

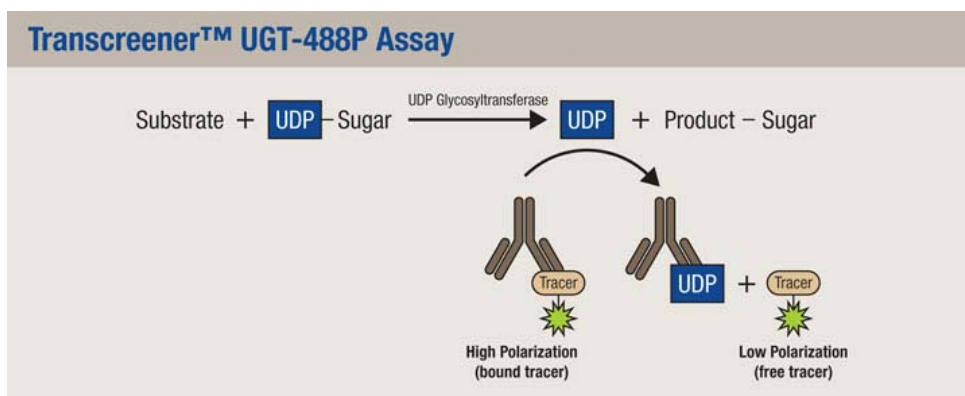
## A Universal HTS Platform for Screening Glycosyltransferase Enzymes

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### Introduction

The Transcreener™ HTS Assay Platform has been designed to detect the invariant product of reactions catalyzed by group transfer enzyme families (e.g.: ADP for Kinases, UDP for UDP-glycosyltransferases). The Transcreener™ UGT Assay is a universal, homogenous, competitive fluorescence polarization immunoassay that enables high-throughput screening of the UDP-glycosyltransferase (UGT) enzyme family using a single set of principle reagents (Figure 1). Here the Transcreener™ UGT Assay is used to screen two UGTs, alpha-1,3-galactosyltransferase (GalT) and UDP-glucuronosyltransferase 2B7 (UGT2B7) with a small, pharmacologically active compound library establishing proof of concept for this widely applicable high throughput screening assay to detect and characterize modulators of this important enzyme family.

**Figure 1. Transcreener™ UGT Assay Principle**



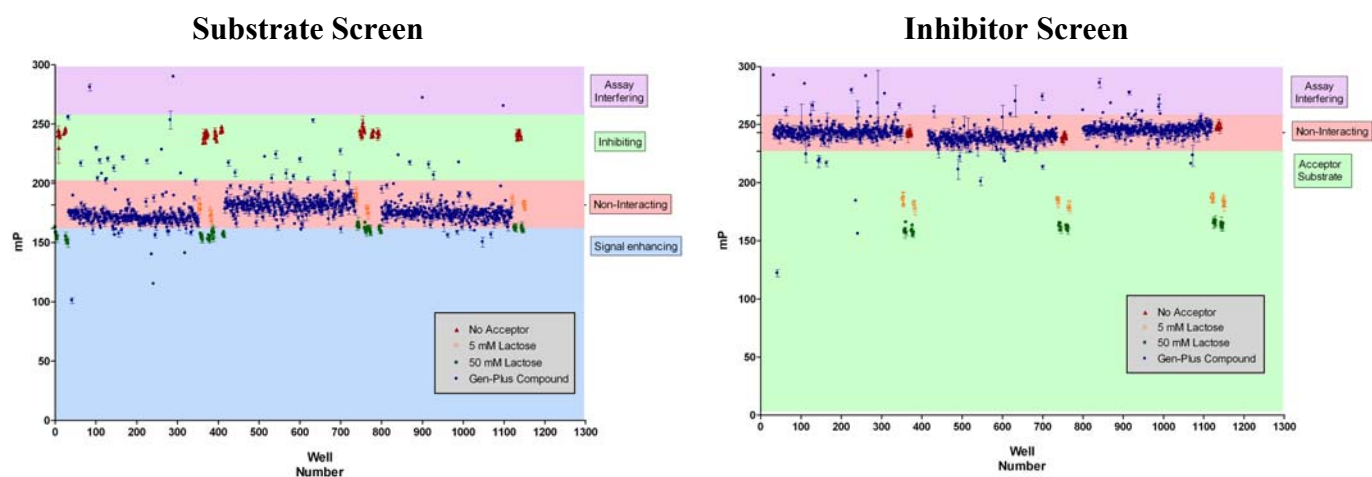
UDP produced during the group transfer from UDP-sugar to acceptor substrate is measured using a competitive fluorescence polarization immunoassay. Because it relies on detection of the invariant reaction product, the same detection reagents can be used for any UGT and any acceptor substrate.

### Inhibitor and Substrate Screens:

A small, structurally-diverse, pharmacologically-active compound library was screened for both inhibitors and substrates of two UDP-glycosyltransferases (UGTs): alpha-1,3-galactosyltransferase (GalT) and UDP-glucuronosyltransferase 2B7 (UGT2B7). Interest in these two glycosyltransferases spans both disease intervention and ADMET with GalT representing a target to mitigate xenotransplant rejection and UGT2B7 an important phase II drug metabolizing enzyme. In addition to their biological relevance, these UGTs were of interest as model proteins to compare assay performance with a purified soluble protein, such as GalT, and a microsomal membrane protein, UGT2B7.

## Figure 2. GalT Substrate and Inhibitor Screens

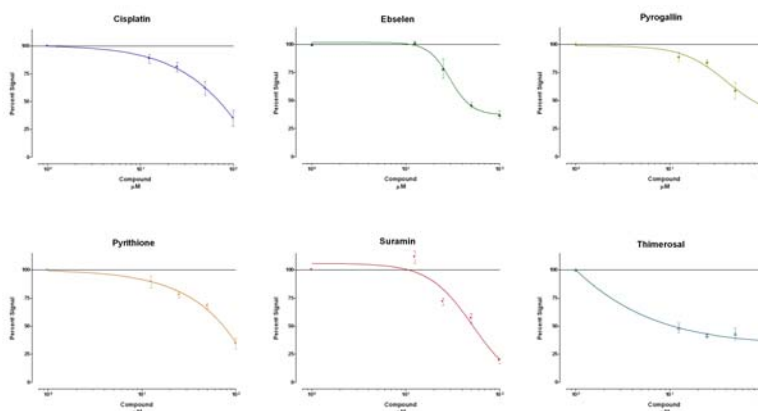
GalT Transcreeper™ assays were performed in 30µl wells with 50 ng/mL alpha-1,3,-GalT, and 50 µM UDP-Gal. The enzyme used in this study was a His-tagged, recombinant alpha-1,3-GalT expressed in and purified from E. coli. The standard assay conditions were: 10 mM Tris-HCl pH 7.0, 10 mM MnCl<sub>2</sub>, 1.25% v/v UGT Assay antibody, 2nM 488-UTP tracer was synthesized in house. Reactions were incubated at 37°C for 1 hour followed by 0.5 hour incubation at room temperature. Fluorescence polarization measurements were taken with a Tecan Ultra plate reader using an EX<sub>485nm</sub>/EM<sub>535nm</sub> filter set at 30°C.



Of the compounds in this library: 1.7% were classified as substrate hits, 94.4% demonstrated no activity, and 2.6% interfered with assay signal. Compounds were assayed at 100 µM.

Of the compounds in the library: 3.2% were inhibitor hits, 92.2% demonstrated no activity, and 1.0% interfered with assay signal. Compounds were assayed at 100 µM with 5 mM Lactose present.

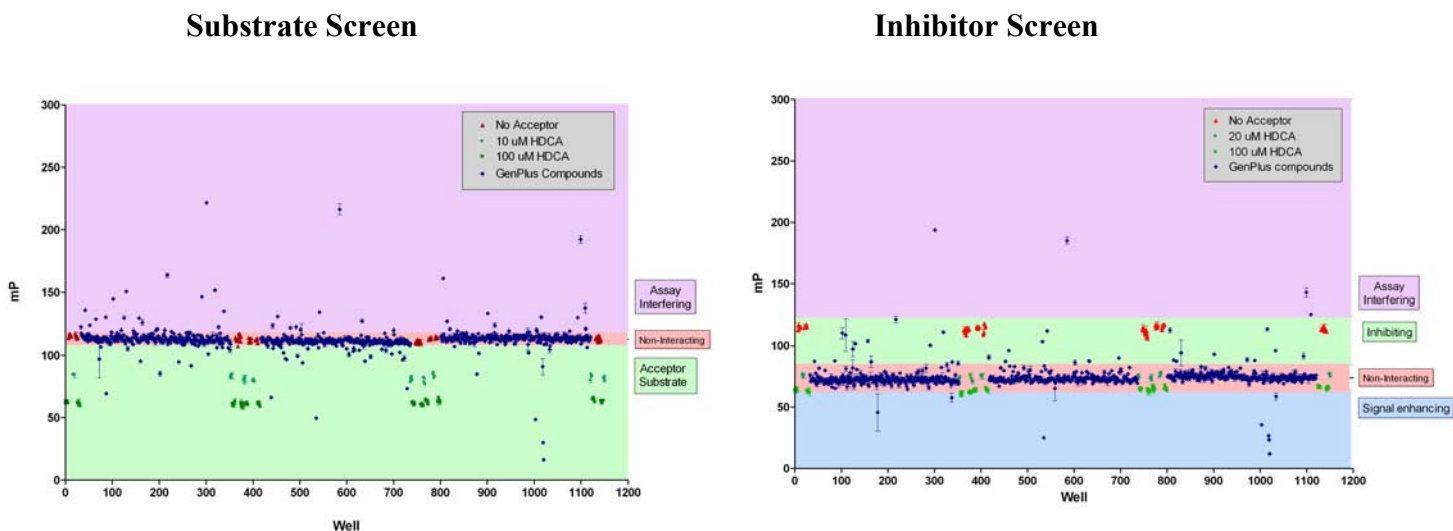
## Dose Dependent Inhibition



Dose dependent inhibition was observed with 6 of the non-substrate compounds identified in the inhibitor screen: Cisplatin, Ebselen, Pyrogallin, Pyriithione, Suramin, and Thimerosal. Compounds were assayed at 0, 12.5, 25, 50, and 100 µM in the presence of 5 mM lactose.

### Figure 3. UGT2B7 Substrate and Inhibitor Screens

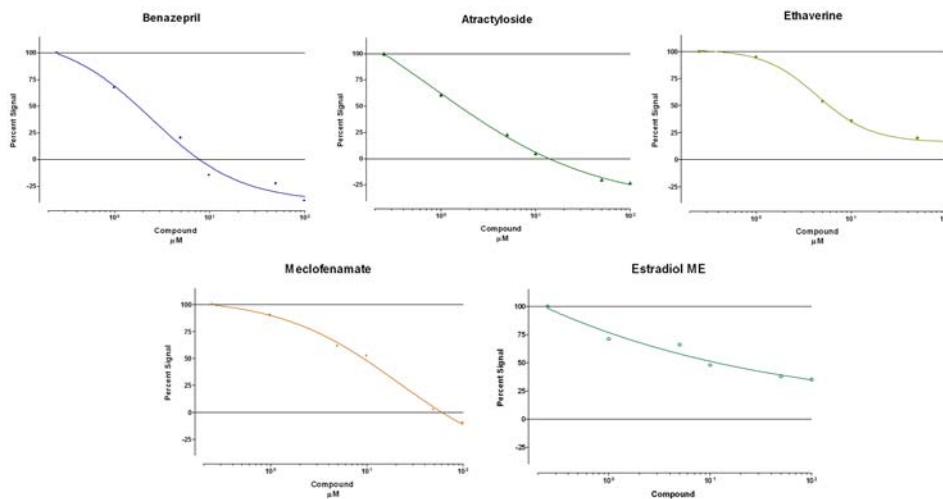
UGT2B7 Transreener™ assays were performed in 30  $\mu\text{L}$  volumes with 75  $\mu\text{g}/\text{mL}$  UGT2B7, and 70  $\mu\text{M}$  UDPGA. Recombinant human UGT2B7 Supersomes™ (BD Biosciences) were used in this study, a microsomal preparation expressed in insect cells. The standard assay conditions were: 50 mM  $\text{KPO}_4$  pH 7.5, 5 mM  $\text{MgCl}_2$ , 0.7% v/v UGT Assay antibody, 2 nM 488-UTP tracer was synthesized in house. Reactions were incubated at 37°C for 1.5 hours followed by 0.5 hour incubation at room temperature and stopped with 25 mM  $\text{Na}_3\text{VO}_4$ . Fluorescence polarization measurements were taken as above.



Of the compounds in this library: 3.5% were classified as substrate hits, 92.2% demonstrated no activity, and 4.3% interfered with assay signal. Compounds were assayed at 10  $\mu\text{M}$ .

Of the compounds in the library: 3.3% were inhibitor hits, 94.7% demonstrated no activity, 0.8% enhanced signal and 1.1% interfered with assay signal. Compounds were assayed at 10  $\mu\text{M}$  with 20  $\mu\text{M}$  HDCA present.

### Dose Dependent Inhibition



Dose dependent inhibition was observed with 5 of the non-substrate compounds identified in the inhibitor screen: Benazepril, Atractyloside, Ethaverine, Meclofenamate, and Estradiol ME. Compounds were assayed at 0, 1, 5, 10, 50, and 100  $\mu\text{M}$  in the presence of 20  $\mu\text{M}$  HDCA.

**Selection Criteria:**

Generation of product (UDP) results in a decrease in polarization and assay interference is observed as an increase in polarization both relative to negative control. Reactions containing no acceptor-substrate (1% DMSO) were used as negative control. A statistically significant polarization (mP) shift is defined as a change in mP greater than 3 standard deviations from control mean.

**Conclusions:**

1. Proof of concept was established for the Transcreener™ UGT Assay as a highly flexible and robust HTS assay for the UGT enzyme family.  $Z'$ -values in excess of 0.5 were obtained for both the purified, soluble drug target, alpha-1,3-galactosyltransferase and the microsomal, membrane-bound, ADME enzyme UDP-glucuronosyltransferase 2B7.  $Z'$ -values were 0.62 and 0.67, respectively.
2. 16 compounds were identified as GalT substrates and 6 non-substrate compounds demonstrated dose dependent inhibition.
3. 34 compounds were identified as UGT2B7 substrates and 5 non-substrate compounds demonstrated dose dependent inhibition.

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Transcreener™ HTS Assay Platform is patent pending. Transcreener™ is a trademark of BellBrook Labs. AlexaFluor® is a registered trademark of Molecular Probes, Inc (Invitrogen). Supersomes™ are a trademark of BD Biosources.

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