PROTOCOL

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Dharmacon™ Edit-R™ synthetic gRNA positive control kits

Product description

The Edit-R Synthetic Positive Control kits provide validated crRNAs and primer pairs for detection of cleavage for the designated human, mouse, or rat positive control gene.

These gene-specific positive controls and 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 crRNA Control primer set utilizing genomic DNA (gDNA) from direct cell lysis. For direct cell lysis, cells were treated for gene editing in a 96-well format.

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 plate with a plate seal to minimize evaporation and cross contamination. Incubate for 15-30 minutes at 56 °C, followed by deactivation for 5 minutes at 96 °C. Briefly centrifuge plate to collect liquid at bottom of wells.
3. Set up 50 µL PCR 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

4. Thermal cycling condition

Cycle step	Temperature	Time	Cycle(s)
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

5. Heat PCR samples to 95 °C for 10 minutes and then slowly (> 15 minutes) cool to room temperature (~ 25 °C).
6. PCR product (~ 5 µL) can be run on 2% agarose gel to confirm amplification.

7. 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.

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

8. Incubate for 25 minutes at 37 °C.
9. Immediately run entire reaction volume with appropriate gel loading buffer on 2% agarose gel, since 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)

Species	Gene target	Species	Primer	Sequence	Tm* (°C)	MW	Extinction coefficient	Expected band sizes (bp)	
								No editing	With editing
UK-007050-01-xx	*PPIB*	Human	Forward Reverse	GAACTTAGGCTCCGCTCCTT CTCTGCAGGTCAGTTTGCTG	64.0 64.3	6044.0 6115.0	176184 178151	505	~ 330, ~ 174
UK-007150-01-xx	*Ppib*	Mouse	Forward Reverse	CCAGCCTGTTTGTTGGATTT ACTGTGAGGCTGCCAGATTT	64.2 64.2	6105.1 6148.1	176905 191297	572	~ 409, ~ 163
UK-007250-01-xx	*Ppib*	Rat	Forward Reverse	TGCCATTTCCTCACTCCCTA GGCTACCGCTACAAATGGAC	60.4 62.4	5938.9 6111.0	165727 197820	524	~ 342, ~ 170
UK-007060-01-xx	*DNMT3B*	Human	Forward Reverse	TGAGAAGGAGCCACTTGCTT GACCAAGAACGGGAAAGTCA	64.4 64.4	6157.1 6193.1	197197 220800	544	~ 335, ~ 209
UK-007160-01-xx	*Dnmt3b*	Mouse	Forward Reverse	ACTTGGTGATTGGTGGAAGC TTAAGCCACACCCTCCTCTG	64.3 64.5	6228.1 5972.9	199164 174218	561	~ 372, ~ 189
UK-007260-01-xx	*Dnmt3b*	Rat	Forward Reverse	GGCATTGATCCCATGTAACC CTCCGAGCTGCTAAGAGGAA	60.4 62.4	6077.0 6151.0	189330 201754	532	~ 319 ~ 212

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

If you have any questions, contact

t +44 (0) 1223 976 000 (UK) **or** +1 800 235 9880 (USA); +1 303 604 9499 (USA)
f + 44 (0)1223 655 581
w horizondiscovery.com/contact-us **or** dharmacon.horizondiscovery.com/service-and-support
Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

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