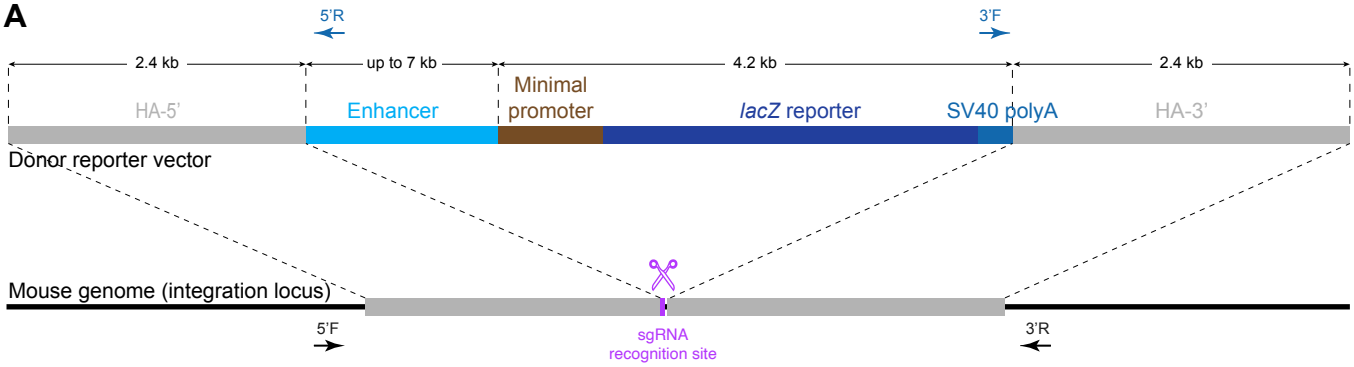
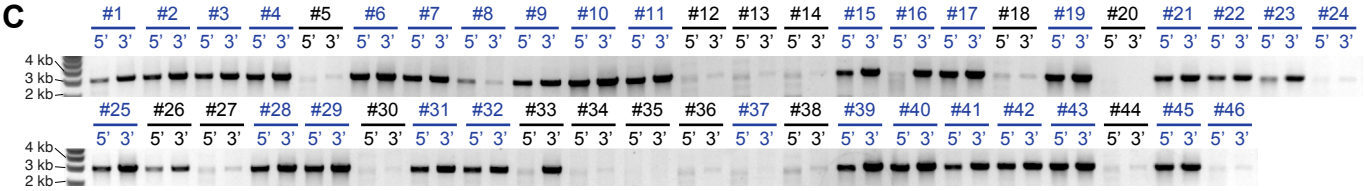
A



B

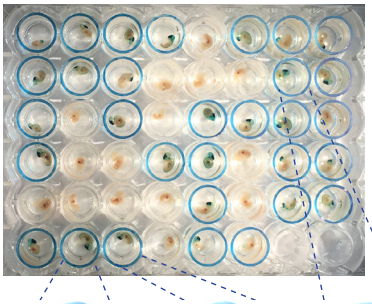
Integration locus	Closest gene	Coordinates (mm10)	Number of E11.5 embryos				% embryos with	
			Total	Knock-in	Knock-in with forebrain expression	Forebrain expression	Knock-in	Forebrain expression
5qB1	*Rbm33*	chr5:28,365,916-28,365,917	44	9	1	5	20.5	11.4
14qE1	*Pcdh17, Diaph3*	chr14:86,043,431-86,043,432	72	9	7	14	12.5	19.4
H11	*Eif4enif1, Drg1*	chr11:3,245,459-3,245,460	46	30	28	31	**65.2**	**67.4**

C

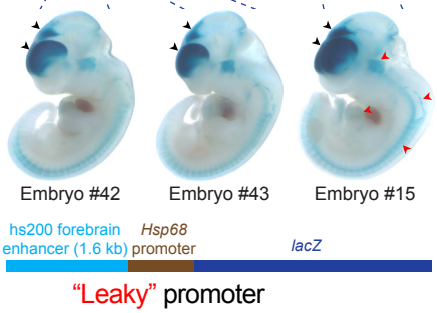


D

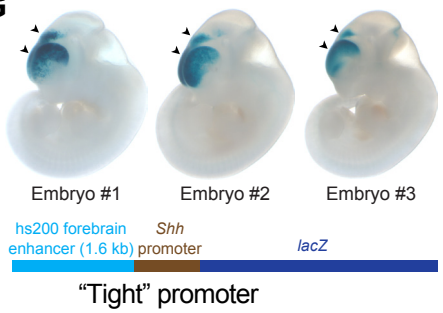
48-well plate with transgenic embryos



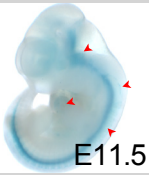
E



G



F

Empty reporter vector (without enhancer) integrated at H11 locus	Number of embryos			Representative LacZ-stained knock-in embryo
	Total	Knock-in (tandem knock-in)	Knock-in with *lacZ* expression	
Hsp68 promoter, *lacZ* — "Leaky" promoter	10	7 (5)	7	 E11.5
Shh promoter, *lacZ* — "Tight" promoter	21	11 (6)	1	E11.5
	6	3 (3)	0	E12.5
	17	1 (1)	0	E13.5
	15	3 (3)	0	E14.5