Optimizing Cell Lysis for Human NF-κB p50/RelA Protein Yield

Ngoc Han Nguyen, Rhosyl Marie Suarez & Dr. Hannah Baughman in TBIOMD 495

INTRODUCTION

- The NF-κB family regulates inflammation and immune responses (Hayden & Ghosh, 2008).
- In cancers and autoimmune disorders, this pathway becomes abnormally activated, contributing to disease progression (Taniguchi & Karin, 2018).
- The most common NF-κB complex, p50/RelA, is a key transcription factor and therapeutic target (Hayden & Ghosh, 2012).
- However, obtaining sufficient, properly folded protein for in-vitro studies remains challenging, particularly due to intrinsically disordered regions (IDRs) in RelA that complicate purification.
- This research focuses on improving extraction efficiency of human RelA/p50 expressed in E. coli, which is a crucial step toward future structural and biochemical studies of NF-κB regulation.

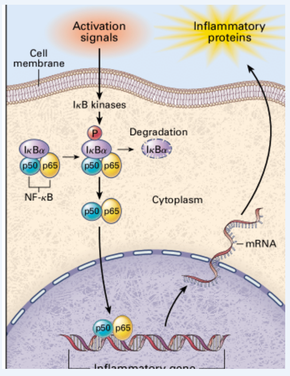


Figure 1: NF-κB activation pathway. Adapted from Chen et al. (1995).

RESEARCH OBJECTIVE

- Determine whether established purification protocols for mouse (M. musculus) p50/RelA can be successfully applied to human (H. sapiens) proteins.
- Optimize extraction efficiency by altering sonication parameters to increase soluble protein recovery in the supernatant.
- Hypothesis: Increasing the number of sonication cycles and adjusting tip depth will improve cell lysis and enhance protein yield.

METHODS

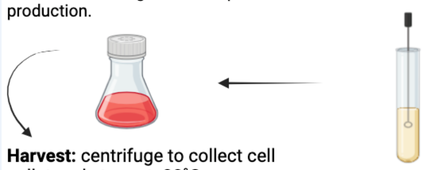
Protein Expression in E.coli

Transform: plasmid DNA containing the gene for mouse or human p50/RelA with competent *E. coli* (BL21), then heat-shock at 42 °C.

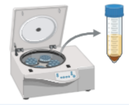
Plate: spread on LB agar + antibiotic, incubate overnight (37˚C).

Expression: transfer into a large LB flask, grow to $OD_{600} \approx 0.6$, add IPTG to induce expression, and incubate the culture overnight to allow protein production.

Starter culture: inoculate 5 ml culture with a single colony and shake overnight.



Harvest: centrifuge to collect cell pellet and store at -80˚C.

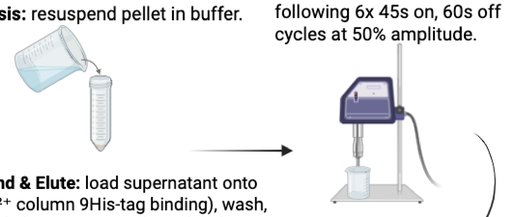


Goal: To express the target recombinant protein in E. coli cells.

Protein Purification

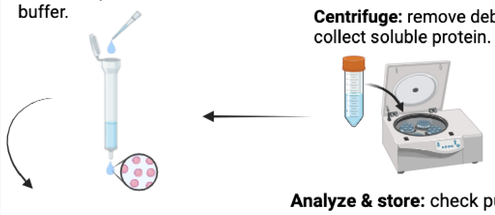
Lysis: resuspend pellet in buffer.

Sonicate cells with large tip following 6x 45s on, 60s off cycles at 50% amplitude.



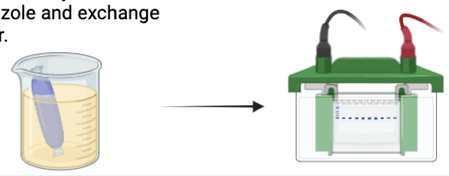
Bind & Elute: load supernatant onto Ni^{2+} column 9His-tag binding), wash, and elutw protein with imidazole buffer.

Centrifuge: remove debris, collect soluble protein.



Dialysis: dialyze to remove imidazole and exchange buffer.

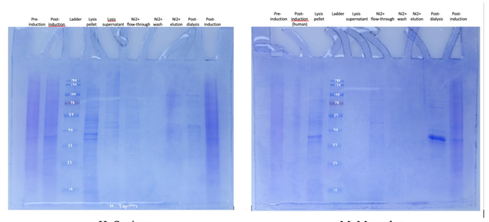
Analyze & store: check purity by SDS-PAGE and store at -80˚C.



Goal: To isolate and verify the purity of the expressed protein.

RESULTS

Stage 1 SDS-PAGE results



H. Sapiens M. Musculus

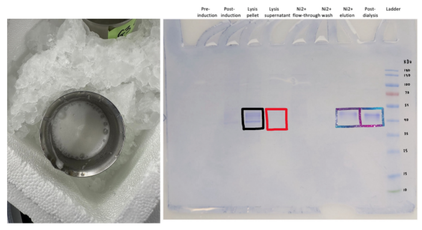
Stage 1 Recap:

- Most protein was lost in the pellet.
- Goal for Stage 2: improve soluble fraction yield.

SDS Stage 2 Control

6x 45s on, 60s off cycles, 50% amplitude. Sonicated with tip closer to bottom of liquid.

SDS Stage 2 Test



12x 45s on, 60s off cycles, 50% amplitude. Sonicated with tip at 20-30% height below the liquid surface.

SDS-PAGE results:

- Original Protocol: protein ~47 kDa
- Altered Protocol: protein ~45 kDa
- Expected MWs (human plasmid): p50 ~42 kDa, RelA ~60 kDa.

Interpretation:

- Increasing sonication cycles and raising tip position did not improve yield.
- The frothing likely occurred because the sonicator tip was positioned too close to the liquid surface, which introduces air and causes foam formation.
- Both human and mouse proteins showed similar loss trends, with most p50 retained in the pellet.

CONCLUSION

- We thought longer sonication and shallower tip depth would help, but it didn't improve protein yield. Instead, it likely caused some proteins to denature and aggregate.

What we noticed:

- Tip placement might not have been ideal, or too many cycles were run
- Foaming in the lysate, which often means proteins are denatured
- SDS-PAGE shows lighter bands in the supernatant, with most protein stuck in the pellet

Next steps:

- Try different tip depths, cycle lengths, on/off times, and number of cycles
- Focus on one variable at a time to see what really makes a difference
- Continue using the same centrifugation and SDS-PAGE methods for consistent comparison

Takeaway: Optimizing sonication is a delicate balance — enough to break cells open, but gentle enough to keep proteins intact.

ACKNOWLEDGEMENTS

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