

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	31783
MMRRC Strain Name	STOCK Tg(DBH)3Dwei <i>Dbh</i> ^{tm1Rpa}
Gene Name(s)	Human Dopamine beta-hydroxylase (DBH)
Breeding Protocol(s)	Random Intra-Strain Mating Backcross to C57B6/J (mice can be all coat colors due to mixed background)
Protocol Date	10/14/13

MMRRC# 31783 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 +: 375
WT: no band
DBH – WT: 375
MUTANT: 255

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

Primers are 10uM with respect to each primer.

Primer sequences 5' to 3':

BAC

Primer 73 (leptin) [TGTCCAAGATGGACCAGACTC]
Primer 74 (leptin) [ACTGGTCTGAGGCAGGGAGCA]
Primer SEQ-R (BAC) [GCGAGAGCATCATAGGGAGT]
Primer 1.3F (BAC) [GCCCTCAGTCTACTTGCGGGA]

DBH

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

Run on 2.0% agarose gel in TAE.

