

FAX TRANSMITTAL FORM

Date: November 8th, 2004

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Number of pages including this page: 2

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Comments:

Here is the genotyping protocol
for MMR C # ~~9976~~
9979

AIH Genotyping Information
Chien-Chang Chen
Modified Oct 21, 2004

-This reaction uses 3 oligo primers to identify wild type or mutant $\alpha 1H$ genotypes. The primers used are:

- Oligo #2194** ATTCAAGGGCTTCCACAGGGTA which is a forward primer of mouse $\alpha 1H$
- Oligo #2293** CATCTCAGGGCCTCTGGACCAC which is a reversed primer of mouse $\alpha 1H$
- Oligo #2158** GCTAAAGCGCATGCTCCAGACTG which is a reverse primer within the *neo* cassette inserted in the $\alpha 1H$ knockout animals. (pair with 2293)

-The wild-type product will be ~480 bp product

-The mutant product will be ~330 bp product

PCR RXN conditions:

2 ul	genomic DNA- tail genomic DNA (1 cm tail clip dissolved in 300 ul TE) processed by isopropanol precipitation method
2.5 ul	10X PCR RXN buffer
1.25 ul	dNTPs (2.5mM Stock)
1 ul	25 mM MgCl ₂
1 ul	Primer 1: #2194 (10 uM stock)
0.6 ul	Primer 2: #2293 (10 uM stock)
0.6 ul	Primer 3: #2158 (10 uM stock)
0.25 ul	TAQ polymerase
15.8 ul	dH ₂ O

25ul overlay with mineral oil

PCR RXN program

2 min 94°C
 30 cycles of:
 94°C 30 sec
 70°C 30 sec
 72°C 30 sec
 Final extension:
 72°C 5 min