**High Throughput RNA-Sequencing**

**RNA extraction for sequencing**. To gather qualitative brain transcriptome information relevant to the ILSXISS recombinant inbred panel, brain total RNA was isolated from the two progentior strains of the LXS recombinant inbred panel (ILS and ISS; 3 mice per strain) using the RNeasy Midi Kit with additional clean-up using the RNeasy Mini Kit (Qiagen, Valencia, CA). Quality of extracted total RNA was assessed on an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA). Ribosomal RNA was depleted from total RNA using the Ribo-Zero Magnetic Kit (Epicentre Biotechnologies, Madison WI) according to the manufacturer’s protocol.

**Sequencing of RNA**. Sequencing libraries were constructed using the Illumina **TruSeq Stranded Total RNA Sample Prep Kit (Illumina, San Diego, CA) according the manufacturer’s protocol. Library quality was assessed using the Agilent Bioanalyzer. The samples were multiplex and all six samples were included in three lanes of the flowcell. Libraries were sequenced using 100 base pair paired end reads and the Illumina HiSeq 2000. Reads were de-multiplexed prior to quality control and alignment.**

**Quality control and alignment of reads. Prior to alignment, reads were trimmed of adaptors and low quality base calls using trim\_galore (**<http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/>**). Entire reads were eliminated if either read fragment was less than 20 bp after trimming. Reads were aligned to the mm10 version of the mouse genome using tophat2 (REF) and its default settings for stranded reads.**

**Transcriptome reconstruction. The brain transcriptomes for the ILS and ISS strains were reconstructed separately using all aligned reads from that strain. Cufflinks was used to reconstruct the brain transcriptome using both a genome guide and a transcriptome guide (REF). The transcriptome used for guidance was the RefSeq mm10 version (Dec 2011) downloaded from the UCSC Genome Bioinformatics Site (**<http://genome.ucsc.edu>**). The two strain-specific transcriptomes were combined into a single transcriptome of high confidence transcripts manually. First, each strain-specific transcriptome was reduced to transcripts with an FPKM value greater than 1 (as calculated in the CuffLinks software) and a length greater than 300 bp (not including introns). Novel, i.e., unannotated, transcripts identified in the strain-specific transcriptomes were combined. Novel transcripts “matched” between strain-specific transcriptomes if: 1) all exon start and stop positions matched, 2) all exon junctions matched, or 3) both transcripts contained only one exon and their transcripts start sites were within 100 bp of each other and their transcription stop sites were within 100 bp of each other. Two transcripts were identified as being from the same gene if: 1) their transcription start sites matched exactly, 2) their transcription stop sites matched exactly, or 3) at least one exon junction matched exactly. This combined transcriptome was pruned further by quantifying the transcript expression levels of the combined transcriptome in either strain using sailfish (REF). Transcripts were retained if at least one of the six samples had a TPM (transcripts per million) value greater than 1. After elimination of low expressing transcripts, TPM values were calculated again. This iteration between quantification and elimination of transcripts continued until less than 1% of transcripts had a TPM value less than 1.**

**Exon Array Probe Mask/Transcript Cluster Quantification. Information gather from high throughput DNA sequencing of the two progenitor strains (RICHARD REF) and the brain transcriptome generated from the two progenitor strains of the LXS panel was used to guide the use of probes/probe sets from the Affymetrix Mouse Exon Array 1.0 ST for quantifying expression in the LXS recombinant inbred panel. First, individual probes were masked due to poor integrity if they did not align uniquely to the mouse genome (mm10) or if they aligned to a region that harbored a SNP between the reference genome (based on the C57BL/6 inbred mouse strain) and either of the ILS or ISS strains. Entire probe sets were eliminated if less than 3 probes remained after masking. The remaining high integrity probe sets were compared to the brain transcriptome derived from the RNA-Seq data. Probe sets that were completely contained within gene, not including intronic regions, were identified and the correlation among probe sets targeted to the same gene were evaluated.**