CENTER FOR AIDS RESEARCH
University of California San Diego

Translational Virology Core Protocols

HIV PCR and Population-based Clonal and Single-genome Sequencing

env Clonal Sequencing

HIV RNA is extracted and undergoes RT-PCR amplification for cDNA synthesis per manufacturer's protocol (Thermoscript RT, Invitrogen, Carlsbad, CA). gp160 is then amplified through "nested" PCR. Cloning is used to identify minority and majority HIV variants in the blood and GS. (Twenty clones increases the detection sensitivity to a theoretical 5% [95%CI = 1–14%]). Amplicons are cloned using the Topo-TA cloning system (Invitrogen,). Twenty randomly selected clones are sequenced using an automated dye-terminator sequencing kit (ABI, Foster City, CA). Terminal dilution PCR with sequencing is performed on the first 10 reactions to determine the degree of disagreement from the cloning system in measuring viral population diversity. All clonal PCR products are sequenced using Prism Dye terminator kits (ABI) on an ABI 3100 Genetic Analyzer. Sequence data are compiled, aligned, and edited using SequencherÔ 4.0 (Gene Codes, Ann Arbor, MI).

pol Sequencing

Extracted HIV RNA from blood plasma from all participants undergoes population-based *pol* sequencing (Viroseq™ version 2.0, Applied Biosystems) for use in the phylogenetic determination of HIV transmission (see Clinical and Specimen Core, PAR Unit). Unused extracted RNA is reverse transcribed using random hexamer priming to generate first strand c-DNA for future genetic studies, then aliquoted and stored at –80°C (RETROscript™, Ambion). The Viroseq™ HIV genotyping kit has been validated for sequencing HIV *pol* from samples with copy numbers of 2000/mL or greater. However, in practice, sequence data are obtainable with viral loads between 200 and 2000 copies/mL using our ABI 3100 Genetic Analyzer (data not shown). Additionally, using mixture experiments, we have validated that we are able to detect resistance-associated mutations when the minority viral population comprises at least 30% of the circulating population [80]. Sequences are manually reviewed and edited using the Viroseq™ genotyping software (Version 2.4.2, ABI). In addition, our lab employs the Sequencher 4.1 software package (Gene Codes, Ann Arbor, MI) for alignment and generation of batch contig files that can then be exported in a variety of formats for further analysis and future GenBank submission. A more sensitive "nested" PCR adaptation of the Viroseq™ system has been published and is incorporated into our laboratory procedures for samples that cannot be amplified using the above methods.

Interpretation of genotypic drug resistance first uses the interpretive algorithm available with Viroseq™. Comparisons also will be made with the online Los Alamos HIV drug resistance database (LANL; http://resdb.lanl.gov/Resist_DB/) and the Stanford HIV Drug Resistance Database (HIVdb) [82]. These latter resources have the advantage of being continuously updated as new drug resistance patterns are recognized. Staff of the AIEDRP Statistical and Coordinating Center have developed software to align, curate, and generate predictions of genotypic resistance based on these algorithms using Viroseq-generated sequence data as input.

Contact

Demetrius dela Cruz, Core Administrator
Tel: 858-642-1620
Fax: 858-552-7445
E-mail: ddelacruz@ucsd.edu
URL: http://cfar.ucsd.edu/core-facilities/translational-virology-core

More

For our analyses, genetic mixtures of drug-sensitive and drug-resistant virus are treated as drug resistant. Those sequences identified with genotypic mixes will be of particular interest for clonal analysis. Additionally, resistance data generated with these studies are made available to the participant's primary care provider with the caveat that resistance testing is not FDA approved in non-blood bodily fluids.

HIV Single Genome Sequencing

Briefly, HIV RNA or DNA is extracted as described above. Reverse transcription of HIV RNA is performed in bulk using the RetroScript kit (Ambion) to produce complementary DNA or cDNA. Dilutions are made of the cDNA based on the known viral load of the original sample. First round PCR is then performed 10 times from each dilution, using gene specific primers (see Table below) and the thermocycling protocol specific for each primer set. PCR product is then visualized by gel electropheresis. The dilution that produces <30% PCR positivity (3/10 positive wells), will then be selected for 98 PCR reactions, with an expected <30 positive PCR reactions. The PCR products then undergo a sequencing reaction, as described above.

Inner Primer Sets for Individual HIV Coding Regions

Primers	Sequence
nef_IN5'	CGTCTAGAACATACCTAIAAGAATAAGACAGG
nef_IN3'	CGGAATCCGTCCCCAGCGGAAAGTCCCTTGTA
Env2_5'	GATCAAGCTTTAGGCATCTCCTATGGCAGGAAGAAG
Env3:_3'	AGCTGGATCCGTCTCGAGATACTGCTCCCACCC
V1-2In_2	CCATGTGTAAAATTAACCCCACTCTGTGT
V1-2In_3	TCAAAGGATACCTTTGGACAGGC
C2-V3In_2	GAACAGGACCAGGATCCAATGTCAGCACAGTACAAT
C2-V3In_3	GACAGGCTAATTTTTTAGG
V4-5In_2	GTACAATGTACACATGGAAT
V4-5In_3	AATTCACTTCTCCAATTGTC
gagP6-Fin	GACAGGCTAATTTTTTAGG
gagP6-B	CTTCTAATACTGTATCATCTGCTCCTG
tat_inF_5'	GTAGCCAAGCTTATGGAACCGGTC
tat_inB_5'	TTACCGCTCGAGGACGCGTCACTGTTTAGA