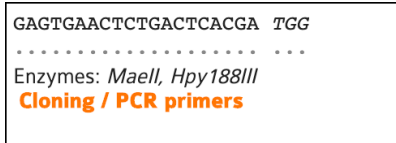


Primer design instructions:

1. For picking primers one can use the primer selection feature on crispor.tefor.net shown below by clicking the “PCR primers” and scrolling down to “PCR to amplify the on-target site”. The link is shown under each sgRNA that is listed, so you can choose an sgRNA and its appropriate primers in one step.



PCR to amplify the on-target site

Use these primers to amplify a genomic fragment around the on-target site:

OntargetGuideRna296fwLeft	TTGAACAGCCCACGAGAGAC	Tm 59.966
OntargetGuideRna296fwRight	ACCCTCAAACAGCTGTCTCG	Tm 59.966

2. For designing better primers than shown on CRISPOR, visit the website “primer 3 plus” <http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>
3. If you are only designing a set of better primers, enter the sequence in the textbox of the primer3plus homepage. Isolate the target sequence by placing brackets “[]” around the sequence. In the “Advanced Settings” you can change the minimum amplicon length from 100 bp to more bp if you need a product that is a specific size. Click the pick primers button and choose the best pair.
4. If you are designing a nested PCR strategy, copy and paste the sequence of the inner PCR, including the inner primers, as well as approximately 100 bp of sequence on each side of the inner primers. Use the brackets “[“ and “] ” to capture the region of interest. For example if I want outer primers in this sequence:
GAAGGCACATTGTGGGCACCAGCATTTGGGTGATGAATGGAACAG

I need to target a sequence within this sequence (i.e. the inner primers/product) so I would put brackets around it like so:

GAAGGCACATTGTGGGCACCAG[CATTTGGGTGATG]AATGGAACAG

Where CATTTGGGTGATG is the inner sequence. Click the green “Pick Primers” button. This will give a list of “Left” and “Right” primers (AKA “Forward” and “Reverse”) that should amplify the target region. These primers then serve as the outer pair of primers in a nested PCR.

5. Order Primers via IDT <https://www.idtdna.com/site/order/oligoentry>
 - o enter the DNA oligos one by one

- select “Lab ready” for each oligo under the “Formulation” dropdown (100 uM solution)