## **Case Study: Environmental Monitoring and Ethinyl Estradiol**

### **Getting Started**

- 1. Create folder on desktop titled SeqAPASS\_Training folder
- 2. Open SeqAPASS using Chrome browser
  - <u>https://seqapass.epa.gov/seqapass/</u>
- 3. Download SeqAPASS User Guide and save it in your training folder

### **Problem Formulation**

Challenge:

- Active endocrine disruptor screening and testing program (EDSP)
- Legislated mandate to US EPA: evaluate ~10,000 chemicals for possible endocrine activity
- Need to prioritize chemicals for resource-intensive Tier 1 screening and Tier 2 testing (EDSP21)
- Explore the utility of U.S. EPA ToxCast to screen chemicals Identify chemicals most likely to be endocrine disruptors (Estrogen receptor, Androgen receptor, Thyroid)
- Goal to protect humans and wildlife though majority of assays are mammalian-based

**Question:** Does this mammalian-based screening/prioritization approach reasonably reflect potential impacts on other vertebrates? Focus on assays that screen for estrogenic chemicals

### Level 1: Choose an appropriate query and request a SeqAPASS run

- 1. Identify appropriate query sequence and run level 1 query in the "Request SeqAPASS Run" tab
  - <u>Query Protein:</u> estrogen receptor isoform 1 (NCBI Accession: NP\_000116.2)
  - <u>Query Species:</u> Human (*Homo sapiens*; Taxid: 9606)
- 2. View "SeqAPASS Run Status"
  - Did your run complete?
- 3. Download Level 1 Primary Report as a CSV file and save to SeqAPASS\_Training folder
- 4. Open Level 1 visualization
  - Click on box plot
  - Add ortholog candidates to visualization
  - Click on Actinopteri box to view summary information for Actinopteri
- 5. Download Level 1 boxplot visualization and save to SeqAPASS\_Training folder

# Level 2: Use NCBI Conserved Domain Database to identify appropriate domain(s) and run level 2 query

- 1. On SeqAPASS, open NCBI Conserved Domain Database link
- 2. Within the Conserved Domain Database, select "Full Reports" and identify the "Specific Hits", scroll over NR\_LBD\_ER, and note the domain Accession
  - In SeqAPASS, use Level 2 drop-down to find accession (cd06949) and "Request Domain Run".
- 3. View "SeqAPASS Run Status"
  - Did your Level 2 run complete?
- 4. Go to "View SeqAPASS Reports" and navigate to the "Level 2" drop down window
  - To view Level 2 Data, click "Choose Domain to View" and select cd06949 from the drop down and click "View Level 2 Data"
- 5. Download Level 2 Primary Report as a CSV file and save to SeqAPASS\_Training folder
- 6. Open Level 2 visualization
  - Click on box plot
  - Add ortholog candidates to visualization
- 7. Download Level 2 boxplot visualization and save to SeqAPASS\_Training folder

# Level 3: Use information gathered from literature to assess the conservation of key amino acids.

In this case study, we are going to use information from publications that identified key residues. In this case, previously determined crystal structures of the estrogen receptor bound with estrogenic and anti-estrogenic compounds guide us to choose six amino acid residues.

Template Sequence: Human estrogen receptor isoform 1

Protein Accession: NP\_000116.2

Key Individual Amino Acid Residues: D351 (Aspartic acid), E353 (Glutamic acid), K362 (Lysine), V364 (Valine), R394 (Arginine), H524 (Histidine)

- 1. Set up Level 3 query for the taxonomic group Actinopteri and Amphibia, performing the alignment with one taxonomic group at a time
- 2. Use "View Combined Report Section" to combine Actinopteri and Amphibia data to push to view the Level 3 data
- 3. Enter the amino acid residues listed above based on the template sequence (Human ESR1) and "Update Report"
- 4. Download Level 3 Report as a CSV file and save to SeqAPASS\_Training
- 5. Open Level 3 Visualization
- Click on heat map visualization
- Choose Taxonomic Groups to view in the Level 3 Heat Map

Application of SeqAPASS to Understand Cross Species Susceptibility to Chemical in the Environment Level 1, Level 2, and Level 3 – Beginner User

- Explore Simple and Full Report Types
- Explore Optional Selections
- Explore Heat Map Settings
- Hover over heat map
- Download custom Level 3 Heat Map visualization and save to SeqAPASS\_Training

#### **Develop a Decision Summary Report**

- 1. Open Level 1 Primary Report and Push Level 1 to DS Report
- 2. Open Level 1 Visualization and Push Level 1 Boxplot to DS Report
- 3. Open Level 2 Primary Report and Push Level 2 to DS Report
- 4. Open Level 2 Visualization and Push Level 2 Boxplot to DS Report
- 5. (Optional) Open Level 3, Select amino acids, Update report and Push Level 3 to DS Report
- 6. (Optional) Open Level 3 Visualization, Select Taxonomic groups, and Push Level 3 Map to DS Report
- 7. Open DS Report, Select Taxonomic Groups
- 8. Choose Level 1 Visualization
- 9. Choose Level 2 checkboxes
- 10. (optional) Choose Level 3 checkboxes
- 11. Download DS Report as PDF and save to SeqAPASS\_Training

#### After completing Levels 1-3, how broadly might we anticipate extrapolating from human ER?