EDITGENE Bingo™ CRISPR Point Mutation Cell Line Generation Kit

Product Manual

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Bingo™ CRISPR Point Mutation Cell Line Generation Kit

► Product Information

Catalog Number	Product Name	Components
EDY003-Y01	Bingo™ CRISPR Point Mutation Cell Line Generation Kit (Basic Version)	Bingo™ Point Mutation Plasmid
EDY003-Y02	Bingo™ CRISPR Point Mutation Cell Line Generation Kit (Advanced Version)	Bingo™ Point Mutation Plasmid
		Cell Lysis Reagents
		PCR Validation Reagents

► Product Overview

This kit is the Bingo™ CRISPR Point Mutation Cell Line Generation Kit, designed for the precise and efficient preparation of cell lines with targeted gene point mutations (substitutions, insertions, deletions). It provides cells, plasmids, and key reagents necessary for the gene point mutation process, offering researchers a highly efficient and convenient tool for one-stop construction of point mutation cell lines.

The Bingo™ CRISPR Point Mutation Cell Line Generation Kit is developed based on the Prime Editing (PE) technology, the most efficient and safe method available today, leveraging over a decade of EDITGENE's gene editing expertise. EDITGENE's PE technology achieves a much higher mutation success rate compared to traditional point mutation systems. Since developing PE technology, EDITGENE has optimized PE through multiple advancements:

1. Enhanced the editing activity of SpCas9 and RT enzymes, upgrading PE to PEmax.
2. Introduced nick sgRNA to improve editing efficiency, upgrading PE2 to PE3.
3. Developed PE5 on top of PE3 to inhibit the cellular mismatch repair (MMR) mechanism, reducing unwanted mutations.

4. Further modified the PE5 editing protein to improve pegRNA stability, thereby increasing gene editing efficiency, resulting in the advanced PE7 system.

The Bingo™ CRISPR Point Mutation Cell Line Generation Kit is equipped with the latest PE7 system, offering 4.7 times higher editing efficiency and a 20% increase in success rate compared to PE5, ensuring high editing activity and stability. The kit includes three sets of Target Plasmids (Bingo™ pegRNA and Bingo™ gRNA) based on EDITGENE's unique PE design logic, ensuring optimal editing efficiency, specificity, and overall performance.

To significantly reduce experimental costs, this kit also includes Cell Lysis and PCR Validation products. Genomic sample preparation takes only 20 minutes and allows downstream PCR experiments without purification. The PCR Validation reagents are compatible with residual cell lysis components in crude genomic samples, enabling rapid and accurate identification of targeted mutation cell genotypes.

► Kit Components

●Bingo™ Point Mutation Plasmid

<table>
<tr><th colspan="3">Components</th><th>Specification</th><th>Storage Temperature</th></tr>
<tr><td>Helper Plasmids (500 ng/μL)</td><td colspan="2">Bingo™ PE</td><td>50 μg</td><td>-20 ℃</td></tr>
<tr><td rowspan="3">Target Plasmids (200 ng/μL)</td><td>Bingo™ pegRNA1</td><td>Bingo™ gRNA1</td><td rowspan="3">4 μg/pegRNA
1.5 μg/gRNA</td><td rowspan="3">-20 ℃</td></tr>
<tr><td>Bingo™ pegRNA2</td><td>Bingo™ gRNA2</td></tr>
<tr><td>Bingo™ pegRNA3</td><td>Bingo™ gRNA3</td></tr>
<tr><td>Positive Control (200 ng/μL)</td><td>Bingo™ pegRNA Control</td><td>Bingo™ gRNA Control</td><td>4 μg/pegRNA
1.5 μg/gRNA</td><td>-20 ℃</td></tr>
<tr><td>Cell (optional)</td><td colspan="2"></td><td>1×10^6/tube</td><td>Liquid nitrogen</td></tr>
</table>

●**Cell Lysis Reagents**

Component	Specification	Storage Temperature
Buffer A	10 mL	RT
Buffer B	200 μL	-20 ℃

●**PCR Validation Reagents**

Component	Specification	Storage Temperature
2× High Fidelity Pfu Mix (+Dye)	1.25 mL	-20 ℃
Genotyping Primer F	1 OD Powder	-20 ℃
Genotyping Primer R	1 OD Powder	-20 ℃

Note:

1. To facilitate the enrichment of successfully transfected cells, Bingo™ PE has blasticidin resistance, and Bingo™ pegRNA has puromycin resistance.
2. The Positive Control is an EDITGENE plasmid with a successful point mutation; see the Appendix for details.
3. This kit is suitable for 10 transfections in a 24-well plate; the recommended transfection mass ratio of Bingo™ PE : Bingo™ pegRNA : Bingo™ gRNA is 15 : 3.3 : 1.1.
4. The 2× High Fidelity Pfu Mix (+Dye) is a high-fidelity Taq enzyme; 2× Taq Plus Master Mix II (Dye Plus) (Vazyme: P213-01) is also compatible with this kit.
5. **It is recommended to use this kit with cells provided by EDITGENE for optimal results.**

► Storage Conditions and Shelf Life

Storage: -20°C, valid for 1 year.

► Additional Required Materials

To complete this experiment, other materials not provided in this kit may be required:

Reagents	Cell culture medium, serum, PBS, trypsin, puromycin, blasticidin, Opti-MEM I, cell freezing medium, transfection reagents, etc.
Consumables	24-well plates, 96-well plates, various sterile tips, centrifuge tubes, etc.
Equipment	Biosafety cabinet/sterile workstation, cell incubator, fluorescence microscope, centrifuge, PCR machine, agarose gel electrophoresis system, gel imaging system, pipettes, etc.

► Workflow Diagram

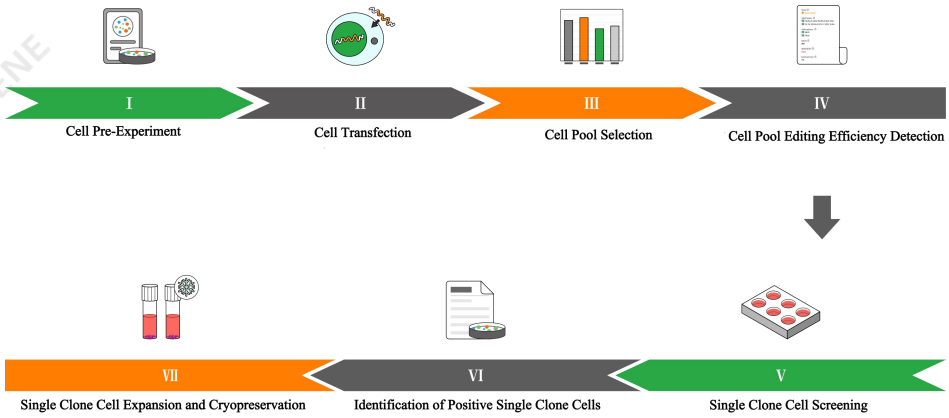

Figure 1. Workflow Diagram for Gene Point Mutation

► Experimental Procedures

●Cell Pre-Experiment

1. Target Site Identification

To ensure the editing effectiveness of the Bingo™ CRISPR Point Mutation Cell Line Generation Kit, it is recommended to conduct SNP identification for the target mutation

site before the experiment. Provide the identification results to EDITGENE for designing the kit's Target Plasmids. EDITGENE offers SNP identification services for point mutation sites as a paid service.

2. Transfection Pre-Experiment

Before starting the main experiment, it is recommended to perform a pre-transfection with a GFP plasmid to optimize transfection conditions for the target cells, facilitating smoother progression of the main experiment.

Note: Efficient transfection is essential for successful editing. Common cell lines like 293T, HeLa, A549, N2A, and CHO-K1 are effectively transfected using lipofection. Transfection of therapeutically relevant cells may be more challenging and vary greatly in optimal methods, so it is advisable to optimize the transfection method before proceeding.

3. Determining Puromycin and Blasticidin Selection Concentration

(1) Seed cells in the logarithmic growth phase into a 12-well plate with 1 mL of medium per well, and incubate for 24 hours.

(2) When the cells reach 60%-70% confluency, replace the medium with selection medium containing various antibiotic concentrations (e.g., Puromycin gradient: 0, 0.5, 1, 1.5, 2, 3, 4, 5 μg/mL).

(3) After 2-3 days, observe the cells under a microscope and select the minimum concentration of Puromycin and Blasticidin that completely kills the cells for use in subsequent experiments (optimal selection concentration).

4. Single Clone Formation Verification

(1) Digest and count cells in the logarithmic growth phase. Resuspend cells in conditioned medium and dilute them to 50-100 cells/mL by limiting dilution.

(2) Add 1 mL of cell suspension to 10 mL of conditioned medium, seed 100 μL per well in a 96-well plate, and incubate.

(3) After cell attachment, count the wells with single cells (Value 1) and continue incubation.

(4) After 7-10 days, observe for cell proliferation and colony formation under a microscope. Count the single clones (Value 2) and calculate the single clone formation rate as follows:

Single Clone Formation Rate = (Value 2 / Value 1) * 100%.

●Main Experiment

1. Cell Transfection

Using Lipofectamine 3000 as an example, if electroporation is used, adjust the parameters based on the equipment's recommended settings.

(1) Digest and count cells in the logarithmic growth phase.

(2) Seed cells in a 24-well plate and incubate.

Note: Due to growth rate differences, aim for 80% confluency after 18-24 hours.

(3) After 18-24 hours, transfect the cells when they reach 80% confluency.

(4) Prepare the plasmid mixture according to the table below:

Component	Amount for 24-well plate
Bingo™ PE	1 μg
Bingo™ pegRNA	0.33 μg
Bingo™ gRNA	0.11 μg
Add opti-MEM I to	21 μL

(5) Add 4 μL of P3000 reagent, gently mix.

(6) Prepare Lipo 3000-Opti-MEM I mixture as follows and gently mix:

Component	Amount for 24-well plate
Lipo 3000	1.5 μL
opti-MEM I	23.5 μL
Add opti-MEM I to	25 μL

(7) Add 25 μL of plasmid mixture to 25 μL of Lipo 3000-Opti-MEM I mixture, gently mix, incubate at room temperature for 15 minutes, then carefully add to the culture medium and shake gently before incubation.

(8) The next morning, transfer cells to a 12-well plate and continue incubation.

Note: Lipofectamine 3000 can be cytotoxic; changing the medium 4-24 hours post-transfection is recommended.

2. Cell Pool Selection

After 48 hours of transfection, replace the medium with selection medium containing Puromycin and Blasticidin at the optimized concentration determined in the pre-experiment. Selection time is 2-3 days, depending on cell type. Stop selection once all control cells have died, and replace with complete medium to expand the gene-edited cell pool.

3. Cell Pool Editing Efficiency Detection

(1) Digest the cell pool with trypsin, centrifuge, and discard the supernatant.

(2) Add 1×10^5 cells to 100 µL DNA Lysis Buffer A, mix, then add 2 µL DNA Lysis Buffer B. Vortex, incubate at 55℃ for 10 minutes, then at 95℃ for 5 minutes.

(3) Centrifuge at 12,000 rpm for 5 minutes and transfer 80 µL of the supernatant to a 1.5 mL tube for PCR.

PCR Reaction System:

Reagent	Volume µL
2 × High Fidelity Pfu Mix (+Dye)	25
Cell lysate	2
Genotyping Primer F（10 µM）	1
Genotyping Primer R（10 µM）	1
Add ddH_2O to	50

PCR Program:

Step	Temperature	Time	Cycles
Pre-denaturation	95 ℃	3 min	1 cycle
Cyclic Amplification	95 ℃	15 s	35 cycles
	60 ℃（Set According to Primer Tm Value）	15 s	
	72 ℃	1 min/kb	
Final Extension	72 ℃	5 min	1 cycle

(4) Run 3 μL of PCR product on an agarose gel (no loading buffer needed). If a single band matching the expected size is observed, send the PCR product for Sanger sequencing.

(5) Analyze the cell pool sequencing results using SnapGene to determine editing efficiency at the target mutation site.

4. Single Clone Cell Screening

(1) Digest the edited cell pool to obtain a single-cell suspension and count the cells.

(2) Dilute and seed cells into a 96-well plate based on the pre-experiment's seeding gradient and incubate at 37℃ with 5% CO_2.

(3) After cell attachment, mark wells containing single clones.

(4) After 7-10 days, when colonies have grown sufficiently, transfer clones from the 96-well plate to a 24-well plate for further expansion.

(5) Once confluent in the 24-well plate, perform sequencing validation on a sample while continuing to expand the remaining cells.

5. Identification of Positive Single Clone Cells

Follow the same method used in cell pool editing efficiency detection.

6. Single Clone Cell Expansion and Cryopreservation

Expand and cryopreserve validated single clone cells, or proceed with downstream experiments.

► FAQs and Solutions

1. Does the Bingo™ CRISPR Point Mutation Cell Line Generation Kit recommend lipofection or electroporation?

A: The Bingo™ PE, pegRNA, and gRNA in the kit are all plasmids, compatible with both lipofection and electroporation. You can choose the appropriate transfection method and parameters based on cell type. Generally, adherent cells can be transfected by either method, while therapeutically relevant cells are recommended to use electroporation

2. What should I do if the transfection efficiency in target cells is low?

A: It is recommended to conduct a pre-transfection experiment before the main experiment. Try different transfection methods to find optimal conditions, such as common chemical transfection methods (e.g., lipofection) or physical methods (e.g., electroporation).

3. How can I verify the editing activity of the Bingo™ CRISPR Point Mutation Cell Line Generation Kit?

A: The kit's Positive Control has been validated in HeLa cells (human gene) and N2a cells (mouse gene). Due to the high heterogeneity of cells, transfection and editing efficiency may vary in different cell types using the same kit.

4. Can cells constructed with the Bingo™ CRISPR Point Mutation Cell Line Generation Kit be stably passaged?

A: Yes. The Bingo™ CRISPR Point Mutation Cell Line Generation Kit targets genomic DNA for gene mutation, allowing the genotype of the target cells to be stably inherited by subsequent generations.

5. Why do cells plated in a 96-well plate for single-clone screening grow slowly or die, even though single-clone formation was good in the pre-experiment?

A: Gene mutations may affect cell viability. Before conducting a gene point mutation experiment, it is advisable to consult relevant literature to understand the function of the target gene. If the target gene plays a critical role in cell proliferation or survival, point mutations may hinder these processes, making it difficult to obtain positive cells.

► Application Example

1. Project Information

Cell Name	Hela
Gene	HSPD1（GeneBank ID：3329）
Mutation Site	c.A391G

2. Bingo™ PE System Plasmid Design

Bingo™ pegRNA	Spacer	ggcttcgagaagattagcaa
	Extension	ttagcaccttCgctaatcttctcga
Bingo™ gRNA	agcttcttcatttgtgttat	

3. Editing Efficiency Detection

The editing efficiency of the cell pool for the HSPD1 gene c.A391G site in HeLa cells is 71%, and homozygous mutant single-clone cells were obtained.

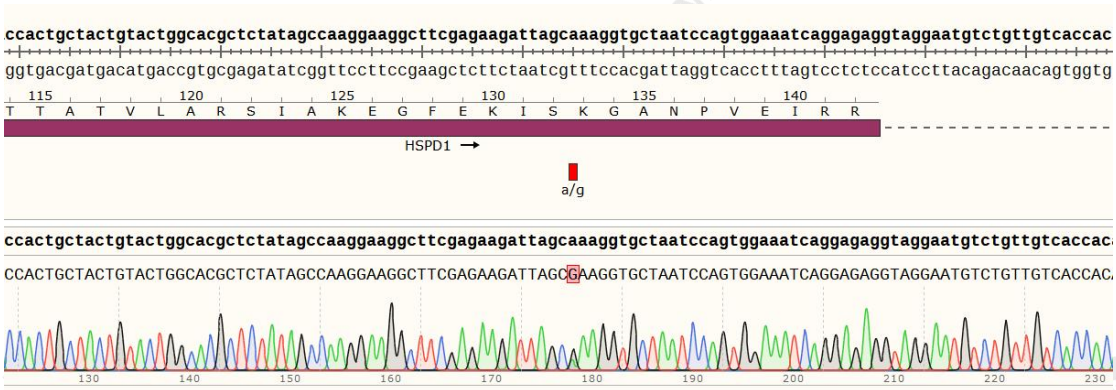

Figure 2. Sanger Sequencing Results of HSPD1 Gene c.A391G Point Mutation in HeLa Cell Pool

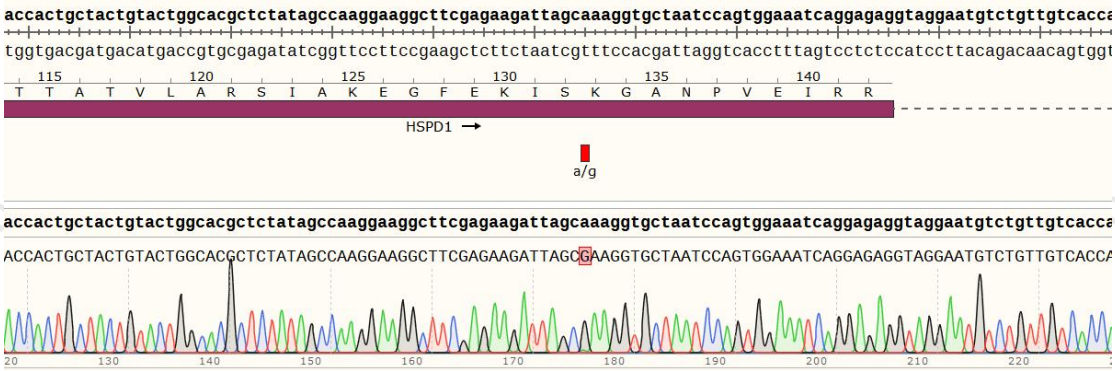

Figure 3. Sanger Sequencing Results of HSPD1 Gene c.A391G Point Mutation in HeLa Single Clone Cells

●Editing Efficiency of Various Point Mutation Cell Pools

Cell Type	Cell Pool Editing Efficiency
293T	★★★
Hela	★★★
A549	★★★★
HepG2	★★★★
MDA-MB-231	★★★★
HCT116	★★★
U2OS	★★★
Ishikawa	★★★
R28	★★★
SN4741	★★★

Editing Efficiency（%） ★★★★ ＞81%，★★★ 51~80%，★★ 21~50%

► Notes

(1) This product is intended for research use only in laboratory settings. Please comply with relevant laws, regulations, and ethical requirements; any consequences from improper use are the sole responsibility of the user.

(2) Follow the guidelines for transportation, storage, and use of reagents. Avoid repeated freeze-thaw cycles unless necessary. The company is not responsible for experimental failures due to improper storage or handling.