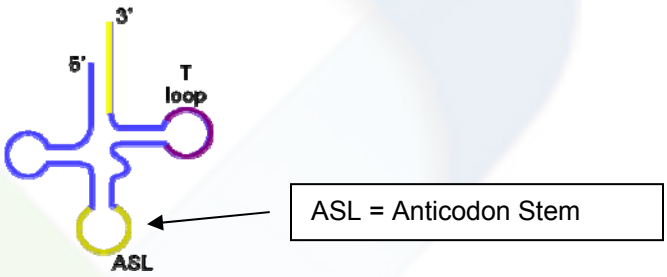
Trana HIV 201 HTS Assay
Product Description

The Trana HIV 201 High-Throughput (HTS) Assay - designed to identify compounds that inhibit the use of tRNA by HIV - has the ability to select compounds with anti-HIV bioactivity. Because tRNA is essential for HIV replication, disruption of the virus' ability to use tRNA would represent a novel target for anti-HIV drug therapy.

Introduction

Scientists at Trana Discovery have invested years of research to develop the patented technology that forms the basis for the assay used to screen molecular libraries for tRNA inhibitors. The technology centers on the anticodon stem loop (ASL) of tRNA and the importance of nucleotide modifications within the ASL. The ability to synthesize copies or mimics of the ASL with the modifications, just as they occur in nature, is what overcomes previous barriers to high-throughput screening and enables further research and the application of Trana Discovery technology to methodically search for compounds that inhibit interactions with tRNA.

The Trana HIV 201 assay targets interactions in specific steps of the life cycle of the HIV virus – that target is the complex formed in the ***pre-binding complex*** of reverse transcription. This occurs when the HIV genomic RNA binds with a hijacked, restructured form of the Human tRNA Lys. The restructured Human $tRNA^{Lys3}_{SUU}$ forms a complex with the HIV genome RNA during HIV replication.



HIV proteins "unwind" tRNA from its form used by Humans, depicted above; the restructured form will ***stabilize binding*** to viral genomic RNA. The interactions at the Anticodon Stem-Loop Binding Sites are dependent on modified bases of the sequence in the Human tRNA Lys.

Assay Components

The bimolecular interaction for the Trana HIV 201 assay uses two synthetic RNA mimics. One RNA oligomer containing 12 bases mimics the Human tRNA Lys sequence (Tool) and the other RNA oligomer mimics HIV genomic sequence (Target). The binding properties of these two RNA oligomers are highly specific.

This assay screens for compounds that inhibit the binding of the fluorescently labeled Human tRNA "tool" and the biotin-labeled HIV genomic RNA "target" in the presence of the Perkin Elmer AlphaScreen™ beads using an HTS format.

AlphaScreen™ is a bead-based chemistry used to study biomolecular interactions in a microplate format. The acronym ALPHA stands for Amplified Luminescent Proximity Homogeneous Assay. Binding of molecules captured on the beads leads to an energy transfer from one bead to the other, ultimately producing a detectable luminescent/fluorescent signal.

The AlphaScreen assay is constructed by capturing one binding partner, such as a receptor, onto the Donor beads and the other partner, such as the ligand, onto the Acceptor beads. For the Trana HIV 201 assay, the Target is attached to the Donor beads and the Tool is attached to the Acceptor beads. When the partners interact, chemical energy is transferred from Donor to Acceptor beads and a signal is produced (see figure 1). Alternatively, competition or cleavage assays can be read as signal reduction (see figure 2). Donor beads have streptavidin (SA) conjugates, since biotinylation of one binding partner provides efficient capture onto the Donor bead. Acceptor beads have a conjugate to antibodies to anti-FITC, the second binding partner has the corresponding FITC attached.

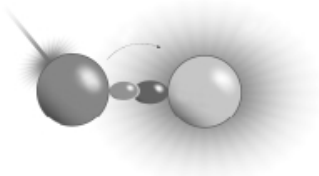


Figure 1: AlphaScreen Binding
Binding of biological "partners" brings Donor (Target) and Acceptor (Tool) beads into close proximity (≤200 nm) and thus a fluorescent signal between 520–620 nm is produced.

Figure 2: No Binding
When there is no binding, Donor and Acceptor beads are not in close proximity. Singlet oxygen decays and no signal is produced.

Illustration source: Perkin Elmer

High Throughput Screening Capability

The Trana HIV 201 assay has been tested and validated in the 384 well format. These results established several important principles:

1) Synthetic mimics of modified tRNAs can be used in a two-component (bimolecular interaction) screening assay.
2) This bimolecular screening assay was validated using a 384 well format, indicating that this and future assays can be automated and scaled.
3) This assay demonstrates the feasibility to use fluorescently-labeled synthetic mimics of tRNA anticodon stem-loops as ligands.

Identification of Active Compounds

At the High Throughput Screening Center at Southern Research, using the HIV 201 assay, which can screen up to 50,000 compounds per day, several compounds were identified from Southern Research's large molecular library that inhibited the assay. In a cell-based study that followed, a subset of these compounds was examined for their inhibition qualities in the replication of the HIV-1 Ba-L virus using live Peripheral Blood Mononuclear Cells (PBMC) and found to have anti-HIV activity. Further testing revealed that antiviral activity was not due to inhibition of reverse transcriptase, the mechanism of action of many of the currently available HIV therapies. Although not definitively confirmed, these early findings suggest that the Trana HIV 201 assay has the ability to select anti-HIV bioactive compounds with a novel mechanism of action.

For more information on the Trana HIV 201 HTS Assay, contact Trana Discovery at info@tranadiscovery.com, tel: 866-390-3452 (toll free), or int'l: +1-919-342-6192.
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