Simple PCR-SSP- rs6656401-In4 - CR1

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Abstract

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

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https://www.protocols.io/view/simple-pcr-ssp-rs6656401-in4-cr1-p44dqyw
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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 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 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.

Step 2.

Add Ultrapure Water to the microtube of each mix.

AMOUNT
4.696 µl : for sample
REAGENTS
Ultrapure Water by Contributed by users

Step 3.

Add Coral Buffer to each mix

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

Step 4.

Add 0.2 mM dNTP

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

Step 5.

Add control primer (forward and reverse) - HGH:

0.08 µl for forward

0.08 µl for reverse

5’-3’ Sequence:

HGH f

TGCCTTCCCAACCATTCCCTTA

HGH r

CCACTCACGGATTTCTGTTGTGTTTC

The size of the fragment is 431bp

AMOUNT
0.08 µl : for sample/ 0.1 µM
REAGENTS
HGH (Control Primer) - Forward and Reverse by Contributed by users

Step 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:

CTCTGTCTCCATCTTCTC**A**

CTCTGTCTCCATCTTCTC**G**

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

CATAGTTGTAGTTGGGGATTG

The size of the fragment is 257bp

AMOUNT
0.24 µl : for sample/ 0.3 µM
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 : for sample
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
0.064 µl : 0.04 Units/ul
REAGENTS
Taq DNA polymerase by Invitrogen - Thermo Fisher

Step 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.

AMOUNT
7 µl : for sample

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 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.

Warnings

Do not leave the Taq polymerase too long outside the freezer