From: Heather Hopkins [hhopkins@u.washington.edu]
Sent: Tuesday, November 21, 2006 5:18 PM
To: sacoord@mmrrc.org
Subject: CMGCC/Ladiges Submission: RecqL4 PCR Genotyping Protocol

Description: Recql4 wildtype band ~ 280 and gene trap band ~ 460. F-Recql4 and R-Recql4 WT detect endogenous wildtype sequence in the intron between exon 20 and exon 21. F-Recql4 and R-Recq14 NEO detects presence of gene trap in the intron between exon 20 and exon 21.

Primers:

F-Recql4 5' –AGA CCC TGG TCA GCT ACT ACT TTG AG – 3'

R-Recql4 WT 5' –TGA AGC TGT GGG AGG ATA GCA TGT – 3'

R-Recq14 NEO 5' –TAG AAG CGG AAG AGG CTG GCG AA – 3'

Reaction Mix (For one 25ul reaction):

H2O	12.85 ul
10X PCR Buffer	2.5 ul
25mM MgCl2	1.5
25 mM dNTP	0.2 ul
25 uM F Recql4	0.75 ul
25 uM R Recql4 WT	0.5ul
25 uM R Recq14 NEO	0.5 ul
5X Rapid-Load Buffer*	5.0ul
Taq DNA Polymerase	0.2 ul
DNA	1.0 ul

*= 5X R.L. Buffer – OriGene Technologies, Catalog # RL-105

Thermocycler Settings:

95C for 2 min

Cycle 35 times

95C for 20 sec

62C for 20 sec

72C for 30 sec

72C for 2 min

10C forever

Electrophoresis:

Run on 2% Gel

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Heather C. Hopkins
Research Coordinator
Department of Comparative Medicine, T-138 HSB
University of Washington
Box 357190
Ph: 206-616-2027
Fax: 206-685-3006