

The CRISPRi plasmid was purchased from addgene (#44249). Resistance was replaced to AmpR. dCas9 is under control of a tet promoter. The plasmid was modified with sponge elements, identical to the sequences of the FtsZ promoters pZ2-4 (2) (see Table S1). The sgRNAs for the pZ2-4 sites were transcribed from a T7 phage promoter. mVenus gene was cloned as reporter protein under the TetR repressible promoter (BBa: R0040). The sequence is:

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ATTCGCAAACGCCCTCTAATCGAAACTAATGGGGAACTGGAGAAATTGCTGGGATAAAGGGCGAGATTTTGCAC
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GCAAAAATATTTCTTTACTCTAATATCATGAACCTCTTCAAACAGAAATTACACTTGCAAATGGAGAG

The high copy number sponge plasmid (backbone: pSB1K3) is compatible with the CRISPRi plasmid and was constructed with an *mrfp1* (BBa_J04450) reporter gene under control of a lac promoter. We found that in LB medium this reporter is constitutively expressed. Furthermore, we added pZ2-4 sponge elements. The sequence is:

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