATAGATAGATAGGTAGATAGAGG
TATAAATAAGGATACAGATATAGNTACAAATGTTGTAAACT
GTGGCTATGATTGGAA*TCACTTGGCTAAAAAGCGC*TNAAGC

Short Tandem Repeat (STR) Analysis



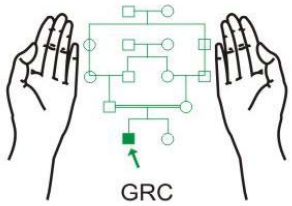
Short Tandem Repeat (STR) Analysis



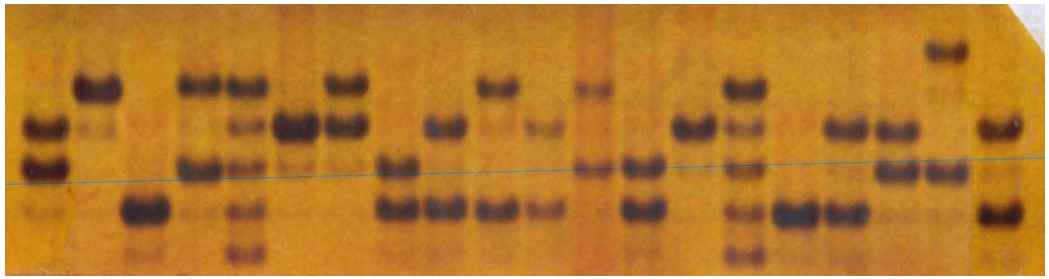


Manual

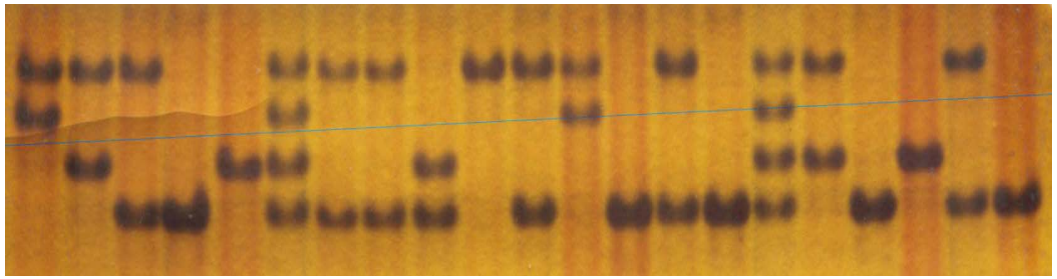
Automated

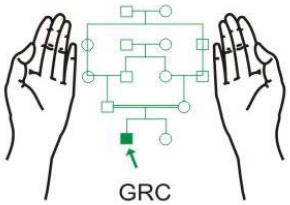


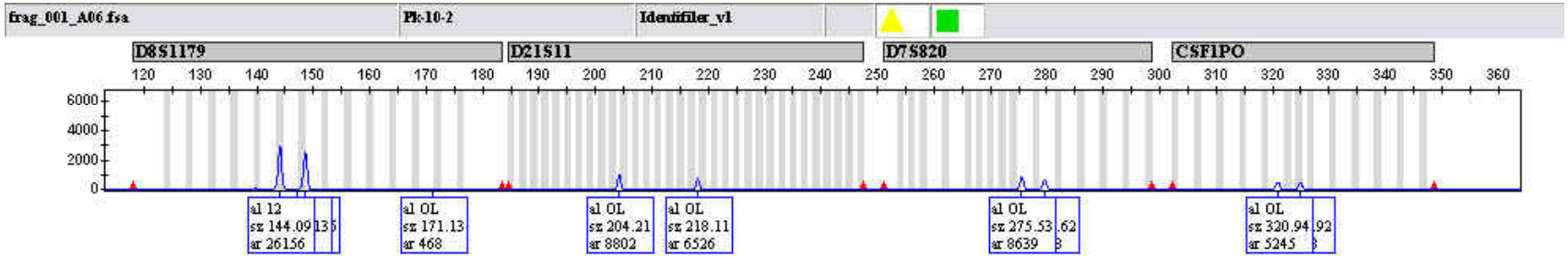
D3S1358

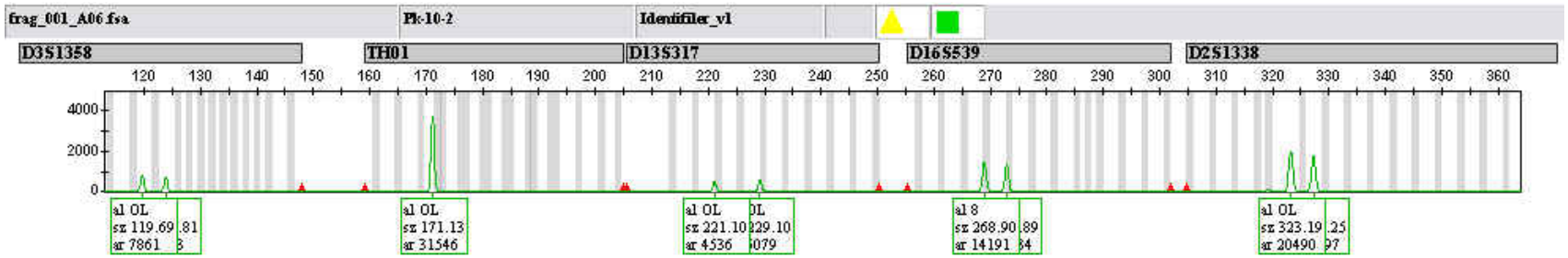


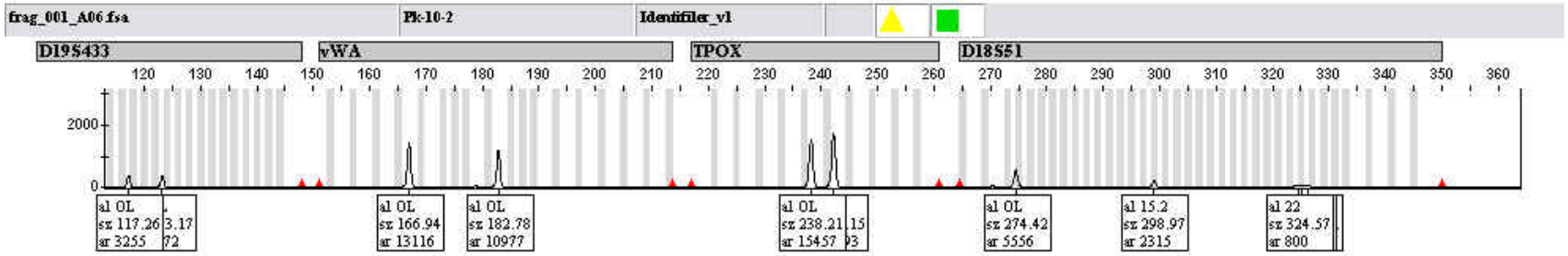
TPOX



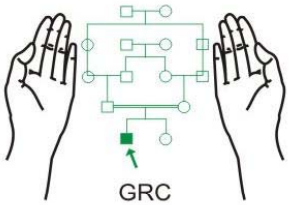

GRC


frag_001_A06.fsa
Pk-10-2
Identifiler_v1
D8S1179
D21S11
D7S820
CSF1PO
al 12
sz 144.09
ar 26156
al OL
sz 171.13
ar 468
al OL
sz 204.21
ar 8802
al OL
sz 218.11
ar 6526
al OL
sz 275.53
ar 8639
al OL
sz 320.94
ar 5245


frag_001_A06.fsa
Pk-10-2
Identifiler_v1
D3S1358
TH01
D13S317
D16S539
D2S1338
al OL
sz 119.69
ar 7861
al OL
sz 171.13
ar 31546
al OL
sz 221.10
ar 4536
al 8
sz 268.90
ar 14191
al OL
sz 323.19
ar 20490


frag_001_A06.fsa
Pk-10-2
Identifiler_v1
D19S433
vWA
TPOX
D18S51
al OL
sz 117.26
ar 3255
al OL
sz 166.94
ar 13116
al OL
sz 182.78
ar 10977
al OL
sz 238.21
ar 15457
al OL
sz 274.42
ar 5556
al 15.2
sz 298.97
ar 2315
al 22
sz 324.57
ar 800

ARMS



IVSI-5 (G-C)

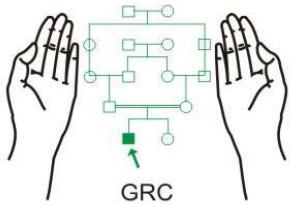
```
A.      5′-GGCCCTGGGCAGGTTGCTATCAAGGTTACAAGACAGGTTTAAGGAG-3′
   Mutation primer  <--3′-GATTGTTCCAATGTTCTGTCCAAATTCCTC-5′

B.      5′-GGCCCTGGGCAGGTTGGTATCAAGGTTACAAGACAGGTTTAAGGAG-3′
   Mutation primer  <--3′-GATTGTTCCAATGTTCTGTCCAAATTCCTC-5′

C.      5′-GGCCCTGGGCAGGTTGGTATCAAGGTTACAAGACAGGTTTAAGGAG-3′
   Normal Primer    <--3′-CATTGTTCCAATGTTCTGTCCAAATTCCTC-5′

D.      5′-GGCCCTGGGCAGGTTGCTATCAAGGTTACAAGACAGGTTTAAGGAG-3′
   Normal Primer    <--3′-CATTGTTCCAATGTTCTGTCCAAATTCCTC-5′
```

Genomic Sequencing



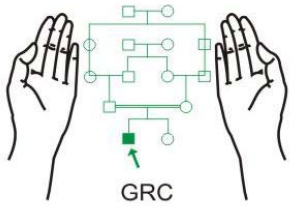
5’-TGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGATCA-3’

3’-ACGGCAATGACGGGACACCCCGTTCCACTTGCACTAGT-5’

dNTPs

- dATP
- dGTP
- dTTP
- dCTP

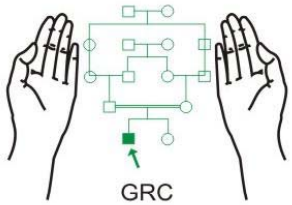
- ddATP
- ddGTP
- ddTTP
- ddCTP



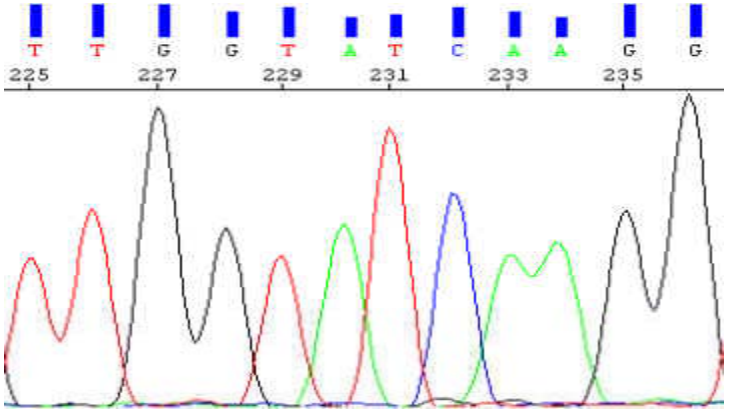
3′-GTACGCTACCATGTCATATCAACCATAGTTCCGATCCTAGTACAA-5′
5′-CATGCGATGGTACAGTATAG-3′→

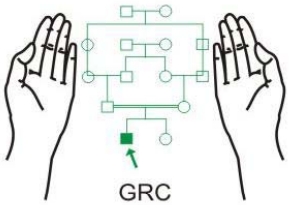
3′-GTACGCTACCATGTCATATCAACCATAGTTCCGATCCTAGTACTAAC-5′
5′-CATGCGATGGTACAGTATAG-dTTP
5′-CATGCGATGGTACAGTATAGT-ddTTP
5′-CATGCGATGGTACAGTATAGTT-dGTP

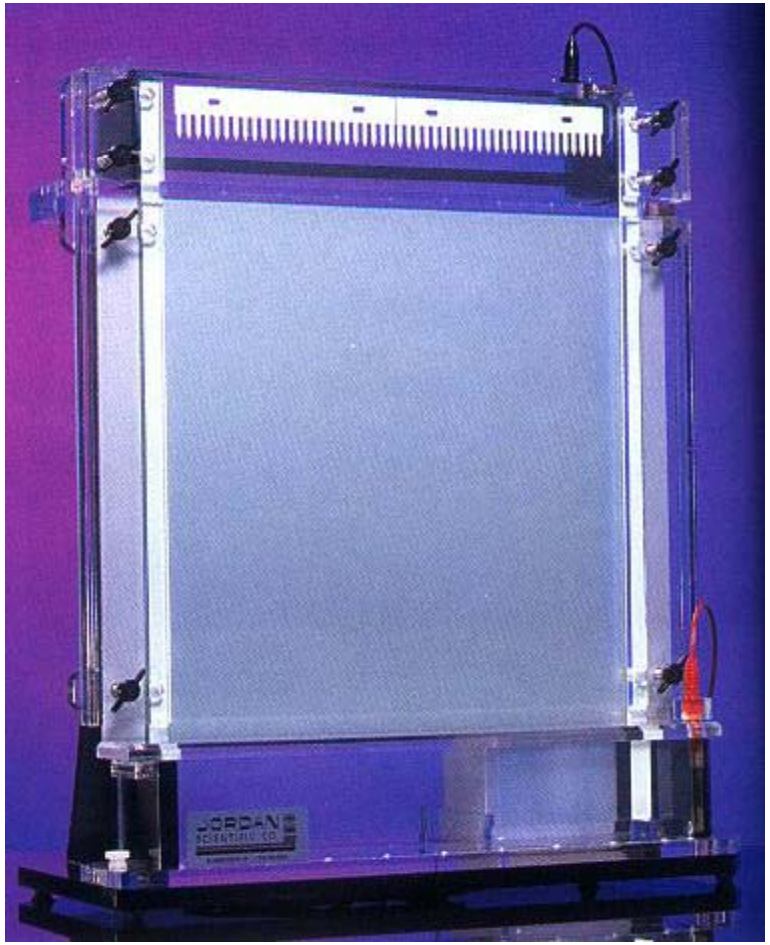
Sequencing

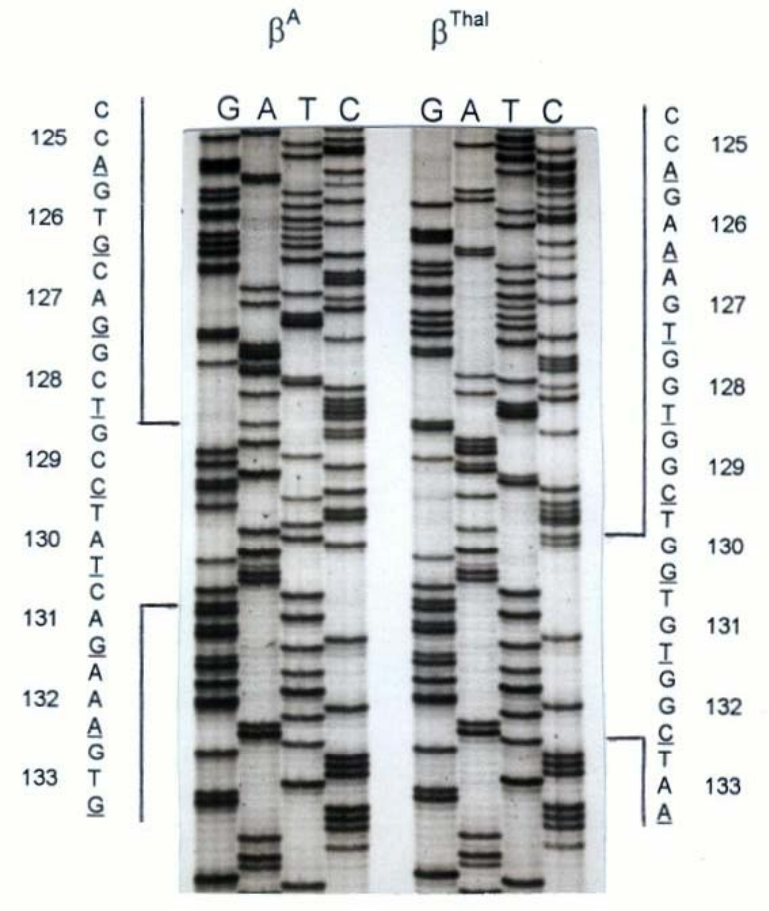


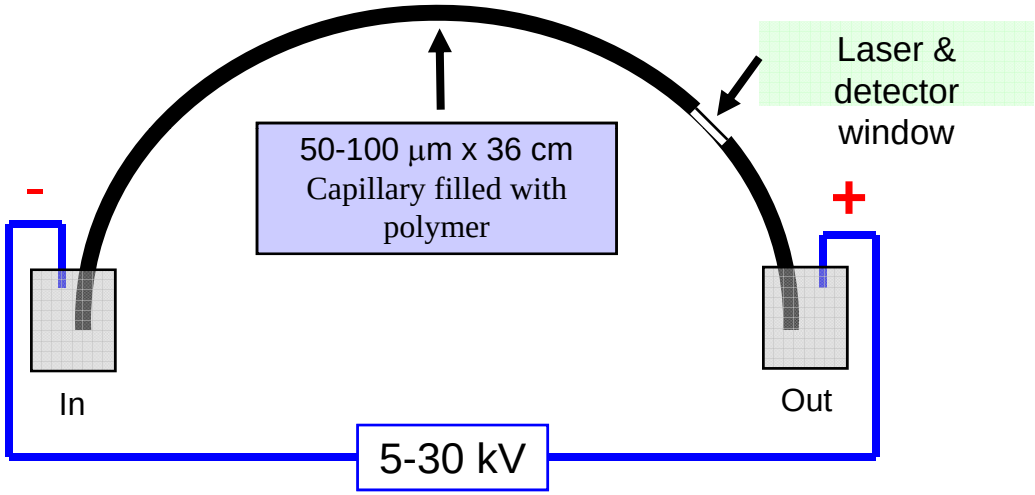
3′-GTACGCTACCATGTCATATCAACCATAGTTCCGATCCTAGTACTAAC-5′
5′-CATGCGATGGTACAGTATAG-3′→
3′-GTACGCTACCATGTCATATCAACCATAGTTCCGATCCTAGTACTAAC-5′
5′-CATGCGATGGTACAGTATAG-ddTTP
5′-CATGCGATGGTACAGTATAGT-ddTTP
5′-CATGCGATGGTACAGTATAGTT-ddGTP
5′-CATGCGATGGTACAGTATAGTTG-ddGTP
5′-CATGCGATGGTACAGTATAGTTGG-ddTTP
5′-CATGCGATGGTACAGTATAGTTGGT-ddATP
5′-CATGCGATGGTACAGTATAGTTGGTA-ddTTP
5′-CATGCGATGGTACAGTATAGTTGGTAT-ddCTP
5′-CATGCGATGGTACAGTATAGTTGGTATC-ddATP
5′-CATGCGATGGTACAGTATAGTTGGTATCA-ddATP
5′-CATGCGATGGTACAGTATAGTTGGTATCAA-ddGTP
5′-CATGCGATGGTACAGTATAGTTGGTATCAAG-ddGTP

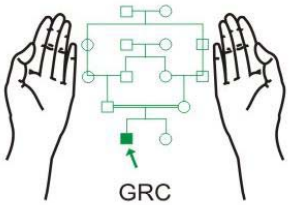


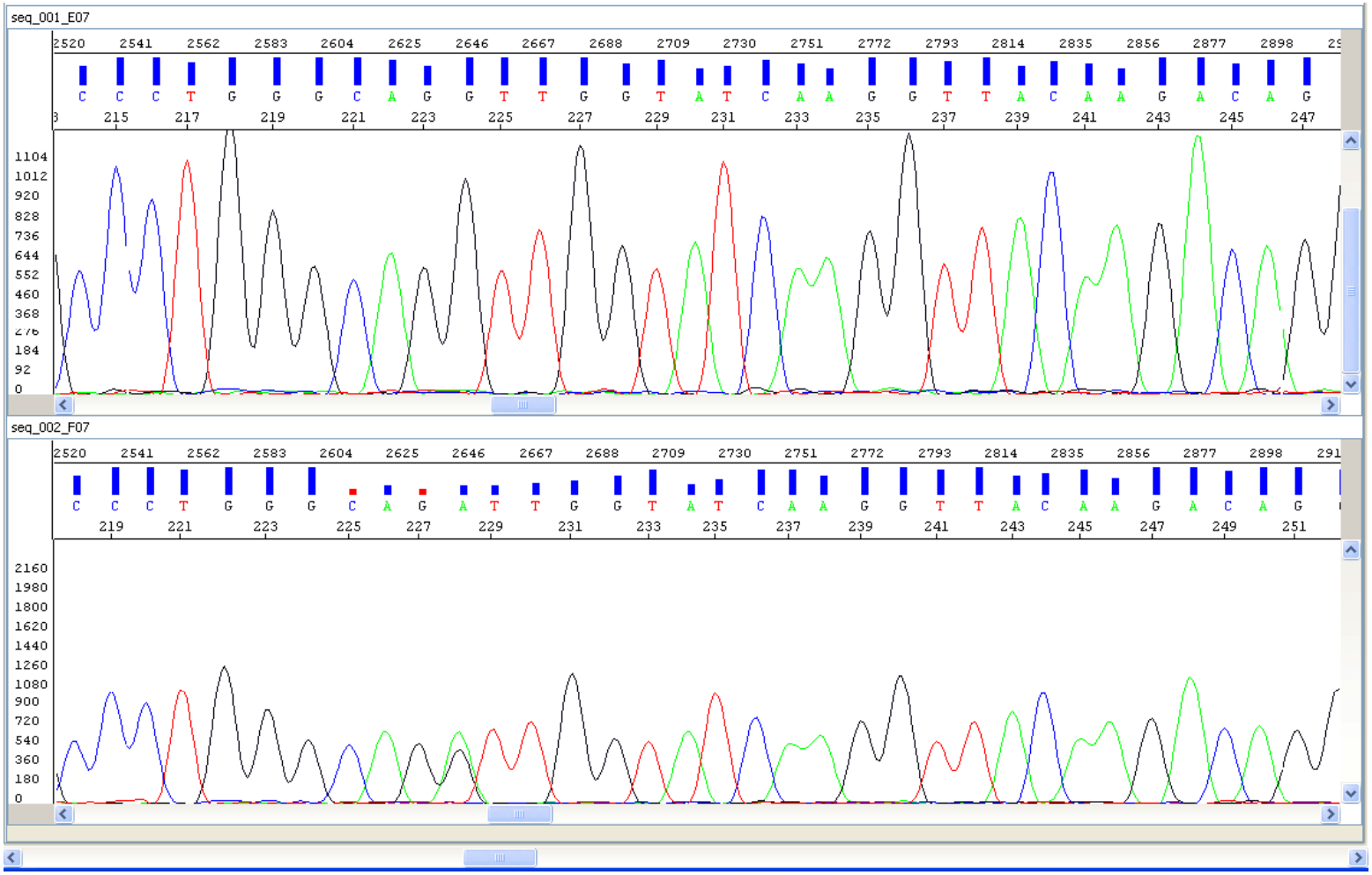

GRC

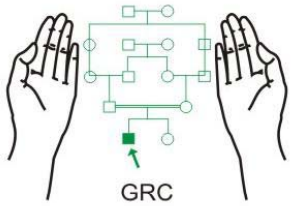



βA
βThal
G A T C
G A T C
125
126
127
128
129
130
131
132
133
C C A G T G C A G G C T G C C T A T C A G A A A G T G
C C A G A A A G T G G T G G C T G G T G T G G C T A A
125
126
127
128
129
130
131
132
133


Laser &
detector
window
50-100 µm x 36 cm
Capillary filled with
polymer
-
+
In
Out
5-30 kV


GRC

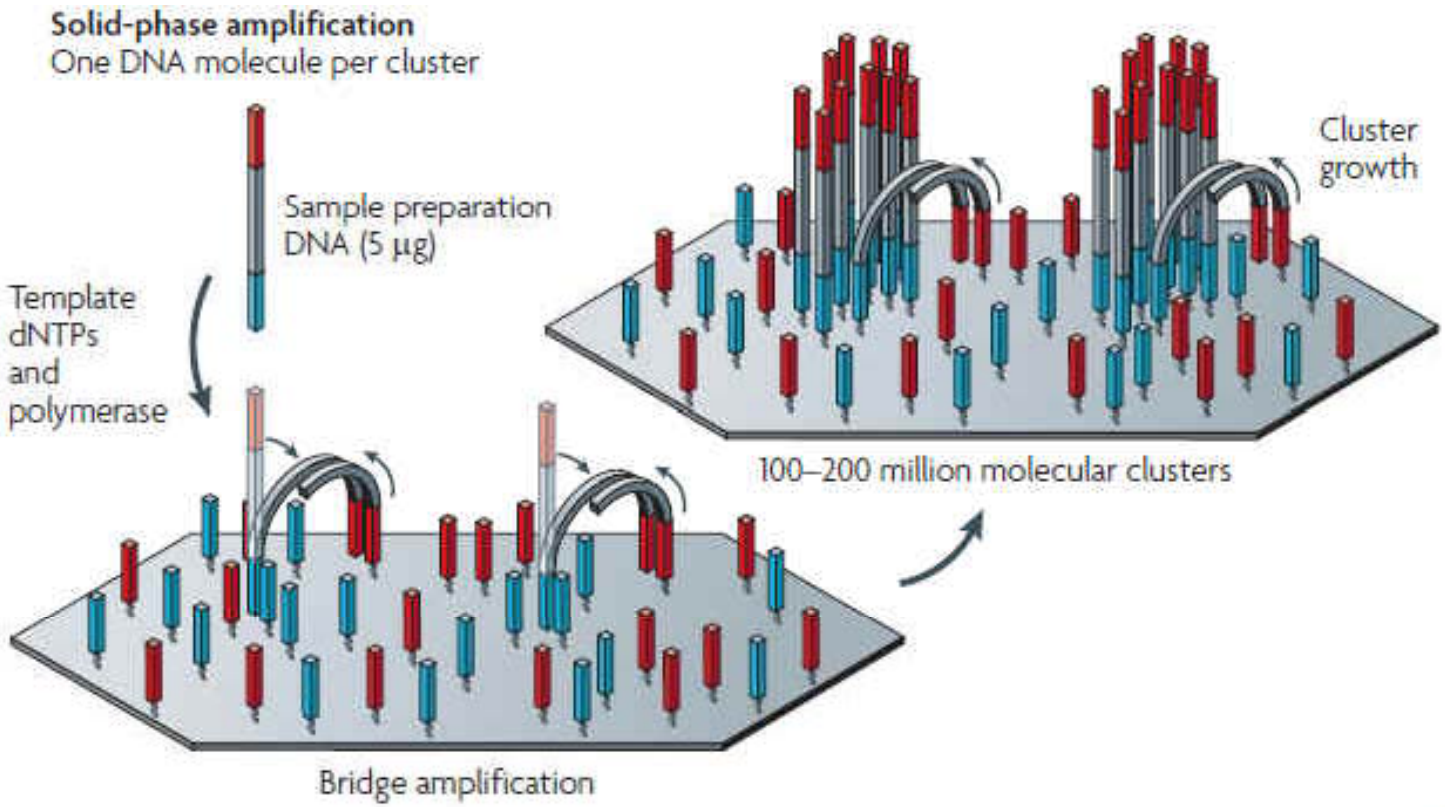

seq_001_E07
seq_002_F07



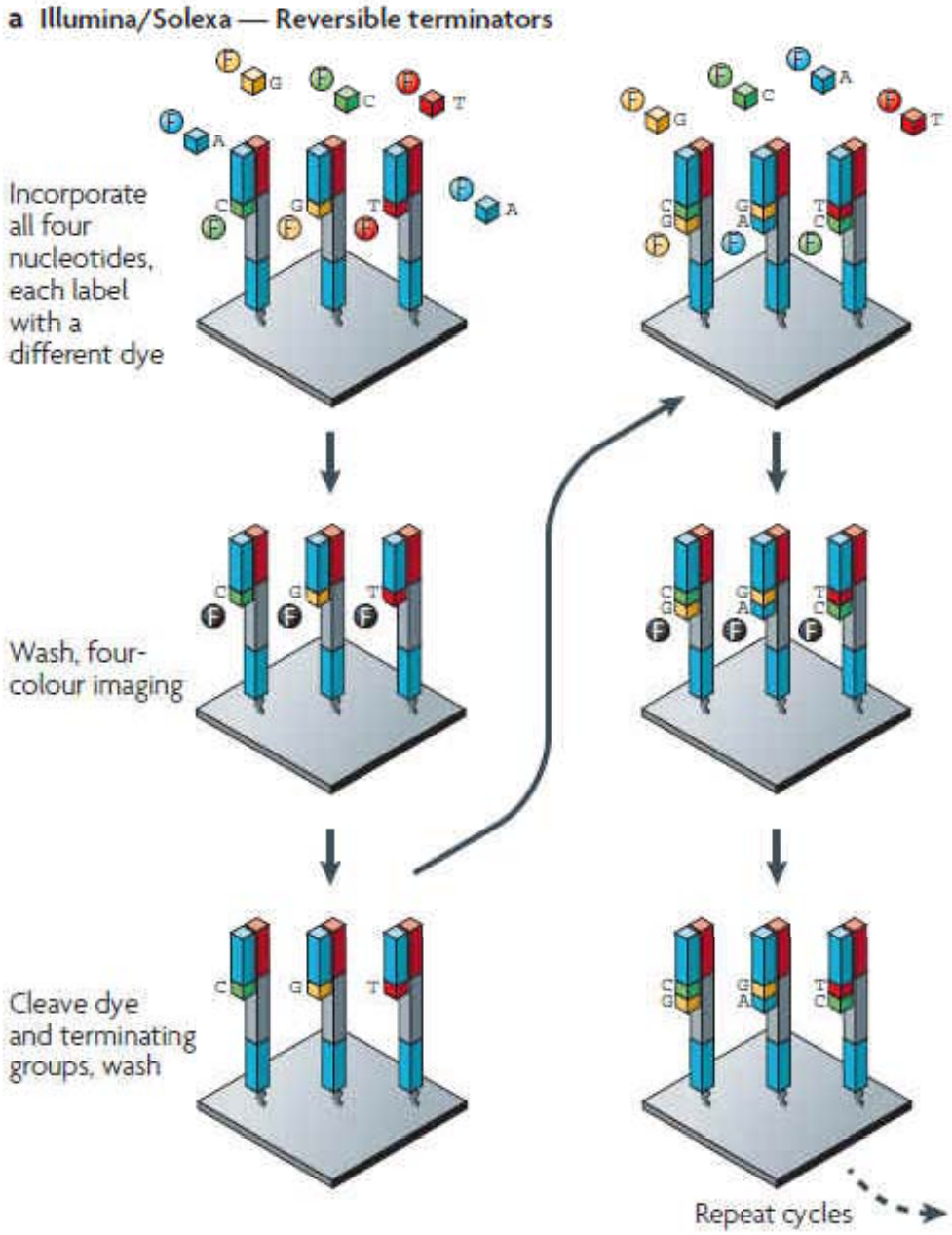
seq_001_E06

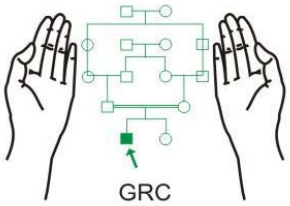
seq_004_H06

Next Generation Sequencing (NGS)



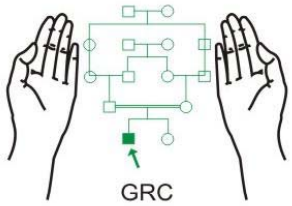
GRC


a Illumina/Solexa — Reversible terminators
Incorporate all four nucleotides, each label with a different dye
Wash, four-colour imaging
Cleave dye and terminating groups, wash
Repeat cycles


GRC

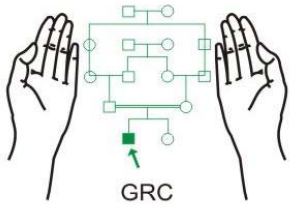
Quality Control in PCR

Pre-analytical Errors



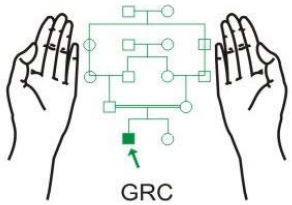
- Sample collection
- Transport
- Extraction of nucleic acids

Inhibitors of PCR



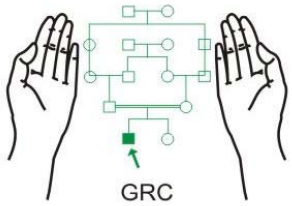
- Heparin, Porphyrin, SDS, Phenol, Proteinase-K, Chocolate
- The effect of inhibitors can be reduced or abolished by diluting the sample as it also dilutes the inhibitor

Errors in PCR



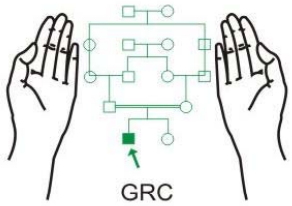
- Taq polymerase
- Primer design
- $MgCl_2$
- Concentration of DNA in PCR
- PCR tubes
- While setting up PCR
  - Premix reagents
  - Avoid inconsistency in pipetting
  - DNA should be added at the end

False Positives

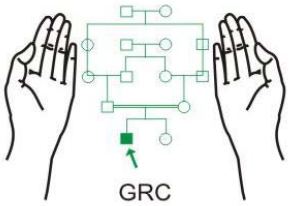


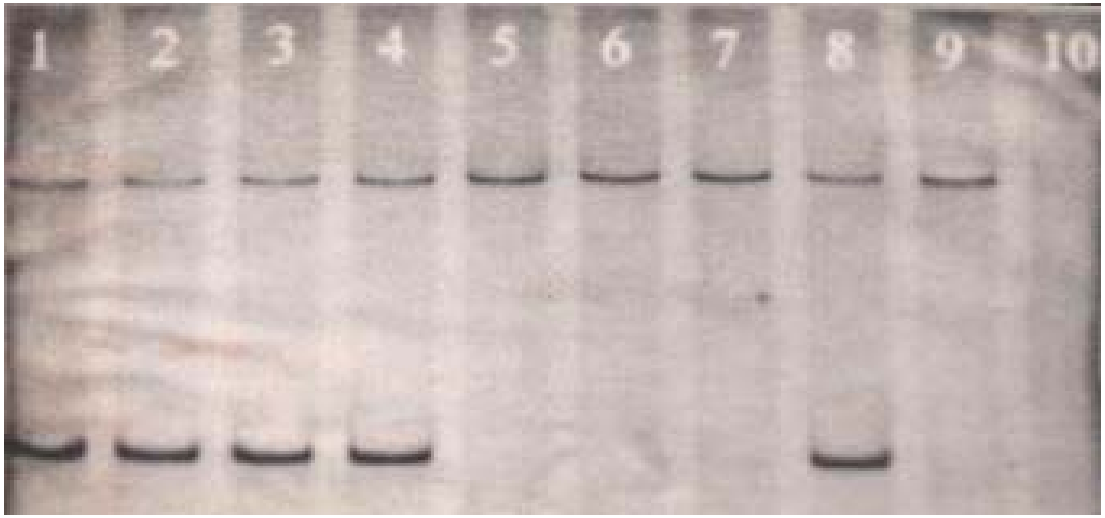
- Physical separation of sample preparation, amplification and end point analysis
- Separate sets of pipettes for the three steps
- Include appropriate
  - Positive Control
  - Negative Control
  - Non Target Control (NTC) or Reagent Blank
- UV irradiation
- Clean lab benches and equipment periodically with freshly diluted 10% bleach solution
- Amplified DNA should be handled with separate pipettes clearly marked "amplified DNA only".
- Barrier filter tips
- Wear disposable gloves
- dUTP instead of dTTP can be rendered un-amplifiable by enzymatic degradation of uracil with UNG (Uracil DNA Glycosylase)

False Negatives



- Degraded, too little or too much DNA
- Poor quality of reagents
- Failure to add any constituent of reaction e.g. primer, Taq polymerase or DNA
- Problem with the PCR machine


GRC


1
2
3
4
5
6
7
8
9
10