

How to design primers

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1. qPCR primers

- use primer3 program
- take gene sequence from pombase (use with introns and unspliced)
- amplification product size: 80-150 bp
- melting T: 57-62 optimal 59
- in the 5' end they should not contain more than 2 C/G's (important!)
- in the 3' end, good when the last 2 are C/G's

2. Deletion/checking/internal primers

First thing is to check whether the mutants are already in the bioneer library. For this look into dropbox-> BraunLab Databases-> Bioneer S. pombe Deletion Library -> Bioneer 384 8-plate library-> updated 384...final array. In any case we want to have them into the right background (65 wt for single mutants and 906 lem2 for double mutants with lem2). For designing the primers for deleting and checking:

- When they are already in the bioneer library
 - Go to Dropbox: BraunLab Databases->PERL->KOprim_ver13.5.7 and copy the whole folder KOprim_ver13.5.7 into your desktop. Alternatively copy and paste the folder from the protocol dropbox directly into your desktop.
 - Open the command line and navigate to your desktop. Then run the script: `cd Desktop, cd KOprim_ver13.5.7 , perl KOprim_ver13.5.7.pl`
 - Enter name of the gene of interest. You will get a .txt with all the information into the folder ORFs and KO primers...
 - Enter a minimal annealing temperature of 48 degrees and then press q to quit
 - The generated excel is ready for a metabion order ☺
- When they are not in the bioneer library
 - Go to http://bahlerweb.cs.ucl.ac.uk/cgi-bin/PPPP/pppp_deletion.pl
 - Get KO primers and checking primers keeping the preset settings
 - For this KO primers order 0.04 micromol since they are very long

For designing primers for the ORF do it manually:

- Copy the sequence from pombase into SeqBuilder
- In the ORF, avoid the first 80 bp
- Amplification product of around 500 bp
- C/G in the 3'

3. C terminal gfp tagging

- Enter http://bahlerweb.cs.ucl.ac.uk/cgi-bin/PPPP/pppp_c_term.pl
- Check the first offered primers, the reverse should be the same as for the deletion when they are not in the library!! Only order the forward primer!