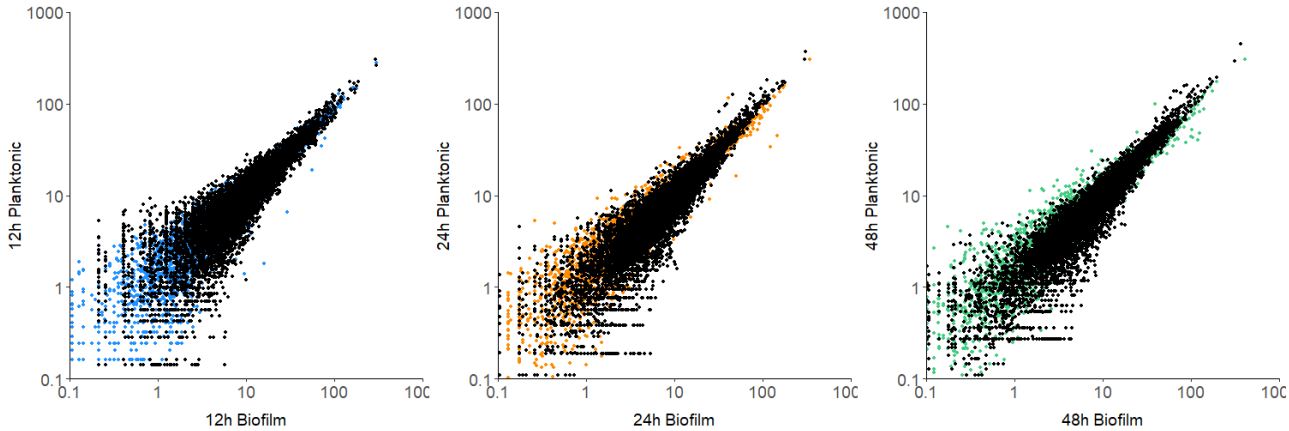
## Supplementary Information

**Supplementary figure 1:** Mean insertion frequencies per gene for each time point. Coloured points show mean insertion frequencies per gene in biofilm conditions compared to planktonic conditions for each time point. Black points show insertion frequencies per gene compared between identical replicates and show the experimental error. Mean insertion frequencies combine all replicates with and without promoter induction with IPTG.



**Supplementary table 1:** Genes determined by TraDIS-*Xpress* to be important for biofilm formation in *E. coli*

| Pathway | Gene | Time point | Difference in insertions in biofilm condition relative to planktonic condition | | Significantly different phenotype from wild type? | | | | | Ref |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Log fold change * | Observed change | Biomass | Aggregation | Curli production | Adhesion | Biofilm architecture | |
| Cell division | *zapE* | 48h | -3.4 | Fewer insertions | No change | Increased | No change | Reduced | No change | [1] |
| c-di-GMP metabolism | *rcdA* | 48h | -0.8 | Fewer insertions | Reduced | | Reduced | | | [2] |
| | *pdeF* | 48h | -0.3 | Fewer insertions | | | | | | [3] |
| Curli biosynthesis | *csgC* | 24h, 48h | -1.6 -0.6 | Fewer insertions | | | | | | [4] |
| | *csgD* | 24h, 48h | -1.4 | Increased expression beneficial at 24h, Fewer insertions at 48h | Reduced | Reduced | Reduced | | | |
| | *csgE* | 12h, 48h | -2.5 -1.6 | Fewer insertions | Reduced | | Reduced | | | |
| | *csgF* | 48h | -4.8 | Fewer insertions | | | | | | |
| DNA housekeeping | *dam* | 24h | -3.9 | Fewer insertions | No change | Reduced | No change | | | [5] |
| | *maoP* | 24h | 1.6 | More insertions | Reduced | Reduced | Reduced | Reduced | Reduced density and biomass | [6] |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Flagella-associated motility | *flhD* | 24h, 48h | -3.9 -2.6 | Increased expression beneficial | No change | No change | No change | | | [7] |
| | *flhC* | 48h | -4.1 | Fewer insertions | No change | Reduced | No change | | | |
| | *flgD* | 24h | -3.0 | Fewer insertions | No change | No change | No change | | | [8] |
| | *fliE* | 48h | -4.7 | Fewer insertions | No change | Reduced | No change | | | |
| | *hdfR* | 12h, 24h | 3.8 2.4 | More insertions | Reduced | No change | Reduced | | | [9] |
| | *lrhA* | 12h, 24h, 48h | 2.0 3.2 2.3 | More insertions | No change | Reduced | No change | Increased | Early microcolony formation, reduced in the mature biofilm | [10] |
| LPS | *wzzB* | 48h | -1.4 | Fewer insertions | Reduced | | No change | | | [11] |
| Oxidised protein repair | *msrQ* | 48h | -0.4 | Fewer insertions | No change | | No change | | | [12] |
| Protein modification | *dsbA* | 12h, 24h | -0.7 -3.0 | Fewer insertions | No change | Increased | Increased | | | [13] |
| Purine ribonucleotide biosynthesis | *purD* | 48h | -4.3 | Fewer insertions | Reduced | No change | Reduced | No change | Reduced microcolony formation | [14] |
| | *purE* | 48h | -5.7 | Fewer insertions | Reduced | Increased | Reduced | | | |
| | *purH* | 48h | -3.2 | Fewer insertions | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *purL* | 48h | -3.1 | Fewer insertions | | | | | | |
| rRNA methyltransferase | *rlmI* | 12h | -3.8 | Fewer insertions | Reduced | No change | No change | | | [15] |
| RNase III regulator | *ymdB* | 24h, 48h | -0.5 -2.5 | Fewer insertions | Reduced | Increased | No change | | | [16] |
| Sugar metabolism and transport | *sgbE* | 48h | -2.5 | Fewer insertions | No change | | No change | | | [17] |
| Toxin-antitoxin system | *tomB* | 12h, 24h, 48h | -0.5 -0.4 -1.6 | Fewer insertions | Reduced | Reduced | Reduced | Increased | Early microcolony formation, reduced in the mature biofilm | [18] |
| Transcriptional regulators and signalling systems | *dksA* | 12h, 24h | 4.4 2.9 | More insertions | Reduced | Reduced | Reduced | Increased | Reduced microcolony formation | [19-21] |
| | *leuO* | 12h, 48h | -0.6 | Increased expression beneficial at 12h, Fewer insertions at 48h | Reduced | Reduced | No change | No change | Reduced microcolony formation | [22] |
| | *marR* | 12h | -4.1 | Fewer insertions | Reduced | No change | No change | | | [23] |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *ompR* | 24h,<br>48h | -0.8<br>-4.7 | Fewer insertions | Reduced | Reduced | Reduced | | | 24 |
| | *lrp* | 48h | -5.9 | Fewer insertions | Reduced | Reduced | Reduced | | | 25 |
| | *gadW* | 48h | -1.1 | Fewer insertions | No change | No change | No change | | | 26 |
| | *rcsC* | 48h | -2.9 | Fewer insertions | Reduced | | No change | | | 27 |
| Transmembrane transport, porins and channels | *mscL* | 48h | -0.9 | Fewer insertions | | | | | | 28 |
| | *tolC* | 48h | -2.9 | Fewer insertions | No change | Reduced | No change | | | 29 |
| | *ompF* | 48h | -2.7 | Fewer insertions | Reduced | | No change | | | 24 |
| | *fadL* | 48h | -1.5 | Fewer insertions | | | | | | 30 |
| tRNA modification | *truA* | 24h,<br>48h | -3.3<br>-5.9 | Fewer insertions | No change | Increased | No change | No change | Increased filamentation after 24- and 48-hours growth | 31 |
| Type 1 fimbriae | *fimB* | 12h,<br>24,<br>48h | -0.4<br>-1.3<br>-2.1 | Fewer insertions and increased expression beneficial at all time points | No change | Reduced | No change | | | 32 |
| | *fimE* | 12h,<br>24,<br>48h | 1.5<br>3.3<br>2.6 | More insertions | Reduced | Reduced | No change | | | |
| | *fimC* | 48h | -1.3 | Fewer insertions | | | | | | 33 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *fimD* | 24h, 48h | -2.3 -2.1 | Fewer insertions | | | | | | [33] |
| Putative fimbrial-like protein | *ydeR* | 48h | -2.4 | Fewer insertions | | | | | | [34] |
| Unknown | *yigZ* | 12h | -2.8 | Fewer insertions | No change | Increased | No change | No change | No change | [35] |
| | *yebB* | 48h | -2.3 | Fewer insertions | | | | | | [36,37] |
| | *yedN* | 48h | -1.7 | Fewer insertions | | | | | | [38] |
| | *yjbL* | 48h | -2.8 | Fewer insertions | Reduced | | No change | | | [15] |
| | *ykgJ* | 12h | | Reduced expression beneficial (increased expression of antisense mRNA beneficial) | No change | Increased | No change | No change | Increased filamentation after 24- and 48-hours growth | [39] |

* Log fold change is only shown for genes where there are differences in insertion frequency inside the coding region. Where the plot files generated by TraDIS-*Xpress* show a difference in insertion frequency between the biofilm and planktonic conditions upstream or downstream of a gene, log fold change cannot easily by quantified and therefore the effect has been described in the column titled ‘observed change’. Significant differences in insertion frequencies have been manually verified with the plot files generated by TraDIS-*Xpress*.

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