JGI JOINT GENOME INSTITUTE
UNITED STATES DEPARTMENT OF ENERGY

HiSeq lane to lane variation related to RNA differential expression experiments

RNASeq lane to lane variation?

- **Problem: Concern that there is lane to lane variation when sequencing RNASeq libraries for expression projects.**

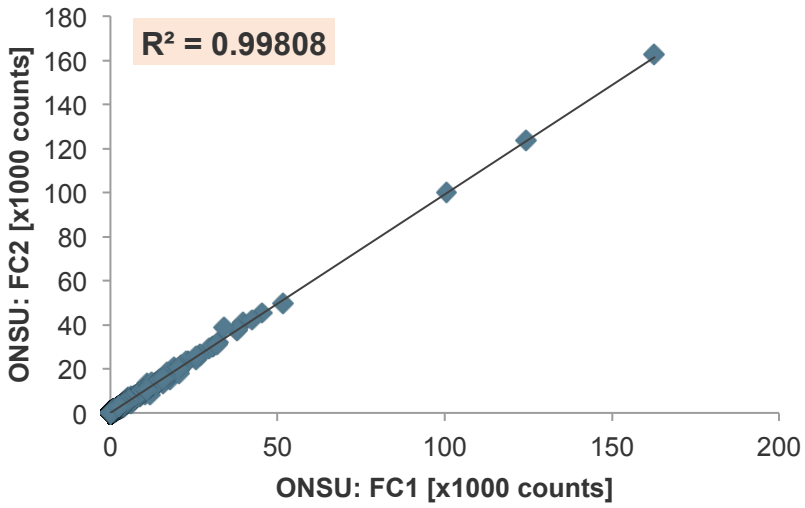
- **Findings: Technical replicates correlated very well. Furthermore, biological replicate example indicated more variation than technical replicate.**

- **Design:**
  - Used reads from Standard RNASeq libraries (Fungal, Plant, Bacterial)
  - For technical replicates, the same library was run on separate Flowcells (FC)
  - RNASeq reads were "counted" using the JGI standard method by alignment of reads to the reference transcriptome (bwa)
  - For biological replicate, separate RNA was used to construct RNASeq libraries

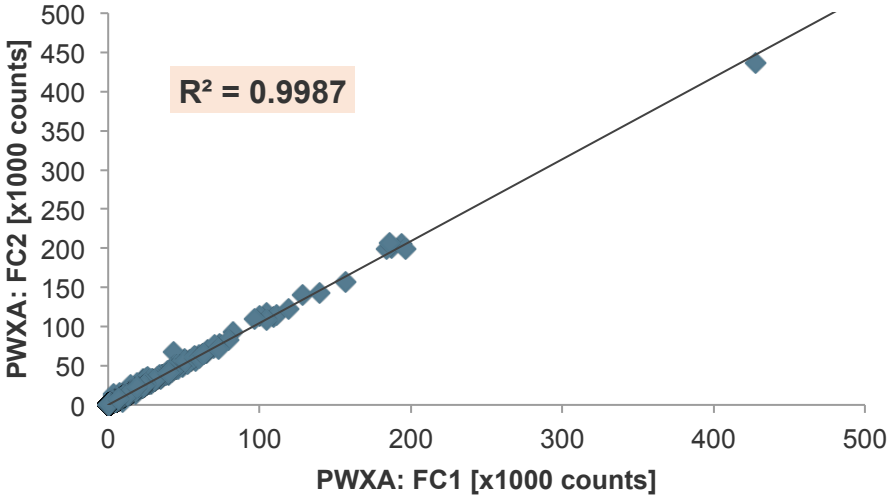
- **Note: in general, literature strongly supports use of biological replicates but not technical replicates.**

Correlations of Technical Replicates

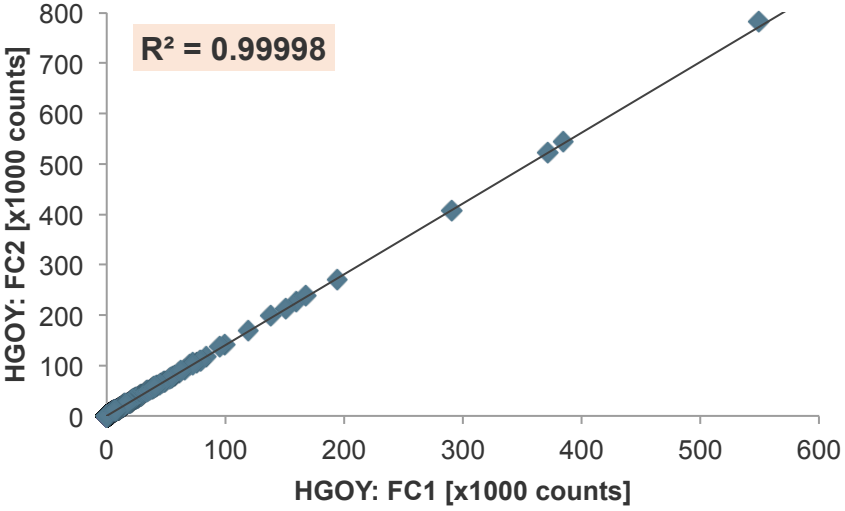
Plant Library ONSU: FC1 vs FC2



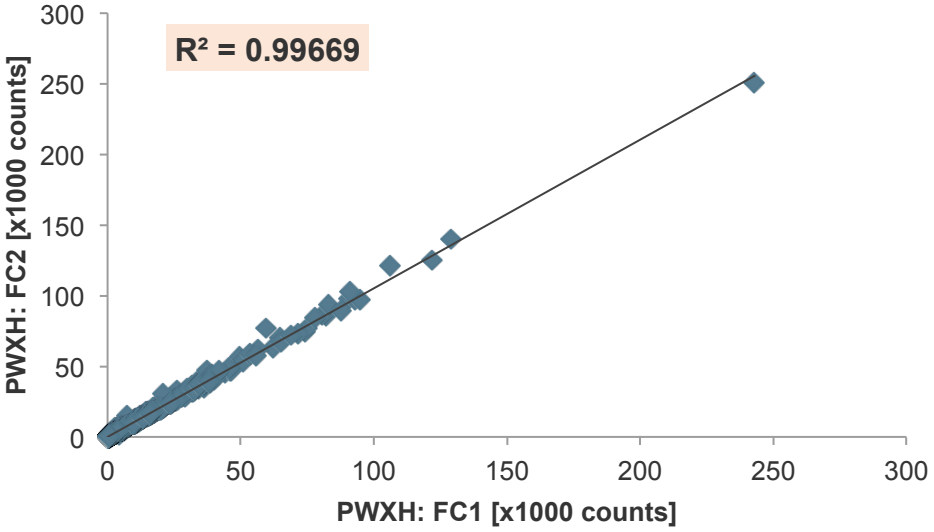
Fungal Library PWXA: FC1 vs FC2



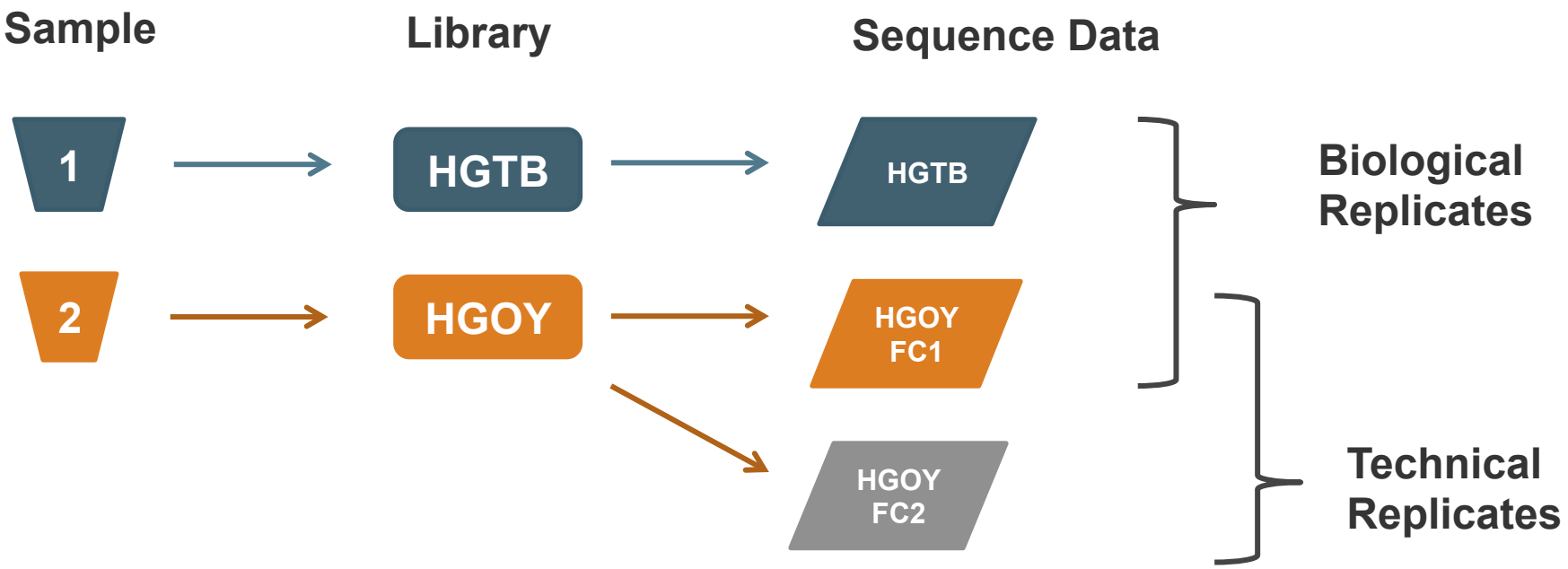
Bacterial Library HGOY: FC1 vs FC2



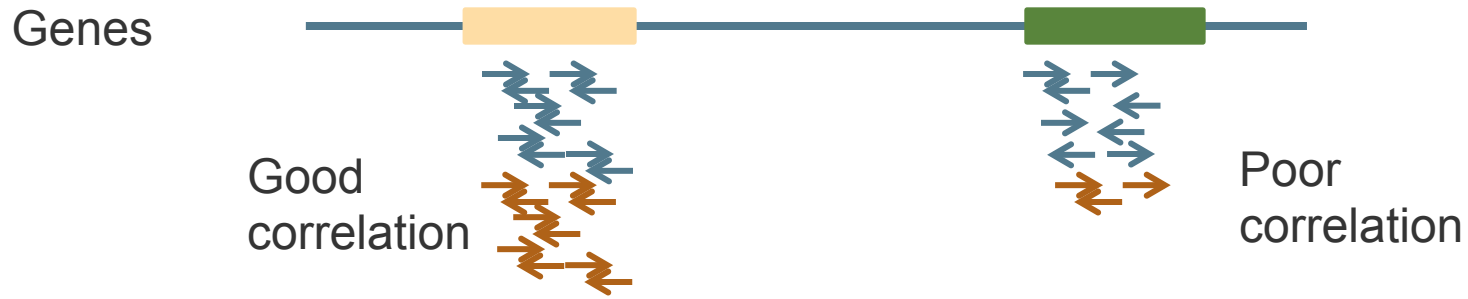
Fungal Library PWXH: FC1 vs FC2



Technical replicates vs Biological replicates

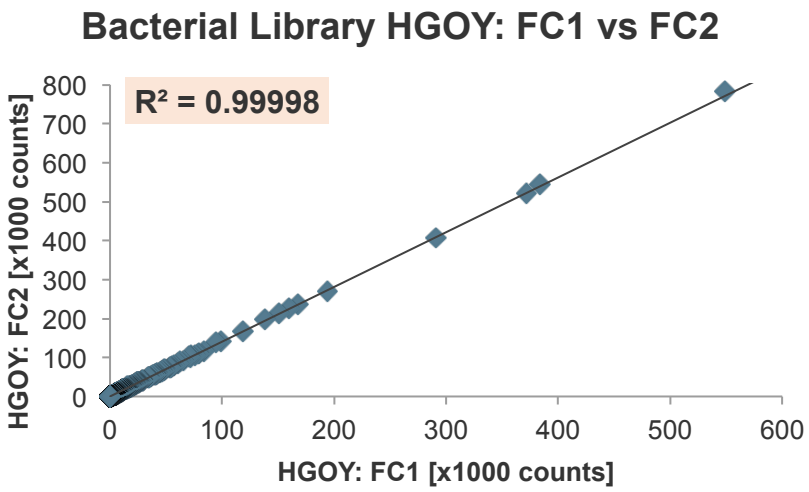


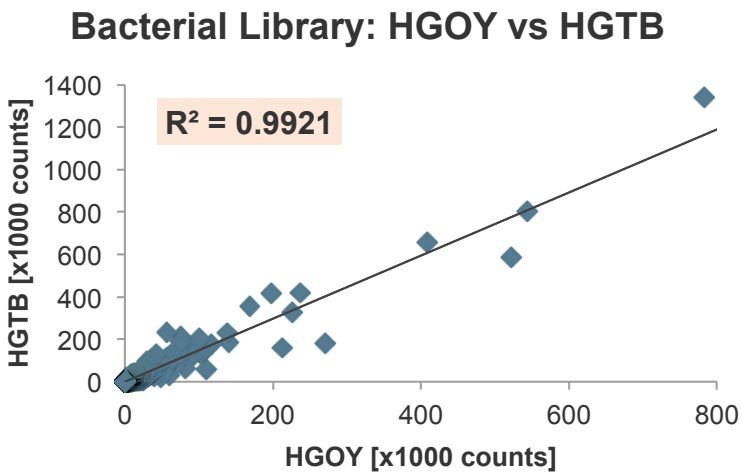
Align sequence reads to reference, count them and evaluate correlation



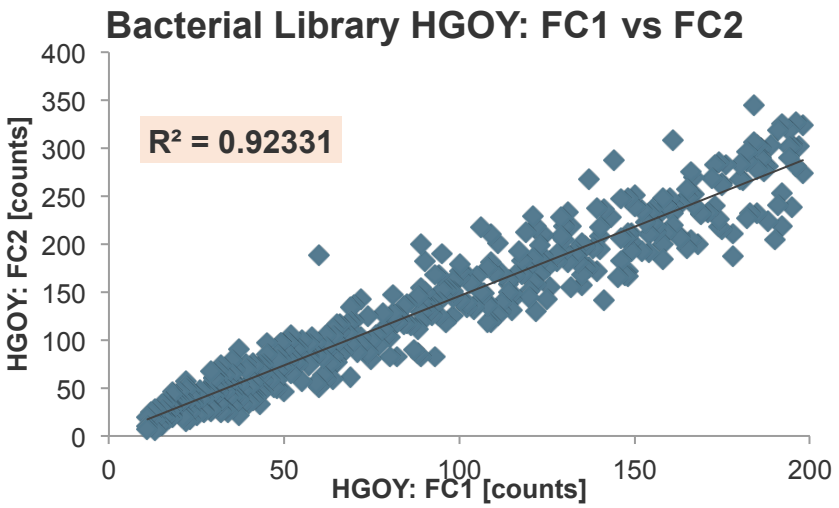
Technical Replicates

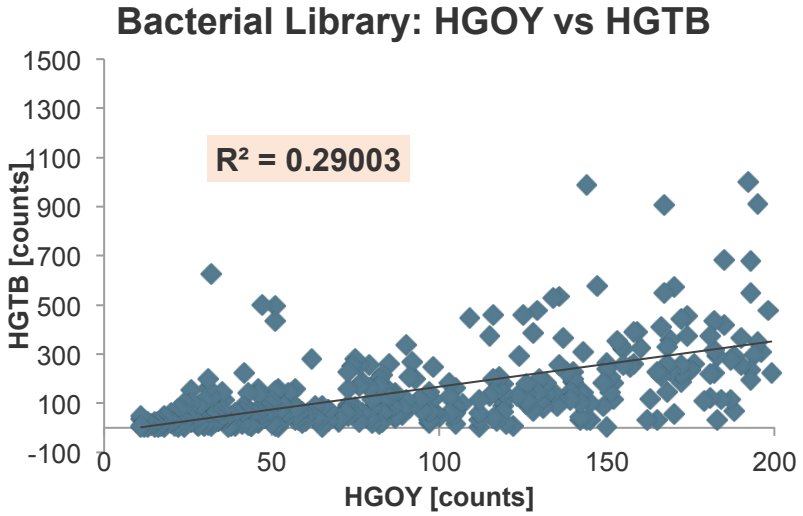
Biological Replicates





Deep Coverage *





Medium Coverage **

* Genes with >200 counts (same as all counts)

** Genes with 11-200 counts