

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	31763
MMRRC Strain Name	STOCK <i>Dbh^{tm1Rpa}</i> Tg(DBH)2Dwei /Mmnc
Gene Name(s)	Human Dopamine beta-hydroxylase (DBH)
Breeding Protocol	Random Intra-Strain Mating (mice can be all coat colors due to mixed background)
Protocol Date	10/14/13

MMRRC# 31763 PCR Reaction

NOTE: There are 2 separate reactions per DNA sample: BAC and DBH. BAC uses a 4-primer mix, DBH a 3-primer mix. Water is adjusted accordingly. Run separately on gels

Thermal Cycler:

94°C for 45 sec
50°C for 45 sec
72°C for 90 sec
35x

Taq: **Apex and Chromataq 10X Buffer**

Bands:

BAC - Tg +: 375bp
WT no band
DBH – MUTANT: 255bp
WT: 375bp

	<u>1X</u>
DNA	1.0
5X Buffer	5.0
10mM dNTPs	0.5
40 uM Primer F/R-BAC	2.0
30 uM DBH	1.5
Taq	1.0
ddH ₂ O - BAC	14.0
- DBH	14.5
25mM MgCl ₂	2.0

Primer sequences 5' to 3': Primers are 10uM with respect to each primer.

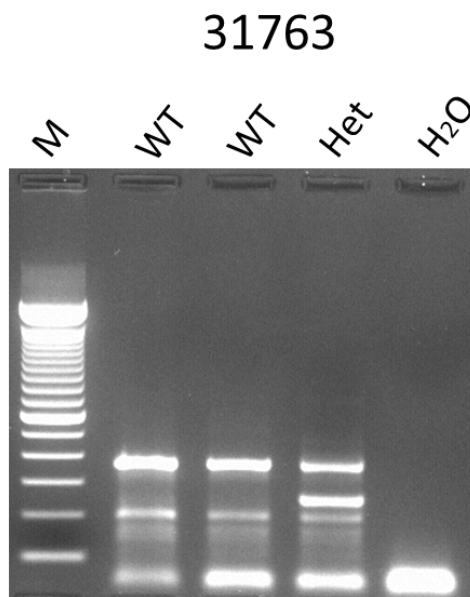
BAC

Primer 73 (leptin) [TGTC AAGATGGACCAGACTC]
 Primer 74 (leptin) [ACTGGTCTGAGGCAGGGAGCA]
 Primer SEQ-R (BAC) [GCGAGAGCATCATAGGGAGT]
 Primer 1.3F (BAC) [GCCCTCAGTCTACTTGC GGGA]

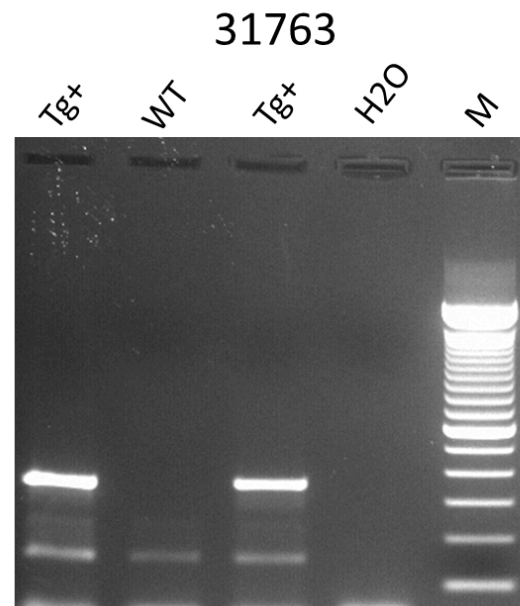
DBH

Primer 1 [GACAGCGCTGGGTACAGCTGACC]
 Primer 2 [CTTTCCTGAGTGACAGTGAGTGG]
 Primer 3 [CCTGTGCTCTAGTAGCTTTACGG]

Run on 2.0% agarose gel in TAE.



DBH reaction
 M: 100 bp DNA ladder (Invitrogen)



BAC reaction
 M: 100 bp DNA ladder (Invitrogen)