James Helzberg

**Folypolyglutamate synthase [EC: 6.3.2.17] found in Scaffold 1575 (query sequence starts at base 17039 on scaffold)**

1)

For Primer AATCCCAATCCACCTATCCTTT

Rev Primer GCATTCAGCAGATGATTACAGC

repeats (ct) x13 PCR product = 136 bp & start at base 16858

2)

For Primer TCGAAAGCAGCTAATTCTCCAT

Rev Primer GGGGCTGAGGTCTTTTAGTTCT

repeats (gt) x12 PCR product = 298 bp & start at base 39327

3)

For Primer TCTTTCTCTCCAATTCTCAGCC

Rev Primer TGGAGCATAAAAGGGGAATAGA

repeats (tc) x10 PCR product = 300 bp & start at base 74834

**Folypolyglutamate synthase [EC: 6.3.2.17] found in Scaffold 343 (query sequence starts at base 203727 on scaffold)**

1)

For Primer GGTTGGTGTAAGATTCGTCCAT

Rev Primer TTCAACTCTCACCAGTCTCGAA

repeats (ga) x10 PCR product = 108 bp & start at base 189380

2)

For Primer CAACACAGATCTGCAGACAACA

Rev Primer CTTGTGTCAGTGTGACTCCCAT

repeats (ac) x8 PCR product = 187 bp & start at base 234573

3)

For Primer AATCAGCATGGCAGAAGGTACT

Rev Primer CCTTCAGCAATATCAACCATCA

repeats (tc) x7 PCR product = 196 bp & start at base 203551

**Folypolyglutamate synthase [EC: 6.3.2.17] found in Scaffold 772 (query sequence starts at base 35323 on scaffold)**

1)

For Primer GAAGAAGCTGATGAAGTGGGTC

Rev Primer TCCTTCAACTCTTCGTTTTTCC

repeats (ga) x10 PCR product = 269 bp & start at base 29847

2)

For Primer TTCAGCACGACAAGCTGATATT

Rev Primer AAGTGGCGGGATACATAAGAGA

repeats (ct) x10 PCR product = 177 bp & start at base 49250

3)

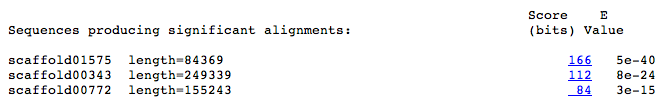
For Primer TGTAACAAGCTGGGATTTTGTG

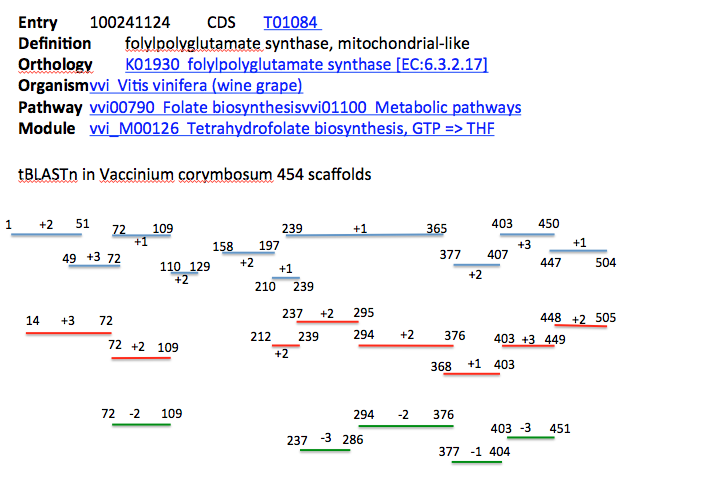
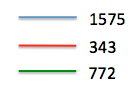
Rev Primer TTTGAGTGGGGTCTTCAAGATT

repeats (tc) x8 PCR product = 233 bp & start at base 25035

**Experiments are shown on pages 3-5.**

**Experiment 1:** tBLASTn against *Vaccinium corymbosum* 454-scaffolds (Query is 521 aa sequence of folypolyglutamate synthase from *Vitis vinifera*)

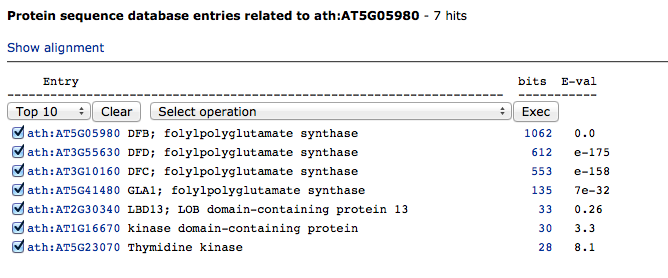




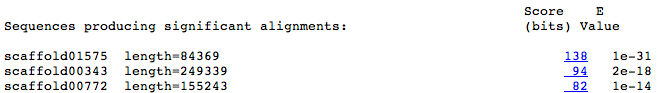
**Legend:** Results of tBLASTn of folypolyglutamate synthase [EC:6.3.2.17] using *Vitis vinifera* as the query against *Vaccinium corymbosum* 454-scaffolds. Results yielded three significant scaffolds (shown above). Scaffold 1575 is drawn as blue lines and contains many fragments due to frame shifts, but covers most of the protein. Scaffold 343 is drawn as red lines and also contains many fragments, but has good coverage as well. Scaffold 772 is shown as green lines and has less coverage.

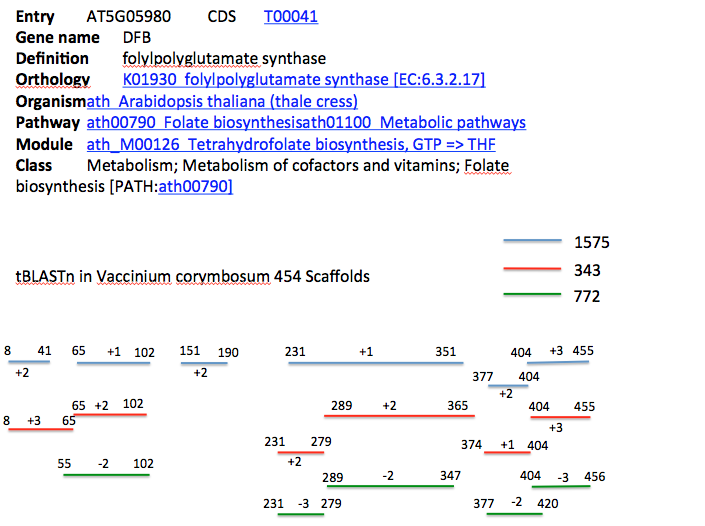
**Experiment 2:**

I also ran a BLASTp using folypolyglutamate synthase [EC:6.3.2.17] from *Vitis vinifera* as the query and searched for the enzyme in *Arabidopsis thaliana*. Results are shown below:



The top hit from *Arabidopsis thaliana* is folypolyglutamate synthase, which has a slightly different amino acid sequence than the enzyme in *Vitis vinifera*. I used the amino acid sequence for folypolyglutamate synthase from *Arabidopsis* *thaliana* (513 amino acids) as the query in a tBLASTn against *Vaccinium corymbosum* 454-scaffolds. Results from this experiment yielded the same three scaffolds (1575, 343, and 772), but with slightly less coverage. Results are shown below and on the next page:





**Legend:** Results of tBLASTn of folypolyglutamate synthase [EC:6.3.2.17] using *Arabidopsis thaliana* as the query (513 amino acids) against *Vaccinium corymbosum* 454-scaffolds. Results yielded three significant scaffolds as shown above. Scaffold 1575 is drawn as blue lines and contains multiple fragments. Scaffold 343 is drawn as red lines and also contains many fragments. Scaffold 772 is shown as green lines.