**Tox21 Phase 3: High-Throughput Transcriptomics and the S1500+ Initiative**

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The Tox21 federal collaboration was established through the signing of a Memorandum of Understanding (MOU) released in February of 2008 between the NTP at NIEHS, the NCCT at the U.S. EPA and the NCGC, then in NHGRI but now in NCATS, re-released in 2010 with the addition of the U.S. FDA and renewed in June 2015 for an additional 5-year period. This multi-agency Federal partnership is committed to pursuing the research, development, validation and translation of new and innovative test methods. An increasing emphasis of the program is on the use of alternative assays for interrogating key pathways, molecular events, and processes linked to disease or injury aid in a visionary paradigm shift in safety science. Phase 1 of Tox21 focused on evaluating the feasibility of the stated goals for the Tox21 program, determining best practices for chemical selection and high throughput screening, as well as for data interpretation. In Phase 2 of Tox21, the program moved into production level quantitative high-throughput screening (qHTS) of the "10 K" chemical compound library using high speed robotics. In the process of evaluating the results of the Phase 2 qHTS program, a number of challenges emerged that needed to be addressed in order to fulfill the goal of the Tox21 program of improving human health through improving human safety and risk assessment from exposures to environmental agents. Tox21 is moving to implement a Strategic Plan for Phase 3 that directly addresses these challenges. Integral to this effort is incorporating a high-throughput transcriptomic (HTT) screening approach that will interrogate the maximal biological response space to toxicological exposures and utilizing this with more physiologically relevant model systems. Tox21 partners have developed and implemented both a targeted whole transcriptome and a targeted gene set, the S1500+, HTT approach that is rapid and economical, utilizing the TempO-Seq technology.

The NTP is utilizing the Human S1500+ gene set platform in a number of initial studies to evaluate performance. A selection process was derived and the performance of the ~ 2900 genes of the S1500+ was evaluated and found to approach that of traditional whole transcriptome coverage provided by microarrays and NextGen sequencing (manuscript in press). A study of the response of human liver cultures of HepaRG cells exposed to 10 concentration levels of 24 reference compounds (known liver toxicants and non-toxicants) in triplicate experiments demonstrated that the S1500+ platform is a powerful new tool for capturing transcriptomic responses in a high-throughput approach. The NTP has been working with Tox21 partners and others to develop the BMDExpress 2.0 informatics tool to analyze HTT data, with the goal to provide quantitative dose response information that can be used toward risk assessment evaluations. To facilitate this process, *In Vitro - In Vivo* Extrapolation (IVIVE) approaches have been developed that can be used to estimate human external doses that would be anticipated to produced changes in the *in vitro* measured targets in an *in vivo* setting.