**BI386 BLAST Activity: Cloning Confirmation**

*This activity asks students to confirm PCR product (GFP) insertion into a plasmid (pET28a). They have sequencing results from two different possible clones.*

* Go to the NCBI website at [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)
* Find the BLAST tool
* Enter your GFPpET28a sequencing results (colony 10 or 14)
* Choose the right database: Human, Mouse, or Other

pGlo derived from this sequence: <https://www.ncbi.nlm.nih.gov/nucleotide/1490531?report=genbank&log$=nucltop&blast_rank=1&RID=7ADUGWW801N>

*pGlo plasmid was our template for GFP PCR, so we can see that plasmid entries in the database do shows genes encoded on the plasmid.*

1. What does the color code on the graphical interface represent?

2. What are the top 10 best “hits”? Anything look strange?

*Answer: All hits are cloning vectors because the GFP sequence used in molecular biology today is a modified version of the jellyfish gene.*

3. Click the Taxonomy button. What do these results tell you?

4. Choose a matching sequence by clicking on the SEQUENCE ID. What information is on this entry page?

5. Did we clone GFP into pET28a? How did you decide?

## Teaching Notes

### By *Erin Morris, Baker University*

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**Course Information**

Department: **Biology & Chemistry**

Level: Lower/Upper Undergraduate (select one) **Upper undergraduate**

Course type: Lab/Lecture/Both (other, please describe) (select one) **Lab**

Students: Majors/Non-majors (select one) **Biology and Biochemistry majors**

Number of Students: **10**

**Module Information**

Original Module Name: **Lesson I - Introduction to Genome Solver**

Link to Original: <https://qubeshub.org/qubesresources/publications/808/1>

[Adapted Module Name: (if applicable). Introduction to Genome Annotation

Link to Adapted Module]

Modified Module Name: **BI386 BLAST Activity: Cloning Confirmation**

Files associated: (ie. Class Worksheet, Summative Quiz, Lecture Powerpoint, etc)

**Class worksheet; Genome solver slide deck used in class: “Basic Local Alignment Search Tool (BLAST)”**

Modification Learning Goals:

**• Become familiar with BLAST and be able to use it for homology searches**

**• Be able to interpret BLAST results**

**• Analyze cloning experiment results**

**Teaching Notes**

*(Think about what you would like to read about this activity if you came back to it in 2 years)*

Suggestions for this section (not all required, and extras always welcome):

1. What did you change and why?

**I focused on one part of the GS activity, Part A that asks the students to look at some of the links on the BLAST result page. I modified it to help students identify an unknown sequence – the sequencer file from PCR of an cloned insert. The sequence should be GFP cloned into a plasmid.**

2. How did the activity go?

**Students were engaged, included screen shots, and were able to interpret results outside of class.**

3. What went well and why?

**We had practiced using BLAST with another GS activity, so they were ready for this.**

4. What went wrong and why?

**This was a 6 week project, so it required lots of prompting to help students connect all the dots…PCR…plasmid…sequence results file. They go there, just a lot of hand holding. And then we had to confirm the insert was in frame.**

5. What was the prep like?

**As with any worksheet involving websites, one must check that each link is live and that the website did not change in appearance. The NCBI website is notorious about changing.**

6. How much time went into prep?

**Not much prep, just have to think through the entire assignment (and do the assignment) so you know all of the directions are up to date.**

7. Did you have to do any prep (i.e. grow cultures, grow seeds, order supplies) ahead of implementation?

**No prep for this one.**

8. Would you do this activity again?

**Yes!**

9. What would you change in the future?

**Not sure yet. Now that I have done it once, the second time through I can really assess how the students engage and if there are any questions/directions to add.**

10. What do you wish you’d known before you ran the activity?

11. Is there anything else you would like to make note of?

**Run through it every year! NCBI makes changes constantly.**

12. How does this activity fit in your overall course curriculum?

**It follows PCR, RE digest, cloning. We use it analyze our results. We did use NCBI to find sequences prior to this, but this was our first BLAST. It was good prep for more complicated sequence comparison/identification in our cloning project.**

13. In what ways, if any, did you modify your teaching practice with this activity?