***Library amplification protocol***

* Following standard plasmid transformation procedures, we introduced 500 ng of plasmid DNA into *One Shot™ TOP10 Electrocomp™ E. coli* using electroporation. All transformed cells were plated on LB agar supplemented with 15 µg/mL chloramphenicol. After incubation for one day, all colonies were collected, and plasmid DNA was extracted using a miniprep procedure.
* Library plasmid DNA was diluted to 1 ng/μL, and PYR1-amplicons were amplified using Q5 High-Fidelity 2X master mix (NEB, USA) using **TH18-lib primers**. 1.5μg of column-purified PCR product was sequenced by Novogene (USA) using 2x250 bp paired-end reads on an Illumina NovaSeq. FastQ files were processed to remove reads with a Q-score lower than 20, and coverage was determined using the code ().

***Primers and PCR protocols to prepare samples of amplified library for deep sequencing verification***

* Library plasmid DNA was diluted to 1 ng/μL, and PYR1-amplicons were amplified using Q5 High-Fidelity 2X master mix (NEB, USA) using TH18-lib primers.
	+ TH18-lib-F1: TCAATCGTACGACGATTCGACAAACCAC
	+ TH18-lib-R1: CGGAGTTACGAGCCATAGCTTCAGCAAC
* Representative Illumina sequencing results (read counts or some representation or visualization thereof) that confirm diversity of the library sample
	+ For TSM library:

|  |  |
| --- | --- |
| Filtered reads (Q>=20) | 4547108 |
| WT | 0.086 % |
| **Library encoded mutations** |  |
| Single | 1.076 % |
| Double | 9.484 % |
| Triple | 69.857 % |
| Quadruple | 9.412 % |
| Quintuple | 1.459 % |
| Sextuple | 0.201 % |
| Septuple | 0.020 % |
| Octuple | 0.002 % |
| Nonuple | 0.000 % |
| Other mutations | 8.403 % |
| Coverage of targeted triple mutations | 95.296 % (309095 / 324352) |

* + For DSM-Hao:

|  |  |
| --- | --- |
| Filtered reads (Q>=20) | 1342295 |
| WT | 1.51 % |
| **Library encoded mutations** |  |
| Single | 4.1 % |
| Double | 81.41 % |
| Triple | 5.01 % |
| Quadruple | 1.95 % |
| Quintuple | 0.10 % |
| Sextuple | 0.01 % |
| Septuple | 0.00 % |
| Other mutations | 5.90 % |
| Coverage of targeted triple mutations | 99.95 % (34657/34675) |