Comparative transcriptomics and host-specific parasite gene expression profiles inform on drivers of proliferative kidney disease

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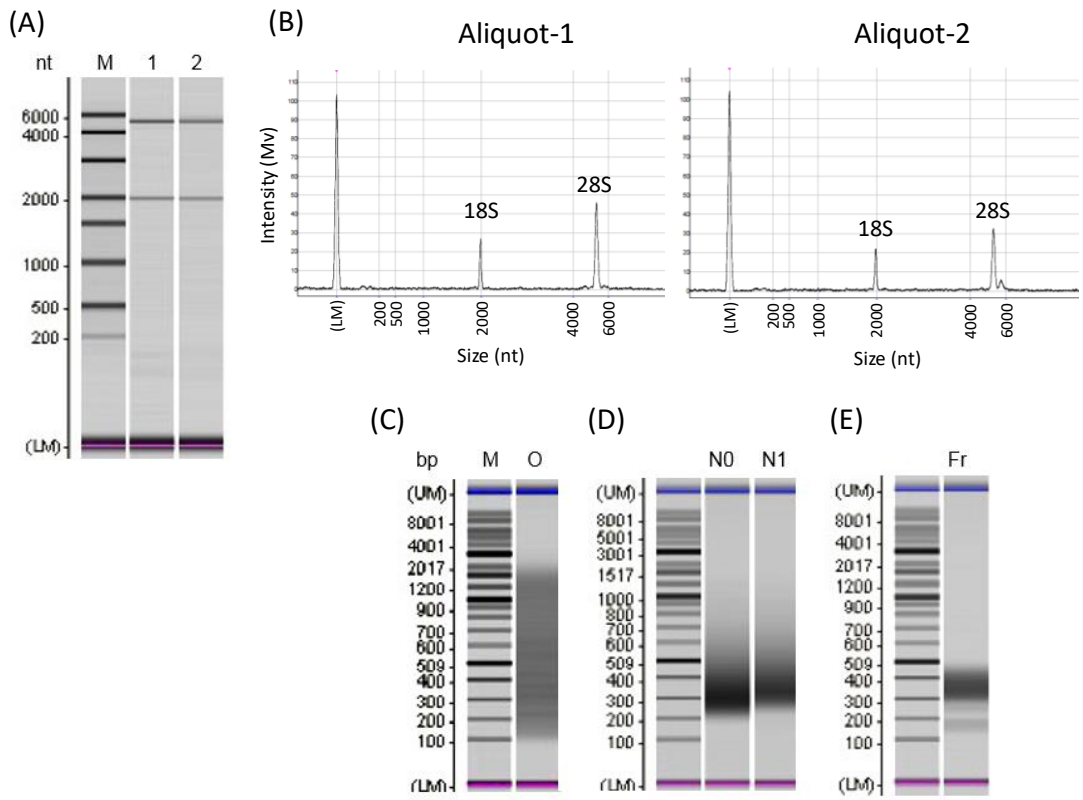
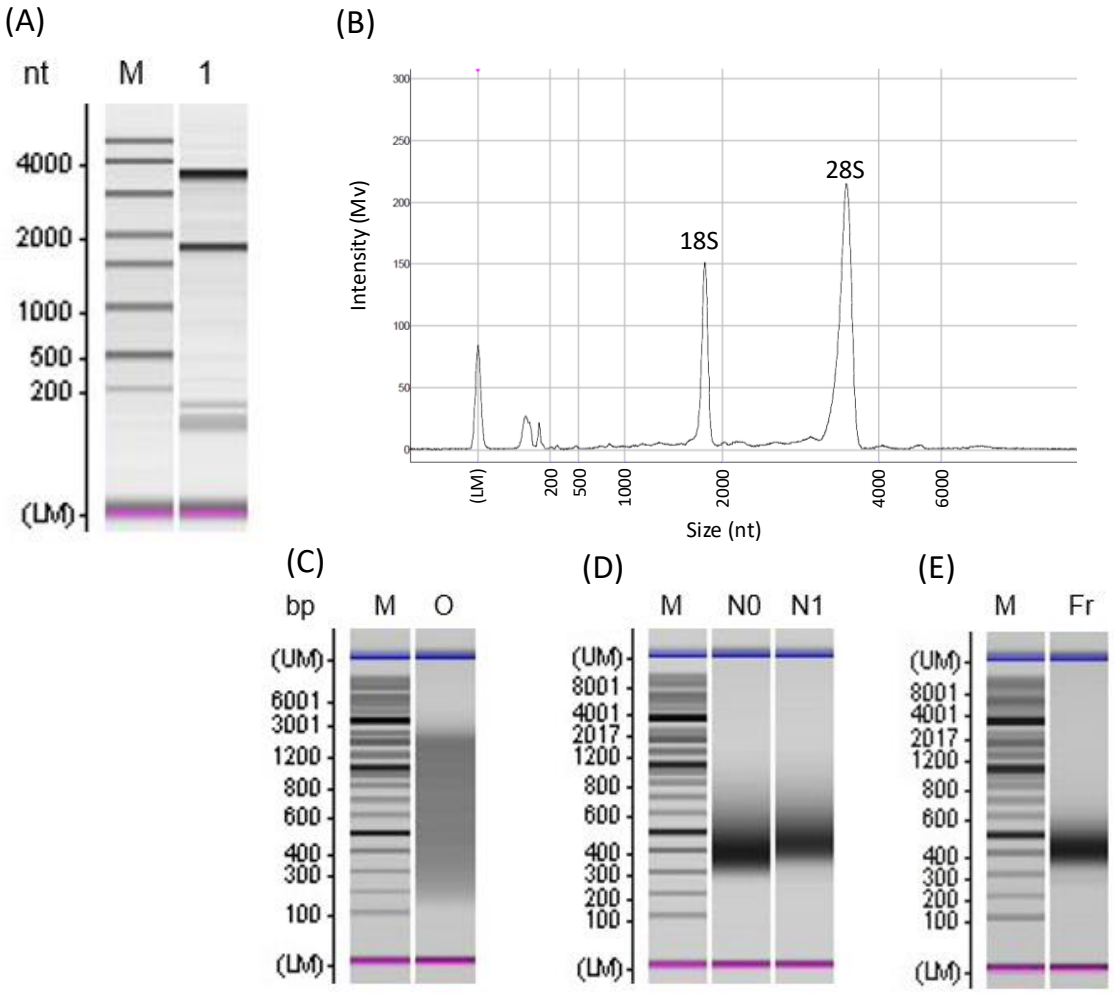
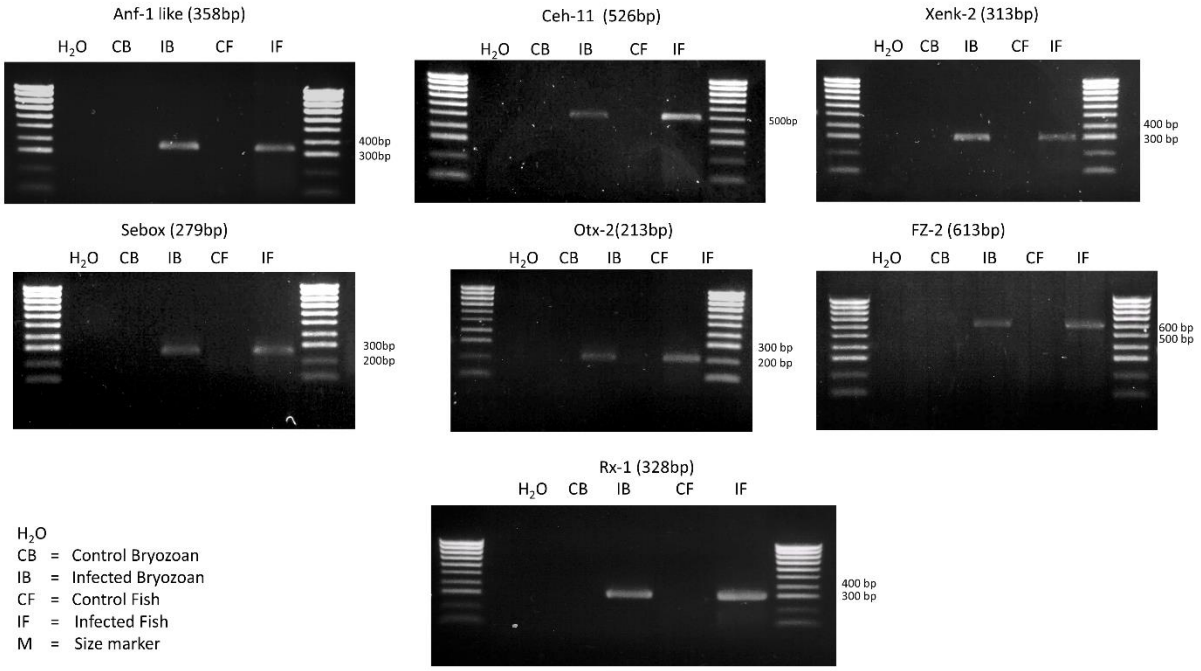


Supplementary Figure S1. Shimadzu MultiNA microchip total RNA and cDNA electrophoresis profiles prior to and during Illumina cDNA library preparation. (A) Spore sac total RNA (total yield = 1.3 μg supplied as 2 aliquots (Aliquot-1 & Aliquot-2) of 0.6 & 0.7 μg in 64 μl and 71 μl RNase-free water respectively) were used to generate the bryozoan-derived spore sac Illumina library. (B) Electropherograms representing the two total RNA aliquots used for cDNA library preparation with 28S rRNA / 18S rRNA ratios of 1.6 (RNA-1) and 1.4 (RNA-2). (C) Poly A+ RNA was purified, and reverse transcribed into cDNA using the Ovation RNA-Seq System V2 kit (NuGEN Technologies Inc). (D-E) Amplified cDNA was fragmented with ultrasound (2 pulses of 30 s at 4°C), end repaired, and TruSeq sequencing adapters ligated to the cDNA fragments. cDNA normalisation was undertaken by one cycle of denaturation and reassociation of the cDNA. Single stranded cDNA was separated from double stranded cDNA by hydroxylapatite chromatography. Normalised cDNA was size fractionated (size range 300-500 bp) using a differential clean-up with an AgenCourt AMPure kit prior to end repair and adapter ligation. M = Size marker. O = cDNA generated using the Ovation RNA-Seq System V2 kit. NO = PCR-amplified cDNA. N1 = normalised cDNA. Fr = size fractionated N1 cDNA.



Supplementary Figure S2. Shimadzu MultiNA microchip total RNA and cDNA electrophoresis profiles prior to and during Illumina cDNA library preparation. (A) Fish kidney total RNA (total yield = 21 μg in 42 μl RNase-free water) used to generate the fish-derived Illumina library. (B) Electropherogram representing fish kidney total RNA used for cDNA library preparation with a 28S rRNA / 18S rRNA ratio of 1.9. (C) Poly A+ RNA was purified, and reverse transcribed into cDNA using the Ovation RNA-Seq System V2 kit (NuGEN Technologies Inc). (D-E) Amplified cDNA was fragmented with ultrasound (2 pulses of 30 s at 4°C), end repaired, and TruSeq sequencing adapters ligated to the cDNA fragments. cDNA normalisation was undertaken by one cycle of denaturation and reassociation of the cDNA. Single stranded cDNA was separated from double stranded cDNA by hydroxylapatite chromatography. Normalised cDNA was size fractionated (size range 300-600 bp) using a differential clean-up with an AgenCourt AMPure kit prior to end repair and adapter ligation. M = Size marker. O = cDNA generated using the Ovation RNA-Seq System V2 kit. NO = PCR-amplified cDNA. N1 = normalised cDNA. Fr = size fractionated N1 cDNA.



Supplementary Figure S3. Uncropped pictures of the PCR profile shown in Figure 8D. Validation of Sebox, Ceh-11, Anf-1-like, Otx-2, FZ-2, Rx-1 and Xenk-2 in negative control (H_20), uninfected bryozoan and fish kidney controls respectively (CB & CF) and *T. bryosalmonae* infected bryozoan and fish genomic DNA respectively (IB & IF). All blots are flanked by the Molecular weight marker.