Protocol for genotyping LE-Tg(EF1a-TetR)1Ottc transgenic rats January 24, 2018

Genomic DNA Preparation by Macherey-Nagel Tissue Spin Columns

Using this kit according to the manufacturer's protocol is the preferred way for preparing genomic DNA at OTTC when it is intended for ddPCR (i.e. copy-number quantification). Typically, 10 to 90 ng of genomic DNA are used in an 11-25uL PCR reaction.

Genomic DNA Preparation by the "Hot Shot" method

This method was adapted by NIDA-OTTC from the protocol originally described in Biotechniques 29(1), 52-54 (2000). It has been used successfully with ear punches and tail clips of rats. This method is quicker and more cost-effective when compared to the MN Tissue Spin columns, but in our experience, it does not produce templates that are reliable for ddPCR and quantitative analysis. Typically, 1uL of template is sufficient for an 11-25uL PCR reaction.

Place the biopsy sample in 1.5ml microfuge tube.
Add 300 microliters of 50mM NaOH
Incubate tubes at 95C for 60 minutes.
Vortex tubes on medium power setting for 5 seconds.
Quick spin the tubes to bring down the condensation.
Neutralize each sample by adding 30 microliters of 1M Tris-HCl (pH 8).
Vortex tubes on medium power setting for 5 seconds.
Quick spin the tubes to bring down the condensation.

Debris (the "undigested" remnant of sample) may remain visible at the bottom of the tube. This is OK, but be sure to take only from the supernatant when setting the PCR reaction.

Use 1uL of this supernatant in a PCR reaction (11uL – 25uL final volume).

LE-Tg(EF1a-TetR)1Ottc

Primer Name	**Primer Sequence (5' to 3')**
TETR-F34	AGC GCA TTA GAG CTG CTT AAT GAG
TETR-R412	CCA CAG CGC TGA GTG CAT ATA ATG

These oligos produce a 378 bp amplicon, using with OneTaq polymerase with 68oC annealing and 1min extension.

This protocol was updated on 11-28-2017 by CR.
Any questions regarding protocol, contact nidatransgenicprojects@mail.nih.gov.