Comparison of NF-κB Heterodimer Protein p50/Rel A from *H. sapiens* and *M. musculus*

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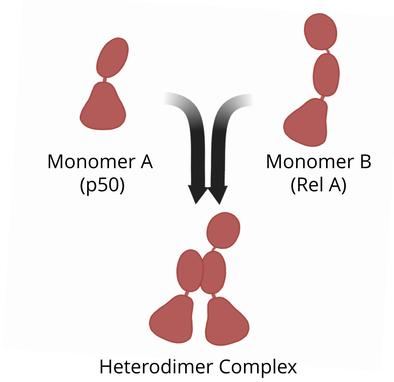
Background

Nuclear Factor-kappa B (NF-κB) is a transcription factor comprising various monomer subunits that is integral in regulating immune response, cell development, and cell survival.[5,6]

p50 and Rel A monomers are the most common subunits and together form a heterodimer that promotes activation of pro-inflammatory responses.[1,2]



p50-RelA heterodimer facilitates DNA binding and gene transcription[4,7]

Research Objective

Our goal was to purify and compare the p50/Rel A heterodimer protein found in *H. sapiens* and *M. musculus*

We can then evaluate the reliability of mice models and reaffirm its continued use in the overall study of human health.

Our research was conducted in two stages:

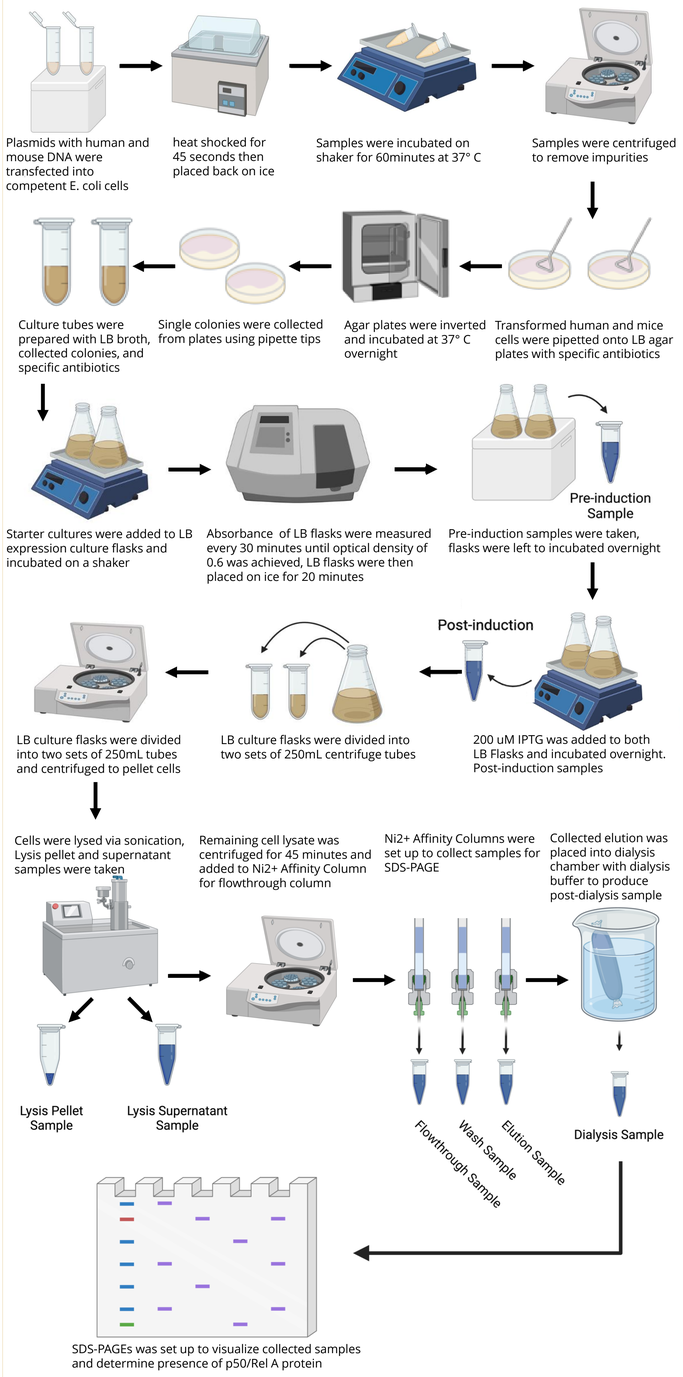
[Stage 1] Express and purify target proteins from *E. coli* cells then observe and analyze efficacy of protocol with SDS-PAGE

[Stage 2] Optimize protocol to yield pure target p50 and Rel A proteins

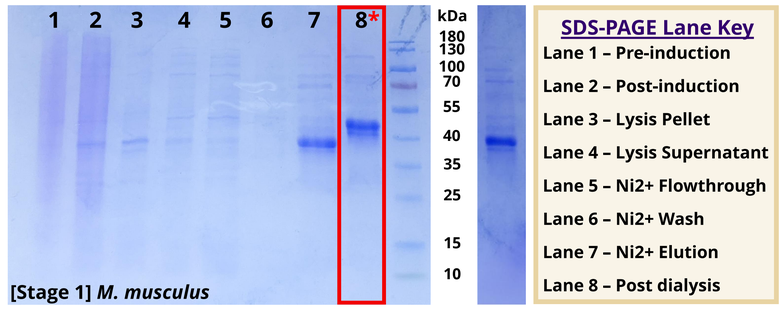
Prior studies indicate increasing imidazole concentration disrupts and elutes weakly bound proteins and impurities, leaving only our proteins of interest.[3]

Methods

Protein Expression Protocol



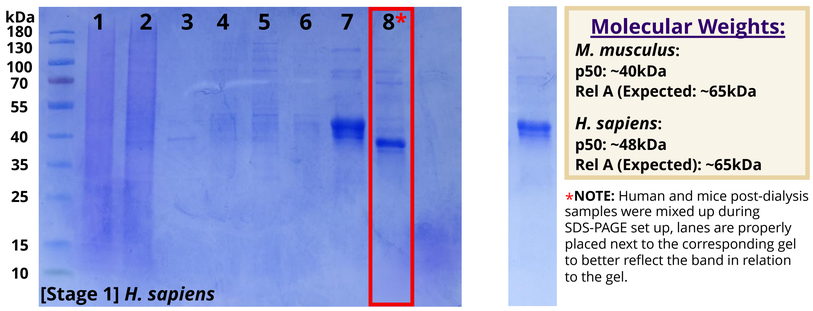
Results



SDS-PAGE Lane Key

- Lane 1 – Pre-induction
- Lane 2 – Post-induction
- Lane 3 – Lysis Pellet
- Lane 4 – Lysis Supernatant
- Lane 5 – Ni2+ Flowthrough
- Lane 6 – Ni2+ Wash
- Lane 7 – Ni2+ Elution
- Lane 8 – Post dialysis

Fig 1. SDS-PAGE results of Stage 1 *M. Musculus* protein purification. Gel electrophoresis performed on *M. musculus* sample depicts vivid p50 bands at roughly 40kDa, Rel A bands are not present.



Molecular Weights:

***M. musculus*:**
p50: ~40kDa
Rel A (Expected: ~65kDa

***H. sapiens*:**
p50: ~48kDa
Rel A (Expected): ~65kDa

***NOTE:** Human and mice post-dialysis samples were mixed up during SDS-PAGE set up, lanes are properly placed next to the corresponding gel to better reflect the band in relation to the gel.

Fig 2. SDS-PAGE results of Stage 1 *H. sapiens* protein purification. Similarly to figure 1, Gel electrophoresis performed on *H. sapiens* sample depicts vivid p50 bands at roughly 48kDa and is higher up on the lane compared to *M. musculus* SDS-PAGE, Rel A bands are also not present here.

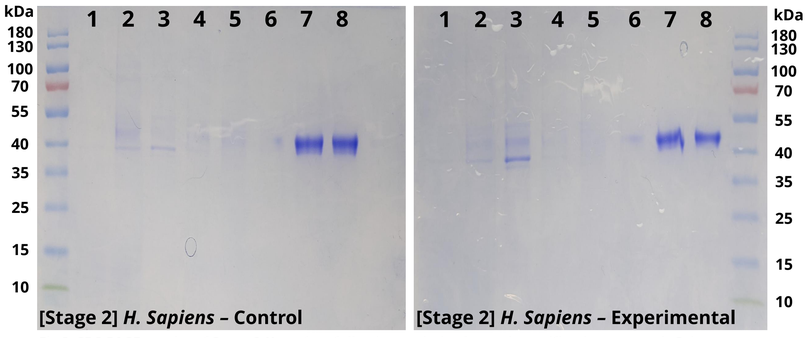


Fig 3. SDS-PAGE results of Stage 2 *H. sapiens* following optimized protein purification protocol. Gel electrophoresis was performed to set up a control gel following the same protocol as stage 1 and an experimental using with optimized wash step. The control gel bands are similar to stage 1, as expected. The experimental gel bands are also very similar to the control gel with impurities present and lack of Rel A band.

Discussion

Based on Stage 1 SDS-PAGEs, both *H. sapiens* and *M. musculus* gels show p50 but not Rel A. This could be due to possible degradation of the Rel A during Ni2+ Affinity Chromatography.

In Stage 2 we aimed to optimize our initial protocol by increasing the imidazole concentration in the wash step of our Ni2+ Affinity Chromatography column to elute weaker binding proteins and remove remaining impurities. The experimental gel displays similar bands to the control gel, indicating unsuccessful optimization of our protocol.

Conclusion

Based on our SDS-PAGEs from Stage 2 of our research, we were unable to obtain a higher purity of p50 and Rel A

The experimental SDS-PAGE visualizing the altered imidazole step depicts bands similar to both the control gel and the *H. sapiens* gel from Stage 1.

Presence of impurities in our Stage 2 gels tell us another step is necessary for increased purification of p50/Rel A from *H. sapiens* and *M. musculus* samples.

Future Steps

Other unknown factors may be present that are contributing more to the retention of impurities in the gels rather than the concentration of imidazole.

The connection between the similarities of the p50/Rel A protein heterodimer in humans and mice still suggest that mouse models can be reliable biological predictors however further research is necessary to solidify and better understand this connection.

Acknowledgements

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References