Study on the effect of the CAG repeat in the ATXN2 gene on expression

Study Rationale

This work supports other efforts in the lab to characterize the ataxin 2 promoter that ultimately will identify therapeutic ways to control ataxin 2 expression

Objective

The objective of my part of this study was to to determine how the polyglutamine alters ataxin 2 expression or mRNA stability.

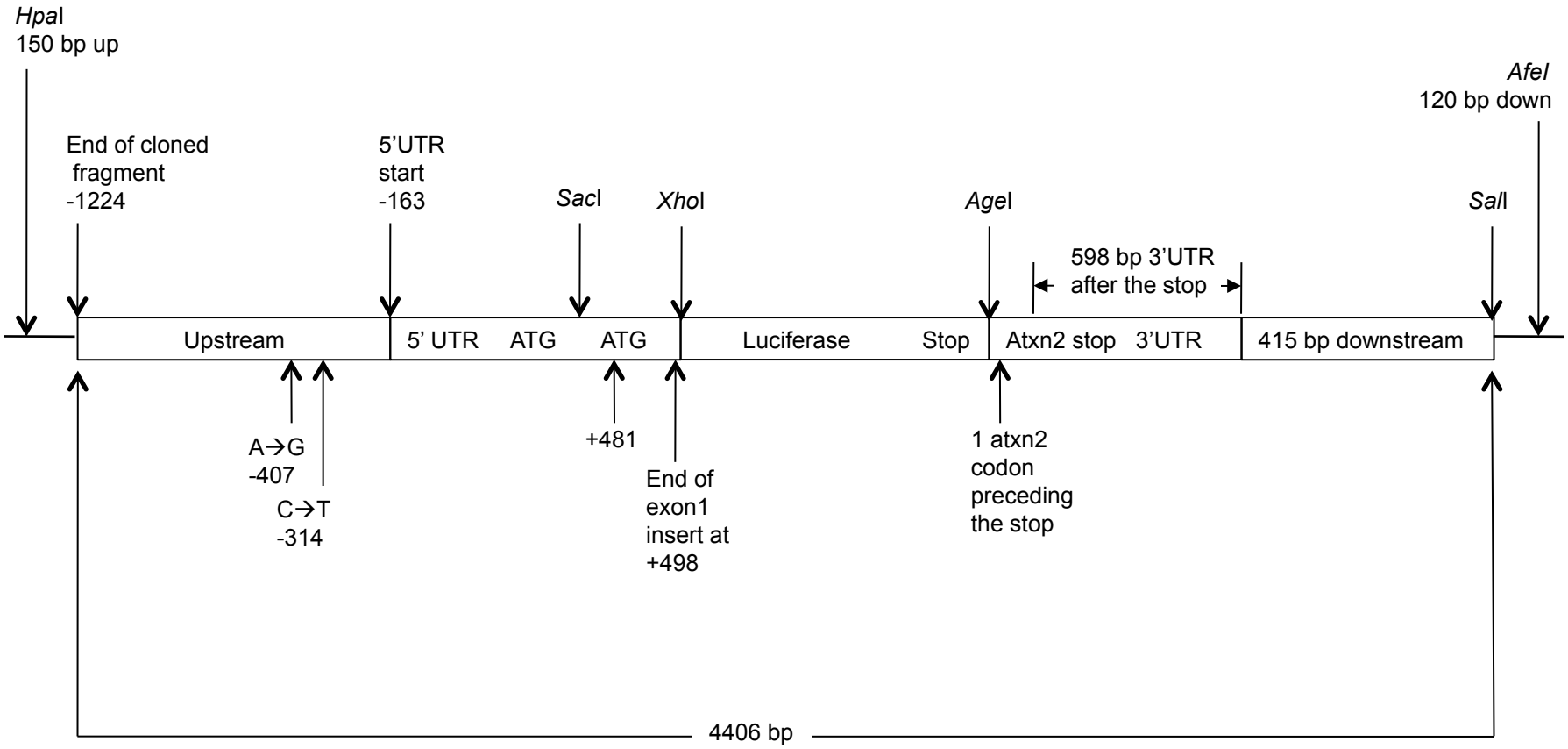
Hypothesis:

We hypothesize that the expression of ataxin 2 is controlled in part by the length of the CAG repeat.

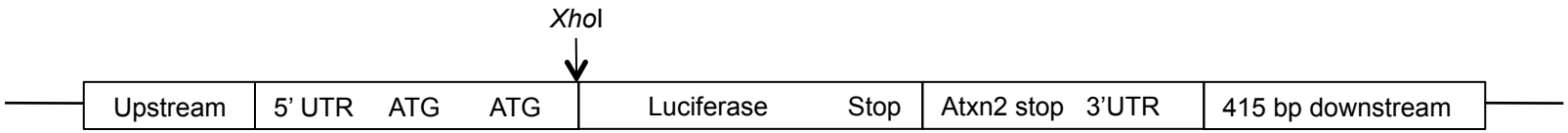
This might be due to an influence by the CAG on the transcription of the gene or on the stability of the mRNA.

To test this hypothesis we cloned different luciferase reporter constructs with varying CAG tracts and tested for differences in expression using luciferase assays.

pGL2c.5A3c


*Hpa*I
150 bp up
End of cloned fragment
-1224
5'UTR
start
-163
*Sac*I
*Xho*I
*Age*I
*Sal*I
*Afe*I
120 bp down
598 bp 3'UTR
after the stop
Upstream
5' UTR
ATG
ATG
Luciferase
Stop
Atxn2 stop
3'UTR
415 bp downstream
A→G
-407
C→T
-314
+481
End of
exon1
insert at
+498
1 atxn2
codon
preceding
the stop
4406 bp

Constructs with different CAG repeats



Previously Cloned

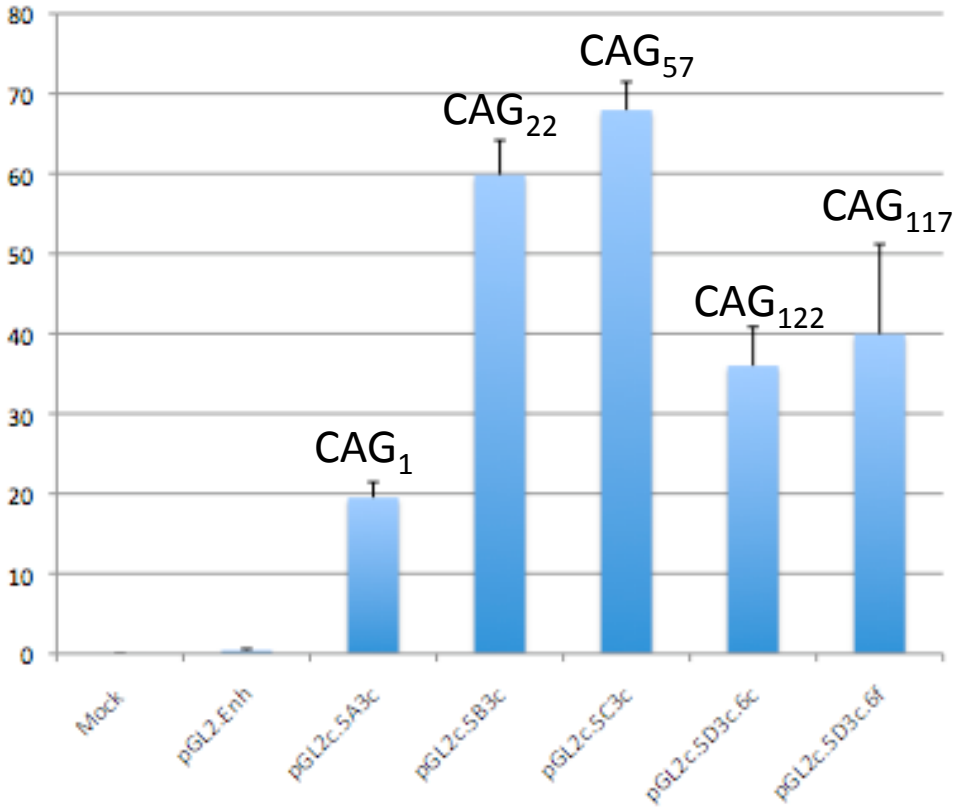
pGL2c.5A3	$MSLKJPQ_{1}$
pGL2c.5B3	$MSLKJPQ_{22}$PPPAAADVRKPGGSGLLASPPAAAPSPSSSSVSSSSA
pGL2c.5C3	$MSLKJPQ_{57}$PPPAAADVRKPGGSGLLASPPAAAPSPSSSSVSSSSA
pGL2c.5D3 #1	$MSLKJPQ_{122}$PPPAAADVRKPGGSGLLASPPAAAPSPSSSSVSSSSA
pGL2c.5D3 #2	$MSLKJPQ_{117}$PPPAAADVRKPGGSGLLASPPAAAPSPSSSSVSSSSA

Cloned by Patrick

pGL2c.5B3-K	$MSLKJPQ_{22}$PPPAAADVRKPGGSGLLASPPAAAPSPSSSSVSSSSA
pGL2c.5C3-K	$MSLKJPQ_{58}$PPPAAADVRKPGGSGLLASPPAAAPSPSSSSVSSSSA
pGL2c.5D3-K #1	$MSLKJPQ_{102}$PPPAAADVRKPGGSGLLASPPAAAPSPSSSSVSSSSA

Comparison of constructs with different CAG repeats.

This assay was done previously by Dan



3/4/09

You can rewrite this…just notes for you…

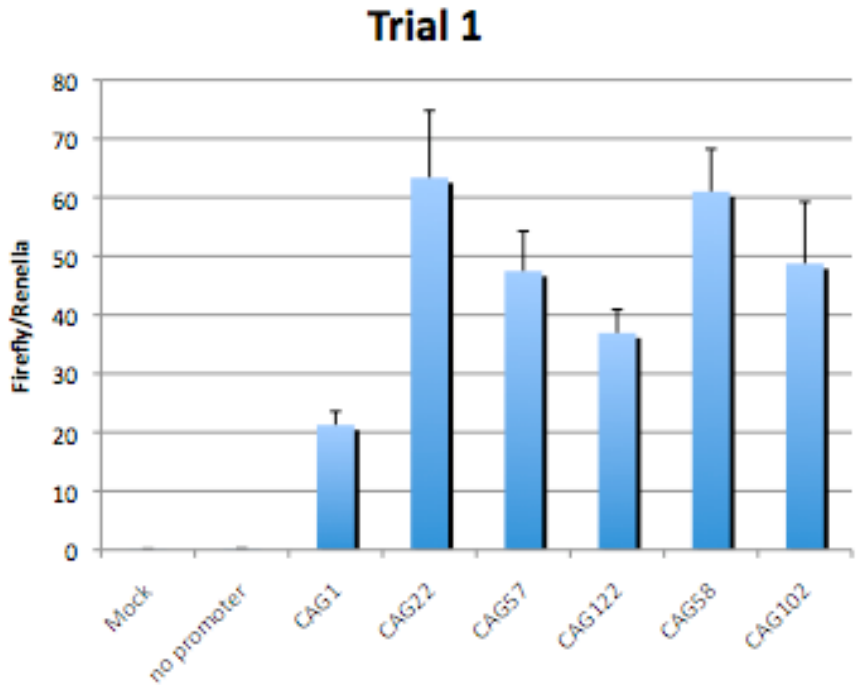
This previous experiment demonstrated that CAG length does indeed change the reporter gene expression.

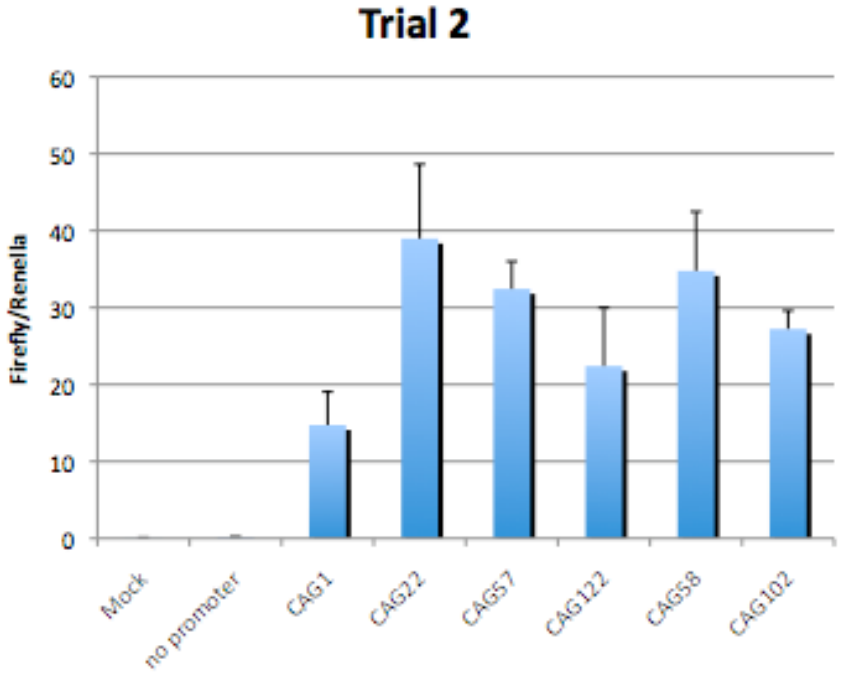
- The addition of 22 CAGs resulted in a more than 3-fold increase in expression.

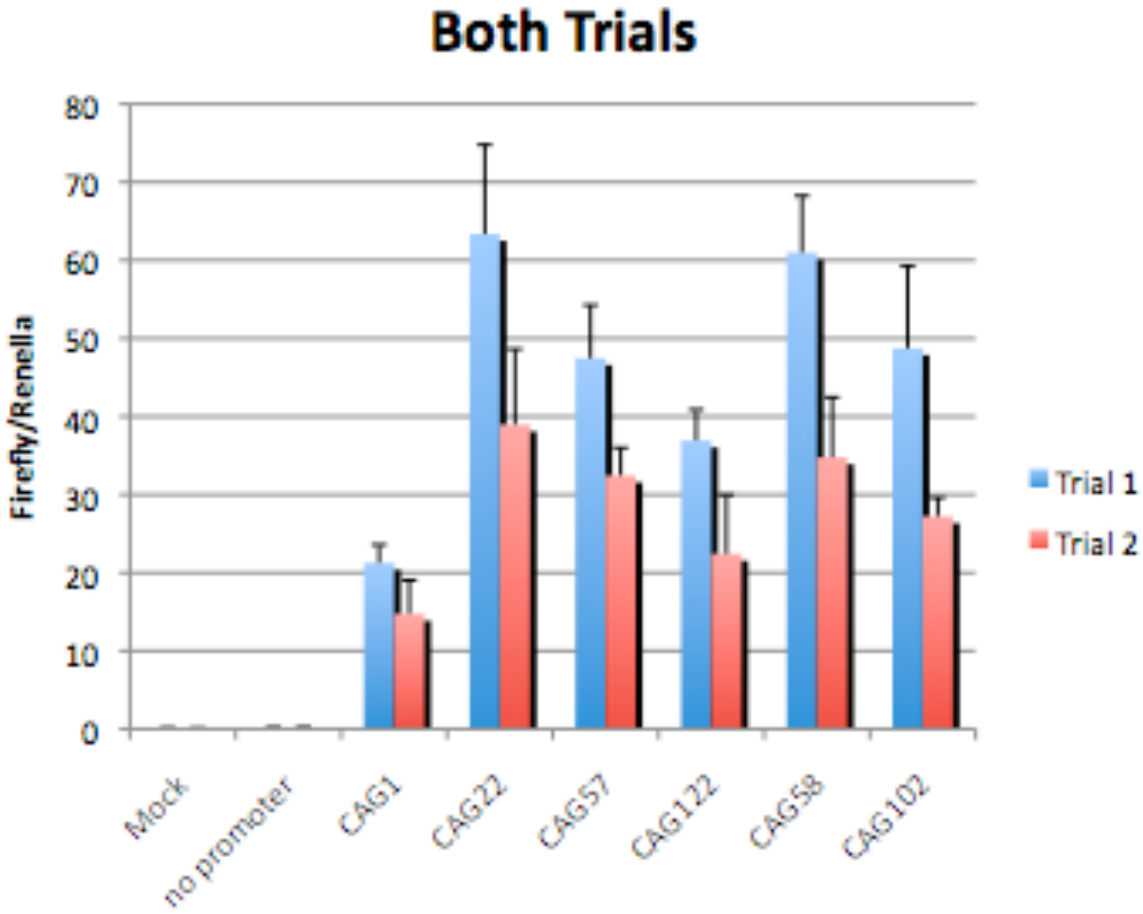
- Lengthing the CAG further to 57 CAGs resulted in a slightly higher expression that was not significantly different from CAG22.

- Further lengthening the CAG to >100 CAGs significantly reduced the expression of the reporter gene.

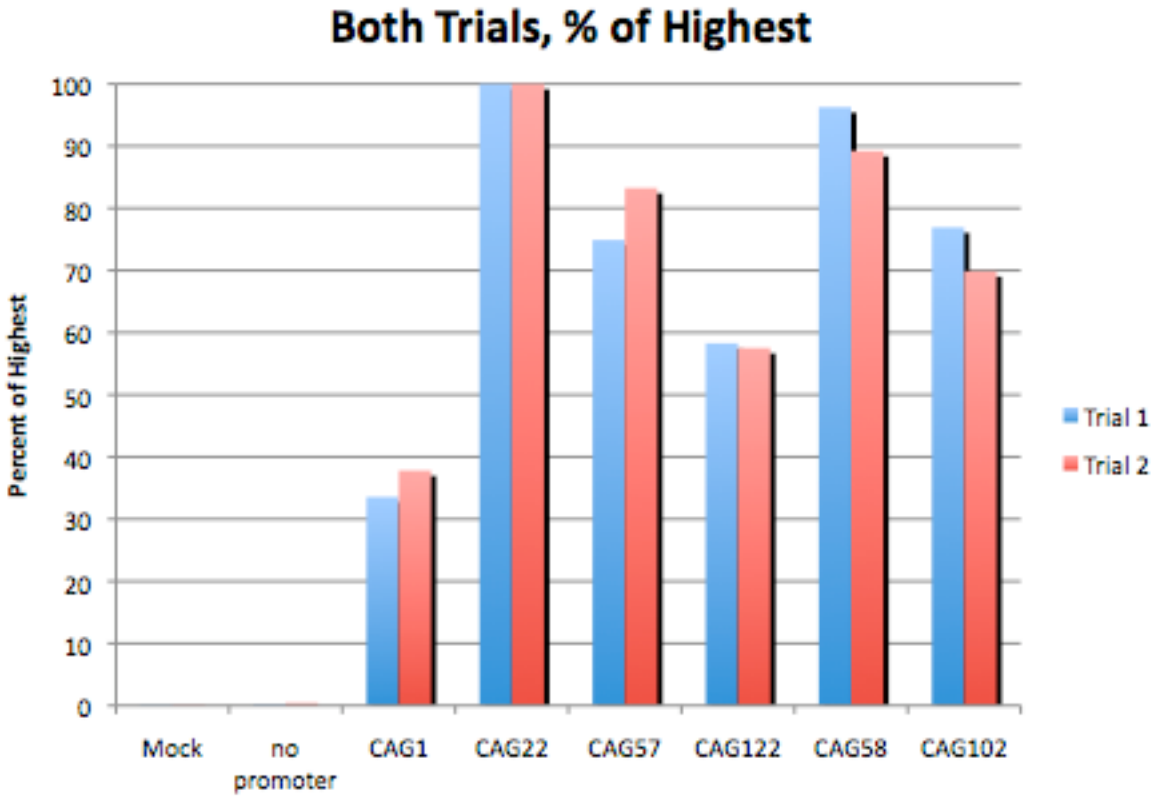
Because as I said earlier the longest CAGs were interrupted with one arginine we recloned and retested in luciferase assays to validate the effect of the CAG length on the reporter gene expression.


Trial 1
Firefly/Renella
80
70
60
50
40
30
20
10
0
Mock
no promoter
CAG1
CAG22
CAG57
CAG122
CAG58
CAG102

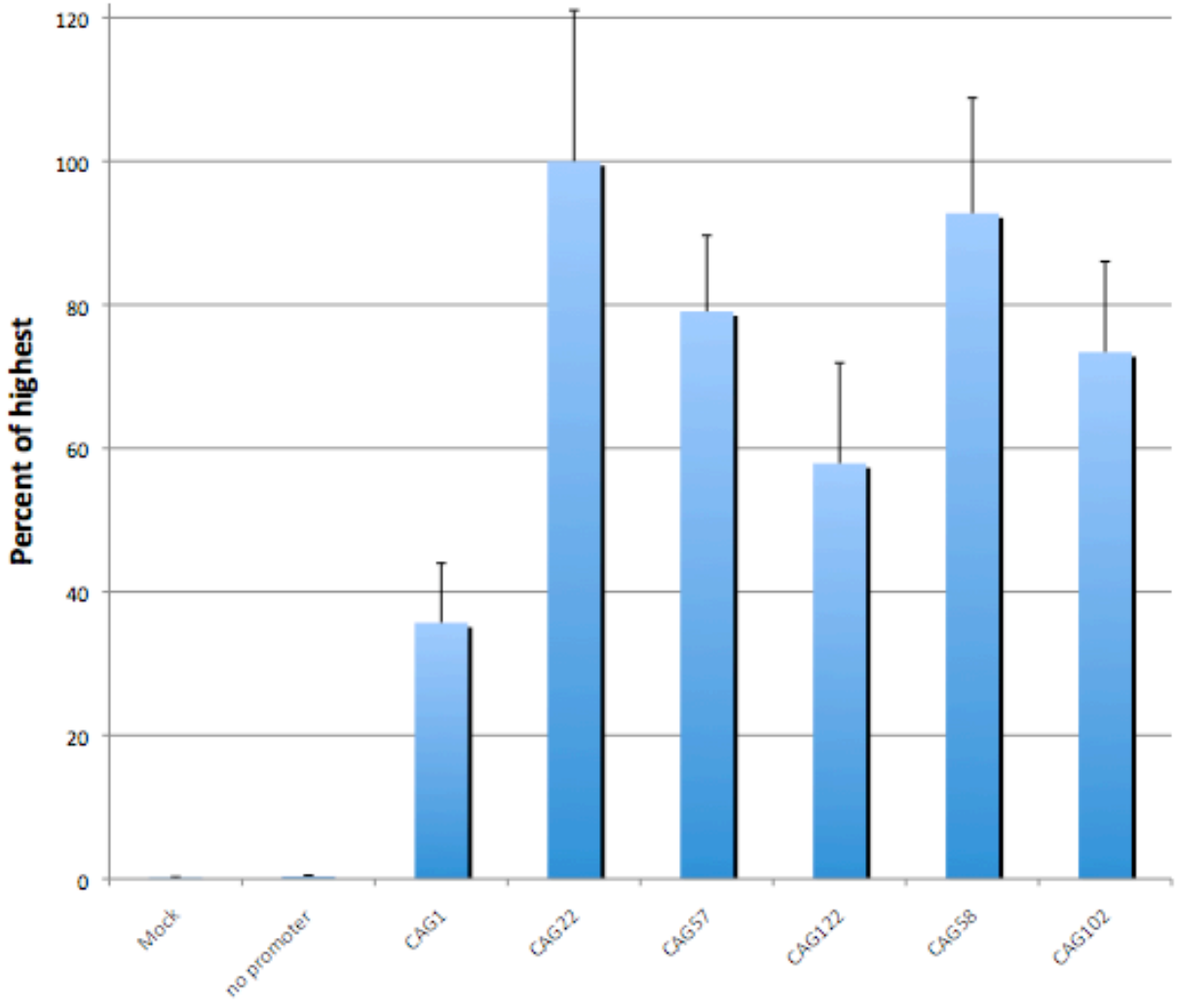

Trial 2
Firefly/Renella
60
50
40
30
20
10
0
Mock
no promoter
CAG1
CAG22
CAG57
CAG122
CAG58
CAG102

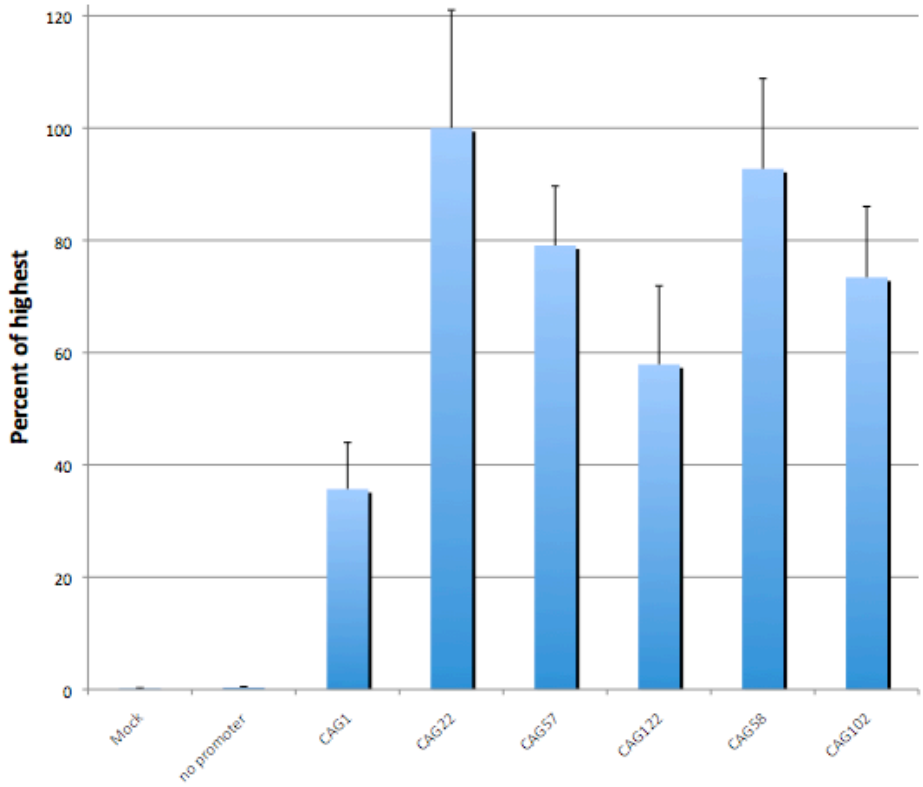

Both Trials
Firefly/Renella
80
70
60
50
40
30
20
10
0
Mock
no promoter
CAG1
CAG22
CAG57
CAG122
CAG58
CAG102
Trial 1
Trial 2

We calculated everything as a percent of the highest value as a means to compare the two trials and found that they were nearly identical…



…so we pooled the data. To do this we went back to the raw data. We set the average value of the highest transfection set to 100 % and coded all the data to that resulting in this chart…





One-way ANOVA
P<0.0001

Bonferroni post-tests

	CAG1	CAG22	CAG57	CAG122	CAG58	CAG102
CAG1	-----					
CAG22	<0.001	-----				
CAG57	<0.001	>0.05	-----			
CAG122	>0.05	<0.001	>0.05	-----		
CAG58	<0.001	>0.05	>0.05	<0.01	-----	
CAG102	<0.001	<0.05	>0.05	>0.05	>0.05	-----

Conclusions

Some conclusions are unchanged compared to Dan's initial study, but some are revised as well…

- The addition of 22 CAGs resulted in a more than 3-fold increase in expression.

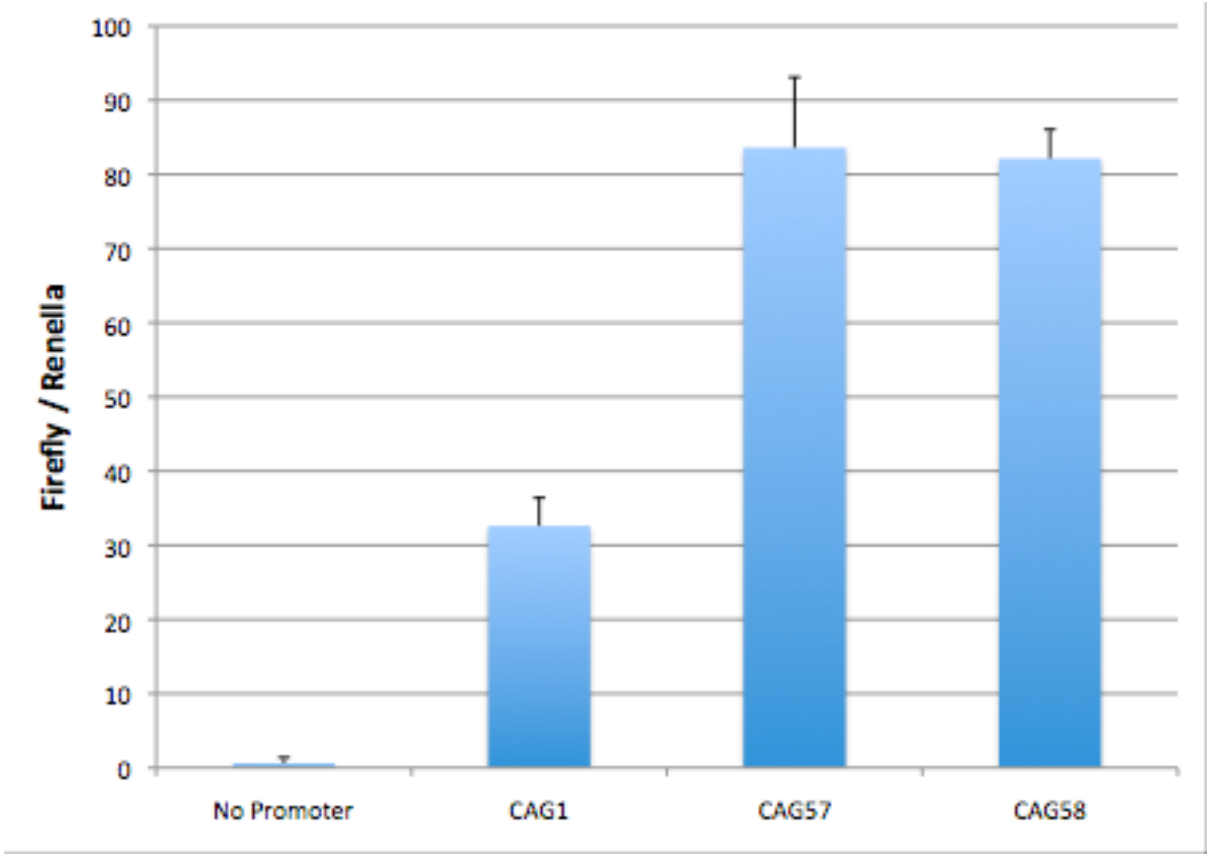
- Lengthing the CAG further to 57 CAGs resulted in a slightly *lower* expression that was not significantly different from CAG22 but showed a trend towards significance.

- Further lengthening the CAG to >100 CAGs significantly reduced the expression of the reporter gene.

- We have not yet found a significant difference for having an arginine in the CAG tract

Dan has begun a series of additional tests to even further validate the relative magnitude of expression among the plasmids, conducting smaller more easily handled experiments.

His first one conducted last week shows no significant difference between the old CAG57 clone and the new CAG58 clone.



Note that Dan still wants to revalidate by comparing the 50s with CAG22, but for now the % magnitude difference is consistent with my experiments:

CAG1 as % of CAG57	39.00907
CAG1 as % of CAG58	39.69948
average % difference	39.35428
Same data from Patrick's last experiment set:	
CAG1 as % of CAG57	45.13291
CAG1 as % of CAG58	38.48194
average % difference	41.80742

This experiment is also important regarding replication and reproducibility, in that it shows that different clones of nearly the same thing prepared by different technicians and maxippreped at different times by different technicians yield the same value.

5/1/09

Overall we conclude that the CAG tract has an effect on the expression of the reporter gene.