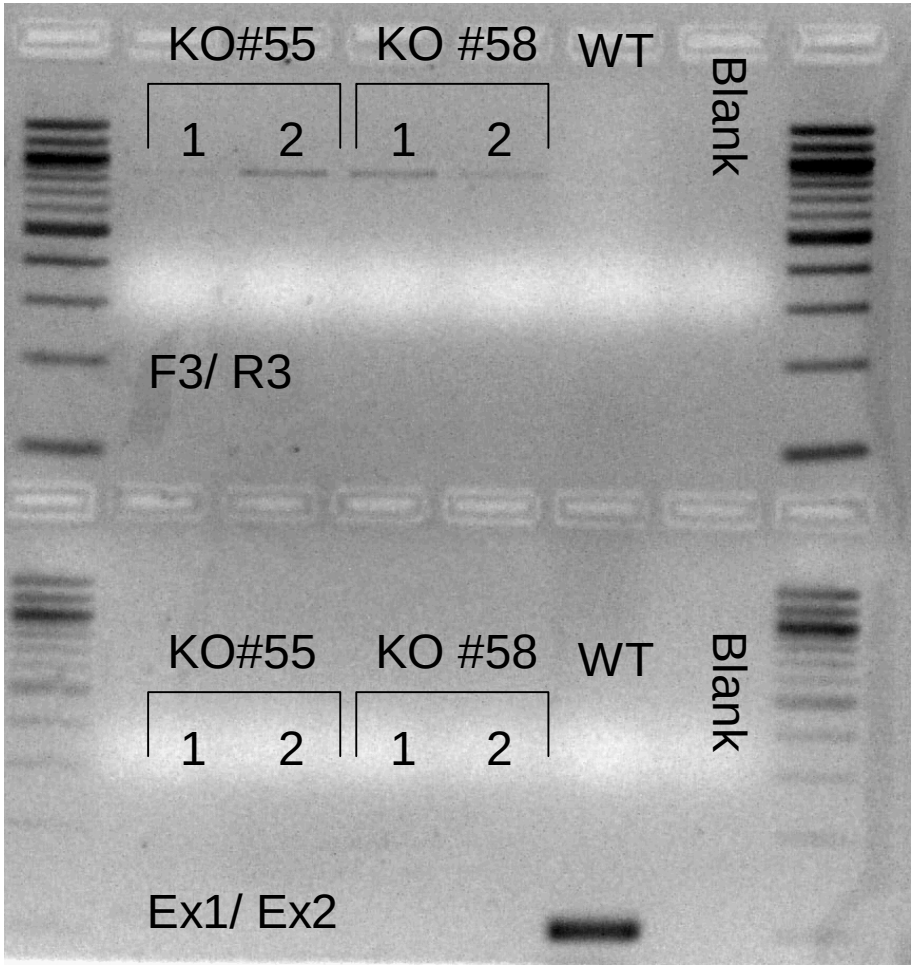
KO PCR

- KO animals #55 and #58 were euthanized on different days
- five tissue types were harvested from each animal for WB
- Tail clips were taken for genotype confirmation
  - Two pieces of tail from each animal were harvested in order to duplicate in parallel genotyping results
- WT sample is DNA extracted from B6 mouse in August of ’09. I keep it as a positive control sample to run on special occasions like this.

Original KO PCR Rxn



- PCR rxn mix
  - 5 ul dilute DNA (30-50ng total)
  - 2 ul 10x buffer
  - 4 ul Q-solution
  - 2 ul dNTPs
  - 4 ul primer pair (2ul each): F3/R3 or EX1/Ex2
  - 0.2ul Hot Taq
  - 2.8 ul H2O

- Thermocycler setup

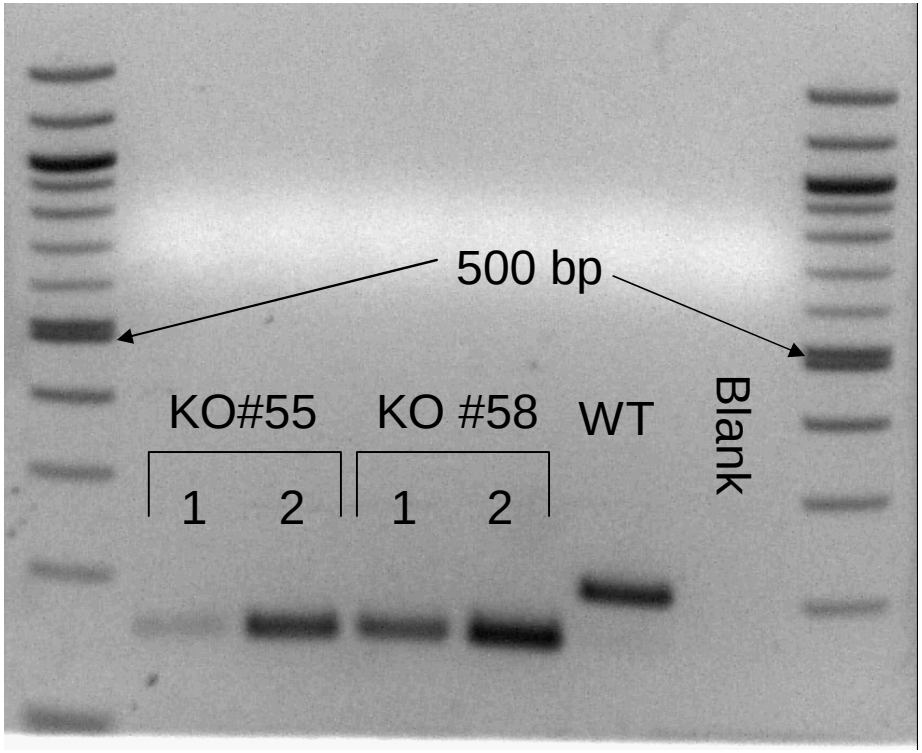
95c	5 min	
95c	60 sec	X 5
65c	30 sec	
72c	2 min	
95c	60 sec	X 40
60c	30 sec	
72c	2 min	
72c	5min	
4c	hold	

- **Run on 1.5% gel**

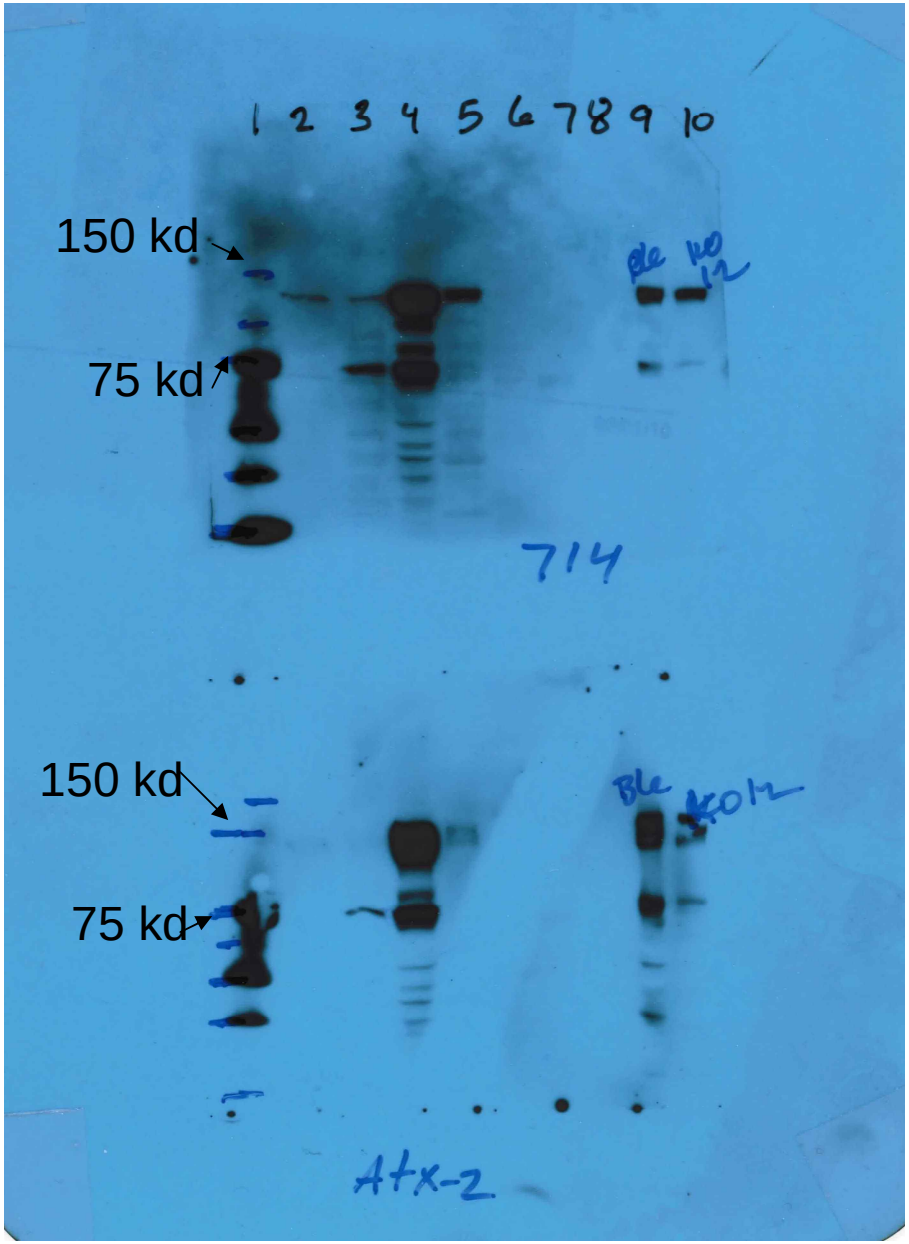
- KO = 830 bp; Het = 830 & 106 bp; WT = 106 bp

Don’s Redesigned KO PCR Rxn

- PCR rxn mix
  - 4 ul 5x multiplex master mix
  - 5 ul 0.6 uM primer mix
  - 5 ul dilute DNA (30-50ng total)
  - 6 ul Water
- Thermocycler setup
  - 95c 60 sec
  - 95c 20 sec } X 30
  - 68c 60 sec }
  - 68c 5 min
  - 10c hold
- Run on 2%



- KO = 157 bp
- Het = 157 & 197 bp
- WT = 197 bp



- Western blot for KO #55
  - 1: Marker
  - 2: cerebellum
  - 3: Heart
  - 4: Muscle
  - 5: Kidney
  - 6: Spleen
  - 7: N2A cells with q108
    - Forgot to add PI to lysate
  - 8: 1x loading buffer
  - 9: B6 cerebellum
    - (from previous wb's)
  - 10: KO 12 cerebellum
    - (from previous wb's)

Top gel: AB 714; Bottom gel: AB commercial Atxn-2