



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 ₂ O | - | 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 µl | 5UI/ ml |
| Primers | 10nM | 2 µl | 10nM |
| DNA | 200ng | 2 | 200ng |

PCR conditions

| HLA-C SNP | Temperature (°C) | 95° | 95° | 70° | 72° | 95° | 65° | 72° | 95° | 55° | 72° | 72° | RT |
|-----------|------------------|-----|------|------|-----|------|------|------|------|-----|-----|-----|----|
| | 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 load 25 μ l of 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.

