



A Potent BALB/c ES Cell Line for Germline Transmission

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inGenious Targeting Laboratory (iTL) has generated a germline-potent BALB/c mouse strain embryonic stem (ES) cell line, iTLb. Germline transmission has been achieved from high and low percentage chimeric mice by injection of wild-type and targeted ES cells. This BALB/c ES cell line provides a new tool for immunology, cancer, cardiovascular and medicinal researchers.

BALB/c is one of the most widely used mouse strains in immunology. Infections with pathogens are traditionally characterized in BALB/c mice. Immunological mouse studies often present challenges from the antigenic or injection requirements needed for phenotype alterations to occur. Genetic modification of BALB/c mice via mouse embryonic stem (ES) cells can overcome those challenges, resulting in a more useful tool to study targeted genes in immunology.

The BALB/c genetic background is becoming more recognized in other research areas including cancer. The first gene targeted tumor suppressor mouse model, Trp53^{+/-}, was generated from a hybrid (C57BL/6 x 129/Sv) background. The animal model was designed to mimic the human Li-Fraumani syndrome where breast cancer is the most frequent cancer types in affected women. However, the Trp53^{+/-} mouse model rarely developed mammary tumors. After backcrossing (11 generations) to the BALB/c background, 55% of the female BALB/c Trp53^{+/-} mice developed mammary carcinomas.¹ The BALB/c Trp53^{+/-} mouse model is now widely used to study pathways of human mammary tumorigenesis.

To date, BALB/c has been utilized less than other popular mouse ES cell lines, such as 129 and C57BL/6, due to the difficulty of establishment and low efficiency of germline transmission.^{2,3} inGenious Targeting Lab (iTL) has derived a robust BALB/c mouse ES cell line, iTLb. This potent iTLb ES cell line has been confirmed to produce high germline transmission efficiencies from wild-type and targeted ES cells.

Materials and Methods

Establishment of ES cell lines and targeted clones

Embryos were flushed from the uterus of BALB/c females with Flushing Holding Medium on the fourth day after natural mating, 3.5 days post coitus (dpc). Well-developed blastocysts were transferred to ES medium in a 96-well plate with γ -irradiated mouse embryonic fibroblasts as feeder layers. ES cell medium contains 15% Fetal Bovine Serum, 1 mM non-essential amino acids, 0.1 mM 2-mercaptoethanol, and 1,000 units/mL Leukemia-inhibiting factor. Presence of the Y-chromosome was determined by PCR for each ES cell line.

ES cell transfection was accomplished by electroporating linearized P53NeoINvi DNA into iTLb ES cells (day 1). Neomycin selection was initiated 24 hours later. Colonies (200) were picked on day 10 and transferred to 96-well plates. Plates were duplicated, one plate for frozen storage and the other for cell DNA extraction. Recombinant clones were identified by PCR.

Chimera generation, germline determination and confirmation

Blastocysts were harvested by flushing the uterus of C57BL/6 females at 3.5 dpc. iTLb ES cells were microinjected into blastocysts to generate chimeras using standard methods.⁴ Percentage chimerism was determined visually by

distribution percentage of albino coat color on mouse body: (low) L=0-39%; (medium) M=40-79%; (high) H=80-100%. For targeted ES cells, germline transmission was confirmed using genomic DNA from tails in a PCR reaction.

Results

Germline transmission of wild-type iTLb BALB/c ES cells

Two injections of wild-type iTLb BALB/c ES cells produced an average of 92% efficiency of chimera formation and 83% male chimera offspring (Table 1).

Injection	1	2	Total
Embryo host strain	C57BL/6	C57BL/6	
Injected & transferred embryos	10	10	20
Pups born	8	5	13
Birth rate	80%	50%	65% avg
Chimeras produced	7	5	12
Chimera generation efficiency	88%	100%	92% avg
Male chimeras	6	4	10
Male chimera generation efficiency	86%	80%	83% avg

Ten male chimeras of varying percent chimerism were mated with wild-type BALB/c female mice to assess germline transmission performance. Germline transmission was determined by visual inspection for albino coat colored pups. Seven out of ten chimeras achieved germline transmission. Six out of the seven germline-transmitting chimeras produced albino pups in their first litter. The chimeras yielding no albino pups either produced no litters at all, or produced no subsequent pups after their first litter. Medium to high percent chimeric mice produced 90-100% albino pups in the first litters (Figure 1, Table 2). Low percentage chimeric mice also produced albino pups in the first litters, but at a lower frequency (Figure 2).

Figure 1. High % chimeric mouse generated from wild-type iTLb BALB/c ES cells with a litter of albino pups.



Table 2. Germline transmission efficiency from iTLb BALB/c ES cells.

Chimera ID	1	2	3	4	5	6	7	8	9	10
Chimerism	L	M	H	L	M	H	M	M	H	L
Litters #	3	3	3	1	0	2	3	0	2	3
Total pups	21	24	26	7	0	18	21	0	7	20
Albino pups	4	23	26	0	0	18	20	0	7	10
%Albino pups	19	96	100	0	0	100	95	0	100	50
%Albino from 1 st litter	17	90	100	0	0	100	100	0	100	14

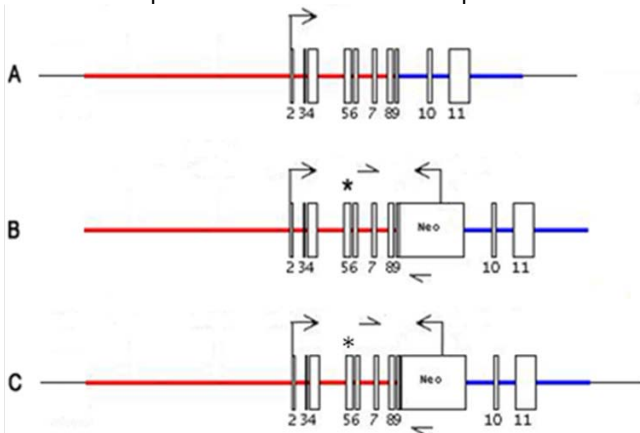
Figure 2. Low % chimeric mouse generated from wild-type iTLb BALB/c ES cells with a litter of albino pups.



Germline transmission of targeted iTLb BALB/c ES cells

The P53NeolNvi targeting vector (Figure 3) was transfected into the iTLb BALB/c ES cells. Three recombinant clones were obtained from the screening of 200 clones.

Figure 3. Targeting vector schematic presenting A) wild-type allele B) targeting construct and C) knockout allele. Half arrows indicate PCR primers and the * indicates a point mutation.



Injections of two independent targeted ES clones produced an average of 58% efficiency of chimera formation and 70% male chimera offspring (Table 3).

Seven male chimeras of varying percent chimerism were mated with wild-type BALB/c female mice to assess germline transmission performance. Germline transmission was determined by visual inspection for albino coat colored pups and PCR analysis. Both clones achieved germline transmission with efficiencies comparable to the wild-type study (data not shown). Similar to the wild-type study, low percentage chimeric mice were able to achieve germline transmission. For example, a low percentage male produced 5 albino pups from a litter of 13 pups (Figure 4).

Table 3. Chimera formation efficiency from injection of targeted iTLb P53 ES cells.

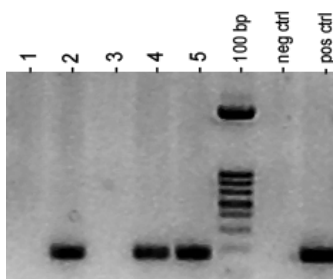
Clone #	172	284	Total
Embryo host strain	C57BL/6	C57BL/6	
Injected & transferred embryos	15	15	30
Pups born	8	10	18
Birth rate	53%	67%	60% avg
Chimeras produced	6	4	10
Chimera generation efficiency	75%	40%	58% avg
Male chimeras	5	2	7
Male chimera generation efficiency	83%	50%	70% avg

Figure 4. Low % chimeric mouse generated from gene targeted iTLb BALB/c ES cells with a litter of albino pups.



Genotyping by PCR analysis of the tails of the albino pups indicated 3 of the 5 pups had undergone germline transmission by the targeted knockin p53 gene (Figure 5).

Figure 5. PCR confirming germline transmission in albino pups from gene targeted iTLb ES cells with an iTL 100 base pair ladder, negative control, and positive control of a recombinant ES clone. Primer locations are shown in Figure 3.



Conclusion

inGenious Targeting Lab (ITL) has derived a robust BALB/c mouse ES cell line, iTLb. This potent iTLb ES cell line has been confirmed to produce high germline transmission efficiencies from wild-type and targeted ES cells.

The BALB/c ES cell line can provide researchers a new tool to manipulate their gene of interest in the appropriate mouse genetic background for their studies.

References

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