# PCR from Genomic DNA

1. Obtain the genomic DNA from the instructors that you extracted on week 2. The TA can provide DNA if you did not isolate any in that laboratory.

2. Dilute the genomic DNA with $dH_2O$ in a 0.5 ml tube. Use between 200 and 500ng, you may need to make a 1:5 or 1:10 dilution (1 µl of DNA into 4 or 9 µl of $dH_2O$, mix, add 1 microliter).

3. Prepare your PCR reaction mix. All components listed below will be added, in order, to one 1.5 ml tube and then mixed. After mixing, the tube should be centrifuged briefly (3-5 seconds) to bring all components to the bottom of the tube.

| Reagent | Amount to add for 1 reaction |
|---|---|
| $dH_20$ | 38.75 µl |
| *Diluted* genomic DNA | 1.0 µl |
| 10X PCR Buffer (includes $MgCl_2$) | 5.0 µl |
| Gene Specific Primer mix | 1.0 µl |
| 10 mM dNTPs (2.5mM each dT, dA, dC, dGTP) | 4.0 µl |

   All of the reaction mix should then be transferred to a 0.2 ml PCR tube containing the 0.25 µl of Taq Polymerase. (Note: this tube is very small and is not marked so you can label the tube with your group number and initials on the top and side of the tube).

   Taq polymerase (this is in the 0.2 ml tube already ) 0.25 µl

4. Your PCR samples should be placed on ice until everyone has finished assembling their reactions.

5. Amplify the reaction mixtures using a MJ Research DNA Engine Peltier Thermal Cycler. The reaction conditions are as follows:

   94°C for 5 minutes
   - 94°C for 30 seconds
   - 55°C for 30 seconds
   - 72°C for 60 seconds

   ] 25 cycles (50 minutes)

   4°C until electrophoresis.

6. Prepare a sample from the PCR reaction for electrophoresis. Obtain your PCR reactions from the TA. The samples should be centrifuged briefly (5-10 seconds). 5 µl of each sample should be added to new tubes. 2 µl of loading dye is then added. The reactions are mixed, centrifuged briefly, and loaded onto a prepared agarose gel as directed by the instructors.

Materials

The primer mix contains both PCR primers at concentrations of 10 pmoles/µl each. The 10X PCR buffer will give a final reaction concentration of 50 mM Tris-HCL pH 9.1, 16 mM ammonium sulfate, 3.5 mM $MgCl_2$ and 150 µg/ml BSA.

Primer F

5'- ACCTCCTCGGGAAAGTTACAAC -3'

Primer R

5'- GGCCTCATGCAACACAAAGC -3'

Gene X DNA and Protein Sequence

atggctacttcaaagttgaaaacccagaatgtggttgtatctctctccctaacc
M A T S K L K T Q N V V V S L S L T
ttaaccttggtactggtgctactgaccagcaaggcaaactcagcggaaactgtttctttc
L T L V L V L L T S K A N S A E T V S F
agctggaacaagttcgtgccgaagcaaccaaacatgatcctccaaggagacgctattgtg
S W N K F V P K Q P N M I L Q G D A I V
acctcctcgggaaagttacaactcaataaggttgacgaaaacggcaccccaaaaccctcg
T S S G K L Q L N K V D E N G T P K P S
tctcttggtcgcgccctctactccacccccatccacatttgggacaaagaaaccggtagc
S L G R A L Y S T P I H I W D K E T G S
gttgccagcttcgccgcttccttcaacttcaccttctatgcccctgacacaaaaaggctt
V A S F A A S F N F T F Y A P D T K R L
gcagatgggcttgccttctttctcgcaccaattgacactaagccacaaacacatgcaggt
A D G L A F F L A P I D T K P Q T H A G
tatcttggtcttttcaacgaaaacgagtctggtgatcaagtcgtcgctgttgagtttgac
Y L G L F N E N E S G D Q V V A V E F D
actttccggaactcttgggatccaccaaatccacacatcggaattaacgtcaattctatc
T F R N S W D P P N P H I G I N V N S I
agatccatcaaaacgacgtcttgggatttggccaacaataaagtagccaaggttctcatt
R S I K T T S W D L A N N K V A K V L I
acctatgatgcctccaccagcctcttggttgcttctttggtctacccttcacagagaacc
T Y D A S T S L L V A S L V Y P S Q R T
agcaatatcctctccgatgtggtcgatttgaagacttctcttcccgagtgggtgaggata
S N I L S D V V D L K T S L P E W V R I
gggttctctgctgccacgggactcgacatacctggggaatcgcatgacgtgctttcttgg
G F S A A T G L D I P G E S H D V L S W
tcttttgcttccaatttgccacacgctagcagtaacattgatcctttggatcttacaagc
S F A S N L P H A S S N I D P L D L T S
tttgtgttgcatgaggccatctaa
F V L H E A I -