# Multiplex 1 PCR-SSP - CR1(rs3737002; rs11118131; rs11118167; rs17047660)

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## Abstract

This is a multiplex PCR-SSP for amplification of the follow SNPs of CR1 gene:

rs3737002;

rs11118131;

rs11118167;

rs17047660

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https://www.protocols.io/view/multiplex-1-pcr-ssp-cr1-rs3737002-rs11118131-p49dqz6
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## Before start

1- Wear clean gloves;
2- Clean pipettes and stand with hypochlorite and 70% alcohol;
3- Defreeze DNA samples and reagents (exception: Taq polymerase);
4- Gently mix the DNA samples and pass the reagents briefly on the vortex.
5- Centrifuge the DNA samples and reagents with a spin;
6- Keep reagents on ice;
7- Leave microtubes ready for preparation of the mix, keep the same ones identified and on ice.
8- Leave application plates properly identified.

## Materials

- 1x Coral Buffer by Qiagen
- dNTP mix by Contributed by users
- custom made primers by Contributed by users
- 20 ng of genomic DNA by Contributed by users
- Ultrapure Water by Contributed by users
- Taq DNA Polymerase Platinum by Invitrogen - Thermo Fisher

## Protocol

***Step 1.***

**The amounts described in this protocol are for one sample. Multiply them for the number of samples to be investigated.**

*Remember:*

- Each sample shall be tested in all four mix reactions 1, 2, 3 and 4 (4 tubes).
- Always make plus 10% mix.

*This means that each sample shall be distributed in 4 microtubes (for 4 reactions).*

- Label properly the microtubes for the mixes, identifying them:
  - **MIX 1** (in28-rs11118167_C/ ex29-rs17047660_A; ex26-rs3737002_C/ in26-rs11118131_C)
  - **MIX 2** (in28-rs11118167_T/ ex29-rs17047660_A; ex26-rs3737002_T/ in26-rs11118131_T)
  - **MIX 3** (in28-rs11118167_T/ ex29-rs17047660_G; ex26-rs3737002_C/ in26-rs11118131_T)
  - **MIX 4** (in28-rs11118167_C/ ex29-rs17047660_G; ex26-rs3737002_T/ in26-rs11118131_C)
- Keep these microtubes in ice.

The sizes of the fragments are:

in28-rs11118167 / ex29-rs17047660 = 746 bp

ex26-rs3737002 / in26-rs11118131 = 457 bp

***Step 2.***

Add Ultrapure Water to the microtube of each mix

AMOUNT
4.864 µl : for sample

REAGENTS
Ultrapure Water by Contributed by users

***Step 3.***

Add Coral Buffer to each mix.

The Coral Buffer containing 1.5 mM of MgCl2

AMOUNT
1 µl : for sample
REAGENTS
1x Coral Buffer by Qiagen

***Step 4.***

Add 0.2 mM dNTP

AMOUNT
1 µl : for sample
REAGENTS
dNTP MIX (mM) 0.2 by Contributed by users

***Step 5.***

Add control primer (forward and reverse) - HLA-E:

0.04 µl for forward

0.04 µl for reverse

**5’-3’ Sequence:**

**HLA-E f**

CGGGACTGACTAAGGGGCGG

**HLA-E r**

GTAGCCCTGTGGACCCTCTTAC

The size of the fragment is 324 bp

AMOUNT
0.04 µl : 0.08 µM
REAGENTS
HLA-E Control primer by Contributed by users

***Step 6.***

Add the specific primers:

- **MIX 1** (in28-rs11118167_C/ ex29-rs17047660_A; ex26-rs3737002_C/ in26-rs11118131_C)
- **MIX 2** (in28-rs11118167_T/ ex29-rs17047660_A; ex26-rs3737002_T/ in26-rs11118131_T)
- **MIX 3** (in28-rs11118167_T/ ex29-rs17047660_G; ex26-rs3737002_C/ in26-rs11118131_T)
- **MIX 4** (in28-rs11118167_C/ ex29-rs17047660_G; ex26-rs3737002_T/ in26-rs11118131_C)

0.5µl of each primer (F and R)

We used 0.5 µM of SSPs for rs3737002 and rs11118131, 0.6 µM for rs11118167 and rs17047660

**5’-3’ Sequence - rs3737002**

CCATTTGCCAGTCCTA**C**

CCATTTGCCAGTCCTA**T**

**5’-3’ Sequence - rs11118131 (reverse primer)**

CAAGAAGAAGGGGTGAT**G**

CAAGAAGAAGGGGTGAT**A**

**5’-3’ Sequence - rs11118167**

GCCAATATGTGAATATTATTATCTTA**T**

GCCAATATGTGAATATTATTATCTTA**C**

**5’-3’ Sequence - rs17047660 (reverse primer)**

TTCTGGAGCTGTGCATT**T**

TTCTGGAGCTGTGCATT**C**

AMOUNT
0.5 µl : for sample

REAGENTS
Specific Primers Forward and Reverse by Contributed by users

***Step 7.***

Add an aliquot of DNA sample to each tube.

Kepp the plate cold.

AMOUNT
1 µl :

REAGENTS
DNA sample by Contributed by users

***Step 8.***
Add the Taq DNA polymerase to the mix.
Briefly vortex and centrifuge for some seconds.

AMOUNT
0.056 µl : 0.2 Units/ul

REAGENTS
Taq Platinum DNA Polymerase by Invitrogen - Thermo Fisher

***Step 9.***

Add an aliquot of the mix to each tube.

Remember, each sample shall be tested in all four mix reactions 1, 2, 3 and 4 (4 tubes)

The final volume for each sample is 10 µl.

AMOUNT
9 µl :

***Step 10.***

Close well the tubes and centrifuge for some seconds

Put in the thermocycler

***Step 11.***

Use:

Thermal cycling begins with 94°C for 5 min and 20 s; followed by 35 cycles, where each cycle began with 94 °C for 20 s and ended with 72 °C for 40 s. The annealing temperatures were 59°C for the initial 8 cycles, 57°C for the following 7 cycles, 55ºC for another 10 cycles and 53°C for the last 10 cycles.

## Warnings

Do not leave the Taq polymerase too long outside the freezer