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Institute for
Critical Technology and Applied Science

Gene sequence and primer design

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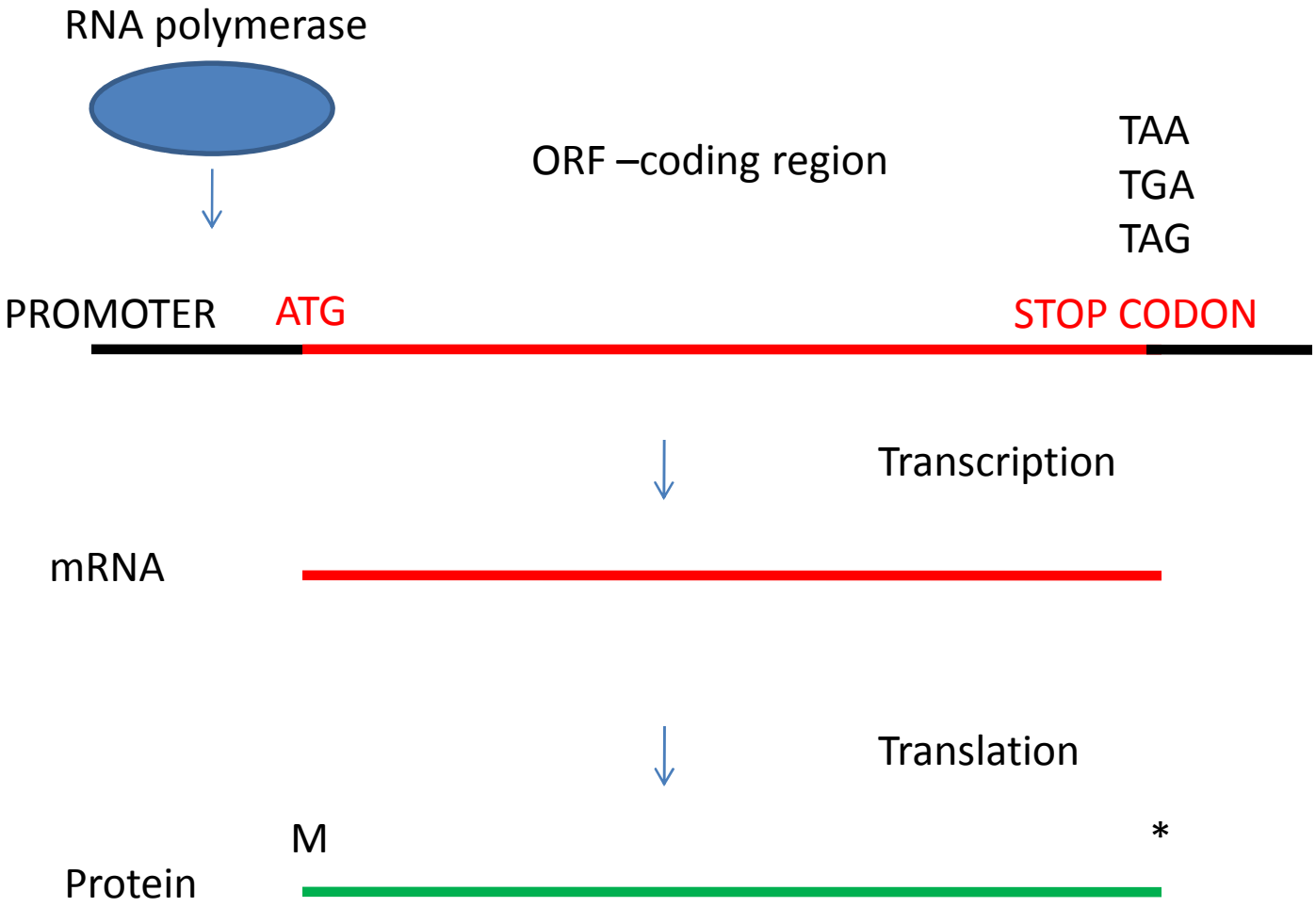
Gene expression



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Primer design for diagnosis and regular amplification

- Previous experiments
- Software
- By hand

Gene sequence

Pubmed

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

Omp25 *Brucella*

```
     gene            1..642
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     CDS             1..642
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ORIGIN
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      241 ggctggaact tccagcagga ccagatcgta tatggtgttg aaggtgatgc aggttattcc
      301 tgggccaaga agtccaagga cggcctggaa gtcaagcagg gctttgaagg ctcgctgcgt
      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
//
```

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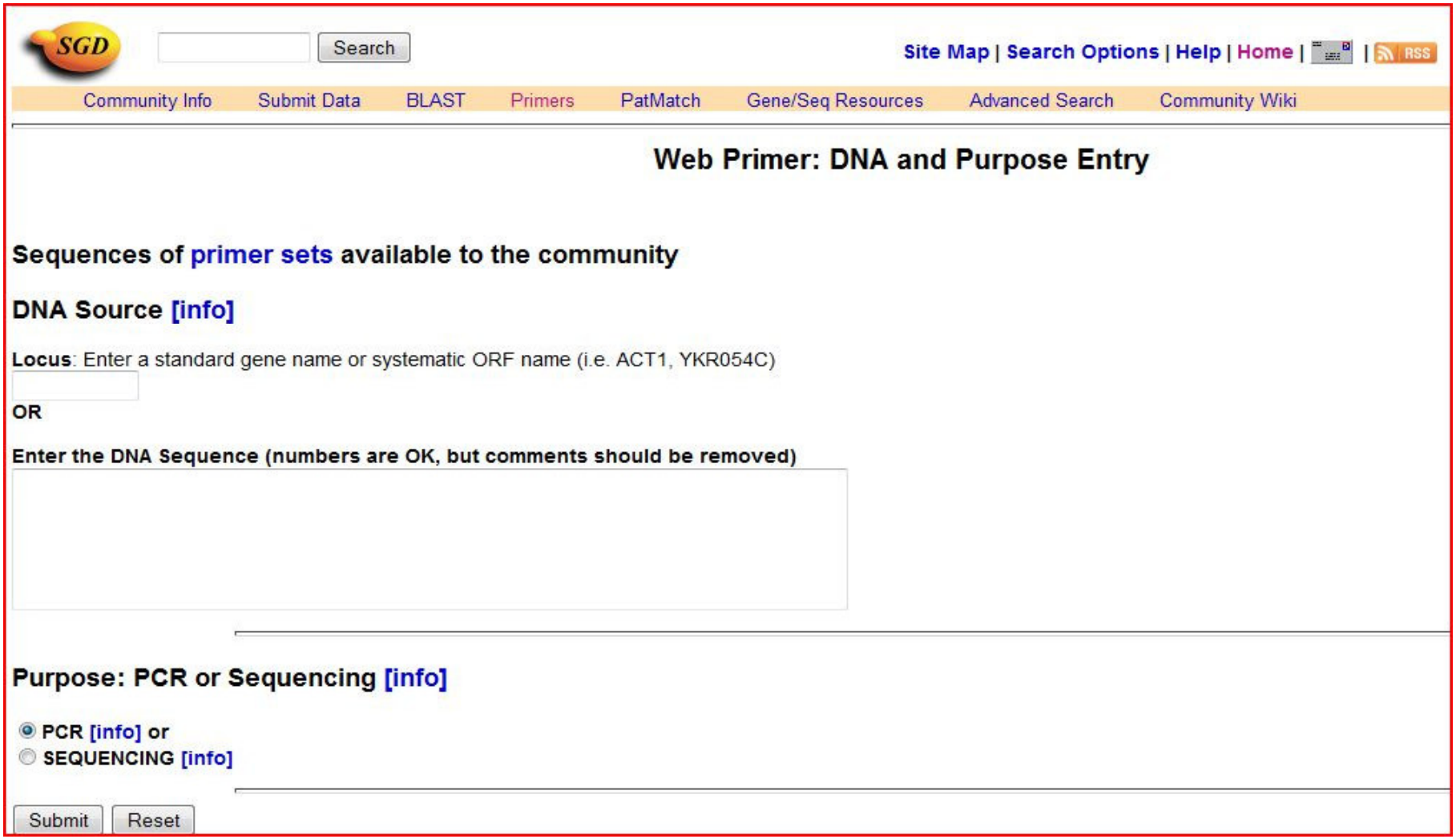
WebPrimer software



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Copy and paste sequence

copy

```
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ORIGIN
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       61 gcttttgctg ccgacgccat ccaggaacag cctccggttc cggctccggt tgaagtagct
      121 ccccagtata gctgggctgg tggctatacc ggtctttacc tcggctacgg ctggaacaag
      181 gccaagacca gcaccgttgg cagcatcaag cctgacgatt ggaaggctgg cgcttttgct
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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

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Options to chose 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

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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
```

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Primer for gene expression

Hand design

- Coding region ATG----------Stop codon
- Frame

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Primer for gene expression

Frame

OLD MEN ARE FUN

LDM ENA REF UN

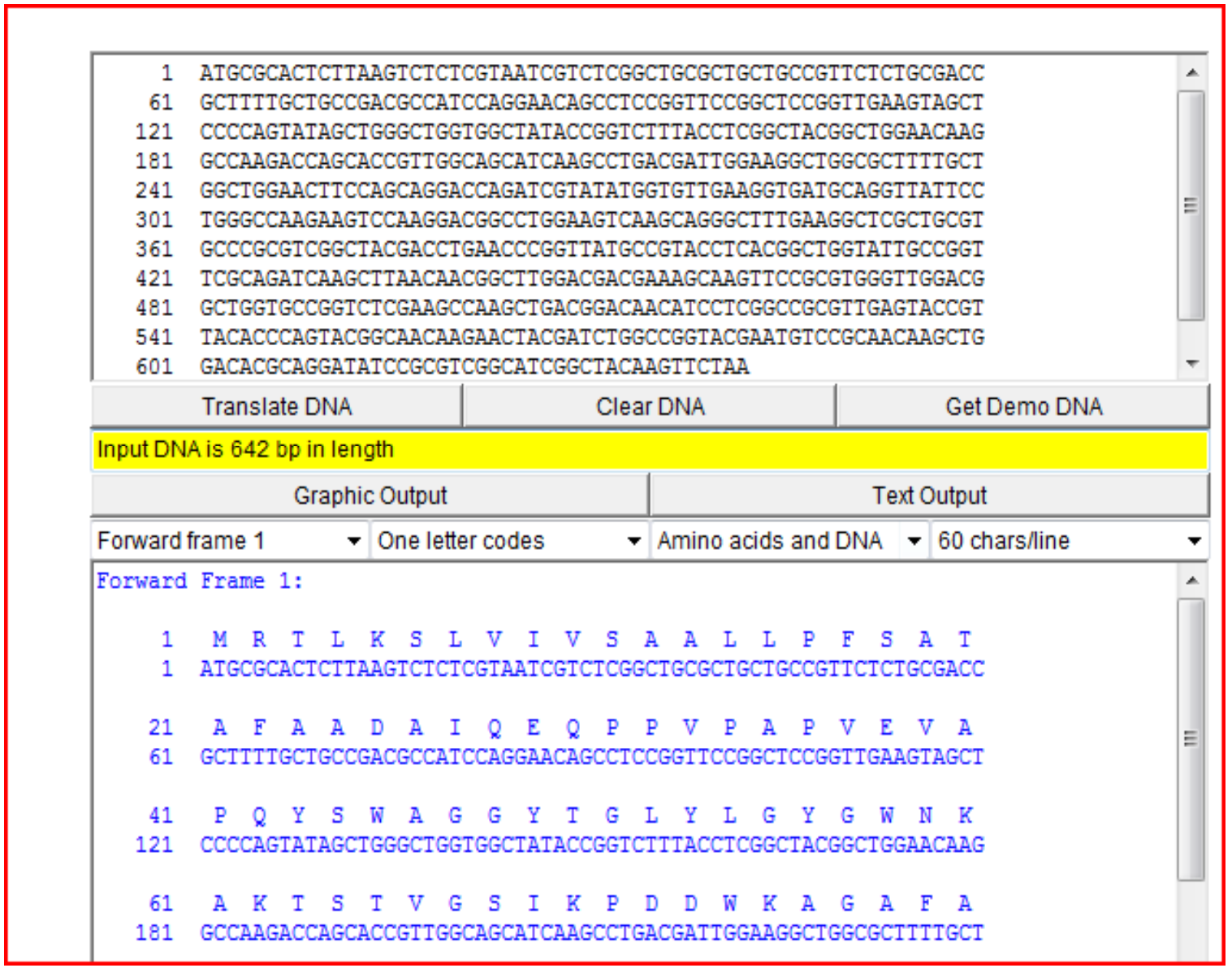
DME NAR EFU N

MEN ARE FUN

Correct frame –ORF

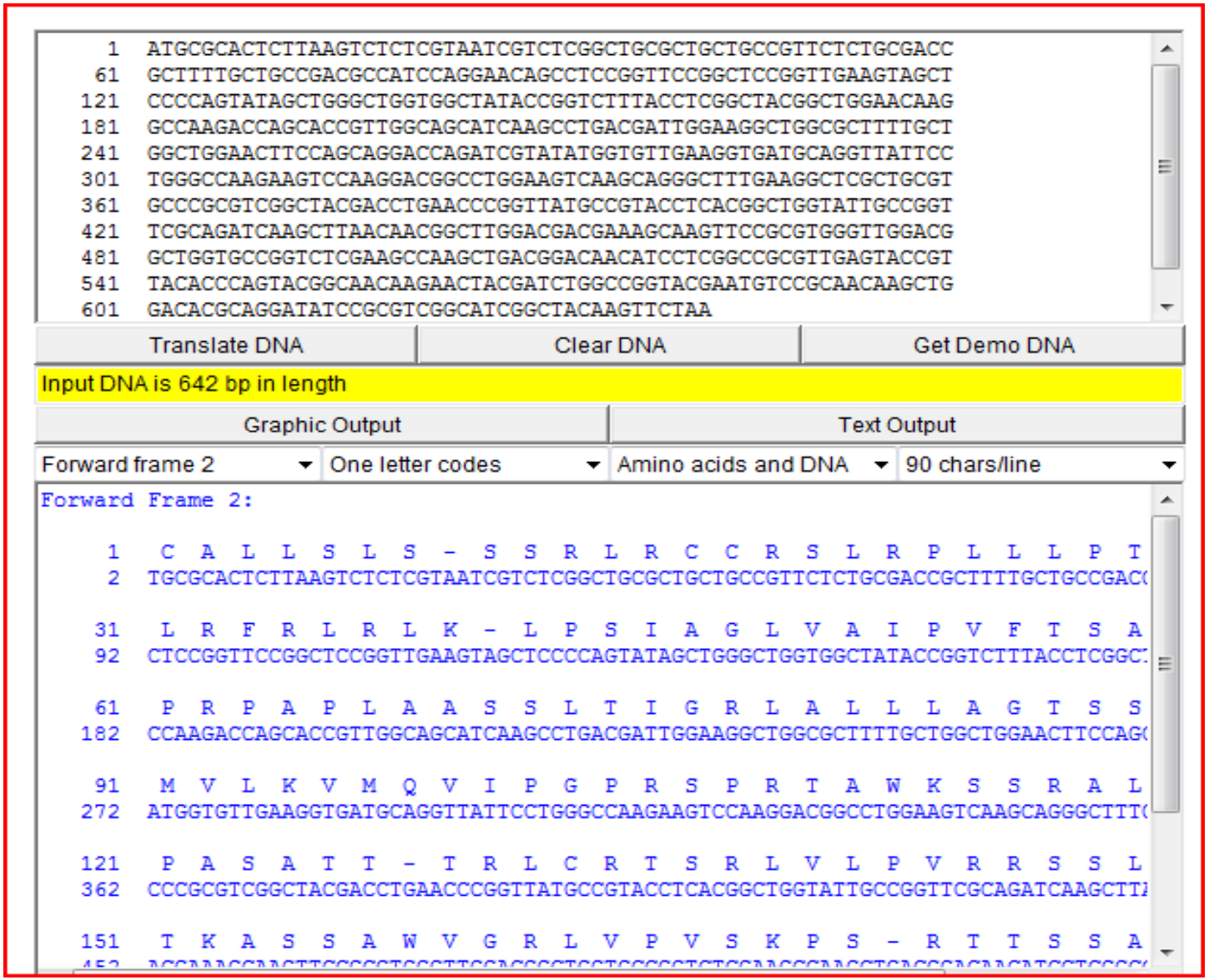
Omp 25

- Correct protein
- ORF – no stop codon



Wrong frame

Omp 25



- Wrong protein
- Non ORF –stop codon

Designing primers by hand

ATGCGCACTCTTAAGTCTCTCGTAATCGTCTCGGCTGCGCTGCTGCCGTCCAGCACCGTTACAAGTTCTAA
TACGCGTGAGAATTCAGAGAGCATTAGCAGAGCCGACGCGACGACGGCAGGTCGTGGCAATGTTCAAGATT

```
     1  ATGCGCACTCTTAAGTCTCTCGTAATCGTCTCGGCTGCGCTGCTGCCGTTCTCTGCGACC
    61  GCTTTTGCTGCCGACGCCATCCAGGAACAGCCTCCGGTTCCGGCTCCGGTTGAAGTAGCT
   121  CCCCAGTATAGCTGGGCTGGTGGCTATACCGGTCTTTACCTCGGCTACGGCTGGAACAAG
   181  GCCAAGACCAGCACCGTTGGCAGCATCAAGCCTGACGATTGGAAGGCTGGCGCTTTTGCT
   241  GGCTGGAACTTCCAGCAGGACCAGATCGTATATGGTGTTGAAGGTGATGCAGGTTATTCC
   301  TGGGCCAAGAAGTCCAAGGACGGCCTGGAAGTCAAGCAGGGCTTTGAAGGCTCGCTGCGT
   361  GCCCGCGTCGGCTACGACCTGAACCCGGTTATGCCGTACCTCACGGCTGGTATTGCCGGT
   421  TCGCAGATCAAGCTTAACAACGGCTTGGACGACGAAAGCAAGTTCCGCGTGGGTTGGACG
   481  GCTGGTGCCGGTCTCGAAGCCAAGCTGACGGACAACATCCTCGGCCGCGTTGAGTACCGT
   541  TACACCCAGTACGGCAACAAGAACTACGATCTGGCCGGTACGAATGTCCGCAACAAGCTG
   601  GACACGCAGGATATCCGCGTCGGCATCGGCTACAAGTTCTAA
```

Translate DNA | Clear DNA | Get Demo DNA

Input DNA is 642 bp in length

Graphic Output | Text Output

Forward frame 1 | One letter codes | Amino acids and DNA | 60 chars/line

```
Forward Frame 1:

     1   M  R  T  L  K  S  L  V  I  V  S  A  A  L  L  P  F  S  A  T
     1  ATGCGCACTCTTAAGTCTCTCGTAATCGTCTCGGCTGCGCTGCTGCCGTTCTCTGCGACC

    21   A  F  A  A  D  A  I  Q  E  Q  P  P  V  P  A  P  V  E  V  A
    61  GCTTTTGCTGCCGACGCCATCCAGGAACAGCCTCCGGTTCCGGCTCCGGTTGAAGTAGCT

    41   P  Q  Y  S  W  A  G  G  Y  T  G  L  Y  L  G  Y  G  W  N  K
   121  CCCCAGTATAGCTGGGCTGGTGGCTATACCGGTCTTTACCTCGGCTACGGCTGGAACAAG

    61   A  K  T  S  T  V  G  S  I  K  P  D  D  W  K  A  G  A  F  A
   181  GCCAAGACCAGCACCGTTGGCAGCATCAAGCCTGACGATTGGAAGGCTGGCGCTTTTGCT
```

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Designing forward primers by hand

ATGCGCACTCTTAAGTCTCTCGTAATCGTCTCGGCTGCGCTGCTGCCGTCCAGCACCGTTACAAGTTCTAA
TACGCGTGAGAATTCAGAGAGCATTAGCAGAGCCGACGCGACGACGGCAGGTCGTGGCAATGTTCAAGATT

FORWARD PRIMER ATGCGCACTCTTAAGTCTCTC

Memorize Forward primer : first 17- 21 nucleotides on the upper strand

ATGCGCACTCTTAAGTCTCTCGTAATCGTCTCGGCTGCGCTGCTGCCGTCCAGCACCGTTACAAGTTCTAA

Forward primer

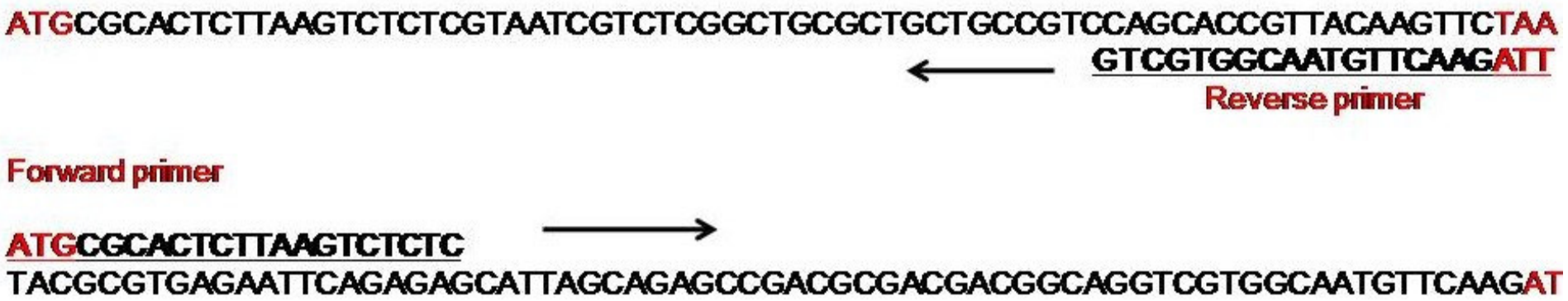
ATGCGCACTCTTAAGTCTCTC
TACGCGTGAGAATTCAGAGAGCATTAGCAGAGCCGACGCGACGACGGCAGGTCGTGGCAATGTTCAAGATT

Designing Reverse primers by hand

ATGCGCACTCTTAAGTCTCTCGTAATCGTCTCGGCTGCGCTGCTGCCGTCCAGCACCGTTACAAGTTCTAA
TACGCGTGAGAATTCAGAGAGCATTAGCAGAGCCGACGCGACGACGGCAGGTCGTGGCAATGTTCAAGATT

REVERSE PRIMER TTAGAACTTGTAACGGTGCTG

Memorize Reverse primer : last17- 21 nucleotides on the lower strand (right to left)



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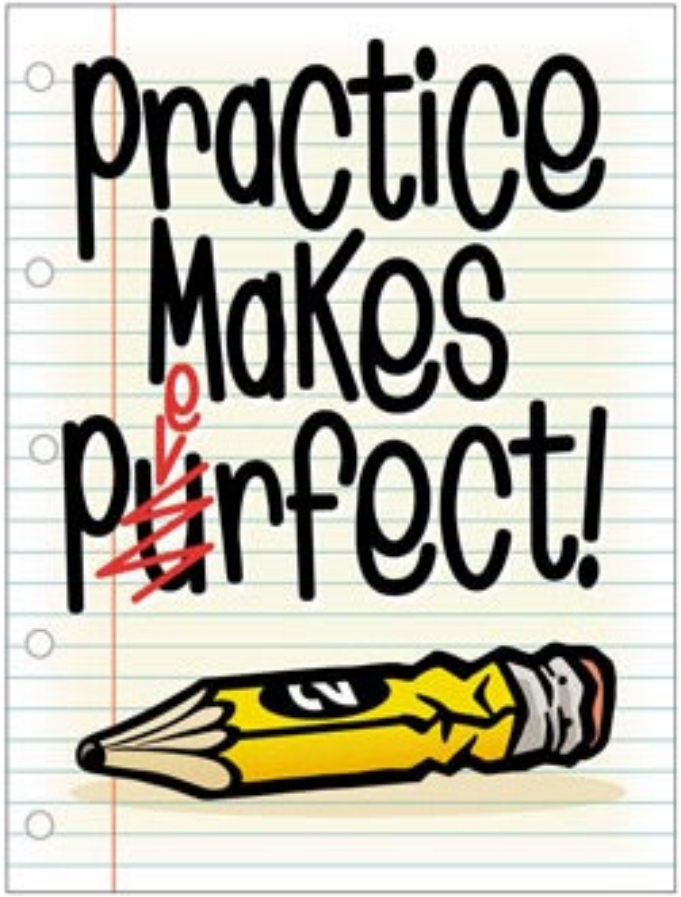
Needs practice



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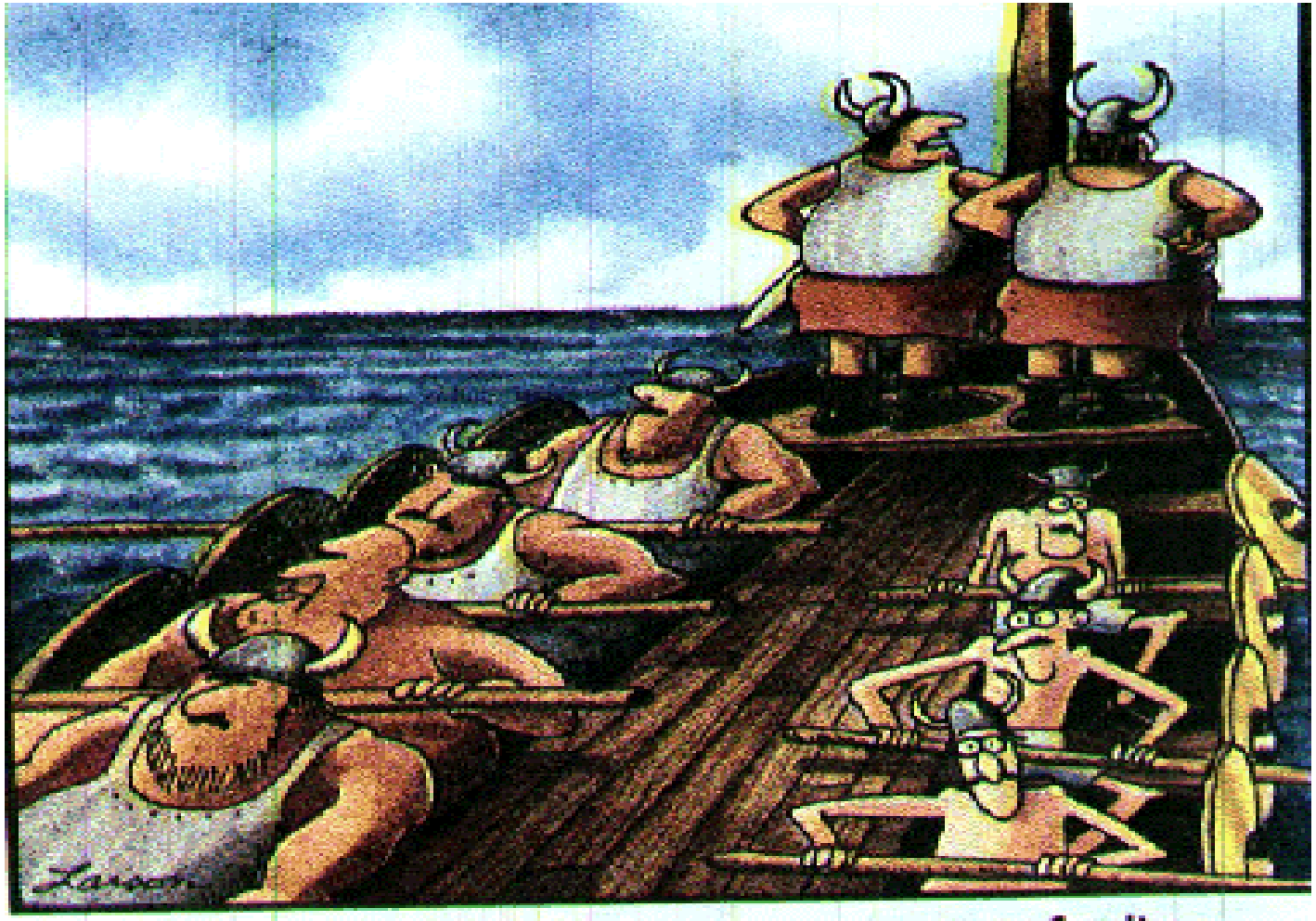
Questions!!!



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