

## **MMP PCR Genotyping**

- A. MMP2 (Gelatinase A)**
- B. MMP3 (Stromelysin -1)**
- C. MMP7 (Matrilysin -(PUMP-1))**
- D. MMP9 (Gelatinase B)**
- E. MMP13 (Collagenase 3)**
- F. MMP14 (MT - MMP -1)**

## Suggested Materials:

1. HotStarTaq Plus DNA Polymerase Cat # 203603 (QIAGEN)
2. GeneAmp dNTP Mix 10mM (2.5 mM each) Cat # N8080260 (Applied Biosystems)

### A. MMP2

#### 1. Primers:

WT	Ex1.S2	CAA CGA TGG AGG CAC GAG TG
	Ex1.A1	GCC GGG GAA CTT GAT GAT GG
KO	Ex2.A2	TGT ATG TGA TCT GGT TCT TG
	Pgkneo.A1	TGC AAA GCG CAT GCT CCA GA

#### 2. Recipe:

	Reaction (25ul)
ddH <sub>2</sub> O	19.75
10X PCR Buffer	2.5
dNTP 10mM	0.5
Ex1.S2 or Ex2.A2 20pmol	0.5
Ex1.A1 or Pgkneo.A1 20pmol	0.5
Taq-polymerase 5u/ul	0.25
DNA	1

#### 3. Program:

1	35 X			5	6
	2	3	4		
95°C	95°C	57°C	72°	72°C	20°C
5'	1'	1'	1'30''	5'	∞

#### 4. Expectation:

WT: 120 bp fragment

KO: 1.1kbp fragment

### B. MMP3

#### 1. Primers:

WT	ST1 391	ACC GGA TTT GCC AAG ACA GAG TG
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	<b>ST1 608</b>	<b>GCA TCT CCA TTA ATC CCT GGT CC</b>
<b>KO</b>	<b>Lab Neo3</b>	<b>GAT GGA TTG CAC GCA GGT TC</b>
	<b>Lab Neo4</b>	<b>CTT CAG TGA CAA CGT CGA GC</b>

## 2. Recipe:

	<b>Reaction (25ul)</b>
<b>ddH2O</b>	<b>19.75</b>
<b>10X PCR Buffer</b>	<b>2.5</b>
<b>dNTP 10mM</b>	<b>0.5</b>
<b>ST1 391 20pmol/ul</b>	<b>0.5</b>
<b>ST1 608 20pmol/ul</b>	<b>0.5</b>
<b>Taq-polymerase 5u/ul</b>	<b>0.25</b>
<b>DNA</b>	<b>1</b>

## 3. Program:

<b>1</b>	<b>35 X</b>			<b>5</b>	<b>6</b>
	<b>2</b>	<b>3</b>	<b>4</b>		
<b>95°C</b>	<b>95°C</b>	<b>60°C</b>	<b>72°</b>	<b>72°C</b>	<b>20°C</b>
<b>5'</b>	<b>30''</b>	<b>30''</b>	<b>45''</b>	<b>5'</b>	<b>∞</b>

## 4. Expectation:

**WT: 325 bp fragment**

**KO: 247 bp fragment**

## C. MMP7

### 1. Primers:

<b>WT</b>	<b>OIMR3575 w</b>	<b>AGA CAG CTT CCC CTT TGA TG</b>
	<b>OIMR3576</b>	<b>CTG CGT CCT CAC CAT CAG T</b>
<b>KO</b>	<b>OIMR1100 ko</b>	<b>GCT ATC AGG ACA TAG CGT TGG</b>
	<b>OIMR3576</b>	<b>CTG CGT CCT CAC CAT CAG T</b>

### 2. Recipe:

	<b>Reaction (25ul)</b>
<b>ddH2O</b>	<b>17.05</b>
<b>10X PCR Buffer</b>	<b>2.5</b>
<b>dNTP 10mM</b>	<b>1</b>
<b>OIMR 3575 20pmol</b>	<b>0.2</b>
<b>OIMR 3576 20pmol</b>	<b>1</b>
<b>OIMR 1100 20pmol</b>	<b>2</b>
<b>Taq-polymerase 5u/l</b>	<b>0.25</b>
<b>DNA</b>	<b>1</b>

### 3. Program:

1	35 X			5	6
	2	3	4		
94°C	94°C	68°C	72°	72°C	20°C
5'	30''	1'	1'	5'	∞

### 4. Expectation:

**WT: 122 bp fragment**

**KO: about 700bp fragment**

### D. MMP9

#### 1. Primers:

WT	1292	GCA TAC TTG TAC CGC TAT GG
	1293	TAA CCG GAG GTG CAA ACT GG
KO	1292	GCA TAC TTG TAC CGC TAT GG
	GelA Neo S	GAC CAC CAA GCG AAA CAT

#### 2. Recipe:

	Reaction (25ul)
ddH <sub>2</sub> O	19.75
10X PCR Buffer	2.5
dNTP 10mM	0.5
1292 20pmol	0.5
1293 20pmol or Neo S 20pmol	0.5
Taq-polymerase 5u/ul	0.25
DNA	1

### 3. Program:

1	35 X			5	6
	2	3	4		
94°C	94°C	57°C	72°	72°C	20°C
5'	1'	1'	1'	5'	∞

### 4. Expectation:

**WT: 300bp fragment**

**KO: about 800bp fragment**

### E. MMP13

### 1. Primers:

<b>WT</b>	<b>In5 anti</b>	<b>GGT GGT ATG AAC AAG TTT TCT GAG C</b>
	<b>Ex5</b>	<b>TGA TGA CGT TCA AGG AAT TCA GTT T</b>
<b>KO</b>	<b>In5 anti</b>	<b>GGT GGT ATG AAC AAG TTT TCT GAG C</b>
	<b>In2</b>	<b>CAG ACC CTA CAG TGC CAG ATT TTA G</b>
<b>FLOX</b>	<b>In5 anti</b>	<b>GGT GGT ATG AAC AAG TTT TCT GAG C</b>
	<b>Neo (flox)</b>	<b>CCA CAC TGC TCG ACA TTG</b>

### 2. Recipe:

	<b>Reaction (25ul)</b>
<b>ddH2O</b>	<b>18.25</b>
<b>10X PCR Buffer</b>	<b>2.5</b>
<b>dNTP 10mM</b>	<b>1</b>
<b>IN5 anti 10 pmol</b>	<b>1</b>
<b>Ex5 10 pmol or In2 or Neo</b>	<b>1</b>
<b>Taq-polymerase 5u/ul</b>	<b>0.25</b>
<b>DNA</b>	<b>1</b>

### 3. Program:

<b>1</b>	<b>35 X</b>			<b>5</b>	<b>6</b>
	<b>2</b>	<b>3</b>	<b>4</b>		
<b>94°C</b>	<b>94°C</b>	<b>57°C</b>	<b>72°</b>	<b>72°C</b>	<b>20°C</b>
<b>5'</b>	<b>45"</b>	<b>45"</b>	<b>1'</b>	<b>5'</b>	<b>∞</b>

### 4. Expectation:

**WT: 572 bp fragment**

**KO: 672 bp fragment**

**Flox: 370bp fragment**

## F. MMP14

### 1. Primers:

<b>KO</b>	<b>MMP14 (KO)</b>	<b>GTG CGA GGC CAG AGG CCA CTT GTG TAG CG</b>
	<b>Int5 rev</b>	<b>AGA TGG AGG AGC AGG AAT GG</b>
<b>MT</b>	<b>Ex2-fw</b>	<b>ATG GTT TAC AAG TGA CAG GCA AGG</b>

Ex4-revA	GCT CGG CAG AAT CAA AGT GG
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## 2. Recipe:

	Reaction (25ul)
ddH <sub>2</sub> O	18.25
10X PCR Buffer	2.5
dNTP 10mM	1
MMP14 -KO 10pmol	0.5
MMP14 -Int5 rev 10pmol	0.5
MMP14 -Ex2 fw 10pmol	0.5
MMP14 -revA 10pmol	0.5
Taq-polymerase 5u/ul	0.25
DNA	1

## 3. Program:

1	35 X			5	6
	2	3	4		
94°C	50°C	94°C	72°	72°C	20°C
5'	45"	45"	1.30'	5'	∞

## 4. Expectation:

**WT: >1000 bp fragment**

**KO: 100~200 bp fragment**