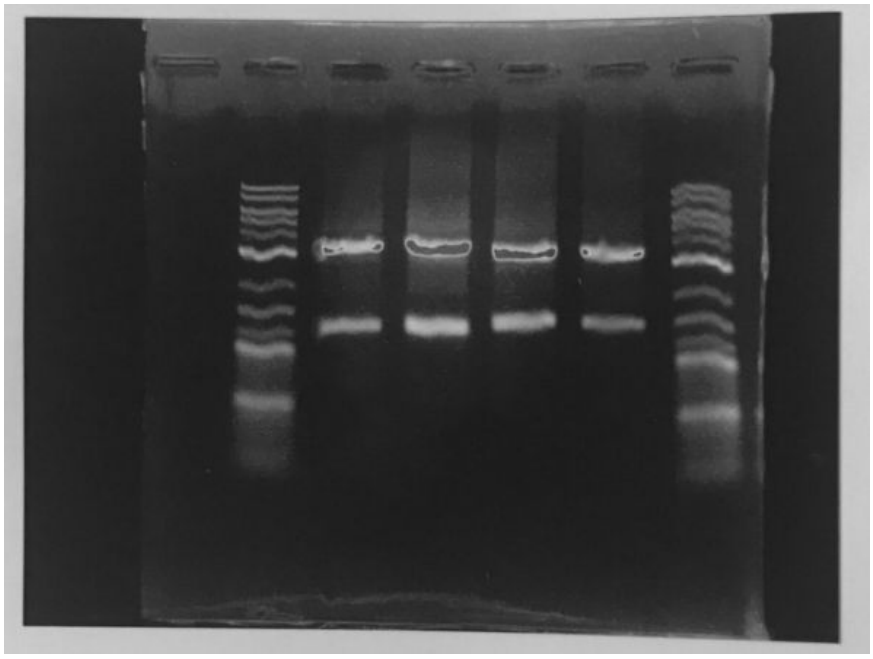
Restriction of AddGene Plasmid:

We submitted a part of the plasmid assembly, Exon 1. In the near future, we hope to complete the full assembly, test its ability to generate HTT protein, and isolate the mRNA and test the toehold exchange mechanism that we designed.

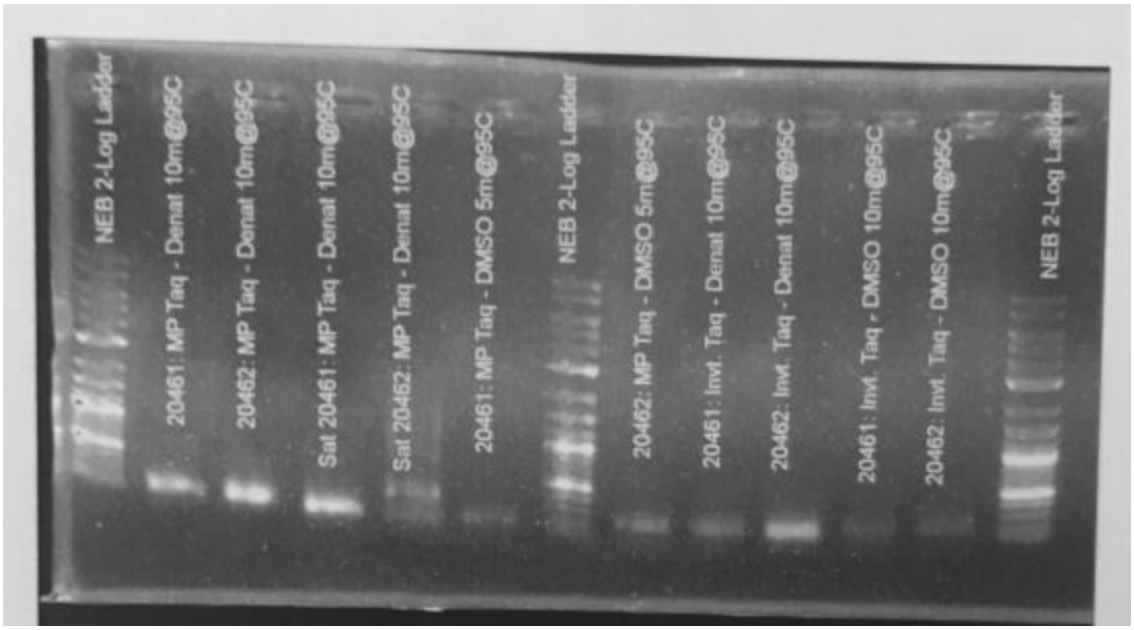
To accomplish this the following steps were performed:

Restriction of the 4.8 K AddGene plasmid to remove the plasmids of interest. The double digest did not produce a strong enough band of the HTT component, so the larger STUI fragment that contained the HTT element was isolated instead

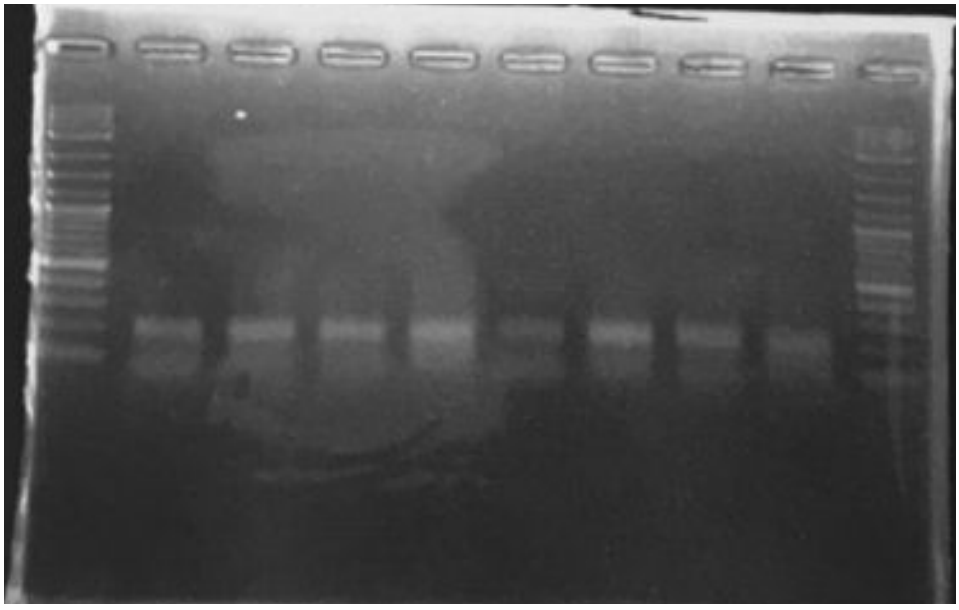
Lane	Name
1	2-Log ladder
2	Plasmid 40261 - group 1 isolation
3	Plasmid 40262 - group 1 isolation
4	Plasmid 40262 - group 2 isolation
5	Plasmid 40261 - group 2 isolation
6	2-Log ladder



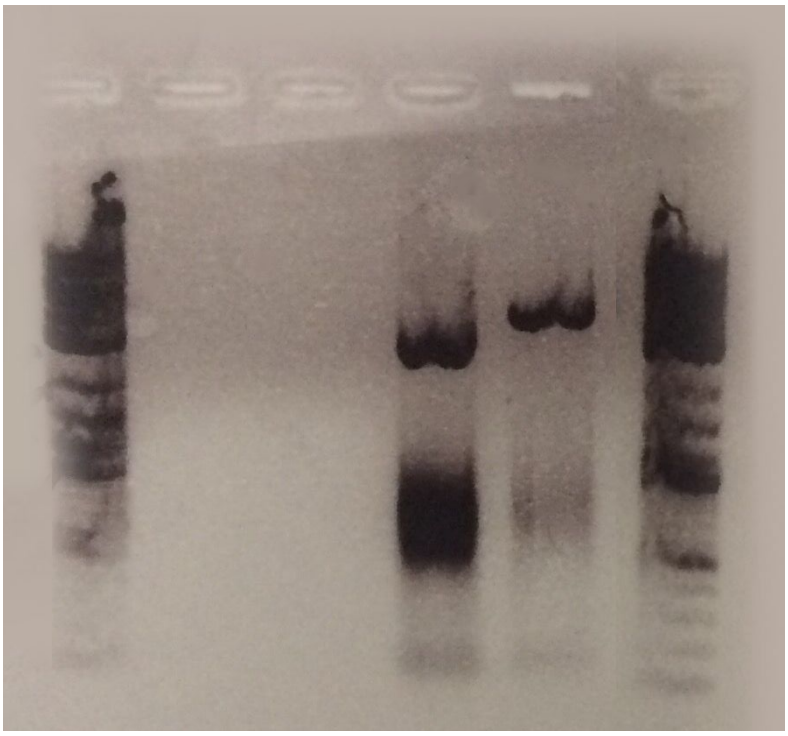
The 144 nucleotide sequence (23Q repeat plasmid) and 296 nucleotide sequence (74Q repeat plasmid) were PCRed out.



The remaining of the upstream 5' UTR and high GC content 3' addition were then added to the amplified plasmid via isothermal assembly (2-log ladder on the outsides, lanes 2,4,6,8 = parts originating in plasmid 40261 and lanes 3,5,7,9 = parts originating in plasmid 40262:



3A assembly was then used to clone Exon 1 into the pSB1C3 plasmid:



Plasmid was then inserted into e coli and shipped to iGEM HQ.