Isolation and Comparison of NF-kB p50/RelA Human & Mouse Homologs

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Background

Nuclear Factor-kappa B (NF-κB) is a transcription factor regulating various genes involved in immune response, inflammation, cell survival, and cell development.

RelA (p65) and p50 are two important subunits of NF-κB. Together they form a heterodimer controlling gene transcription. RelA promotes gene expression and p50 acts as a repressor.

In humans, p50 and RelA (p65) help regulate the NF-κB signaling pathway. While mouse homologs, which share significant similarities with humans, provide a valuable model for studying these processes.

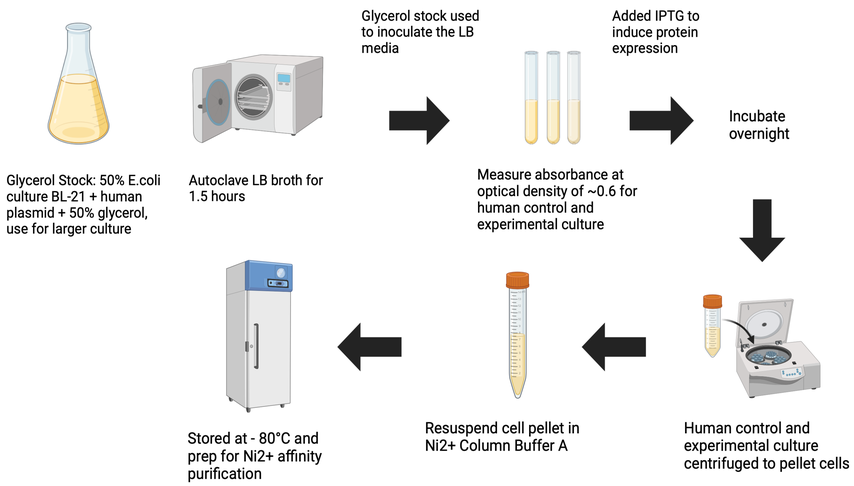
Research Objective

The goal is to compare the biochemical properties of RelA and p50 in mice and human samples. Achieving clearer separation will help compare functionality between species, making it easier to translate findings from mouse models to human health.

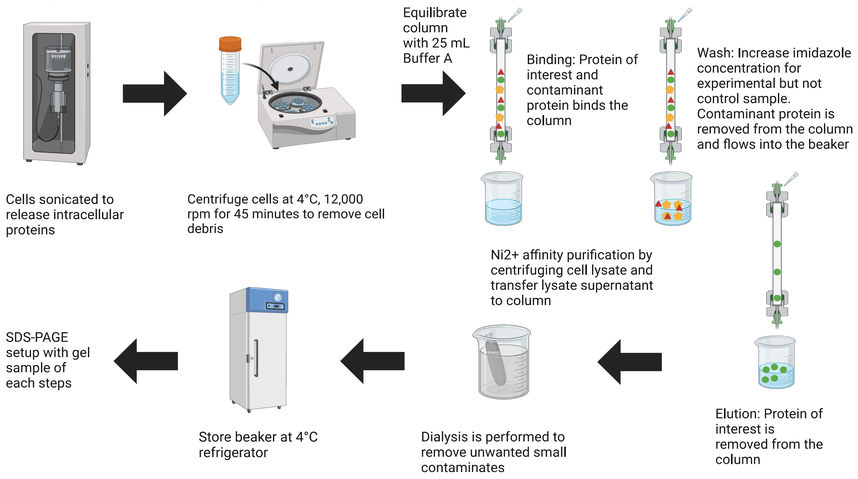
Hypothesis

Increasing the imidazole concentration in the wash buffer during Ni2+ affinity chromatography will enhance the removal of non-specifically bound contaminants without significantly affecting the binding or recovery of the target proteins.

Method



Stage 1- Protein Expression



Stage 2- Protein Purification

Continued Research

- Further studies aim to modify various aspects of the protocol to increase visualization of target proteins.
- Would benefit from using larger aliquots of each sample and possibly decrease the imidazole concentration instead to further purify proteins.
- The similarities between the p50/RelA heterodimers in mouse and human cells suggest shared health implications for both species.

Result

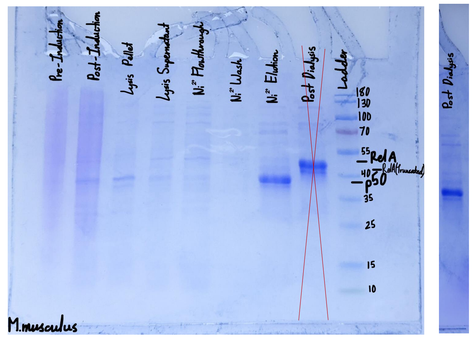
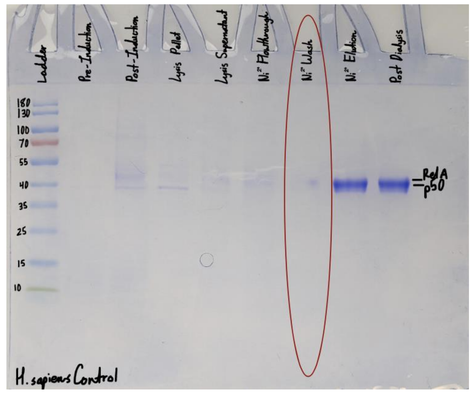
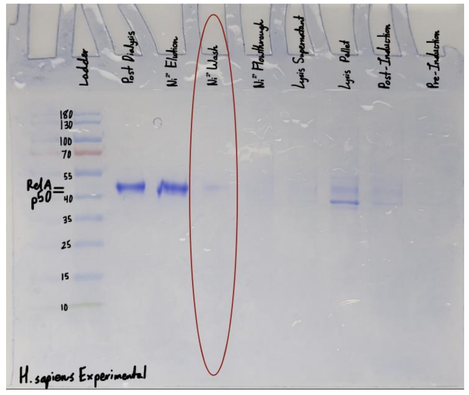


Figure 2: SDS-PAGE results of altered wash buffer. Comparing the original protocol using low imidazole concentrations to the altered protocol with increase imidazole concentration in the wash buffer.





Conclusion

- The p50/RelA proteins in mice and humans were highly similar.
- Truncated RelA proteins were found in all samples, suggesting that different conditions may lead to the production of shorter versions of the protein.
- Change to the protocol by increasing imidazole did not improve our yield or purity in SDS-PAGE gels.
- Increasing the imidazole concentration might not selectively remove contaminants but instead could also cause the loss of p50/RelA protein during the wash step.

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References