Progress Towards Overexpression of CHO Stabilization Genes

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Introduction
- Chinese hamster ovary (CHO) cells industrially generate monoclonal antibodies and other biopharmaceuticals and are the preferred organism for making protein therapeutics.
- Quickly mutated genome leads to problems
  - genetic instability
  - inefficient antibody production
  - departure from clonality over time
- Lee Group, University of Delaware (UD) indicated to evaluate Lig4
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- Xrcc6

Methods
- Extract DNA from CHO cells
- Amplify Lig4 and Xrcc6 from the CHO genome via Polymerase Chain Reaction (PCR)
- PCR amplify backbone of pcDNA3.1/Zeo
- Ligate in enzyme digestion sites (Not1 and Kpn1)
- Restriction digest with enzymes on both sequences and plasmid
- Ligate Lig4 or Xrcc6 into plasmid
- Transform plasmids into E. coli cells
- Sequence confirmation
- Transfect plasmids into CHO cells using linear Polyethyleneimine (PEI)
- Isolate single cells with positive transfection quality
  - Initial tests used EGFP marker in pcDNA3.1/Zeo
- Later taken out for favor of Zeocin selection marker
- Grow cultures from single cell and analyze results

Challenges
- CHO VRCO1 cell line grows faster than K1
- K1 cells lump together and grow slower
- Very low transfection efficiency after 4 days

Successful Cloning of Lig4 into Vector
- Gel Electrophoresis revealed Lig4 and Xrcc6 in CHO genome (there was doubt after amplification)
- Confirmed complete pcDNA3.1/Zeo backbone after removal of EGFP, digestion, and desphosphorylation
- Colony PCR showed Lig4 successfully ligated into vector after transformation into E. coli

Flow Cytometry Analysis
- Characterizes CHO cells after transfection
  - Daily fluorescence measurements show small population with higher fluorescence on Day 4
  - Number of cells with baseline fluorescence decreases

Results
- CHO Lig4 gene successfully inserted into overexpression plasmid
- CHO-VRCO1 cells must be passaged before they reach 8 x 10^6 cells/mL to maintain high viability
- K1 cells lump together and grow slower
- Very low transfection efficiency after 4 days

Future Work
- Clone Xrcc6 into vector after amplification via RT-PCR
- Investigate best transfection procedure
  - branched PEI vs. linear PEI
  - Zeocin selection marker
- Analyze long-term transfection effects

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References