Inter-lab validation of EcoToxChips using reference cDNA samples

ecotoxchip

BACKGROUND INFORMATION

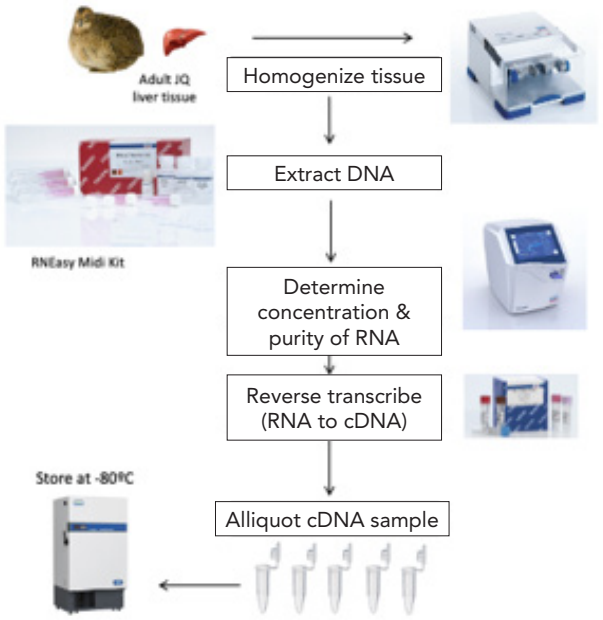
To ensure confidence in, and reliability of EcoToxChips, it is critical to conduct multiple validation steps. One important criteria is to evaluate the performance and reproducibility of EcoToxChips in different laboratories and ensure the SOPs are sufficiently robust to be transferrable regardless of different lab bench workflows and qPCR instruments.

OBJECTIVE

Three sets of reference cDNA samples (referred to as A, B, C) were prepared from RNA derived from adult Japanese quail (JQ) liver to run on JQV0.1 EcoToxChips as part of this inter-laboratory validation study. The overall objective was to evaluate inter-laboratory CVs for Ct values of the target and housekeeping genes.

APPROACH & RESULTS

The extracted RNA came from liver samples of two treatment groups (solvent and chlorpyrifos high dose, 10 mg/kg body weight), which was later reverse transcribed into cDNA to create 3 blinded sample types (A, B, and C) consisting of a solvent, CPF HD, and CPF HD-diluted (1:2) groups (Figure 1). These samples were shared with the 3 core labs: Environment and Climate Change Canada, McGill University, University of Saskatchewan.



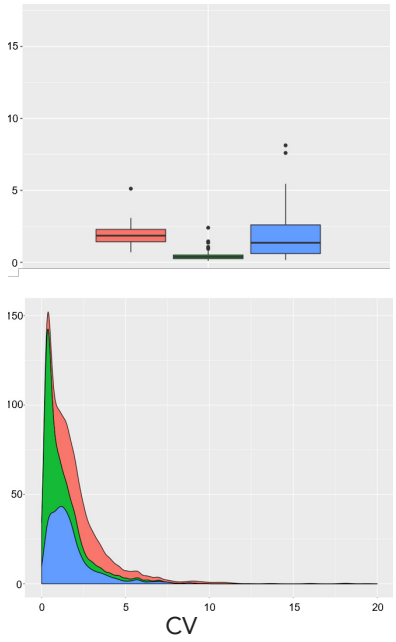


Figure 2. Boxplot of CV variance of housekeeping genes from test labs (red – ECCC; green – McGill; blue – U Sask)
→ **Median value <2.5%**

Figure 3. Density plot of CV variance of gene targets from the 3 different test labs.
→ **Largely <5%**

TAKEAWAYS

Intra- and inter-lab variability of the reference cDNA samples run on JQ v0.1 EcoToxChips was lower than the criteria established by the MAQC program (e.g., intra and inter-lab variation in Ct values less than 20%). This provides confidence in the performance and reproducibility of EcoToxChips regardless of where they are run.

Notes

Reference: MAQC Consortium. The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. Nat Biotechnol 24, 1151–1161 (2006).