Mycoplasma pneumoniae Genome Editing Based on Oligo Recombineering and Cas9-Mediated Counterselection

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SUPPORTING INFORMATION

Seven supplementary figures (Figure S1: Multiple sequence alignment of RecT proteins. Figure S2: Western blot analysis of the expression of the different recombinases used in the screening. Figure S3: Chromosome locations of the different recombineering sensors. Figure S4: Screening of optimal inducer concentrations for Cas9-based counterselection on each recombineering sensor strain. Figure S5, S6 and S7: Screening of edited clones of the M129MutCm+50GP35, M129MutCm+750GP35 and M129MutCm+1800GP35 strains, respectively upon eiCas9-mediated counterselection) and ten supplementary tables (Table S1, Table S2 and Table S3: Crude data from the transformations performed for the screening of recombinases, for the protocol optimization and for the different recombineering sensor strains, respectively. Table S4: Screening of optimal Cas9 inducer concentration, and determination of Cas9 evader rate in the different recombineering sensor strains. Table S5: Strains generated in this work. Table S6: Plasmids generated in this work with detailed information of the sequences present in them. Table S7: Oligonucleotides employed for plasmid assembly and details on vector assembly. Table S8: Editing oligonucleotides employed in this work. Table S9: Oligonucleotides employed for A-PCR protocol. Table S10: Oligonucleotides employed for the screening of edited clones).

Supplementary Figures

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CLUSTAL O(1.2.4) multiple sequence alignment


RecT         MTKQPPIAKADLQKTQGNRAPAAVKNSDVISFINQPSMKEQLAAALPRHMTAERMIRIAT  60
RecT-sm      -------------------------MSNIVEFLKTDKATDWIKNKFSNENEIARFKSNLV  35
RecT-sc      -------------------------MTNIVEFLKTDKVNEWIKSKFSNENEIARFKSNIV  35
RecT-sp      -------------------------MANIVEFLRTDRTTEWIKSKFSNENEIARFKSNII  35
                                       ::::.*:.    .: :   : ..    *:


RecT         TEIRKVPALGNCDTMSFVSAIVQCSQLGLEPGSALGHAYLLPFGNKNEKSG---------  111
RecT-sm      AISNSNDLLQKADPRTIMTACYQGVLLNLPMEKQFGYAYVVPYNTKIERKDDYGKTIFGK  95
RecT-sc      AISNSNDLLQKANPRTIMTACYQGVLLNLPMEKQFGYAYVVPYNTKITRI---------I  86
RecT-sp      AISNSNELLQKADPKTIMTACYQGVLLNLPMEKQFGYAYVVPYNTKITRI---------I  86
             :  ..   * :.:  ::::*  *   *.*   . :*:**::*:..*  :


RecT         --------KKNVQLIIGYRGMIDLARRSGQIASLSARVVREGDEFSFEFG--LDEKLIHR  161
RecT-sm      DGKPLYDWINQAQFQMGYKGYIQLAQRSGQYLDMSVSDVRTGELVIYDRLKGTTFNWIQN  155
RecT-sc      NGREIQEWVNQAQFQMGYKGYIQLAQRSGQYLDMSVSDVRVGELVNYDRLKGTSFNWVQN  146
RecT-sp      NGREIQEWVNQAQFQMGYKGYIQLAQRSGQYLDMSVSDVKENELVNYDRLKGTSFNWIQN  146
                      ::.*: :**:* *:**:****  .:*.  *: .: . ::       : ::.


RecT         PGENEDAPVTHVYAVARLKDGGTQFEVMTRKQIEL---VRSLSKAGNNGPWVTHWEEMAK  218
RecT-sm      EDEREKLPIIGYVAYFKMVNGFEKTLYMTKEQMENHFMKYSKTYAKNKSFYIASFDEMAL  215
RecT-sc      EDEREKLQIIGYVAYFKMVNGFEKTLYMTKEQMENHFMKYSKTYAKNKSFYIATFDEMAL  206
RecT-sp      EDEREKLPIIGYVAYFKMVNGFEKTLYMTKEQMENHFMKYSKTYAKNKSFYIASFDEMAL  206
              .*.*.  :    *  :: :*  :   **::*:*      * : * *:. ::: ::***


RecT         KTAIRRLFK-YLPVSIEIQRAVSMDEK------EPLTIDPADSSVLTGEYSVIDNSEE--  269
RecT-sm      KTVLTSLLRKWGIMSVELQQAYKSDQAVITTNDEKIYIDNDNNTVVENKPQISNKNKKVE  275
RecT-sc      KTVLTSLLRKWGIMSVELQQAYKSDQSVITTNDENIYIDNDTTVA-KNKGQMSDEPLLNN  265
RecT-sp      KTVLTSLLRKWGIMSVELQQAYKSDQAVITTNDEKIYIDNDATFT-QNKGHINDEILSNN  265
             **.:  *:: :  :*:*:*:* . *:       * : **   . .  .:  : ::


RecT         -----------------------------------------    269
RecT-sm      QPI-IIDQDSYNNQINNVVVEDDISNVVPTVNNDE--DIYSW-    314
RecT-sc      IN--TPSYPEIADTNDNVVKEEDISNVVPAVNN--NDEWAKWE    304
RecT-sp      IKLYTPPNDEIVDINDNVVKEEDISNVVPTVNNNNDEEWATW-    307
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Figure S1. Multiple sequence alignment of RecT proteins. The sequences of the *E. coli*-derived RecT recombinase and the three Mycoplasma-derived RecT-like proteins were aligned by the Clustal Omega Server. Asterisks (*) indicate positions with a single fully-conserved residue in all sequences; colons indicate positions at which the residue is conserved between amino acids sharing strongly similar properties; and periods indicate positions at which the residue is conserved between amino acids sharing weakly similar proteins.

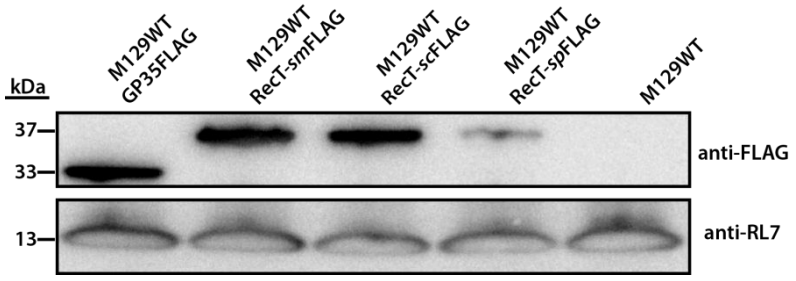

Figure S2. Western blot analysis of the expression of the different recombinases used in the screening. Bacterial lysates of the indicated Mycoplasma strains were subjected to analysis. All recombinases were immunodetected with anti-FLAG tag mAb. Ribosomal protein RL7 was used as loading control (lower panel) and detected with anti-RL7 pAb

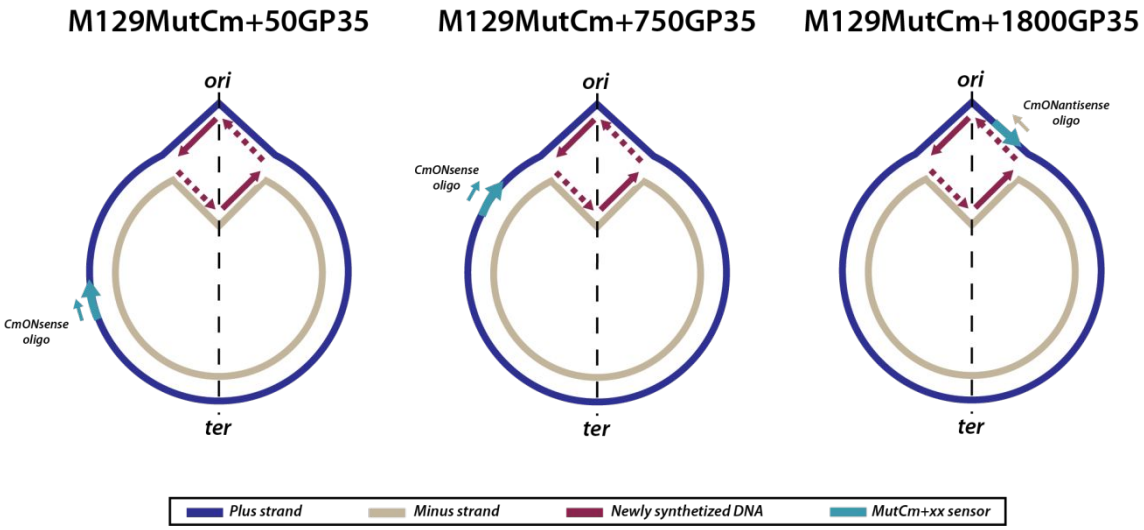

Figure S3. Chromosome locations of the different recombineering sensors. Schemes depict the chromosomes of the M129MutCm+50GP35, M129MutCm+750GP35, and M129MutCm+1800GP35 strains, showing the bidirectional replication fork that would start from the origin of replication (ori) and enlarge until reaching the terminus of replication (ter). The plus and minus strands follow a color code as indicated, as well as the newly synthesized DNA in either continuous (solid line) or discontinuous way (dashed line). Green arrows shown at each chromosome indicate the approximate locations of the MutCm+50, MutCm+750, and MutCm+1800 recombineering sensors at the plus strand of the *MPN493*, *MPN582*, and *MPN034* loci, respectively. The appropriate editing oligonucleotide for each strain is depicted with a color code if its sequence is the same as that present on the plus or minus strand of the chromosome

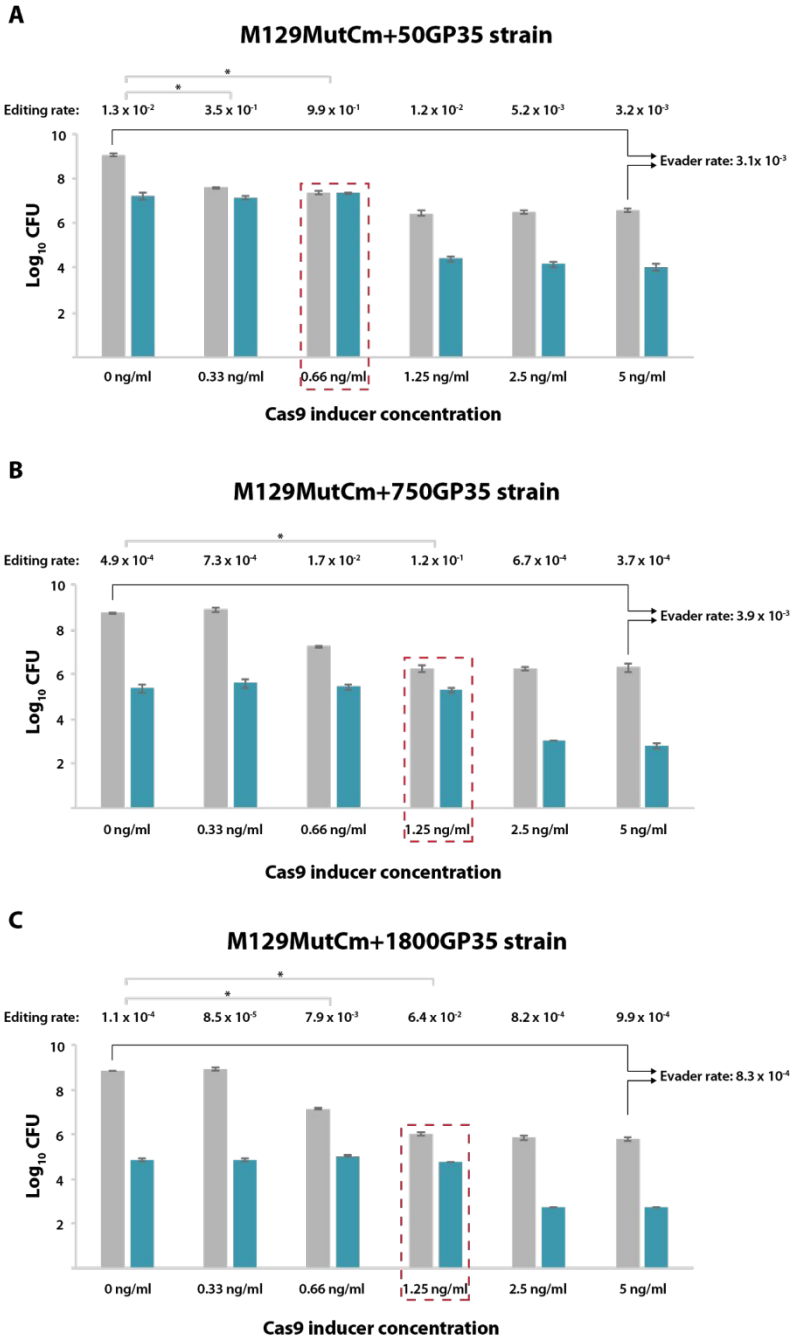

Figure S4. Screening of optimal inducer concentrations for Cas9-based counterselection on each recombineering sensor strain. (A–C) Barplots showing in logarithmic scale (left side) the CFU obtained for the recombineering sensor strains M129MutCm+50GP35 (A), M129MutCm+750GP35 (B), and M129MutCm+1800GP35 (C). Strains were transformed with their respective editing oligos following the conditions established in the optimization screening, and seeded on non-selective medium (grey bars) or Cm-selective medium (green bars) supplemented with different aTc concentrations, as indicated on the x-axis. The editing rate (edited cells / total cells) obtained for each strain and experimental condition is shown on top of each group of bars. The optimal inducer concentration, that is the one triggering a higher editing rate for each strain is highlighted by a red dotted line square. Also the evader rate obtained for each strain is shown in the right side of each barplot, calculated as indicated by the arrows as the ratio between the number of survivor cells at the highest aTc concentration and the total cells when no induction is applied. Those differences in terms of editing rate that were found to be statistically significant ($P < 0.05$) after conducting a paired t-test are indicated with an asterisk (*). Error bars represent the mean of the standard deviation (SEM) of three different biological replicas.

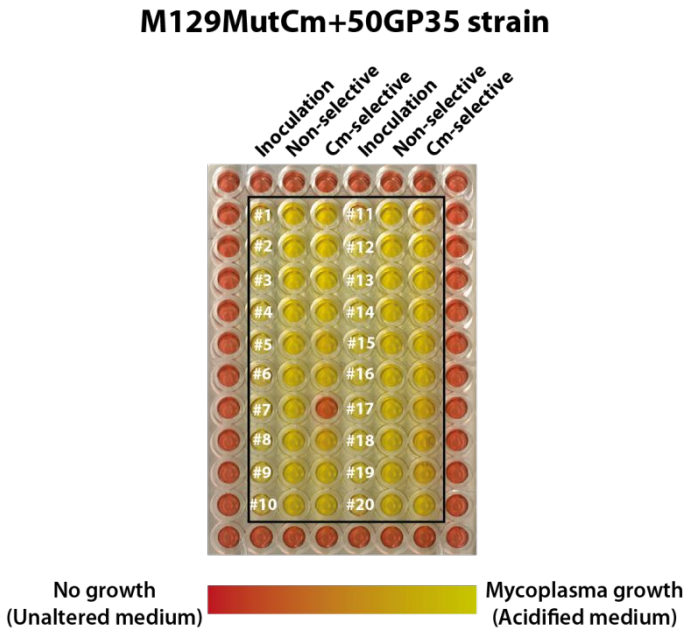

Figure S5. Screening of edited clones of M129MutCm+50GP35 strain upon eiCas9-mediated counterselection. Picture of the screening plate where 20 colonies were inoculated and assessed for Cm resistant phenotype. Colonies were picked from the non-selective plate supplemented with the optimal aTc dose found for this strain. The growth of each clone in non-selective or Cm-selective medium was monitored by the ability of *M. pneumoniae* cells to acidify and thus mediate a color change on the medium containing phenol red as indicated in the color scale below the picture of the plate.

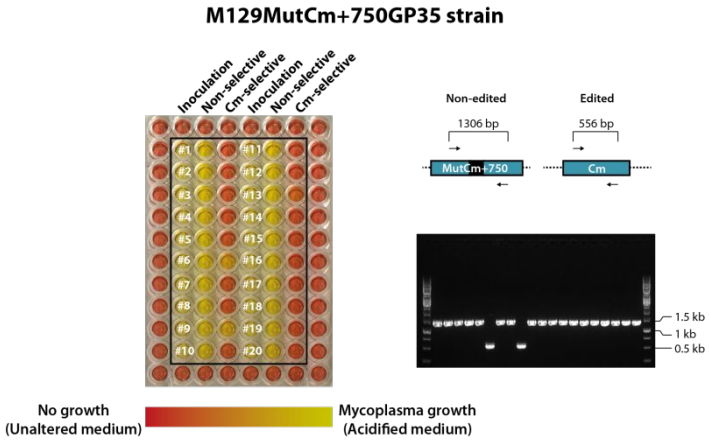

Figure S6. Screening of edited clones of M129MutCm+750GP35 strain upon eiCas9-mediated counterselection. On the left side**,** picture of the screening plate where 20 colonies were inoculated and assessed for Cm resistant phenotype. Colonies were picked from the non-selective plate supplemented with the optimal aTc dose found for this strain. The growth of each clone in non-selective or Cm-selective medium was monitored by the ability of *M. pneumoniae* cells to acidify and thus mediate a color change on the medium containing phenol red as indicated in the color scale below the picture of the plate. On the right side picture of the electrophoresis gel analyzing the size of the MutCm sensor in the same 20 clones analyzed on the multiwall plate. On top of the gel picture, a scheme of the PCR analysis conducted is shown together with the expected sizes of the different PCR products that could be generated depending on the status of the sensor.

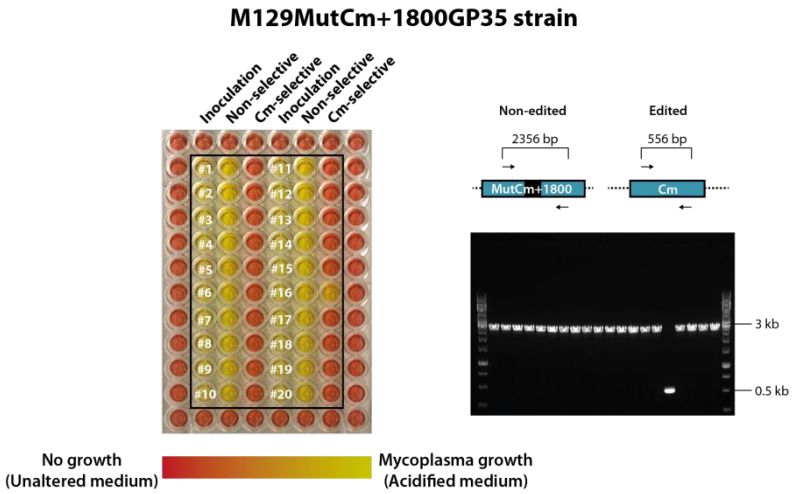

Figure S7. Screening of edited clones of M129MutCm+1800GP35 strain upon eiCas9-mediated counterselection. On the left side**,** picture of the screening plate where 20 colonies were inoculated and assessed for Cm resistant phenotype. Colonies were picked from the non-selective plate supplemented with the optimal aTc dose found for this strain. The growth of each clone in non-selective or Cm-selective medium was monitored by the ability of *M. pneumoniae* cells to acidify and thus mediate a color change on the medium containing phenol red as indicated in the color scale below the picture of the plate. On the right side picture of the electrophoresis gel analyzing the size of the MutCm sensor in the same 20 clones analyzed on the multiwall plate. On top of the gel picture a scheme of the PCR analysis conducted is shown together with the expected sizes of the different PCR products that could be generated depending on the status of the sensor.

Supplementary Tables (Excel file)