Protocol

Dharmacon™ Edit-R™ guide RNA control kits via T7EI assay

Version: 2

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For research only. Not for use in diagnostic procedures.

Product Description

The Edit-R control kits provide validated guide RNAs and primer pairs for detection of cleavage for the designated housekeeping gene or safe harbor region.

These gene-specific positive control kits and region-specific cutting control kits are validated for mismatch detection assays to verify gene editing experiments. The following protocol is an example of thermal cycling and cleavage conditions for a DNA mismatch detection assay using T7 Endonuclease I (T7EI) and the Edit-R control primer set utilizing genomic DNA (gDNA) from direct cell lysis. The same primer pairs can also be used for "Tracking of Indels by Decomposition" (TIDE) analysis. The volumes in this protocol are for direct cell lysis in a 96-well plate format.

T7EI Protocol

1. Lyse cells in 100 µl of 1x Phusion High-Fidelity buffer with additives.

Reagent	Volume	Final concentration
5x Phusion HF Buffer	20 µL	1x
Proteinase K (~ 20 mg/mL)	5 µL	~ 1 mg/mL
RNase A (10 mg/mL)	5 µL	0.5 mg/mL
Water, nuclease free	70 µL	N/A

2. Seal the 96-well tissue culture plate with a plate seal to minimize evaporation and cross contamination. Incubate for 15-30 minutes at 56 °C.

3. Transfer cell lysate into a 96-well PCR-plate, seal plate with a plate seal, and place into a thermocycler for 5 minutes at 96 °C. Briefly centrifuge the plate to collect liquid at the bottom of each well.

***Note:* Due to variable depth of thermocycler heat blocks, deactivation time may need to be increased to 15 or 30 minutes.**

4. Set up 50 µl PCR reaction for each sample to be analyzed.

***Note:* Other PCR reagents can be used provided that PCR optimization is performed for mismatch detection conditions and testing compatibility with direct cell lysis or purified gDNA.**

Reagent	Volume	Final Concentration
5x Phusion HF buffer	10 µL	1x
Forward primer (50 µM)	0.5 µL	500 nM
Reverse primer (50 µM)	0.5 µL	500 nM
dNTPs (10 mM)	1 µL	200 µM each
Phusion Hot Start II High-Fidelity DNA Polymerase (2 U/µL)	1 µL	0.04 U/µL
Water, nuclease free	32 µL	N/A
Cell lysate	5 µL	N/A

5. Thermal cycling conditions:

Cycle step	Temperature	Time	Cycles
Initial denaturation	98 °C	3 min	1
Denature	98 °C	10 s	10
Touchdown annealing	72 °C – 1 °C/cycle	15 s	
Extension	72 °C	30 s	
Denature	98 °C	10 s	25
Annealing	62 °C	15 s	
Extension	72 °C	30 s	
Final extension	72 °C	10 min	1

6. Heat PCR samples to 95 °C for 10 minutes and then slowly (>15 min) cool to room temperature (~25 °C).

7. PCR product (~5 µl) can be run on a 2% agarose gel to confirm amplification.

8. Set up 15 µl reactions for mismatch detection assay using T7EI. Be sure to include at least one sample without the T7EI enzyme (No enzyme negative control and/or untreated (no gene editing negative control).

Note: Other mismatch detection assays can be used following the manufacturer's recommended protocol.

9. Incubate for 25 minutes at 37 °C.

10. Immediately run entire reaction volume with appropriate gel loading buffer on 2% agarose gel, since the T7EI enzyme cannot be heat inactivated. Compare samples with expected gene editing to the no enzyme and/or untreated negative controls.

Materials

- Thermo Scientific Phusion Hot Start II High-Fidelity DNA Polymerase, 2 U/µL (Cat #F-549S)
- T7 Endonuclease I, 10 U/µL (NEB, Cat #M0302S)
- Thermo Scientific Proteinase K,~ 20 mg/mL (Cat #EO0492)
- Thermo Scientific RNase A, 10 mg/mL (Cat #EN0531)
- Thermo Scientific 5x Phusion HF Buffer (Cat #F-518L)
- NEBuffer 2 (NEB, Cat #B7002S)

Reagent	Volume	Final concentration
PCR from gDNA (300-500 ng/reaction)	10 µL	variable
Water, nuclease free	3 µL	N/A
NEBuffer 2 (10x)	1.5 µL	1x
T7 Endonuclease I (10 U/µL)	0.5 µL	0.33 U/µL

Target gene/ region	Species	Guide RNA target sequence	Primer	Primer sequence	Tm* (°C)	Expected band sizes (bp)	
						No editing	With editing
PPIB	Human	GUGUAUUUUGACCUACGAAU	Forward	GAACTTAGGCTCCGCTCCTT	64.0	505	~ 330
			Reverse	CTCTGCAGGTCAGTTTGCTG	64.3		~ 174
DNMT3B	Human	GCUGAAUUACUCACGCCCCA	Forward	TGAGAAGGAGCCACTTGCTT	64.4	544	~ 335
			Reverse	GACCAAGAACGGGAAAGTCA	64.4		~ 209
AASVI_sg1	Human	GACGCAAGGGAGACATCCGT	Forward	ACTAGCTGAGCTCTCGGACC	63.1	481	~272
			Reverse	GCTGCCCAAGGATGCTCTTT	68.0		~209
AASV1_sg2	Human	GCGGCTCCAATTCGGAAGTG	Forward	GGCCAGGCAGATAGACCAGA	66.9	514	~303
			Reverse	CCAACGCCGACGGTATCAG	69.1		~211
AASV1_sg3	Human	GAGTTCCGGCGGCACAGCAA	Forward	GAACCCAGCGAGTGAAGACG	67.6	590	~427
			Reverse	CTCTTCCGATGTTGAGCCCC	68.4		~163
Ppib	Mouse	GUAUACUUUGAUUUACAAAU	Forward	CCAGCCTGTTTGTTGGATTT	64.2	572	~409
			Reverse	ACTGTGAGGCTGCCAGATTT	64.2		~163
Dnmt3b	Mouse	GCUGAAUUAUACCCGCCCCA	Forward	ACTTGGTGATTGGTGGAAGC	64.3	561	~372
			Reverse	TTAAGCCACACCCTCCTCTG	64.5		~189
Rosa26_sg1	Mouse	ATGTCTTTAATCTACCTCGA	Forward	AGAACTGCAGTGTTGAGGCC	59.6	586	~358
			Reverse	TTCTGGGCAGGCTTAAAGGC	59.6		~228
Rosa26_sg2	Mouse	TGGGAGGATAGGTAGTCATC	Forward	AGAACTGCAGTGTTGAGGCC	59.6	586	~428
			Reverse	TTCTGGGCAGGCTTAAAGGC	59.6		~158
Rosa26_sg3	Mouse	CGACAAAACCGAAAATCTGT	Forward	AGAACTGCAGTGTTGAGGCC	59.6	934	~494
			Reverse	CGTTTCCGACTTGAGTTGCC	58.8		~440

***Tm calculated specifically for use with Phusion DNA polymerases. Tm should be calculated for specific DNA polymerases and PCR reagents using appropriate parameters.**

For more information

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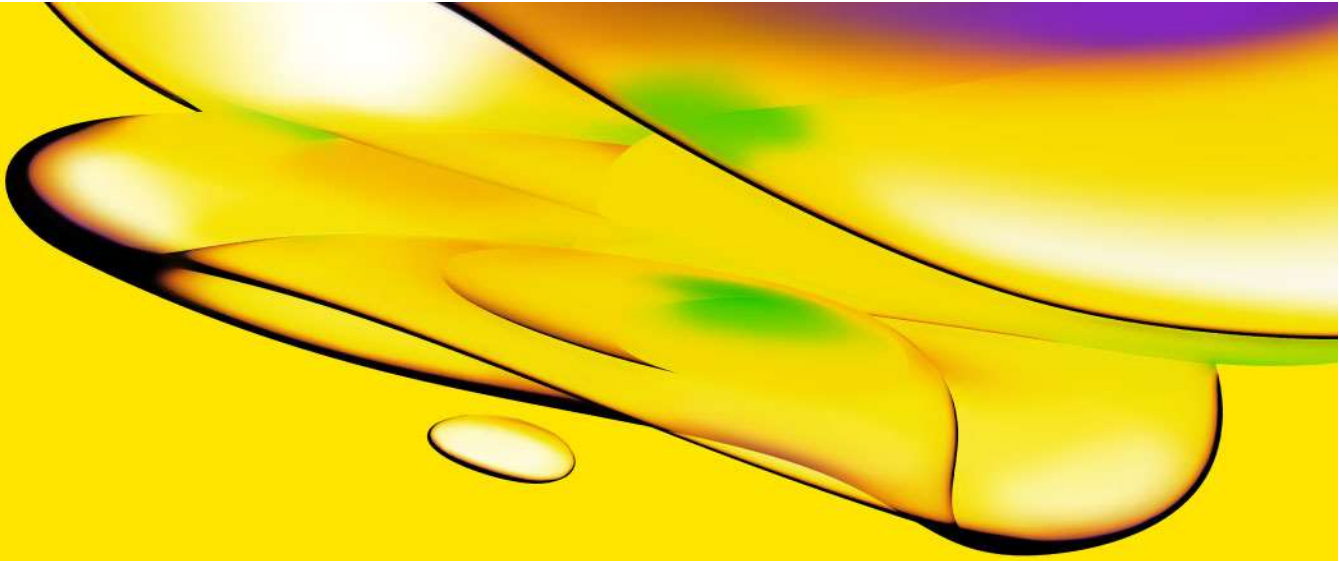

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