Mutation Detection Kit

Intact Genomics®

Catalog #	3173	3176
Package Size	25 Reactions	100 Reactions

Description

Double-stranded breaks (DSBs) generated by CRISPR/ TALEN/ZFN at desired target sites can be PCR-amplified, and the PCR products can be denatured and re-annealed to form mismatched DNA. If the mismatched DNA length position is more than 1 bp, T7 endonuclease I can recognize and cleave it. It is useful for quantitatively estimating the nuclease-induced mutation frequency of gene edited cells.

Product Includes

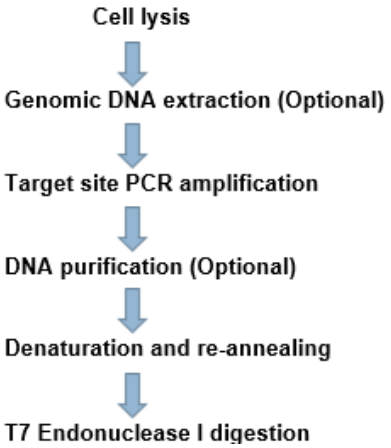
- i7™ high fidelity 2x PCR master mix
- T7 Endonuclease I
- 10x T7 Endonuclease I reaction buffer

Storage Temperature: –20 °C

Protocol

This protocol describes how to determine genome targeting efficiency by digesting annealed mismatched PCR products with T7 Endonuclease I. In the first step, PCR products are produced from the genomic DNA of cells whose genomes were targeted using Cas9, TALEN, ZFN etc. In the second step, the PCR products are annealed and digested with T7 Endonuclease I. If two shorter fragments of the expected size are generated, which means that it has successfully introduced mutations at the targeted chromosomal site. Fragments are analyzed to determine the efficiency of genome targeting.

Description



Procedure

I. Sample Preparation

Option 1: Genomic DNA extraction

Harvest cells and extract genomic DNA according to the manufacturer extraction protocol.

Option 2: Cell lysate preparation

Collect cells and add 50-100 µL Lysis buffer and lyse cells at 95° C for 5-10 min.

II. PCR Amplification

1. Thaw kit components, mix and pulse-spin in micro-centrifuge before use.
2. Assemble the following reaction:

Reagent	50 µL reaction
Genomic DNA	100-200 ng
Forward primer (10 µM)	2.5 µL
Reverse primer (10 µM)	2.5 µL
i7 2x PCR Master Mix	25.0 µL
H_2O up to	50.0 µL

3. Mix the reaction and run PCR with the following thermocycling conditions:

PCR cycling conditions:			
Steps	Temperature	Time	Cycles
Initial denaturation	95° C	3 min	1
Denaturation	95° C	10 sec	30
Annealing	Tm-3° C	20 sec	
Extension	72° C	30-45 sec	
Final extension	72° C	5 min	1

4. Run 3-5 µL of the PCR product on a 1-2 % agarose gel. The final PCR product should be within the range of 600 –1000bp.
5. (Optional) If necessary, purify the DNA by using either gel extraction kit or ampure XP beads according to the manufacturer protocol

III. Hybridization

1. Assemble reactions as follows:

Component	50 µL reaction
DNA (wt)	15.0 µL
DNA (mutant)	15.0 µL
10x Endonuclease I buffer	5.0 µL
H_2O	15.0 µL
Total	50.0 µL

2. Mix the reaction gently.
3. Heat at 95° C for 5 min by using a heat block. Thermocyler can also be used for hybridization.
4. Turn off the heat block and cool down gradually to room temperature.

IV. T7 Endonuclease I Digestion

1. Assemble reactions as follows:

Hybridized DNA	5.0 µL
10x Endonuclease I buffer	1.0 µL
T7 Endonuclease I (10 U)	1.0 µL
H_2O up to	10.0 µL

2. Incubate at 37° C for 15-30 min.
3. Stop the reaction by adding 1.0 µl of 0.5 M EDTA.
4. Run 1-2 % agarose gel to see the cleavage efficiency. A typical gel for Endonuclease I (Endo I) cleavage is shown below:

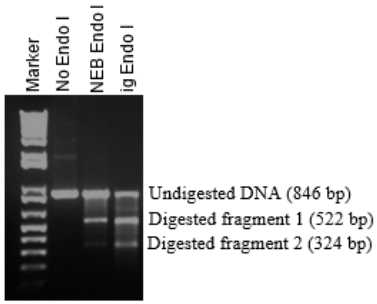


Figure 1: T7 endonuclease I activity testing: Double-stranded (ds) DNA fragments containing two types of sequences are PCR amplified, denatured and annealed to produce mismatched (heterologous) dsDNA. Purified mismatched dsDNA digested with Endonuclease I from NEB and Intact Genomics (ig) to compare the enzyme efficiency.

Related Products

- Taq DNA Polymerase(Cat.# 3243)
- Taq DNA Polymerase 2x Premix(Cat.# 3249)
- T4 DNA Ligase(Cat.# 3212)
- ig® 10B Chemically Competent Cells(Cat.# 1011-12)

Technical Support

Intact Genomics is committed to supporting the worldwide scientific research community by supplying the highest quality reagents. Each new lot of our products is tested to ensure they meet the quality standards and specifications designated for the product.

Please follow the instructions carefully and contact us if additional assistance is needed. We appreciate your business and your feedback regarding the performance of our products in your applications.

Troubleshooting

Problems	Possible Causes	Recommendations
Non-specific cleavage	Non-specific PCR amplification	▪ Purify DNA ▪ Optimize PCR primers ▪ Optimize PCR conditions.
No cleavage	Low T7 Endonuclease I activity	▪ Add more T7 Endo I
No expected bands	Incomplete lysis Poor PCR primer	▪ Adjust the volume of lysis buffer according to the cell number ▪ Increase the lysis time ▪ Optimize PCR primers