NT-3lac Zneo ID 191

Description: NT-3 mutant generated at UCSF by Kevin Jones. LacZ inserted in frame at ATG in coding region followed by neo casette. Neo casette not excised.
Received From:
Genotyping:

I recommend to use Eric's primer because it works nicer.

wildtype allele
primers: MNTIF, MNTIR
conditions:
hot start before cycling (94-95^{o}C for 2 to 3 minutes)
30 cycles(94^{o}C 1', 65^{o}C 1', 72^{o}C 1')
extra extension time at the end (10 minutes at 72^{o}C) before going to 4^{o}C

band size: 150 base pairs
MNTIF: ACT ACG GCA ACA GAG ACG CTA C
MNTIR: ACA GGC TCT CAC TGT CAC ACA C

mutant allele
primers: MN3PS7, LACZN2
conditions:
hot start before cycling (94-95^{o}C for 2 to 3 minutes)
33 cycles(94^{o}C 1', 65^{o}C 1'30", 72^{o}C 2')
extra extension time at the end (10 minutes at 72^{o}C) before going to 4^{o}C

band size: 1700 base pairs
MN3PS7: TAG AAG ATT GTC GGA AAT GTC ACT TTC C
LACZN2: GGG ATG TGC TGC AAG GCG ATT AAG TTG

NT-3 lac Z (Eric's primers)

wildtype allele
primers: MNT3-IF, MNT3-IR
conditions:
hot start before cycling (94-95^{o}C for 2 to 3 minutes)
25 cycles(95^{o}C 1', 72^{o}C 1')
extra extension time at the end (10 minutes at 72^{o}C) before going to 4^{o}C

band size: 200 base pairs

mutant allele
primers: PGK2, SV40F
conditions:
hot start before cycling (94-95^{o}C for 2 to 3 minutes)

28 cycles(95^{o}C 1', 65^{o}C 1', 72^{o}C 1')
extra extension time at the end (10 minutes at 72^{o}C) before going to 4^{o}C

band size: 150 base pairs

Background:
Original: 129/Bl6
Maintenance:
Reference: Farinas I, Jones KR, Backus C, Wang XY, Reichardt LF. Severe sensory and sympathetic deficits in mice lacking neurotrophin-3. Nature. 1994 Jun 23;369(6482):658-61.
PMID: 8208292 [PubMed - indexed for MEDLINE]