

MMRRC UNC – Genotyping Protocol

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|-----------------------------|---|
| MMRRC Strain ID | 36976 |
| MMRRC Strain Name | B6N.129P2-Scp2 ^{tm1Fsch} Fabp1 ^{tm1Bin} /Mmnc |
| Gene Name(s) | sterol carrier protein 2, liver (Scp2) fatty acid binding protein 1, liver (Fabp1) |
| Breeding Protocol(s) | Sib-mating |
| Protocol Date | 8/18/14 |

MMRRC #36976 PCR Reactions

Scp2 Reaction

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 58°C for 30 sec

Step 4: 72°C for 45 sec

Step 2 to 4 Cycles: 30

Step 5: 72°C for 7 min

Taq: **Apex Taq and Chromataq 5X Buffer**

| | |
|------------------------|-----------|
| | <u>1X</u> |
| ddH ₂ O | 11 |
| 5X Buffer | 5.0 |
| 25mM MgCl ₂ | 2 |
| 10mM dNTPs | 0.5 |
| 10uM WT Primer | 1 |
| 10uM Mutant Primer | 1 |
| 10uM Common Primer | 2 |
| Taq | 0.5 |
| DNA | 2 |

Bands: MUTANT: 325bp
WT: 550bp

Primer sequences 5' to 3':

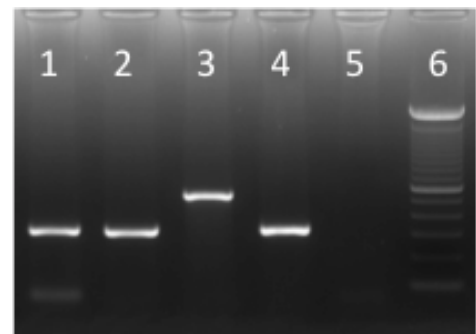
Scp2(36974)Com: TCAAAGCAGCAGTGCAGGCA

Scp2(36974)WT: GTA GAG AGC CCC AAG TGT TAG GAG AAG

Scp2(36974)Mut: GCT TCC TCG TGC TTT ACG GT

Run on 2% agarose gel.

1. Homozygous Sample
2. Homozygous Sample
3. Wild-type Control
4. Homozygous Positive Control
5. Negative Control
6. 100bp Marker



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Fabp1 Reaction

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 58°C for 30 sec
 Step 4: 72°C for 45 sec
 Step 2 to 4 Cycles: 35
 Step 5: 72°C for 7 min

| | <u>1X</u> |
|------------------------|------------------|
| ddH ₂ O | 9 |
| 5X Buffer | 5 |
| 25mM MgCl ₂ | 2 |
| 10mM dNTPs | 0.5 |
| 10 mM WT 1 & 2 | 2/2 |
| 10 mM KO 1&2 | 1/1 |
| Taq | 0.5 |
| DNA | 2 |

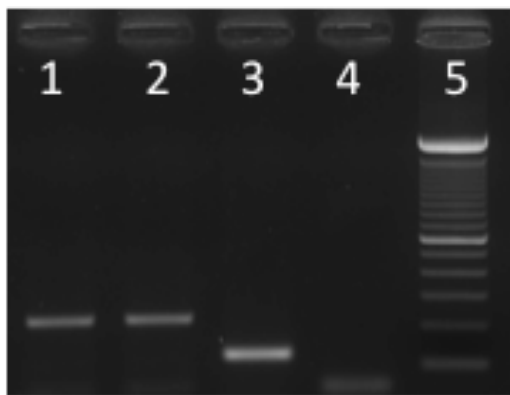
Taq: **Apex Taq and Chromataq 5X Buffer**

Bands: MUTANT: 227bp
 WT: 123bp

Primer sequences 5' to 3':

Fab(36976)WT1: caaggggtgtcagaaatcgtgc
 Fab(36976)WT2: ccagtcatggtctccagttcgca
 Fab(36976)KO1: aagagcttggcggcgaatgg
 Fab(36976)KO2: tggccatttggctgtgctc

Run on 2% agarose gel.



1. Homozygous Sample
2. Homozygous Sample
3. Wild-type Control
4. Negative Control
5. 100bp Marker