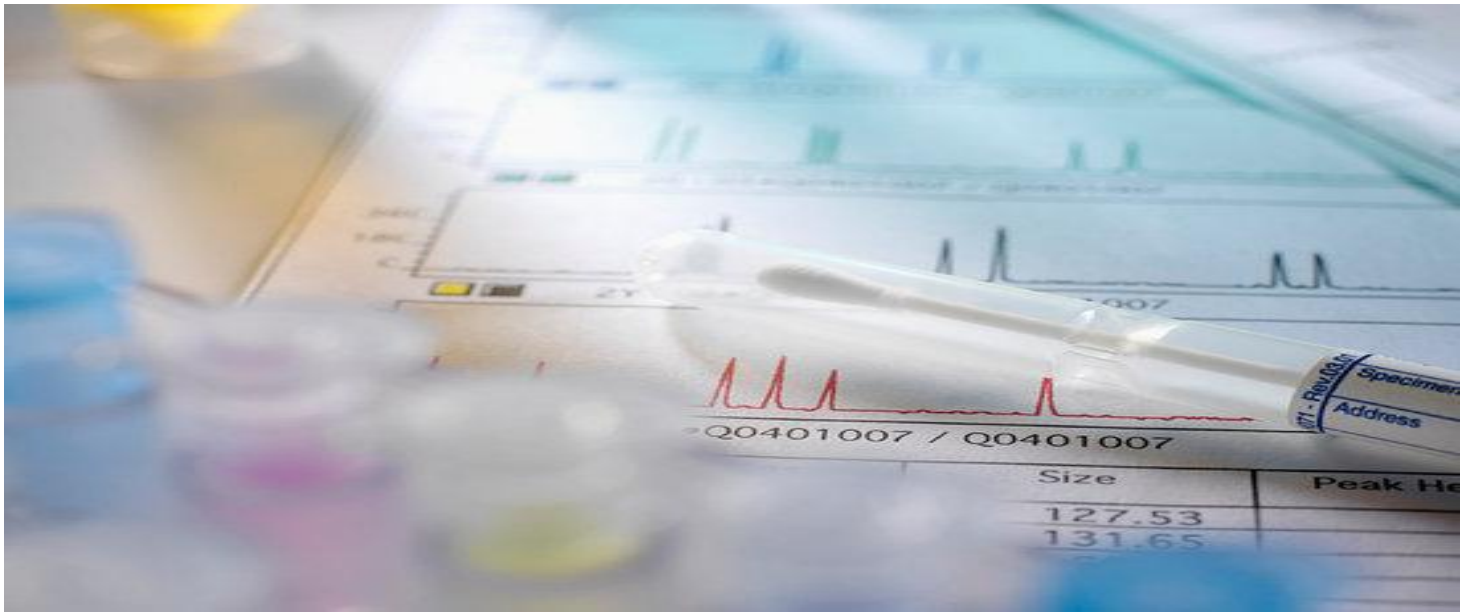
Gene sequence and primer design



Mohamed N. Seleem

What is primer?

Primer design for diagnosis and regular amplification

- Previous experiments
- Software
- By hand

How to design your Primer

Free software

http://frodo.wi.mit.edu/primer3/

http://www.humgen.nl/primer_design.html

Many others

Gene sequence

Pubmed

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

Omp25 *Brucella*

```
     gene            1..642
                     /gene="omp25"
     CDS             1..642
                     /gene="omp25"
                     /codon_start=1
                     /product="outer membrane protein 25"
                     /protein_id="CAQ68393.1"
                     /db_xref="GI:206596726"
                     /db_xref="GOA:B5U6Y0"
                     /db_xref="InterPro:IPR011250"
                     /db_xref="UniProtKB/TrEMBL:B5U6Y0"
                     /translation="MRTLKSLVIVSAALLPFSATAFAADAIQEQPPVPAPVEVAPQYS
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                     AGAGLEAKLTDNILGRVEYRYTQYGNKNYDLAGTNVRNKLDTQDIRVGIGYKF"
ORIGIN
        1 atgcgcactc ttaagtctct cgtaatcgtc tcggctgcgc tgctgccgtt ctctgcgacc
       61 gcttttgctg ccgacgccat ccaggaacag cctccggttc cggctccggt tgaagtagct
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      361 gcccgcgtcg gctacgacct gaacccggtt atgccgtacc tcacggctgg tattgccggt
      421 tcgcagatca agcttaacaa cggcttggac gacgaaagca agttccgcgt gggttggacg
      481 gctggtgccg gtctcgaagc caagctgacg gacaacatcc tcggccgcgt tgagtaccgt
      541 tacacccagt acggcaacaa gaactacgat ctggccggta cgaatgtccg caacaagctg
      601 gacacgcagg atatccgcgt cggcatcggc tacaagttct aa
//
```
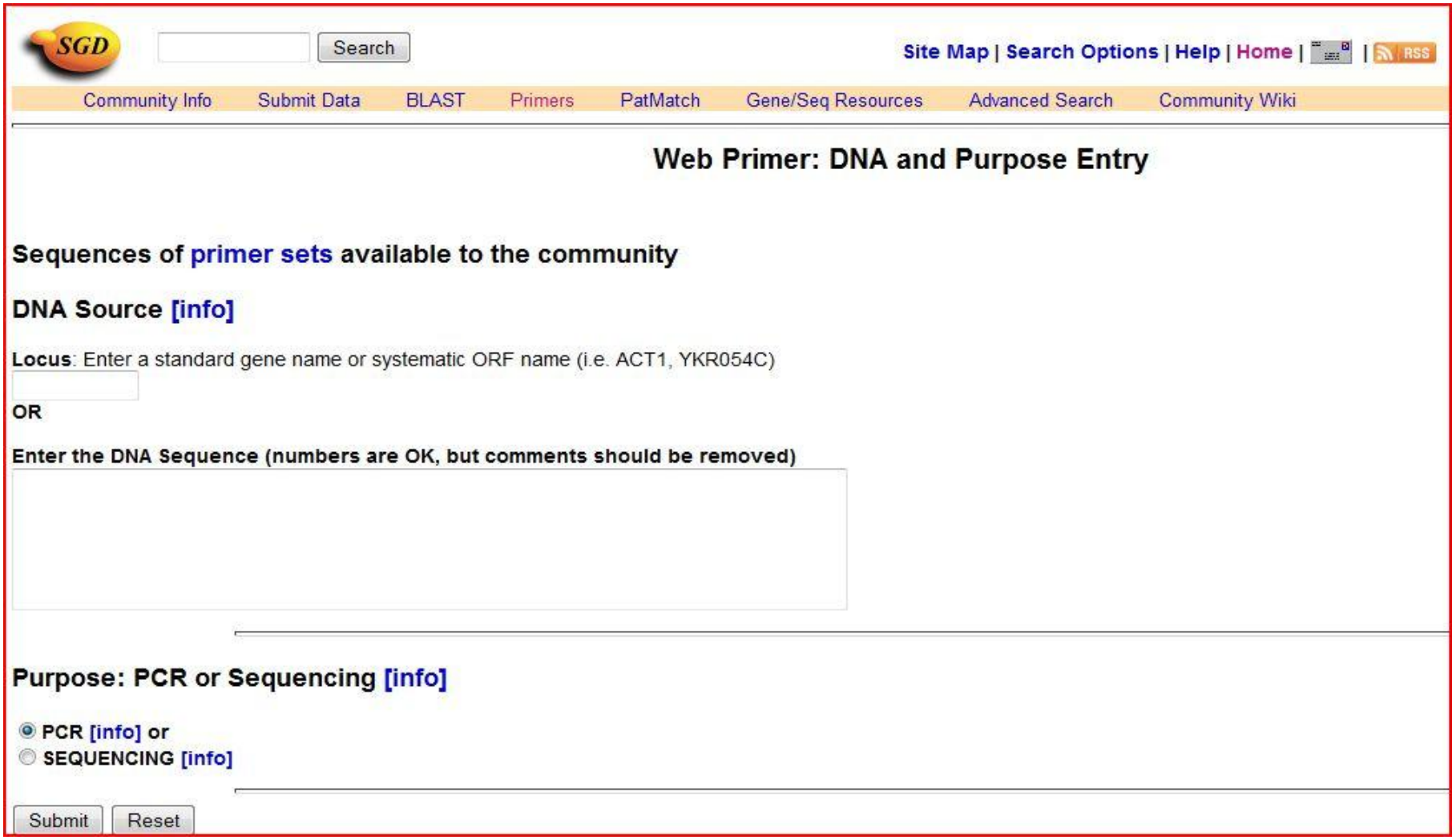

WebPrimer software



Copy and paste sequence

copy

```
                     /db_xref="GOA:B5U6Y0"
                     /db_xref="InterPro:IPR011250"
                     /db_xref="UniProtKB/TrEMBL:B5U6Y0"
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                     AGAGLEAKLTDNILGRVEYRYTQYGNKNYDLAGTNVRNKLDTQDIRVGIGYKF"
ORIGIN
        1 atgcgcactc ttaagtctct cgtaatcgtc tcggctgcgc tgctgccgtt ctctgcgacc
       61 gcttttgctg ccgacgccat ccaggaacag cctccggttc cggctccggt tgaagtagct
      121 ccccagtata gctgggctgg tggctatacc ggtctttacc tcggctacgg ctggaacaag
      181 gccaagacca gcaccgttgg cagcatcaag cctgacgatt ggaaggctgg cgcttttgct
      241 ggctggaact tccagcagga ccagatcgta tatggtgttg aaggtgatgc aggttattcc
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      361 gcccgcgtcg gctacgacct gaacccggtt atgccgtacc tcacggctgg tattgccggt
      421 tcgcagatca agcttaacaa cggcttggac gacgaaagca agttccgcgt gggttggacg
      481 gctggtgccg gtctcgaagc caagctgacg gacaacatcc tcggccgcgt tgagtaccgt
      541 tacacccagt acggcaacaa gaactacgat ctggccggta cgaatgtccg caacaagctg
      601 gacacgcagg atatccgcgt cggcatcggc tacaagttct aa
//
```

paste

Sequences of primer sets available to the community

DNA Source [info]

Locus: Enter a standard gene name or systematic ORF name (i.e. ACT1, YKR054C)

OR

Enter the DNA Sequence (numbers are OK, but comments should be removed)

```
      481 gctggtgccg gtctcgaagc caagctgacg gacaacatcc tcggccgcgt
tgagtaccgt
      541 tacacccagt acggcaacaa gaactacgat ctggccggta cgaatgtccg
caacaagctg
      601 gacacgcagg atatccgcgt cggcatcggc tacaagttct aa
```

Purpose: PCR or Sequencing [info]

- (•) **PCR [info] or**
- ( ) **SEQUENCING [info]**

Submit Reset

Options to choose from!

Location [info]

Select YES to Amplify a region with **EXACT** endpoints of the DNA sequence entered ◉ NO ○ YES
Length of DNA in which to search for valid primers: 35

Melting Temperature [info]

Optimum Tm: 56 Minimum Tm: 52 Maximum Tm: 60
Optimum primer length: 20 Minimum length: 18 Maximum length: 21

Primer Composition [info]

Optimum percent GC content: 45 Minimum GC: 30 Maximum GC: 60

Primer Annealing [info]

Self Anneal: 24 Self End Anneal: 12 Pair Anneal: 24 Pair End Anneal: 12

Submit Reset

Primers for PCR

There were 66 forward primers in the valid range for GC content.
There were 23 reverse primers in the valid range for GC content.
There were 43 forward primers in the valid range for melting temperature.
There were 22 reverse primers in the valid range for melting temperature.
There were 43 forward primers with valid self anneal values.
There were 22 reverse primers with valid self anneal values.
There were 33 forward primers with valid self end anneal values.
There were 22 reverse primers with valid self end anneal values.
Some primers were not evaluated

There were 250 pairs of valid primers.

This is the BEST pair of primers

List of all valid pairs of primers

VirginiaTech
Invent the Future

Best choice of primer pair

Primer Pair Data

Primers for 1 (Entered)

These primers are for PCR

Forward-Primer	ATGCGCACTCTTAAGTCTCT	**Reverse-Primer**	TTAGAACTTGTAGCCGATGCC
Length:	20	Length:	21
Percent GC:	45	Percent GC:	47
Tm:	48	Tm:	53
Self-Anneal:	20	Self-Anneal:	10
End-Anneal:	6	End-Anneal:	4
Offset:	1	Offset:	642

Pair-Anneal: 16
Pair-End-Anneal: 8
Total valid primer pairs: 250

These primers will amplify a fragment of 642 basepairs
The sequence of DNA that will be amplified by these primers is

```
1   ATGCGCACTC TTAAGTCTCT CGTAATCGTC TCGGCTGCGC TGCTGCCGTT
51  CTCTGCGACC GCTTTTGCTG CCGACGCCAT CCAGGAACAG CCTCCGGTTC
```

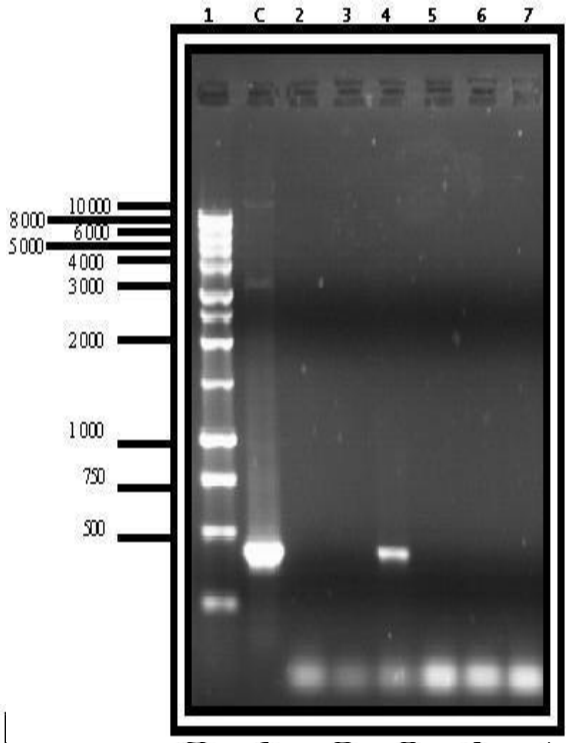
Things to avoid in Primer Design

A primer may be self-complementary and fold into a **hairpin**:

5-GTTGACTTGATATTATCAAG-3

5´-GTTGACTTGATA
|||||| T
3´-GAACTAT

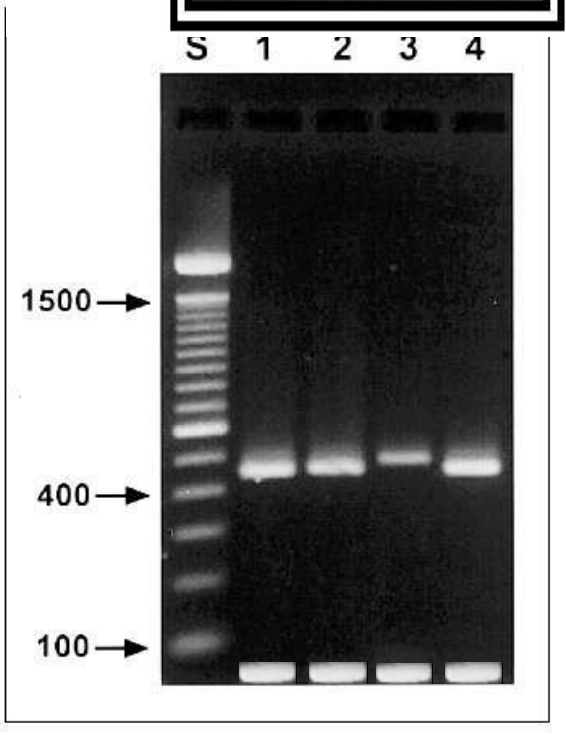
Things to avoid in Primer Design



A primer may form a **dimer** with itself or with the other primer.

5´-ACCGGTAGCCACGAATTCGT-3´

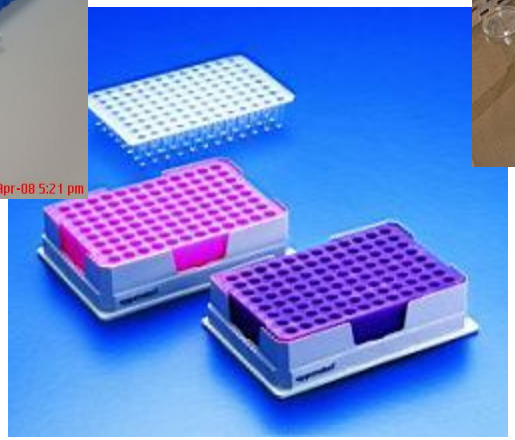
5´-ACCGGTAGCCACGAATTCGT-3´
||||||||||||
3´-TGCTTAAGCACCGATGGCCA-5´



Order primers commercially

- 3.5 - 4 Egyptian Pounds for base
- Powder
- 5,000 reactions

Set up PCR reaction



Set up PCR reaction

Reagents in a tube

1- 5 µl (200 ng)	•Sample (DNA)
1 µl F and 1 µl R 20 pmol	•Primers (forward and reverse)
25 µl – 50 µl	•Enzyme (DNA polymerase, *Taq)* •dNTPs (A, T, G, C) •Buffer (50 mM KCl; 10 mM Tris-HCl; 1.5 mM MgCl2)
If needed	•Water DNAase free water

Thermocycler

PCR Cycles

Time and temperature

- Initial denature
- denature
- annealing
- extension
- Final extension

25-40 cycles

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PCR Cycles

		Time and Temperature
•Initial denature		**94 °C for 2-5 minuets**
•denature	**25-40 cycles**	**94 °C for 30 sec**
•annealing		**55 °C for 30 sec**
•Extension		**68-72 °C for xxx sec**
•Final extension		**72 °C for 5 minuets**

Overnight Thermocycler

Last step incubation

- 10 °C for gene cloning
- Room temperature for diagnosis

How to chose your enzyme And your company

taq vs *pfu*

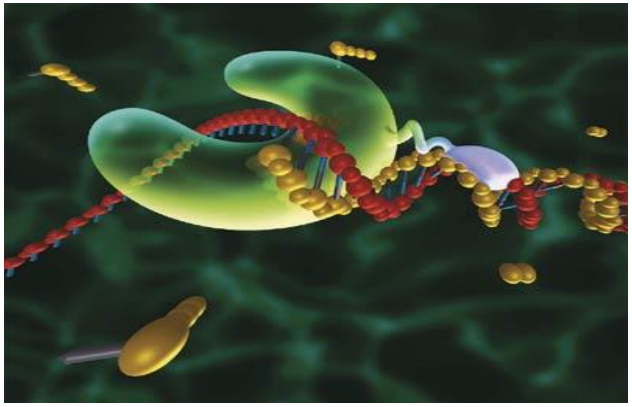
Diagnosis vs molecular cloning

Promega GoTaq green mastermix (cat no. M7122)
Qiagen mastermix

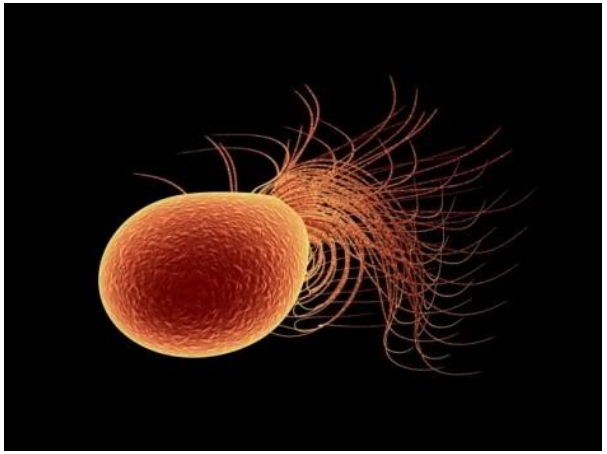
DNA Polymerase

***T**hermus **aq**uaticus*
Taq enzyme
Heat stable
1988

Error 1:10,000



Hot spring

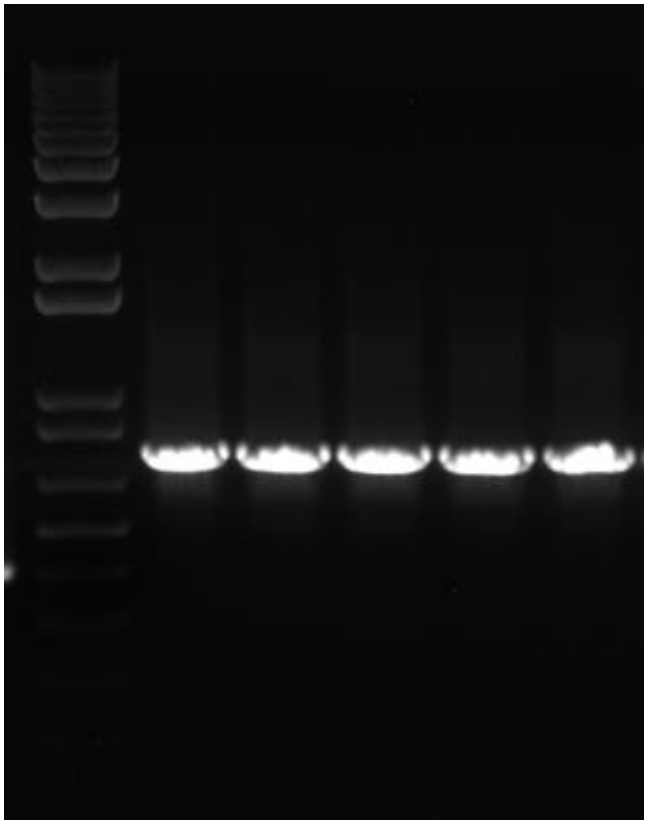


***P**yrococcus **fu**riosus*
Pfu enzyme
More stable
1991

Error 1: Million

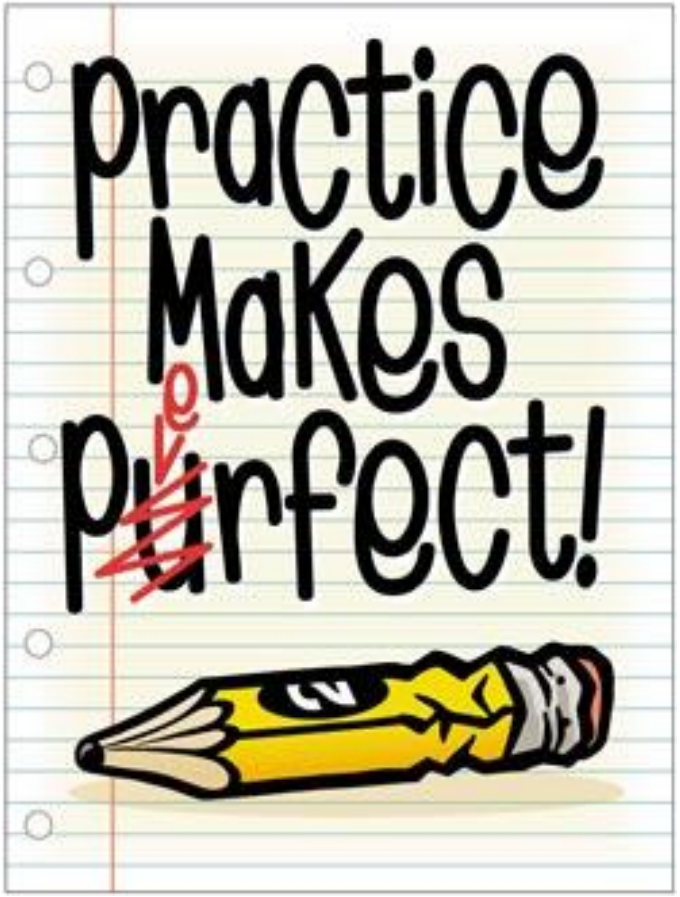
3' to 5' exonuclease proofreading activity

Marker PCR products

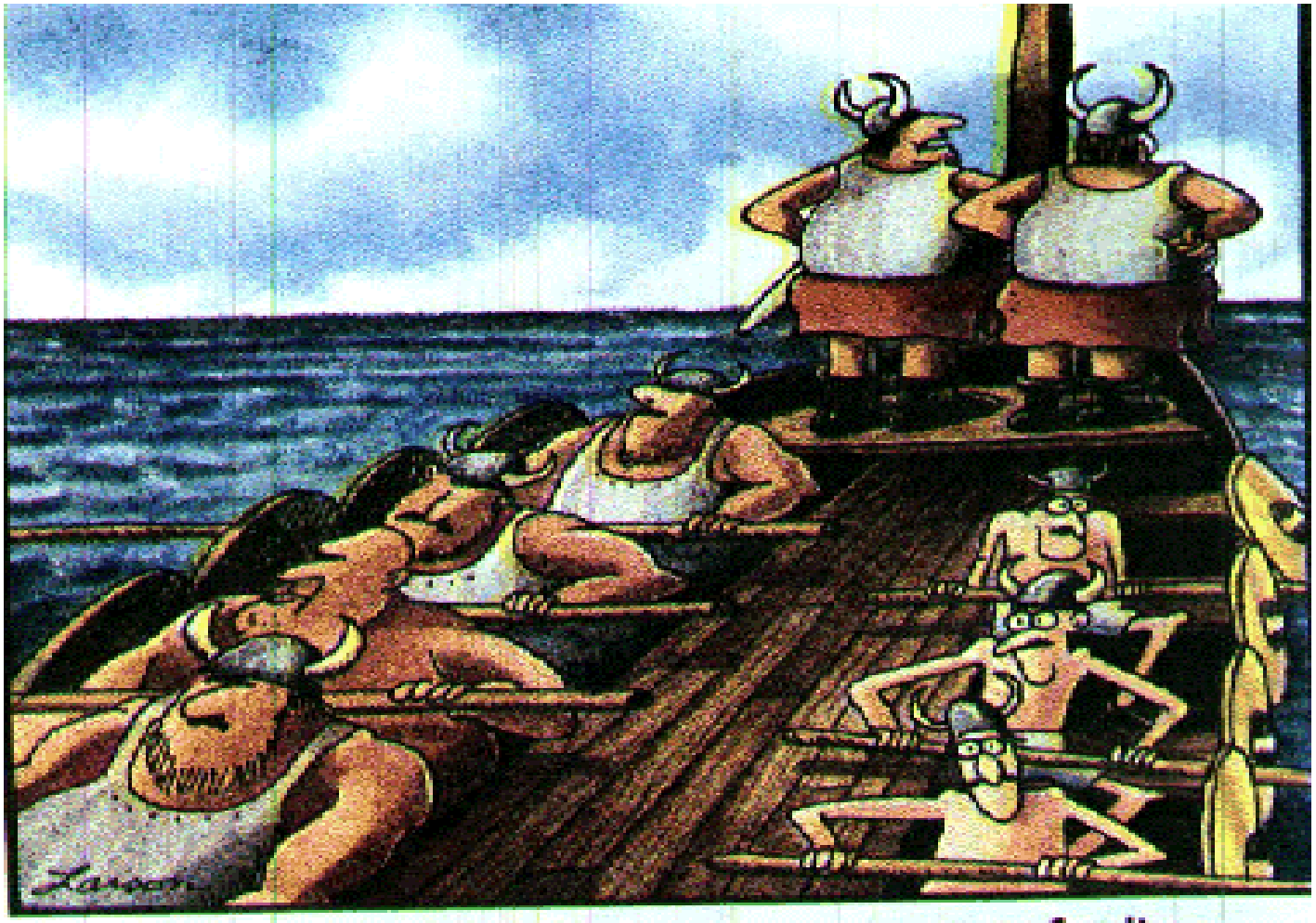


Real time PCR

Needs practice



Questions!!!



"I've got it, too, Omar ... a strange feeling like we've just been going in circles."