## Results S3 - Evaluation of results of the TSS prediction method.

The performance of the TSS prediction method was evaluated using experimentally determined TSSs. Recent publications were selected that report experimentally determined transcription start sites (TSSs) in *Lactobacillus plantarum*. In all publications, TSSs were determined by primer extensions on total RNA from *L. planatrum* grown on rich medium (MRS or CDM). If the TSSs were determined in strains of this species other than *L. planatrum* WCFS1, sequence information on the TSSs were used to locate their genomic positions in genome sequence of *L. planatrum* WCFS1 reference. In the table below, the genomic positions of the TSSs of 15 genes determined by primer extension are provided. They were compared to the TSSs determined in this study. For three genes, TSSs could not be assigned on basis of the tiling array data because the expression signals were too low to detect any transcript, which is most likely due to the culture conditions employed. For the other 12 genes, the differences of genomic positions of the TSSs determined by primer extension and by tiling array data for 11 genes were smaller than 28 nt, likely within the resolution of our tiling probe design (probes were 14 nt apart) whereas one gene showed a difference of more than 50 nt. Out of these 12 genes, 8 genes were highly expressed in our experimental data (averaged expression signal belonged to the upper 40% percentile of the averaged signals of all TARs). The references to the publications in which the primer extension results were reported are listed below.

**Comparison of TSS positions determined by primer extension and inferred from tiling-array data.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **TSS position (in nt), primer extension**  | **TSS position (in nt), tiling array**  | **Absolute difference (in nt)** | **Expression** | **Reference** |
| groES (lp\_0727) | 664949 | 664930 | 19 | Highly expressed | 1 |
| cggR (lp\_0788) | 723751 | 723765 | 14 | Highly expressed | 2 |
| ftsH (lp\_0547) | 496342 | 496337 | 5 | Highly expressed | 3 |
| mntH2 (lp\_2992) | 2661975 | 2661999 | 24 |  | 4 |
| padA (lp\_3665) | 3286749 | 3286766 | 17 | Highly expressed | 5 |
| padR (lp\_3664) | 3286727 | 3286741 | 14 | Highly expressed | 5 |
| bglH (lp\_3525) | 3141965 | 3141944 | 21 |  | 6 |
| flmA (lp\_0593) | 567863 | - |  | No expression | 7 |
| flmB (lp\_0290) | 263020 | - |  | No expression | 7 |
| flmC (lp\_1000) | 924045 | 923991 | 54 |  | 7 |
| malE (lp\_0175) | 151184 | - |  | No expression | 8 |
| ccpA (lp\_2256) | 2040383 | 2040394 | 11 | Highly expressed | 9 |
| pyrR1 (lp\_2696) | 2407985 | 2408011 | 26 |  | 10 |
| Hsp1 (lp\_0129) | 114959 | 114986 | 27 | Highly expressed | 11 |
| Hsp3 (lp\_3352) | 2982027 | 2982050 | 23 | Highly expressed | 11 |

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