BTX Applications

Tech-Trends

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A bacterial artificial chromosome (BAC) transgene targeted to the Hprt locus can be stably maintained in the genome of transgenic mice

Tissue-specific expression of a BAC transgene targeted to the *Hprt* locus in mouse embryonic stem cells

Cell Preparation: The HM-1 ESC line, derived from HPRT-deficient 129/OlaHsd mice (129), has been described previously [25]. ESCs were grown on murine embryonic fibroblasts in DMEM-H (Life Technologies/Invitrogen) supplemented with 15% fetal bovine serum (Atlanta Biologicals), 0.1 mM β-mercaptoethanol, 2 mM Glutamax, and LIF conditioned supernatants (~1000 U/ml).

Electroporation Settings:

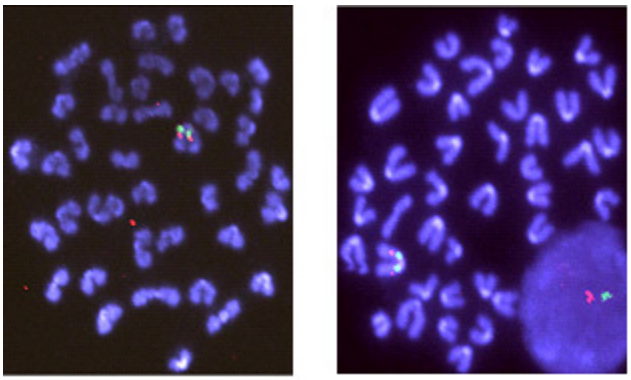
Choose Mode:	LV
Set Voltage:	270 V
Capacitance:	50 µF
Resistance:	360 Ω
Cuvette gap size:	2 mm
Desired Field Strength:	1350 V/cm

Electroporation Procedure:

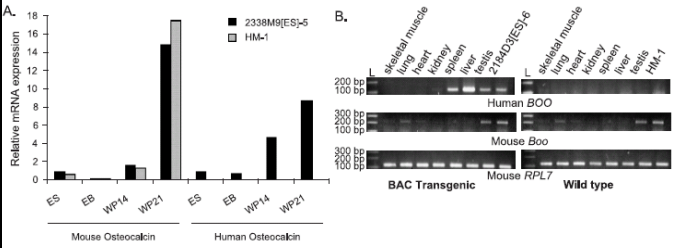
Total sample volume:	400 µl
Transfectant conc:	2 nM linearized BAC DNA in 400µl of 1X PBS and TE
Cell density:	4 x 10^7 cells/ml
Pulse:	Press **Start** to activate Charge and Pulse Sequence
Post-pulse:	Following electroporations, homologous recombinants were selected in ESC medium supplemented with HAT (0.016 mg of hypoxanthine/ml, 0.01 mM aminopterin, and 0.0048 mg of thymidine/ml) for 10 to 12 days, at which time individual colonies were picked for expansion and verification of the desired recombination events.

Results: We have shown that it is possible to target efficiently a human BAC as large as 146 kb into the *Hprt* locus of mouse ESCs, that a targeted BAC transgene can be conditionally excised from the genome with *Cre* recombinase, that a BAC transgene targeted to the *Hprt* locus can be stably maintained in the genome of transgenic mice, and that the expression of genes on targeted BACs showed tissue-specific expression in vitro and in vivo. We had initial concerns that the targeting efficiency of BACs into the *Hprt* locus would be low due to the large size of the BAC relative to the short *Hprt* homologies; however, we observed only a seven- to eightfold decrease in the number of HAT-resistant ESC colonies from electroporations with BAC DNA versus a base vector with identical homology regions.

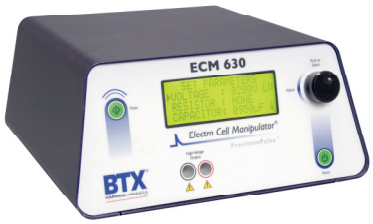
Reference: Heaney, J.D. et. al. Tissue-specific expression of a BAC transgene targeted to the Hprt locus in mouse embryonic stem cells, 2004, *Genomics*



Localization of the BAC transgene to the X chromosome of clones (A) 2338M9[ES]-4 and (B) 2338M9[ES]-9 by FISH. Metaphase chromosome spreads were hybridized with labeled X-chromosome centromeric repeat probe DXwas70 (green) and labeled modified or unmodified BAC DNA (red). Chromosomes were counterstained with DAPI (blue). Arrows indicate chromosomes with detectable signals from the probes.



In vitro and in vivo tissue-specific expression of targeted BAC transgenes. (A) Expression of human osteocalcin from a BAC transgene targeted to the Hprt locus. Levels of mRNA in ESCs, day 2 EBs, and cultures after 14 or 21 days, in which ~60% of the colonies were mineralized bone nodules, were quantified by QRT-PCR. Data for human and mouse osteocalcin expression are plotted relative to the expression of human and mouse osteocalcin from 2338M9[ES]-5 ESCs, respectively. The ratio of both human and mouse osteocalcin expression to Rpl7 expression was approximately 1:1 in ESCs. (B) Expression of the human BOO BAC transgene and mouse Boo in transgenic and wild-type 12-week-old mice,2184D3[ES]-6 ESCs, and HM-1 ESCs. First-strand cDNAs made from DNase-treated RNA samples from various tissues were PCR-amplified using human BOO- and mouse Boo-specific primers. Primers for Rpl7 were used as a control for amplification.



450001 Electroporation System includes ECM 630 Generator, 630B Safety Stand, Cuvettes 1 mm, 2 mm, 4 mm pkg of 30 (10 ea) and Cuvette Rack 660

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