Multiplex 2 PCR-SSP - CR1(rs3849266; rs2274567; rs4844610; rs12034383)

Gabriela Canalli Kretzschmar,Luana Caroline Oliveira,Angelica Beate Winter Boldt

Abstract

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

rs3849266;

rs2274567;

rs4844610;

rs12034383.

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https://www.protocols.io/view/multiplex-2-pcr-ssp-cr1-rs3849266-rs2274567-rs4844-p5sdq6e
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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

- MgCl2 by Contributed by users
- 1x Coral Buffer by Qiagen
- custom made primers by Contributed by users
- 20 ng of genomic DNA by Contributed by users
- Ultrapure Water by Contributed by users
- dNTP mix by Contributed by users
- Taq DNA Polymerase 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** (in21-rs3849266_C/ ex22-rs2274567_A; in37-rs4844610_A / in37-rs12034383_G)
  - **MIX 2** (in21-rs3849266_C/ ex22-rs2274567_G; in37-rs4844610_A / in37-rs12034383_A)
  - **MIX 3** (in21-rs3849266_T/ ex22-rs2274567_A; in37-rs4844610_C / in37-rs12034383_G)
  - **MIX 4** (in21-rs3849266_T/ ex22-rs2274567_G; in37-rs4844610_C / in37-rs12034383_A)
- Keep these microtubes in ice.

The sizes of the fragments are:

in21-rs3849266 / ex22-rs2274567 = 667bp

in37-rs4844610 / in37-rs12034383 = 1080bp

Step 2.

Add Ultrapure Water to the microtube of each mix

AMOUNT
3.928 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
0.8 µl : for sample
REAGENTS
1x Coral Buffer by Qiagen

Step 4.

Add 0.5 mM MgCl2 to each mix.

(Total of MgCl2 is 2 mM to each mix).

AMOUNT
0.32 µl : for sample
REAGENTS
MgCl2 by Contributed by users

Step 5.

Add 0.2 mM dNTP

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

Step 6.

Add control primer (forward and reverse) - HGH

0.064 µl for forward

0.064 µl for reverse

5’-3’ Sequence:

HGH f

TGCCTTCCCAACCATTCCCTTA

HGH r

CCACTCACGGATTTCTGTTGTGTTTC

The fragment size is: 431bp

AMOUNT
0.064 µl : (0.08 µM)

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

Step 7.

Add the specific primers:

- **MIX 1** (in21-rs3849266_C/ ex22-rs2274567_A; in37-rs4844610_A / in37-rs12034383_G)
- **MIX 2** (in21-rs3849266_C/ ex22-rs2274567_G; in37-rs4844610_A / in37-rs12034383_A)
- **MIX 3** (in21-rs3849266_T/ ex22-rs2274567_A; in37-rs4844610_C / in37-rs12034383_G)
- **MIX 4** (in21-rs3849266_T/ ex22-rs2274567_G; in37-rs4844610_C / in37-rs12034383_A)

For in21/ex22 - add 0.144µl of each primer (F and R) for sample, (0.18 µM)

For in37/in37 - add 0.32µl of each primer (F and R) for sample (0.4 µM)

5’-3’ Sequence - rs3849266

CTGATGGCTTGGGGTA**T**

CTGATGGCTTGGGGTA**C**

5’-3’ Sequence - rs2274567 (reverse primer)

CTCAATCTGCATTGATCCA**C**

CTCAATCTGCATTGATCCA**T**

5’-3’ Sequence - rs4844610

CTACACAAAACAGCCTTGT**A**

CTACACAAAACAGCCTTGT**C**

5’-3’ Sequence - rs12034383 (reverse primer)

AGATGTCCATGCCTTAA**C**

AGATGTCCATGCCTTAA**T**

REAGENTS
Specific Primers Forward and Reverse by Contributed by users

Step 8.

Add an aliquot of DNA sample to each tube.

Kepp the plate cold.

AMOUNT
1 µl :

REAGENTS
DNA sample by Contributed by users

Step 9.
Add the Taq DNA polymerase to the mix.
Briefly vortex and centrifuge for some seconds.

AMOUNT
0.096 µl : for sample/ 0.3 Units/ul

REAGENTS
Taq DNA Polymerase by Invitrogen - Thermo Fisher

Step 10.

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 8 µl.

AMOUNT
7 µl : for sample

Step 11.

Close well the tubes and centrifuge for some seconds

Put in the thermocycler

Step 12.

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 63 °C for the initial 6 cycles, 61 °C for the
following 16 cycles, 59 ºC for the last 11 cycles.

Warnings

Do not leave the Taq polymerase too long outside the freezer