Expression, Purification & Characterization of Recombinant Heparan Sulfate Glucuronyl Epimerase

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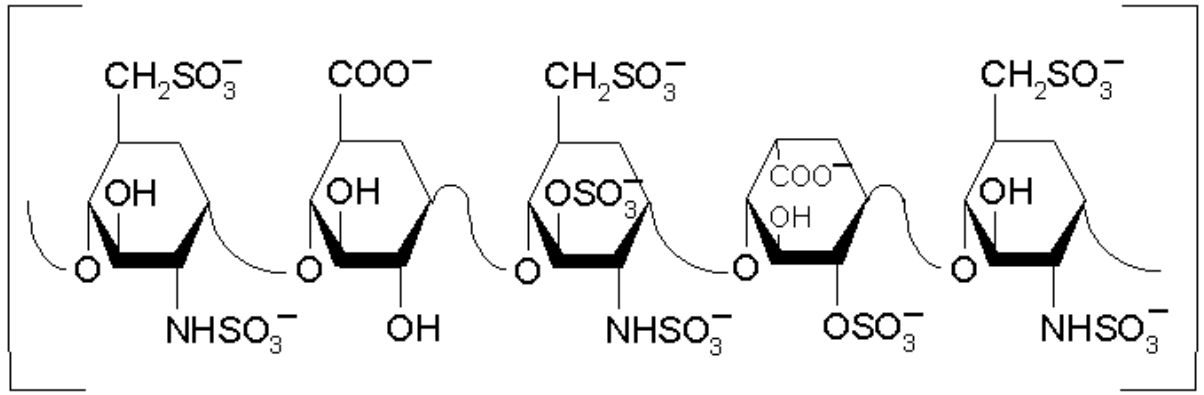
Heparin

- Heparin is an anticoagulant used to prevent and treat blood clots.
- Typically derived from animal tissues which causes inconsistencies in production.
- Laboratory based synthesis is more reliable.
- C5 epimerase, is an essential enzyme for lab-synthesized heparin.

https://www.mountainside-medical.com/products/heparin-sodium-injection-multiple-dose-glass-fliptop-vials-10-ml-25-tray

Function of C5 epimerase

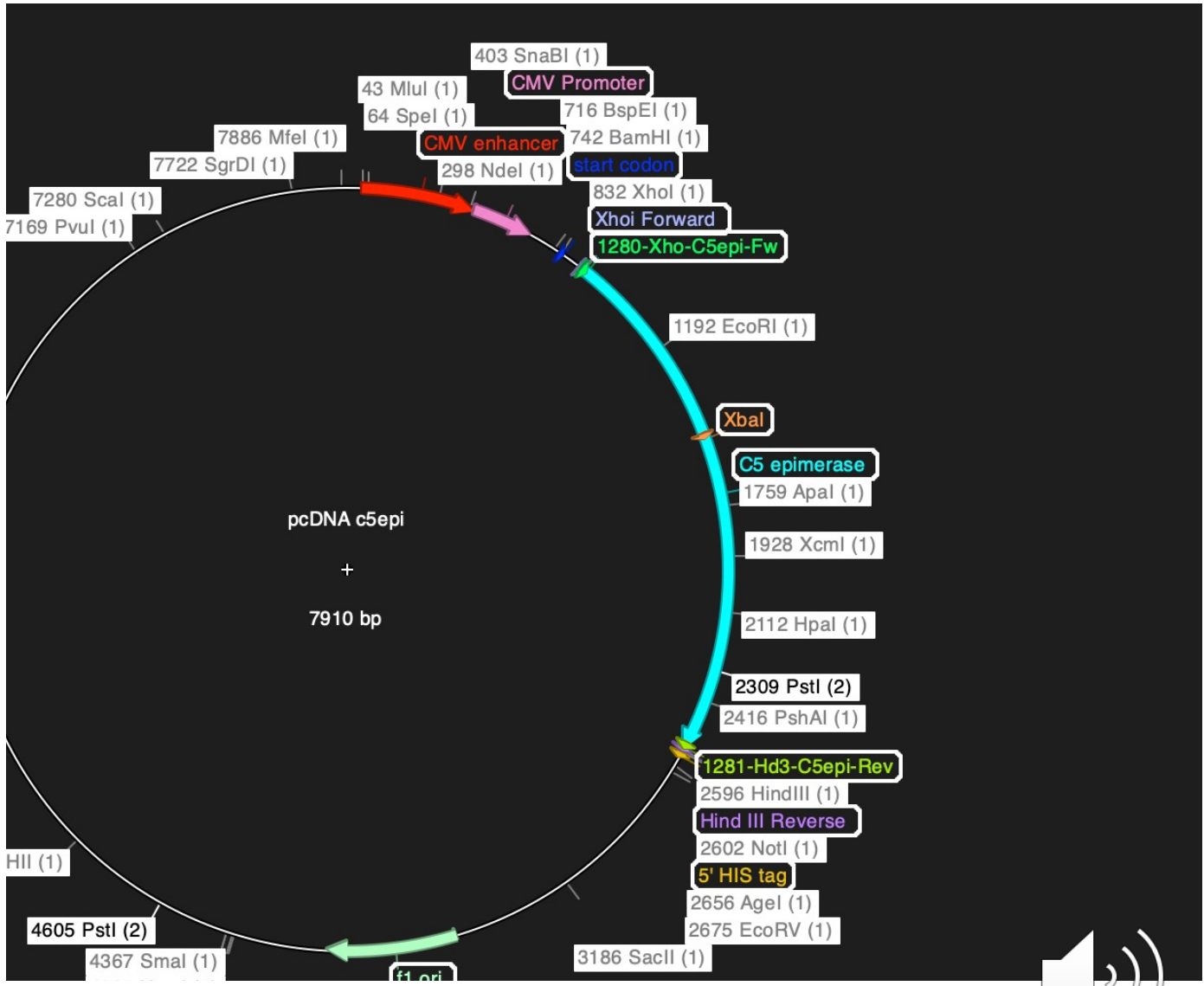
- Heparin is comprised of repeating units of either iduronic or glucuronic acid and glucosamine.
- The conversion of glucuronic acid to iduronic acid is catalyzed by the c5 epimerase enzyme



https://www.people.vcu.edu/~urdesai/hep.htm

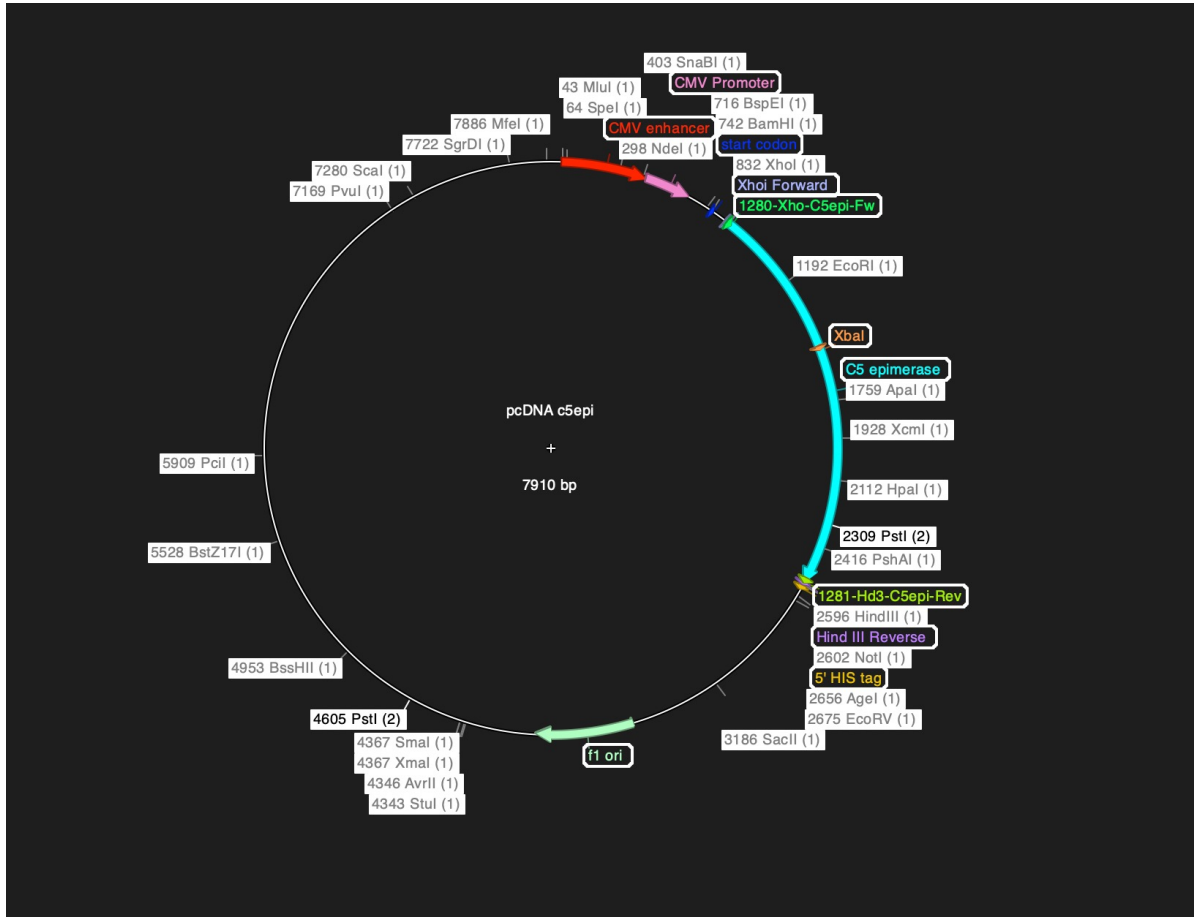
Sequencing

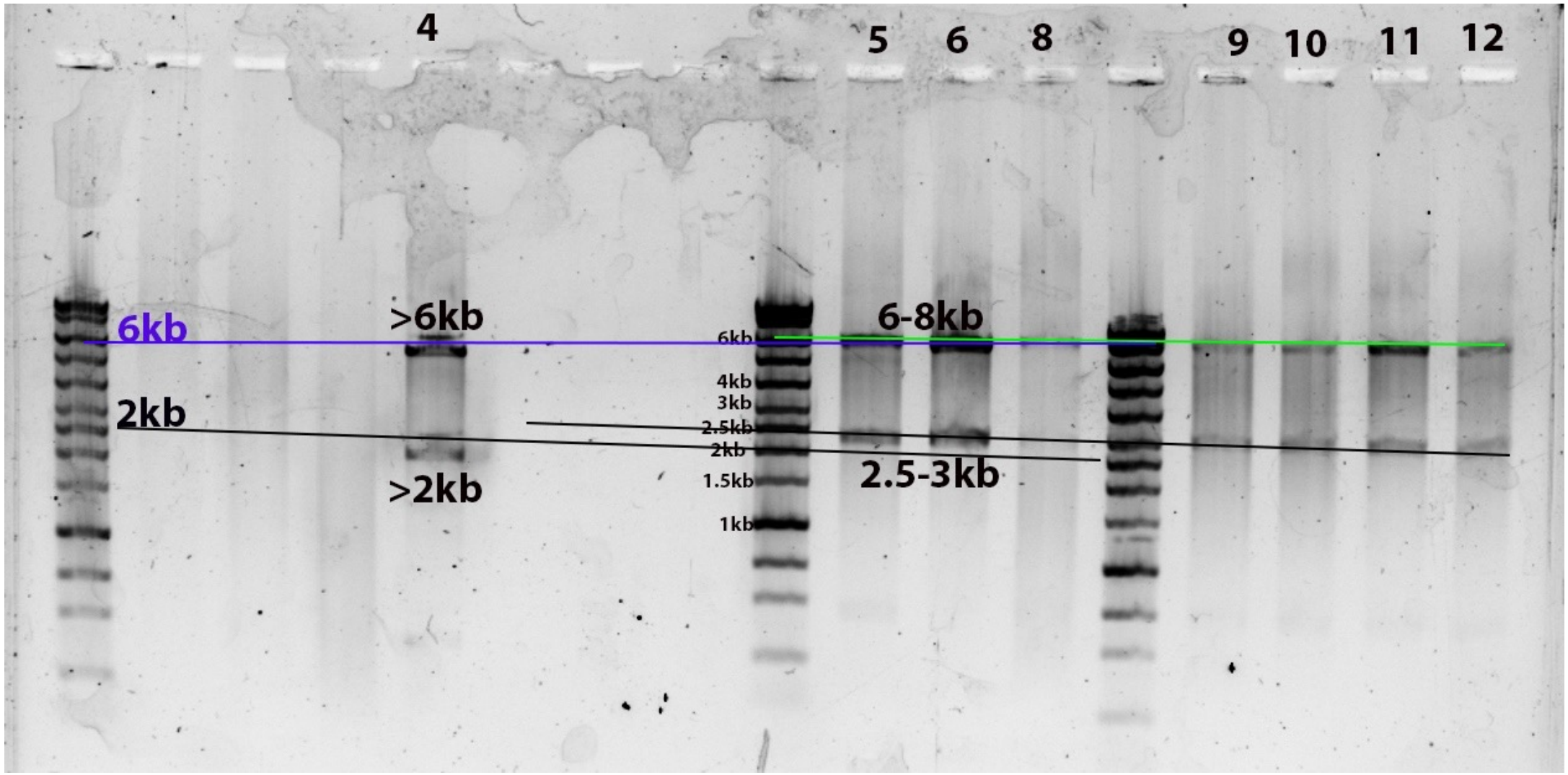
- The plasmid (pcDNA) and insert were sent for sequencing and analyzed to determine the restriction enzymes we were going to use for this process.



Molecular Cloning

PCR, gel electrophoresis & Purification
Digestion using XhoI and HindIII restriction enzymes
Ligation of Plasmid and Insert.
Transformation of E.coli by recombinant plasmid
Plating, Culturing and plasmid Purification
Second Digestion using PstI
Large Scale culture & Endotoxin Free Plasmid Purification


403 SnaBI (1)
CMV Promoter
43 MluI (1)
64 SpeI (1)
716 BspEI (1)
7886 MfeI (1)
742 BamHI (1)
CMV enhancer
7722 SgrDI (1)
298 NdeI (1)
start codon
7280 ScaI (1)
832 XhoI (1)
7169 PvuI (1)
Xhoi Forward
1280-Xho-C5epi-Fw
1192 EcoRI (1)
XbaI
C5 epimerase
1759 ApaI (1)
pcDNA c5epi
+
7910 bp
1928 XcmI (1)
5909 PciI (1)
2112 HpaI (1)
2309 PstI (2)
5528 BstZ17I (1)
2416 PshAI (1)
1281-Hd3-C5epi-Rev
2596 HindIII (1)
Hind III Reverse
4953 BssHII (1)
2602 NotI (1)
5' HIS tag
2656 AgeI (1)
4605 PstI (2)
2675 EcoRV (1)
3186 SacII (1)
4367 SmaI (1)
f1 ori
4367 XmaI (1)
4346 AvrII (1)
4343 StuI (1)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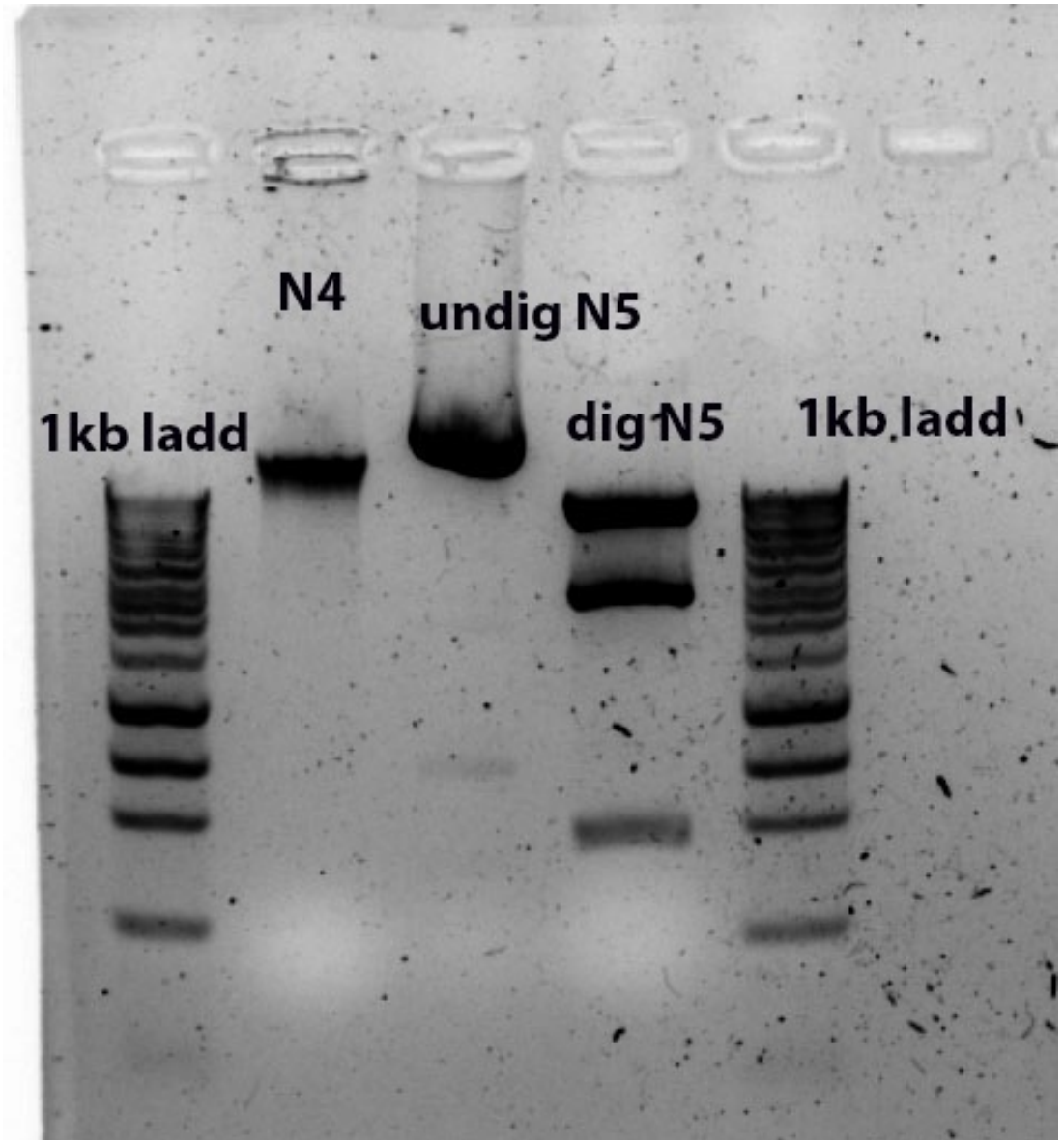
Concentrations of purified plasmids

Sample#	Concentration ng/ul
4	20.4
5	1836.1


N4
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Results, Analysis & Next Steps

- The sequencing results showed expected sequence for one of the clones of C5 epimerase which allowed us to conclude that we had at the least one correct clone.
- We have started transfection of cells and culturing.
- Next, we will conduct the purification of recombinant enzyme and characterization
- Finally, we will evaluate the enzyme's activity and demonstrate its ability to generate iduronic acid-containing sequences using NMR and mass spectrometry analysis.


Questions

References

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