

HLA-C -35Kb single nucleotide polymorphism (rs9264942) genotyping

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This protocol describes PCR-SSP genotyping components and conditions for the identification of HLA-C -35Kb rs9264942 SNP. The rs9264942 SNP (C/T) lies 35 kb upstream (in the 5' region) of the HLA-C gene promoter (transcription initiation site). Individuals with T/T variant have up to 90% less human immunodeficiency virus (HIV) loads than those who with other variants (C/C or C/T). About 10% of European caucasoids are T/T, which leads to an average 90% viral load reduction. About 50% of European caucasoids bear one copy (C/T), which gives a 60% reduction. By comparison, less than 40% of people of African descent appear to carry a single copy of the polymorphism. This SNP is also reported [PMID 176411650] to account for 6.5% of the 15% variation in viral load set point in asymptomatic HIV infected individuals. rs2395029 can also be associated with reduced HIV viral load set point.

Oligonucleotide primers

Name	Sequence	bp	%GC	Tm	Amplicon (bp)	Reference
HLA-C SNP Fwd	5′-ggg-Tgg-TgC-CAA-gTA-TgA-g-3′	19	73,5	60,1		1
HLA-C SNP Rev T	5'-AgA-AAg-TCC-CAC-AgT-gCC-TA-3'	20	76,5	63,5	334bp	1
HLA-C SNP Rev C	5′-AgA-AAg-TCC-CAC-AgT-gCC-Tg-3′	20	63,2	58,7	334bp	1

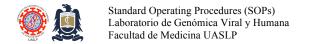
PCR components

	CI	1x (μl)	Cf	
dH_20	-	13 μl	-	
Buffer A	10X	2 μl	1x	
$MgCl_2$	50mM	0.4 µl	1mM	
dNTP's	10mM	0.4 µl	10mM	
Taq DNA Pol	5UI/ ml	0.2 μ1	5UI/ ml	
Primers	10nM	2 μ1	10nM	
DNA	200ng	2	200ng	

PCR conditions

	Temperature (°C)	95°	95°	70°	72°	95°	65°	72°	95°	55°	72°	72°	RT
HLA-C SNP	Time	1'	25"	45"	45'	25"	50"	45''	25"	1'	2'	10'	∞
	Cycles	1	5			15			4			1	1







Electrophoresis

Add 5 μ l Orange G loading buffer to each reaction and oad 25 μ l of the this mixture to each well of 2% agarose gel and run electrophoresis for 60 minutes at 100 VDC.

References

- 1. Corrah TW, Goonetilleke N, Kopycinski J, Deeks SG, Cohen MS, Borrow P, McMichael A, Brackenridge S. Reappraisal of the relationship between the HIV-1-protective single-nucleotide polymorphism 35 kilobases upstream of the HLA-C gene and surface HLA-C expression. J Virol. 2011 Apr;85(7):3367-74. doi: 10.1128/JVI.02276-10. PMID: 21248048
- 2. Fellay J, Shianna KV, Ge D, Colombo S, Ledergerber B, Weale M, Zhang K, Gumbs C, Castagna A, Cossarizza A, Cozzi-Lepri A, De Luca A, Easterbrook P, Francioli P, Mallal S, Martinez-Picado J, Miro JM, Obel N, Smith JP, Wyniger J, Descombes P, Antonarakis SE, Letvin NL, McMichael AJ, Haynes BF, Telenti A, Goldstein DB.A whole-genome association study of major determinants for host control of HIV-1. Science. 2007 Aug 17;317(5840):944-7. PMID: 17641165

Revision history

- 1.0 Original document.
- 2.0 Changes to document format only.

