

## **One step engineering of the small-subunit ribosomal RNA using CRISPR/Cas9**

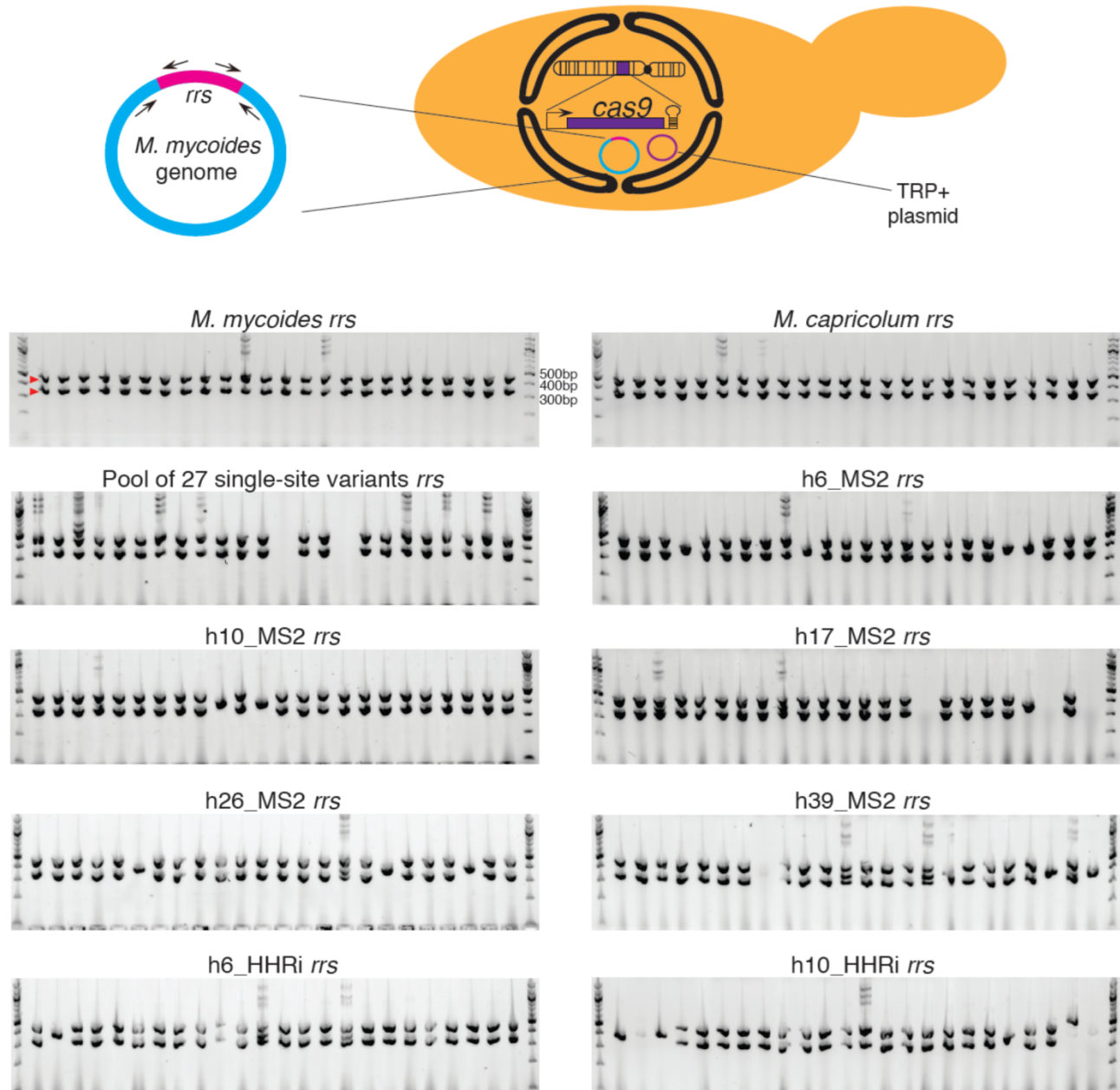
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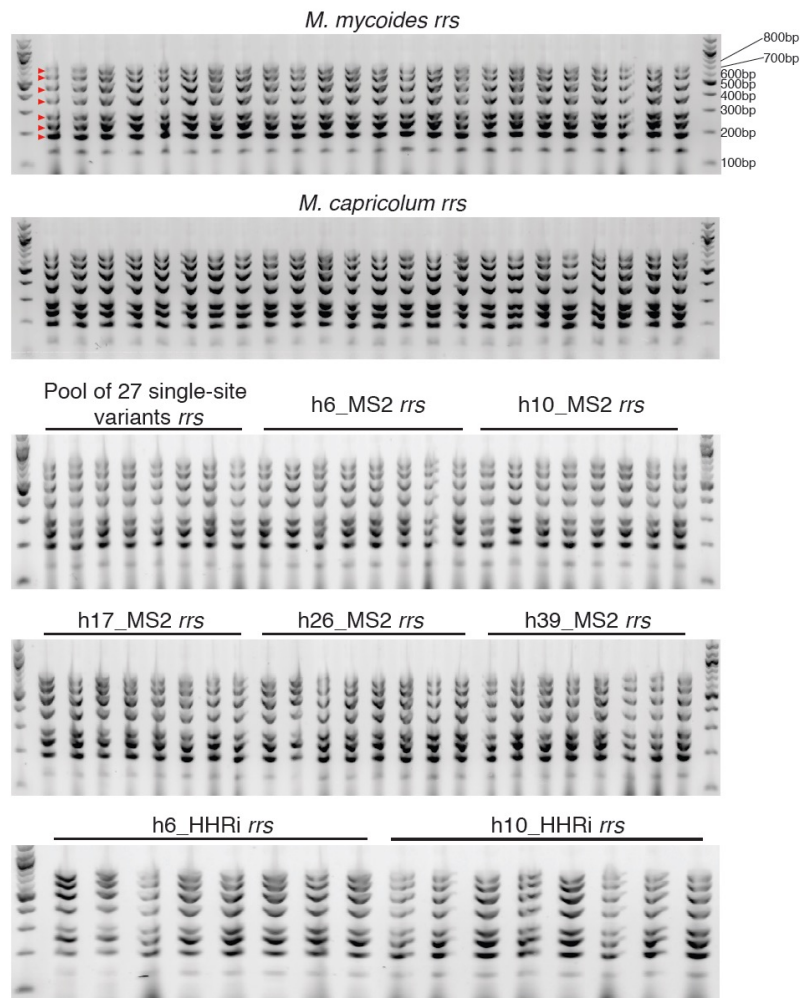
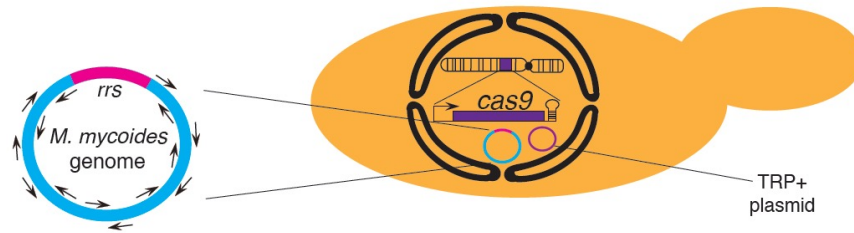
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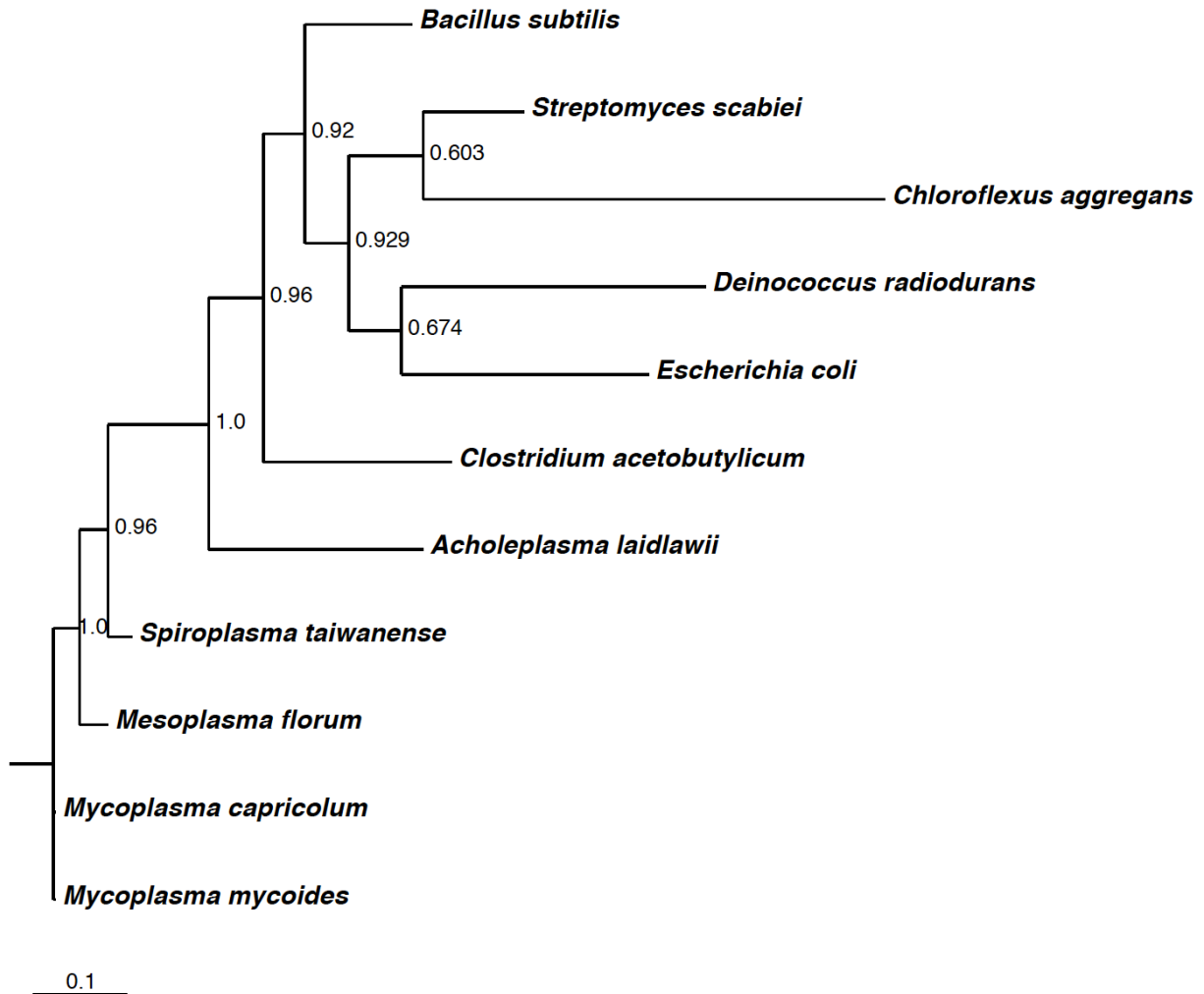
**Supplementary figure 1. Detecting *M. mycooides* genome editing event using diagnostic multiplex PCR.**

VL6-48N-Cas9 yeast strain carrying *M. mycooides*  $\Delta rrs::ura3$  genome was electroporated with two *in vitro* transcribed gRNAs (Supplementary Table 3), “empty” pYAC\_TRP1 plasmid and donor *rrs* DNA. Transformants were selected on plates lacking histidine and tryptophan and screened by multiplex PCR (Primers listed in Supplementary Table 5) for the replacement of *ura3* cassette with *rrs* donor. Data from select donor transformations (*M. mycooides* and *M. capricolum rrs* and single-site variant donors used individually or as a pool) are shown after screening 24 transformants that were patched on to selection plate lacking histidine. The expected bands are indicated by red arrows (353bp and 474bp).



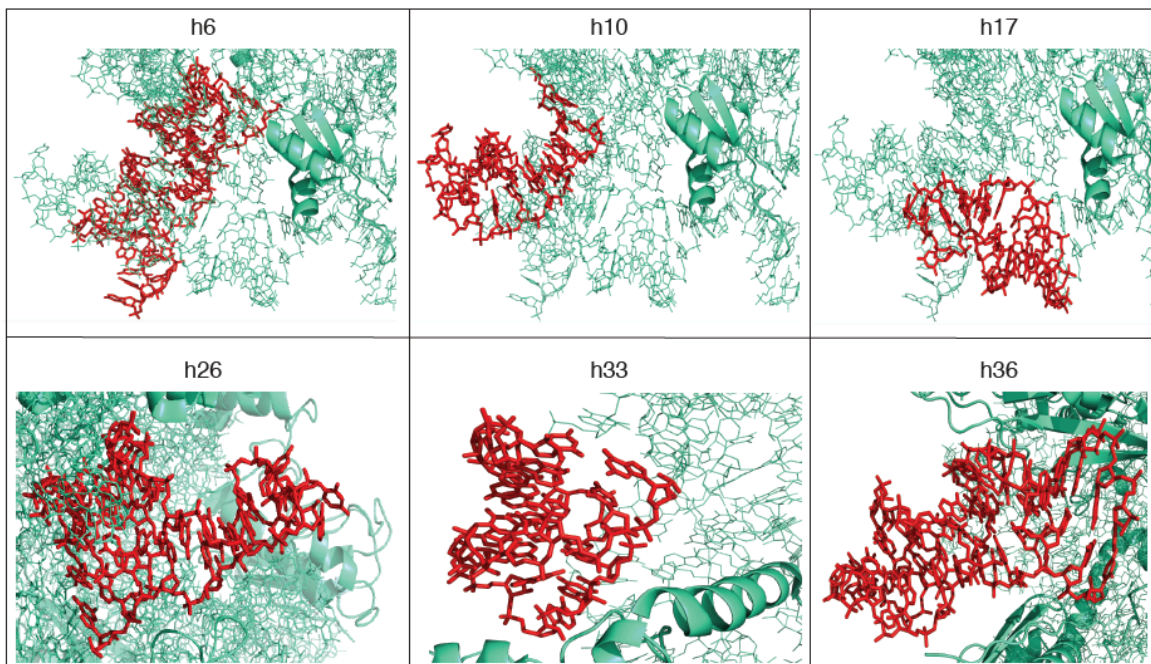
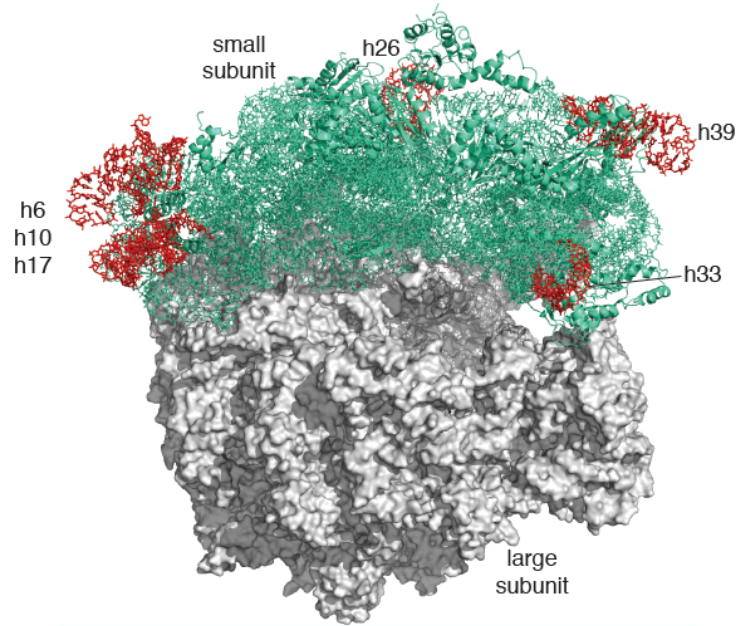
**Supplementary figure 2. Testing the integrity of the edited *M. mycooides* genome using diagnostic multiplex PCR.**

Twenty four (*M. mycooides* and *M. capricolum rrs* donors) or eight (single-site variants used individually or as a pool for donors) positive clones that were confirmed for *M. mycooides* genome editing at the  $\Delta rrs::ura3$  locus (Supplementary Fig. 1) were tested for rearrangements in within the YAC carrying the genome as a result of this editing using multi-locus multiplex PCR (Supplementary Table 4). Expected bands are indicated by red arrows.



**Supplementary figure 3. Phylogenetic analysis of heterologous *rrs* that were used to replace *M. mycoides* *rrs* and test viability.**

In the *M. mycoides* genome carrying single rDNA operon, the *rrs* gene was substituted with *rrs* genes with variable phylogenetic-distances. Resulting genome was tested for viability by genome-transplantation and only *M. capricolum* *rrs*, carrying seven mutations compared to *M. mycoides* *rrs*, was able to support viability. Sequence alignment was performed using MUSCLE (<http://www.ebi.ac.uk/Tools/msa/muscle/>); phylogenetic analysis was performed using PHYML (<http://phylogeny.lirmm.fr/phylo.cgi/phylogeny.cgi>) and the tree was rendered using Phylodendron (<http://iubio.bio.indiana.edu/treeapp/treeprint-sample1.html>).



**Supplementary figure 4. Location of helices 6, 10, 17, 26, 33 and 36 in *E. coli* ribosome.**

Solvent-exposed helices 6, 10, 17, 26, 33 and 36 were chosen for engineering due of their lack of functional interactions with ribosomal proteins. Structure of the 70S ribosome with the large and the small subunits are shown (PDB IDs: 3OAR - 30S subunit and 3OAS – 50S subunit) and the locations of the six helices (red) on the 30S subunit (cyan) are highlighted.

**Supplementary Table 1. *Mycoplasma mycoides* genome editing efficiency using CRISPR/Cas9.**

Editing efficiency was calculated for select donor transformations (*M. mycoides* and *M. capricolum rrs*, single-site variant donors used individually or as a pool) from Supplementary Fig. 1. Twenty-four transformants from each transformation were screened.

<b>Donor</b>	<b>Edited clones (%)</b>
<i>M. mycoides rrs</i>	100
<i>M. capricolum rrs</i>	100
Pool (27 single-site variants)	92
h6_MS2	83
h10_MS2	92
h17_MS2	95
h26_MS2	87
h39_MS2	83
h6_HHRi	92
h10_HHRi	78

All 24 positive clones for *rrs* replacement when *M. mycoides rrs* or *M. capricolum rrs* was used as a donor, were tested for the integrity of the edited *M. mycoides* genome. For the other donors, eight positive clones were subsequently tested for genome integrity. None of these *rrs* positive clones showed any genome-rearrangements decipherable by multiplex PCR (Supplementary Fig. 2).

**Supplementary Table 2. Sequences of primers used for *rrs* engineering.**

Sequence	Name
AAGCTTGCTCTGTCTCTTGTACAAGAGACAGAAGCTTGCTCACCTCAGTG GCGAACGGGT	h6_SH_R
AGCAAGCTTCTGTCTCTTGTACAAGAGACAGAGCAAGCTTCACCCCGTT CGACTTGCAT	h6_SH_F
CCTTGGGGCCTGTCTCTTGTACAAGAGACAGCCTTGGGGCGGTTCACTA TGAGATGGGGA	h10_SH_R
GCCCCAAGGCTGTCTCTTGTACAAGAGACAGGCCCAAGGGGTTCTTTT GATCTTTTCTC	h10_SH_F
AGTTAATACCTGTCTCTTGTACAAGAGACAGAGTTAATACGACTTTATCTT GACAGTACC	h16_SH_R
GTATTAACTCTGTCTCTTGTACAAGAGACAGGTATTAACTACTCTATTTTT TCTTCCCTT	h16_SH_F
CCCTTGAGGCTGTCTCTTGTACAAGAGACAGCCCTTGAGGCTCAGCGCT GCAGCTAACGC	h26_SH_R
CCTCAAGGGCTGTCTCTTGTACAAGAGACAGCCTCAAGGGCCCAACT TAGTACTCATC	h26_SH_F
CCACGGAAGCTGTCTCTTGTACAAGAGACAGCCACGGAAGTATAGTAGA GGTTAACATTG	h33_SH_R
CTCCGTGGCTGTCTCTTGTACAAGAGACAGCTTCCGTGGTATAGCTTTG CACTGGATGT	h33_SH_F
GGAGACTGCCCTGTCTCTTGTACAAGAGACAGGGAGACTGCCTGAGAAC TCTAACGAGAC	h39_SH_R
GGCAGTCTCCCTGTCTCTTGTACAAGAGACAGGGCAGTCTCCTGTTAGT AACTAACGACA	h39_SH_F
TGGAATCCAGGACGCGCCGAAGCGCGTCCGTCTATTTGGGACTCATCA GCTGGATGTACCACCCCGTTTCTGACTTGCAT	h6_HHRi_F
GTACATCCAGCTGATGAGTCCCAAATAGGACGGACGCGCTTCGGCGCGT CCTGGATTCCACACCTCAGTGGCGAACGGGT	h6_HHRi_R
TGGAATCCAGGACGCGCCGAAGCGCGTCCGTCTATTTGGGACTCATCA GCTGGATGTACGGTTCTTTTCTGACTTTTCTC	h10_HHRi_F
GTACATCCAGCTGATGAGTCCCAAATAGGACGGACGCGCTTCGGCGCGT CCTGGATTCCAGGTTCACTATGAGATGGGGA	h10_HHRi_R
TGGAATCCAGGACGCGCCGAAGCGCGTCCGTCTATTTGGGACTCATCA GCTGGATGTACTCTATTTTTTCTTCCCTT	h17_HHRi_F
GTACATCCAGCTGATGAGTCCCAAATAGGACGGACGCGCTTCGGCGCGT CCTGGATTCCAGACTTTATCTTGACAGTACC	h17_HHRi_R
TGGAATCCAGGACGCGCCGAAGCGCGTCCGTCTATTTGGGACTCATCA GCTGGATGTACCCCAACTTAGTACTCATC	h26_HHRi_F
GTACATCCAGCTGATGAGTCCCAAATAGGACGGACGCGCTTCGGCGCGT CCTGGATTCCACTCAGCGCTGCAGCTAACGC	h26_HHRi_R
TGGAATCCAGGACGCGCCGAAGCGCGTCCGTCTATTTGGGACTCATCA GCTGGATGTACTATAGCTTTGCACTGGATGT	h33_HHRi_F
GTACATCCAGCTGATGAGTCCCAAATAGGACGGACGCGCTTCGGCGCGT CCTGGATTCCATATAGTAGAGGTTAACATTG	h33_HHRi_R
TGGAATCCAGGACGCGCCGAAGCGCGTCCGTCTATTTGGGACTCATCA GCTGGATGTACTGTTAGTAACTAACGACAAG	h39_HHRi_F

GTACATCCAGCTGATGAGTCCCAAATAGGACGGACGCGCTTCGGCGCGT CCTGGATTCCATGAGAACTCTAACGAGACTG	h39_HHRi_R
ACTAGTAAAGATGGGTAATCCTCATCAAACTAGTCACCCCGTTCGACT TGCAT	h6_MS2_F
ACTAGTTTTGATGAGGATTACCCATCTTTACTAGTCACCTCAGTGGCGAA CGGGT	h6_MS2_R
ACTAGTAAAGATGGGTAATCCTCATCAAACTAGTGGTTCTTTTGATCTTT TCTC	h10_MS2_F
ACTAGTTTTGATGAGGATTACCCATCTTTACTAGTGGTTCACCTATGAGATG GGGA	h10_MS2_R
ACTAGTAAAGATGGGTAATCCTCATCAAACTAGTACTCTATTTTTCTTC CCTT	h17_MS2_F
ACTAGTTTTGATGAGGATTACCCATCTTTACTAGTGACTTTATCTTGACAG TACC	h17_MS2_R
ACTAGTAAAGATGGGTAATCCTCATCAAACTAGTCCCAACACTTAGTAC TCATC	h26_MS2_F
ACTAGTTTTGATGAGGATTACCCATCTTTACTAGTCTCAGCGCTGCAGCT AACGC	h26_MS2_R
ACTAGTAAAGATGGGTAATCCTCATCAAACTAGTTATAGCTTTGCACTG GATGT	h33_MS2_F
ACTAGTTTTGATGAGGATTACCCATCTTTACTAGTTATAGTAGAGGTTAAC ATTG	h33_MS2_R
ACTAGTAAAGATGGGTAATCCTCATCAAACTAGTTGTTAGTAACTAACGA CAAG	h39_MS2_F
ACTAGTTTTGATGAGGATTACCCATCTTTACTAGTTGAGAACTCTAACGAG ACTG	h39_MS2_R
GCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCATG TATTAGGCATG	h6_Bs_F
GTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGAACGG GTGAGTAACACG	h6_Bs_R
TTATCGCATGAGAAAAGATCAAAAGGTGGCTTCGGCTACCACTATGAGAT GGGGATGCGG	h10_Bs_F
GGTGGCTTCGGCTACCACTATGAGATGGGGATGCGG	h10_Bs_R
TTGTAAGGGAAGAAAAGTACCGTTCGAATAGGGCGGTACCTTGACAGT ACCTTACCAGA	h17_Bs_F
GTACCGTTCGAATAGGGCGGTACCTTGACAGTACCTTACCAGA	h17_Bs_R
ACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGGTTGCGT TCGTTGCG	h39_Bs_F
ATCTTAGTTGCCAGCATTAGTTGGGCACTCTAAGGTGACTGCTAGTGTA AGCTAGA	h39_Bs_R
GCCGCTCTTTACCGAAGTAAATCGCTCAACTTGCATGTATTAGGCATGCC	h6_Cd_F
GTTGAGCGATTTACTTCGGTAAAGAGCGGCGAACGGGTGAGTAACACGT A	h6_Cd_R
ATCTTATTATCGCATGAGAAAAGATCAAAAGGTGAGCCACTATGAGATGG GGATGCGGCG	h10_Cd_F
GGTGAGCCACTATGAGATGGGGATGCGG	h10_Cd_R
TCTGTTGTAAGGGAAGAAAAGATAATGACCTTGACAGTACCTTACCAGA AAGCCACGGC	h17_Cd_F
GATAATGACCTTGACAGTACCTTACCAGA	h17_Cd_R



TCTCTAGAGTGCCCAACTTAATGATGGCAACTAAAGACAAGGGTTGCGTT CGTTGCG	h39_Cd_F
TCTTTAGTTGCCATCATTAAAGTTGGGCACTCTAGAGAGACTGCTAGTGTA AGCTAGA	h39_Cd_R
GCCACTCGTCAGCAAAGAAGCAAGCTTCTTCCTGTTACCGTTGACTTGC ATGTATTAGGCATGCC	h6_Ec_F
GTCGAACGGTAACAGGAAGAAGCTTGCTTCTTTGCTGACGAGTGGCGAA CGGGTGAGTAACACGTA	h6_Ec_R
CATGAGAAAAGATCAAAAAGAGGGGGACCTTCGGGCCTCTTACTATGAGA TGGGGATGCGG	h10_Ec_F
GAGGGGGACCTTCGGGCCTCTTACTATGAGATGGGGATGCGG	h10_Ec_R
TTGTAAGGGAAGAAAAAGAGTAAAGTTAATACCTTTGCTCCTTGACAGTA CCTTACCAGA	h17_Ec_F
GAGTAAAGTTAATACCTTTGCTCCTTGACAGTACCTTACCAGA	h17_Ec_R
TCCTTTGAGTTCCCGGCCGGACCGCTGGCAACAAAGGACAAGGGTTGC GTTGCGTTGCG	h39_Ec_F
TCCTTTGTTGCCAGCGGTCCGGCCGGGA ACTCAAAGGAGACTGCTAGTG TAAGCTAGA	h39_Ec_R
GGTAAGACTAATTAAGATGGTAGTGAATATAAAATGTCTAAAAG	Cas9_donor_F
GAACAATCTATATTCAAAGATGTGTGACGATCTCTG	Cas9_donor_R

**Supplementary Table 3. Sequences of ultramers used for *rrs* engineering.**

T1 and T6 ultramers were used to generate gRNAs for replacing the native *rrs* with the *ura3* cassette while gRNAs resulting from T9 and T10 ultramers were used for replacing the *ura3* cassette with wild-type *rrs* or engineered *rrs*\*. Target sequences are indicated in red.

F_T1	TAATACGACTCACTATAGGTATCCGTACGGGAACGTGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTT
R_T1	AAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTA TTTTAACTTGCTATTTCTAGCTCTAAAACACAGTTCCTCGTACGGATACCTATAGT GAGTCGTATTA
F_T6	TAATACGACTCACTATAGCATACTGAGCATAATAAACGTTTTAGAGCTAGAAATA GCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG GTGCTTTTTTT
R_T6	AAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTA TTTTAACTTGCTATTTCTAGCTCTAAAACGTTTATTATGCTCAGTATGCTATAGTG AGTCGTATTA
F_T9	TAATACGACTCACTATACATAGTTAAGCCAGCCCAACGTTTTAGAGCTAGAAATA GCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG GTGCTTTTTTT
R_T9	AAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTA TTTTAACTTGCTATTTCTAGCTCTAAAACGTTGGGCTGGCTTAACTATGTATAGT GAGTCGTATTA
F_T10	TAATACGACTCACTATACACAGATGCGTAAGGAGGTGGTTTTAGAGCTAGAAAT AGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTC GGTGCTTTTTTT
R_T10	AAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTA TTTTAACTTGCTATTTCTAGCTCTAAAACCACCTCCTTACGCATCTGTGTATAGT GAGTCGTATTA

**Supplementary Table 4. Diagnostic primers used to confirm the integrity of the *M. mycoides* genome through multiplex PCR.**

When the genome is intact, seven bands, 204bp, 256bp, 301bp, 408bp, 515bp, 612bp and 724bp, are obtained after the diagnostic multiplex PCR.

Sequence	Name
GTAGAACATGTTGCAGATAAG	MPCR9-2F
TGATGGTGCATAACGTAATC	MPCR9-2R
ATTTAGTTGCTGATTGAGTTTG	MPCR9-3F
CTAGGACCAGTGGCTGC	MPCR9-3R
AGTTGACTGAAATTCTCAGTC	MPCR9-4F
AAGATCGTTTAGCTAAAGGAC	MPCR9-4R
TGGTTATATAACTTGGAGTG	MPCR9-5F
GCAGGTTTTCTCCTCTAC	MPCR9-5R
CATGATTGATATGTTTAAAGATGC	MPCR9-6F
TGCAAAGACTAAAGTATATGTG	MPCR9-6R
TTGATGTATCTCACTTATTTAGA	MPCR9-7F
CAACAAGTAGAGATTTAAACAG	MPCR9-7R
GCTTGTTCAACATTATAAGCTC	MPCR9-8F
TTGATGGTGAAGTCTAATAACT	MPCR9-8R

**Supplementary Table 5. Primers used to confirm genome-editing event through multiplex PCR.**

In the presence of an editing event, 353bp and 474bp bands would be observed, while a ~1.8kb amplicon would be obtained if the *ura3* cassette is not replaced by *rrs*\*.

Sequence	Name
GCAGGTAGTCACGTCCTTCTTCGG	FJ1-Diagn16S
GTCGCGGTGAATACGTTCTCGG	RJ1-Diagn16S
GCTTGGTTTAAAATTGAATGACATCTAGCAGTAGC	FJ2-Diagn16S
AAATTAATTAATGTTTGAGGTGGAGATCATCATGG	RJ2-Diagn16S

**Sequences of the heterologous 16S rRNA genes used to substitute the entire wild-type *M. mycoides* 16S rRNA gene**

*E. coli*

AGATAAGGAGGTGATCCAACCGCAGGTTCCCTACGGTTACCTTGTACGACTTCACCCCAGTCATGAATCACAAAG  
TGGTAAGCGCCCTCCCGAAGGTTAAGCTACCTACTTCTTTTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTAC  
AAGGCCCGGAACGTATTACCGTGGCATTCTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCA  
GACTCCAATCCGGACTACGACGCACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGCCATT  
GTAGCACGTGTGTAGCCCTGGTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCAGTTTATCACTGG  
CAGTCTCCTTTGAGTTCCCGGCCGACCGCTGGCAACAAAGGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAAC  
ATTTTACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCACGGTTCCCGAAGGCACATTCTCATCTCTGAAAA  
CTTCCGTGGATGTCAAGACCAGGTAAGGTTCTTCGCGTTGCATCGAATTAACCACATGCTCCACCGCTTGTGCGGG  
CCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTIONCCCCAGGCGGTGACTTAACGCGTTAGCTCCGGAAG  
CCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTC  
CCCACGCTTTTCGACCTGAGCGTCAGTCTTCGTCCAGGGGGCCGCTTCGCCACCGGTATTCTCCAGATCTCTACG  
CATTTACCGCTACACCTGGAATTCTACCCCCCTCTACGAGACTCAAGCTTGCCAGTATCAGATGCAGTTCCAGGT  
TGAGCCCGGGGATTTACATCTGACTTAACAAACCGCTGCGTGCCTTTACGCCAGTAATTCGATTAACGCTTG  
CACCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATGAGCAAAGGT  
ATTAACTTTACTCCCTTCCTCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGC  
ATCAGGCTTGCGCCATTGTGCAATATTTCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTG  
TGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCACCTACTAGCTAATCCCATC  
TGGGCACATCCGATGGCAAGAGGCCCGAAGGTACCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTT  
CCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCAGACATTACTCACCCGTCCGCCACTCGTCAGCAAAGAAGCAAG  
CTTCTTCTGTTACCGTTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCTGAGCCATGATCAAACCTCTTC  
AATTT

*Streptomyces scabiei*

AGAAAGGAGGTGATCCAGCCGCACCTTCCGGTACGGCTACCTTGTACGACTTCGTCCCAATCGCCAGTCCCACCTT  
CGACAGCTCCCTCCCTTACGGGTTGGGCCACCGGCTTCGGGTGTTACCGACTTTCGTGACGTGACGGGCGGTGTGTA  
CAAGGCCCGGAACGTATTACCGCAGCAATGCTGATCTGCGATTACTAGCAACTCCGACTTCATGGGGTCGAGTTG  
CAGACCCCAATCCGAAGTACGACAGGCTTTTTGAGATTGCTCCGCCTCACGGCTTCGACGCTCATTGTACCTGCCA  
TTGTAGCACGTGTGACGCCAAGACATAAGGGGCATGATGACTTGACGTCGTCCCCACCTTCCTCCAGTTGACCCC  
GGCAGTCTCCTGTGAGTCCCCATCACCCGAAGGGCATGCTGGCAACACAGAACAAGGGTTGCGCTCGTTGCGGGAC  
TTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACCTGTACACCGACCACAAGGGGGCGACCATCT  
CTGGCCGTTTTCCGGTGTATGTCAAGCCTTGGTAAGGTTCTTCGCGTTGCGTTCGAATTAAGCCACATGCTCCGCTGCC  
TGTGCGGGCCCCGTCAATTCCTTTGAGTTTTAGCCTGCGGCCGTACTIONCCCCAGGCGGGGAACCTAATGCGTTAGCTG  
CGGCACCGACGACGTGGAATGTGCGCAACACCTAGTTCCCACCGTTTACGGCGTGGACTACCAGGGTATCTAATCCT  
GTTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTAATGGCCCAGAGATCCGCCTTCGCCACCGGTGTTCTCCTCTGA  
TATCTGCGCATTTACCGCTACACCAGGAATTCGATCTCCCCTACCACACTCTAGTCTGCCCGTATCGAATGCAGA  
CCCGGGGTTAAGCCCCGGGCTTTCACATCCGACGCGACAGACCGCTACGAGCTCTTTACGCCCAATAATTCGGAC  
AACGCTCGCGCCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGCGCTTCTTCTGCAGGTACCGTCACTTT  
CGCTTCTTCCCTGCTGAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCACGCGGCGTGCCTGCATCAGGCTTTCG  
CCCATTGTGCAATATTTCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGGTGC  
CTCTCAGGCCGGCTACCCGTCGTCGCCTTGGTGGAGCCGTTACCTCACCAACAAGCTGATAGGCCGCGGGCTCATCCT  
TCACCGCCGAGCTTTCACACTCATCGGATGCCCGAGAGTGTGCTATCCGGTATTAGACCCGTTTTCCAGGGCTTG  
TCCCAGAGTGAAGGGCAGATTGCCACGTGTTACTCACCCGTTGCCACTAATCCCCACCGAAGTGGTTTCATCGTTC  
GACTTGCATGTGTTAAGCACGCCCGCCAGCGTTCGTCTGAGCCAGGATCAAACCTCTCCGTGAATGTTT

*Bacillus subtilis*

AGAAAGGAGGTGATCCAGCCGCACCTTCCGATACGGCTACCTTGTTACGACTTCACCCCAATCATCTGTCCCACCTT  
CGGCGGCTGGCTCCTAAAAGGTTACCTCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTAC  
AAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCACGCAGTCGAGTTGCA  
GACTGCGATCCGAACCTGAGAACAGATTTGTGGGATTGGCTTAACCTCGCGGTTTTCGCTGCCCTTTGTTCTGTCCATT  
GTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTTGTCACCGG  
CAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACA  
TCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACCTCGCCCCGAAGGGGACGTCCATCTCTAGGATT  
GTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAACCACATGCTCCACCGCTTGTGCGGGC  
CCCCGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACT  
AAGGGGCGGAAACCCCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTGCGCTC  
CCCACGCTTTGCGCTCCTCAGCGTCAGTTACAGACCAGAGAGTCGCTTCGCCACTGGTGTTCCTCCACATCTCTACG  
CATTTACCGCTACACGTGGAATTCACCTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCCCGGT  
TGAGCCGGGGCTTTCACATCAGACTTAAGAAACCGCTGCGAGCCCTTACGCCAATAATTCCGGACAACGCTTG  
CCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACCGCCCT  
ATTCGAACGGTACTTGTCTTCCCTAACACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTC  
CGTCAGACTTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCGGTGTCTCAGTCCCAGT  
GTGGCCGATCACCTCTCAGGTCGGCTACGCATCGTCGCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCC  
GCGGGTCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGTTTGAACCATGCGGTTCAAACAACCATCCGGTATTA  
GCCCCGTTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACATCA  
GGGAGCAAGCTCCCATCTGTCCGCTCGACTTGATGTATTAGGCACGCCGCCAGCGTTTCGTCTGAGCCAGGATCAA  
ACTCTCCGATAAA

*Clostridium acetobutylicum*

AGAAAGGAGGTGATCCAGCCGCAGGTTCTCCTACGGCTACCTTGTTACGACTTCACCCCAATTATCAACCCACCTT  
CGACCGCTGGTTCAAAAGGTTACCTCACGGGCTTCGGGTGTTGCCGACTCTCATGGTGTGACGGGCGGTGTGTACA  
AGACCCGGGAACGTATTCACCGCGACATTCTGATTGCGGATTACTAGCAACTCCGGCTTCATGTAGGCGGATTTTCAG  
CCTACAATCCGAACCTGGGATGGGGTTTTGAGTTTTGCTCCACCTTGCGGTATTGCATCTTTTTGTCCCCACCATTGT  
AGCACGTGTGTAGCCCTAGACATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCCGGTTAACCCGGGCA  
GTCTCACTAGAGTGCTCAACTAAATGTTAGCAACTAATGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATC  
TCACGACACGAGCTGACGACAACCATGCACCACCTGTCATCCTGTCCCCGAAGGGACTTCATCCATTACGGACTAAT  
TCAGGAGATGTCAAGTCTAGGTAAGGTTCTTCGCGTTGCTTCGAATTAACCACATGCTCCGCTGCTTGTGCGGGTC  
CCCGTCAATTCCTTTGAGTTTTAATCTTGCAGCCGACTTCCCAGGCGGAATACTTATTGTGTTAACTGCGGCACAG  
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CCACGCTTTCATGCCTCAGCGTCAGTTACAGTCCAGAAGGCCGCTTCGCCACTGGTATTCTTCCTAATCTCTACGC  
ATTTACCGCTACACTAGGAATTCTGCCTTCTCCTGCACTCCAGACATCCAGTTTGAAATGCAGCCCCCAAGTT  
AAGCCCGGGGATTTACATCTCACTTAAATATCCGCCTACACATCCTTTACGCCAGTAAATCCGGACAACGCTTGC  
CACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTCCTCCTATGGTACCGTCATTATCGTCCCATA  
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*Acholeplasma laidlawii*

AGAAAGGAGGTGATCCATCCCCACGTTCCCGTAGGGATACCTTGTTACGACTTAACCCCAATCATGGACCCTACCTT  
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*Spiroplasma taiwanense*

AAAATGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCATGCCTAATACATGCAAGTCGAACGGGGTGCTTGC  
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*Mycoplasma capricolum*

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ACTTGCATGTATTAGGCATGCCGCCAGCGTTTATCTGAGCCAGGATCAAACCTCTCATT

*Mesoplasma florum*

AGAAAGGAGGTGATCCATCCGCACGTTCCCGTACGGATACCTTGTTACGACTTCACCCCAATCGCTAGTCCTACCTT  
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*Chloroflexus aggregans*

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*Deinococcus radiodurans*

AGAAAGGAGGTGATCCAACCGCACCTTCCGGTACAGTTACCTTGTTACGACTTCACCCAGTCATAAACCACAGTCT  
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