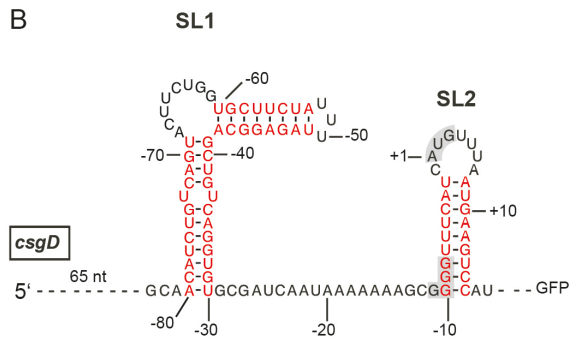
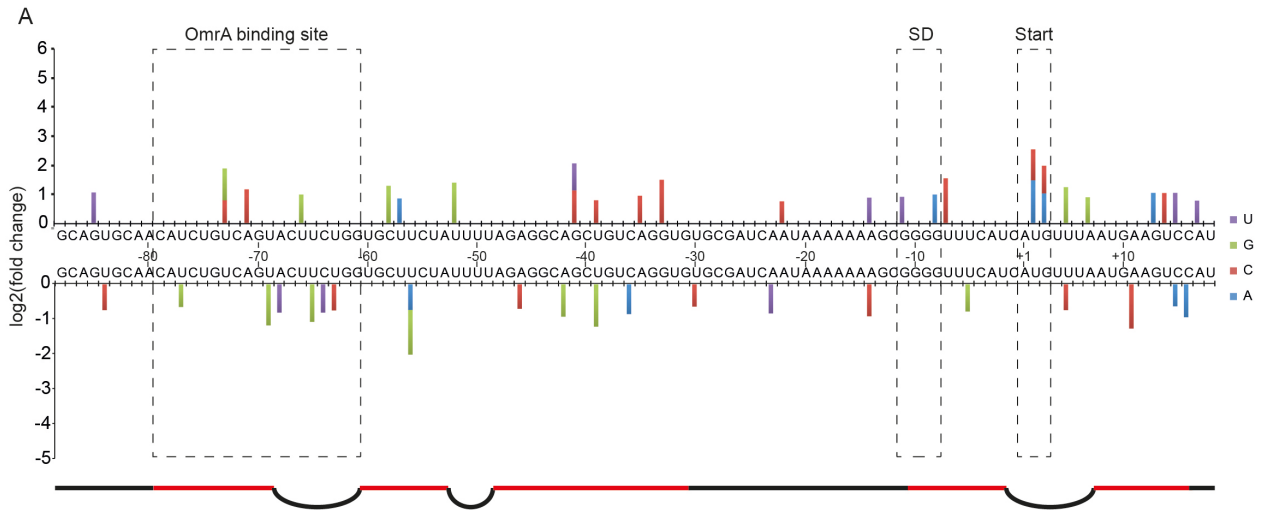


Supplementary information for:

**Massive functional mapping of a 5'UTR by saturation mutagenesis,
phenotypic sorting and deep sequencing**

Erik Holmqvist, Johan Reimegård and E. Gerhart H. Wagner



Supplementary Figure 1. Mutations associated with lowered *csgD-gfp* expression. (A) Significantly under- and overrepresented mutations in samples that were sorted for low fluorescence in the absence of OmrA compared to samples sorted for all fluorescence levels. Data were generated by Illumina sequencing. The RNA sequence of the *csgD* 5'UTR is shown in the middle of the graph in 5' to 3' direction. Numbers next to the sequence indicate nucleotide positions relative to A in the AUG start codon (set as +1). The OmrA binding site, the SD sequence, and the start codon are indicated with dashed boxes. A schematic under the graph represents the secondary structure of the *csgD* 5'UTR where black lines and bulges indicate unpaired nucleotides while red lines indicate paired nucleotides (same coloring as in (B)). (B) Sequence and secondary structure of the 5'UTR of *csgD* mRNA. Coloring and numbering as in (A).

Supplementary Table 1. Oligodeoxyribonucleotides used in this study.

Name	Sequence (5' to 3')	Used for
EHO-560	ccggagcgcgccagatcgcagctctaagaaacc	pCsgD::GFP construction
EHO-561	ccggagcgcgcggcggttgcctact	pCsgD::GFP construction
EHO-570	ggccgaattccgcttccagtcgggaaacc	pOmrA construction
EHO-571	ggccggatccgacgggcttctgctcc	pOmrA construction
EHO-572	ggccggatccccacctgacgtctaagaaacat	pOmrA construction
EHO-573	ggccgaattcagcgcagtaaattaggcgcaa	pOmrA construction
EHO-522	cccatttagggctgatttactactaca	Error-prone PCR
EHO-575	caacaataatgtatgacctgaatacta	Error-prone PCR
EHO-423	acatagtattcatggtcatacattattgttg	SDM: C+15A fwd
EHO-424	aaatagtattcatggtcatacattattgttg	SDM: C+15A, C+16A fwd
EHO-425	acttcattaacatgatgaaacccc	SDM: rev for EHO-423 and EHO-424
EHO-628	cacagcagtgcaacatcagtcagctactctgggtgc	SDM: T-75A fwd
EHO-629	gcaccagaagtactgactgatgtgactgctgtg	SDM: T-75A rev
EHO-630	cacagcagtgcaacatccgctcagctactctgggtgc	SDM: T-75C fwd
EHO-631	gcaccagaagtactgacggatgtgactgctgtg	SDM: T-75C rev
EHO-632	cacacagcagtgcaacatctatcagctactctgggtg	SDM: -74 G:A fwd
EHO-633	caccagaagtactgatagatgtgactgctgtgtg	SDM: -74 G:A rev
EHO-634	cacacagcagtgcaacatcttcagctactctgggtg	SDM: -74 G:T fwd
EHO-635	caccagaagtactgaaagatgtgactgctgtgtg	SDM: -74 G:T rev
EHO-636	gtgcaacatctgtcagctacatctgggtcttctattttag	SDM: -66 T:A fwd
EHO-637	ctaaaatagaagcaccagatgactgacagatgttgac	SDM: -66 T:A rev
EHO-638	agcagtgcaacatctgtcagctactatgggtcttatt	SDM: -64 C:A fwd
EHO-639	aatagaagcaccataagtactgacagatgttgactgct	SDM: -64 C:A rev
EHO-640	caacatctgtcagctactcagggtcttctatttttagagg	SDM: -63 T:A fwd
EHO-641	cctctaaaatagaagcacctgaagtactgacagatgttg	SDM: -63 T:A rev
EHO-642	caacatctgtcagctactctagcttctatttttagaggca	SDM: -62 G:A fwd
EHO-643	tgctctaaaatagaagcactagaagtactgacagatgttg	SDM: -62 G:A rev
EHO-644	caacatctgtcagctactctgtgcttctatttttagaggca	SDM: -62 G:T fwd
EHO-645	tgctctaaaatagaagcacagaagtactgacagatgttg	SDM: -62 G:T rev
EHO-646	aacatctgtcagctactctgatgcttctatttttagaggcag	SDM: -61 G:A fwd
EHO-647	ctgctctaaaatagaagcatcagaagtactgacagatgtt	SDM: -61 G:A rev
EHO-648	aacatctgtcagctactctgtgcttctatttttagaggcag	SDM: -61 G:T fwd
EHO-649	ctgctctaaaatagaagcaacagaagtactgacagatgtt	SDM: -61 G:T rev
EHO-650	aacatctgtcagctactctcgtgcttctatttttagaggc	SDM: -62 G:C fwd
EHO-651	gcctctaaaatagaagcacgagaagtactgacagatgtt	SDM: -62 G:C rev
EHO-662	gtgtgcatcaataaaaaaagcgaggttcatcatgttaaatgaagt	SDM: -10 G:A fwd
EHO-663	acttcattaacatgatgaaacctcgcttttttattgatcgcacac	SDM: -10 G:A rev
EHO-664	tgtgcatcaataaaaaaagcgaggttcatcatgttaaatgaagtc	SDM: -9 G:A fwd
EHO-665	gacttcattaacatgatgaaactccgcttttttattgatcgcacaca	SDM: -9 G:A rev

SDM: Site-directed mutagenesis