# Genotypic HIV coreceptor tropism assay

**Maurizio Zazzi**

## Abstract

The assay is intended to amplify and sequence the HIV-1 gp120 V3 domain and infer if the virus can use the CXCR4 coreceptor for cell entry. The use of this coreceptor renders the virus not susceptible to the CCR5 coreceptor antagonist maraviroc.

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## Protocol

### Step 1.

Depending on which kind of sample you have to analyze, extract DNA from whole blood by using the High Pure Viral Nucleic Acid Kit (Roche Applied Science, catalogue number 11858874001) OR extract RNA from plasma by using the QIAamp Viral RNA Mini Kit (Qiagen, catalogue number 52904).

### Step 2.

Amplify the HIV-1 gp120 V3 coding region by the following RT-nested PCR protocol:

### RT (when starting from plasma RNA)

The reaction mix contains 20 µL RNA extract pre-heated at 70°C for 5 min, 6 µL Improm II 5x Reaction buffer (Promega), 4 µL 1.25 mM each dNTP, 1.8 µL $MgCl_2$, 1 µL 50 ng/µL random Hexanucleotides (Promega), 0.5 µL RNasin RNase inhibitor and 1 µL Improm II Reverse Transcriptase (Promega). Run at 37°C for 45 min, 80°C for 5 min, hold at 4°C.

### First PCR

The reaction mix contains 5 µL RT reaction mixture (when starting from plasma RNA) or 2-5 µL DNA extract (when starting from whole blood), 28 µL distilled water, 10 µL 5X Colorless GoTaq Flexi Buffer, 3.2 1.25 mM each dNTP, 0.2 µL 5U/µL GoTaq Hot Start Polymerase (Promega), 5 µL 25 mM $MgCl_2$, 0.6 µL 10 pmol/UL each primer P150 (5'-AATGTCAGCACAGTACAATGYACACAT-3', coordinates 6945-6971 in the reference HXB2 genome) and P151 (5'-CTACTTTATATTTATATAATTCAYTTCTC-3', 7661-7689) . Run 25 cycles at 49°C for 30 sec, 72°C for 50 sec, 93°C for 30 sec, followed by a final extension at 50°C for 1 min and 72°C for 10 min.

**Second PCR**

The reaction mix contains 1 µL of the first PCR mixture, 18 µL distilled water, 6 µL 5X Colorless GoTaq Flexi Buffer, 0.8 1.25 mM each dNTP, 0.2 µL 5U/µL GoTaq Hot Start Polymerase (Promega), 3 µL 25 mM $MgCl_2$, 0.3 µL 10 pmol/UL each primer P537 (5'-CAGTACARTGYACACATGGAAT-3', coordinates 6955–6976 in the reference HXB2 genome) and P538 (5'-TAGAAAAATTCYCCTCYACAATTAAA-3', 7353–7373) . Run 25 cycles at 49°C for 30 sec, 72°C for 50 sec, 93°C for 30 sec, followed by a final extension at 50°C for 1 min and 72°C for 10 min.

**Step 3.**

**Visualization of the amplified product**

Load 5 µL of the second PCR mixture on a 1.5% standard agarose gel, run elecrophoresis at 5-10 V/cm for 20-40 min and stain with ethidium bromide or equivalent DNA stain. Estimate visually the expected 421-bp band since the product concentration dictates the dilution factor for the following step.

**Step 4.**

**Sequencing reaction**

Dilute the PCR product down to 1-2 ng/µL. If the nested PCR ran as expected a 1/50 to 1/200 dilution factor should apply. Note that when the dilution factor is higher than 20 you do not need to purify the PCR product before the sequencing reaction because the nested PCR step used low amounts of both primers and dNTPs and residual amounts following such dilution will not interfere with the sequencing step. If necessary, purify the PCR product with your system of choice.

Use 3 µL of diluted/purified PCR product as the template for a cycle sequencing reaction with BigDye 1.1 or BigDye 3.1 or equivalent. Run the reaction for 25 cycles made of 96°C for 10 sec, 50°C for 5 sec, 60°C for 2 min. Setup two identical reactions, one driven by primer P537 and one driven by primer P538.

AT the end of the sequencing reaction, purify the reactions from unincorporated labelled ddNTPs with your system of choice. We recommend the BigDye X-terminator system from Applied Biosystems because of its ease of use and complete preservation of the sequencing products. Once the

purification step has been completed, load the reactions on the capillary elctrophoresis apparatus of your choice and run the migration as per instrument instructions.

**Step 5.**

**Interpretation**

Process the raw sequencing files with the software of your choice, edit the contig to generate a sequence around 370 bp devoid of primer sequences at both ends. Submit the sequence to the geno2pheno server with the false positive rate threshold of your choice to infer if the sequence obtained is derived from a CCR5 or CXCR4 using virus.