CRISPR LifePipe®: tools for the design of gRNAs and donor sequence required for genome editing using CRISPR/Cas9 system

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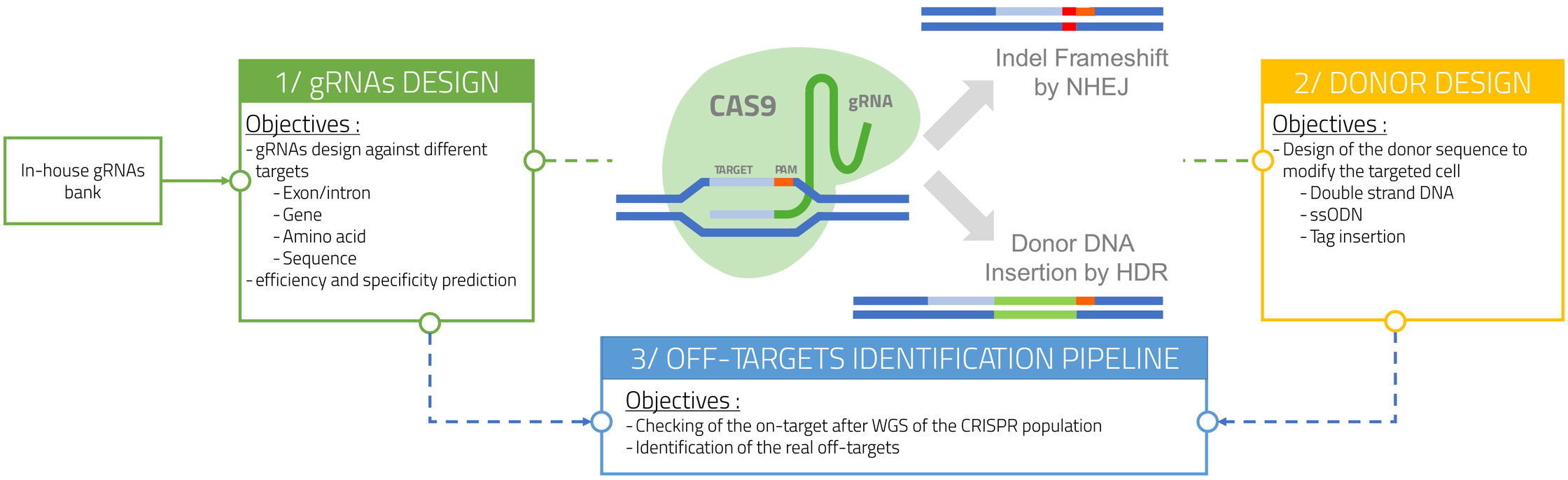
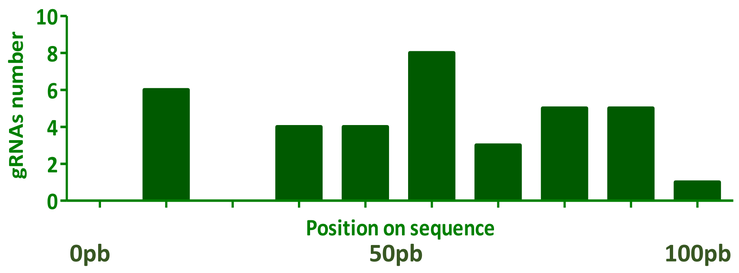
CRISPR Lifepipe tools: from the sequence design to the experiment validation



An experiment design example: insert a stop mutation in TP53 gene at position P153

1st step: the gRNA design and filter

Design and select most efficient and specific gRNA close to the P153 amino acid used by sp. Cas9.

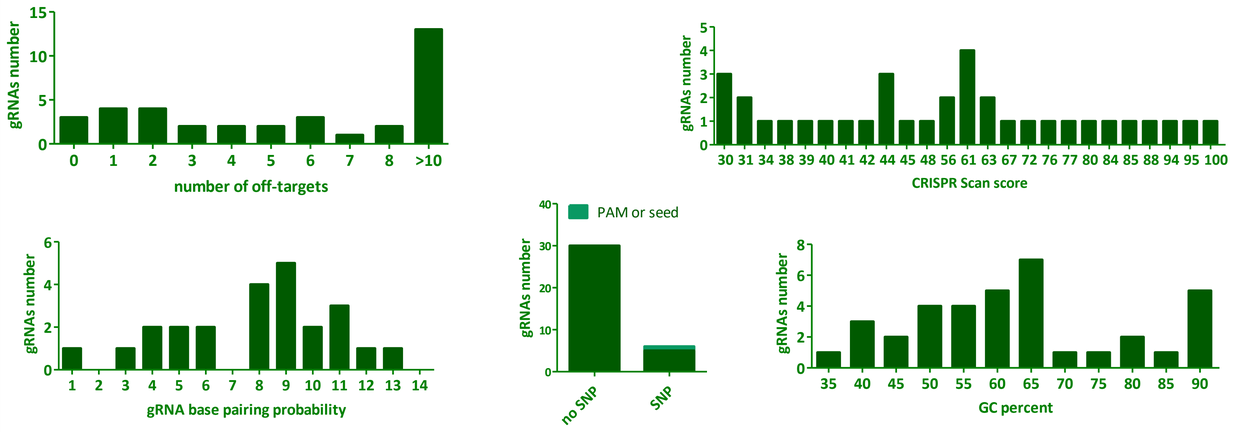


Lots of candidates in the 100nt sequence around the amino acid to target:

How to choose the best gRNA?

The best gRNA is the one with the highest efficiency and specificity.

Computing scores helps predicting efficiency and specificity.



We have integrated all these annotations in the design tool and some additional ones to have a unique tool that gather all the score that information you need for your selection.

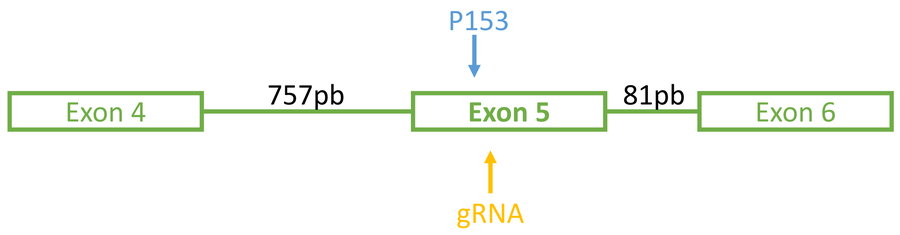
A ranking system helps to sort the gRNA based on each score annotation to keep the best candidates for your experiment.

Amino acid target
1. **Distance between the cut site and the targeted amino acid.**
2. Number of off-targets
3. Efficiency prediction score (CrisprScan, Doench, GC%)

gRNA	GC	CrisprScan	Doench	Distance CutSiteAA	Offtargets Number	SNP Number	Restriction Site
GCTTGTAGATGGCCATGGCG	60	45	88	20	3	0	EaeI,NcoI,MscI,BtgI
GTGCTGTGACTGCTTGTAGA	50	44	27	31	4	0	.
GCAGTCACAGCACATGACGG	60	41	11	52	1	1	.
GAGCGCTGCTCAGATAGCGA	60	63	33	98	0	0	AfeI,HaeII
GATAGCGATGGTGAGCAGCT	55	11	3	110	3	0	.
GACGACAGGGCTGGTTGCCC	70	47	7	141	4	0	.

2nd step: Donor template design

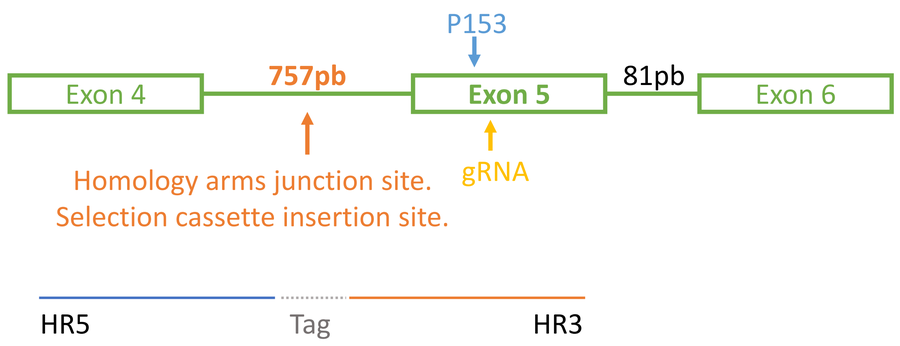
Design a DNA fragment that can be used by the HDR mechanism to insert P153 mutation inside the host DNA and insert an intronic selection cassette.



Depending on the design mode the donor template is centered on the closest intron and large enough (mutation + cassette) or on the gRNA cut site (ssODN).

In addition to the desired mutation, we silently mutated the gRNA's PAM (or seed) to prevent any Cas9 cut in the donor template during the lab's experiment.

Eventually, a selection cassette is inserted inside an intron with caution in order to preserve splicing.



TTATCCATCCCATCACACCCTCAGCATCTCTCCTGGGGATGCAGAACTTTTCTTTTTCTTCATCCACGTGTATTCCTTGGCTTTT
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TGCCGTCTTCCAGNNN