

## Genomic optimisation of cell line performance in bioproduction

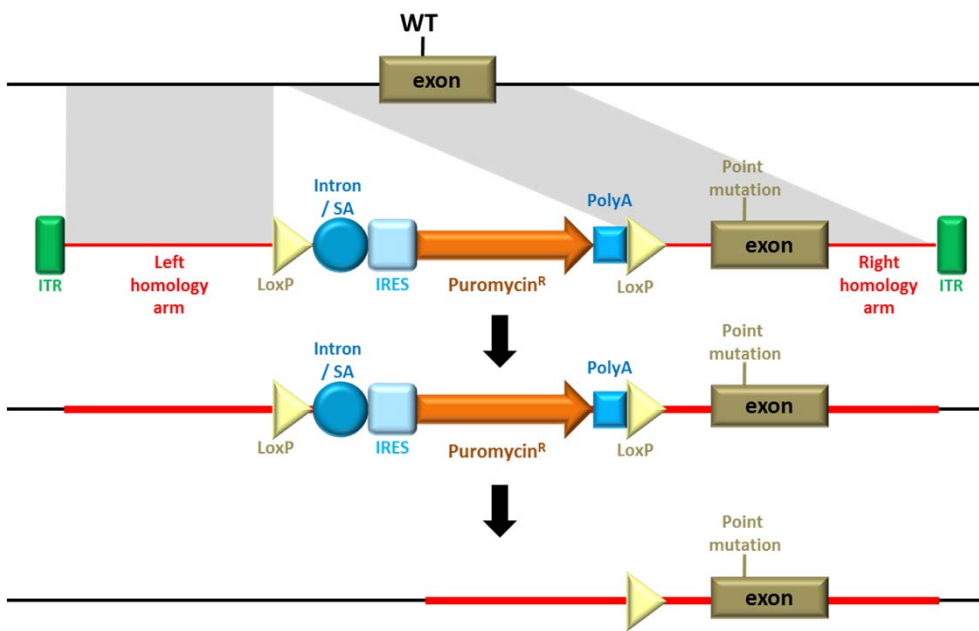
### INTRODUCTION

Horizon Discovery specialised in the precision engineering of *in vitro* mammalian somatic cell line genomes. Employing its proprietary homologous recombination-based genome editing platform GENESIS™, Horizon applies this expertise to the field of bioproduction through custom engineering of host cell lines to address some of the key issues facing the industry including cell selection, confounding glycosylation and variable expression.

### SELECTED APPLICATIONS

- Knock-out of glutamine synthetase
- Knock-out of confounding glycosylation genes
- Knock-in recombinant proteins at high expression loci

### THE KEY FEATURES OF GENESIS™



**1. Targeted knock-in, knock-out or base-pair substitution anywhere in the genome, one allele at a time**

Figure 1: schematic of GENESIS™ recombinant adeno associated virus vector design

TARGETING METHOD	% INCIDENCE OF RANDOM INTEGRATION	STUDY
Zinc Fingers	42.6, n=349	Hockemeyer D <i>et al.</i> 2009 Nat Biotechnol 27: 851–857.
I-SceI	61, n= 31	Miller <i>et al.</i> 2010 Mol Ther 18: 983-986
AAV	0, n=52	Russell <i>et al.</i> 2010 Mol Ther 18: 1192-1199

**2. High fidelity homologous recombination based genome editing yields no off-target effects**

Figure 2: No off-target effects with rAAV. Alternative approaches can lead to issues with long term genome stability

### 3. High stability of cell genomes modified using GENESIS™

Figure 3: Analysis of GENESIS™ engineered cancer cell model drift using SNP6 profiling reveals >99.5 % genomic conservation across clones

Horizon MT or WT	Kras WT	Kras WT	MCF10a OCB	MCF10a OCB	PTEN -/- clone 2	PTEN -/- clone 2	PTEN WT	PTEN WT	PTEN -/- clone 1	Kras G12V	PI3K E545K exon 9	PI3K E545K exon 9	PI3K H1047R exon 20
Kras WT	1.000	0.999	0.993	0.993	0.997	0.997	0.998	0.998	0.997	0.998	0.998	0.997	0.998
Kras WT	0.999	1.000	0.993	0.993	0.997	0.997	0.998	0.998	0.997	0.998	0.997	0.997	0.998
* MCF10a	0.993	0.993	1.000	0.999	0.991	0.991	0.992	0.992	0.991	0.992	0.992	0.992	0.992
* MCF10a	0.993	0.993	0.999	1.000	0.991	0.992	0.992	0.992	0.992	0.992	0.992	0.992	0.992
PTEN -/- clone 2	0.997	0.997	0.991	0.991	1.000	0.999	0.998	0.998	0.999	0.998	0.998	0.998	0.998
PTEN -/- clone 2	0.997	0.997	0.991	0.992	0.999	1.000	0.999	0.999	0.999	0.999	0.998	0.998	0.999
** PTEN WT	0.998	0.998	0.992	0.992	0.998	0.999	1.000	1.000	0.999	1.000	0.999	0.999	0.999
** PTEN WT	0.998	0.998	0.992	0.992	0.998	0.999	1.000	1.000	0.999	0.999	0.999	0.999	0.999
PTEN -/- clone 1	0.997	0.997	0.991	0.992	0.999	0.999	0.999	0.999	1.000	0.999	0.999	0.999	0.999
Kras G12V	0.998	0.998	0.992	0.992	0.998	0.999	1.000	0.999	0.999	1.000	0.999	0.999	1.000
PI3K E545K exon 9	0.998	0.997	0.992	0.992	0.998	0.998	0.999	0.999	0.999	0.999	1.000	1.000	0.999
PI3K E545K exon 9	0.997	0.997	0.992	0.992	0.998	0.998	0.999	0.999	0.999	0.999	1.000	1.000	0.999
PI3K H1047R exon 20	0.998	0.998	0.992	0.992	0.998	0.999	0.999	0.999	0.999	1.000	0.999	0.999	1.000

\* Another lab's MCF10a \*\* HD MCF10a Parental

CELL LINE	TISSUE	GENE	MUTATION
MCF10A	Colon	PI3K	H1047R, E545k, E542k
Various	Various	IDH1	R132C/+ to R132H/+
U87MG	Glioblastoma	IDH1	R132H/+
HCT116	Colon	mTOR	D2338A/D2338A
SW48	Colon	Rac1	Q61L/+
Cal12T	Lung	K-Ras	G12C/+
NCI-H838	Lung	K-Ras	G12C/+, G12D/+
HCT116	Colon	HSF1	-/+/+
HCT116	Colon	mir21	-/- (miRNA KO)
HCT116	Colon	Smo	W535L/+
NALM6	B-cell	DNA repair	Single and double KO's
HCT116	Colon	Multiple	Flag and CBP/SBP fusion tags
HCT116	Colon	Confidential	shRNA resistance allele/+
SW48	Colon	K-Ras	G12V/+, G12C/+ & WT Halotag fusion
HCT116	Colon	XBP1	Luciferase tag (Stress transcript)

### 4. Proven expertise in endogenous gene targeting with a catalogue of over 350 isogenic cell models.

Figure 4: Recent projects Horizon has completed using GENESIS™

### SUMMARY ON GENESIS™ HOST CELL LINE OPTIMIZATION SERVICES

- Simple and rapid method to perform any endogenous genome alteration in mammalian cells
- Service delivers a fully engineered and characterized host cell line
- Enables flexible gene engineering approaches such as; gene ablation; gene defunctionalisation, gene knock-in; inducible gene expression; point mutations; fusion tags
- Zero off-target effects guaranteed
- Proven technology in generating hundreds of isogenic 'knock-in' and 'knock-out' disease models

