CRISPR Information Sheet & FAQs

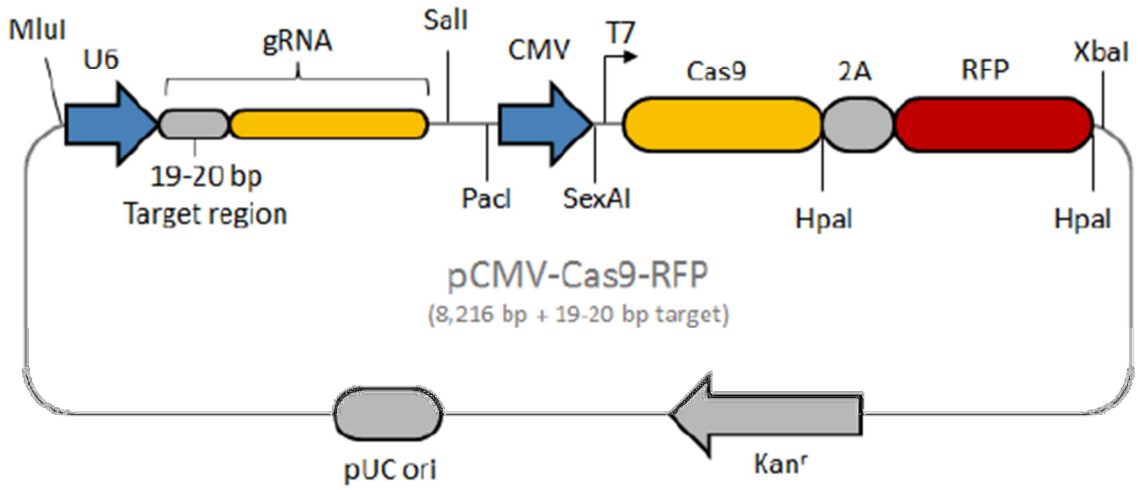
Features

- CRISPR stands for “Clustered Regularly Interspaced Short Palindromic Repeats”
- CRISPR off-the-shelf products available for Human, Mouse & Rat - NZ$725 per clone (List)
- We recommend customers to buy 3 or more clones per gene for knockout or knock-in because CRISPRs are not validated (unlike ZFNs)
- CRISPR – Custom design– for all other organisms other than above - NZ$795 per clone (List)
- CRISPRs come in plasmid DNA form (mRNA form available by mid-2014)
- Plasmid DNA has a GFP or RFP for cell sorting the cells that have taken up the CRISPRs
- The target sequence of the CRISPR is shorter (19-20 bp) than ZFNs (36 bp), therefore the specificity and efficiency of recognition in genome is less than ZFNs

URL

- http://www.sigmaaldrich.com/technical-documents/articles/biology/crispr-cas9-genome-editing.html

CRISPR/Cas9 Plasmid



Competitors

- Life Technologies has a CRISPR (do it yourself kit) but customer has to design sequence and sub-clone CRISPR into their own vector and it does not have a guide RNA, the cost of this kit is approx. $550 for 10 RXN
- Addgene Consortium – do it yourself vector sharing site, $300 - $700, customer needs to buy rest of reagents and design their own sequence

- Gencopoeia – Design and Cloning service only - $350 - $600

FAQs

- What are the benefits of using CRISPR/Cas9 system for genome-editing over other technologies?
    - CRISPRs are a RNA-guided endonucleases (as oppose to DNA-guided) and a simple system with a vector backbone that has a number of Key features including a guideRNA and a GFP selection cassette (this is different to Life Technologies CRISPR offering
    - They are cheaper (about 10x cheaper than ZFNs), so any lab can begin their gene editing project using a reliable system that has almost as much of the benefits of the ZFNs
- Does it come in mRNA format like the ZFNs?
    - No, but it is in R&D pipeline and should be available by mid-2014

- How does the cutting occur?
    - Via the Cas9 endonucleases
- How do you know your cells have taken up the CRISPRs / how do you measure transfection efficiency?
    - Because the cells that have taken up the CRISPR have also taken up the corresponding GFP they will show a green colour under the microscope so it is safe to assume that some of these green cells have had the double stranded break occur in them.

- Is there any off-target effects?
    - There has been some publications reporting on the off-target effects of the home made CRISPRs
    - Sigma's CRISPRs are in a solid backbone with an improved Cas9

Any other technical questions please forward to myself farhad.shafiei@sial.com

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