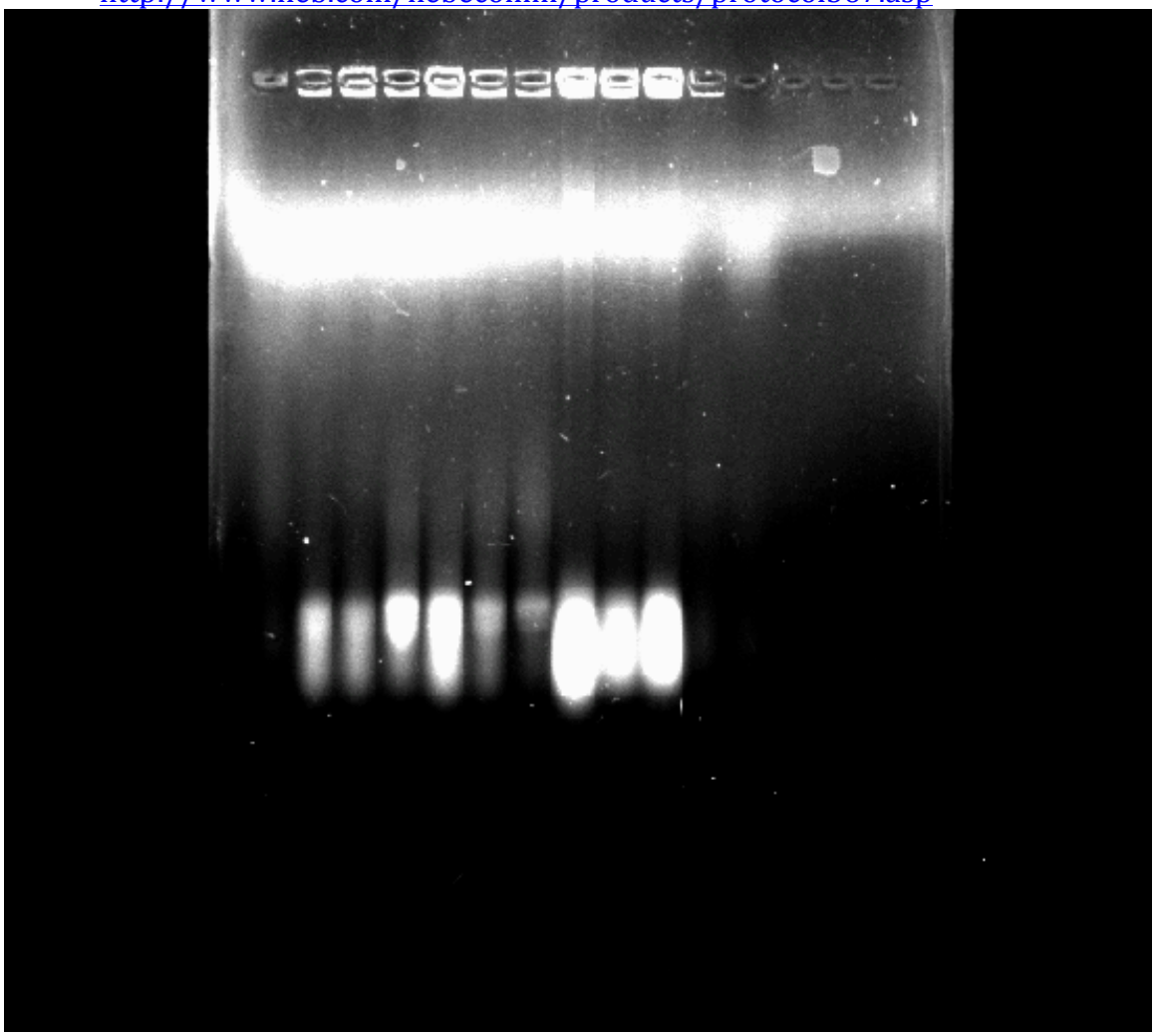
Thursday, 7/5

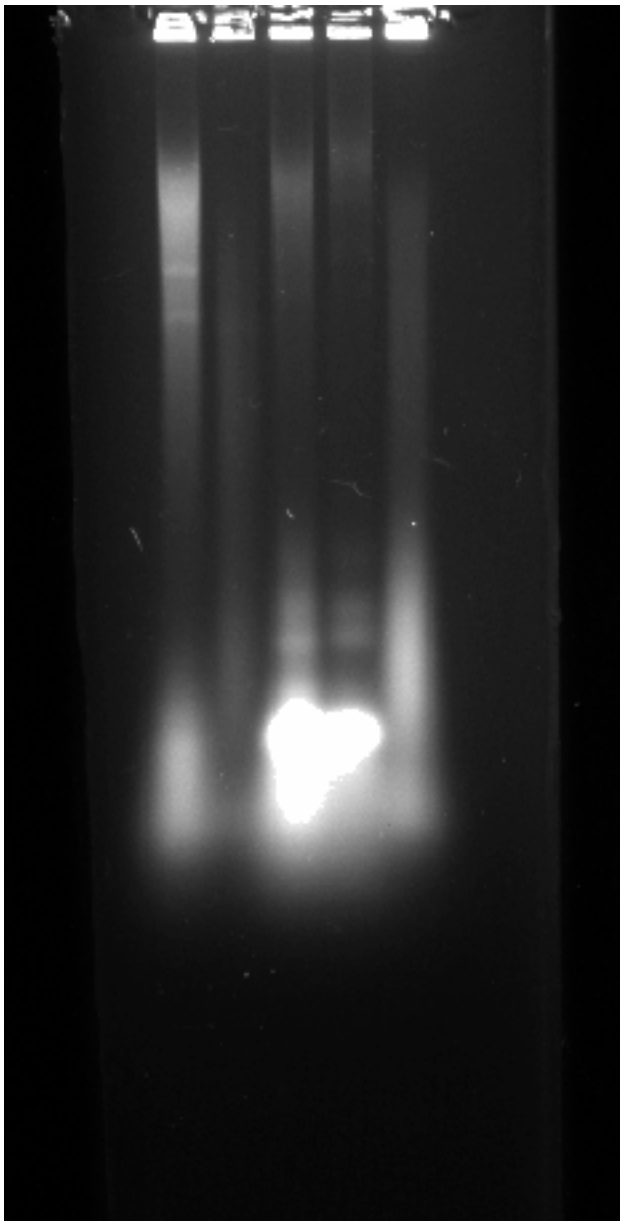
- Redid colony PCR, and imaged the resulting gel with no apparent success: Protocol/guidelines for Taq DNA polymerase used: http://www.neb.com/nebecomm/products/protocol567.asp



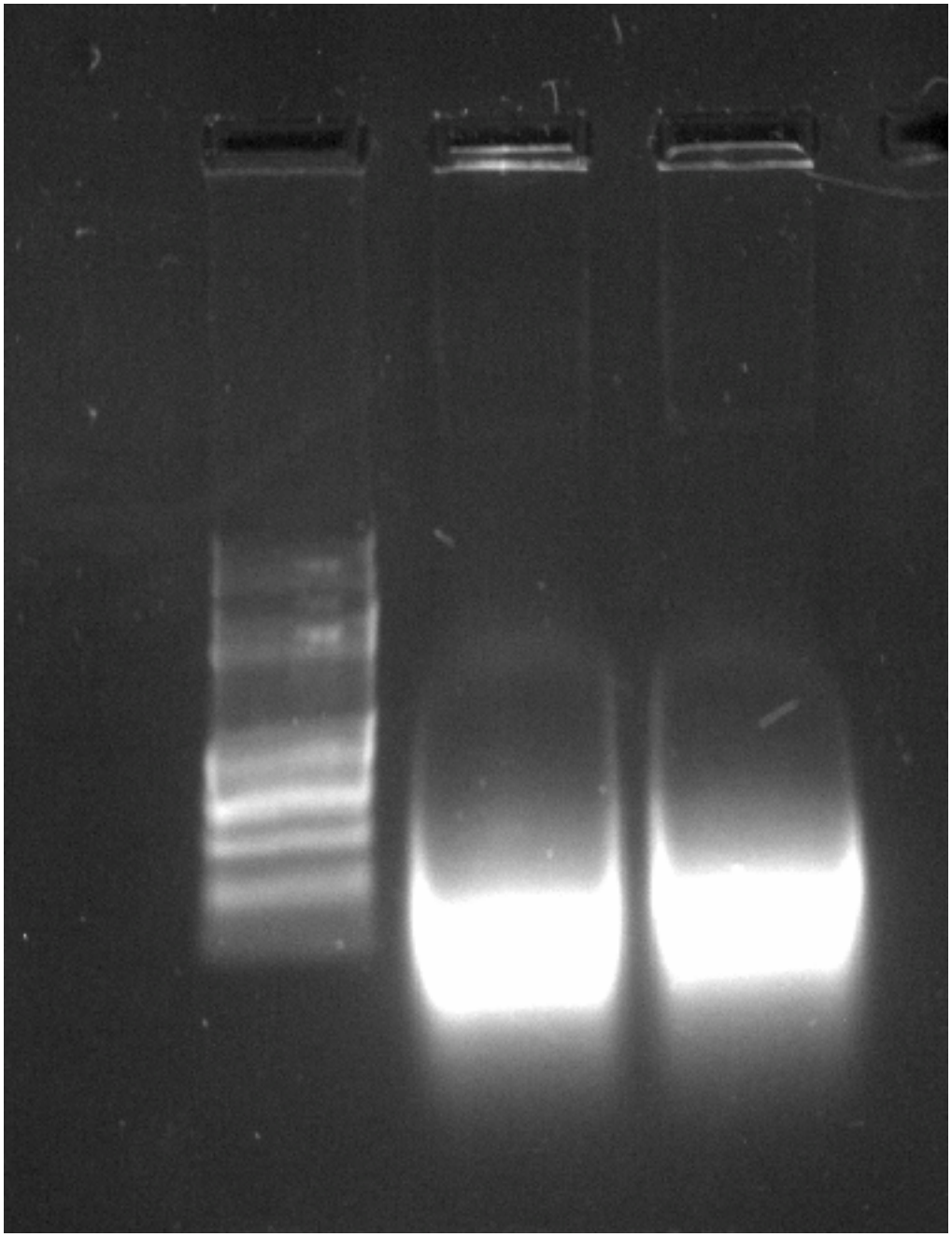
- Carried out multiple-primer PCR using new site-directed mutagenesis protocol by adding .5 ul of primers 31-40
- Redid the gel to image the remaining PCR reactions that looked most promising, but still unsuccessfully because TBE buffer with which the gel is made must be of same concentration as the buffer in the gel box:

Friday, 7/6

- Carried out one more PCR with the cells from pellets of the most promising colonies: 3, 5, and 6 as well as 2 more colonies from the original plate named A and B.
- Ran another gel with the PCR reactions, but still inconclusive since the ladder did not show (from left to right A, B, 3, 5, 6):



- Ran 2 most promising samples: 3 and 5 using all materials and equipment from Isaacs lab, but still not successfully:



- Transformed a 1 ml aliqot of competent XL1 blue with 25ul of row mix PCR reaction diluted with 100ul sterile, RNAse –free water. Transferred the transformation mixture into a culture tube with 2ml SOC and incubated for an hour at 37C as usual. Tranferred the solution to 3 microcentrifuge tubes, and spun down to form pellets. Decanted the excess supernatant, resuspended the pellets, and plated the samples (2 on amp/kan, 1 on amp).
- Carried out 2 more multiplex PCR's, using .5 ul of ~ 6uM primers 11-20 and 21-29.
- DPN digest of deletion and insertion PCR's for 140 min.

Saturday, 7/7

- Miniprepped double-digest cultures (old) 9+10, 21+22, 29+30 since sequences came back without desirable changes
- Transformed XL1B with the insertion and deletion mixes and plated the cells on amp/kan

Sunday, 7/8

- Transformed another 1ml aliquot of XL1B with remaining row PCR
- Cultured 4 deletion mutant colonies
- Cultured the suspected correct 3 and 5 T7 clones

Component	Volume (µl)	Final Concentration
Standard or ThermoPol Taq Reaction Buffer (10X)	5 µl	1X
Deoxynucleotide Solution Mix (10 mM)	1 µl	200 µM
Upstream Primer (10 µM stock)	0.5–2.5 µl	0.1–0.5 µM
Downstream Primer (10 µM stock)	0.5–2.5 µl	0.1–0.5 µM
DNA Template	determined by user	0.1-1 ng/ml plasmid DNA 1-10 µg/ml genomic DNA
Taq DNA Polymerase*	0.2 µl	0.02 units/µl
Nuclease free water	Bring reaction to a final volume of 50 µl	

Initial Denaturation	**95°C**	**30 seconds**
30 Cycles	95°C	15–30 seconds
	45–68°C	15–60 seconds
	68°C	1 minute per kb
Final Extension	68°C	5 minutes
Hold	4°C	∞

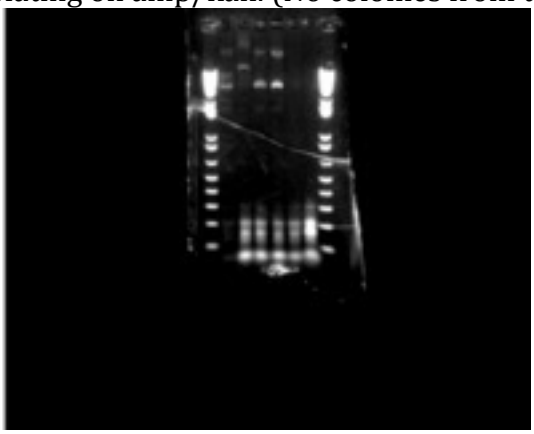
Monday, 7/9

- Remade primer design file
- Prepared a powerpoint of current work and challenges and presented to Isaacs
- miniprepped double digest samples and the possible RiA clones
- washed culture tubes and dry cycle autoclaved

Tuesday, 7/10

- Worked on remaking gel buffer and gel's until decent resolution
- Ran 31+32 25ul PCR's according to manufacturer and Jen's protocol (slightly different cycling conditions and DPN1 digest times)
- DPN1 digested the PCRs, transformed, and plated
- Autoclaved all lab trash

Wednesday, 7/11

- Ran gels until discovered that right ones should be made with non-low-melt agarose, heated for 2 or more minutes, and that used PCR reactions are blurry themselves due to cellular gunk
- Redid Taq "colony" PCR with positive and negative controls, 2 possible plasmids purified via miniprep, and 2 colonies off the plate. Ran them with 2 ladders in the flankinglanes
- SDM PCRs with mixes of primers, DPN1 digest for 1 hr, transformation, and plating on amp/kan. (No colonies from transformation the previous day.)
- 
  Analysis of full colony PCR with positive and negative control – something is wrong with the PCR reaction conditions because 600bp band is not present.

Thrusday, 7/12

- Still no colonies for mix plates, sequences are still not back = ☹
- Used leftover PCR reactions to thermocycle with highest possible annealing temperature – 68C, for Taq via 2-step PCR, but gel indicated no success.
- Primer mix concentration gradient PCR, digest, transformation, and plating
- Gel test for insert presence or RiA from a long time ago showed that first 5+6 digestion had very little insert, and second digestion had none.

Friday, 7/13

- 5+6 PCR and row mix using Jen's directions, 50 cycles, and 55 melting temperature.