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Supplemental Material to:

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Revisiting the coding potential of the E. coli genome through Hfq co-immunoprecipitation

2012; 11(5)
http://dx.doi.org/10.4161/rna.29299

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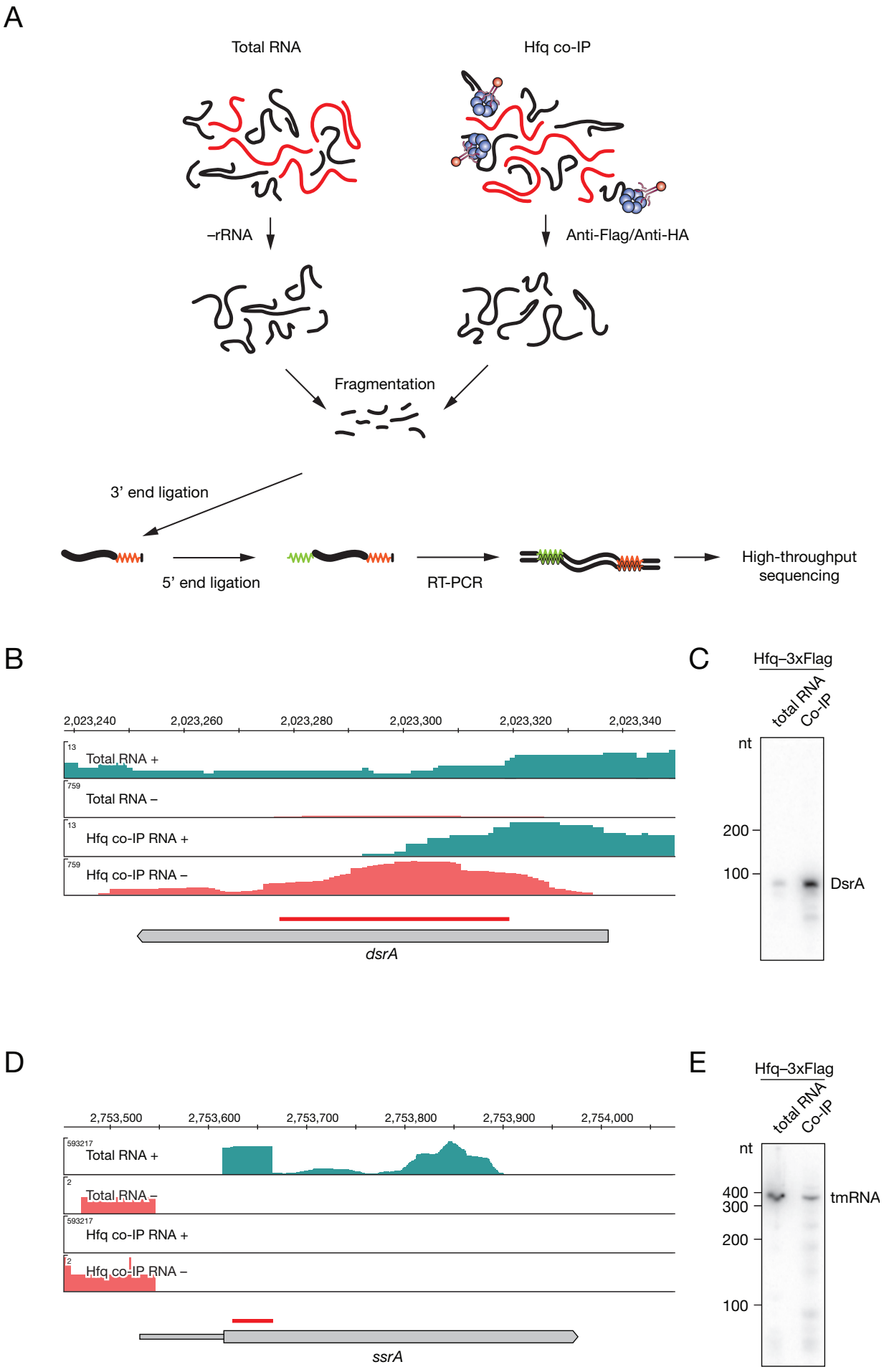
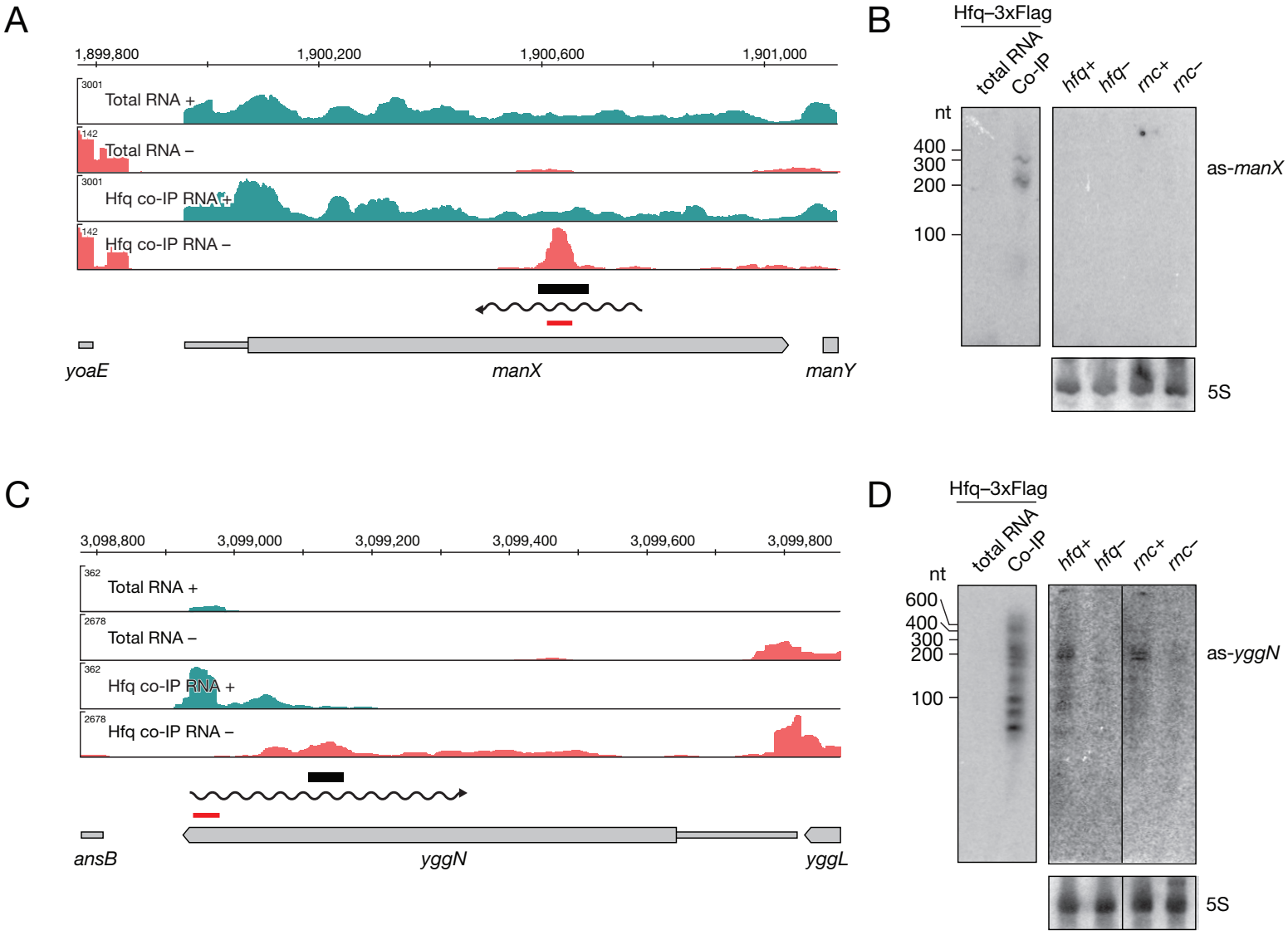
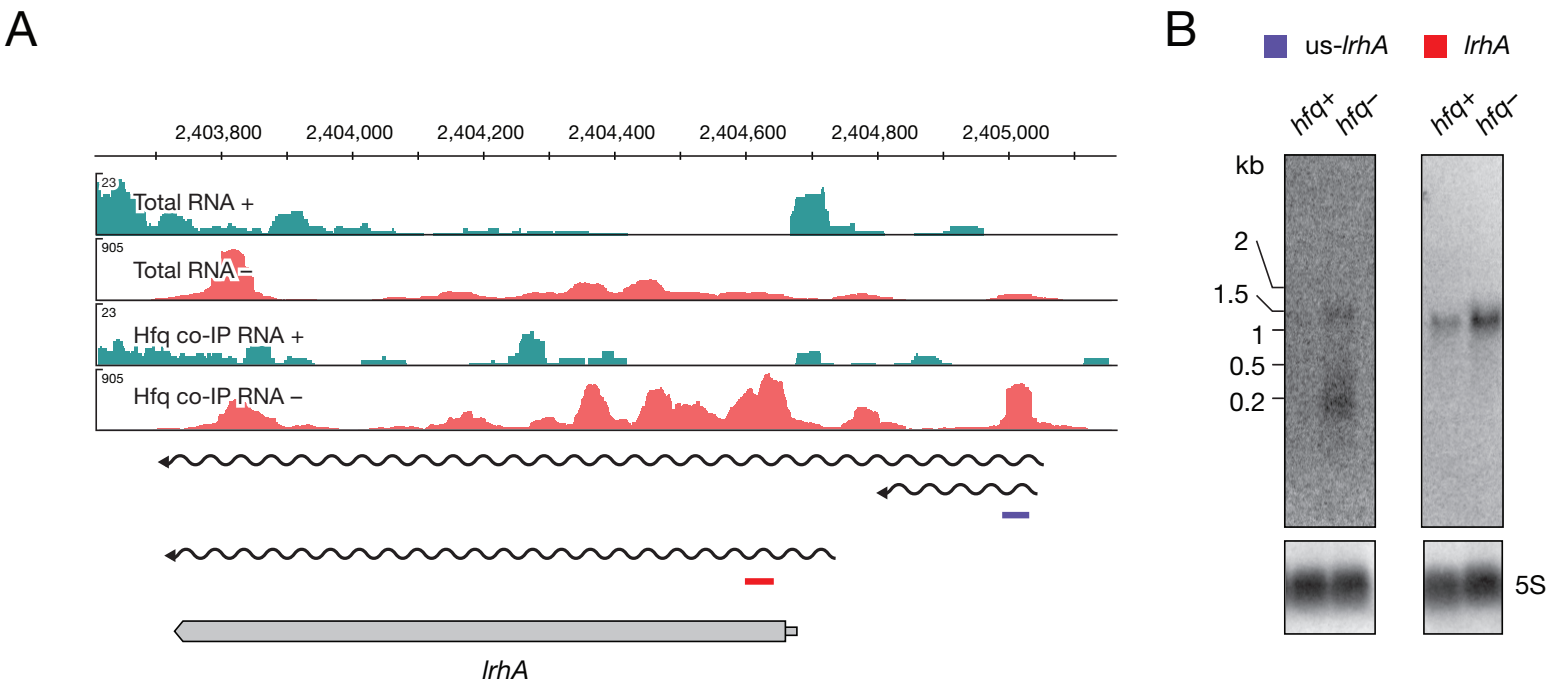


Bilusic et al. Figure S1



Bilusic et al. Figure S2



Bilusic et al. Figure S3

Figure S1. **Library preparation and validation of results.**

(A) Cells with C-terminally tagged Hfq bearing either 3xFlag or HA were grown to exponential phase. Total RNA depleted of rRNA or RNA co-immunoprecipitated with Hfq were fragmented, size-selected and subjected to subsequent RNA ligation. RNA was converted into cDNA, amplified and subjected to high-throughput sequencing. Deep-sequencing results for DsrA and tmRNA (B and D) represented as averaged coverage maps of 3xFlag- and HA total RNAs and 3xFlag- and HA-Hfq co-immunoprecipitated RNAs (Hfq Co-IP). Genomic strands are shown in blue (+) and red (-). Note that scales for + and – strand differ. Genomic location is depicted on top. The red bar indicates position of the oligonucleotide probe. Northern blot analyses of DsrA and tmRNA (C and E). Total RNA and RNA co-immunoprecipitated with 3xFlag were fractionated on a denaturing 8% polyacrylamide gel, electro-blotted onto a nylon membrane and hybridized with radioactively labeled oligonucleotide. Note different amounts of RNA were loaded; DsrA 10 μg total RNA and 1.5 μg Co-IP RNA; tmRNA 3 μg total RNA and 3 μg Co-IP RNA. 5S RNA was used as a loading control.

Figure S2. **Antisense RNAs containing Hfq aptamers bind Hfq.**

Deep sequencing and Northern blot analyses of as-*manX* and as-*yggN* as described in Figure 3A and 3B. The black bar indicates the position of the Hfq aptamer reported by Lorenz et al.[9]

Figure S3. **Hfq destabilizes *lrhA*.**

(A) Deep sequencing of *lrhA* region as described in Figure 3A. (B) Northern blot analyses of ig-*lrhA*-*alaA* and *lrhA*. 10 μg of total RNA isolated from *hfq*-deletion (*hfq*-) and corresponding isogenic wild-type (*hfq*+) cells were fractionated on a denaturing 1% formaldehyde-agarose gel, blotted onto a nylon membrane and hybridized with radioactively labeled oligonucleotide. Violet and red bars indicate the positions of the oligonucleotide probes used in the corresponding Northern blots. Ladder sizes in kb are indicated. 5S RNA was used as a loading control.

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