Generating a Monoclonal Antibody Against the Centromeric Protein, HCP-3, in *C. elegans*

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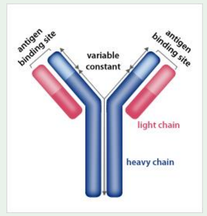
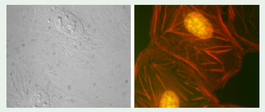
Introduction:



Antibodies
- Have a broad range of research applications in cell biology.
- Ability to bind to specific molecules makes them ideal probes for detecting the presence of an antigen and for localization studies inside the cell.

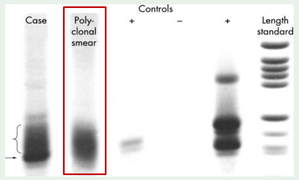
Visualization without antibody. Visualization with antibody.



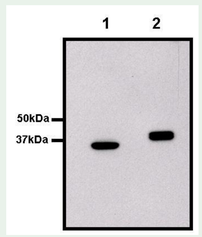
Polyclonal Antibodies
- Recognize multiple regions, or epitopes, on an antigen.
  - This can create background signals.
- Polyclonal antibodies are much cheaper to produce than monoclonals, but creating monoclonals in-house can offset their cost, and be much more beneficial.

Monoclonal Antibodies
- Recognize a specific epitope.
- A monoclonal antibody is preferred in this instance due to its specificity.
  - Monoclonal antibodies result in less background and are less likely to cross-react with other proteins.
- Consistency of monoclonal antibodies makes their assays highly reproducible.
- Once a monoclonal hybridoma has been generated, antibodies can be easily and consistently generated.

Polyclonal antibody causing background smearing on Western Blot.



Monoclonal antibody provides much clearer picture.



Objective:
The focus of this research is to produce a monoclonal antibody for the HCP-3 gene in *C. elegans*. HCP-3 is the first of potentially many antibodies which can be generated to study the processes of cell division and embryogenesis. This entire process is reproducible for other proteins of interest as the need arises.

Isolating HCP-3 Gene:

mRNA Preparation

cDNA Preparation
-via reverse transcription
PCR

Gene Amplification
-via PCR
-gel purification

Insert Gene into Vector
-via NEBuilder Hi-Fi Assembly

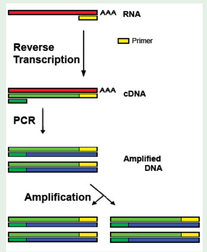
Plasmid Miniprep

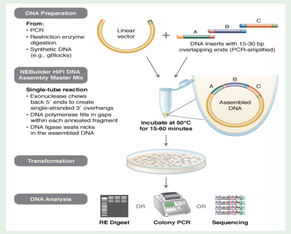
Diagnostic Restriction Enzyme Digest
-using EcoRV and SacI

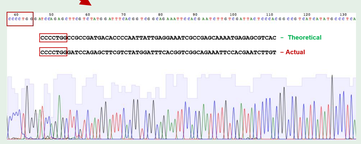
Sequencing

Transformation into BL21

Protein Expression Test







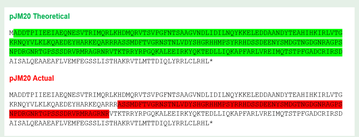
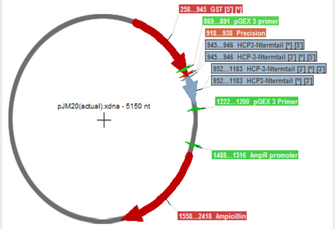
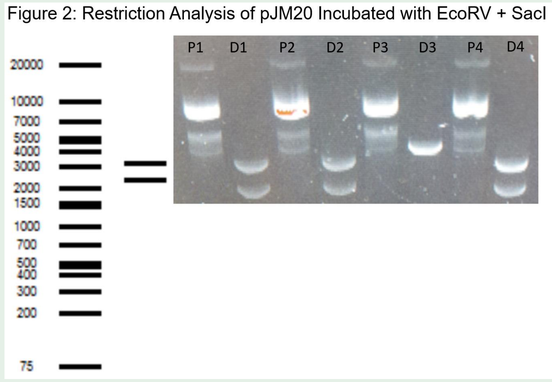


Figure 1: pJM20 Plasmid Graphic Map



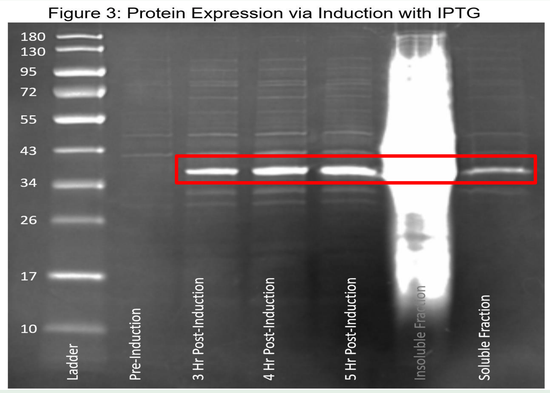
Sequencing Results:
- Sequencing revealed actual plasmid differed from prediction; small segments of HCP-3 gene were deleted (5150 bp vs 5506 bp).
- Still in frame - does not affect gene product.
- Plasmid generated contains HCP-3 gene and ampicillin resistance.

Figure 2: Restriction Analysis of pJM20 Incubated with EcoRV + SacI



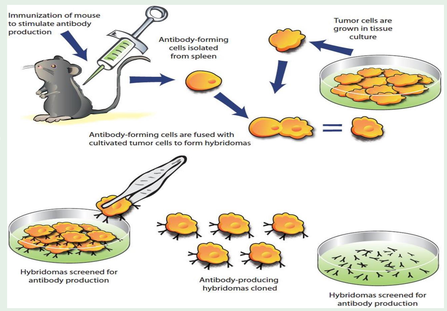
- Confirmed structure of pJM20 plasmid by comparing virtual and actual digest.

Figure 3: Protein Expression via Induction with IPTG



- Verified that protein with weight of 36 kD was being produced.

Next Steps:
- Increase protein production.
  - Protein purification via Affinity Chromatography.
- Inoculate mice and begin antibody production.
- Obtain, purify, and clone monoclonal HCP-3 antibodies.



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References:
Antibody Structure. June 12, 2000; [March 29, 2017]. The Biology Project: Immunology.
GST Tag Monoclonal (8-326) Antibody. [March 29, 2017]. ThermoFisher Scientific.
NEBuilder HiFi DNA Assembly Cloning Kit. [March 29, 2017]. New England BioLabs.
Speaking of Research. August 7, 2014; [March 22, 2017]. Antibody Production Illustration provided by Alice Ra'anan and Bill Yates.