# Cloning of Atrolysin A from *Crotulas atrox*

AJ Goos and Kayla Ohrt

# Atrolysin A

- This venom comes from *Crotalus atrox* which is the Western Diamondback Rattlesnake.
- We have found a tissue sample coming from the Kentucky Reptile Zoo.
- Atrolysin A works by not allowing platelet adhesion, and works only in a neutral pH.
- This is called a hemorrhagic toxin.
- In acidic conditions the venom denatures.

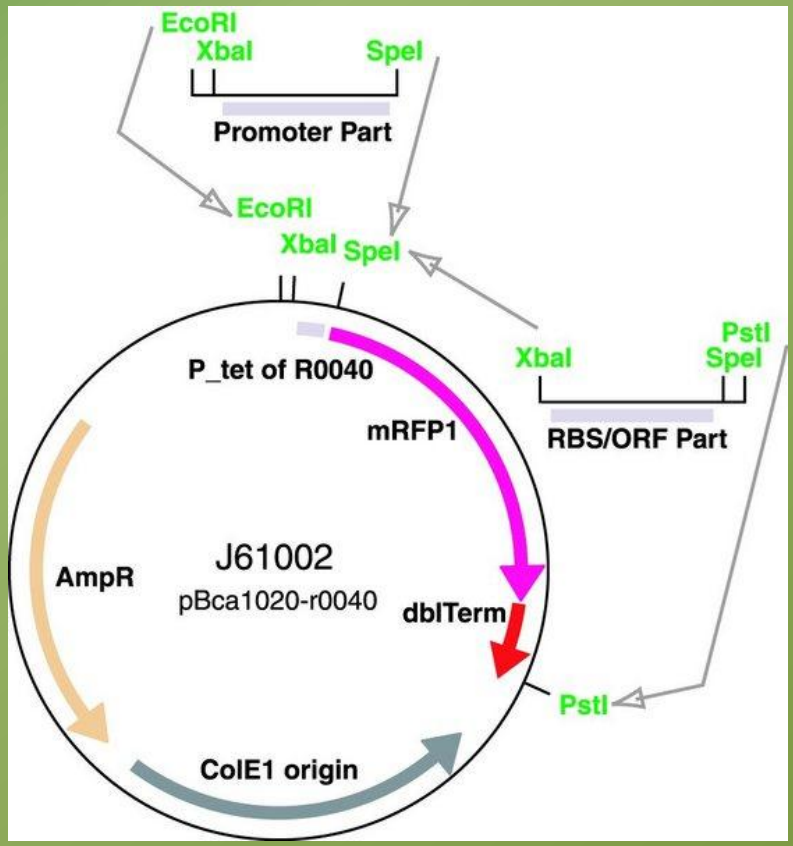
# Western Diamondback

- The Western Diamondback is an ambush hunter.
- Its venom falls in the PIII-SVMP class which accounts for 27.3% of total venom protiens.
- This venom is a protein.

- The Atrolysin A has been sequenced in mRNA form. The Genbank accession number is U01234.
- The mRNA is 1640 base pairs and the coding region is 1263 base pairs. The coding region starts at base pair 1 and codes to 1263.
- We must clone the gene with PCR and make sure it does not grow more than the 1260 base pairs.

# Vector

- We decided to use *E. coli* as the bacteria of choice for the vector.



# Promoters

- J23100 (Unregulated)
- J23109 (Unregulated)
- Bba_10500  (Inducible by agarose)
- We chose these promoters due to the high yield of protein and the high range of variability.

# Primers Sequence

- - 5‘gaattcgcggccgcttctagag atg gaaagactca ccaaaagata tgttgacctt gtcatagtt 3'
- 5' gaaagactca ccaaaagata tgttgagctt gtcatagttg cggatcaccg aatgttcacg aaatacaacg gcaatttaaa aaagataaga aaatggatat atcaaattgt caacactata aatgagattt acatacctttt gaatattcgt gtcgcactgg ttcgcctaga aatttggtcc aacggagatt tgattgatgt gacatcagca gcaaatgtta ctttgaagtc atttggaaac tggagagtga caaatttgct gaggcgcaaa agtcatgata atgctcagtt actcacggcc attgatcttg atgaagaaac tttaggattg gctccttttgg gcaccatgtg tgacccgaag ctttctatag gaattgttca ggatcatagt ccaataaatc ttttggttgc agttacaatg gcccatgagc tgggtcataa tctgggcatg gttcatgatg aaaatcggtg tcattgcagt actcccgcat gcgttatgtg tgctgtgcta aggcaacgac cttcctatga gttcagcgat tgtagtctga atcactatcg aacgtttatt atcaattata acccacaatg cattctcaat gaacccttgc aaacagatat aatttcacct ccagtttgtg gaaatgaact tttggaggtg ggagaagaat gcgactgtgg ctctcctaga acttgtcgag atccatgctg tgatgctgca acctgtaaac tacactcatg ggtagagtgt gaatctggag agtgttgtca gcaatgcaaa tttacgagtg caggaaatgt atgccggcca gcaaggagtg agtgtgacat tgctgaaagc tgcactggcc aatctgctga ctgtcccaca gatgacttcc ataggaatgg aaaaccatgc ctacacaact tcggttactg ctacaatggg aattgcccca tcatgtatca ccaatgttat gctctctggg ggtcaaatgt aactgtggct ccagatgcat gttttgatat taaccagagc ggcaataatt ctttctactg cagaaaggaa aatggtgtaa atattccatg
- Forward Mutagen 5'ggcaataattctttctactgcagaaaggaaaatggtgtaa 3'
- Reverse Mutagen 5'ttacaacattttcctttctgcagtagaaagaattattgcc 3'
- tgcacaagag gatgtaaagt gtggcaggtt attctgcaat gttaatgatt ttctatgccg acacaaatat tcagatgatg gaatggttga tcatggaaca aaatgcgcag atggaaaggt ctgcaaaaac aggcagtgtg ttgatgtgac tacagcctac aaatcaacct ctggcttctc
- 3'atgtcggatg tttagttggag acggaagag
- tcagatttga 3'
- Agtctaaact gacgtcgccggcgatgatcat 5'-suffix

# PCR Primer Sequence

- Forward: 5’ ATG GAA AGA CTC ACC AAA AGA TAT GTT GAC CTT GTC ATA GTT G 3’
- Reverse Primers: 5’ TCA AAT CTG AGA GAA GCC AGA GGT TGA TTT GTA GGC TGT A 3’
- Mutagen Forward Site 5’- GGC AAT AAT TCT TTC TAC TGC AGA AAG GAA AAT GTT GTA A - 3’
- Mutagen Reverse Site 5'- TTA CAA CAT TTT CCT TTC TGC AGT AGA AAG AAT TAT TGC C -3'

# Finding introns

- If in the cloning process the gene grows longer than its initial 1260 base pairs it has introns.
- We then have to sequence the gene from mRNA and see where these are coming into play and see how many there are.

# Procedure

- 1. Extract DNA
  - Using the sample of snake skin we plan on using a technique that yields high amounts of DNA
  - Procedure http://www.springerlink.com/content/kw1716u555371764/fulltext.pdf

# Procedure

- 2. To see if there is DNA present, we will need to run some of the sample on agarose gel electrophoresis
- AGE procedure:

http://openwetware.org/wiki/Agarose_gel_electrophoresis

# Procedure

- 3. Once we know that the DNA is present we need to amplify it with PCR. Since we are working with a eukaryote organism, will need to see if there are introns in the sequence.
- PCR Procedure: http://openwetware.org/wiki/PCR

# Procedure

- 4. If introns are present we must remove them with site directed mutagensis. We also have a restriction site (PstI) in the gene sequence so it also has to be removed.
- Procedure of Site Directed Mutagenesis
- http://en.wikipedia.org/wiki/Site-directed_mutagenesis

# Procedure

- 5. Once we have the final piece of the gene, we will amplify it along with the biobrick parts through PCR.
- 6. Then the gene can be added into the *E. coli* vector and grown.

# Testing

- For testing we plan on finding the length of the control *E. coli* cell on SDS PAGE gel and compare it to the length of the experiment *E. coli* cell.

# References


- http://scholar.google.com/scholar?hl=en&q=getting+DNA+from+snake+skin&btnG=&as_sdt=1%2C16&as_sdtp
- http://en.wikipedia.org/wiki/Site-directed_mutagenesis
- http://www.springerlink.com/content/kw1716u555371764/fulltext.pdf
- http://partsregistry.org/wiki/index.php?title=Part:BBa_J23100