CC-Chemokine receptor (CCR5)-delta32 genotyping.

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This protocol describes the optimized components and conditions for the genotyping of CCR5 wild type and its mutant (Δ32) variant, using Polymerase Chain Reaction with specific sequence primers (PCR-SSP). C-C chemokine receptor type 5 (also known as CCR5 or CD195) is a leukocyte surface protein that acts as a receptor for chemokines. This allows for the process by which T cells are attracted to specific target organs and sites. HIV requires CCR5 co-receptor binding to enter and infect host CD4 T cells. Individuals carrying a mutation known as CCR5-Δ32 are protected from most HIV infections. With this technique it is possible to amplify one or two PCR products: CCR5 native or wild-type or full-length of 174 bp and the 32bp deletion variant of 142 bp.

Oligonucleotide primers

Name	Specificity	Sequence	Base	%GC	Tm[b]	Product (bp)	Reference
CCR5-F	Generic	5'-gCTCTCTCCCAggAATCATC-3'	20	55	54.7	174 / 142	Veloso S, 2010
CCR5-R	Generic	5'-TTCCCgAgTAgCAgATgACC-3'	20	55	56.3		Veloso S, 2010

The oligonucleotide reverse sequence showed here is the reverse complement in its annealing.

PCR components

PCR		1 rx (µl)
dH_2O	C*f*	7.525
10x Buffer PCR	1X	1.25
$MgCl_2$ 50 mM	1.5 mM	0.375
4x dNTPs 10 mM	200 µM	0.25
2x Primers 10 µM	800 nM	1
Taq 5 UI/µl	0.04 UI/µl	0.1
DNA	-	2.00
	Vf	12.5

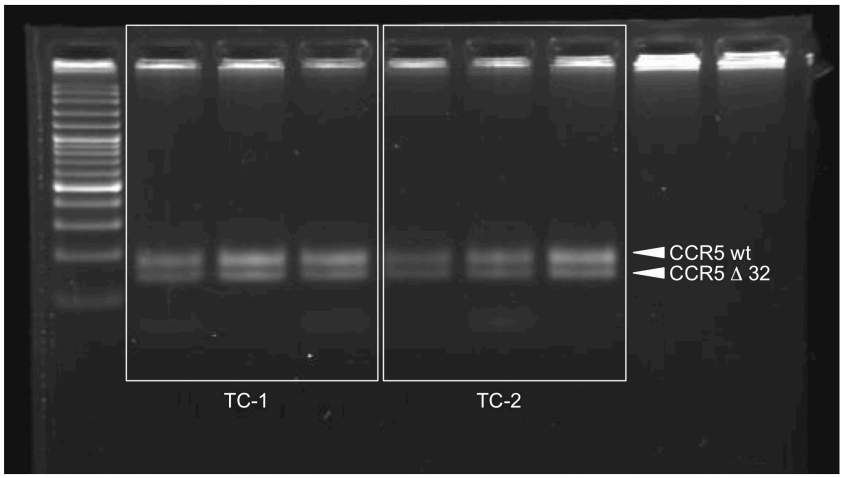
PCR conditions

Time: 1 hr 40 min		Denaturation		Annealing	Extension		
CCR5	Temperature	94°	94°	59°	72°	72°	RT
	Time	1 min	30 sec	20 sec	15 sec	1 min	5 min
			x 30 cycles				

Optimized conditions for Axygen thermocyclers (TC-1 y TC-2).

Electrophoresis

Add 4 µl of Orange G loading buffer to each PCR reaction and load 15 µl of this mixture per well in a 3% agarose gel. Run the gel during 75 minutes at 100 VDC.



Notes

1. Clean workbench with 0.1% NaOCl and 70% ethanol before and after the PCR preparation.
2. Use iceboxes or *Eppendorf Ice Coolers* for PCR preparation.
3. Vortex all reagents (except DNA) before preparing the master mix. Vortex briefly after preparing the master mix.
4. Use the most appropriate micropipette for the required volume.

5. Add 10.5 µl of the master mix to each PCR tube and then add 2 µl of DNA (± 100 ng/µL).

References

1. Alkhatib G, The Biology of CCR5 and CCXR4. *Curr Opin HIV AIDS*. USA 2009 March ; 4(2): 96–103

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3. Lederman MM, Biology of CCR5 and Its Role in HIV Infection and Treatment. *JAMA* USA 2006;296:815-826

4. Mahajan SD, Role of chemokine and cytokine polymorphisms in the progresión of HIV-1 disease. *Biochem Biophys Res Commun* USA 2010 MAY 28: 396(2): 348-352.

5. Veloso S, Effect of *TNF-α* genetic variants and *CCR5Δ32* on the vulnerability oto HIV-1 infection and disease progression in Caucasian Spaniards. *BMC Medical Genetics* Spain 2010, 11:63

Revision history

1.0 Original document.

2.0 Changes to document format only.