AUG 10, 2018

OPEN ACCESS

DOI:
[dx.doi.org/10.17504/protocols.io.p49dqz6](https://doi.org/10.17504/protocols.io.p49dqz6)

External link:
<https://doi.org/10.1371/journal.pntd.0006705>

Protocol Citation: Gabriela GCK Canalli Kretzschmar, Luana Caroline Oliveira, Angelica Beate Winter Boldt 2018. Multiplex 1 PCR-SSP - CR1(rs3737002; rs11118131; rs11118167; rs17047660). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.p49dqz6>

MANUSCRIPT CITATION: Kretzschmar GC, Oliveira LC, Nisihara RM, Velavan TP, Stinghen ST, Stahlke ERS, Petzl-Erler ML, Messias-Reason IJTD, Boldt ABW (2018) Complement receptor 1 (CR1, CD35) association with susceptibility to leprosy. PLoS Negl Trop Dis 12(8): e0006705. doi: [10.1371/journal.pntd.0006705](https://doi.org/10.1371/journal.pntd.0006705)

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

PLOS Neglected Tropical Diseases

Gabriela GCK Canalli Kretzschmar¹, Luana Caroline Oliveira¹, Angelica Beate Winter Boldt¹

¹Universidade Federal do Paraná

Gabriela GCK Canalli Kretzschmar
 Universidade Federal do Paraná

ABSTRACT

This is a multiplex PCR-SSP for amplification of the follow SNPs of CR1 gene: rs3737002; rs11118131; rs11118167; rs17047660

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

Created: May 14, 2018

Last Modified: Aug 10, 2018

PROTOCOL integer ID: 12161

MATERIALS

MATERIALS

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

STEP MATERIALS

- Ultrapure Water Contributed by users
- 1x Coral Buffer Qiagen
- dNTP MIX (mM) 0.2 Contributed by users
- HLA-E Control primer Contributed by users
- Specific Primers Forward and Reverse Contributed by users
- DNA sample Contributed by users
- Taq Platinum DNA Polymerase Invitrogen - Thermo Fisher
- Ultrapure Water Contributed by users
- 1x Coral Buffer Qiagen
- dNTP MIX (mM) 0.2 Contributed by users
- HLA-E Control primer Contributed by users
- Specific Primers Forward and Reverse Contributed by users
- DNA sample Contributed by users
- Taq Platinum DNA Polymerase Invitrogen - Thermo Fisher

SAFETY WARNINGS

Do not leave the Taq polymerase too long outside the freezer

BEFORE START INSTRUCTIONS

- 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.

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

2 Add Ultrapure Water to the microtube of each mix

Ultrapure Water Contributed by users

4.864 µL for sample

3 Add Coral Buffer to each mix.

The Coral Buffer containing 1.5 mM of MgCl₂

1x Coral Buffer Qiagen

1 µL for sample

4 Add 0.2 mM dNTP

dNTP MIX (mM) 0.2 Contributed by users

1 µL for sample

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

HLA-E Control primer Contributed by users

0.04 µL 0.08 µM

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

CCATTGCGCAGTCCTAC

CCATTGCGCAGTCCTAT

5'-3' Sequence - rs11118131 (reverse primer)

CAAGAAGAAGGGGTGATG

CAAGAAGAAGGGGTGATA

5'-3' Sequence - rs11118167

GCCAATATGTGAATATTATTATCTTAT

GCCAATATGTGAATATTATTATCTTAC

5'-3' Sequence - rs17047660 (reverse primer)

TTCTGGAGCTGTGCATTT

TTCTGGAGCTGTGCATTG

Specific Primers Forward and Reverse Contributed by users

0.5 µL for sample

- 7 Add an aliquot of DNA sample to each tube.

Keep the plate cold.

DNA sample Contributed by users

1 µL

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

Taq Platinum DNA Polymerase Invitrogen - Thermo Fisher

0.056 µL 0.2 Units/ul

- 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.

9 µL

- 10 Close well the tubes and centrifuge for some seconds

Put in the thermocycler

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, 55°C for another 10 cycles and 53°C for the last 10 cycles.