Introduction

- The mission of the U.S. Tox21 program is to develop, validate, and translate innovative chemical testing methods for the characterization of pathway toxicities. Tox21 integrates Federal resources and expertise from the following offices:
  - Environmental Protection Agency
  - National Institutes of Environmental Health Sciences/National Toxicology Program
  - National Institutes of Health/National Center for Advancing Translational Sciences
  - Food and Drug Administration
- The use of robotics platforms to screen thousands of chemicals provides a cost-effective approach to prioritize further testing of potentially toxic chemicals. Exposure to ‘endocrine active chemicals’ (EACs) may result in developmental or reproductive problems.
- EACs may affect growth and development through a variety of mechanisms. One such mechanism is estrogenic signaling.
- Estrogenic signaling pathways are well-characterized, and a number of test methods that target them have been developed. Two estrogen receptor (ER) transactivation assays, the BG1Luc and ERα HTS, have been adapted to a high-throughput screening (HTS) platform and incorporated into the Tox21 program.

Comparison to ICCVAM Performance Standards

- The U.S. National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods coordinated an international validation study of the BG1Luc assay for the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM).
- A test method evaluation report summarizing the study (ICCVAM 2011) contained performance standards for detecting functional and mechanistically similar test methods and for demonstrating the ability to distinguish between agonist and antagonist modes. These standards include response variability for agonist or antagonist with and without a positive or negative control, specificity reference values for both agonist and antagonist modes with expected positive and negative outcomes for each test. The HTS assay was compared to the ICCVAM performance standards (Table 4.1) and the HTS data were compared to the standards (Table 4.5).
- The assay correctly identified all of the test chemicals. The assay had a sensitivity of 100% (3/3) and specificity of 100% (3/3) in the Tox21 HTS data. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the ER alpha agonists tested as agonists, but result of tests of four chemicals were inconclusive. The assay correctly identified all of the ER alpha antagonist with expected positive and negative outcomes for each test. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the ER alpha agonists tested as agonists, but result of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identified all of the EACs tested as agonists, but results of tests of four chemicals were inconclusive. The assay correctly identifi...