Generation of *Mycoplasma hominis* gene-targeted mutants by targeting-induced local lesions in genomes (TILLING)

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**Abstract**

**Background:** *Mycoplasma hominis* is a human urogenital pathogen involved in gynaecological, neonatal and extragenital infections. However, no versatile genetic tools are currently available to study the pathogenicity of this bacterium. Targeting-Induced Local Lesions IN Genomes (TILLING) is a reverse-genetic method that combines point mutations induced by chemical mutagenesis with a DNA screening technique. We used ethyl methanesulfonate (EMS) that introduces C-G to T-A transition mutations to generate a library of *M. hominis* mutants. As a proof of concept, mutagenized organisms were screened for mutations in two target genes previously associated with the mycoplasma pathogenicity, the *vaa* gene encoding an adhesin lipoprotein and the *oppA* gene encoding the main ectoATPase of the bacterium. The resulting mutants were evaluated using functional assays, an adhesion to HeLa cell assay for *vaa*-mutants and an ATPase activity test for *oppA*-mutants.

**Results:** A 1200-clone library was generated by exposing *M. hominis* PG21 to 9 mg/mL EMS for 3 h. To identify mutants of interest, targeted gene fragments were amplified, heat-denatured, slowly reannealed and digested with the mismatch-specific endonuclease ENDO1. If multiple alleles were present in the PCR amplicons, these alleles formed heteroduplexes during reannealing that were specifically cleaved by ENDO1 at mismatching positions. A total of four *vaa*-mutants and two *oppA*-mutants harbouring missense mutations were obtained and fully sequenced. Zero to eight additional mutations were identified in the genomes of each mutant. The *vaa*-mutants were tested for adhesion to immobilized HeLa cells but their adhesion was not significantly different from the adhesion of *M. hominis* PG21. One of the two *oppA*-mutants that were tested for ATPase activity presented a higher affinity for its ATP substrate than the parental strain.

**Conclusion:** For the first time, we demonstrated that *M. hominis* gene-targeted mutants could be successfully obtained using this TILLING strategy. In the absence of robust genetic tools for studying *M. hominis*, the TILLING strategy that can target any gene of the genome could help to elucidate gene functions and to better understand the pathogenesis of this human pathogenic species.

**Keywords:** *Mycoplasma hominis*, Chemical mutagenesis, Ethyl methanesulfonate, TILLING
Background

*Mycoplasma hominis* is a human urogenital pathogen involved in gynaecological, neonatal and extra-genital infections [1]. The genome of the reference strain, *M. hominis* PG21, is the second smallest genome among self-replicating free-living organisms [2]. Although a conjugal transfer of the transposon Tn916 from *Streptococcus faecalis* to *M. hominis* was reported 30 years ago [3], no versatile genetic tools are currently available for this species. Whereas *Mycoplasma arthritidis*, a phylogenetically closely related species belonging to the same Hominis group, has successfully been transformed using the PEG method [4], all attempts to transform *M. hominis* using either PEG or electroporation methods, as well as transposons, suicide plasmids or replicative plasmids have failed. Only one study reported the transformation of *M. hominis* by the plasmid pAM120 using electroporation in the 2000s [5], but the experiments could not be reproduced. A cutting-edge synthetic biology approach, which consists of cloning and subsequent engineering of *M. hominis* genome in yeast is currently under development but the back transplantation of the genome from yeast to a recipient bacterium has not been achieved yet [6]. The lack of genetic engineering tools for *M. hominis* has limited our capacity to modify its genome in order to elucidate gene functions and to understand its pathogenesis.

Chemical mutagenesis methods may offer an interesting alternative method to modify the genome of this intractable species. Targeting-Induced Local Lesions IN Genomes (TILLING) combines point mutations induced by standard chemical mutagenesis with a sensitive DNA screening technique that identifies single nucleotide polymorphisms (SNPs) in the targeted gene. This reverse-genetic strategy has mainly been used in plants such as *Arabidopsis thaliana* [7], tomato [8] and *Cucurbita pepo* [9] but also in parasites such as *Toxoplasma gondii* [10]. Only one publication reported its use in a bacterium, the intracellular *Chlamydia trachomatis* species [11].

In this study, we used ethyl methanesulfonate (EMS) that introduces C-G to T-A transition mutations to generate a library of *M. hominis* mutants. As a proof of principle, mutagenized organisms were screened for mutations in two target genes, the vaa gene encoding the variable adherence-associated (Vaa) adhesin lipoprotein [12] and the oppA gene encoding the substrate binding subunit of an oligopeptide permease and the main ectoATPase of the bacteria [13, 14]. The generated vaa- and oppA-mutants were fully sequenced and evaluated using two functional assays, an adhesion test to HeLa cells for vaa-mutants and an ATPase activity test for oppA-mutants.

Results

Generation of a *M. hominis* mutant library

The effect of EMS on *M. hominis* survival was assessed after contact times of 1.5 h and 3 h (Fig. 1). A positive correlation was observed between the concentration of EMS and the killing of the bacteria. The contact time with EMS also slightly affected the survival of *M. hominis*. For the generation of the mutant library, a concentration of 9 mg/ml EMS applied for 3 h that resulted in 75% killing was chosen according to the bibliography [11]. After culture filtration to avoid bacterial aggregates and plating on agar medium, 1200 individual colonies were picked, grown and frozen.

Screening of the *M. hominis* mutant library for vaa- and oppA-mutants

To screen the library for vaa-mutants, a 1692-bp PCR fragment of the *M. hominis* genome encompassing the 1295 nucleotides at the 5′-end of the vaa gene (MHO_3470, 1416 bp [2]) was targeted (Table 1, Fig. 2). To screen for oppA-mutants, a 1144-bp PCR fragment of the oppA gene encompassing the regions encoding the CS3, Walker B and Walker A domains involved in ATPase activity [14] of the OppA protein (MHO_1510, 2886 bp) was amplified (Table 1, Fig. 2).

After the screening procedure using ENDO1 nuclease, a total of six vaa and four oppA *M. hominis* mutants were obtained (Table 2). Four mutants were discarded: (i) two vaa-mutants that harbour a C to T transition located upstream of the vaa gene (these mutants were initially screened because the amplified DNA fragment encompassed 397 nucleotides upstream of the gene) and (ii) two oppA-mutants that harboured a G181068A and a G180439A silent substitution, respectively. Overall, four vaa-mutants (Mho66, Mho119, Mho787 and Mho1133) and two oppA-mutants (Mho369 and Mho940), each harbouring one amino-acid substitution in the targeted region of the vaa and oppA genes, respectively, were selected. The genomes of these six selected mutants were fully sequenced. The expected mutation was found in every mutant and zero to eight additional mutations were retrieved in the whole genome of each mutant (Table 3). Notably, mutant Mho66 harboured no additional mutation and mutant Mho369 harboured only one additional mutation in an intergenic region.

Functional assays applied to vaa- and oppA-mutants

The four vaa-mutants, Mho66, Mho119, Mho787 and Mho1133, and the parental PG21 *M. hominis* strain, which has a functional in-frame vaa gene, were tested for adhesion to HeLa cells immobilized on microtiter plates to quantify bacterial adherence. In all cases, the number of adherent *M. hominis* cells increased with the amount of *M. hominis* inoculum used in the assay.
The adhesion of the four vaa-mutants and the non-mutated *M. hominis* PG21 strain was not significantly different ($p = 0.73$, Kruskal-Wallis test).

The two oppA-mutants, Mho369 and Mho940, and the parental PG21 strain were tested for ATPase activity (Fig. 4) during which the kinetics of ATP hydrolysis were analysed by measuring the release of free phosphate [14]. Both mutants had a higher $V_{\text{max}}$ than *M. hominis* PG21 and the Mho369 mutant had the lowest Michaelis-Menten $K_m$ constant, although the 95% confidence intervals were overlapping.

**Discussion**

Because the transformation of *M. hominis* PG21 has so far been elusive, chemical mutagenesis using EMS appears to be an alternative option to create gene-targeted
mutants. In several organisms, a positive correlation was observed between the concentrations of EMS applied, the killing of the organism and the mutation frequency obtained [8, 11]. Only one study has reported the generation of EMS mutants and TILLING strategy in bacteria. Indeed, to generate \textit{C. trachomatis} mutants using EMS, the concentration of EMS that generated a single C-G base pair mutation per genome corresponded to a 75\% killing. Because we also observed a positive correlation between the concentration of EMS and the killing of \textit{M. hominis}, we decided to choose, for the generation of the library, the concentration of EMS that killed approximately 75\% of the \textit{M. hominis} population, i.e. 9 mg/ml applied for 3 h.

Using these conditions, we demonstrated, after screening the 1200-clone library, that four and two \textit{M. hominis} mutants were successfully generated in two targeted genes previously associated with the bacterium’s pathogenicity, the \textit{vaa} gene involved in cytadherence [12] and the \textit{oppA} gene involved in oligopeptide binding and the \textit{ectoATPase} activity [13, 15], respectively. Because the ATPase activity of the OppA protein is due mainly to the CS3, Walker B and Walker A gene regions [14], a fragment of 1144 bp encompassing these regions was chosen. Regarding the \textit{vaa} gene, a 1692-bp fragment encompassing the 5′-end conserved region of the gene was targeted for this proof of concept experiment because an early premature stop in protein synthesis would have had the greatest effect on loss of Vaa-mediated cytadhesion. To our knowledge, the substitutions that were found in the mutated Vaa and OppA have never been reported in the alleles present in other \textit{M. hominis} strains.

The four \textit{vaa}-mutants were tested for adhesion to immobilized HeLa cells [14]. Unfortunately, the

| Primer target and designation | Primer sequence | Product size (bp) |
|------------------------------|-----------------|-------------------|
| \textit{vaa} gene \footnote{\textit{VaaPG21-FE} and \textit{VaaPG21-RE} primers are located upstream and downstream the \textit{vaa} gene, respectively} | \textit{vaaPG21-FE} 5′-AATGGGATAGTTAGTAAAGTCGG-3′ | 1983 |
| \textit{vaaPG21-RE} 5′-AACCTACATTGCTGCTAAGA-3′ | 1692 |
| \textit{VaaPG21-FE-IRD700} 5′-IRD700-AATGGGATAGTTAGTAAAGTCGG-3′ | 1144 |
| \textit{VaaPG21-RI-IRD800} 5′-IRD800-GAAAAATCTCTCCTGCTGATG-3′ | |
| \textit{oppA} gene | \textit{OppAPG21-FE} 5′-GACAGTGAAAGCAGACAC-3′ | 1524 |
| \textit{OppAPG21-RE} 5′-AGGAACTAAATGTCCGGGT-3′ | |
| \textit{OppAPG21-FI-IRD700} 5′-IRDye700-CATCTCTGGCCAAAGCAACT − 3′ | |
| \textit{OppAPG21-RI-IRD800} 5′-IRDye800-CAGTCTTGATGAATCAAC-3′ | |

Fig. 2 Schematic representation of the TILLING strategy used in this study
The adhesion of the four mutants did not show any significant difference with the adhesion of the parental *M. hominis* PG21 strain. However, because *M. hominis* harbours several adhesin proteins such as the P120 lipoprotein and the Lmp-related proteins [16], this adhesion assay may not be sensitive enough to detect a potential adhesion decrease caused by the mutation of the Vaa protein. A control strain with an inactivated Vaa protein or a phase variable “OFF” variant would be necessary to determine whether the sensitivity of this assay was

### Table 2: *vaa*- and *oppA*-mutants obtained by screening the 1200 EMS-generated *M. hominis* clone library

| Mutant designation | Targeted gene | Nucleotide substitution | Amino-acid substitution | Comments |
|---------------------|---------------|-------------------------|-------------------------|----------|
| Mho66               | vaa           | G410726A                | Glu54Lys                |          |
| Mho119              | vaa           | C411332T                | Leu328Phe               |          |
| Mho787              | vaa           | C410526A                | Pro59His                |          |
| Mho864              | vaa           | C410197T                | none                    | Mutation located upstream of the vaa gene (intergenic region) |
| Mho1055             | vaa           | C410154T                | none                    | Mutation located upstream of the vaa gene (intergenic region) |
| Mho1133             | vaa           | G410726A                | Gly126Ser               |          |
| Mho258              | oppA          | G181068A                | Leu813Leu               | Silent mutation |
| Mho369              | oppA          | G180439A                | Asp604Asn               |          |
| Mho785              | oppA          | C181239T                | Asp870Asp               | Silent mutation |
| Mho940              | oppA          | G180634A                | Glu669Lys               |          |

*Nucleotide substitutions are numbered according to the *M. hominis* PG21 genome (GenBank accession number FP236530)*

### Table 3: Mutations harboured by EMS-generated *M. hominis* mutants in comparison to the sequence of the *M. hominis* PG21 strain not exposed to EMS

| Mutant designation | Expected mutation | Other mutations in the genome | Number of additional mutations |
|--------------------|-------------------|-------------------------------|------------------------------|
| Mho66              | G410726A (vaa, 376) | none                          | 0                            |
| Mho119             | C411332T (vaa, 982)  | - C230929T (intergenic)        | 4                            |
|                    |                   | - C316577T (MHO_2640, CHP)    |                              |
|                    |                   | - C465933A (MHO_3770, licA, Protein LicA homolog) | |
|                    |                   | - C494526A (MHO_3990, nuxG, Transcription antitermination protein) | |
| Mho787             | C410526A (vaa, 176) | - C5000T (MHO_0050, dnaA, DNA polymerase III beta chain) | 8 |
|                    |                   | - C44025T (MHO_0340, knrA, Potassium uptake protein KnrA) | |
|                    |                   | - C172081T (MHO_1460, rpsG, 30S ribosomal protein S7) | |
|                    |                   | - C258548T (MHO_2150, rpoD, RNA polymerase sigma factor) | |
|                    |                   | - C283090T (MHO_2320, ABC transporter, ATP-binding protein) | |
|                    |                   | - G288972A (MHO_2370, tRNA/tRNA methyltransferase) | |
|                    |                   | - C441828T (MHO_3650, asrA, Aspartate-ammonia ligase) | |
|                    |                   | - C71686T (MHO_4600, pyrH, Uridylate kinase) | |
| Mho1133            | G410726A (vaa, 376) | - G179389A (MHO_1510, oppA)  | 6                            |
|                    |                   | - C206418T (MHO_1700, recU, Recombination protein U homolog) | |
|                    |                   | - C381191T (MHO_3200, CHP, Hypothetical lipoprotein) | |
|                    |                   | - G462316A (MHO_3760, gyrB, DNA gyrase subunit B) | |
|                    |                   | - G490546A (intergenic)       | |
|                    |                   | - C644067T (MHO_5190, pip, Proline iminopeptidase) | |
| Mho369             | G180439A (oppA, 1813) | - G230779A (intergenic)       | 1                            |
|                    |                   | - G2030779A (intergenic)      | |
| Mho940             | G180634A (oppA, 2008) | - G136127A (MHO_1080, ATB-binding protein) | 8 |
|                    |                   | - G138607A (MHO_1090, HP)    | |
|                    |                   | - G203092A (MHO_1670, cmk, Cytidine kinase) | |
|                    |                   | - G211597A (MHO_1740, oppB, Oligopeptide transport system permease protein) | |
|                    |                   | - A227310G (MHO_1880, aspS, Aspartyl-tRNA synthetase) | |
|                    |                   | - G357674A (MHO_3060, leuS, Leucyl-tRNA synthetase) | |
|                    |                   | - G371725A (MHO_3130, atpA, F1-likeX0) | |
|                    |                   | - G649646C (MHO_5220, hsdS, Type I restriction enzyme specificity protein) | |

*mutations are numbered according to the *M. hominis* PG21 genome (GenBank accession number FP236530)*

CHP conserved hypothetical protein, HP hypothetical protein
accurate. Analysis of the vaa gene sequence of the \textit{M. hominis} PG21 genome showed that C-G to T-A EMS-generated transitions could theoretically create 23 stop codons, leading to truncated proteins. Thus, enlarging our EMS library may generate null mutants.

Regarding the two opp\textit{A}-mutants, mutant Mho369 was of interest because (i) it harboured only one additional mutation in an intergenic region of its genome, suggesting that the observed phenotype could be attributed to the EMS-generated opp\textit{A} mutation with a high
degree of certainty and (ii) it had a higher $V_{\text{max}}$ and a lower $K_m$ than \textit{M. hominis} PG21, although 95% confidence intervals were overlapping. This finding suggests that Mho369 may have the capacity to hydrolyse ATP with a higher turnover. This mutant harboured an aspartic acid-to-asparagine substitution located upstream of the CS3 motif, known to be involved in the ATPase activity of the protein [14]. Further studies such as cloning, expression and analysis of the mutated OppA protein could be informative in future works. Nevertheless, although recombinant OppA-mutant proteins have previously been generated [14], oppA-mutated \textit{M. hominis} cells had never been obtained before. Since OppA is involved in \textit{M. hominis} pathogenicity [13, 15], such mutated \textit{M. hominis} cells will allow for the characterization of the effect of single amino-acid changes in OppA on the pathogenicity of the mycoplasma.

The TILLING strategy used in this study presents some advantages and some drawbacks. The principal advantage is its capacity to generate gene-targeted mutants in species such as \textit{M. hominis} that are difficult to genetically manipulate. The ultimate goal of the method is to generate null mutants. All genes could be potentially targeted except those genes that are essential for growth in vitro, and thus cannot be targeted for null mutations. In addition, once a library has been generated, it can be reused to search for mutants in other genes of interest. However, this method is labour-intensive, both for the generation of the library and its handling. The generation of the library and its handling. The

**Conclusion**

Although genetic manipulations are still hardly achievable in \textit{M. hominis}, targeted mutants were successfully generated using the TILLING strategy. Further experiments are needed to adjust the conditions of EMS mutagenesis to limit the mutation frequency to one mutation per genome, but this strategy will be helpful to elucidate the gene functions and to better understand the pathogenesis of this human urogenital species.

**Methods**

**EMS mutagenesis of \textit{M. hominis} PG21**

The \textit{M. hominis} PG21 reference strain (ATCC 23114) was grown in Hayflick broth supplemented with arginine (hereafter named Hayflick arginine medium) [17]. One millilitre of a culture of \textit{M. hominis} PG21 ranging between $1 \times 10^8$ and $3 \times 10^8$ colony forming unit (CFU)/ml was exposed to concentrations of EMS ranging from 0 to 24 mg/ml for 1.5 h and 3 h at room temperature, including a 30-min centrifugation step at 15,000 g. Pellets were resuspended in 1 ml of Hayflick arginine medium, and \textit{M. hominis} concentration was determined by plating on Hayflick agar plates to assess \textit{M. hominis} survival.

To generate the mutant library, a $10^8$ CFU/ml \textit{M. hominis} culture was exposed to EMS, filtered on 0.45 µm sterile mixed cellulose ester membrane (Millipore, Billerica, MA, USA), and 10−1 to 10−4 serial dilutions in Hayflick arginine medium were plated on Hayflick agar plates. After 48 h at 37 °C under 5% CO₂, individual colonies were picked and grown in 1 ml of Hayflick arginine medium until the colour changed. A total of 800 µl of the latter culture was frozen at −80 °C, and 200 µl was used for DNA extraction.

**Screening for vaa- and oppA-mutants in \textit{M. hominis}**

Two hundred microliters from four distinct clone cultures were pooled together before DNA extraction using the MagNA Pure 96 DNA and Viral NA Small Volume kit on the MagNA Pure 96 instrument (Roche Diagnostics, Meylan, France). DNA extraction was also performed on 200 µl of each of four single clone cultures when a mutated pool of clones was identified.

PCR fragments of 1692 bp and 1144 bp in regions of interest of the \textit{vaa} and the \textit{oppA} genes were amplified by semi-nested and nested PCR, respectively, using primers presented in Table 1. The first round of PCR was performed on a CFX96 real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA, USA) in a final volume of 25 µl using 2.5 µl of tartrazine 10X buffer (50 mM Tris HCl pH 8, 500 µg/ml bovine serum albumin, 0.5% Ficoll, 1% sucrose, 30 mM KCl, 3 mM MgCl₂, 1 mM tartrazine), 0.1 µM of each primer, 120 µM dNTP, 0.0005 U of GoTaq® qPCR Master Mix (Promega, Madison, WI, USA), and 1 µl of DNA extract.
The amplification conditions were 3 min at 95 °C followed by 35 cycles of 15 s at 95 °C, 20 s at 58–68 °C, 2 min at 72 °C then 10 min at 72 °C. The second round of PCR was performed using 0.5 μl from the first PCR and IRD700- and IRD800-labelled primers (Table 1). After checking accurate amplification by agarose gel electrophoresis, 5 μl of the PCR products was used to screen for vaa- and oppA-mutants. PCR amplicons were heat-denatured 3 min at 95 °C and slowly reannealed using a temperature gradient from 95 °C to 8 °C (0.1 °C/s) before a 4 °C final step. The reannealed amplicons were digested using ENDO1 nuclease at 0.33 U/l, prepared as previously reported [8], for 20 min at 45 °C and cooled to 4 °C to stop the enzyme activity. At this step, if multiple alleles were present in the PCR amplicons, these allelic forms were sorted out by heteroduplexes during reannealing that were specifically cleaved by ENDO1 at mismatching positions. The digested amplicons were purified and dried as described previously [18].

SNP screening was performed using a LiCOR* DNA analyser as previously described [19]. Pools of clones containing vaa- and oppA-mutants were identified by the appearance of lower-mass DNA digestion products after electrophoresis on a 6.5% acrylamide gel. The PCR products of vaa or oppA genes from the four individual clones of the mutated pool were sequenced by Sanger and IRD700- and IRD800-labelled primers (Table 1). Fifty microliters of each dilution was incubated in Hayflick arginine medium and serially diluted 1:5 in Hayflick arginine medium. CFU/ml, was concentrated 10 times by centrifugation with concentrations ranging between 1 × 10⁸ and 4 × 10⁸ CFU/ml, was concentrated 10 times by centrifugation and serially diluted 1:5 in Hayflick arginine medium. Fifty microliters of each dilution was incubated in triplicate with HeLa cells that had been lysine-coated on 96-well microplates in 50 μl of Dulbecco’s Modified Eagle’s medium (DMEM) supplemented with 1% fetal bovine serum (DMEM-FBS) for 4 h at 37 °C. Unbound M. hominis cells were removed by three washings with DMEM-FBS. Adherent M. hominis cells were lysed using 20 μl of proteinase K 20 mg/ml from the NucleoSpin® Tissue kit (Macherey-Nagel) for 1 h at 56 °C and DNA was extracted using the MagNA Pure 96 DNA and Viral NA Small Volume kit on the MagNA Pure 96 instrument (Roche Diagnostics). A quantitative real-time PCR targeting the yidC gene of M. hominis was then performed as previously described [21]. The non-parametric Kruskal-Wallis test was used to compare the adhesion of mutants and the PG21 parental strain. A significant p-value was set at < 0.05.

ATPase activity test
The ATPase assay was conducted by incubating M. hominis cells with ATP and subsequently using an ammonium molybdate solution to quantify the emerging free phosphates [13]. M. hominis cells were collected from 10 ml of logarithmic growth culture by centrifugation (6700 g for 10 min at 6 °C), washed twice in buffer A (120 mM NaCl, 5 mM KCl, 20 mM Tris-HCl, pH 7.5) and resuspended in 1.2 ml of buffer A. M. hominis cells were quantified by Taqman PCR as published earlier [22]. Triplicates of 20 μl of mycoplasma cell suspension and 20 μl of buffer A (as negative control) were each adjusted to 0, 0.2, 0.4, 1, 2 or 3 mM ATP and immediately assayed for free phosphate (to estimate phosphate background of mycoplasma suspension) or incubated for 4 h at 37 °C.

Hydrolysis of ATP was terminated by adding 200 μl of malachite green reagent (5.72% [w/v] ammonium molybdate in 6 N HCl, 3.32% [w/v] polyvinyl alcohol, 0.0812% [w/v] malachite green, and distilled water at a ratio of 1:1:2:2). The absolute absorbance of the samples was measured in relation to a blank at 620 nm (Tecan Rainbow, SLT Labinstruments, Crailsheim, Germany). Inorganic phosphate at concentrations ranging from 1 to 20 nmol was used as quantification standard. Values of ATPase activity of M. hominis expressed as the release of nmol phosphate were corrected for the phosphate background of ATP, adjusted to 10¹⁰ M. hominis cells and used in the Michaelis-Menten equation to calculate the values of Vmax and Km using the Graph Pad Prism 6.01 software.

Abbreviations
C. trachomatis: Chlamydia trachomatis; CHP: Conserved hypothetical protein; EMS: Ethyl methanesulfonate; HP: Hypothetical protein; M. hominis: Mycoplasma hominis; TILLING: Targeting-Induced Local Lesions IN Genomes
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Availability of data and materials
The PG21 M. hominis reference strain and the EMS-generated M. hominis mutant sequence information were deposited in NCBI Sequence Read Archive (SRA) with the following accession numbers: PG21 (ATCC 23114) SRX4325267, Mho66 SRX4325266, Mho119 SRX4325269, Mho369 SRX4325268, Mho787 SRX4325264, Mho940 SRX4325263 and Mho1133 SRX4325265.

Authors’ contributions
SP, PSP, BH and CBéb conceived and designed the study. SP, CBr, CLR, JPM, FR and BH conducted the experiments and performed data analysis. SP wrote the manuscript with contributions from CBr, PSP, BH and CBéb. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable. No human samples and no animals were used. The M. hominis PG21 reference strain was from ATCC (ATCC 23114). The HeLa cells were Human cervical carcinoma cell line HeLa S3 (ATCC CCL2.2).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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