1. What was the total amount of raw sequence data generated (in gigabases)?

26.8 Gb

2. What's the name of the software program that the investigators used to assemble the genome?

Celera assembler

3. What are "axenic" cultures?

Culture that have no contamination from other organisms.

4. In their PCR testing, where Boothby et al. tested cases when a non-metazoan gene (something from a bacterium or fungus) was near a metazoan gene in their assembly, how many times was the PCR product the correct size?

58 out of 59, which is 98.3%.

5. How many contigs from the PacBio assembly failed to match the Illumina assembly?

4,440 contigs.

A total of 4,440 (10.72%) of the PacBio contigs, making up 7.91% of the assembled sequence, do not have direct homologous matches in the Illumina assembly.

6. How large was the genome of the tardigrade H. dujardini estimated to be, based on flow cytometry?

110Mh

Using propidium iodide flow cytometry, we estimated the genome of ${\it H.}$ dujardini to be ~110 Mb, similar to a previous estimate.

7. In the nHd.2.3 assembly, how many scaffolds had either no genes or only bacterial genes?

195 scaffolds, 1.5 Mb.

Some scaffolds (195 spanning 1.5 Mb) had only bacterial or no genes and were very likely to be contamination.

8. In their nHd.2.3 assembly, how many genes did the authors find, as an upper bound, for the number of "hard" candidates for horizontal gene transfer?

196 genes.

Most of these (196, 0.9% of all genes) had expression >0.1 tpm (Fig. 3D) and are an upper bound of "hard" candidates for fHGT.

9. How many of the PCR products from Boothby et al. were determined not to assess HGT because they only probed relationships between bacterial genes?

49 products.

We found no expression of the 49 bacterial—bacterial junction loci, supporting assignment as contaminants rather than examples of fHGT.