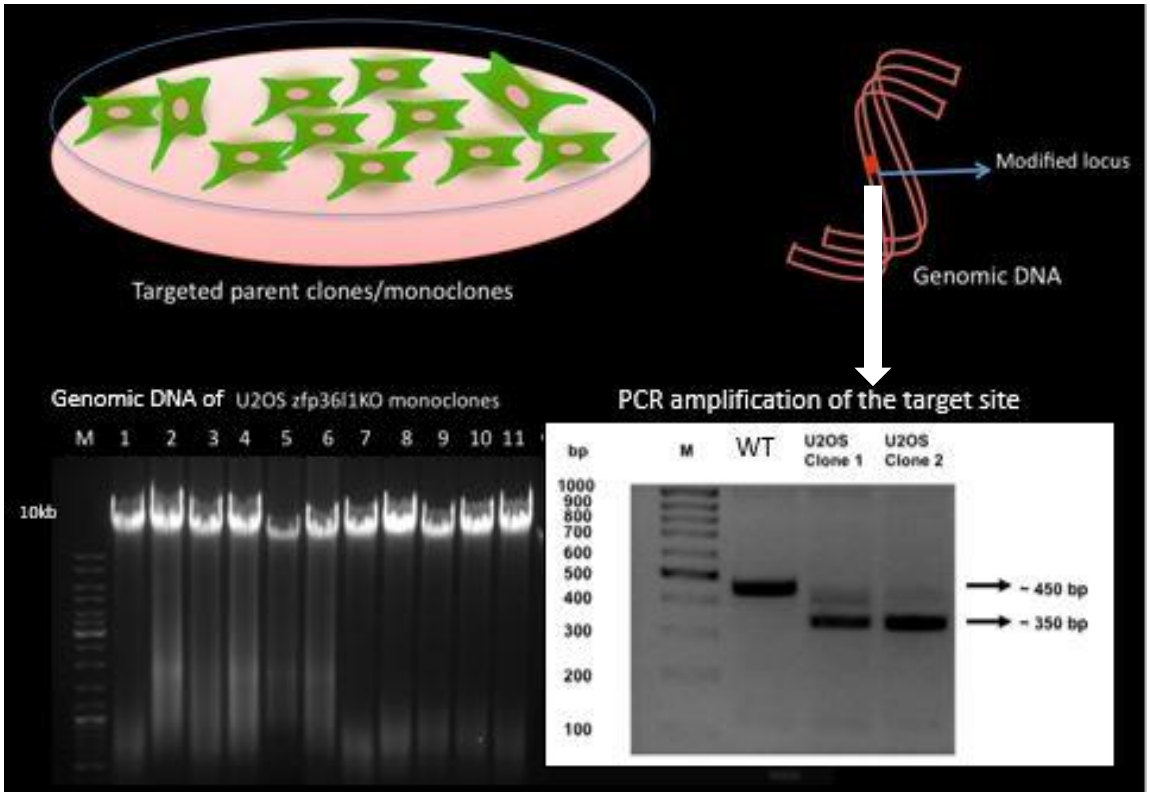
Gene Editors of the Future - Research internship Lab session 1

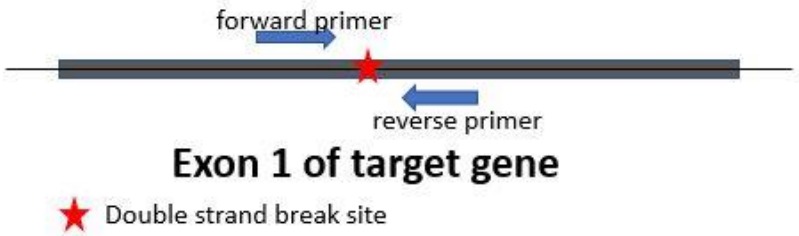
Genomic PCR and CRISPR gene editing screens



Let's begin with a few simple questions

Why do we need genomic PCR in CRISPR clonal screen? What information do we get from the Genomic PCR products?

Once you have successfully transfected plasmids into the cells of your interest and selected the cells in your gene editing experiment, it is time to monitor the result? It is time to verify and characterize the edit. Please remember the editing (insertions/deletions) introduced by CRISPR tool at the target site can be from ~1bp to 2000bp. Depending on your experimental purpose and the nature of the gene edit, a variety of biochemical assays and sequencing analysis may be used, yielding various amounts and types of information. For instance. If you are editing exon 1 of a certain gene the forward and reverse primers (https://primer3.ut.ee/ https://www.eurofinsgenomics.eu/en/ecom/tools/pcr-primer-design/https://www.ncbi.nlm.nih.gov/tools/primer-blast/) must be designed to amplify a region of <500 bp surrounding the double strand break site.



Setting up a Genomic PCR

Amplification of PCR Products Up to 0.5kb(500bp)

Before assembling the amplification mixture. Preparation of master mixes recommended for large number of reactions.

1) Thaw the components indicated in table below and set them on ice.
2) Set up the PCR reaction in a sterile microfuge tube on ice. A detailed pipetting scheme is given the table below.
3) Pipette together on ice and prepare Q5 master mix.
4) Place the amplification mixture in the pcr tubes and start thermocycler immediately. A typical profile for Biorad thermocyclers can be found here: https://www.bio-rad.com/featured/en/thermocycler.html
5) Analyse 10ul of the amplification products on a 1% agarose gel.

Preparation of Taq PCR reaction

COMPONENT	25 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
Template DNA	variable	variable	<1,000 ng
Taq 5X Master Mix	5 µl	10 µl	1X
Nuclease-free water	to 25 µl	to 50 µl	

Try preparing a 100µl Mastermix which will be split in to 10x50µl pcr reactions, load a 5µl of each reaction on to a 1% agarose gel

★ You must create a record of today's experiment for qualifying in the research internship

Thermocycling conditions

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C 45-68°C 68°C	15-30 seconds 15-60 seconds 1 minute/kb
Final Extension	68°C	5 minutes
Hold	4-10°C	

More information @New England Biolabs link below:

https://international.neb.com/protocols/2012/12/07/protocol-for-q5-high-fidelity-2x-master-mix-m0492

Try writing the cycling conditions for amplifying a 1kb fragment and 1.5kb fragment in the genomic pcr, record in your notes

What next??

Sanger sequencing	Analyze the genotype of your single cell clones, such as allelic frequency and sequence of the edit.	Information on nucleotide sequence of each allele.
NGS	Analyze the genotype of your cells, such as allelic frequency and sequence of the edit. Identify off-targets. In context of a pooled CRISPR screen, identify enriched or depleted genes in a cell population.	High throughput, information on nucleotide sequence

We shall continue soon …