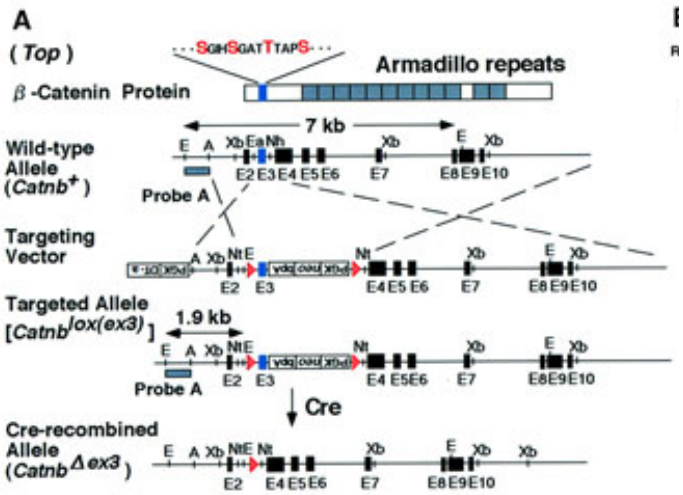
# *Ctnnb1*$^{tm1Mmt}$ (*Ctnnb1*$^{lox(ex3)}$, Beta-Catenin Gain of Function) Genotyping

JDW 3/12

From:
Harada N, Tamai Y, Ishikawa T, Sauer B, Takaku K, Oshima M, Taketo MM. Intestinal polyposis in mice with a dominant stable mutation of the beta-catenin gene. EMBO, 1999.

**MGI#:**1858008

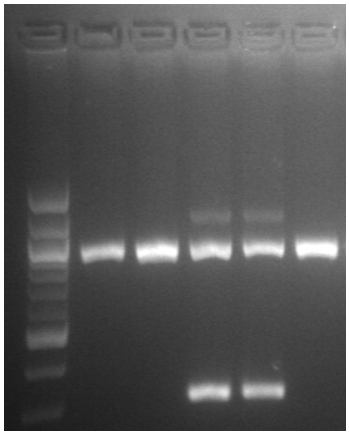



**Primers:**
**JDW 31** (MUT allele / 5'Ex2BCAT):
5'-TGCGTGGACAATGGCTACTCA
**JDW 32** (Common Primer/ p85):
5'-CTAAGCTTGGCTGGACGTAAACTC
**JDW 33** (WT allele/ BCAT-AS5):
5'-ACGTGTGGCAAGTTCCGCGTCATCC

WT allele: 894
MT allele: 300

**Reaction Conditions:**

| | | |
|---|---|---|
| 10x CL buffer (Qiagen) | 2.5µl | |
| Q solution (QIagen) | 2.5µl | |
| dNTPs (10mM each stock) | 0.5µl | |
| Fwd primer-AKA "common" (20mM stock) | 0.5µl | WT-> |
| Rev-Mutant-AKA "5'Ex2Bcat" (20mM stock) | 0.5µl | |
| Rev-WT-AKA "BCAT-AS5" (20mM stock) | 0.5µl | |
| DNA | 1µl | |
| Taq (Qiagen) | 0.25µl | |
| H2O | 16.75µl | GOF-> |



*Note: Original paper recommends two separate reactions for wildtype and GOF allele. We multiplexed this reaction and find that it works great using the Qiagen Taq and homemade Taq / reagents.

**PCR Rxn:**
95˚x3 min
95˚x40 sec
60˚x1 min
72˚x 90 sec
Repeat 34 additional cycles
72˚x 5 min
12˚ for ever

From the paper:
BCAT-F1: 5'-GGTAGTGGTCCCTGCCCTTGACAC-3'
P85: 5'-CTAAGCTTGGCTGGACGTAAACTC-3'

for 35 cycles of 94°C for 30 s, 60°C for 1 min and 72°C for 1 min.