Isolation and Purification of *M. musculus* & *H. Sapiens* NF-κB Proteins

Rhosyl Marie Suarez & Dr. Hannah Baughman

TBIOMD 495

INTRO & BACKGROUND

- The **NF-κB** (Nuclear factor kappa B) pathway is involved in regulating many cellular processes, and is particularly critical for regulating inflammation and immune responses
- In cancers and autoimmune disorders, this pathway is often aberrantly activated
- The most common NF-κB protein is the **p50/RelA** heterodimer, an essential transcription factor and promising therapeutic target
- Obtaining a detailed biochemical understanding of p50/RelA remains challenging due to **intrinsically disordered regions (IDRs)** in the RelA subunit
- Historically, IDRs have not been well studied due to their complex nature and lack of stable structure, but better understanding IDR function could be vital to treating NF-κB dysregulation

RESEARCH OBJECTIVE

- Finding ways to deactivate abnormal NF-kB activation could lead to developing treatments for diseases
- Part of the goal of this project is to learn more about what these regions are doing, which will help us understand how the protein works as a whole
- Most prior biochemical studies and established protocols for NF-kB proteins have been conducted using the *M. musculus* (mouse) version of p50/RelA
- The first step in studying NF-kB proteins is isolating and purifying them
- Since we care about *H. sapiens* (human health), we aimed to answer the question:

☆ Can we translate experimental findings in *M. musculus* NF-κB proteins to *H. sapiens* and better understand how they work? ☆

PROTEIN SEQUENCE ALIGNMENT

Score	Expect	Method	Identities	Positives	Gaps
929 bits(2402)	0.0	Compositional matrix adjust.	471/535(88%)	491/535(91%)	6/535(1%)

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Sbjct  2    .....................S.....................................  61

Query  79   KDPPHRPHPHELVGKDCRDGFYEAELCPDRCIHSFQNLGIQCVKKRDLEQAISQRIQTNN  138
Sbjct  62   .....................Y...D.....S.............................  121

Query  139  NPFQVPIEEQRGDYDLNAVRLCFQVTVRDPSGRPLRLPPVLSHPIFDNRAPNTAELKICR  198
Sbjct  122  ...H.............................A....L.T.....................  181

Query  199  VNRNSGSCLGGDEIFLLCDKVQKEDIEVYFTGPGNEARGSFSQADVHRQVAIVFRTPPYA  258
Sbjct  182  ..............................................................  241

Query  259  DPSLQAPVRVSMQLRRPSDRELSEPMEFQYLPDTDDRHRIEEKRKRTYETFKSIMKKSPF  318
Sbjct  242  ..............................................................  301

Query  319  SGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFPSGQIS-QAS  377
Sbjct  302  N...E....T......T.N.T..........T.PA......F...SP.LL......N..L  361

Query  378  ALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAPKPTQAGEGTLS  437
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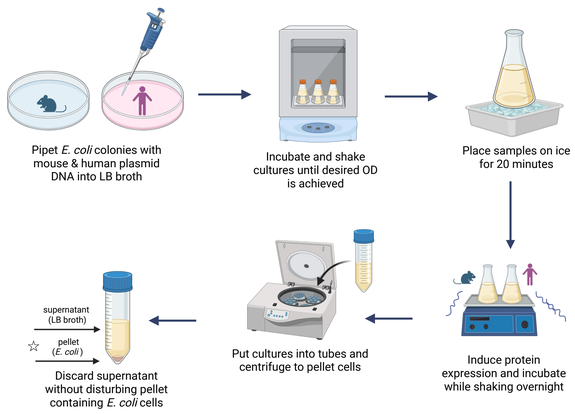
Sbjct  479  ..........S........T...TS.....-.........................  532
```

RelA Protein Alignment in BLAST between *M. musculus & H. sapiens*. Differences in the *M. musculus* sequence from the *H. sapiens* are indicated by the red letters. Most of the sequence is well conserved until the intrinsically disordered region where there are many amino acid differences and gaps. (1)

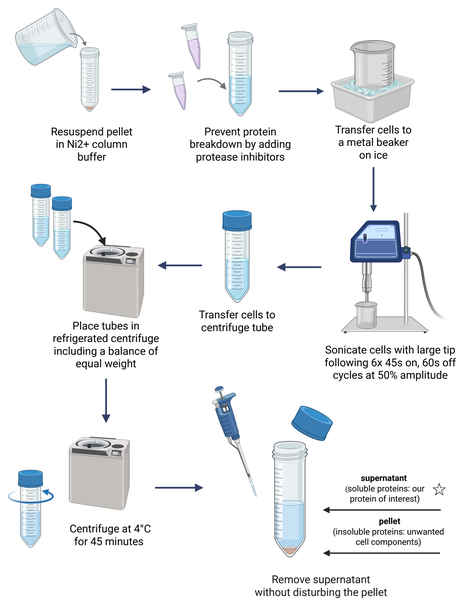
METHODS

Protein Expression in *E. coli*



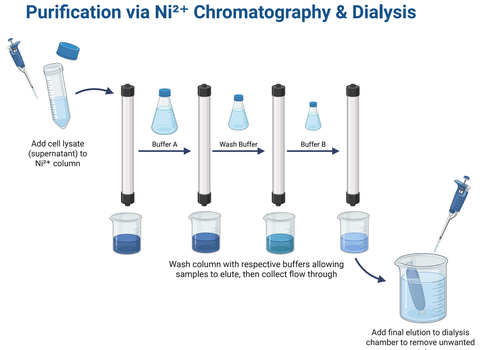
Protocol Objective: Transform *E. coli* bacteria and induce expression of desired proteins: human and mouse p50/RelA. Then isolate them via centrifugation.

Cell Lysis via Sonication



Protocol Objective: Break open *E. coli* cells via sonication to free proteins of interest (p50/RelA).

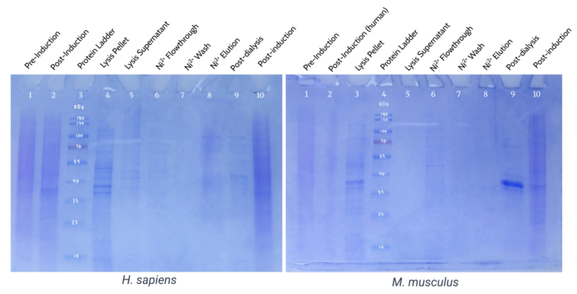
Purification via Ni²⁺ Chromatography & Dialysis



Protocol Objective: Further purify protein sample by getting rid of undesired proteins through affinity chromatography and dialysis.

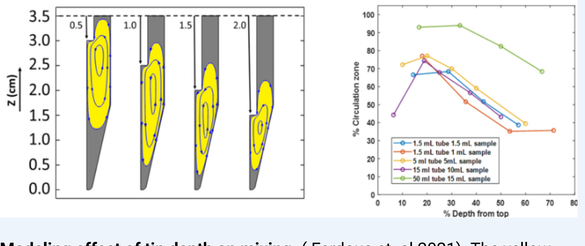
RESULTS

Stage 1 SDS-PAGE Results



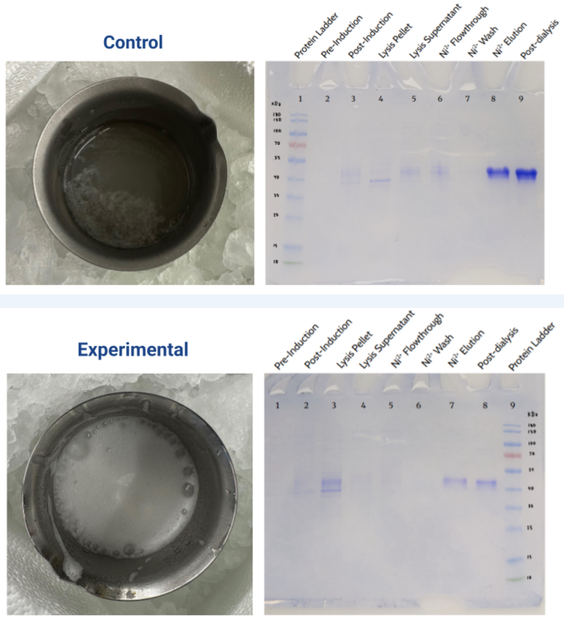
Stage 1 SDS-PAGE Results. Comparison between *H. sapiens* and *M. musculus* at each stage of the isolation and purification protocols.

☆ Stage 2 Hypothesis: Altering the sonication protocol will result in a greater amount of NF-kB proteins in the soluble fraction and greater overall yield. ☆



Modeling effect of tip depth on mixing. (Ferdous et. al 2021). The yellow area indicates circulation zone. The line graph illustrates the percentage of volume in circulation zone with changing tip height for four sizes. This figure is from a sonication optimization study that led us to alter our protocol to try and increase lysis efficacy based on their results. (2)

Stage 2 Cell Lysate & SDS-PAGE Results



Stage 2 Cell Lysate and SDS-PAGE Results. The cell lysate in the control group is the expected result. The foaming in the experimental group indicates denatured proteins. The altered protocol for sonication doubled the amount of on/off cycles (6->12) and had the tip placed closer to the surface instead of toward the bottom as it was in the original.

EXPERIMENTAL STAGES

- Our capstone was divided into two stages:
  - **Stage 1:** Learning about NF-kB protein isolation and purification, then applying established protocols for *M. musculus* to *H. sapiens*
  - **Stage 2 :** Utilizing these protocols to carry out an experimental hypothesis
- Looking at our Stage 1 SDS PAGE results, we noticed that our proteins of interest appeared to have been mostly lost in the pellet when it should have been in the supernatant
- A possible explanation for this is that our *E. coli* cells were not properly lysed during sonication
- This led us to create an altered sonication protocol in an attempt to improve cell lysis efficacy and protein yield
- Based on previous protein extraction optimization research, we decided to alter the protocol from the original by:
  - doubling the amount of on and off cycles
  - changing depth of the sonication tip to sit closer to the surface of the liquid instead of at the bottom

CONCLUSION

- Protocols for isolating and purifying *M. musculus* NF-kB proteins appear to also work for *H. sapiens*
- We attempted to maximize NF-kB protein yield by altering the sonication protocol, but this did not result in any improvement
- This leads to our conclusion that tweaking sonication parameters too much can further negatively affect protein yield
- Our results illustrate the complexity of optimizing sonication for protein extraction and further highlights the need and potential for more investigation into the balance between effective cell lysis and protein yield
- Once an optimized protocol is established, downstream studies can better examine the structure and function of p50/RelA IDRs to work towards therapeutic treatments for NF-kB dysregulation

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REFERENCES

1. Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., and Madden, T.L. 2009. BLAST+: architecture and applications. BMC Bioinformatics, 10, 421.
2. Ferdous S, Dopp JL, Reuel NF. 2021. Optimization of E. coli tip-sonication for high-yield cell-free extract using finite element modeling. AIChE Journal. 67(10). doi:https://doi.org/10.1002/aic.17389.
3. Schematics created with BioRender.com