

Protocol for genotyping LE-Rosa26Tm1(LSL-Cas9)Ottc transgenic rats February 27, 2019

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 a 25uL PCR reaction.

General PCR reaction setup:

- 12.0 uL 2x Q5 master mix (New England Biolabs)
- 12.0 uL 2x specific oligos (1 uM Forward + 1uM Reverse; in water)
- 1.0 uL genomic DNA
- 25.0 uL PCR reaction

Thermocycler Programs:

PCR Program CR2232		
<u>Line</u>	<u>Temp</u>	<u>Time</u>
Step 1	98oC	HOLD (hot start)
Step 2	98oC	30 sec
Step 3	98oC	10 sec
Step 4	70.8oC	30 sec
Step 5	72oC	2 min
Step 6	Go to Step 2	Repeat 34x
Step 7	72oC	5 min
Step 8	12oC	HOLD

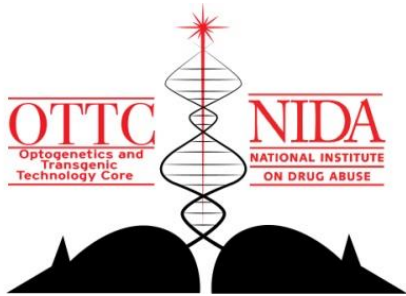
PCR Program CR1943		
<u>Line</u>	<u>Temp</u>	<u>Time</u>
Step 1	98oC	HOLD (hot start)
Step 2	98oC	30 sec
Step 3	98oC	10 sec
Step 4	60oC	30 sec
Step 5	72oC	2 min
Step 6	Go to Step 2	Repeat 34x
Step 7	72oC	5 min
Step 8	12oC	HOLD

Primer Sequences

rRosa26 F64755	GCCTAAAGAAGAGGCTGTGCTC	5' junction
CAG R366	CCATGGTAATAGCGATGACTAATACGTAGATG	5' junction
Cas9 F4889	CAGGCCGAGAATATCATCCACC	3' junction
rRosa26 R67001	TTCTGCATTCCAGAAGGAACCTTTTATAGAG	3' junction

This protocol was updated on 02-27-2019 CR.

Any questions regarding protocol, contact nidatransgenicprojects@mail.nih.gov.



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Assay for the 5' junction

These oligos (rRosa26 F64755 and CAG R366) produce a 1540 bp amplicon and work with Q5 polymerase and program CR2232.

Assay for the 3' junction

These oligos (Cas9 F4889 and rRosa26 R67001) produce a 1600 bp amplicon and work with Q5 polymerase and program CR1943.

This protocol was updated on 02-27-2019 CR.

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