

## 7/7/17- Alkaline Phosphatase and BCIP detection mechanism, test to see if it will bind to the paper

AIM: The first step is to produce a mechanism that will signal if Factor C undergoes autocatalysis in the presence of gram-negative bacteria. Alkaline phosphatase (AP) and BCIP is widely used as a detection system. An added benefit of using Alkaline Phosphatase is that it is a part in the registry (BBa\_K1470004). We intended to fuse the factor C with AP and bind the fusion factor c to the top of the nitrocellulose paper and BCIP to the bottom and place in a saline solution, so when factor c undergoes autocatalysis the AP will release and bind to BCIP. When AP and BCIP combine it produces an intense black color. The aim of this protocol was to see if the BCIP or AP will bind to nitrocellulose paper and produce a signal.

### Materials:

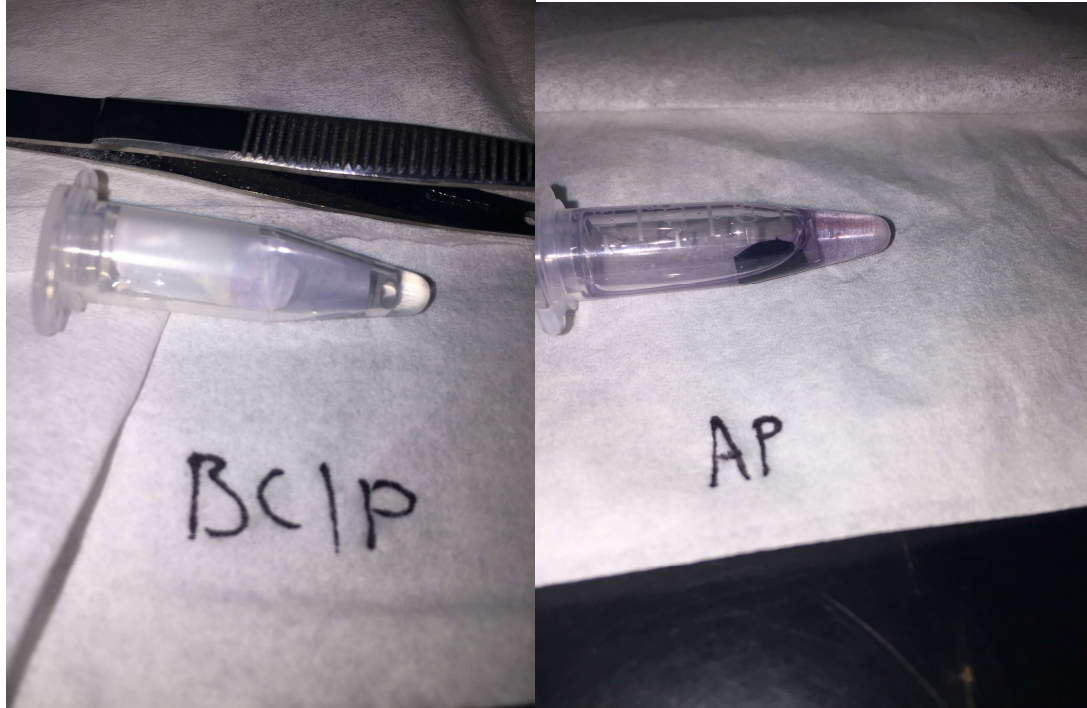
- Cut small strips of nitrocellulose paper (Nitran)
- Promega Alkaline Phosphatase (Conc: 1ng/ul)
- Alkaline 10X buffer diluted
- BCIP
- UV Cross-linker

### Protocol:

1. Cut Nitran into small strips
2. Add 2ul of AP to one strip and allow to dry
3. Add 2ul of BCIP to the 2nd strip and allow to dry
4. Uv crosslink the two strips of paper and add each to its own eppendorf tube
5. Make up a solution of 50ul AP buffer and 450 water =500ul in the eppendorf tube
6. In the tube with the Alkaline Phosphatase bound paper add 450ul of the BCIP solution
  - a. Answer the questions: does the AP stay in one spot?
  - b. Does the AP spread all over the paper?
    - i. ---- Answers: The Ap spread outward but stayed confined meaning the crosslinking worked
7. Make up a solution of 500ul AP 10X buffer and 1ul of AP enzyme
8. Add the solution to the tube with the paper with BCIP bound to it
  - i. Observation: There was a slight discoloration

### Results:

Adding the BCIP solution to the tube with the paper produced a noticeable discoloration. Adding the enzyme to the BCIP only produced a small discoloration.



Conclusion:

The binding of the alkaline phosphatase and BCIP to the paper was successful. The reaction worked only in the intended areas meaning that if the AP is bound to the factor c it may produce a signal only if it is cut, the AP nor BCIP disperses in the solution after being bound to the paper.