AUG 10, 2018

OPEN ACCESS

DOI:
[dx.doi.org/10.17504/protocols.io.p44dqyw](https://doi.org/10.17504/protocols.io.p44dqyw)

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

Protocol Citation: Gabriela GCK Canalli Kretzschmar, Luana Caroline Oliveira, Angelica Beate Winter Boldt 2018. Simple PCR-SSP- rs6656401-In4 - CR1. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.p44dqyw>

MANUSCRIPT CITATION: Kretzschmar GC, Oliveira LC, Nisihara RM, Velavan TP, Stinghen ST, Stahlke ERS, Petzl-Erler ML, Messias-Reason JLTd, 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)

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Simple PCR-SSP- rs6656401-In4 - CR1

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 simple PCR-SSP for amplification of the rs6656401, located in intron 4 (CR1 gene).

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 **Invitrogen - Thermo Fisher**

STEP MATERIALS

- ☒ Ultrapure Water **Contributed by users**
- ☒ 1x Coral Buffer **Qiagen**
- ☒ dNTP MIX (mM) 0.2 **Contributed by users**
- ☒ HGH (Control Primer) - Forward and Reverse **Contributed by users**
- ☒ Specific Primers Forward and Reverse **Contributed by users**
- ☒ DNA sample **Contributed by users**
- ☒ Taq DNA polymerase **Invitrogen - Thermo Fisher**
- ☒ Ultrapure Water **Contributed by users**
- ☒ 1x Coral Buffer **Qiagen**
- ☒ dNTP MIX (mM) 0.2 **Contributed by users**
- ☒ HGH (Control Primer) - Forward and Reverse **Contributed by users**
- ☒ Specific Primers Forward and Reverse **Contributed by users**
- ☒ DNA sample **Contributed by users**
- ☒ Taq DNA polymerase **Invitrogen - Thermo Fisher**

Protocol status: Working

SAFETY WARNINGS

Created: May 14, 2018

Do not leave the Taq polymerase too long outside the freezer

Last Modified: Aug 10, 2018

PROTOCOL integer ID:
12156

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 two mix reactions 1 and 2 (2 tubes).
- Always make plus 10% mix.

For this PCR we use 2 microtubes because are 2 mixes.

1. Label properly the microtubes for the mixes, identifying them: mix 1 (with A allele of SNP of rs6656401) and mix 2 (with G allele SNP of rs6656401).
2. Keep these microtubes in ice.

2

Add Ultrapure Water to the microtube of each mix.

Ultrapure Water **Contributed by users**

4.696 µL for sample

3

Add Coral Buffer to each mix

1x Coral Buffer **Qiagen**

0.8 µL for sample

4

Add 0.2 mM dNTP

dNTP MIX (mM) 0.2 **Contributed by users**

0.8 µL for sample

- 5 Add control primer (forward and reverse) - HGH:
0.08 µl for forward
0.08 µl for reverse

5'-3' Sequence:

HGH f

TGCCTTCCCAACCATTCCTTA

HGH r

CCACTCACGGATTTCTGTTGTGTTTC

The size of the fragment is 431bp

HGH (Control Primer) - Forward and Reverse **Contributed by users**

0.08 µL for sample/ 0.1 µM

- 6 Add the specific primers:
- MIX 1 - In4_rs6656401_A_f + in4_G_r
 - MIX 2 - In4_rs6656401_G_f + in4_G_r
- 0.24µl to each primer (F and R)

5'-3' Sequence - rs6656401 - primers forwards:

CTCTGTCTCCATCTTCTCA

CTCTGTCTCCATCTTCTCG

5'-3' Sequence - intron 4 - primer reverse:

CATAGTTGTAGTTGGGGATTG

The size of the fragment is 257bp

Specific Primers Forward and Reverse **Contributed by users**

0.24 µL for sample/ 0.3 µM

- 7 Add an aliquot of DNA sample to each tube

Kepp the plate cold.

DNA sample **Contributed by users**

1 µL for sample

- 8 Add the Taq DNA polymerase to the mix.

Briefly vortex and centrifuge for some seconds.

Taq DNA polymerase **Invitrogen - Thermo Fisher**

0.064 µL 0.04 Units/ul

9 Add an aliquot of the mix to each tube.

Remember, each sample shall be tested in two mix reactions 1 and 2 (2 tubes).

The final volume for each sample is 8 µl.

7 µL for sample

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 33 cycles, where each cycle began with 94 °C for 20 s and ended with 72 °C for 40 s. The annealing temperatures were 58°C for the initial 11 cycles, 55°C for the following 11 cycles and 52°C for the last 11 cycles.