Generation of BAC-SCA2 Transgenic Mice

Warunee Dansithong

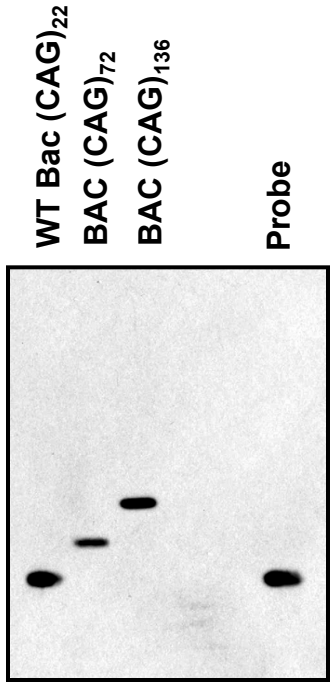
Bacterial Artificial Chromosome (BAC) Transgenic Mice

Traditional transgenic mice:

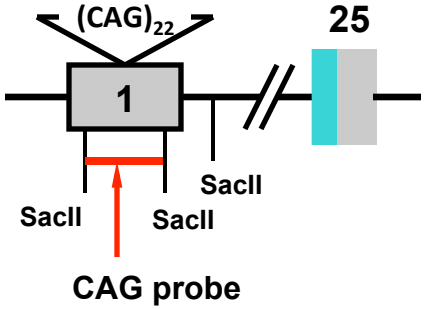
- **Show incorrect temporal and cell specific transgene expression.**
- **Missing regulatory elements of native gene which may be essential for transgene expression.**

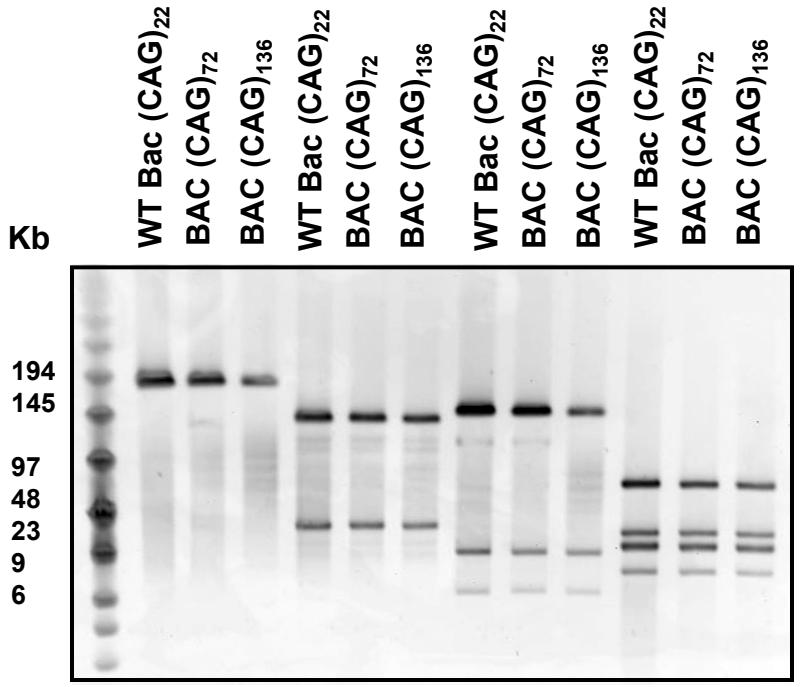
BAC transgenic mice

- **Large DNA (on average~ 200 kb) with high stability.**
- **Contain all the regulatory elements necessary for transgene expression *in vivo*. Therefore, BAC transgenic mice accurately reflect endogenouse gene expression.**
- **Now widely used to study as disease models [BAC-HD (Gray et al., 2008), BAC-SCA8 (Moseley et.al., 2008) and BAC-LRRK2^{R1441G} (Li et al.,2009)].**



Southern Blot





Restriction enzyme digestion mapping

BAC transgenic mice (FVB strain)

BAC-ATXN2-Q22: 7 founders

- They look fine and healthy. 5 founders had offspring.
- Expression of human ATXN2 was verified at RNA and protein levels.

BAC-ATXN2-Q136: 10 founders

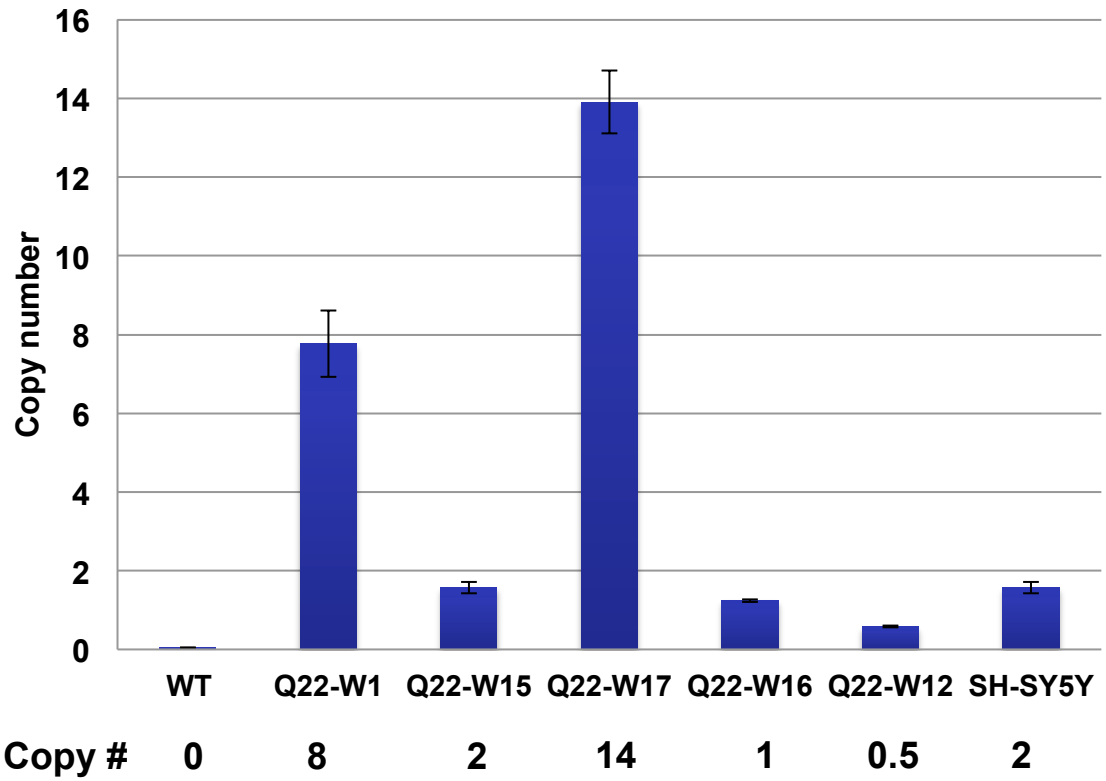
- One founder shows the CAG repeat tract deletion to be **CAG 37 repeats**. This animal had 21 offspring. Of these, 2 are transgenes (2/21=10%).

- 9 founders containing CAG 136 repeats are sterile.
  There were 5 animals looked sick and started to die at 3 months old.
  All of Q136 founders were euthanized since they couldn't breed.

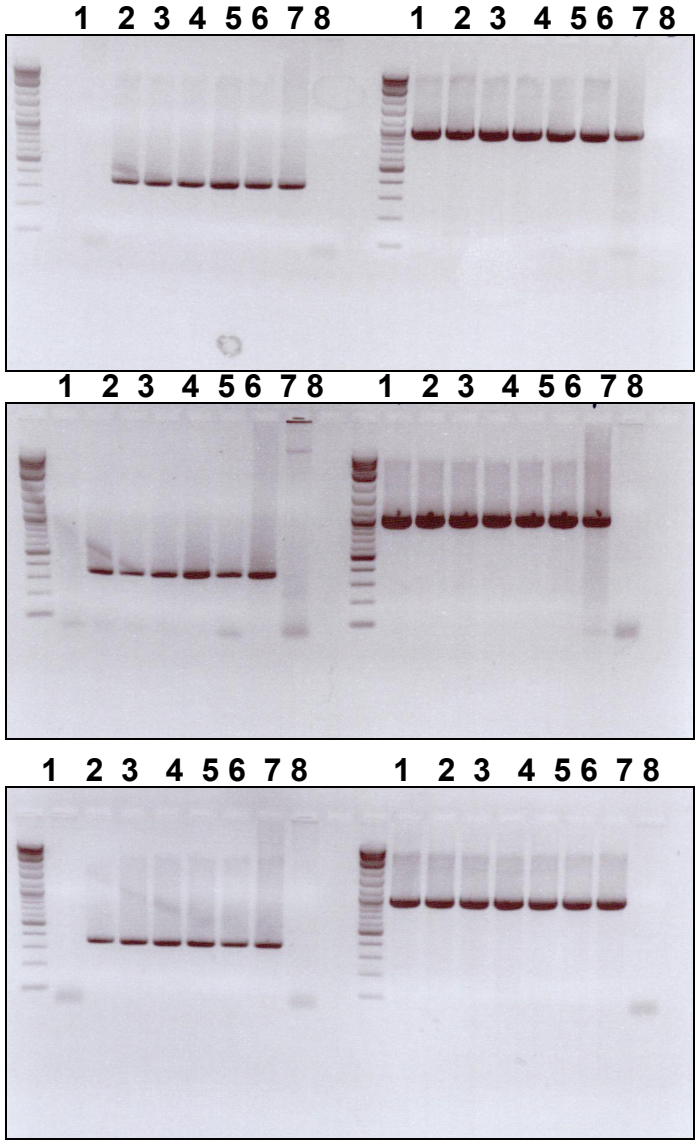
BAC-ATXN2-Q72 :

- DNA was injected at UCI on Jan 3, 2011.
- There are 49 pups from this injection. Of these, **there are 10 transgenes**.

Determine the genomic DNA copy number of BAC Q22 by qPCR



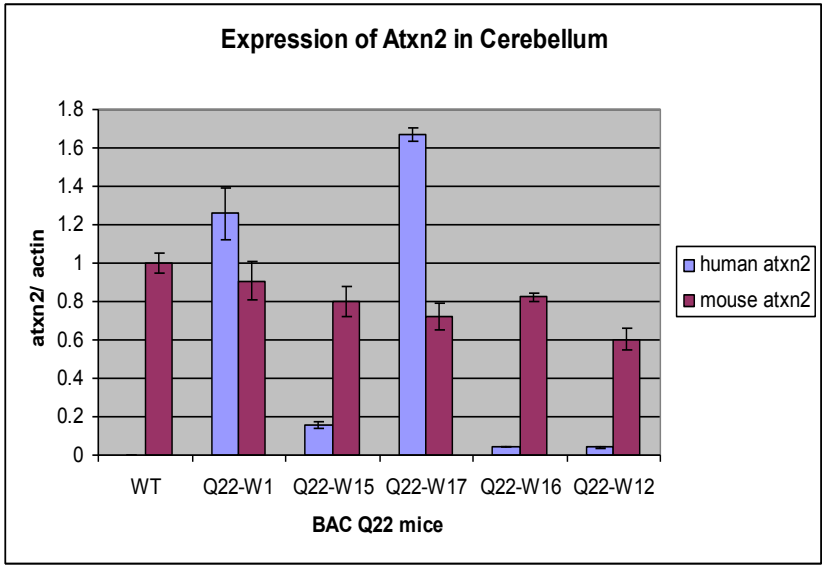
All BAC Q22 lines express human ataxin 2 RNA by RT-PCR

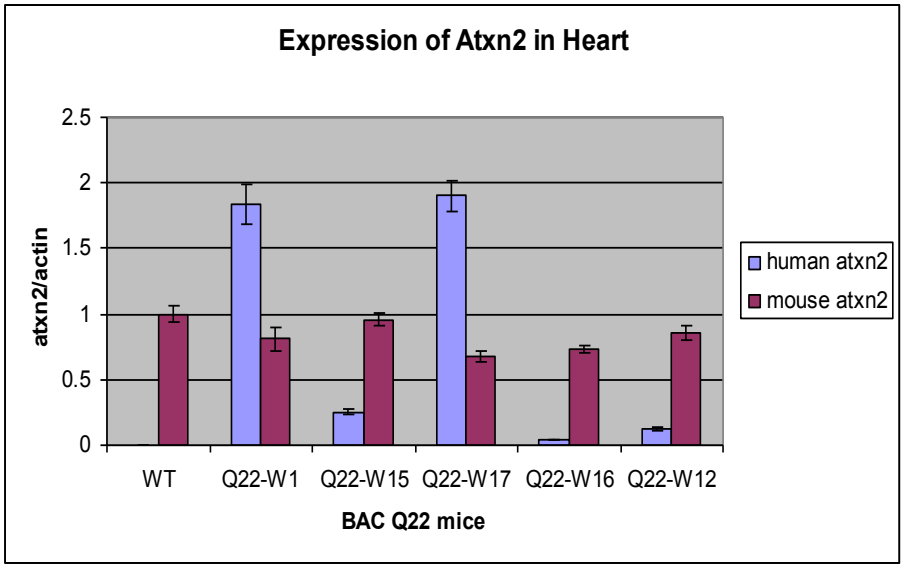


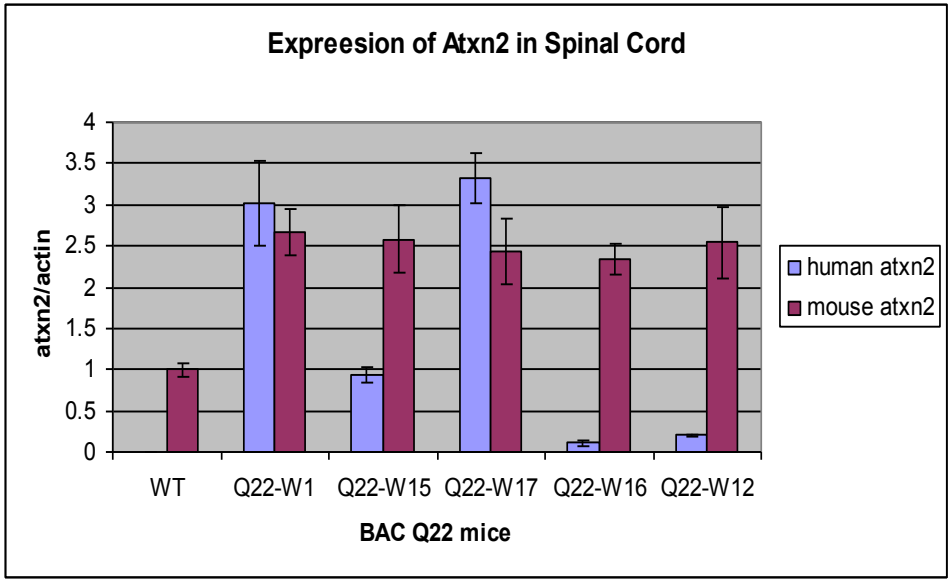
cDNA from

1. Non-TG (WT)
2. Line W1
3. Line W15
4. Line W16
5. Line W17
6. Line W12
7. Lymphoblast-control
8. Water (Neg Control)

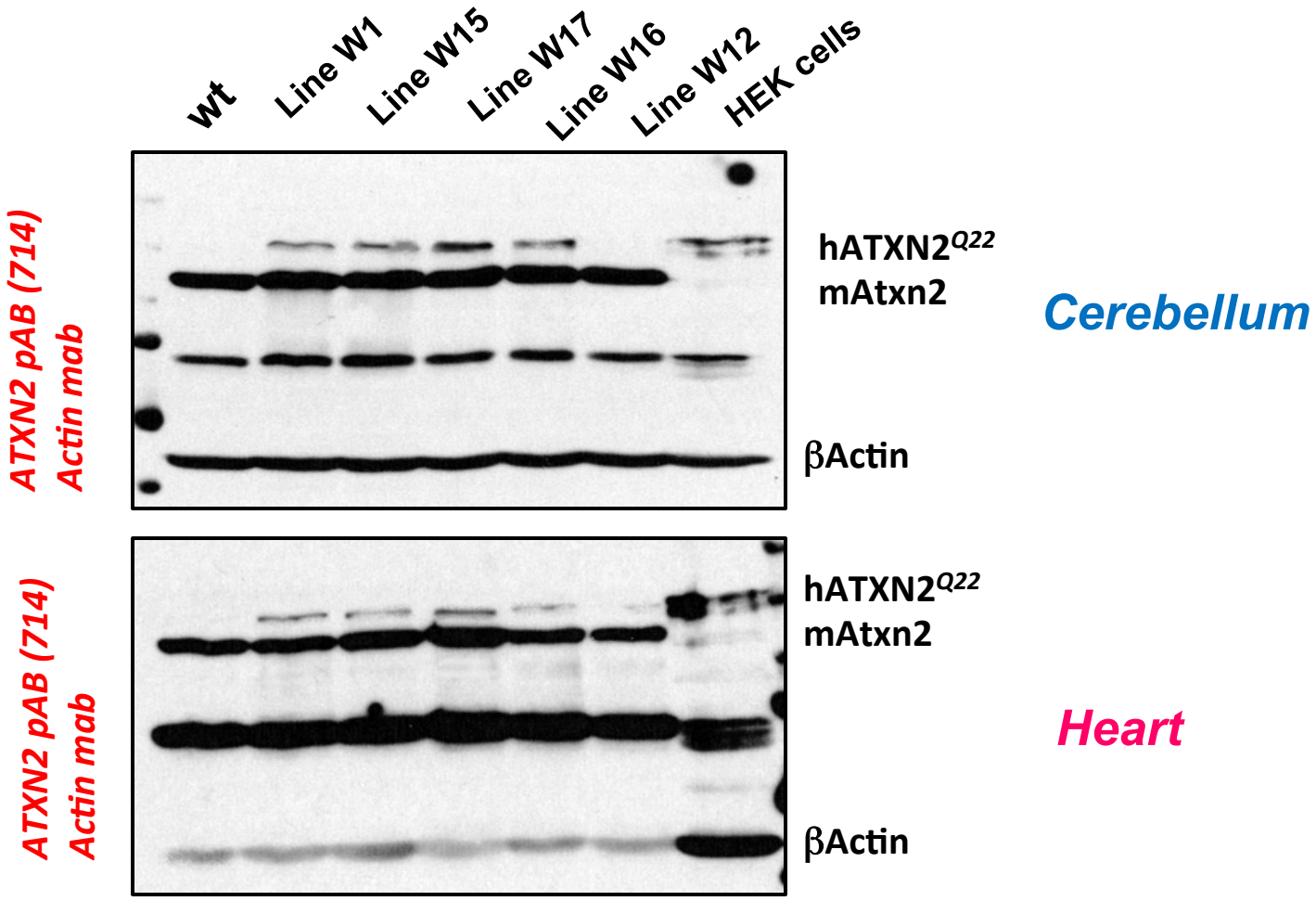
Expression of human ataxin 2 RNA is high in BAC Q22 line W17 and W1 by qPCR







Human ATXN2 is expressed in BAC Q22 mice by Western Blot



Conclusion:

1. **BAC-Q22 line W17 shows the highest genomic DNA copy number and expression of RNA and protein of human ataxin 2.**

2. **There is correlation between genomic DNA copy number and expression of human ataxin 2 RNA and protein in BAC Q22 mice.**

3. **BAC-Q136 founders show severe neurological phenotype which is different from tissue specific transgenic mice (PCP2-SCA2-Q127).**