Polymerase Chain Reaction (PCR-I)

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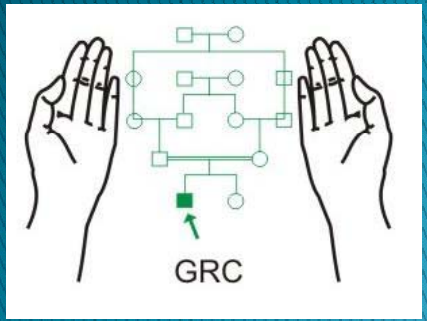
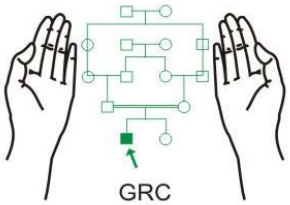
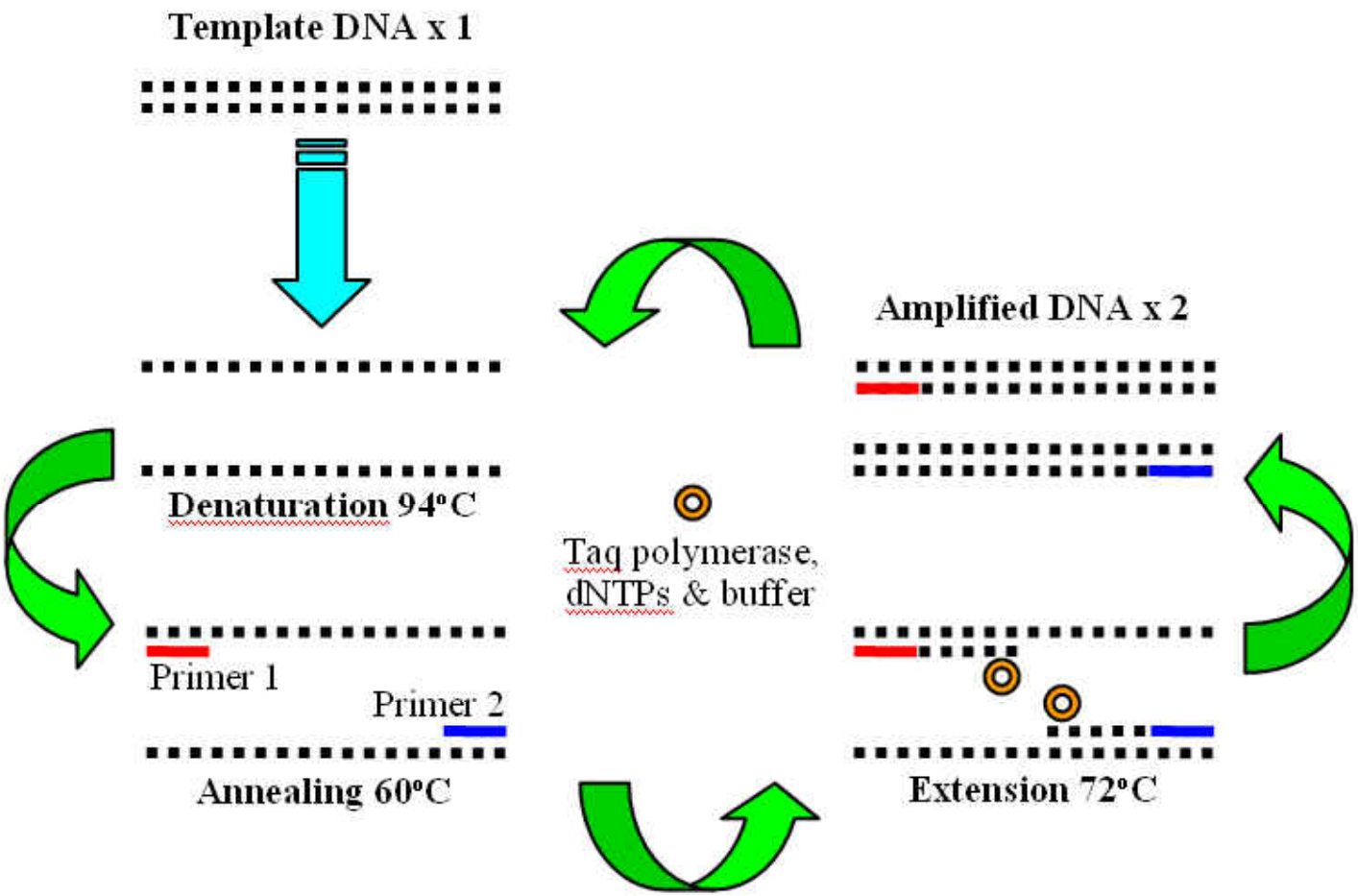
Genetics Resource Centre (GRC)

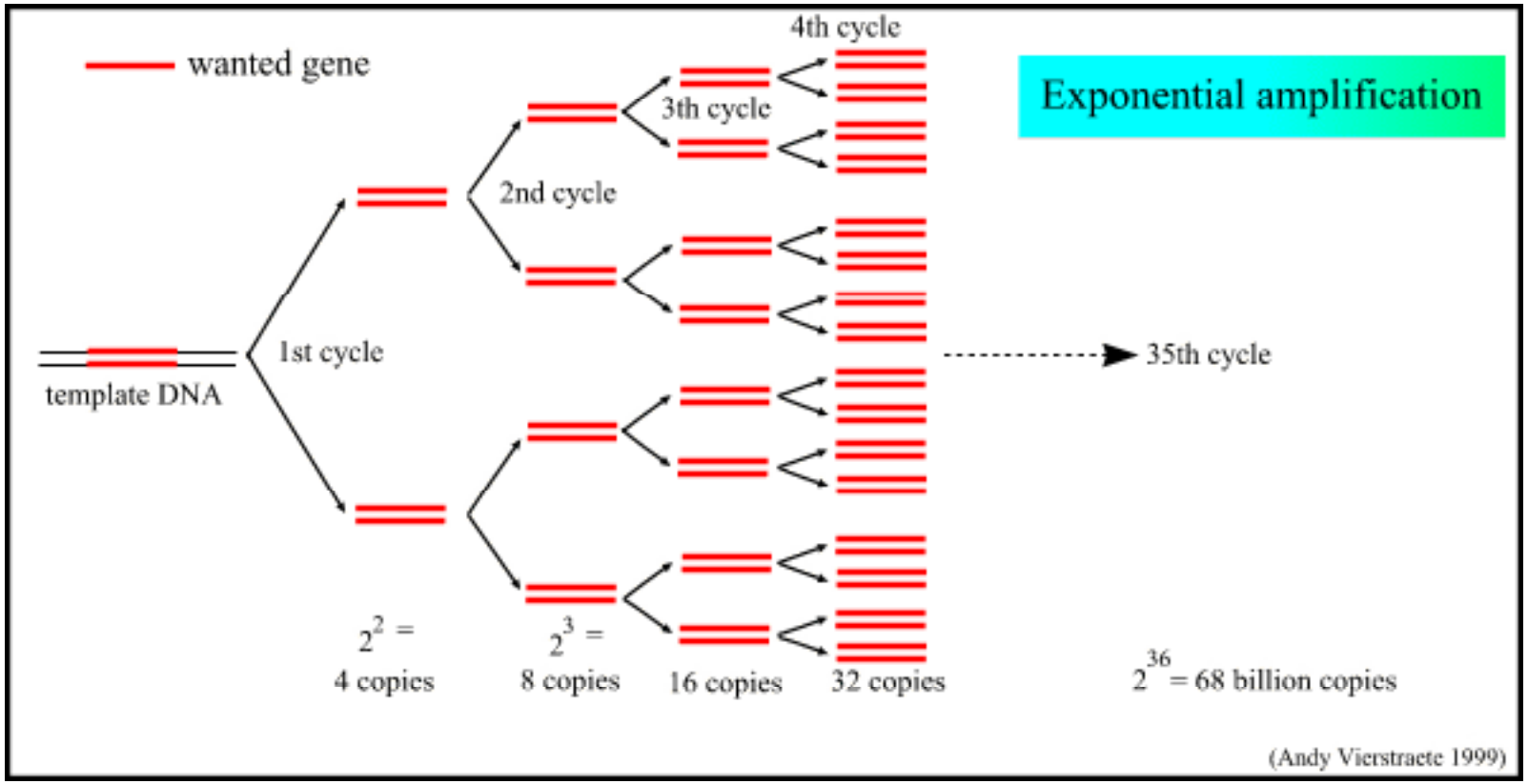


www.grcpk.com

PCR

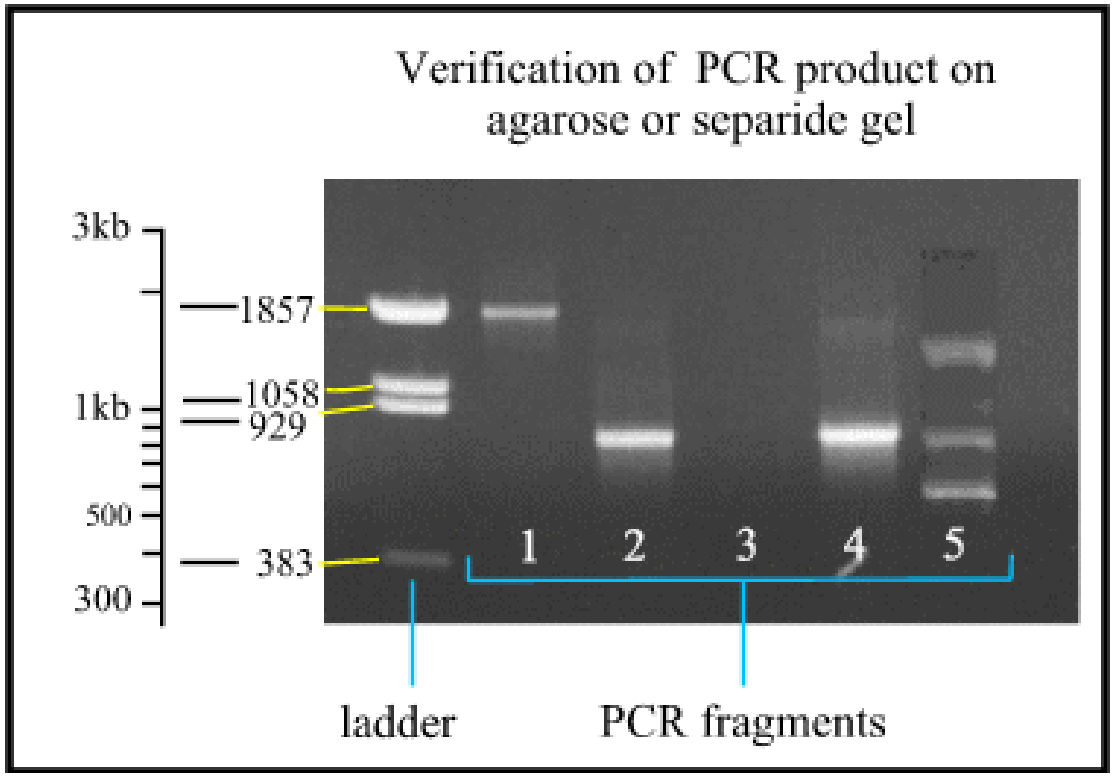

GRC

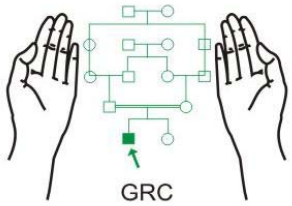

Template DNA x 1
Denaturation 94°C
Primer 1
Primer 2
Annealing 60°C
Taq polymerase,
dNTPs & buffer
Amplified DNA x 2
Extension 72°C


wanted gene
Exponential amplification
4th cycle
3th cycle
2nd cycle
1st cycle
template DNA
35th cycle
2^2 =
4 copies
2^3 =
8 copies
16 copies
32 copies
2^{36} = 68 billion copies
(Andy Vierstraete 1999)


Clear
Enter


Clear

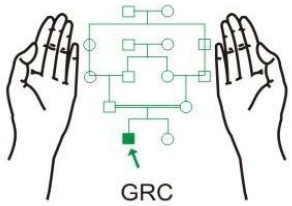

Verification of PCR product on agarose or separide gel
3kb
1857
1058
929
1kb
500
383
300
1
2
3
4
5
ladder
PCR fragments



5’-TGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGATCA-3’

3’-ACGGCAATGACGGGACACCCCGTTCCACTTGCACTAGT-5’

Primer




```
5′-GGCCCTGGGCAGGTTGCTATCAAGGTTACAAGACAGGTTTAAGGAG-3′
     Primer       <--3′-GTTCCAATGTTCTGTCCAAATTCCTC-5′
```

Primer Designing

- Runs of identical nucleotides especially “Gs” should be avoided.
- Should have no more than two G+C at the 3’ end.
- Should have no G at the 5' end (A or C is preferred).
- The G+C content should be 30-80%.
- There should be more Cs than Gs.

AAATCTGTATTAAATAATCGTACGTACTCGTTTA

ATTCTTTTTGTTGTTGTTTACTTACGTATATATA

AAAATTAGGTTTATTCCTCTTCTATACGAATCTT

CCGAGGTAGAGTTTTCATCCATTCTGTCCTGTAA

GRC

AAATCTGTATTA**AATAATCGTACGTACTCGTTT**A

ATTCTTTTTGTTGTTGTTTACTTACGTATATATA

AAAATTAGGTTTATTCCTCTTCTATACGAATCTT

CCGAGGTAGA**GTTTTCATCCATTCTGTCCTG**TAA

Forward: 5′-AATAATCGTACGTACTCGTTT

Reverse: ?

AAATCTGTATTA**AATAATCGTACGTACTCGTTT**A

TTTAGACATAATTTATTAGCATGCATGAGCAAAT

ATTCTTTTTGTTGTTGTTTACTTACGTATATATA

TAAGAAAAACAACAACAAATGAATGCATATATAT

AAAATTAGGTTTATTCCTCTTCTATACGAATCTT

TTTTAATCCAAATAAGGAGAAGATATGCTTAGAA

CCGAGGTAGA**GTTTTCATCCATTCTGTCCTG**TAA

GGCTCCATCTCAAAAGTAGGTAAGACAGGACATT

Forward: 5′-AATAATCGTACGTACTCGTTT

Reverse: ?

AAATCTGTATTA**AATAATCGTACGTACTCGTTT**A

TTTAGACATAAT**TTATTAGCATGCATGAGCAAA**T

ATTCTTTTTGTTGTTGTTTACTTACGTATATATA

TAAGAAAAACAACAACAAATGAATGCATATATAT

AAAATTAGGTTTATTCCTCTTCTATACGAATCTT

TTTTAATCCAAATAAGGAGAAGATATGCTTAGAA

CCGAGGTAGA**GTTTTCATCCATTCTGTCCTG**TAA

GGCTCCATCT**CAAAAGTAGGTAAGACAGGAC**ATT

Forward: 5′-AATAATCGTACGTACTCGTTT

Reverse: ?

AAATCTGTATTA**AATAATCGTACGTACTCGTTT**A

TTTAGACATAATTTATTAGCATGCATGAGCAAAT

ATTCTTTTTGTTGTTGTTTACTTACGTATATATA

TAAGAAAAACAACAACAAATGAATGCATATATAT

AAAATTAGGTTTATTCCTCTTCTATACGAATCTT

TTTTAATCCAAATAAGGAGAAGATATGCTTAGAA

CCGAGGTAGAGTTTTCATCCATTCTGTCCTGTAA

GGCTCCATCT**CAAAAGTAGGTAAGACAGGAC**ATT

Forward: 5′-AATAATCGTACGTACTCGTTT

Reverse: 5′-CAGGACAGAATGGATGAAAAC

AAATCTGTATTA**AATAATCGTACGTACTCGTTT**A

ATTCTTTTTGTTGTTGTTTACTTACGTATATATA

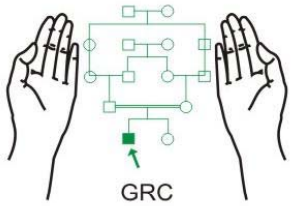
AAAATTAGGTTTATTCCTCTTCTATACGAATCTT

CCGAGGTAGA**GTTTTCATCCATTCTGTCCTG**TAA

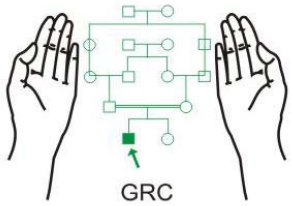
Forward: 5′-AATAATCGTACGTACTCGTTT

Reverse: 5′-CAGGACAGAATGGATGAAAAC

Primer



- Length
- 5’ & 3’ ends
- Direction (forward and reverse primers)
- Tm & annealing temperature
- Dimers and secondary structures
- Cross-homology



$$Tm = 2 \times AT + 4 \times GC$$

Primer Dimers

- Self Dimers

```
5' AAGGGCGAACGCGATTTTA 3'
          | |||| |
   3' ATTTTAGCGCAAGCGGGAA 5'
```

- Cross Dimers

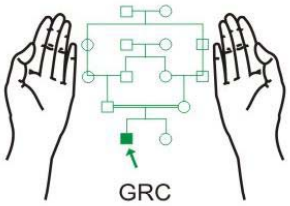
```
    Forward and Reverse Cross Dimer
     5' AAGGGCGAACGCGATTTTA 3'
           ||||
3' ACACCCCACCGCAATTAGC 5'
```

Secondary Structures

- Hairpin loop
- Complex loops

Primer Designing

- Manual
- Softwares
- Primer3
  - http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi



Primer3Plus

pick primers from a DNA sequence

Primer3Manager | Help | About | Source Code

Task: Detection

Select primer pairs to detect the given template sequence. Optionally targets and included/excluded regions can be specified.

Pick Primers | Reset Form

Main | General Settings | Advanced Settings | Internal Oligo | Penalty Weights | Sequence Quality

Sequence Id:

Paste source sequence below Or upload sequence file: Choose File No file chosen Upload File

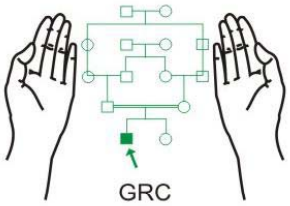
```
CTGCGCGCCTGAGCCCGCTGGCGGACCTGACGCTCGTTGCGCGTGGACCCAGCCAACTTTCCGGTGAGGC
CTTTCCGGCCGGGGCAATGGTGCATCGCCTAGCCGGGATGGGGGGGCTCTGGGGGTCCCTAGCGGGGCAG
ACCCCGTCTCACCGGCCCCTTCTCCTGCAGCTGCTAATCCAGTGTTTCCACGTCGTGCTGGCCTCCCACC
TGCAGGACGAGTTCACCGTGCAAATGCAAGCGGCGTGGGACAAGTTCCTGACTGGTGTGGCCGTGGTGCT
GACCGAAAAATACGCTGAGCCCTGTGCTGCGAGGCCTTGGTCTGTGCATGTCAATAAACAGAGGCCCGAA
CCATCTGCCCCTGCCTGTGTGGTCTTTGGGGAGCTAGCAAAGCGAGGTCACTATTGTTGGCCAGTAAGCT
CAGGGACCTAAAGGGAGCCTCCTAGAACTCTCAAATGCGCCCCACCCCCGGAGGTTTGTCCTCCCATGGC
GAGGAGTGCGATGGGGCAGAGGGAGCAGTGTGATATGGCGGGGGTAGAGAGGGTGGCCTTCGACTTCAAA
CCCTTGACTCGGGCTTCGAACCATACTCGTTCGCAAAGCAGTTCCCCATTCATGCATTTATTCAGTTCAT
TCCTTCCCTCCATCCCCATTTCCTGCTGGGACCTGTAGATGCTAATCCTGGCCCTTTTTGCAGAGAGATG
CAGAAACTGAGGTCCCAGAGCCAAATGTGCAACCTAATTCGTTGGCCAGAGCAGAGGGCCGCAGACCTGT
TCCTTTCCCCTTCCTTCCCCCATGGACACTTCCTCAGTGGCAAACCTGCGCTAGCCTGGTTAGCCCTCCC
```

Mark selected region: < > [ ] { } Clear Save Sequence

Excluded Regions: < >

Targets: [ ]

Included Region: { }



Primer3Plus

pick primers from a DNA sequence

Primer3Manager | Help | About | Source Code

< Back

Pair 1:

Left Primer 1: Primer_F

Sequence: CCTAAGCTGATTCGGCCATA

Start: 225 Length: 20 bp Tm: 60.2 °C GC: 50.0 % ANY: 5.0 SELF: 3.0

Right Primer 1: Primer_R

Sequence: ACATAGCCTTGCCAACATCC

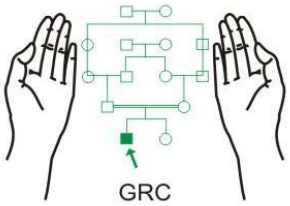
Start: 472 Length: 20 bp Tm: 60.0 °C GC: 50.0 % ANY: 5.0 SELF: 0.0

Product Size: 248 bp Pair Any: 4.0 Pair End: 1.0

Send to Primer3Manager | Reset Form

```
1      GGATCCTCAC ATGAGTTCAG TATATAATTG TAACAGAATA AAAAATCAAT
51     TATGTATTCA AGTTGCTAGT GTCTTAAGAG GTTCACATTT TTATCTAACT
101    GATTATCACA AAAATACTTC GAGTTACTTT TCATTATAAT TCCTGACTAC
151    ACATGAAGAG ACTGACACGT AGGTGCCTTA CTTAGGTAGG TTAAGTAATT
201    TATCCAAAAC CACACAATGT AGAACCTAAG CTGATTCGGC CATAGAAACA
251    CAATATGTGG TATAAATGAG ACAGAGGGAT TTCTCTCCTT CCTATGCTGT
301    CAGATGAATA CTGAGATAGA ATATTTAGTT CATCTATCAC ACATTAAACG
351    GGACTTTACA TTTCTGTCTG TTGAAGATTT GGGTGTGGGG ATAACTCAAG
401    GTATCATATC CAAGGGATGG ATGAAGGCAG GTGACTCTAA CAGAAAGGGA
451    AAGGATGTTG GCAAGGCTAT GTTCATGAAA GTATATGTAA AATCCACATT
501    AAGCTTCTTT CTGCATGCAT TGGCAATGTT TATGAATAAT GTGTATGTAA
551    AAGTGTGCTG TATATTCAAA AGTGTTTCAT GTGCCTAGGG GTGTCAAATA
601    CTTTGAGTTT GTAAGTATAT ACTTCTCTGT AATGTGTCTG AATATCTCTA
651    TTTACTTGAT TCTCAATAAG TAGGTATCAT AGTGAACATC TGACAAATGT
```

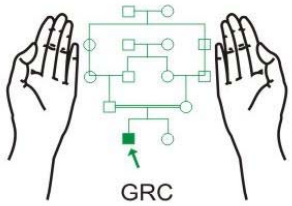
ARMS Primer



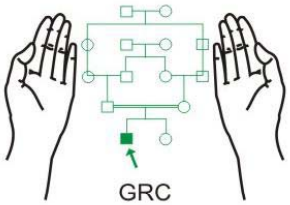
IVSI-5 (G-C)

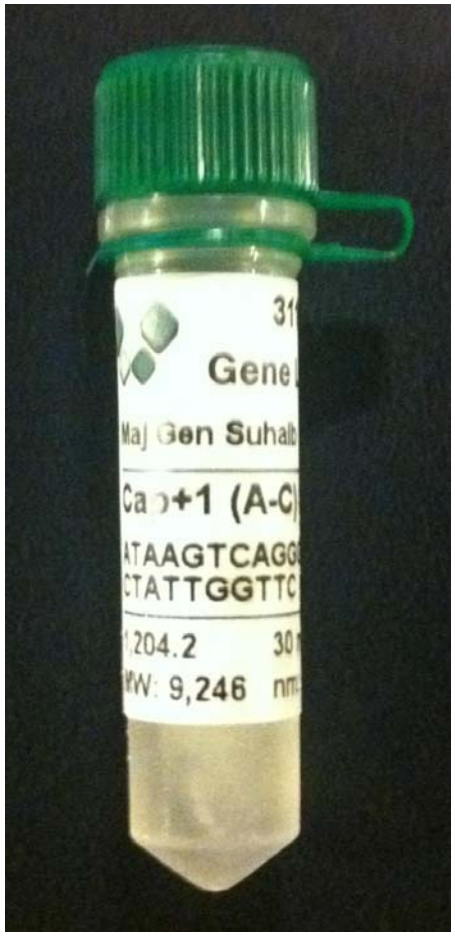
```
A.      5′-GGCCCTGGGCAGGTTGCTATCAAGGTTACAAGACAGGTTTAAGGAG-3′
   Mutation primer  <--3′-GATTGTTCCAATGTTCTGTCCAAATTCCTC-5′

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′
```

Primer



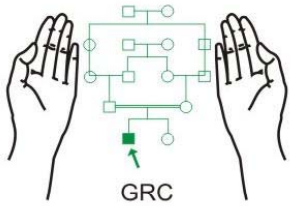
- Labeling
- Synthesis scale
- Purification
- Concentration
- Dilution


GRC


Gene
Maj Gen Suhail
+1 (A-C)
ATAAGTCAGG
CTATTGGTTC
204.2
MW: 9,246

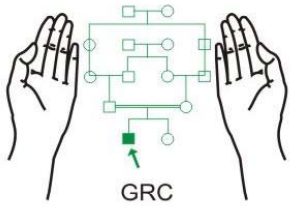

28
Nov 4, 2012
GAGCCAT
O.D. 38.55
Tm: 64.0

Labeling



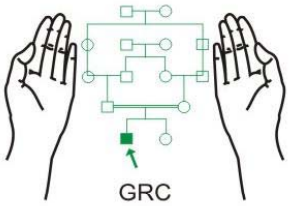
5’ FAM-CAGTAGCATCTGACTTTGAGCCTCAGGGTCT-3’

Synthesis scale



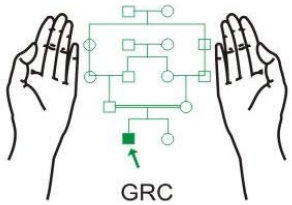
- 50 nmol
- 100 nmol
- 200 nmol
- 1000 nmol

Purification



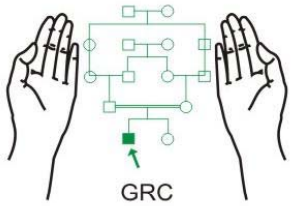
- Gel
- HPLC

Primer Concentration



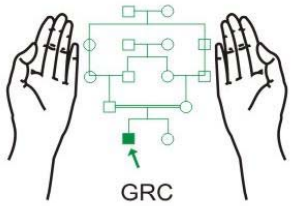
- Reconstitute stock primer in 1 ml DNAse free water
- 5 pM/reaction
- Vol of stock primer: 1/OD x Vol required
- Add distilled water equal to 100 – volume of the stock primer used
- Use 1µl per reaction

DNA Polymerase



- *Taq* Polymerase
  - DNA polymerase
  - 5’ to 3’ exonuclease activity
- *Tth* DNA polymerase
  - Reverse transcriptase and Taq polymerase
- High fidelity DNA polymerase (*pfu*)
  - 3’ to 5’ proof reading

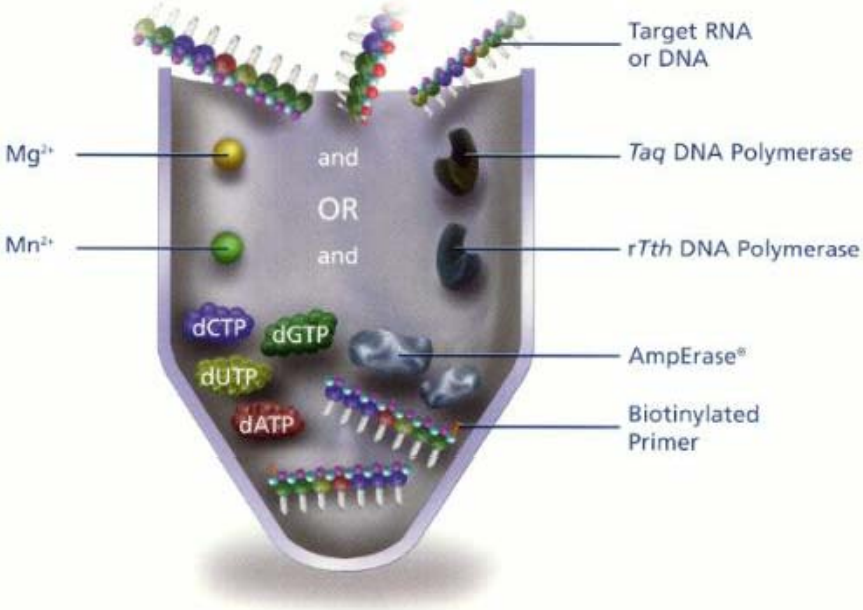
Deoxy-nucleotide Phosphates (dNTPs)



- dATP. dGTP, dTTP, dCTP
- dUTP
- Concentration (10 or 100 mmol)
- $MgCl_2$ concentration

PCR Buffer

Setting up PCR




1 2 3 C
4 5 6
7 8 9 E
0