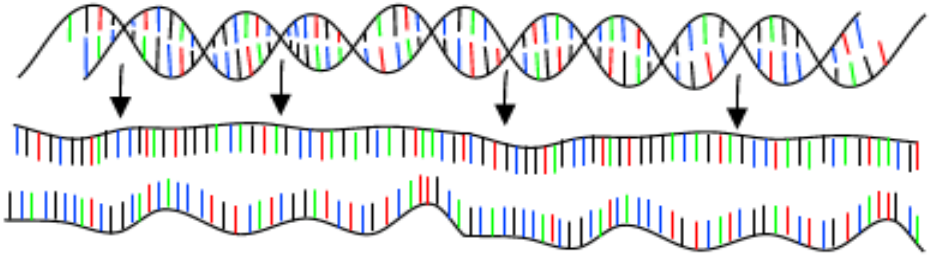
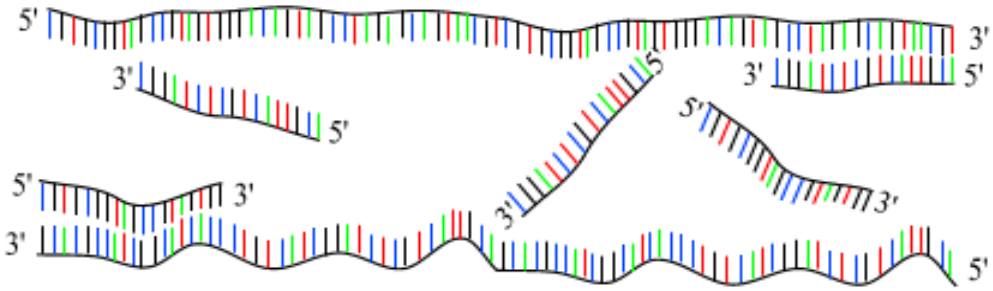
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

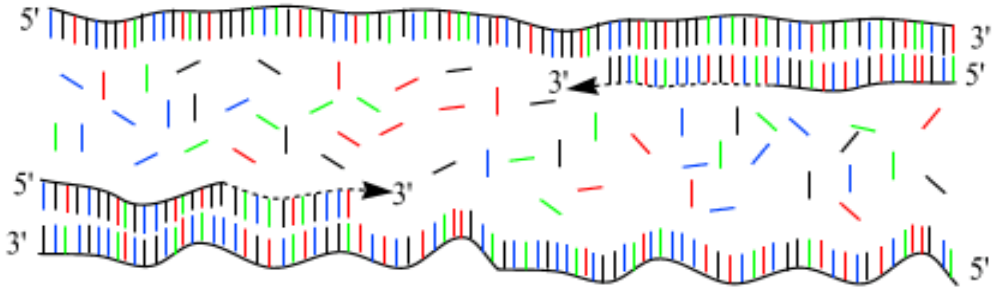
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



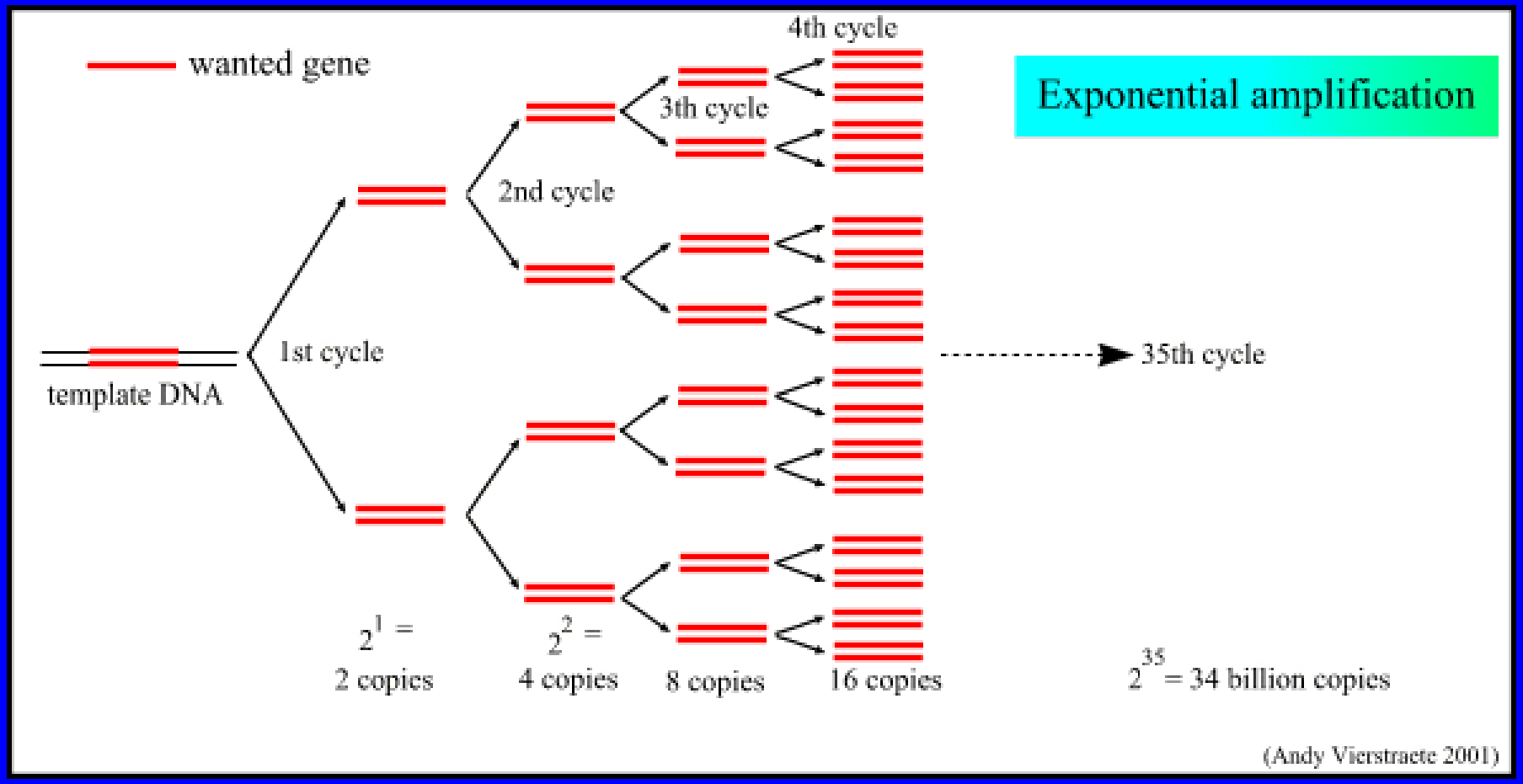
Step 3 : extension

2 minutes 72 °C

only dNTP's

(Andy Vierstraete 1999)

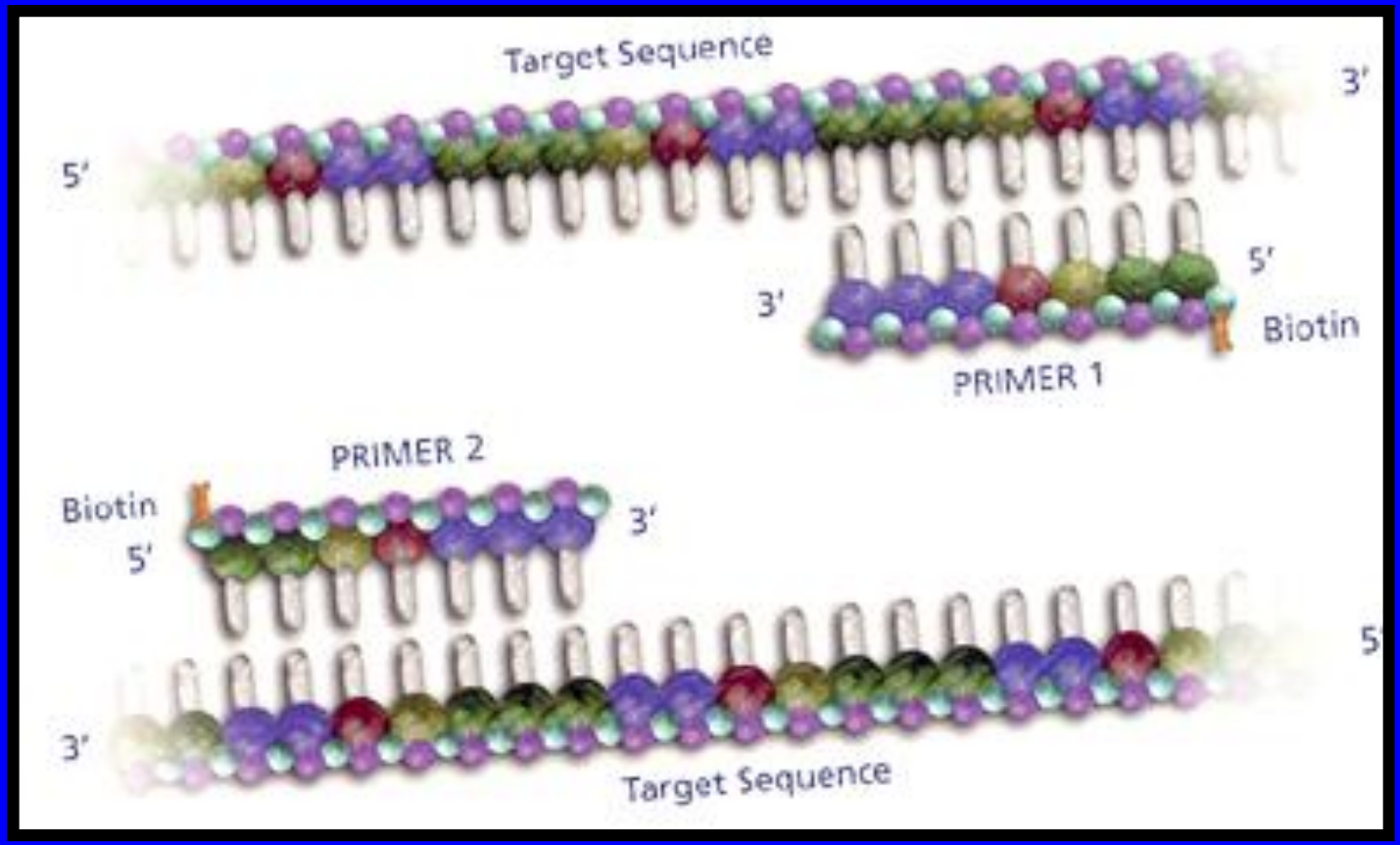
PCR


wanted gene
4th cycle
3th cycle
2nd cycle
1st cycle
template DNA
35th cycle
Exponential amplification
2^1 =
2 copies
2^2 =
4 copies
8 copies
16 copies
2^{35} = 34 billion copies
(Andy Vierstraete 2001)

Primer (molecular biology)

- A primer is a strand of short nucleic acid sequences (generally about 10 base pairs) that serves as a starting point for DNA synthesis.
- The polymerase starts replication at the 3'-end of the primer, and copies the opposite strand.
- PCR primer design is the creation of short nucleotide sequences for use in amplifying a specific region of DNA.

PCR Primer Design



http://www.modares.ac.ir/elearning/mnaderi/Genetic%20Engineering%20course%20II/images/2a.jpg

Summary ~ Primer Design Criteria

1. **Primer Length:**

It is generally accepted that the optimal length of PCR primers is 18-22 bp. This length is long enough for adequate specificity and short enough for primers to bind easily to the template at the annealing temperature.

The Base composition: average (G+C)

- **Optimal G/C content: 45-55%**
- **Common G/C content range: 40-60%** ,
- and avoid long (A+T) and (G+C) rich region if possible;

Summary ~ Primer Design Criteria

2. Primer Melting Temperature:

the one half of the DNA duplex will dissociate to become single stranded and indicates the duplex stability. Primers with melting temperatures in the range of 52-58 ^{o}C generally produce the best results. The GC content of the sequence gives a fair indication of the primer T_m.

- The melting temperature (Tm) of the primers used are not more than 5°C different from each other.

- Calculate Tm with this formula:

$$Tm = 4(G + C) + 2(A + T)°C$$

Summary ~ Primer Design Criteria

3. Primer Annealing Temperature:

The primer melting temperature is the estimate of the DNA-DNA hybrid stability and critical in determining the annealing temperature. Too high T_a will produce insufficient primer-template hybridization resulting in low PCR product yield. Too low T_a may possibly lead to non-specific products caused by a high number of base pair mismatches

$T_a = 0.3 \times T_m(\text{primer}) + 0.7\ T_m\ (\text{product}) - 14.9$

Summary ~ Primer Design Criteria

4. GC Content: The GC content (the number of G's and C's in the primer as a percentage of the total bases) of primer should be 40-60%.

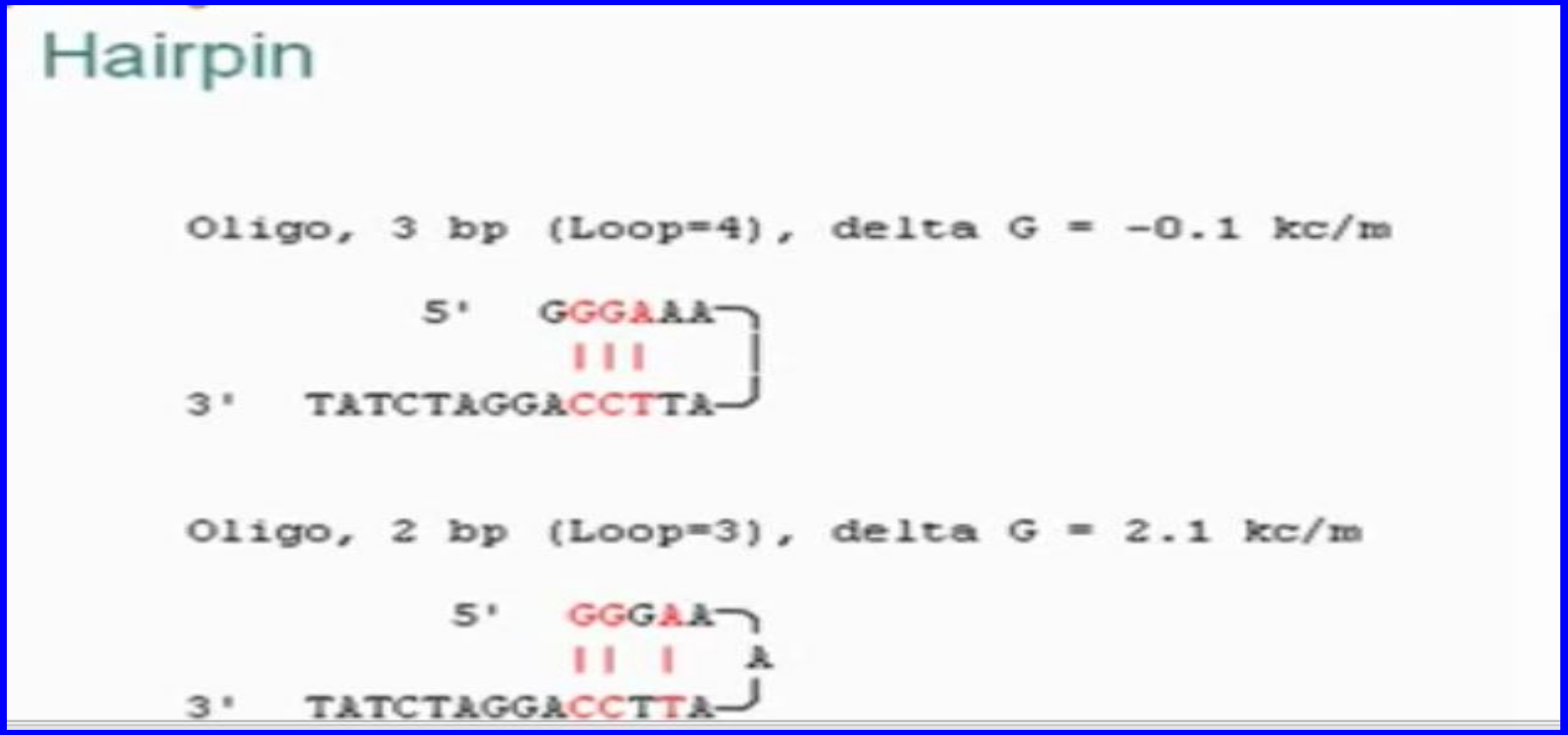
5. GC Clamp: The presence of G or C bases within the last five bases from the 3' end of primers (GC clamp) helps promote specific binding at the 3' end due to the stronger bonding of G and C bases. More than 3 G's or C's should be avoided in the last 5 bases at the 3' end of the primer.

Summary ~ Primer Design Criteria

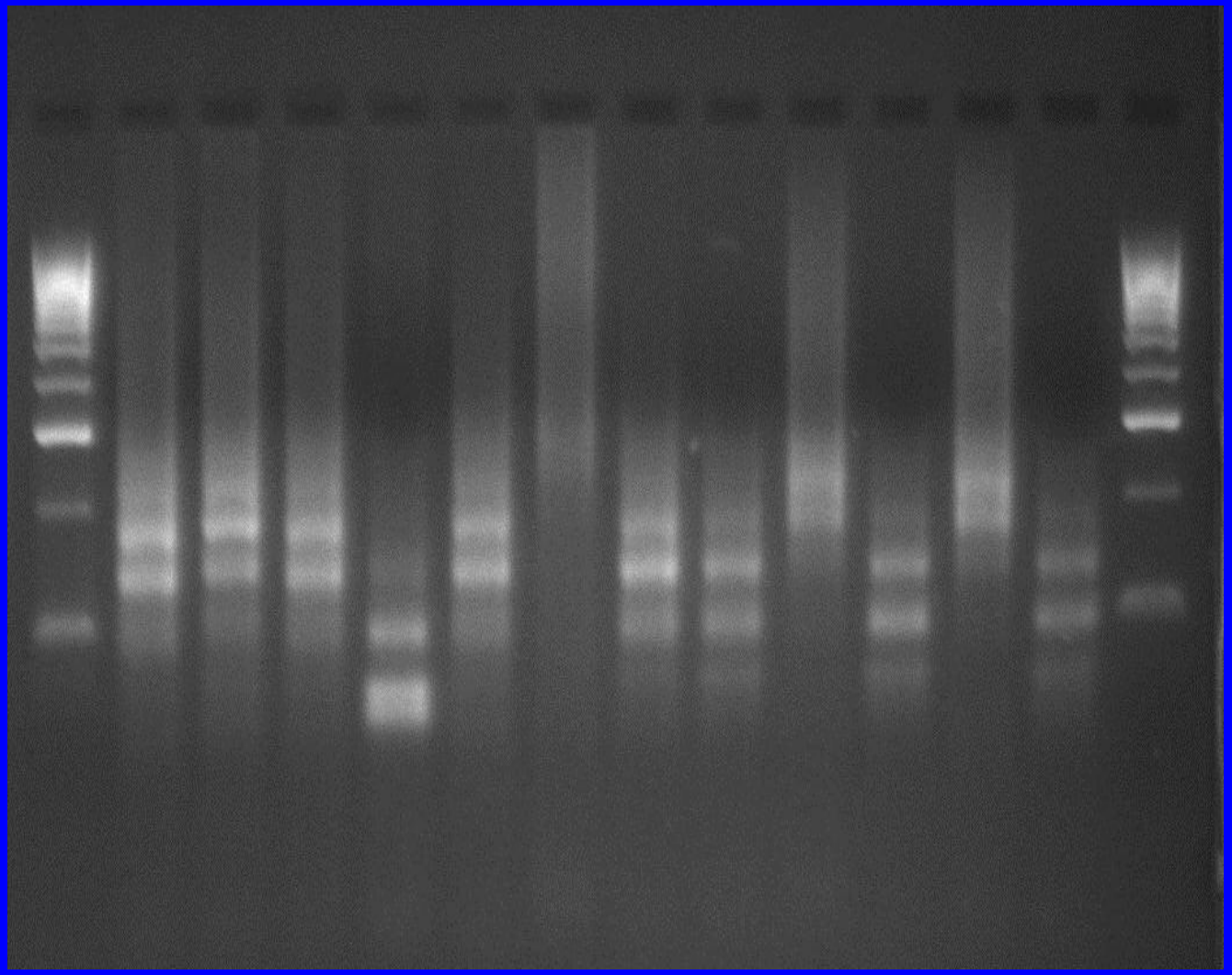
6. Primer Secondary Structures: Presence of the primer secondary structures produced by intermolecular or intramolecular interactions can lead to poor or no yield of the product. They adversely affect primer template annealing and thus the amplification. They greatly reduce the availability of primers to the reaction.

6. Primer Secondary Structures

A.) Hairpins: It is formed by intramolecular interaction within the primer and should be avoided.



A- Hairpins



6. Primer Secondary Structures

B) Self Dimer: A primer self-dimer is formed by intermolecular interactions between the two (same sense) primers, where the primer is homologous to itself. Generally a large amount of primers are used in PCR compared to the amount of target gene. When primers form intermolecular dimers much more readily than hybridizing to target DNA, they reduce the product yield.

B- Self-dimer

Self-Dimer

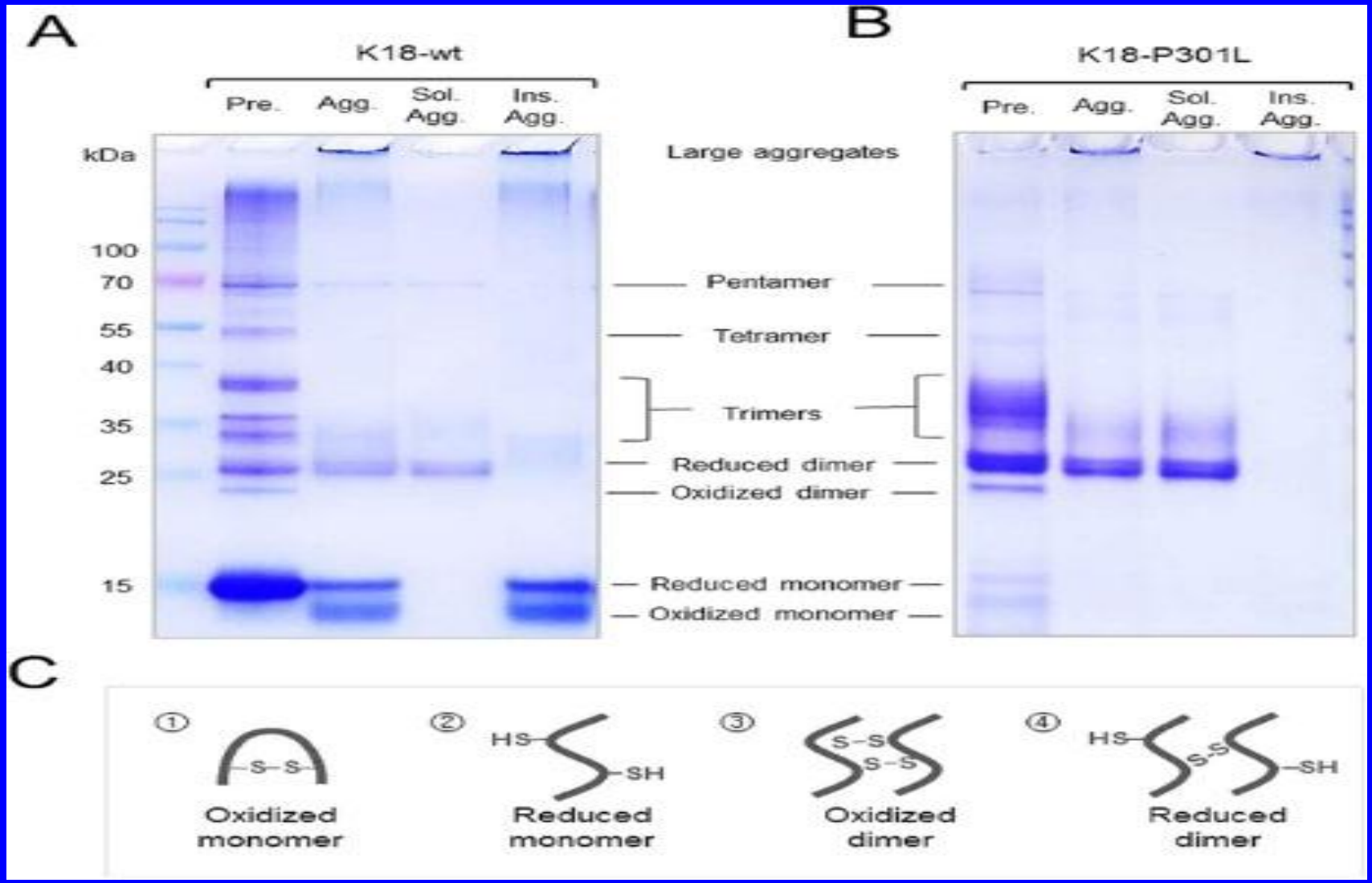
```
4 bp, delta G = -6.6 kc/m (bad!) (worst= -36.6)
       5' GGGAAAATTCCAGGATCTAT 3'
             ||||  ||||
3' TATCTAGGACCTTAAAAGGG 5'


4 bp, delta G = -5.4 kc/m (bad!) (worst= -36.6)
      5' GGGAAAATTCCAGGATCTAT 3'
            ||||
3' TATCTAGGACCTTAAAAGGG 5'
```

6. Primer Secondary Structures

C) Cross Dimer: Primer cross dimers are formed by intermolecular interaction between sense and antisense primers, where they are homologous.

C- Cross Dimer



Computer-Aided Primer Design

Some primer design programs are :-

- **Primer3**
- **Primer3Plus**
- **PrimerZ**
- **PerlPrimer**
- **Vector NTI Advantage 10**

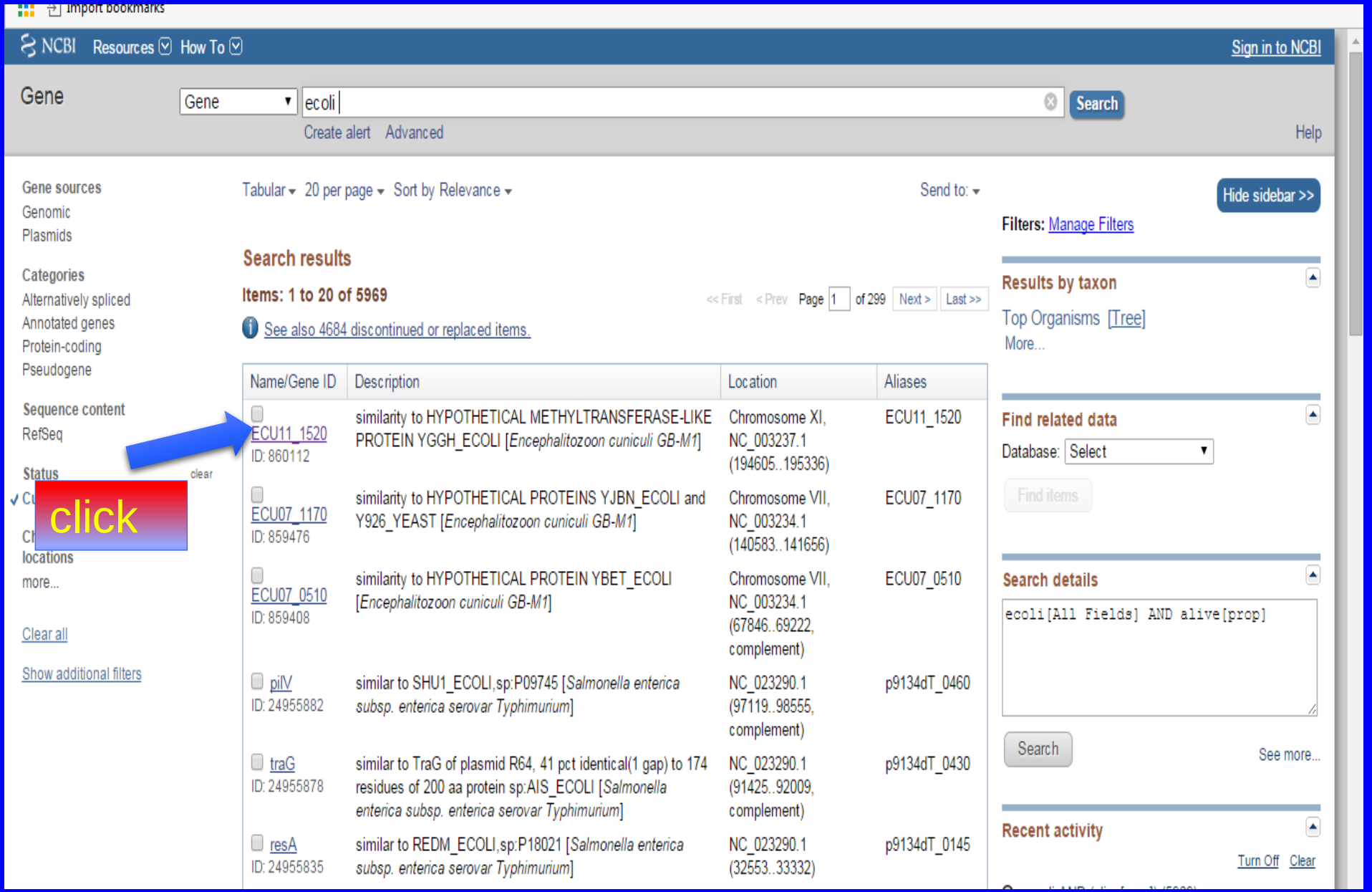
Steps of primer design

http://www.ncbi.nlm.nih.gov/

http://primer3plus.com/cgi-bin/dev/primer3plus.cgi

http://www.mutationdiscovery.com/md/MD.com/screens/optimase/OptimaseInput.html?action=none

http://www.ncbi.nlm.nih.gov



ECU11_1520 similarity to HYPOTHETICAL METHYLTRANSFERASE-LIKE PROTEIN YGGH_ECOLI [*Encephalitozoon cuniculi GB-M1*]

Gene ID: 860112, updated on 26-Jun-2015

Summary

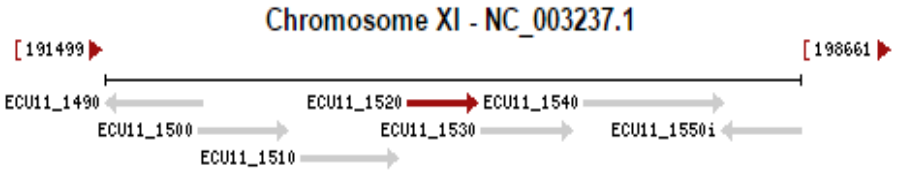
Gene symbol	ECU11_1520
Gene description	similarity to HYPOTHETICAL METHYLTRANSFERASE-LIKE PROTEIN YGGH_ECOLI
Locus tag	ECU11_1520
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Encephalitozoon cuniculi GB-M1 (strain: GB-M1)
Lineage	Eukaryota; Fungi; Microsporidia; Unikaryonidae; Encephalitozoon

Genomic context

Location: chromosome: XI

Exon count: 1

Sequence: Chromosome: XI; NC_003237.1 (194605..195336)



click

Genomic regions, transcripts, and products

Genomic Sequence: NC_003237.1

Go to referen sequence details

Go to nucleotide: Graphics FASTA GenBank

Advanced

FASTA ▾ Send: ▾

Encephalitozoon cuniculi GB-M1 chromosome XI, complete sequence

NCBI Reference Sequence: NC_003237.1

GenBank Graphics

>gi|17158053:194605-195336 Encephalitozoon cuniculi GB-M1 chromosome XI, complete sequence

ATGCAGATGGGAAATGGGATCAAGAAAAGCCTTTACAGACAAAGAGCCCACGCCAATCCATTCAAGGACA
GCAACATCACCGTTCCTCCGAGCCCTCAGCTTATAGACTGGTCTTCTTACTTCAGAATGAATAGAAGGCC
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AGAGGAGTATTGGGCAAAGCAGCTCCTTTTGA

copy to Primer3Plus

http://primer3plus.com/cgi-bin/dev/primer3plus.cgi

Primer3Plus

pick primers from a DNA sequence

More... | Source Code | Help | About

Load server settings: Default ▼ Activate Settings

Task: generic ▼

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

Pick Primers | Reset Form

click

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

Sequence Id:

Paste template sequence below

Or upload sequence file: Choose File No file chosen Upload File

```
sequence
ATGCAGATGGGAAATGGGATCAAGAAAAGCCTTTACAGACAAAGAGCCCACGCCAATCCATTCAAGGACA
GCAACATCACCGTTCCTCCGAGCCCTCAGCTTATAGACTGGTCTTCTTACTTCAGAATGAATAGAAGGCC
GGATTTCGTTGATATCGGGTGTGGATACGGAAAGTTCCTTATGAAGGTTGCCGAGAAGAATCCAGAGCAC
AGCATACTGGGCCTCGAGATCAGAGACAAAGTGTGTGAGTATGTTAAGGCAAGGATAGAAGCCGCCGGGA
TTCCAAATGCCGGGGTCATGAGAACAAATGCATTGATTTTCCTCCCGAACATATTCAGTAGAGGCCAGCT
GACCAAGATCTTTATTTTATTCCCCGACCCTCACTTCAAAAAGAGGAAGCAAAAGGGGCGCATTGTGTGC
CGGCAGATGATGGAGATGTACGAGTACCTGCTAGCGGACGGGGGAAAGTTGTATATATCTACCGACGTGA
AGGAGTTGTTTAACTGCATGATGGAGGCCGTTCTGGGACATGGAGCATTCAGGGCATTGTCTGAGAACGA
AGTGGATGGAGATGAGTTGTTCCATATGATTTCTAGGGACACGGACGAGGCGCTGAGGGCCGGAGCCAAG
GCAGGAAAGGTGTTTTCGAAGGTTTTTGAAATAAAGAAAGATTGGCACTACTCCTTTCATCTTCGACTCC
AGAGGAGTATTGGGCAAAGCAGCTCCTTTTGA
```

Mark selected region: < > [ ] { } Clear

Save Sequence

Excluded Regions: < >

Targets: [ ]

Included Region: { }

Primer overlap positions: -

< Back

1

2

Pair 1: Primer

Left Primer 1: GCATACTGGGCCTCGAGATC

Start: 220 Length: 20 bp Tm: 60.0 C GC: 60.0 % [illegible] End: 0.0 TB: 6.0 HP: 0.0 3' Stab: 2.8 Penalty: 0.038

Right Primer 1: CAAAAGGAGCTGCTTTGCCC

Start: 739 Length: 20 bp Tm: 60.3 C GC: 55.0 % Any: 21.3 End: 0.0 TB: 7.0 HP: 0.0 3' Stab: 5.4 Penalty: 0.320

Pair: Product Size: 520 bp Any: 0.0 End: 0.0 TB: 13.0 Penalty: 0.358

Send to Primer3Mana Reset Form

3

```
1      quenceAT  GCAGATGGGA  AATGGGATCA  AGAAAAGCCT  TTACAGACAA
51     AGAGCCCACG CCAATCCATT  CAAGGACAGC  AACATCACCG  TTCCTCCGAG
101    CCCTCAGCTT ATAGACTGGT  CTTCTTACTT  CAGAATGAAT  AGAAGGCCGG
151    ATTTCGTTGA TATCGGGTGT  GGATACGGAA  AGTTCCTTAT  GAAGGTTGCC
201    GAGAAGAATC CAGAGCACAG  CATACTGGGC  CTCGAGATCA  GAGACAAAGT
251    GTGTGAGTAT GTTAAGGCAA  GGATAGAAGC  CGCCGGGATT  CCAAATGCCG
301    GGGTCATGAG AACAAATGCA  TTGATTTTCC  TCCCGAACAT  ATTCAGTAGA
351    GGCCAGCTGA CCAAGATCTT  TATTTTATTC  CCCGACCCTC  ACTTCAAAAA
401    GAGGAAGCAA AAGGGGCGCA  TTGTGTGCCG  GCAGATGATG  GAGATGTACG
451    AGTACCTGCT AGCGGACGGG  GGAAAGTTGT  ATATATCTAC  CGACGTGAAG
501    GAGTTGTTTA ACTGCATGAT  GGAGGCCGTT  CTGGGACATG  GAGCATTCAG
551    GGCATTGTCT GAGAACGAAG  TGGATGGAGA  TGAGTTGTTC  CATATGATTT
601    CTAGGGACAC GGACGAGGCG  CTGAGGGCCG  GAGCCAAGGC  AGGAAAGGTG
651    TTTTCGAAGG TTTTTGAAAT  AAAGAAAGAT  TGGCACTACT  CCTTTCATCT
701    TCGACTCCAG AGGAGTATTG  GGCAAAGCAG  CTCCTTTTGA
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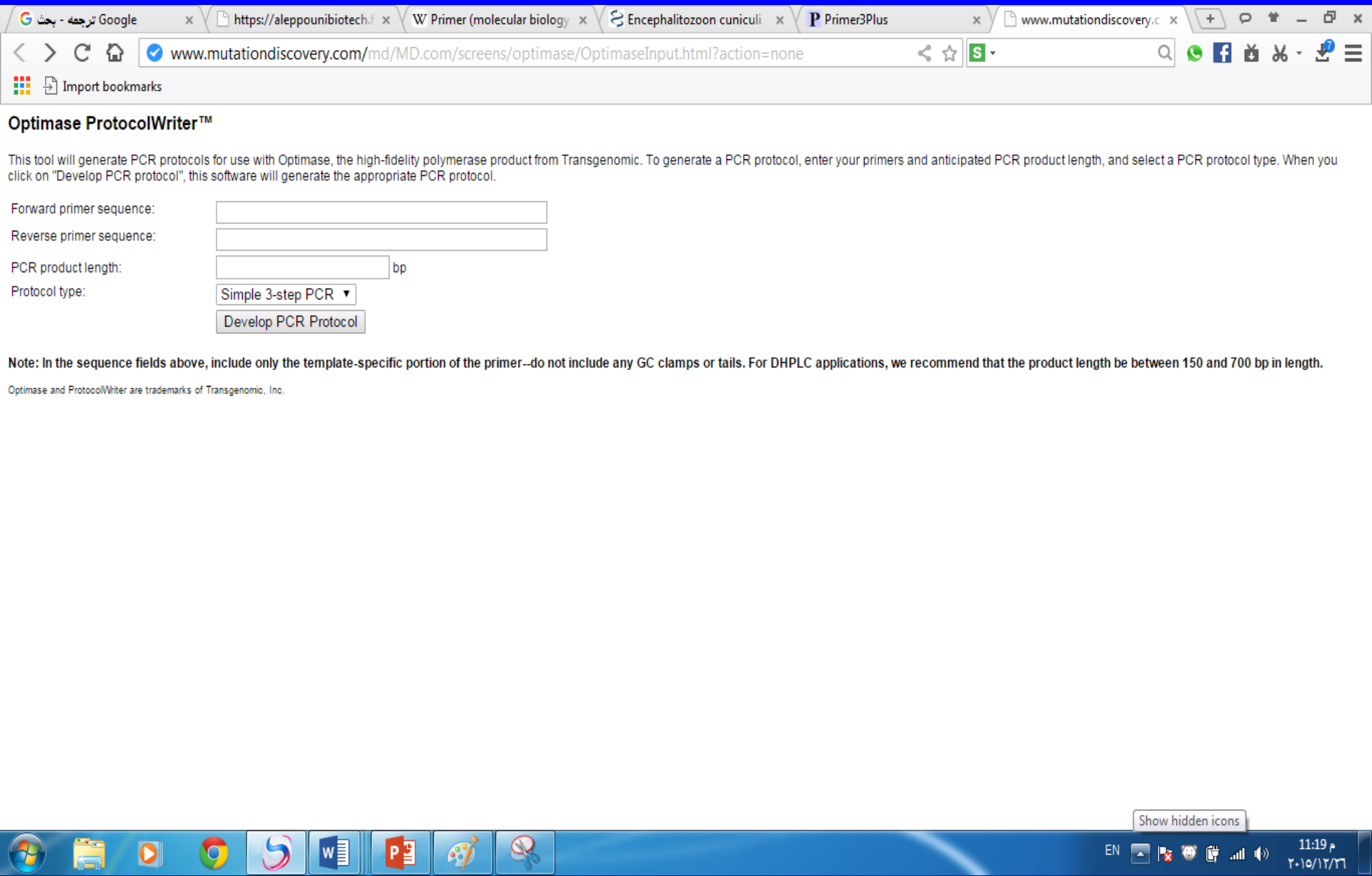
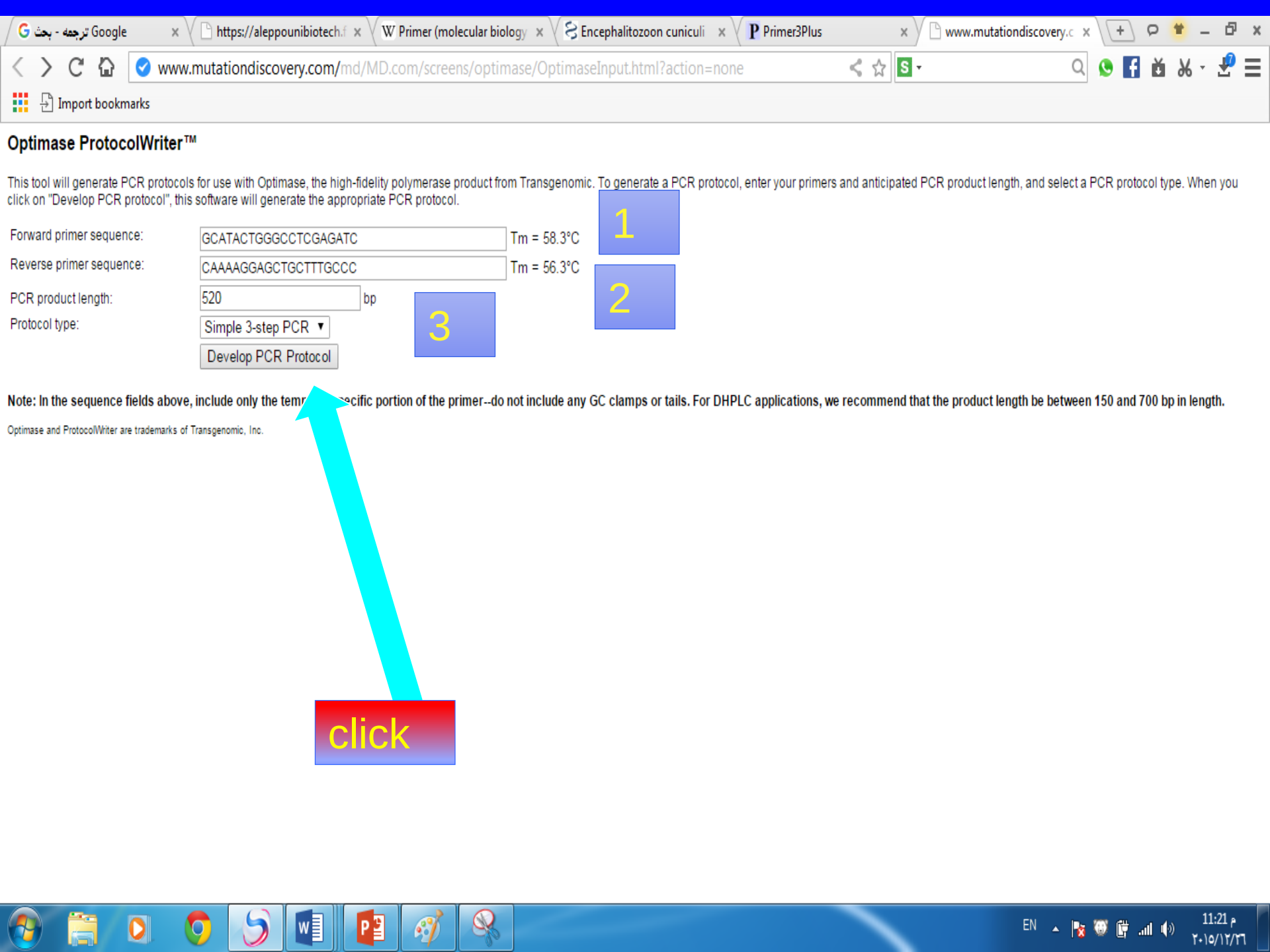
Copy to Optimase ProtocolWriter™

Select all Primers

http://www.mutationdiscovery.com/md/MD.com/screens/optimase/OptimaseInput.html?action=none




Google ترجمه - بحث
https://aleppounibiotech.f
Primer (molecular biology
Encephalitozoon cuniculi
Primer3Plus
www.mutationdiscovery.c
www.mutationdiscovery.com/md/MD.com/screens/optimase/OptimaseInput.html?action=none
Import bookmarks
Optimase ProtocolWriter™
This tool will generate PCR protocols for use with Optimase, the high-fidelity polymerase product from Transgenomic. To generate a PCR protocol, enter your primers and anticipated PCR product length, and select a PCR protocol type. When you click on "Develop PCR protocol", this software will generate the appropriate PCR protocol.
Forward primer sequence:
GCATACTGGGCCTCGAGATC
Tm = 58.3°C
Reverse primer sequence:
CAAAAGGAGCTGCTTTGCCC
Tm = 56.3°C
PCR product length:
520
bp
Protocol type:
Simple 3-step PCR
Develop PCR Protocol
1
2
3
Note: In the sequence fields above, include only the tem
cific portion of the primer--do not include any GC clamps or tails. For DHPLC applications, we recommend that the product length be between 150 and 700 bp in length.
Optimase and ProtocolWriter are trademarks of Transgenomic, Inc.
click
EN
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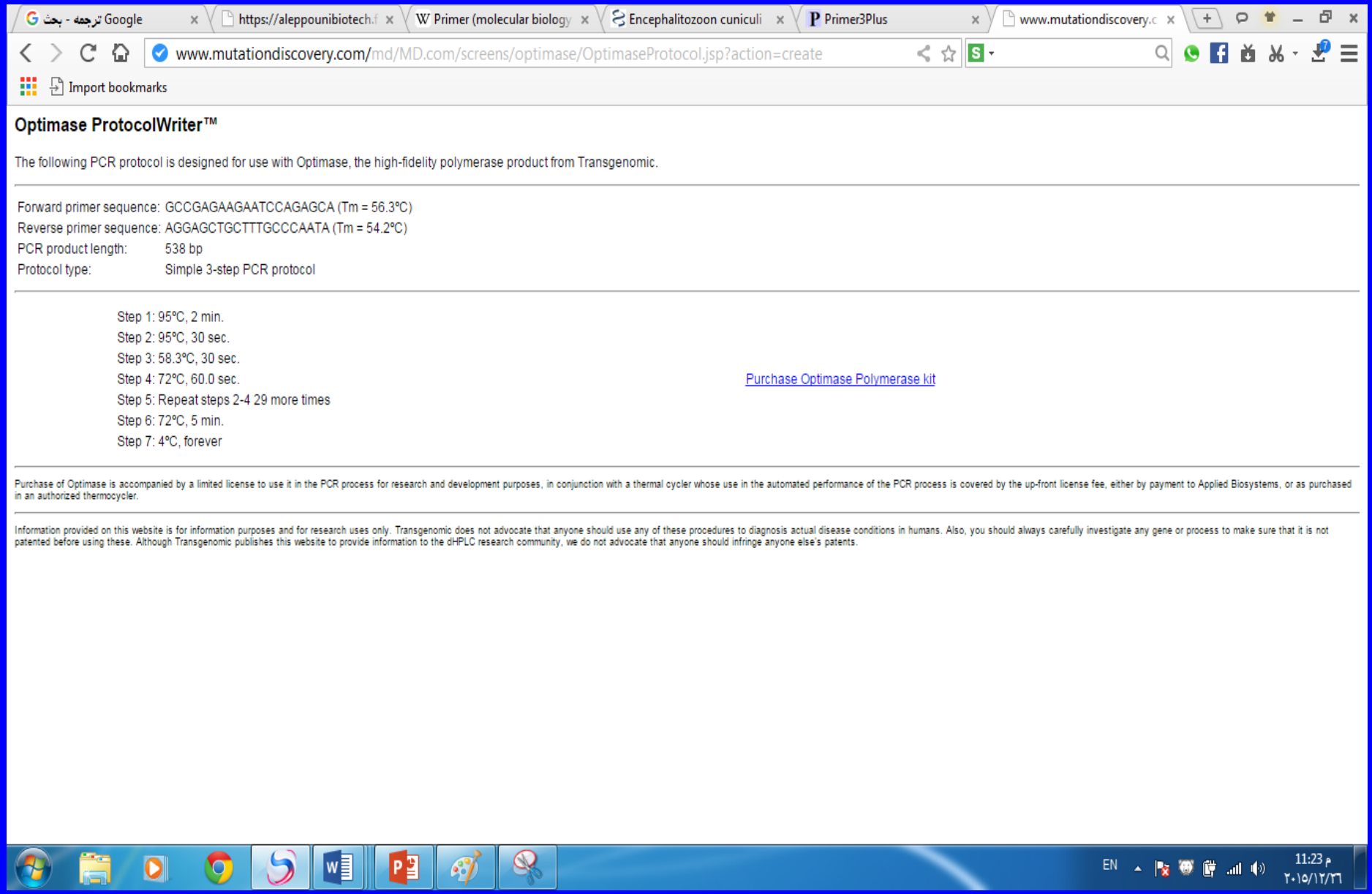
PCR protocol



Optimase ProtocolWriter™

The following PCR protocol is designed for use with Optimase, the high-fidelity polymerase product from Transgenomic.

Forward primer sequence: GCCGAGAAGAATCCAGAGCA (Tm = 56.3°C)
Reverse primer sequence: AGGAGCTGCTTTGCCCAATA (Tm = 54.2°C)
PCR product length: 538 bp
Protocol type: Simple 3-step PCR protocol

Step 1: 95°C, 2 min.
Step 2: 95°C, 30 sec.
Step 3: 58.3°C, 30 sec.
Step 4: 72°C, 60.0 sec.
Step 5: Repeat steps 2-4 29 more times
Step 6: 72°C, 5 min.
Step 7: 4°C, forever

Purchase Optimase Polymerase kit