Your name here. Date here.

Background

Indian hedgehog (Ihh) is an important signaling molecule that is responsible for a number of events during postnatal bone growth. Deletion of the Ihh gene in mice decreases the growth of new bone cells, causes bone cells to become mature at the wrong time, and prevents the development of specialized bone cells called osteoblasts (St-Jacques *et al.*, 1999). This week, I will clone the Ihh gene into an expression plasmid to determine which downstream molecule(s) Ihh signals to that causes the growth of new bone cells.

Methods

To make the plasmids for this study, I cloned a 500 bp fragment from the pUC19/insert plasmid (which contains the Ihh gene) into the pUC19/vector plasmid using the restriction enzymes *EcoRI* and *XbaI*. The cloned plasmids were transformed into competent *E. coli* cells and plated on LB agar plates with ampicillin. Colonies were picked and tested to see whether they contained the plasmid of interest by extracting DNA and performing restriction digestion and gel electrophoresis on the samples.

Results

I got no colonies on my negative control plate, 10 colonies on my vector alone plate, and 126 colonies on my vector + insert plate. This suggests that I have gotten the correct clone, or else the vector + insert plate would have had the same number of colonies as the vector alone plate. After extracting the plasmid DNA from my colonies, digesting the plasmids with *EcoRI* and *XbaI*, and running them on a gel, I got two bands of 6702 and 500 bp in four of the five colonies I picked to test. The last colony had a single band of about 6800 bp. This indicates that my insert was correctly cloned into the four colonies with two bands, and that the colony with the single band is incorrect. I decided to use the first clone, Clone 1, for the rest of my experiment because it had the most DNA out of the four correct clones.

Discussion

The newly cloned plasmid can now be used for the rest of the study to produce Ihh in large quantities. We will use the DNA from the cloned plasmid to transfect tissue-cultured bone cells and test how the production of the Ihh protein affects growth of cultured bone cells and which specific step(s) in the development of bone tissue that Ihh affects. This will help us understand how loss of Ihh causes a decrease in the growth of new bone tissue.

References

St-Jacques, B., Hammerschmidt, M., and McMahon, A. P. 1999. Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. *Genes Dev.* **13**: 2072-2086.