AROSA: an *ATXN2*-promoter associated antisense long non-coding RNA (lncRNA) that regulates *ATXN2* expression.

AROSA = antisense regulator of spinocerebellar ataxia

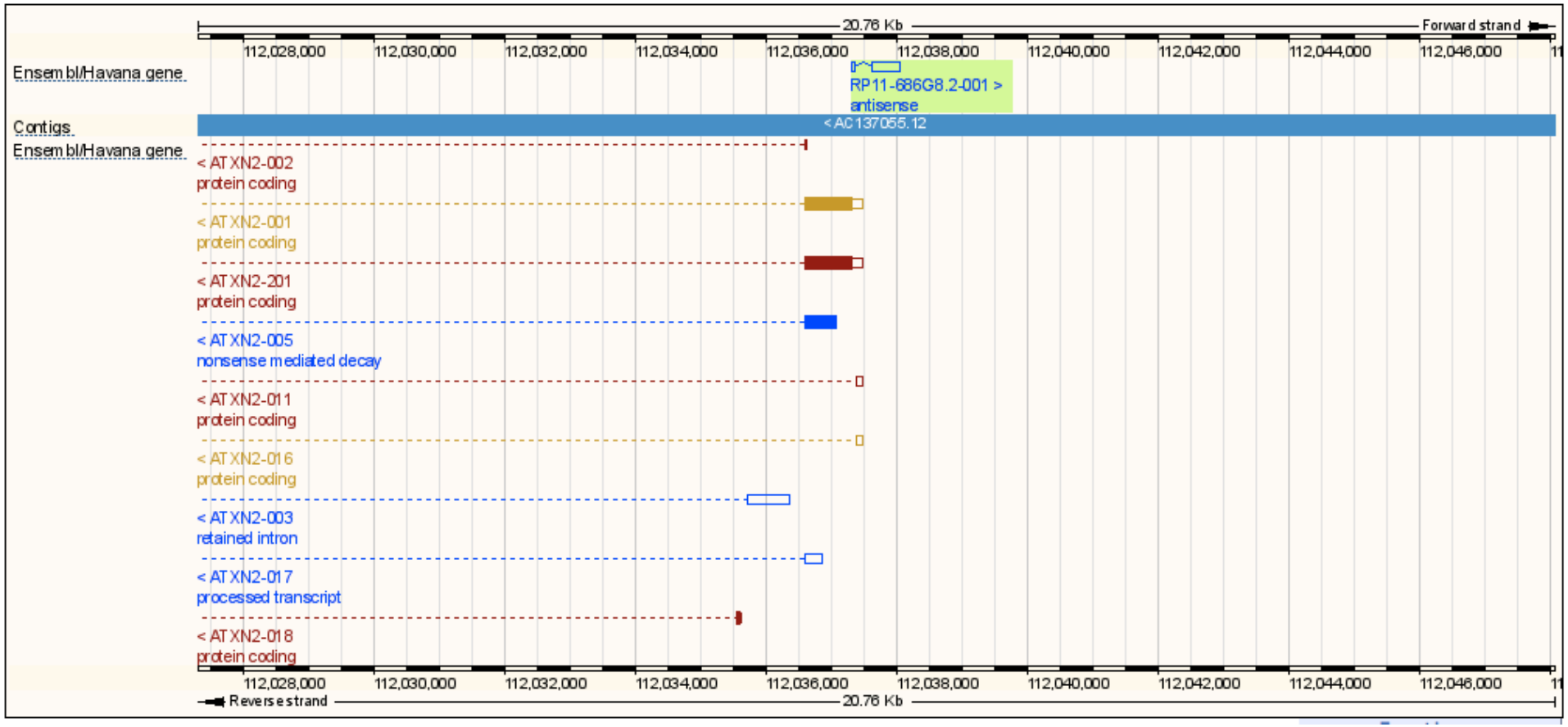
Abstract

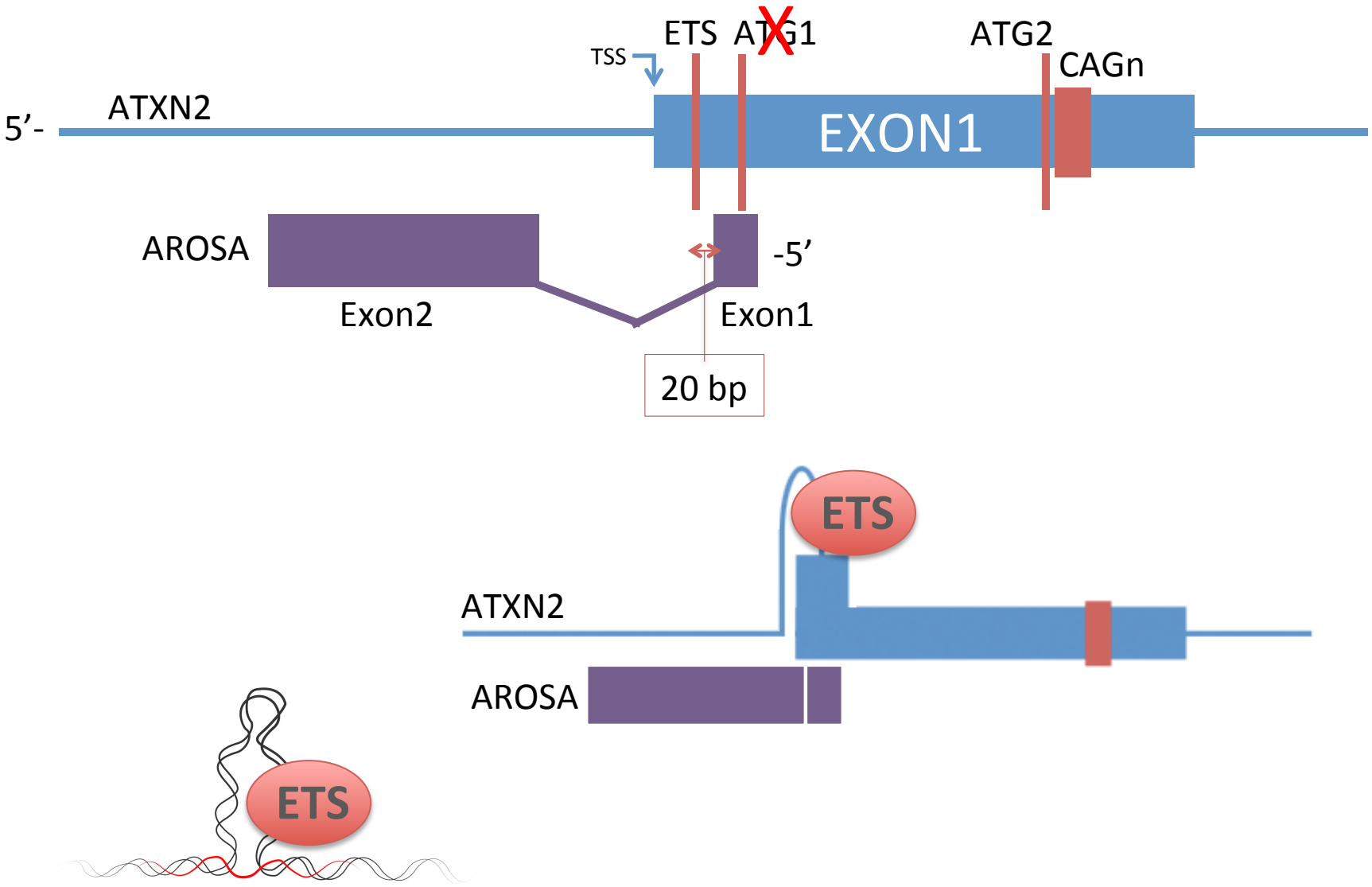
Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant inherited disorder caused by CAG repeat expansion in the *ATXN2* gene. The *ATXN2* gene protein product ataxin-2 is characterized by gains of function upon *ATXN2* mutation, caused by expansion of the CAG-encoded polyglutamine tract in ataxin-2. Based on evidence from studies on the polyglutamine diseases SCA1, SCA3, and Huntington's disease where disease phenotypes were resolved by lowering the disease gene expression in reversible mouse models, we hypothesize that reduction of *ATXN2* gene expression may be therapeutic for SCA2. In seeking ways to reduce *ATXN2* expression we discovered a long non-coding RNA (lncRNA) encoded by a portion of the *ATXN2* antisense strand corresponding to the *ATXN2* upstream sequence and 5'-UTR. We designated this lncRNA AROSA for antisense regulator of spinocerebellar ataxia. AROSA is a spliced transcript with two exons of 56 bp and 429 bp separated by a 274 bp intron. Over-expression of AROSA in HEK293 cells resulted in 60% average reduction of endogenous *ATXN2* expression. The intronic region of AROSA includes the *ATXN2* transcription start site (TSS) as well as a binding site for ETS transcription factors that is required for *ATXN2* expression. We present a hypothesis whereby the annealing of AROSA with its complementary component of the *ATXN2* promoter is inhibitory of *ATXN2* expression due to induction of a promoter conformation that prevents *ATXN2* transcription. This study demonstrated AROSA as a potential therapeutic target for SCA2 whereby its increased expression will inhibit *ATXN2* expression.

Antisense transcript at *ATXN2* exon 1

Show/hide columns | Filter

Name	Transcript ID	Length (bp)	Protein ID	Length (aa)	Biotype	CCDS
RP11-686G8.2-001	ENST00000547021	485	No protein product	-	Antisense	-




ETS
ATG1
ATG2
TSS
CAGn
5'-
ATXN2
EXON1
AROSA
-5'
Exon2
Exon1
20 bp
ETS
ATXN2
AROSA
ETS

Nucleic Acids Research, 1993, Vol. 21, No. 24 5547–5553

Stability of triple helices containing RNA and DNA strands: experimental and molecular modeling studies

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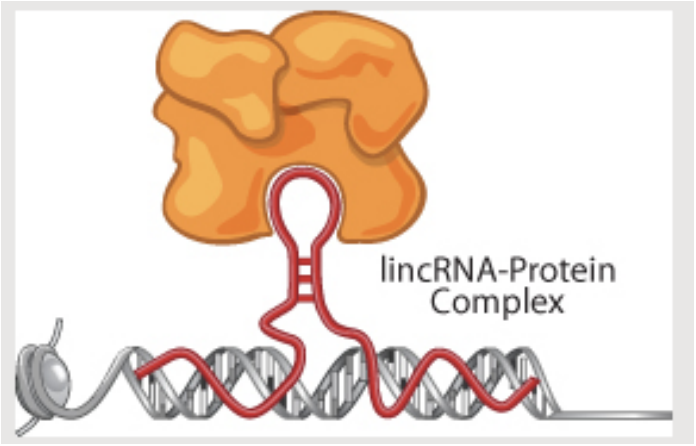
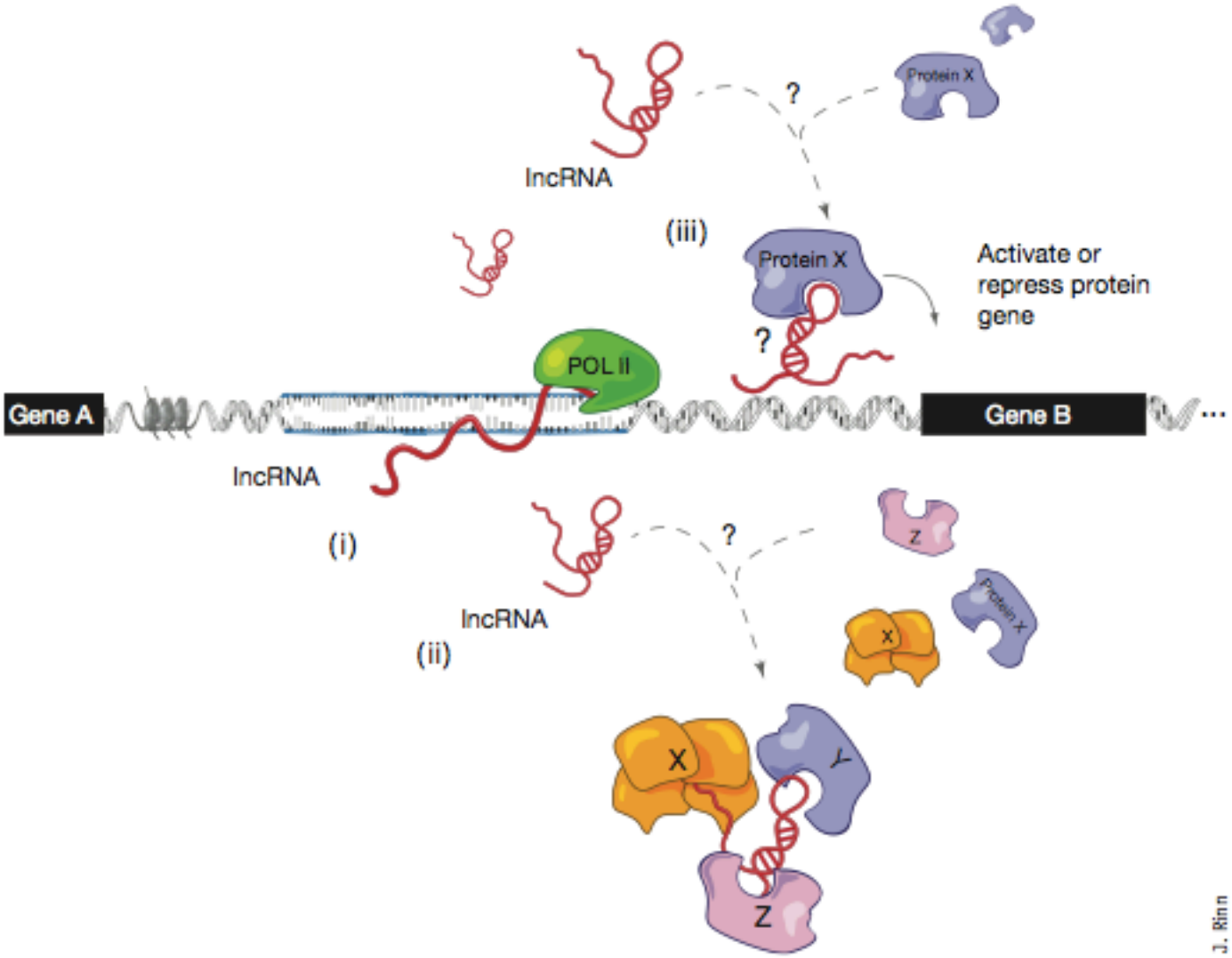
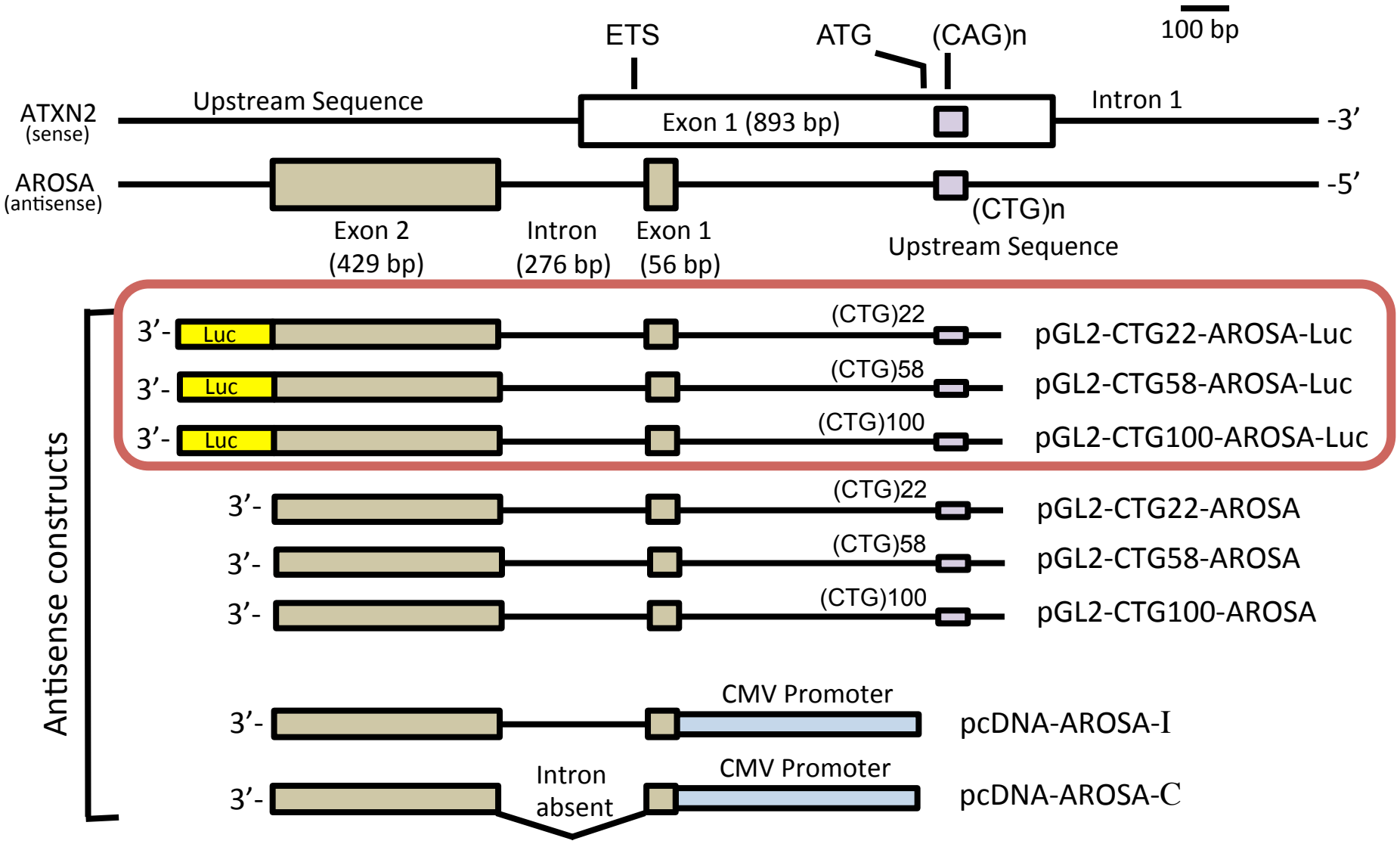




Long noncoding RNAs (lncRNAs) could be a byproduct of transcription (i), a scaffold linking proteins (ii) or a guide bringing proteins to specified parts of the genome (iii). The same lncRNA can function simultaneously as a scaffold and a guide. POL II, RNA polymerase II.

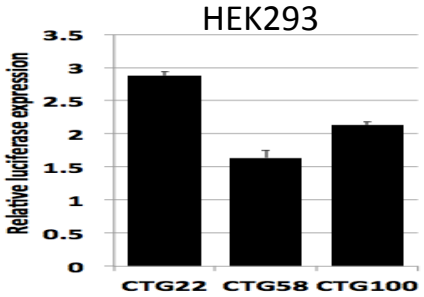
AROSA and the constructs that we have made.



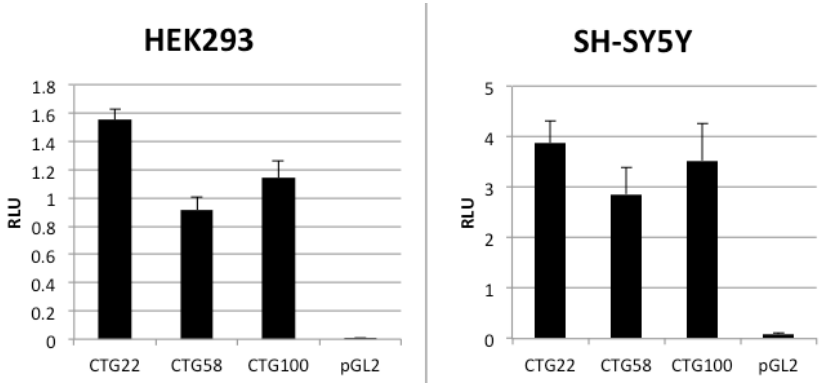
Ability of AROSA with different CTG repeats to drive luciferase expression…

…this provides evidence AROSA is expressed and shows differences among the repeats.

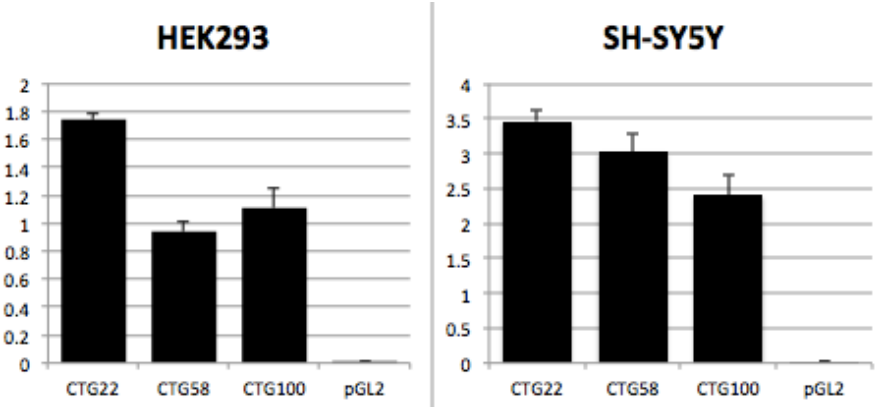
Trial 1



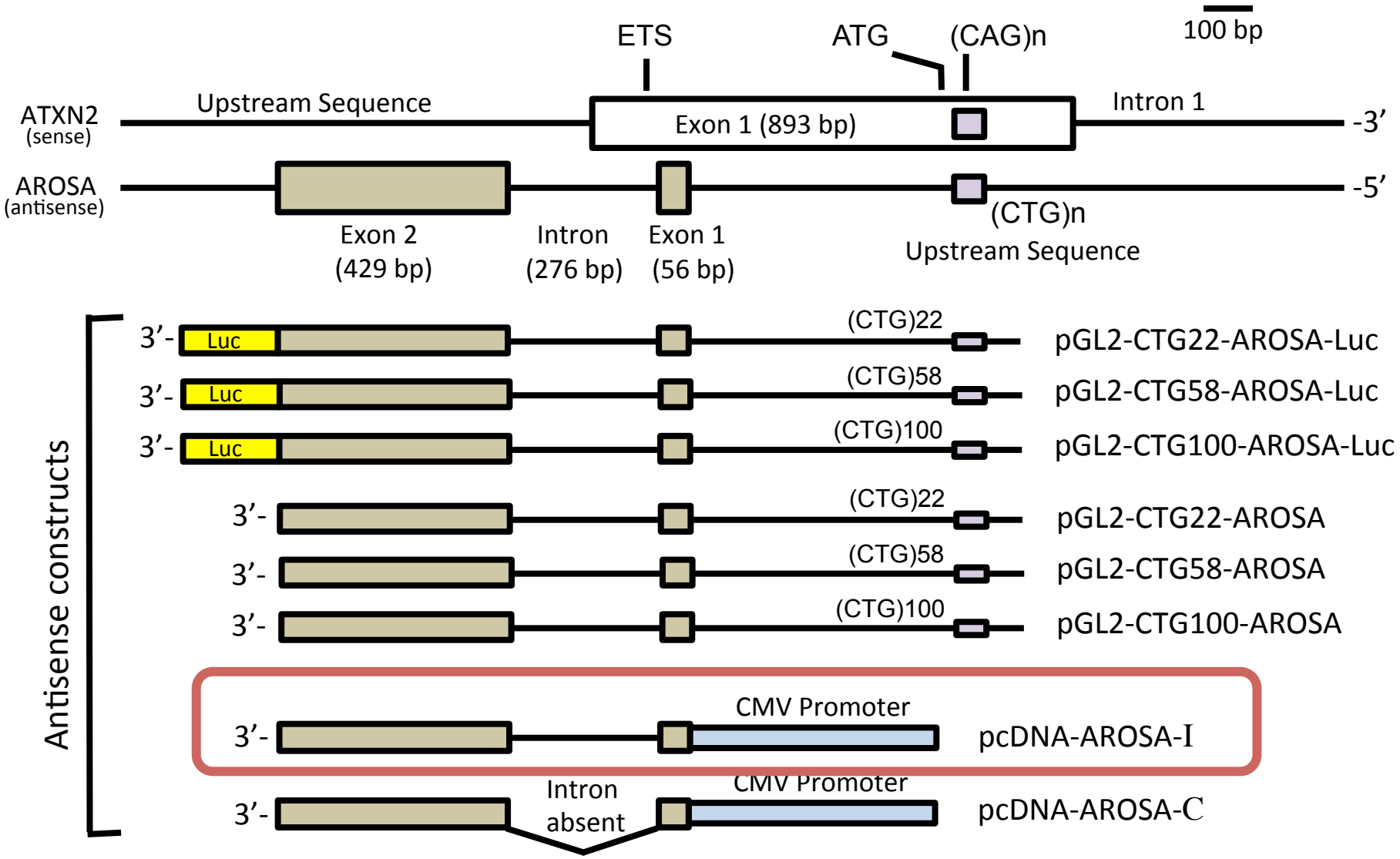
Trial 2



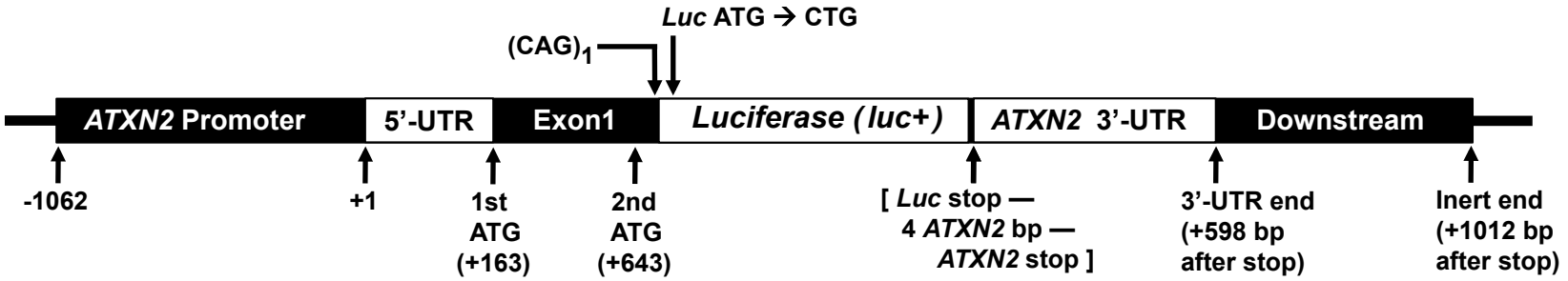
Trial 3



AROSA constructs that we have made.



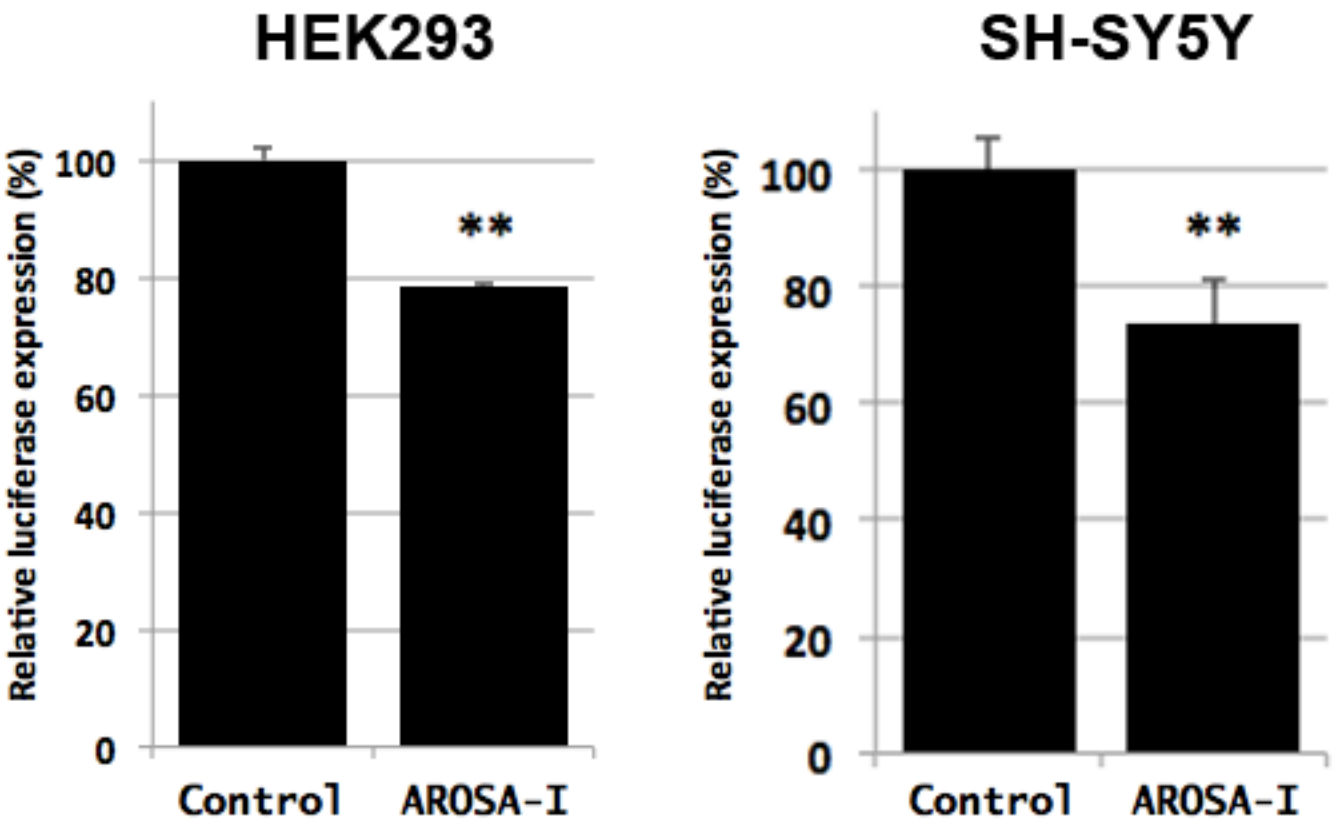
ATXN2-Luciferase construct



From Scoles et al., 2012

We are looking at the effects of AROSA transfection of cotransfected or stably transfected *ATXN2*-luc (in a few slides later), or endogenous ataxin-2.

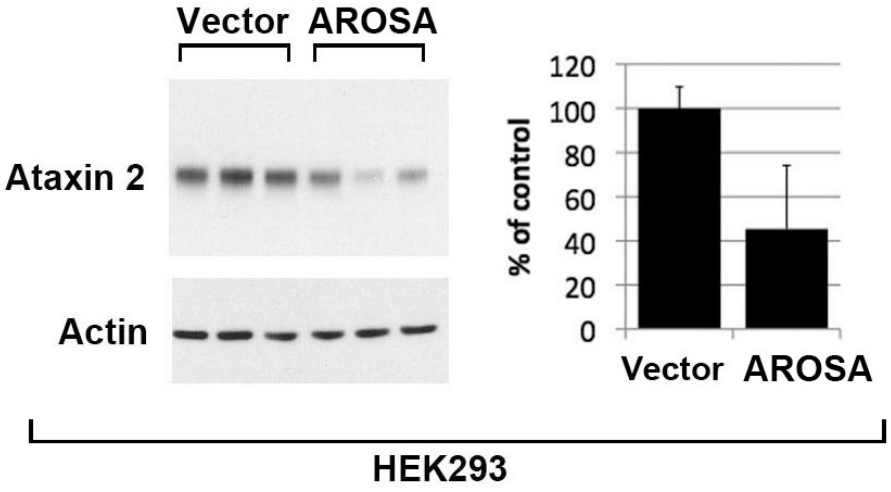
pcDNA-AROSA-I overexpression reduced exogenous *ATXN2*-luciferase*

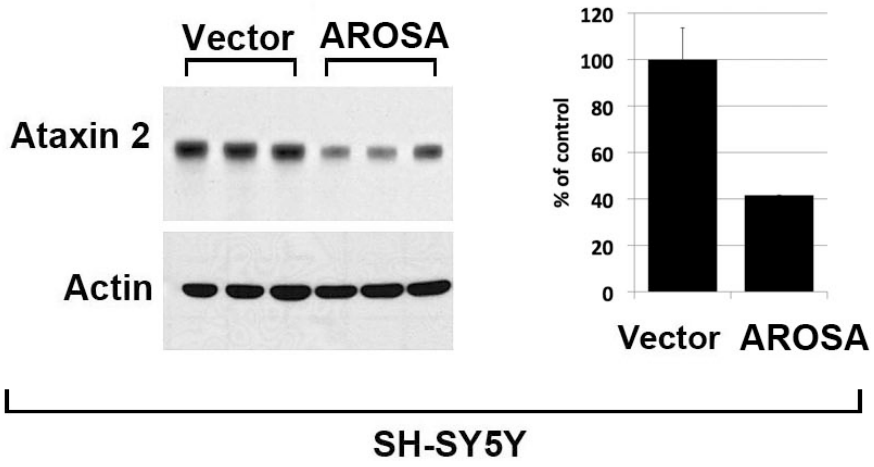


*Cotransfection followed by luc assay 48 hours later, relative to SV40-Renilla

Lance P

pcDNA-AROSA-I overexpression reduced endogenous *ATXN2* expression



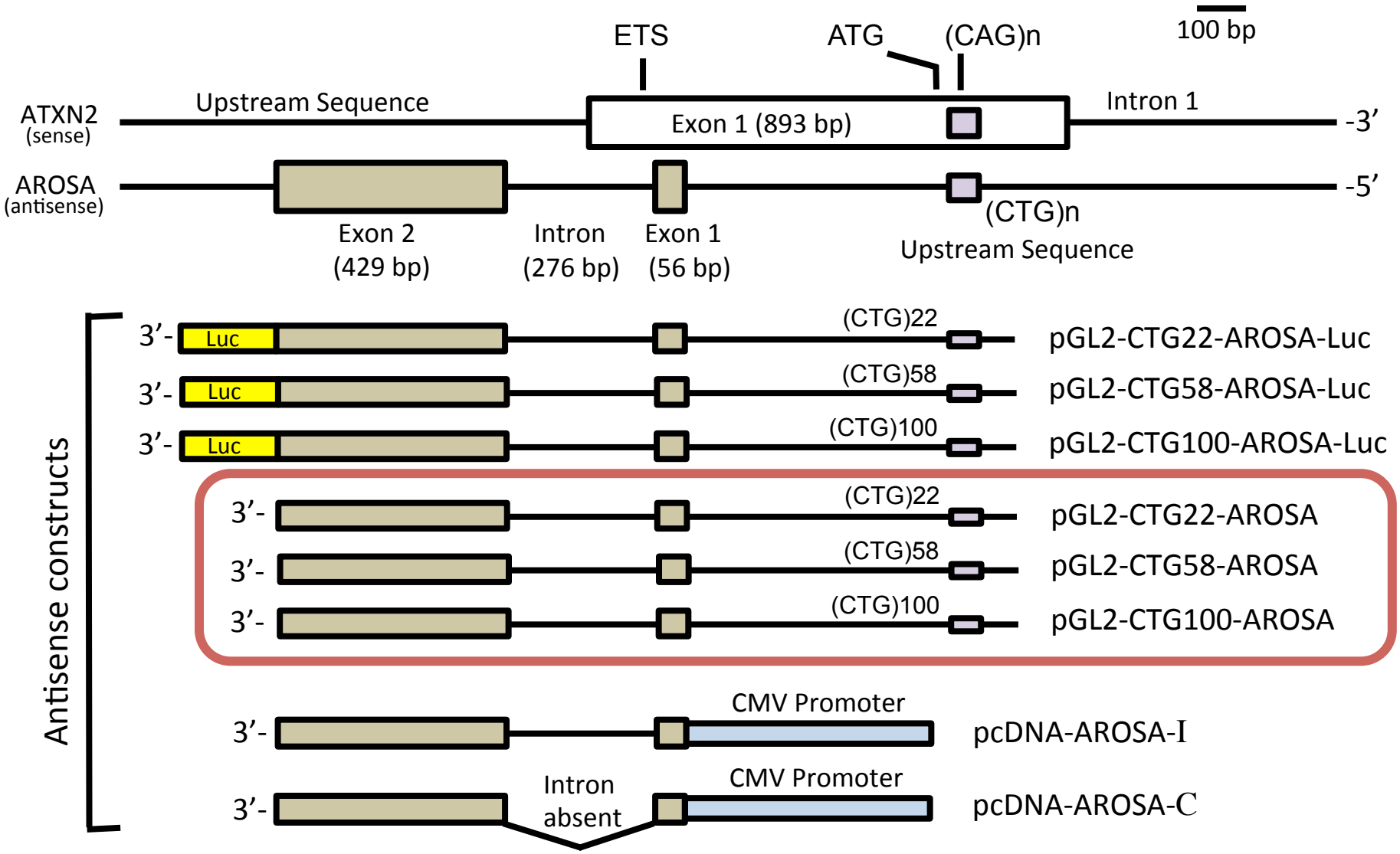


Each lane represents an independent transfection

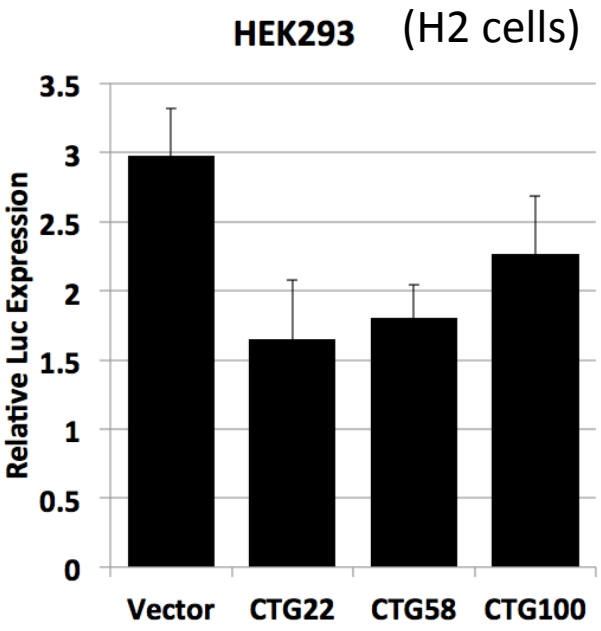
Note KK is also trying this with the pGL2-AROSA-CTGn plasmids

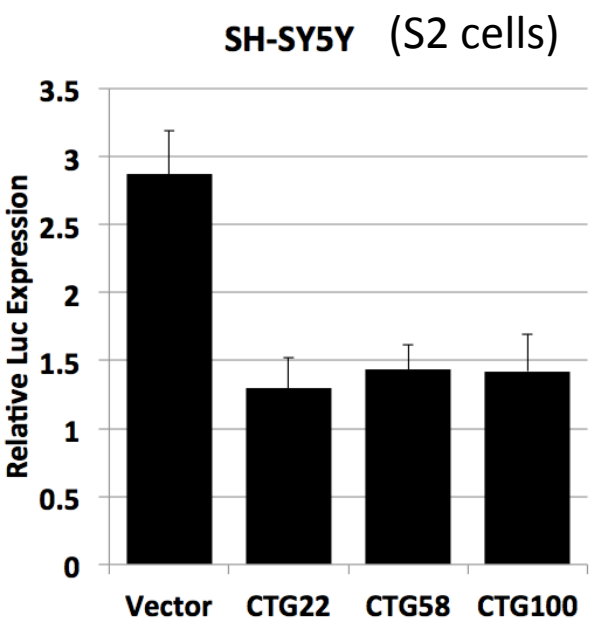
Western blots KK's Data

AROSA constructs that we have made.

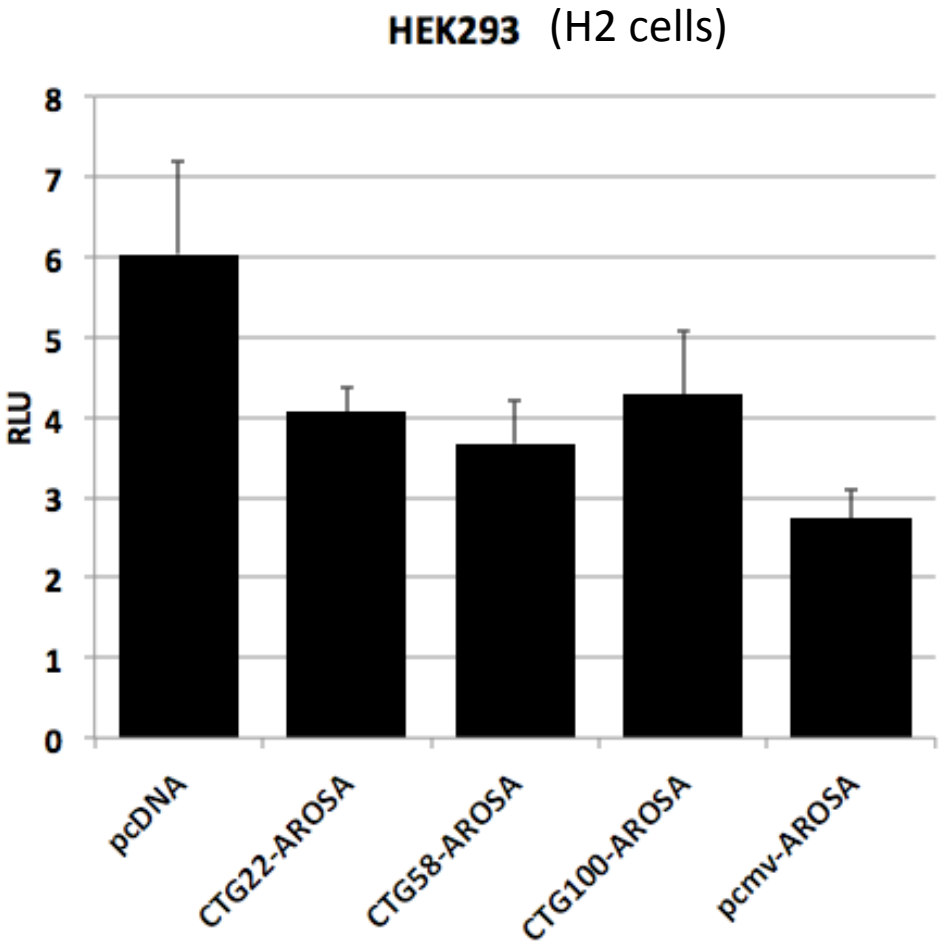


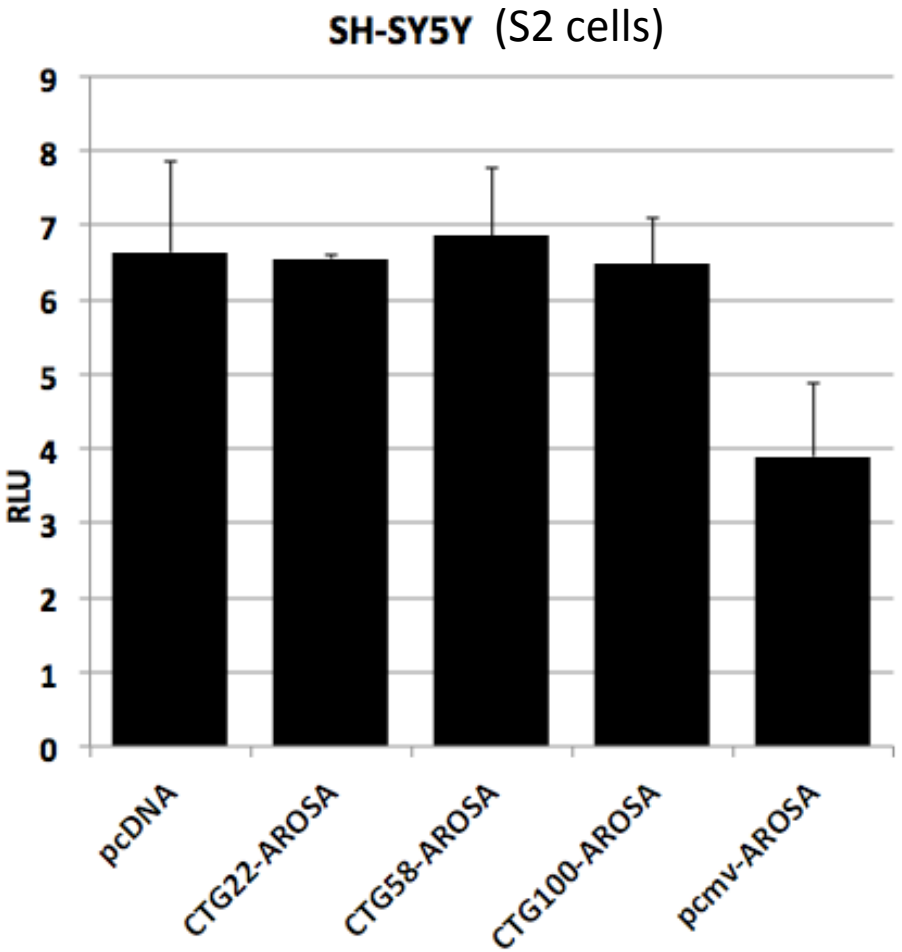
pGL2-AROSA-CAGn effect on stably transfected ATXN2-luc expression





Lance P 8/28/2012





* For S2 cells the transfection was as much as 16 times lower than for H2 so we think this was just the result of poor transfection efficiency.

In Progress:

- Effect of pGL2-*AROSA*-CTGn on endogenous *ATXN2* by
  - Western blotting
  - qPCR

- Effect of the presence of the *AROSA* intron

- LCM

Future Work:

- We made a cell line expressing CTG22-AROSA and will test whether compounds from our qHTS that inhibit *ATXN2-luc* act to elevate *AROSA*.