## File S1

## Supplemental Methods

### *Simulation parameters*

RNA-seq reads were simulated from the CAST inbred strain and from a reconstructed DO individual using the Flux Simulator (version 1.2) and the parameters below.

Command line argument: flux-simulator –lsp Parameter_filename.txt

Single-end sequence parameters

```
REF_FILE_NAME      path/to/Gene_annotations.gtf
GEN_DIR            path/to/Genome.fa
LIB_FILE_NAME      filename.lib
SEQ_FILE_NAME      filename.bed
PRO_FILE_NAME      filename.pro
RT_PRIMER                   PDT
READ_NUMBER        10000000 (or 30000000)
READ_LENGTH        100
FILTERING                   true
SIZE_DISTRIBUTION   N(280,50)
FASTA              true
TSS_MEAN                    NaN
POLYA_SCALE        NaN
POLYA_SHAPE        NaN
ERR_FILE           76
```

Paired-end sequence parameters

```
REF_FILE_NAME      path/to/Gene_annotations.gtf
GEN_DIR            path/to/Genome.fa
LIB_FILE_NAME      filename.lib
SEQ_FILE_NAME      filename.bed
PRO_FILE_NAME      filename.pro
RT_PRIMER                   PDT
READ_NUMBER        60000000
READ_LENGTH        100
PAIRED_END                  YES
FILTERING                   true
SIZE_DISTRIBUTION   N(280,50)
FASTA              true
TSS_MEAN                    NaN
POLYA_SCALE        NaN
POLYA_SHAPE        NaN
ERR_FILE           76
```