horizon

precision genome editing

Improved gene editing efficiencies using AAV- donors in combination with nuclease based approaches

SCRIP AWARDS 2013 WINNER

THE QUEEN'S AWARDS FOR ENTERPRISE: 2012

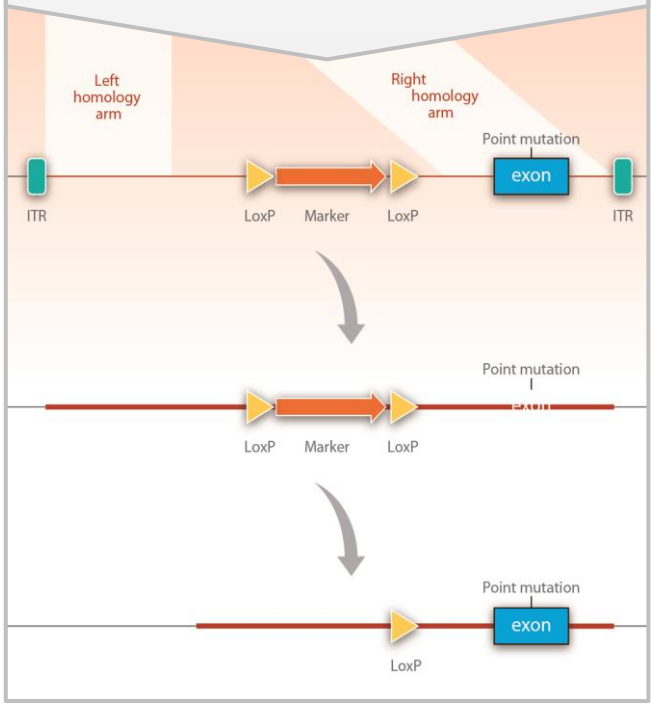
GENESIS™: Comprehensive genome editing

Horizon is the ***only source of rAAV expertise*** and is ***uniquely capable of exploiting multiple platforms***: CRISPR, ZFNs and rAAV singularly or combined

*Horizon's scientists are **experts at all forms of gene editing** and so have the experience to **help guide customers** towards the approach that best suits their project*

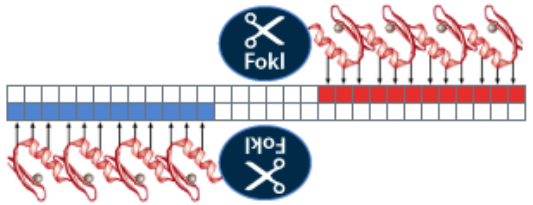
rAAV

- High precision / low thru-put
- Any locus, wide cell tropism
- Well validated, KI focus



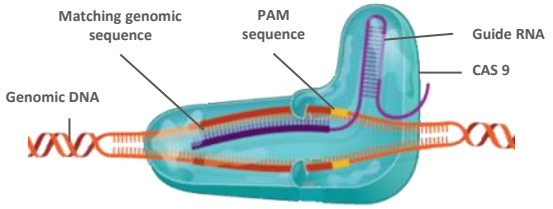
Zinc Fingers

- Med precision / med thru-put
- Good genome coverage
- Well validated / KO Focus



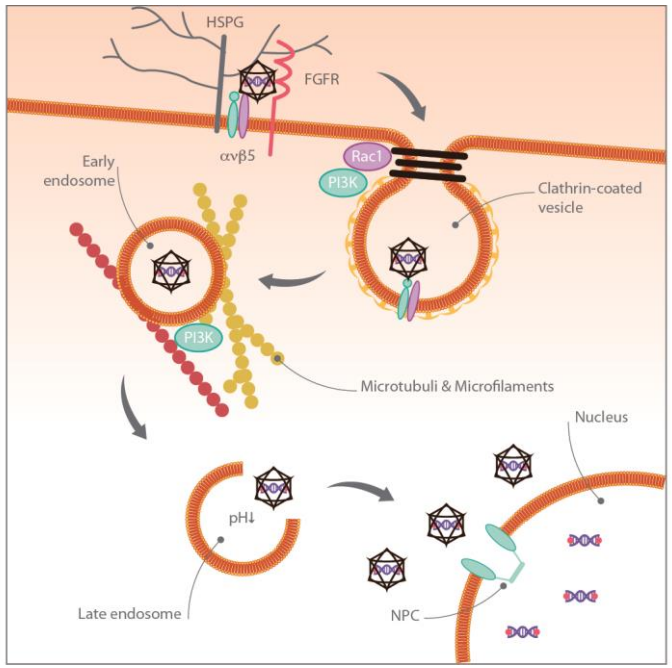
CRISPR

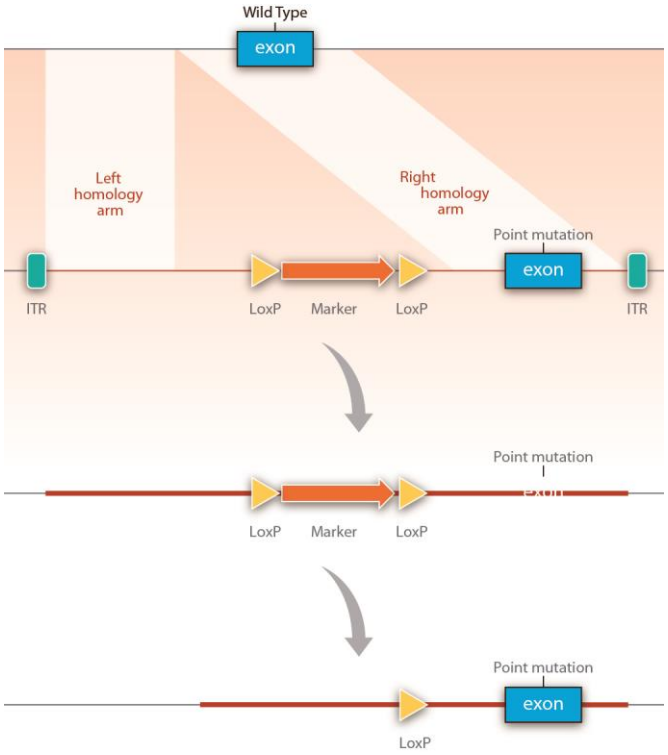
- New but high potential
- Capable of multi-gene targeting
- Simple RNA-directed cleavage



- Point Mutations
- Gene Knockouts
- Deletions
- Insertions
- Translocations
- Amplifications

rAAV method: Modify any genomic loci, in any way, with perfect precision





Homologous Recombination (HR) using single-stranded DNA recombinant Adeno Associated Viruses

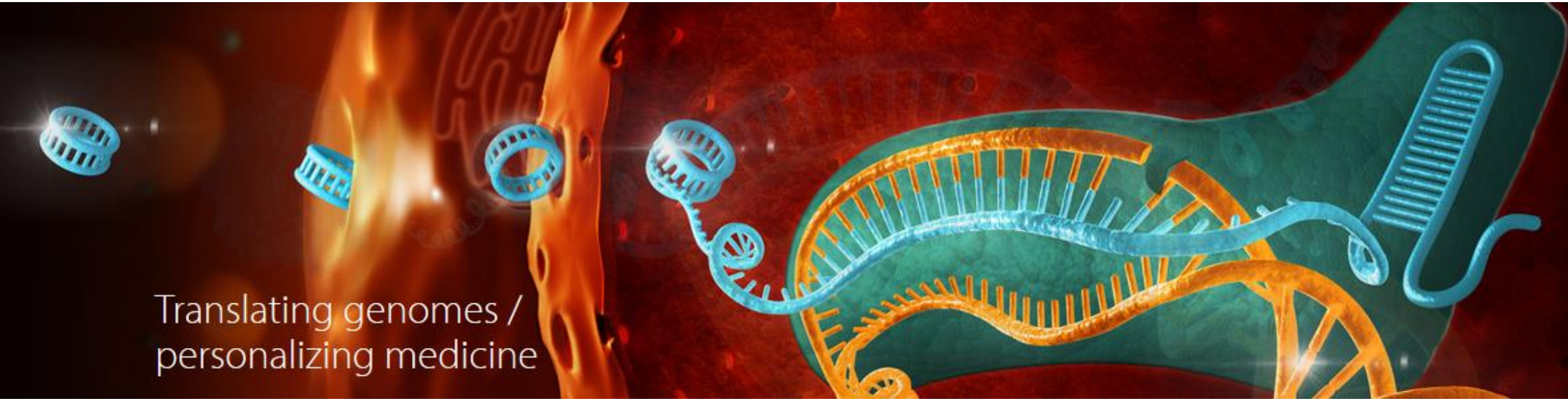
Nature Genetics 18, 325- 330 (1998)

- rAAV alone naturally stimulates homologous recombination in cells to alter genomic sequences
- No DNA-breaks created ……rAAV stimulates HR directly (100x higher efficiency than plasmids)
- Highly precise due to dependence on HR
- Wide tropism for mammalian cell-type; works well on hard to transfect cell-lines
- But, lower efficiency compared to nucleases…..

Nucleases: CRISPR/ Cas9 System

RNA-guided platform to introduce either a double strand DNA break or a single strand nick at a specified location in the genome.

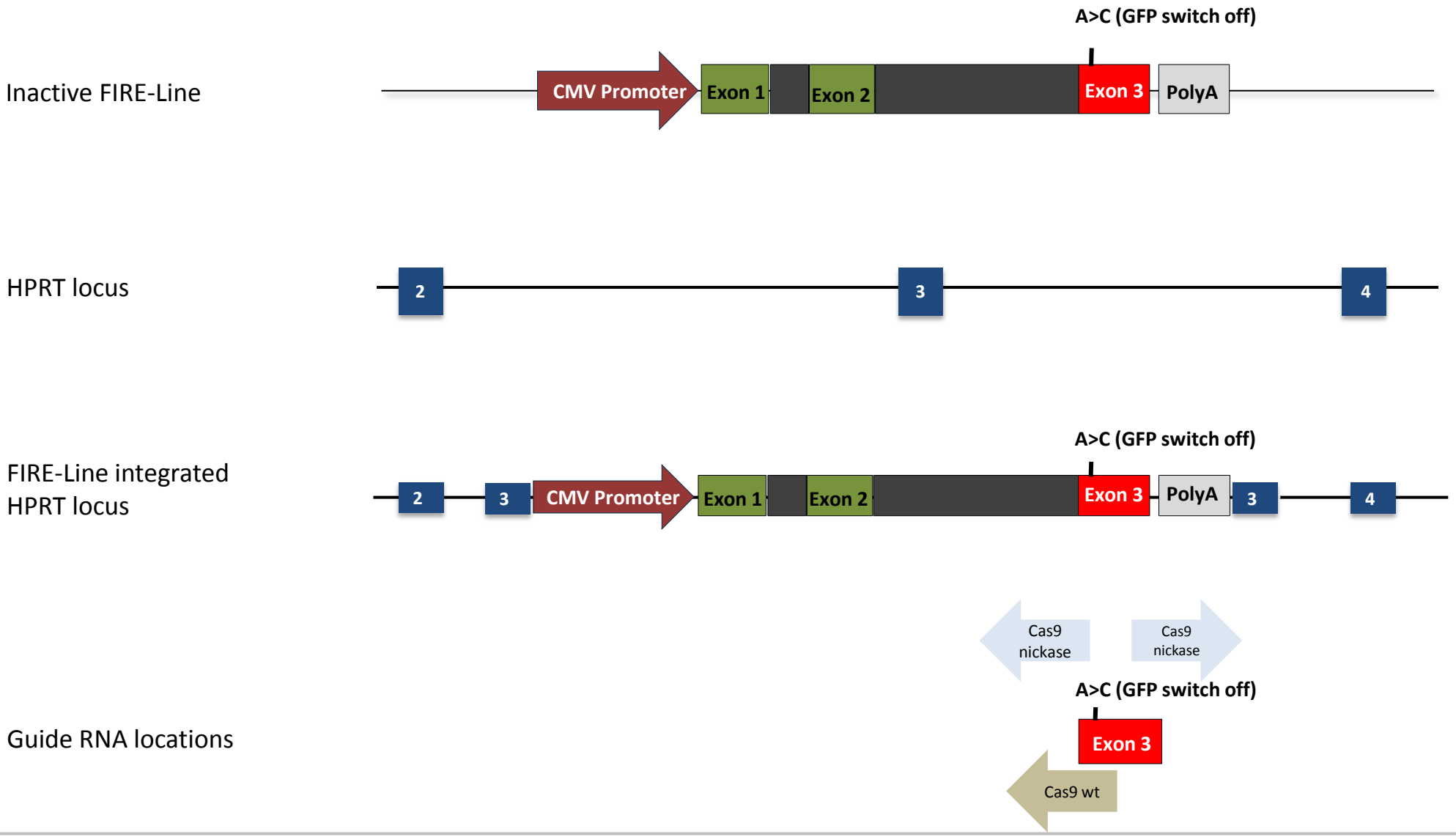
- Short ‘guide’ RNAs with homology to target loci direct a generic nuclease (Cas9)
- Two versions of Cas9 (wt & nickase)
- Guide RNA + Cas9 are delivered into the cell
- Cas9 cleavage is repaired by either NHEJ, or HDR in tandem with a donor
- KO efficiencies are high; specificity concerns now starting to be addressed
- **HR using CRISPR is proving challenging**



Use of rAAV in combination with CRISPR in gene targeting

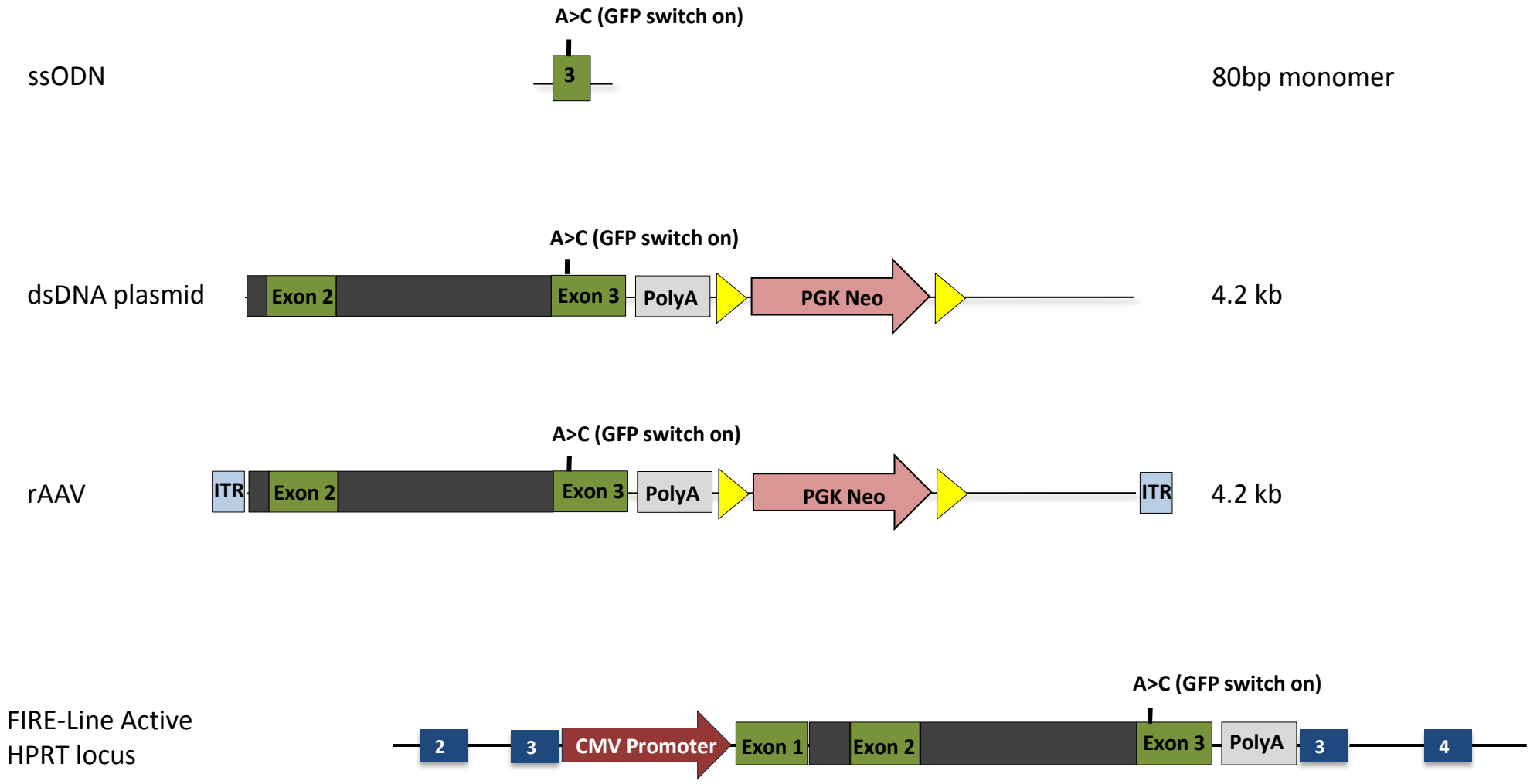
Creation of a FIRE-Line reporter system

FIRE-Line (Fluorescence Indicator of Recombination efficiency cell line)



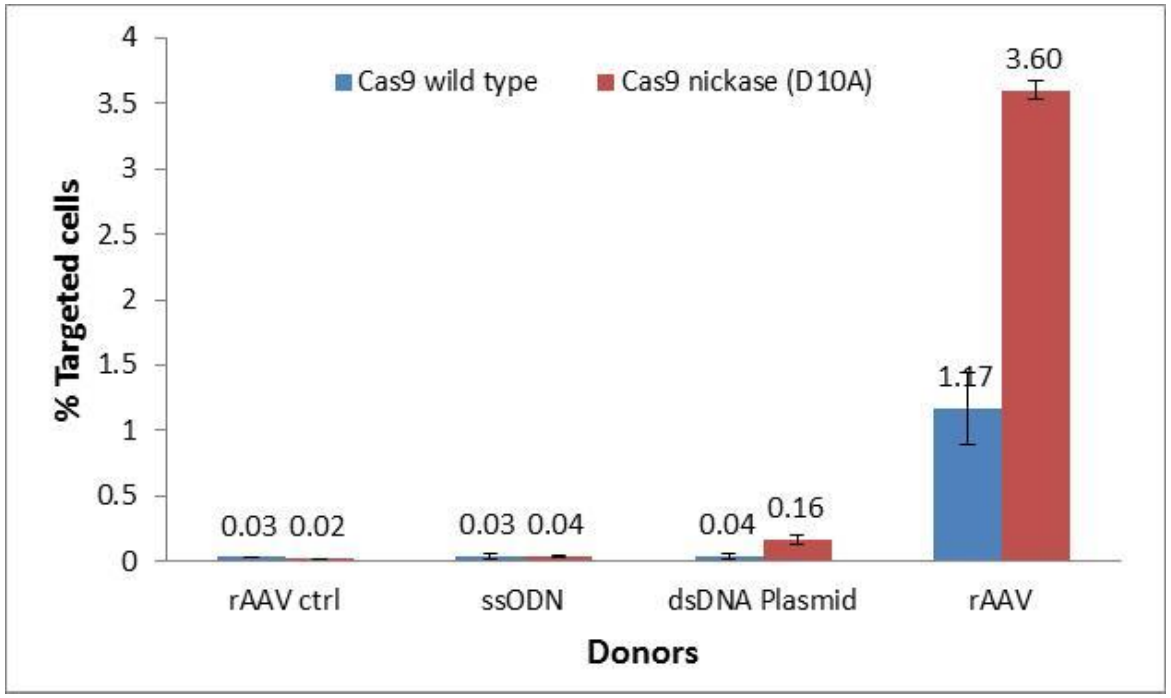
Use of rAAV in combination with CRISPR in gene targeting

Donors used for activating the GFP switch



Use of rAAV in combination with CRISPR in gene targeting

- Donors encode a correction for functional GFP when recombined correctly.
- Cells nucleofected with Cas9+guide RNA along with each separate donors
- On day6 the transfected cells were analysed by FACS to measure the proportion of correctly targeted cells.



- Targeted efficiency increases by 30-100 fold when rAAV is used as a donor.
- We believe this may be due to the natural ability of rAAV to stimulate HR.

Advantages of rAAV as donor

- Reduces the number of clones to be screened to identify correctly targeted clones.
- Significant reduction in resource time and cost to create a cell line.
- rAAV is a Highly efficient donor, especially in hard to transfect cell lines.
- Applicable to wide range of different cell backgrounds

GENASSIST: CRISPR and rAAV enabled gene editing

Cas9
- Wild type and **nickase**
- Separate or **combined with guide**
- Available in cloning and transfection volumes

Guide RNA
- Single or **double guides**
- Available OTS for in-lab cloning
- **Custom guide generation** available with **validation**

Donors
- Available OTS for in-lab cloning
- Plasmid or **rAAV** format
- **Custom donor generation** available

Cell Lines
- **CRISPR-ready** cell lines
- **500+ OTS cell line menu** available for further gene editing

Services
- **Viral encapsulation** of rAAV donor
- Project design support
- On-going **expert scientific support**

Free CRISPR Knockout Generation Program

- Open to all academic researchers
- Free guide design using gUIDEbook, Horizon's *in silico* guide design software
- Free synthesis and cloning into an all-in one vector expressing Cas9.

- Please let Horizon know which guides are active in your cell line
- Horizon would like to license back your cell line in return for a royalty
- Only pay cost of shipping.

Launching next week 30/06/2014!

Horizon would like to license your cell lines!

Acknowledgements

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Questions ????

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