Investigating the Relation Between INI-1 and Mononucleosome Movement

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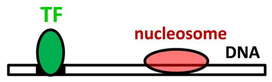
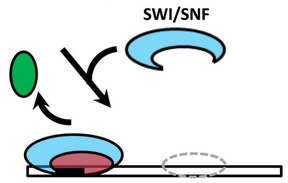
Introduction

- Atypical teratoid/rhabdoid tumors (AT/RT) cause a pediatric brain cancer that is known to contain a mutated version of INI-1.
- INI-1 is a core protein involved in the SWI/SNF chromatin remodeling complex.
- INI-1 can bind to both the free DNA and the mononucleosome DNA.
- SWI/SNF's function is to move a mononucleosome up and down a strand of DNA, allowing the DNA to be properly read.



- A mononucleosome (MN) is a strand of free DNA wrapped around a histone octamer.
- **Hypothesis:** Due to its role within the SWI/SNF chromatin remodeling complex, INI-1 will play a vital role in proper mononucleosome movement.

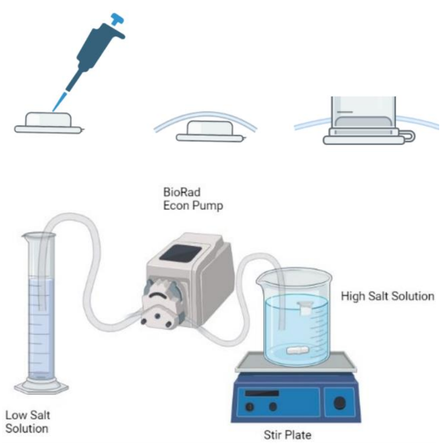


Methods

Plasmid Preparation:
- Combine LB broth, Ampicillin, and designated plasmid.
- Incubate at 37°C overnight and then complete various extraction steps.
- The final plasmid pellet was dissolved in water to create the desired plasmid DNA.

Polymerase Chain Reaction (PCR):
- Combine plasmid DNA with dNTPs, primers, TAQ enzyme, buffer, betaine, and water.
- Run in a thermocycler to allow for denaturation, annealing, and extension to copy DNA.
- Run on 1.5% agarose gel to ensure correct PCR product.

Mononucleosome Assembly:
- Load buffer, PCR DNA, and histones into the lid and seal to create a membrane capsule.
- With each of the capsules in a high salt buffer, begin a dialysis gradient with the low salt buffer and a pump for 22-26 hours. Transfer all of the capsules to a no salt buffer for at least 4 hours.
- Ran on a 4% Native PAGE gel.

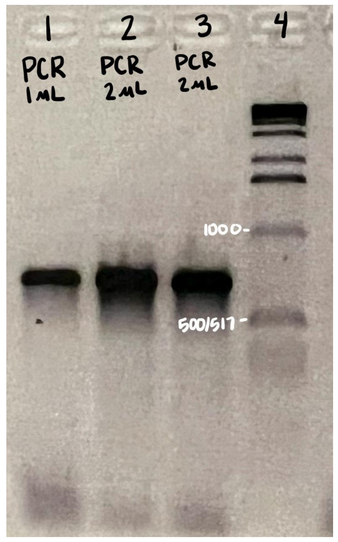


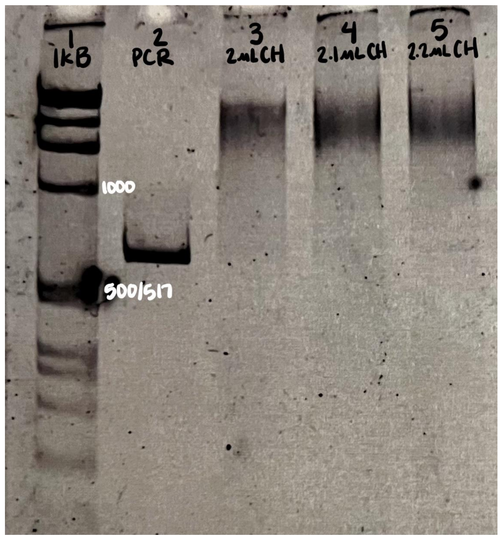
Enzyme Digest:
- DNA and mononucleosomes were combined with Hha1 and cutsmart buffer and incubated at 37°C for 30 minutes.
- Ran on a 4% Native PAGE gel.

Results

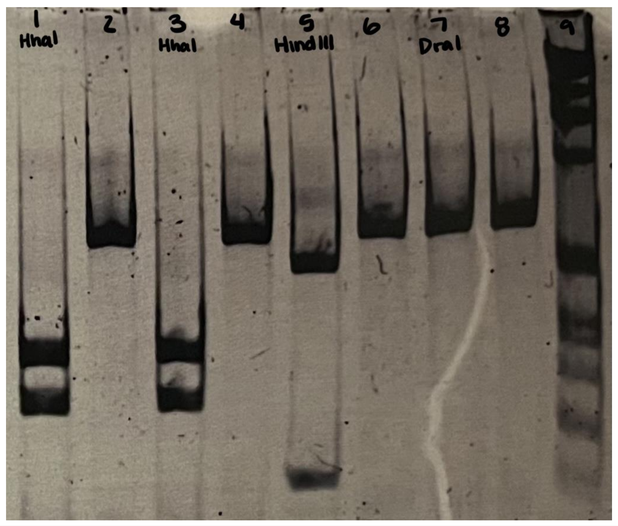
Polymerase Chain Rection:
- 1.5% agarose gel to ensure the correct PCR DNA was created from our plasmid prep and PCR protocol.
- 1kB ladder was used to ensure the correct PCR product was produced.
- 1uL and 2uL concentrations of plasmid were tested to compare band density and concentration.
- Our PCR product should be just under 750 bp in length.

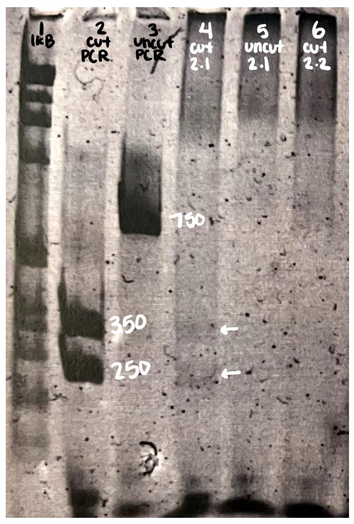
Mononucleosome Assembly:
- 4% Native PAGE gel to ensure the mononucleosome was assembled.
- The size of the PCR and MN are compared using a 1kB ladder.
- Titration of histone concentrations is done to determine the optimal concentration.
- Mononucleosome are around 1200 bp in length.
- Significantly larger than PCR due to the addition of histones.





Enzyme Digest:
- 4% Native PAGE gel to ensure enzyme cut the strand of DNA at the enzyme cut sites.
- Left: PCR that was cut by 3 different enzymes, Hha1, Hind III, and Dra1, separated by uncut PCR.
- Right: PCR and mononucleosomes that were cut by Hha1, separated by uncut PCR and mononucleosomes, respectively.
- Both were compared to a 1kB ladder was used to measure the size of the fragments.
- PCR should be cut into several pieces.
- MN should have a larger piece towards the top of the gel and then several small fragments of around 100 bp.





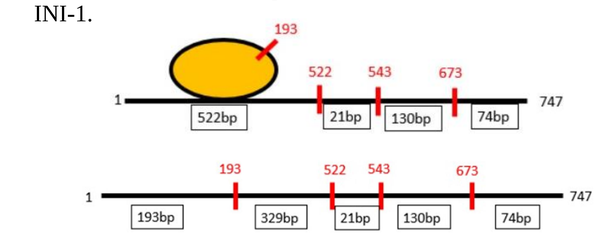
Discussion

DNA Concentration:
- Important to have the correct PCR product for future experiments.
- Our DNA contains a nucleosome positioning element which will allow for mononucleosome assembly in future experiments.

Mononucleosome Assembly:
- During assembly, the perfect concentration of histones is needed to ensure that the DNA is slowly and correctly wrapped around the histone.
- Titrations were completed to ensure the optimal concentration of histones was identified.
- Mononucleosomes are found to stay assembled in vitro for around 30 days.

Enzyme Digest:
- Hha1 was found to be the best cutting enzyme due to the amount of cut sites and their location relative to the mononucleosome positioning element within our DNA.
- The enzyme correctly cut the fragments surrounding the mononucleosome but couldn't access the cut site that is wrapped around the histone.

Next Steps:
- Add SWI/SNF to understand how SWI/SNF typically moves the mononucleosome along our strand of DNA that was replicated during PCR.
- Track where SWI/SNF is moving the MN.
- Use a version of SWI/SNF without INI-1 to compare if/where the MN is moved in comparison to SWI/SNF that contains INI-1.



Acknowledgements

Thank you to the Wentz Research Scholars Program for awarding me this scholarship which has encouraged me throughout my research project. Thank you to the Oklahoma State University College of Arts and Sciences, Ferguson College of Agriculture, and the Honors College for giving me the opportunity to conduct research and to further my education through the completion of the Honors Degree. Thank you to Bailey, Kayla, and Landon for helping to run these experiments. Lastly, thank you to Dr. Wiggins for serving as my second reader for my thesis, and thank you to Dr. Ruhl for providing me with an opportunity to conduct research through his laboratory and for being an exceptional mentor to me throughout the last half of my undergraduate experience.

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