

Figure S4

a

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Ft11-001_NCBIM37 1 AGGTCCCCTGGATCTGTGTCTTGCTTCAACAGTGTTTGAACGGAACAGACCCGGGGATTC
Ft11-001_CAST 1 .....
Ft12-001_NCBIM37 1 -----
Ft12-001_CAST 1 -----

Ft11-001_NCBIM37 61 CCACTGTACTCGCTTCCAGCCGCCTTTACAAGTCTCTCCAGTCGCAGCCTCCGGGACCAT
Ft11-001_CAST 61 .....
Ft12-001_NCBIM37 1 -----
Ft12-001_CAST 1 -----

Ft11-001_NCBIM37 121 CTCCTCGCTGCCTTCAGCTCCTAGGACCAGTCTGCACCGTCTCTTCGCGGTTAGCTCCTA
Ft11-001_CAST 121 .....G.....
Ft12-001_NCBIM37 1 -----
Ft12-001_CAST 1 -----

Ft11-001_NCBIM37 181 CTCCGGATCAGCCATGACCTCTCAGATTTCGTCAGAATTATCCACCGAGGTGGAAGCTGC
Ft11-001_CAST 181 .....
Ft12-001_NCBIM37 1 -----
Ft12-001_CAST 1 -----

Ft11-001_NCBIM37 241 CGTGAACCGCCTGGTCAACTTGCACCTGCGGGCCTCTACACCTACCTCTCTCTGGGCTT
Ft11-001_CAST 241 .....
Ft12-001_NCBIM37 48 .....
Ft12-001_CAST 48 .....

Ft11-001_NCBIM37 301 CTTTTTGTATCGGGATGACGTGGCTCTGGAGGGCGTAGGCCACTTCTTCCGCGAATTGGC
Ft11-001_CAST 301 .....
Ft12-001_NCBIM37 108 .....
Ft12-001_CAST 108 .....

Ft11-001_NCBIM37 361 CGAGGAGAAGCGCGAGGGCGGGAGCGTCTCCTCGAGTTTCAGAACGATCGCGGGGGCCG
Ft11-001_CAST 361 .....
Ft12-001_NCBIM37 168 .....
Ft12-001_CAST 168 .....

Ft11-001_NCBIM37 421 TGCACTCTTCCAGGATGTGCAGAAGCCATCTCAAGATGAATGGGGTAAAACCCAGGAGGC
Ft11-001_CAST 421 .....
Ft12-001_NCBIM37 228 .....
Ft12-001_CAST 228 .....
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Figure S4 (continued)

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Ft11-001_NCBIM37 481 CATGGAAGCTGCCTTGGCCATGGAGAAGAACCTGAATCAGGCCCTCTTGATCTGCATGC
Ft11-001_CAST    481 .....T.....
Ft12-001_NCBIM37 288 .....C.....
Ft12-001_CAST    288 .....C.....

                                     *
Ft11-001_NCBIM37 541 CCTGGGTTCTGCCCGCGCGGACCCTCATCTCTGTGACTTCCTGGAAAGCCACTATCTGGA
Ft11-001_CAST    541 .....C.....
Ft12-001_NCBIM37 348 .....C.....C.....TC.....
Ft12-001_CAST    348 .....C.....C.....TC.....

Ft11-001_NCBIM37 601 TAAGGAGGTGAAACTCATCAAGAAGATGGGCAACCATCTGACCAACCTCCGCAGGGTGGC
Ft11-001_CAST    601 .....
Ft12-001_NCBIM37 408 .....
Ft12-001_CAST    408 .....

                                     *                                     *
Ft11-001_NCBIM37 661 GGGGCCACAACCAGCGCAGACTGGCGCGCCCCAGGGGTCTCTGGGCGAGTATCTCTTTGA
Ft11-001_CAST    661 A.....A.....
Ft12-001_NCBIM37 468 A.....A.....
Ft12-001_CAST    468 A.....A.....

Ft11-001_NCBIM37 721 GCGCCTCACTCTCAAGCACGACTAGGAGGCCTCTGTACCTTCCAAGGGGCTCCCCCTCT
Ft11-001_CAST    721 .....
Ft12-001_NCBIM37 528 .....-----
Ft12-001_CAST    528 .....-----

Ft11-001_NCBIM37 781 GCTCTGCACCAGCCCGCCCTGGGACCTCCACCTGAATGAACCTCTCAAGCCACTAGGCAG
Ft11-001_CAST    781 .....
Ft12-001_NCBIM37 .....-----
Ft12-001_CAST    .....-----

Ft11-001_NCBIM37 841 CTTTGTAACCGCCCTGGAGCCTCTGTCAAGTCTTGGACCAAGTAAAAATAAAGCTTTTG
Ft11-001_CAST    841 .....
Ft12-001_NCBIM37 .....-----
Ft12-001_CAST    .....-----

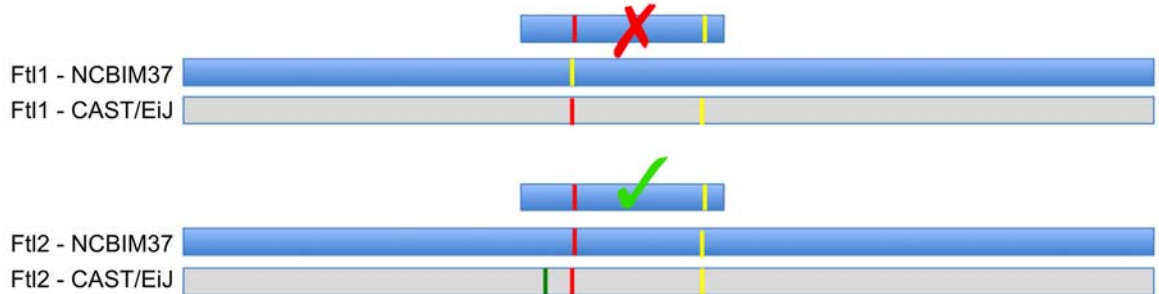
Ft11-001_NCBIM37 901 AGACAGC
Ft11-001_CAST    901 .....
Ft12-001_NCBIM37 .....-----
Ft12-001_CAST    .....-----

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Figure S4 (continued)

b

Alignment of CAST reads to NCBIM37



Alignment of CAST reads to CAST/EiJ



Figure S4 Strain polymorphisms between NCBIM37 and CAST in *Ft1* and *Ft2* transcript sequences can bias alignment of CAST-derived *Ft1* reads. (A) Multiple alignment of *Ft1*-001 and *Ft2*-001 transcript sequences from NCBIM37 and the individualized CAST genomes. Variation in *Ft1*/*Ft2* abundance estimates in CAST liver RNA-seq stems mainly from 3-4 SNPs (starred). (B) Schematic showing how CAST polymorphisms in RNA-seq reads can cause misalignments in NCBIM37. CAST *Ft1* reads that overlap any of these SNPs will align preferentially to *Ft2* if aligned to NCBIM37 (upper panel). Accounting for CAST strain variation in *Ft1* reduces spurious alignments to the *Ft2* pseudogene (lower panel).