

Bry-GFP/Ainv-Notch4 ES cells. The Bry-GFP/Ainv-Notch4 ES cell line contains an *EGFP* cDNA targeted to *brachyury* locus, a *reverse tet transactivator* (rt-TA) targeted to *ROSA26* locus and a *tetracycline responsive element* (TRE) with lox modification in the 5' untranslated region of the *HPRT* gene. An activated form of *Notch4* cDNA, *int-3*, with a *hemagglutinin epitope* (HA) sequence tagged at the carboxyl terminus was inserted into the *Lox* sequence by the Cre-induced specific recombination.



Troponin T

Cardiogenic effects of Notch1 over-expression on the EB-derived Bry-GFP⁺/Flk-1⁺ population. Analysis of cardiac potential of the Bry-GFP⁺/Flk-1⁺ cells derived from the Notch1-inducible ES cell line after Dox induction. The TnT⁺ cells differentiated from day 3.25 Bry-GFP⁺/Flk-1⁺ cells were analyzed by flow cytometry.



Colony counts for mixed and compact colonies. 1 x 10⁵ Bry-GFP⁺/Flk-1⁺ cells were cultured for 2 day in the blast colony assay in the presence of Dox. Following this induction step, the entire contents of the methylcellulose culture was harvested, washed, and replated in the same volume in the blast colony assay without Dox. Epo and IL-3 were added to promote hematopoiesis within the colonies. Colonies were scored 5 days following removal of Dox). Mixed colonies: colonies with an inner core surrounded by hematopoietic-like cells, Compact colonies: tight colonies without surrounding cells.



Quantitative RT-PCR analysis of Bry-GFP⁺/FIk-1⁺ cells following Dox induction. Cells derived from Day 3.25 of Bry-GFP⁺/FIk1⁺ cells in cardiac differentiation conditions in the absence or presence of Dox were harvested at 4, 12, 24, 48, and 96 hours following induction for gene expression analysis by quantitative RT-PCR. X axis: time points, Y axis: relative expression intensity; open squares: without Dox, closed squares: with Dox. The names of genes are indicated on top of the graphs. The average expression normalized to cyclophinin is shown.