**Supplement**



**Figure S1: Size-exclusion chromatography of the maltose-binding protein (MBP).** Size-exclusion chromatography on a Superose12 column (GE Healthcare) was performed for MBP (17 µM)*.* Absorbance at 280 nm is shown. Gelfiltration was performed with a linear flow rate of 0.5 ml/min and the elution volume (ml) is indicated. Two peaks could be identified belonging to an aggregation of proteins with a size of >2,000 kDa (peak 7.3 ml) and to the monomeric MBP with 42 kDa (peak 13.7 ml). Calibration of the column was performed using the gel-filtration mass standard (Bio-Rad Laboratories) containing thyroglobulin (670 kDa), IgG (158 kDa), ovalbumin (44 kDa), myoglobulin (17 kDa) and vitamin B12 (1.35 kDa).



**Figure S2:** **Size-exclusion chromatography of the Cas6b-IIIC endonucleases of *M. mazei* Gö1 in the absence and presence of repeat RNA substrate.** Size-exclusion chromatography on a Superose12 column (GE Healthcare) was performed with His-fusion protein Cas6b-IIIC from *M. mazei* Gö1 (10 µM)*.* Absorbance at 280 nm is shown for the respective proteins in blue and in red for a sample containing the respective protein pre-incubated in the presence of the deoxy-repeat-IB variant (5 µM). Gel filtration was performed with a linear flow rate of 0.5 ml/min using buffer A (see Material and Methods) and elution volume (ml) is indicated. The eluted protein was identified with a silver-stained SDS-gel and co-eluting RNA was detected *via* northern dot blot analysis using a 5’ radioactive labeled repeat-IB probe.



**Figure S3:** **Endonuclease activity of heterologously expressed Cas6b-IB and Cas6b-IIIC proteins.** The cleavage assay was performed with 5` radioactively labeled repeat-IB RNA in the presence of Cas6b-IB (0 ng, 10 ng, 50 ng, 100 ng, 500 ng, 1 mg, 2.5 mg) and Cas6b-IIIC (0 ng, 10 ng, 50 ng, 100 ng, 500 ng, 1 mg, 2.5 mg) protein. Cleavage patterns were analyzed with an 8 % acrylamide gel with 7 M urea on a sequencing gel.



**Figure S4: Quantification of absolute transcript numbers of *cas6b*-IB (dark grey) and c*as6b*-IIIC (light grey) in the wild type under standard conditions.** Indicated are the respective molecule numbers per 50 ng of total RNA with technical standard deviations of three biological replicates (RNA1, RNA2 and RNA3).



Figure S5: Effects of the deletion of one *M. mazei cas6b* gene on the transcription of the respective other or the crRNA in general*.* Quantitative (q) RT-PCR of the crRNA-IB and crRNA–IIIC and the genes *cas6b*-IB or c*as6b*-IIIC of the *M. mazei* strains ∆*cas6b*-IB and ∆*cas6b*-IIIC respectively compared to the wt. Indicated are the respective log2 fold changes with standard deviations of two biological replicates under control conditions and high salt stress (500 mM NaCl).



Figure S6: Effects of high salt stress on the transcription of crRNA and *cas6b* genes in *Methanosarcina mazei.* Quantitative (q) RT-PCR of the crRNA-IB and crRNA–IIIC and the genes *cas6b*-IB and c*as6b*-IIIC of the *M. mazei* wild type strain. Indicated are the respective log2 fold changes with standard deviations of biological replicates under high salt stress (500 mM NaCl) in comparison to control condition.



Figure S7: Putative ribosome binding sites of *cas6b*-IB and *cas6b*-IIIC*.* Depicted are the 5’ untranslated regions of the two genes encoding CRISPR endonucleases *cas6b*-IB and *cas6b*-IIIC. Based on the ribosome binding site (RBS) consensus sequence for *M. mazei ‘*AGGAGG’ as reported by Jäger *et al.* [[1](#_ENREF_1)] a RBS was identified for *cas6b*-IB (highlighted in bold). Only a weak RBS (underlined) was identified for *cas6b*-IIIC.

**Table S1**: **Strains and plasmids**

|  |  |  |
| --- | --- | --- |
| Strain or plasmid | Genotype or description | Source or reference |
| Strain |  |  |
| *M.mazei* Gö1 | wild type | DSMZ No. 3647 |
| *M. mazei*\* | potential cell wall mutant | [[2](#_ENREF_2)] |
| *M. mazei* ∆*cas6b*-IB | *M. mazei*\* ∆*cas6b*-IB::pac, purR | This study |
| *M. mazei* ∆*cas6b*-IIIC | *M. mazei*\* ∆*cas6b*-IIIC::pac, purR | This study |
| *E. coli* DH5a | general cloning strain | [[3](#_ENREF_3)] |
| *E. coli* JM109 λpir | general cloning strain | [[4](#_ENREF_4)] |
| *E. coli* BL21-CodonPlus®-RIL | general cloning strain,containing the pRIL plasmid (*ileW*, *leuY*, *proL*) | Stratagene |
| E. coli **Rosetta™** | general cloning strain,containing the pRARE plasmid | Novagen |
| Plasmid |  |  |
| pBlueskript SK+ | general cloning vector | Stratagene |
| pCR4-TOPO | general cloning vector | Invitrogen |
| pDrive | general cloning vector | Qiagen |
| pMAL-c2 | general cloning vector, *malE* under the control of the *tac* promoter | New England BioLabs  |
| pWM321 | shuttle vector | [[5](#_ENREF_5)] |
| pRS207 | pBlueskript SK+ containing *pac*-cassette | [[2](#_ENREF_2)] |
| pRS714 | Cas6b-IB in pET28a | [[6](#_ENREF_6)] |
| pRS833 | Cas6b-IIIC in pET28a | [[6](#_ENREF_6)] |
| pRS1040 | pBlueskript SK+ containing upstream and downstream fragment of *cas6b*-IB | This study |
| pRS1041 | pBlueskript SK+ containing up- and downstream fragments with *pac*-cassette replacing *cas6b*-IB to construct ∆*cas6b*-IB | This study |
| pRS1042 | pBlueskript SK+ containing upstream and downstream fragment of *cas6b*-IIIC | This study |
| pRS1043 | pBlueskript SK+ containing up- and downstream fragments with *pac*-cassette replacing *cas6b*-IIIC to construct ∆*cas6b*-IIIC | This study |
| pRS1078 | pCR4-TOPO containing *cas6b*-IB | This study |
| pRS1080 | pCR4-TOPO containing *cas6b*-IIIC | This study |
| pRS1079 | pMAL-c2 containing *cas6b*-IB | This study |
| pRS1081 | pMAL-c2 containing *cas6b*-IIIC | This study |

**Table S2.: Primer pairs used for cloning, RT-PCR and sequencing.** Additional attached cleavage sites of restriction enzymes are underlined.

|  |  |
| --- | --- |
| **Primer designations** | **5`→3`** |
| **Mutant construction** |  |
| ∆MM560\_1 \_for | GGGCCCTTCCTTCAAAAAAATACCTC |
| ∆MM560\_1 \_rev | GAGCTCGGATCCAAACAAGTCTCCATATCA |
| ∆MM560\_2\_ for | GGATCCGGGGGTGATATTGTGC |
| ∆MM560\_2\_ rev | GAGCTCCATTCAAGAGCAAGAGAGTC |
| ∆MM3359\_1\_ for | GGGCCCAAATCGGGCTCAAGAATGTT |
| ∆MM3359\_1\_ rev | GAGCTCGGATCCAAAATCTATAAAGAATTTTTTAC |
| ∆MM3359\_2 \_for | GGATCCCATTTGTATTATTTGATCC |
| ∆MM3359\_2 \_rev | GAGCTCTACAATGAGAATAATACGCC |
| MM560\_ for | GAATACTTTCAATGTATATAAACTACACTTG |
| MM560\_ rev | AAGCTTTTATATTTTTTTAATTGAACCATATCC |
| MM3359\_ for | GAATACTTTCAATGAGTCCAGGGAATATTAAG |
| MM3359\_ rev | AAGCTTCTAAATATCATTGAAGTTAGAATAATTCC |
| **Northern blot analysis** |  |
| crRepeat-IB | CTTGTTTTAATGGATCTTGCTCGC |
| **RT-PCR analysis** |  |
| qRT\_MM1215\_for | TCAAGAGCGAGGGCATGAATG |
| qRT\_MM1215\_rev | GCACTACCGAGAACAATAGCC |
| qRT\_MM1621\_for | TAGGAGGTTTTCTCGGAAGCG |
| qRT\_MM1621\_for | AAGCGTATCTCCATCAAGCCC |
| qRT\_MM2181\_for | GCCTCCATGAGAAGAATGCTC |
| qRT\_MM2181\_for | CTTCAAGGTCTCCAACTCCTG |
| qRT\_MM560\_for | CGGATTAGACGAAGGTTCAA |
| qRT\_MM560\_rev | TAAAGCGAGCCAAGGAGTT |
| qRT\_crRNA-IB\_for | AGGGTTTGATAATTTTCCAG |
| qRT\_ crRNA-IB\_rev | AAAAGCGGTGTTAAGTCAG |
| qRT\_MM3359\_for | ATGAGTCCAGGGAATATTAAG |
| qRT\_MM3359\_rev | TACTGCACAAGAGGGTAGC |
| qRT\_crRNA-IIIC\_for | TCCAAACCACTAAAAAAACC |
| qRT\_crRNA-IIIC\_rev | AAGGATCTCATACGTGAATTAG |

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