



Pyruvate Kinase Replication

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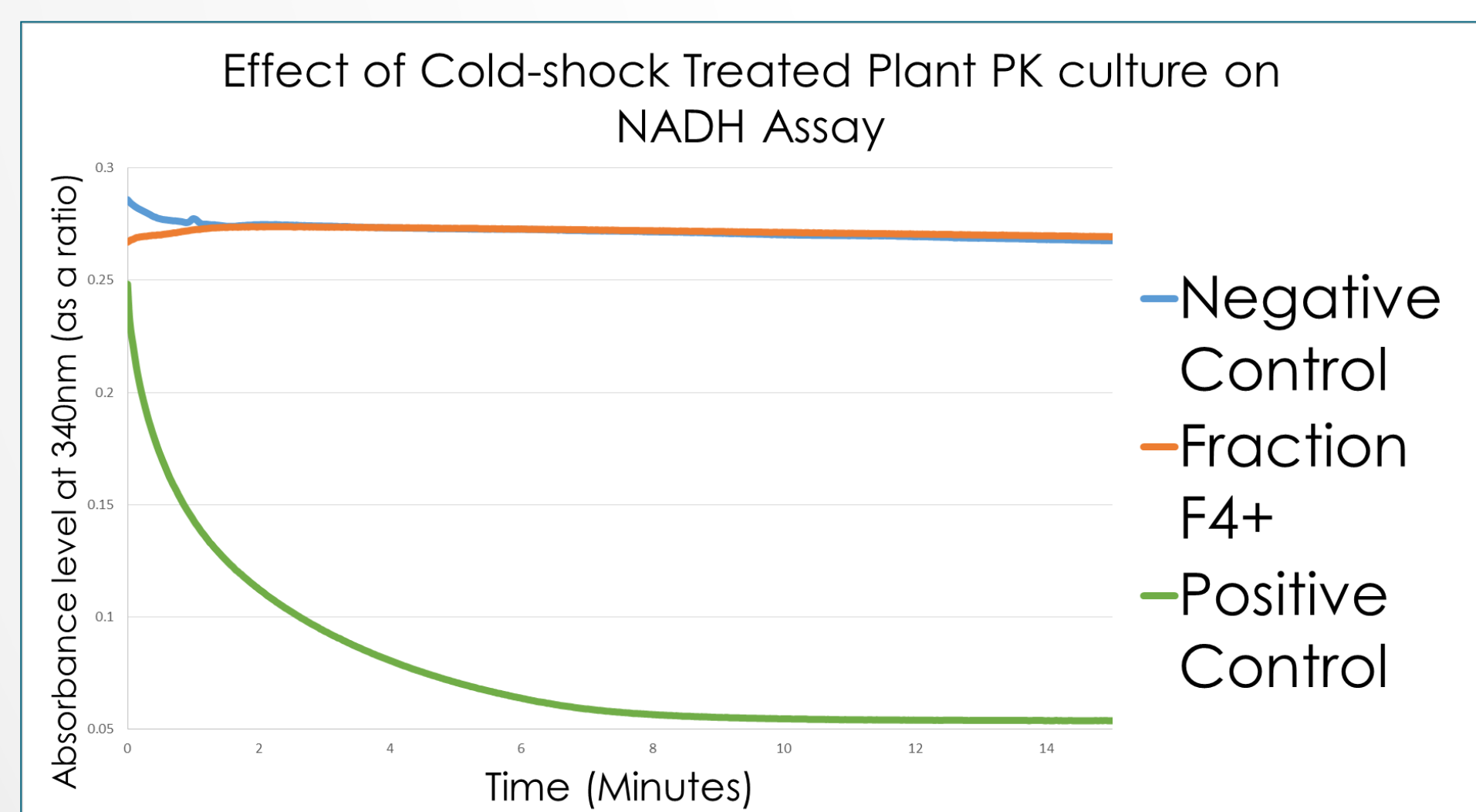
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Background and Objectives

Glycolysis allows production of a variety of important compounds. Because these different possible end products share the same starting materials and pathways, formation of each product is in competition. Plants produce appropriate ratios of the products to support plant health and growth, but these same ratios are not ideal for humans. For example, precursors to amino acids, which are used to build proteins, help humans whereas precursors to carotenoids, which aid in photosynthesis, are completely unnecessary. Plants grown for human consumption could be influenced to produce different ratios of glycolysis products so they better suit human needs.

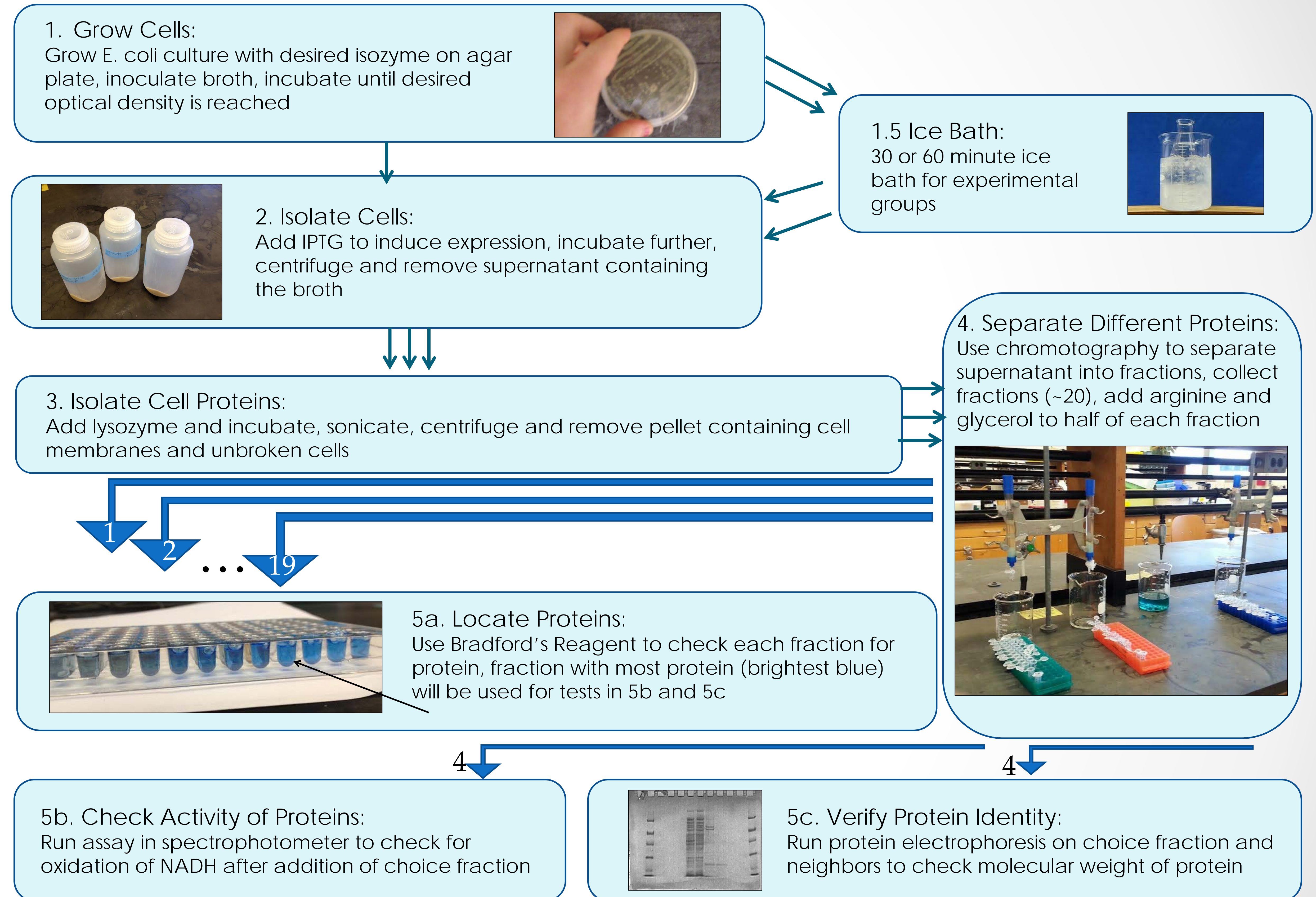
Pyruvate kinase(PK) catalyzes the final reaction of glycolysis, and different isozymes of PK direct glycolysis to form different products. Manipulating the activity of different isozymes of PK would allow humans to influence the nutritional composition of plant seeds for human consumption, such as corn kernels. Our goal was originally to replicate two different isozymes of PK from the model plant *Arabidopsis thaliana* and then to experiment with the effects of different activators and inhibitors on the activity levels of the isozymes. However, we found our PK was misfolding, as evidenced by its insolubility and consequent precipitation from solution. First a reliable way to replicate PK that results in the proper tertiary structure had to be established. We altered our aim for the summer to encompass replicating PK in a way that did not lead to its crash out of solution and to ascertaining its ability to demonstrate its viability through oxidation of NADH in a laboratory setting.



Results

For our first isozyme, the protein precipitated out of solution almost as soon as the fractions were separated (step 4) on multiple trials. The protein electrophoresis (step 5c) indicated that these fractions contained proteins other than our desired PK. For our second isozyme, we were able to isolate the desired PK and found that for this isozyme the PK remained soluble. The absorption of an NADH solution remained at a constant level after the addition of the suspended PK (step 5b). For this isozyme, the sample that underwent a one hour ice bath during the replication stage contained three times as much PK as indicated by a quantitative Bradford's test.

Methods



Discussion

The *E. coli* produced additional proteins with the tag used to separate out PK (step 4) in our first isozyme and so precluded any further experimentation. We learned from our second isozyme that replicating and isolating the PK is insufficient to allow study, because the PK was inactive (step 5b, see graph). The PK remained soluble so it either folded incorrectly or was unable to oxidize NADH because an additional protein or ion was needed. The PK should be mixed with molecules from *Arabidopsis* to elicit activity. We established that a one hour ice bath results in greater production of PK and the ice bath will be added to the protocol for future PK replication.

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