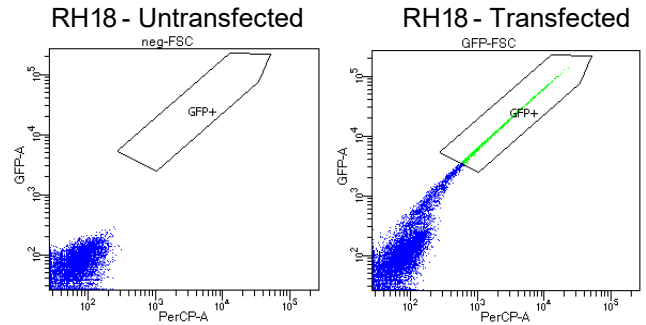
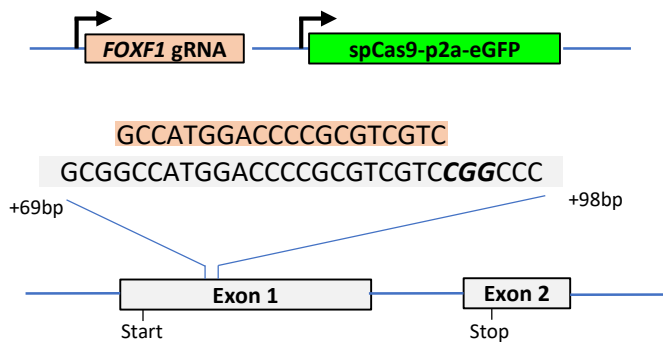


(A)



Transient transfection of RH18 with gRNA/Cas9/GFP plasmid

GFP+

Single Cell plating



4 x 96 well plates

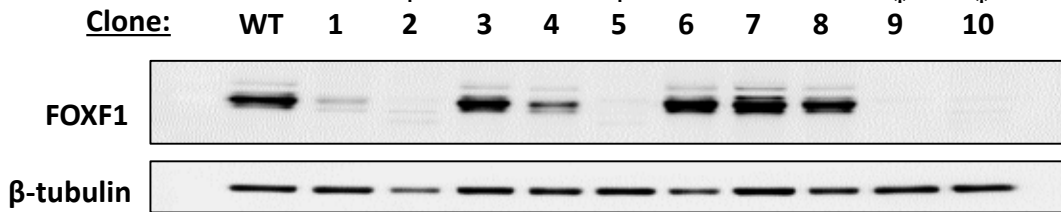
186 single-cell wells

Expand

Screen by Western/ Sequencing

10 clones

(B)



(C)

Clone	FOXF1 Allele (3 total)	Mutation	Predicted effect on protein
KO clone 2	1	c.-4_+109del.	Frameshift
	2	c.88_94del.	Frameshift
	3	c.70_86del.	Frameshift
KO clone 5	1	c.88_89insT	Frameshift
	2	c.-13_+20del.; c.53_62.del;c.64G>A;c.67G>A; c.77_82del.; c.87G>A; c.88_92del.; c.98C>A; c.99del.	Frameshift
	3	c.83_117del.	Frameshift
KO clone 9	1	c.87insA	Frameshift
	2	c.87insT	Frameshift
	3	c.87insG	Frameshift
KO clone 10	1	c.33-94 deletion	Frameshift
	2	c.87 del.	Frameshift
	3	Unknown	Unknown