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 400 µl of 1X PBS and TE
- Cell density: 4 x 10⁷ 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