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Protocol status: Working
We use this protocol and it's working

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Abstract

Primers

- CLYBL insertion of hNIL
- CLYBL insertion of mag-hNIL

Attachments

PDF

fernandopulle2018.pd...

1.7MB

Guidelines

Primers

CLYBL insertion of hNIL:

C1	5'	TGACTAAACACTGTGCCCCA	3'
C2	5'	AGGCAGGATGAATTGGTGGA	3'
C3	5'	CAGACAAGTCAGTAGGGCCA	3'
C4	5'	AGAAGACTTCCTCTGCCCTC	3'

Run PCR products on 1 % agarose gel (Voytas, 2001). The C1/C2 primer pair will yield a 790-bp band if the cells have at least one wild-type allele (i.e., no insertion or heterozygous insertion). With homozygous insertion, no band will be produced. The C3/C4 pair will yield a band of 1080 bp with hNIL insertion to the CLYBL site; this band will be lost with successful Cre excision.

CLYBL insertion of mag-hNIL:

C1	5'	TGACTAAACACTGTGCCCCA	3'
C2	5'	AGGCAGGATGAATTGGTGGA	3'
C3	5'	CAGACAAGTCAGTAGGGCCA	3'
CM4	5'	AGGCCTTCCATCTGTTGCT	3'
CM5	5'	TGCCAAGTGGGCAGTTTAC	3'

Run PCR products on 1 % agarose gel (Voytas, 2001). The C1/C2 primer pair will yield a 790-bp band if the cells have at least one wild-type allele (i.e., no insertion or heterozygous insertion). With homozygous insertion, no band will be produced. The C3/CM4 pair will yield a band of 1410 bp with mag-hNIL insertion. Upon successful Cre excision, the C1/CM5 primer pair will amplify a product of 816 bp, with an amplicon at 4139 bp if editing does not occur.

AAVS1 insertion of NGN2:

A1	5'	GGAATCTGCCTAACAGGAGGT	3'
A2	5'	CGGTTAATGTGGCTCTGGTT	3'
A3	5'	CCCCCAGAATAGAATGACACC	3'

Run PCR products on 1 % agarose gel (Voytas, 2001). The A1/A2 primer pair will yield a 163-bp band if the cells have no or heterozygous insertion of hNGN2 into AAVS1. With homozygous insertion, no band will be produced. The A2/A3 pair will yield a band of 1100 bp with hNGN2 insertion to the AAVS1 site that will be reduced to 229 bp with successful Cre excision at the loxP sites.

Safety warnings

Please see SDS (Safety Data Sheet) for hazards and safety warnings.