**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.

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.

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

- **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.

**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:**