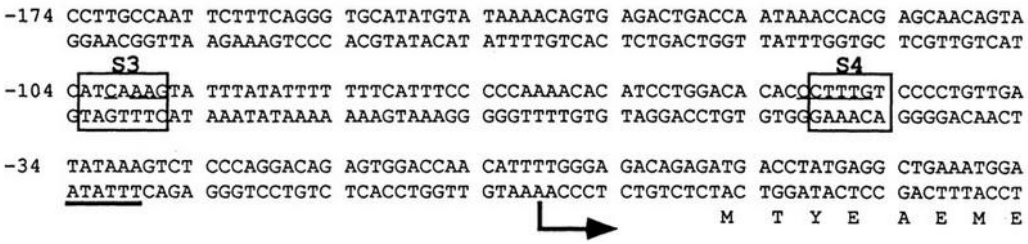
Tg(BAT-nlacZ)3Picc
aka BAT-gal

JDW 7/2012

MGI ID: 3697064

From:

Mapping Wnt/B-catenin signaling during mouse development and in colorectal tumors
Silvia Maretto*, Michelangelo Cordenonsi*, Sirio Dupont*, Paola Braghetta*, Vania Broccoli†, A. Bassim Hassan‡, Dino Volpin*, Giorgio M. Bressan*, and Stefano Piccolo*§. PNAS.

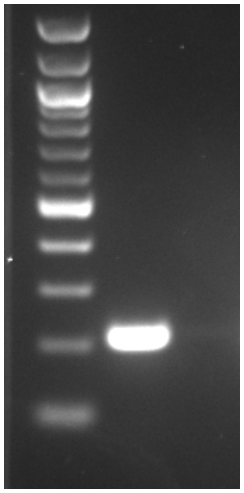


The endogenous siamois promoter. The TATA box is underlined, the transcriptional start is represented by an arrow, and two TCF/LEF sites are boxed (S3, S4).

Primers:

OLIGO	start	len	tm	gc%	any	3'	seq
JDW 50 (Bat-gal FOR)	269	20	60.03	45.00	3.00	0.00	catttccccaaaacacatc
JDW 51 (Bat-gal REV)	486	20	60.01	50.00	5.00	3.00	gttttcccagtcacgacgtt

Transgene: 218 bp



Reaction Conditions:

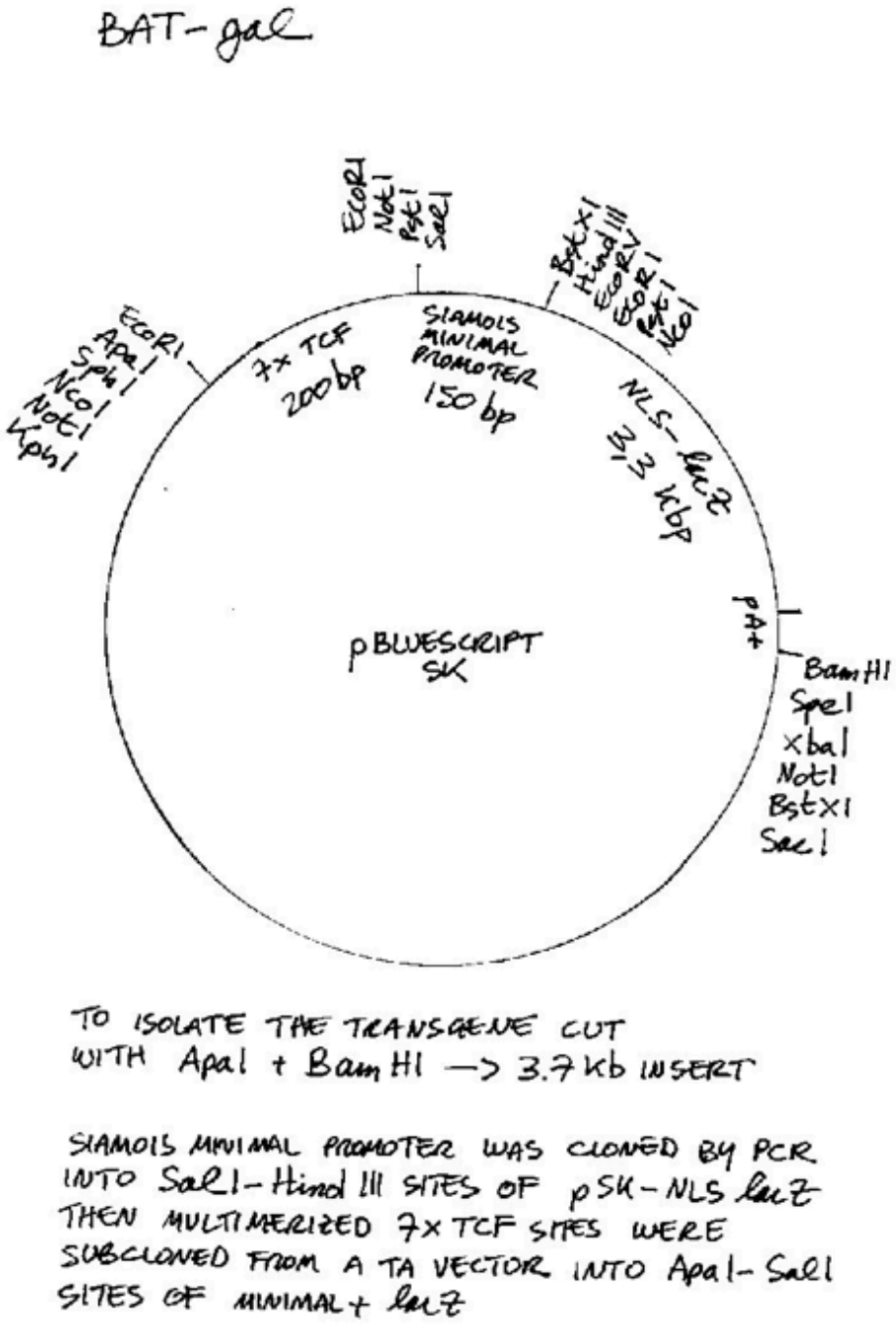
10x CL buffer (Qiagen)	2.5µl
Q solution (QIagen)	2.5µl
dNTPs (10mM each stock)	0.5µl
Fwd primer-AKA “common” (20mM stock)	0.5µl
Rev-Mutant-AKA “5’Ex2Bcat” (20mM stock)	0.5µl
Rev-WT-AKA “BCAT-AS5” (20mM stock)	0.5µl
DNA	1µl
Taq (Qiagen)	0.25µl
H2O	16.75µl

*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 reagents.

PCR Rxn:
95˚xX min
95˚xX sec
60˚xX sec
72˚xXX sec
Repeat XX additional cycles
72˚xXX min
16˚ for ever

Generation and Analyses of Transgenic Mice. BAT-gal was constructed by fusing seven TCF/LEF-binding sites upstream of a 0.13-kb fragment containing the minimal promoter–TATA box of the gene siamois (11). Details of the synthesis of this construct and the initial controls for β-catenin-specific activation of BAT-gal can be found in Supporting Text and Fig. 6, which are published as supporting information on the PNAS web site, www.pnas.org. Transgenic BAT-gal mouse lines and embryos were produced from B6D2F1 females mated with B6D2F1 males (Charles River Breeding Laboratories) by using standard procedures (12). DNA was microinjected into the pronuclei of one-cell embryos, and the surviving embryos were implanted into CD1 pseudopregnant foster mothers. Transgenic mice were identified by analysis of genomic DNA from tail biopsies by PCR and Southern blot to detect lacZ. lacZ primers: forward, 5′-CGGTGATGGTGCTGCGTTGGA-3′; reverse, 5′-ACCACCGCACGATAGAGATTC-3′. Other experimental procedures are published as supporting information on the PNAS web site.

BAT-gal construct. BAT-gal was constructed by fusing 7 TCF/LEF-binding sites upstream of a 0.13 kb (0.13-sia) fragment containing the minimal promoter-TATA box of *siamois* (1). We used this minimal promoter for two reasons: first, this fragment has already been shown to be inactive *in vivo* during the development of *Xenopus* embryos (1); second, *siamois* is not present in mammals, making BAT-gal a completely heterologous reporter system that may be less subject to regulatory mechanisms in mammalian cells. BAT-gal drives the expression of β-gal in the nucleus to enhance detection of the reporter. Multimerized LEF/TCF-binding sites were generated by ligating synthetic double-stranded oligonucleotides (5'- CAGAA TCA TCAAAGGACCT-3'). The mutant LEF/TCF reporterwas generated similarly to BAT-gal but using oligonucleotides mutated in the LEF consensus (2).



Sequence

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a

Generic LacZ Primers from Jeremy Reiter's lab
BatgalFor: CGGTGATGGTGCTGCGTTGGA
BatgalRev: ACCACCGCACGATAGAGATTC

94°C for 12min; 94°C for 30s, 50°C for 30s, 72°C for 30s, 40 cycles; 72°C for 7min. 4°C forever.

If the mouse carries the Batgal allele, you will see a PCR product. No band= No Batgal allele.