EZ-editor™ Monoclone Geneotype Validation Kit (extraction free)

Product Info

This kit is developed for monoclone genotype validation in the process of generating gene-editing cell lines. The validation kit can be used in early growth stage of clones and can screen qualified single clones in advance, and eliminate the cost of culturing large numbers of non-positive clones. It can also shorten the turnaround of 2-4 weeks. The preparation of the genome samples of single-cell clones only takes 15-20 mins. The cells are directly lysed to release the genome and the cell lysate can be used directly in following PCR experiments without purification.Utilizing 96-well plates to simultaneously identify up to hundreds of clones makes high-throughput validation of single-cell clones.

Preferred PCR validation reagent, CloneAmp Taq Mix (+ Dye), are compatible with residual media from crude extracted genomic samples and components after cell lysis that may affect PCR reactions, ensuring validation results can be obtained accurately and quickly.

After a large number of internal tests, the kit can be used for adherent cells, suspension cells, including but not limited to cells of human, mouse, rat or other animal origin. The recommended sample size is $3x10^3$ ~ 10^5 cells (generally $1x10^4$/well for 96-well plate), and the minimum sample size is not less than 100 cells.

Additionally, Ubigene has released two useful tools (1) the primer design tool for gene editing target site identification (EZ-editor™ high efficiency primer design platform) provided in the Red Cotton™ system and (2) EZ-editor™ Genotype Analysis System (GAS) can assist you more rapidly through the entire single-cell clone validation process.

Kit Components

Components		YK-MV-100	YK-MV-250	YK-MV-1000
MicroCell DNA Lysis	Buffer A	10 mL	25 mL	100 mL
	Buffer B	1 mL	2.5 mL	10 mL
PCR Validation	CloneAmp Taq Mix (+Dye)	1.25 mL	3.2 mL	12.5 mL
	CloneAmp Ctrl	200 μL	500 μL	2 mL

*The kit has a shelf life of 12 months, please pay attention to the kit storage temperature, the lysis effect of the Tail Lysis could be affected if placed at -20 ° C.

Experimental Preparation

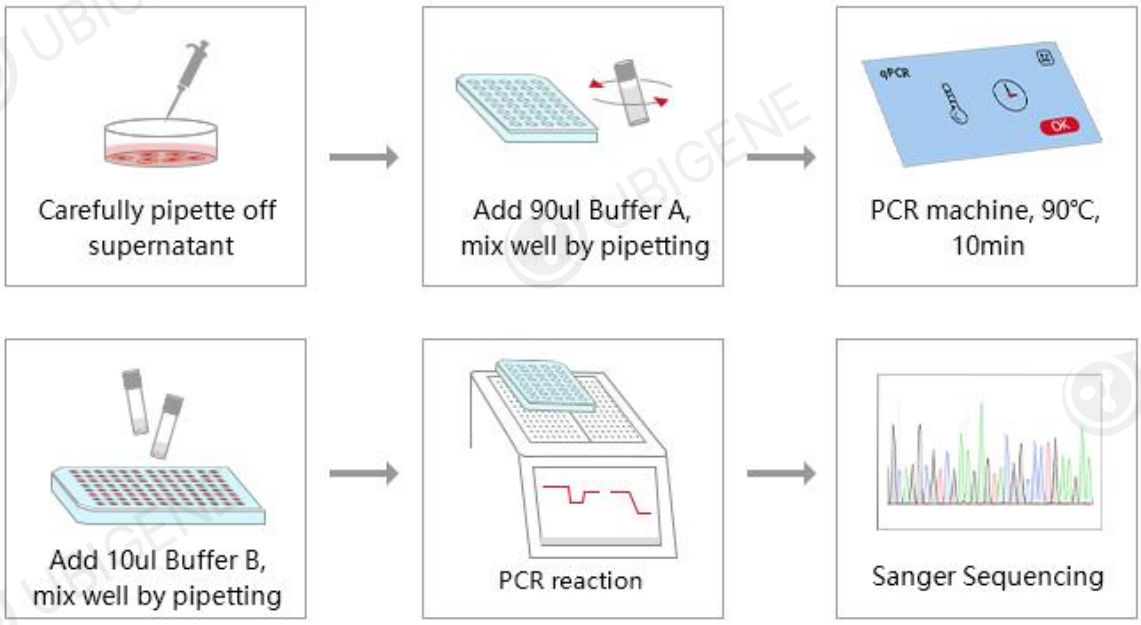
PCR tube, strips of 8/ 96-well PCR plate

Single pipette / multi-channel pipette

PCR machine

Operating batch suspension cells needs a Tabletop Centrifuge (Configure rotor for plates).

Work Flow and Monoclone Validation



Monoclone Cell Sample Preparation

Cell Preparation

① Adherent cells: Carefully pipette supernatant, try to aspirate all the supernatant

② Suspension cells: If the suspended cells are in a EP tube, centrifuge cell sample in the EP tube at 3000 rpm for 10 mins at room temperature in compact centrifuge, and carefully aspirate all the supernatant. If the suspended cells are in 96-well plate, centrifuge cell sample at 3000 rpm for 10 mins at room temperature in Tabletop Centrifuge, and carefully aspirate all the supernatant.

*Optional: Add PBS buffer, centrifuge the cell sample at 3000 rpm for 5-10 mins at room temperature. This step can reduce impurities in the sample, but if there are few cells, there is no need to do this step.

Cell Lysis

① Add 90μl MicroCell DNA Lysis Buffer A to each cell sample, well mixed by pipetting 10-15 times;

② Transfer the mixed cell sample to a 96-well PCR plate, covered with a silica membrane; Or transfer to a PCR tube, strips of 8, well sealed by cap;

③ Set up the program on PCR machine, 95℃, 10 mins, and load the sample.

Stop Lysis

Remove the lysed sample from PCR machine, add 10μl MicroCell DNA Lysis Buffer B, well mixed by pipetting. Lysed sample can be stored at -20℃ or directly used for PCR reaction.

PCR Validation

Take CloneAmp Taq Mix (+Dye) and CloneAmp Ctrl from the - 20 ℃ refrigerator, place them in an ice box for thawing, and prepare the PCR reaction solution according to the following table:

Table 1 Sample preparation method (Experiment group)

Reagent	Volume (each reaction)
CloneAmp Taq Mix（+Dye）, 2x	12.5 μL

Monoclone cell lysate	2 μL
Forward primer（10 μM）	1 μL
Rerver primer（10 μM）	1 μL
ddH_2O	8.5 μL
Total volume	25 μL

Table 2 Sample preparation method (PCR control group)

Reagent	Volume (each reaction)
CloneAmp Taq Mix（+Dye），2x	12.5 μL
Monoclone cell lysate	2 μL
CloneAmp Ctrl	2 μL
ddH_2O	8.5 μL
Total volume	25 μL

Note: The primers should be designed according to the location of gene-editing target site and the knockout size, **EZ-editor™ high efficiency primer design platform**, developed by Ubigene can help you design the primers. The band amplified from PCR control group was about 330 bp.

PCR reaction parameter

Table 3 PCR reaction

Step	Temperature	Time	Cycle
Pre-denaturation	95℃	3 min	1 cycle
Cyclic amplification	95℃	15 s	35~40 cycles
	60℃	15 s	

	72℃	1 min/kb	
Extension compensation	72℃	5 min	1 cycle

PCR products can be either directly perform agarose gel electrophoresis (without loading buffer), or directly used for Sanger sequencing. Sequencing results can be efficiently analyzed and interpreted by using **EZ-editor™ Genotype Analysis System (GAS)**, developed by Ubigene.

Sample Case

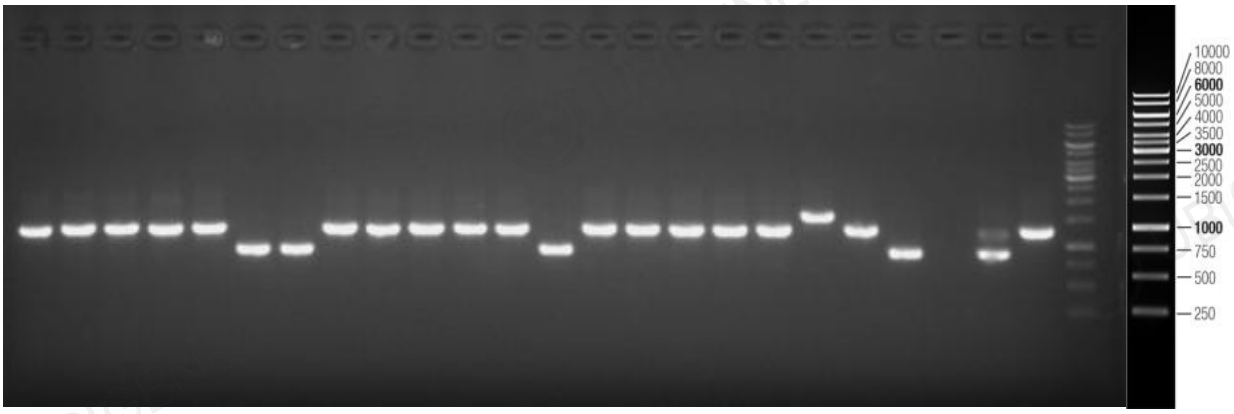


Figure1. Monoclone validation results of gene Cd180-KO Raw264.7 cell line project. From the validation, a total of 4 positive KO clones were detected (KO theoretical size about 902bp), including 1 single-allelic KO clone (two bands, KO clones size about 902bp, WT band size about 1284bp). The DNA template required for PCR reaction in this experiment was obtained from cell lysis (single-cell clones from 96-well plate) by MicroCell DNA Lysis. The amplified bands in this figure were bright and clear to see, indicating that the DNA template lysed by our MicroCell DNA Lysis is perfect to use for genotype validation, and the amplification effect is good.

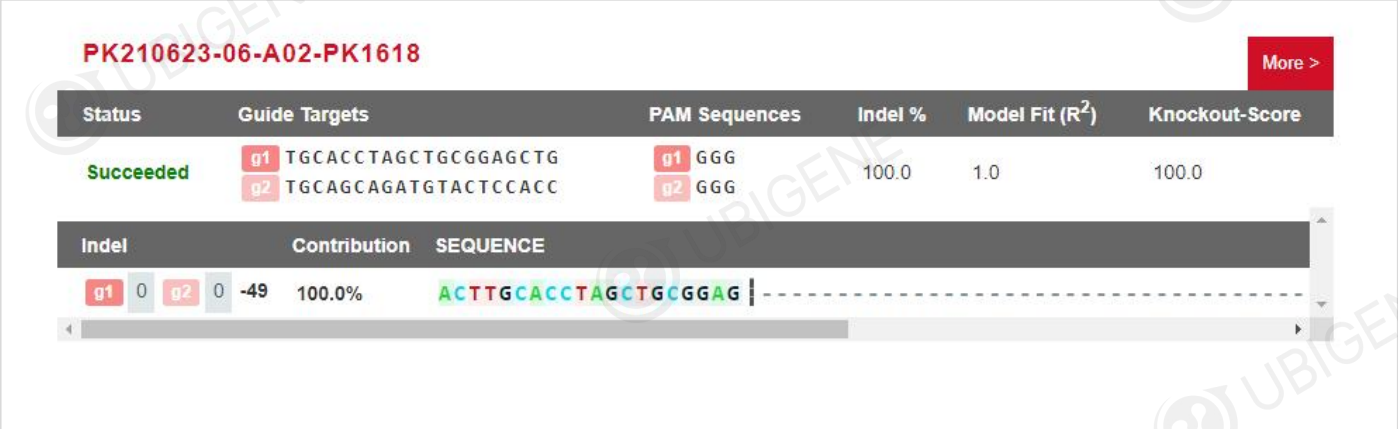


Figure 2. Sequencing analysis results of a KO clone by EZ-editor™ Genotype Analysis System (GAS). Inputting the sequencing results of the gene-edited single clone with that of wild-type cells into **EZ-editor ™ Genotype Analysis System**, plus the gRNA sequences, the system will automatically analyse the genotype of the gene-edited single clone. As shown in the figure, the genotype of the clone is a homozygous KO clone with 49bp KO.

Q&A

① If the cell amount is less than 3x10^3 cells, should I still use the aforementioned volume of MicroCell DNA Lysis Buffer to treat the cells?

If few cell amount is available, try reducing the MicroCell DNA Lysis Buffer A to 10μL~50 μL, at the same time, MicroCell DNA Lysis Buffer B needs to be reduced in equal proportion to give a monoclonal cell lysate concentration of 25 cells/μL。Also to ensure the amount of PCR amplification product, the amount of monoclonal cell lysate for PCR can be adjusted to 5μL。

② Can I use this kit to process the cell sample from the 48-well plate, 24-well plate, 12-well plate, or even larger amount of cell sample?

Aforementioned sizes of cell sample can be proceeded with this kit by proportionally increasing the volume of MicroCell DNA Lysis Buffer A, while an equal increase in MicroCell DNA Lysis Buffer B is required.

③ Can I use other PCR enzymes for cell monoclone validation?

The sample treated with MicroCell DNA Lysis Buffer in this kit is a crude nucleic acid sample, which is easy to use for genomic extraction and high-throughput manipulation from a small number of cells. Because the solutions from this kit are not purified, it is not compatible with

all PCR reagents and the Taq enzyme in this kit is recommended.

④ What is the role of the CloneAmp Ctrl?

Act as a positive control of PCR, it determines if there are problems with PCR system or operation. This control is mainly against human cells, mouse cells, and rat cell templates, and it is not available for cells of other species.

⑤ How can I adjust when amplification of high GC sequences fails?

Add denaturants such as GC Buffer or DMSO to PCR reaction solution, it helps unwind the strands of the template and reduce the difficulty of PCR amplification.

⑥ How can I analyze if no band after PCR amplification?

First, check whether the control group can amplify bands. If the control group can amplify bands, there is no problem with the template (monoclonal cell lysis product), which may be the problem of primers in the experimental group, or the PCR conditions need to be adjusted.

If there is no band in the amplification of the control group, there may be a problem with the preparation of the PCR reaction solution or the program setting. The experiment can be repeated, can only verify using the control group, and different amounts of templates are set, such as 0.5 μL，1 μL，2 μL，5 μL. If there is still a problem in the second amplification control group under the condition of ensuring that the PCR reaction solution is correct and the procedure is correct, there may be a problem with the template (monoclonal cell lysate). The template needs to be prepared again.