Testing theoretical minimal genomes using whole-cell models

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Abstract

The minimal gene set for life has often been theorised, with at least ten produced for *Mycoplasma genitalium* (*M. genitalium*). Due to the difficulty of using *M. genitalium* in the lab, combined with its long replication time of 12 - 15 hours, none of these theoretical minimal genomes have been tested, even with modern techniques. The publication of the *M. genitalium* whole-cell model provided the first opportunity to test them, simulating the genome edits *in-silico*. We simulated eight minimal gene sets from the literature, finding that they produced *in-silico* cells that did not divide. Using knowledge from previous research, we reintroduced specific essential and low essential genes *in-silico*; enabling cellular division. This reinforces the need to identify species-specific low essential genes and their interactions. Any genome designs created using the currently incomplete and fragmented gene essentiality information, will very likely require *in-vivo* reintroductions to correct issues and produce dividing cells.

30 Introduction

31 Genome engineering builds on historical gene essentiality research. The sequencing of small
 32 bacterial genomes (Fleischmann et al., 1995; Fraser et al., 1995) led to comparative genomics;
 33 then, as genome sequences completed increased, minimal gene sets (Forster & Church, 2006;
 34 R. Gil et al., 2004; Rosario Gil, 2015; J. I. Glass et al., 2006; Huang et al., 2013; Hutchison et
 35 al., 1999; Karr et al., 2012; Mushegian & Koonin, 1996; Shuler et al., 2012; Tomita et al., 1999)
 36 were hypothesised. Minimal genomes are reduced genomes where no single gene can be
 37 removed without loss of reproduction (John I. Glass et al., 2017), given an appropriately rich
 38 medium and no external stresses, and focusing solely on protein-coding genes. For a recent
 39 review of gene essentiality, see Rancati *et al.* (Rancati et al., 2018).

40

41 *M. genitalium* is the focal point of minimal gene set creation due its naturally small genome size
 42 (0.58mb and 525 genes) and early sequenced genome (Fraser et al., 1995). Minimal gene sets
 43 are designed using three different approaches: protocells, comparative genomics, and single
 44 gene knockouts. Protocell designs (Dzieciol & Mann, 2012) are not expected to function as true
 45 biological cells, instead functioning as a self-replicating, membrane-encapsulated collection of
 46 biomolecules (Forster & Church, 2006). Comparative genomics (Koonin, 2003) compares
 47 multiple species to identify common genes. This is complicated (John I. Glass et al., 2017;
 48 Lagesen et al., 2010) by non-orthologous gene displacements, i.e. independently evolved or
 49 diverged proteins that perform the same function but are not recognisably related (John I. Glass
 50 et al., 2017; Mushegian & Koonin, 1996), which can result in the removal of a large number of
 51 genes essential to one species. Design using single gene essentiality classifications should, in
 52 theory, not remove any essential genes; but if transposon mutagenesis is used, variance from
 53 different transposon variants, antibiotic resistance genes, and growth periods can result in
 54 differing essentiality classifications (J. I. Glass et al., 2006; Juhas et al., 2011).

55 Ten minimal gene sets were found in the literature that were designed with *M. genitalium* genes
 56 (Forster & Church, 2006; R. Gil et al., 2004; Rosario Gil, 2015; J. I. Glass et al., 2006; Huang et
 57 al., 2013; Hutchison et al., 1999; Karr et al., 2012; Mushegian & Koonin, 1996; Shuler et al.,
 58 2012; Tomita et al., 1999). Due to the difficulty of using *M. genitalium* in the lab (Reich, 2000),
 59 combined with its long replication time of 12 - 15 hours (Benders et al., 2010; John I. Glass et
 60 al., 2017; Hutchison et al., 2016), none of these theoretical minimal genomes have been tested,
 61 even with modern techniques (Benders et al., 2010). The publication of the *M. genitalium*
 62 whole-cell model (Karr et al., 2012) provided the first opportunity to test them, simulating the
 63 genome edits *in-silico*.

64

65 The *Mycoplasma genitalium* whole-cell model (Karr et al., 2012) was the first existing model of a
 66 cell's individual molecules that includes the function of every known gene product (401 of the
 67 525 *M. genitalium* genes), making it capable of modelling genes in their genomic context.
 68 Previously, it has been used to investigate discrepancies between the model and real-world
 69 measurements (Karr et al., 2012; Sanghvi et al., 2013), design synthetic genetic circuits in the
 70 context of the cell (Purcell et al., 2013), make predictions about the use of existing antibiotics
 71 against new targets (Kazakiewicz et al., 2015), and produce *in-silico* minimal genomes
 72 (Rees-Garbutt et al., 2020).

73

74 Of the ten sets, two of the sets (Gil et al. (2004)(R. Gil et al., 2004)and Shuler et al.(Shuler et al.,
 75 2012)) were excluded as they were considered derivative of the Gil (2014) set (Rosario Gil,
 76 2015); four genes differ in the Shuler et al. set (MG_056, MG_146, MG_388, MG_391) and four
 77 genes are absent in the Gil et al. (2004) set (MG_009, MG_091, MG_132, MG_460). Of the
 78 other eight sets, two (Tomita et al. (Tomita et al., 1999) and Church et al. (Forster & Church,
 79 2006)) were designed as protocells, three (Mushegian and Koonin (Mushegian & Koonin, 1996),

80 Huang *et al.* (Huang *et al.*, 2013), and Gil (R. Gil *et al.*, 2004; Rosario Gil, 2015)) from
81 comparative genomics, and three (Hutchison *et al.* (Hutchison *et al.*, 1999), Glass *et al.* (J. I.
82 Glass *et al.*, 2006), and Karr *et al.* (Karr *et al.*, 2012)) from single gene essentiality experiments.

83

84 We simulated eight minimal gene sets from the literature (Forster & Church, 2006; Rosario Gil,
85 2015; J. I. Glass *et al.*, 2006; Huang *et al.*, 2013; Hutchison *et al.*, 1999; Karr *et al.*, 2012;
86 Mushegian & Koonin, 1996; Tomita *et al.*, 1999), testing them for the first time. This was to
87 assess their functionality as extant and untested genome designs, but also to produce new
88 data for designing genomes to evaluate the differing approaches of focusing on
89 species-specific data or focusing on identifying core genes and common functions across
90 species.

91

92 **Results**

93 We adapted eight minimal gene sets for simulation within the *M. genitalium* whole-cell model.
94 To increase clarity, we renamed the sets after the main location where the set was constructed
95 (Table 1). The Bethesda set directly compared *M. genitalium* and *Haemophilus influenzae*
96 genomes (gram-positive and gram-negative bacteria, respectively) (Mushegian & Koonin, 1996).
97 The Rockville set applied global transposon mutagenesis to *M. genitalium in-vivo* to identify
98 non-essential genes (Hutchison *et al.*, 1999). The Fujisawa set constructed an *in-silico*
99 hypothetical cell from 127 *M. genitalium* genes using the E-Cell software (Tomita *et al.*, 1999).
100 The Rockville 2 set reapplied global transposon mutagenesis *in-vivo*, isolating and
101 characterising pure clonal populations (J. I. Glass *et al.*, 2006). The Nashville set listed 151
102 *E.coli* genes (compared to *M. genitalium* genes within the paper) theorised to produce a
103 chemical system capable of replication and evolution (Forster & Church, 2006). The Stanford
104 set was the result of *in-silico* single gene knockouts using the *M. genitalium* whole-cell model

(Karr et al., 2012). The Guelph set compared 186 bacterial genomes (Huang et al., 2013), whereas the Valencia set compared *M. genitalium* with genetic data of five insect endosymbionts (Rosario Gil, 2015). The Nashville, Fujisawa, and Stanford sets were unchanged, but the others had between 6 and 44 genes removed (Table 1) either because the genes were unmodelled (the genes' function were unknown (Karr et al., 2012)) or genes had duplicate entries.

111

112 **Table 1.** Minimal Gene Sets from the literature.

Minimal gene set	Code name	Design approach	In-vivo genome design size*	Unmodelled genes^ in genome design	Unmodelled genes^ in gene deletions	Predicted in-silico genome size
Forster and Church 2006 (Forster & Church, 2006)	Nashville	Protocell	89	0	-	131
Tomita et al. 1999 (Tomita et al., 1999)	Fujisawa	Protocell	98	0	-	140
Huang et al. 2013 (Huang et al., 2013)	Guelph	Comparative Genomics	123	5	-	160
Gil 2014 (Rosario Gil, 2015)	Valencia	Comparative Genomics	180	6	-	216
Mushegian and Koonin 1996 (Mushegian & Koonin, 1996)	Bethesda	Comparative Genomics	253	12	-	283
Karr et al. 2012 (Karr et al., 2012)	Stanford	Single Gene Deletions	242	-	0	284
Glass et al. 2006 (J. I. Glass et al., 2006)	Rockville 2	Single Gene Deletions	258	-	44	344
Hutchison et al. 1999 (Hutchison et al., 1999)	Rockville	Single Gene Deletions	265	-	41	348
-	<i>M. genitalium</i> whole-cell model*	-	359	124	-	401
-	<i>M. genitalium</i> in-vivo *	-	483	-	-	525

113 Comparing minimal gene sets with *M. genitalium* in-vivo and the *M. genitalium* whole-cell
114 model. * = protein-coding genes. *M. genitalium* has 42 RNA-coding genes that are not included
115 in this column, to enable comparison across genome designs. ^ = due to unknown function.

116 Unmodelled genes in genome design reduces the size of the in-vivo genome design.

117 Unmodelled genes in gene deletions increases the size of the in-vivo genome design.

118

119

120 The protocell designs (Nashville, Fujisawa) predicted the smallest in-silico genome. Guelph

121 contained substantially fewer genes than Valencia and Bethesda, due to comparing 186
122 species (Huang et al., 2013). Stanford, Rockville, Rockville 2 had similar numbers of *in-vivo*
123 deletions, but Rockville and Rockville 2 had the highest numbers of unmodelled genes (as
124 genes can be disrupted *in-vivo* without knowing the gene's function). The *in-silico* genomes
125 (and associated gene deletions) of the minimal gene sets are listed in Supplementary Data 1
126 and 2.

127

128 Prior to simulations, we analysed the sets produced by comparative genomics and single gene
129 essentiality and found 96 genes they had in common (Supplementary Data 1 (col L) and 3). We
130 excluded the protocell designs from this comparison due to their much reduced size. 87 of the
131 shared genes were classified as essential (genes that when removed *in-silico* stopped the cell
132 from dividing, classified *in-silico* previously (Karr et al., 2012; Rees-Garbutt et al., 2020)), eight
133 were non-essential (removal did not prevent successful cell division), and one gene was
134 unmodelled.

135

136 The 87 essential genes affect a range of cellular functions including: DNA (repair, supercoiling,
137 chromosome replication, nucleotide synthesis/modification, sigma factors, ligation, transcription
138 termination, and DNA polymerase); RNA (ribosome proteins, translation initiation factors, tRNA
139 modification, ribonucleases, and RNA polymerase); and cellular processes (protein
140 folding/modification, protein shuttling, protein membrane transport, metabolic substrates
141 production/recycling, redox signalling, oxidation stress response, and the pyruvate
142 dehydrogenase complex). Of the eight non-essential genes, four (MG_048, MG_072, MG_170,
143 MG_297) are associated with the SecYEG complex (du Plessis et al., 2011) (protein transport
144 across or into the cell membrane), while MG_172 removes protein synthesis targets from
145 synthesised proteins, MG_305 and MG_392 assist in late protein folding, and MG_425

146 processes ribosomal RNA precursors. Although these eight genes are singly non-essential (by
147 single gene deletion *in-silico* (Karr et al., 2012) and *in-vivo* (J. I. Glass et al., 2006)) they all play
148 a part in essential functions, hence their inclusion.

149

150 We also identified 14 genes deleted by all eight minimal gene sets (Supplementary Data 2 (col
151 L) and 4). The functions of these genes include: fructose import, host immune response
152 activation, chromosomal partition, amino acid transport, antibody binding, phosphonate
153 transport, external DNA uptake, DNA repair, rRNA modification, membrane breakdown, toxin
154 transport, quorum sensing, and a restriction enzyme. These had been previously classified as
155 non-essential by single gene deletion *in-silico* (Karr et al., 2012) and *in-vivo* (J. I. Glass et al.,
156 2006). We placed these 14 common genes in an 'Agreed set' and a genome with these genes
157 removed was also simulated.

158

159 We simulated each minimal gene set in the *M. genitalium* whole-cell model and found that
160 every set, including the Agreed set, produced a non-dividing *in-silico* cell (30 *in-silico* replicates,
161 Supplementary Data 5).

162

163 Analysis found that every one of the sets deleted essential genes (classified *in-silico* previously
164 (Karr et al., 2012; Rees-Garbutt et al., 2020)): Nashville deleted 121, Fujisawa deleted 112,
165 Guelph deleted 107, Valencia deleted 69, Bethesda deleted 34, Rockville and Rockville 2 both
166 deleted 9, and Stanford deleted 3 (Supplementary Data 6-14). This is especially surprising for
167 the single gene essentiality minimal gene sets. For the Rockville sets, this is likely due to
168 transposon mutagenesis issues. Rockville labelled six genes as non-essential in 1999,
169 subsequently labelled essential in 2006. Additionally, Rockville grew cells in mixed pools with
170 DNA isolated from these mixtures rather than from isolated pure colonies of cells (J. I. Glass et

171 al., 2006). Whereas for Rockville 2, the engineers of *JCVI-Syn3.0* used the same transposon
172 mutagenesis protocol (Hutchison et al., 2016), but found that they had to improve upon it due
173 to incorrect identification of essentiality. For the Stanford set, the removal of MG_203, MG_250,
174 and MG_470 is likely due to averaging multiple simulation's data together before computational
175 assessment, genes later found to be essential (Supplementary Table 3(Rees-Garbutt et al.,
176 2020)).

177

178 In an attempt to restore *in-silico* division, we reintroduced essential genes to the minimal gene
179 sets (Figure 1). Based on previous research (Rees-Garbutt et al., 2020), we also reintroduced
180 low essential genes (i.e. genes dispensable in some contexts, such as redundant essential
181 genes and gene complexes (Rancati et al., 2018)). We did this by comparing the gene content
182 of the individual minimal gene sets with a complete list of the *M. genitalium in-silico* genes and
183 their essentiality classifications (Rees-Garbutt et al., 2020) (Supplementary Data 6-14). For
184 example, the original Agreed set removed low essential genes MG_291 (phosphonate
185 transport) and MG_412 (phosphate transport); by disrupting both these processes, the *in-silico*
186 cell has no functioning source of phosphate, which has been established previously
187 (Rees-Garbutt et al., 2020). By reintroducing set-specific genes (Table 2, Supplementary Data
188 15), each modified set, including the Agreed set, was able to produce a dividing cell *in-silico* (30
189 *in-silico* replicates, Supplementary Data 5).

190

191 **Figure 1.** *Testing and restoring the minimal gene sets.*

193 124 genes were unmodeled in the *M. genitalium* whole-cell model due to unknown function.

194 Essential genes are required by the cell to enable survival until successful division. Low

195 essential genes are required by the cell in certain genomic and environmental contexts to

196 enable survival to successful division. The restored minimal gene sets produced dividing

197 in-silico cells. Greater detail on the minimal gene sets is provided in Table 1 and 2.

198

199 **Table 2.** *Reintroducing genes to produce division*

Code name	<i>In-silico</i> gene deletions (cell did not divide)	<i>In-silico</i> gene deletions (cell divided)	Essential genes reintroduced	Low essential genes reintroduced	Size of <i>in-silico</i> genome *
Nashville	270	142	121	7	259
Fujisawa	261	142	112	7	259
Guelph	241	128	107	6	273
Valencia	185	110	69	6	291
Stanford	117	109	3	5	292
Bethesda	118	82	34	2	319
Rockville 2	57	45	9	3	356
Rockville	53	43	9	1	358
Agreed	14	13	0	1	388

200

201 *Minimal gene sets before and after the reintroduction of essential and low essential genes*

202 *dividing in-silico cells. *Size of in-silico genome includes both the 359 protein-coding genes and*

203 *the 42 RNA-coding genes in the M. genitalium whole-cell model, to provide a better*

204 *comparison for in-vivo experiments and future work.*

205

206 In an attempt to gain further understanding, we investigated what processes the nine repaired

207 minimal gene sets removed using gene ontology (GO) biological process terms, and we

208 compared the repaired minimal gene sets to the *in-silico* *M. genitalium* minimal genomes we

209 produced previously (Rees-Garbutt et al., 2020). The smallest repaired *in-silico* genomes

210 (Nashville and Fujisawa, 259 genes) were larger than the prior novel *in-silico* minimal genomes

211 (256 and 237 genes), and both removed the low essential genes related to phosphonate

212 transport, relying on inorganic phosphate being transported into the cell (Supplementary Table

213 6(Rees-Garbutt et al., 2020)). The other sets had different designs that, due to not

214 systematically targeting non-essential genes, resulted in non-essential genes remaining in the

215 genome, making them subsets of the repaired protocell sets and the prior *in-silico* minimal

216 genomes (Supplementary Data 16). As such, the GO terms were also subsets and did not
217 deviate from what we would expect to produce a dividing *in-silico* cell (Supplementary Data
218 17-25).

219

220 Analysis of the repaired sets found that 31 genes were reintroduced into five or more of the
221 minimal gene sets (Supplementary Data 26). 26 were essential and 5 were low essential
222 (Supplementary Table 5(Rees-Garbutt et al., 2020)). The corresponding cellular functions
223 included: DNA (polymerase subunits, thymidine insertion, recycling of pyrimidine, chromosome
224 segregation); RNA (polymerase subunit, tRNA modification, the 50S and 30S ribosomal
225 subunits); transporters (cobalt, phosphonate, potassium); production (NAD, flavin, NADP, fatty
226 acid/phospholipids); and dehydrogenation (glycerol and alpha-keto acids). Of the 26
227 reintroduced essential genes, 19 were already present in the single gene deletion minimal gene
228 sets (Stanford, Rockville, Rockville 2). MG_137 (mycobacteria cell wall production) and MG_517
229 (plasma membrane stability) are genes specifically essential for *Mycoplasma* species, which
230 were only identified as essential by the single gene deletion minimal gene sets. A further five
231 genes were involved in cobalt transport, which increases the rates of DNA synthesis, fatty acid
232 metabolism, and amino acid metabolism, and were also not identified as essential by the other
233 design methodologies. Of the five reintroduced low essential genes, Bethesda did not delete
234 four, likely due to the direct comparison of two genomes resulting in low essentiality genes
235 being conserved to a greater degree.

236

237 We also looked at reintroductions to the protocell sets, as they could outline additional cellular
238 requirements for the successful future unification of independent protocell systems. The genes
239 reintroduced to the Nashville set repaired functions that had been reduced (translation,
240 glycolytic process, protein folding) and restored functions that had been removed including: cell

(division, cycle, transport, redox homeostasis), DNA (topological change, transcription), rRNA processing (including pseudouridine synthesis), protein transport, and cellular processes (carbohydrate metabolic, glycerol metabolic, fatty acid biosynthesis, UMP salvage) (Supplementary Data 27). The glycolytic process had the most change, with 10 of 11 genes being reintroduced, and DNA repair had the least, with only one gene being reintroduced (MG_254), however, this did reintroduce single strand DNA break repair. The genes additionally reintroduced to the Fujisawa set included tRNA processing and protein folding (Supplementary Data 28), with 8 out of 10 DNA replication genes reintroduced.

249

250 Discussion

In conclusion, the repaired protocell minimal gene sets (Nashville and Fujisawa) produced the smallest genomes *in-silico* (Table 2), differing by 6 genes (Supplementary Data 29), but required the most gene reintroductions. The repaired comparative genomics minimal gene sets (Guelph, Valencia, Bethesda) required fewer gene reintroductions, presumably because fewer genomes were compared during their original design. Interestingly, Stanford (a single gene essentiality set) produced a smaller *in-silico* genome than Bethesda (a comparative genomics set), as it did not target unmodelled gene deletions and only required eight genes to be reintroduced.

258

This research has limitations associated with the use of the *M. genitalium* whole-cell model. Through necessity the *M. genitalium* whole-cell model bases some of its parameters on data from other bacteria⁸ and is only capable of modelling a single generation, missing subgenerational gene expression and subsequent essentiality which affects >50% of the genes in some species (Macklin et al., 2020). Additional uncertainty exists around the unknown impact of the unmodelled genes on *in-vivo* experiments, as stated previously (Rees-Garbutt et al., 2020).

266 The computational predictions we have produced need to be tested in living cells, but with the
 267 advancement of gene synthesis and genome transplantation in other *Mycoplasma* species
 268 (Benders et al., 2010; Gibson, Benders, Andrews-Pfannkoch, et al., 2008; Gibson, Benders,
 269 Axelrod, et al., 2008; Gibson et al., 2010; Karas et al., 2013; Tsarmpopoulos et al., 2016) this is
 270 becoming a more realistic proposition for *Mycoplasma* researchers.

271

272 Without the ability to identify species-specific low essential genes, any minimal gene sets
 273 designed with the current incomplete and fragmented gene essentiality information will require
 274 gene reintroductions to produce dividing cells. New research is required to generate the
 275 knowledge required to fill these gaps. Currently, genome-scale engineering has not combined
 276 *in silico* and *in vivo* research (Jiang et al., 2013; Karr et al., 2012; Rees-Garbutt et al., 2020).
 277 With the recent publication of an *E.coli* whole-cell model (Macklin et al., 2020) and the
 278 introduction of a synthetic, recoded *E.coli* genome into a cell (Fredens et al., 2019), this will
 279 quickly change. The current nature of species-specific gene essentiality information is,
 280 potentially, the only remaining barrier to producing custom genomes.

281

282 **Methods**

283 These methods have been published previously [27].

284

285 **Experimental Design and Statistics**

286 Each simulation was replicated 30 times. Prior publications have completed five repetitions [8],
287 ten or one hundred repetitions [27], depending on data storage capacity and supercomputer
288 usage. These were the main factors in our choice for the number of replications.

289 The initial conditions for each simulation are randomised within a natural range for *M. genitalium*
290 cells, introducing variation in the behaviour of simulated cells [8], as previously shown [27].

291 Statistical tests were not used in this research.

292

293 **Code Availability**

294 All code created as part of this paper will be made available on Github under a GNU General
295 Public License v3.0 (gpl-3.0). For more information see choosealicense.com/licenses/lgpl-3.0/.

296

297 **Data Availability**

298 The databases used to design the *in-silico* experiments, and compare the results to, includes
299 Karr *et al.* (Karr *et al.*, 2012) and Glass *et al.* (J. I. Glass *et al.*, 2006) Supplementary Tables, and
300 Fraser *et al.* *M. genitalium* G37 genome (Fraser *et al.*, 1995) interpreted by KEGG (Kanehisa &
301 Goto, 2000) and UniProt (Apweiler *et al.*, 2004) as strain ATCC 33530/NCTC 10195. The output
302 .fig files for all simulations referenced will be made available at the group's Research Data
303 Repository (data-bris) at the University of Bristol.

304

305 **Model Availability**

306 The *M. genitalium* whole-cell model is freely available: github.com/CovertLab/WholeCell. The
307 model requires a single CPU and can be run with 8GB of RAM. We run the *M. genitalium*
308 whole-cell model on Bristol's supercomputers using MATLAB R2013b, with the model's
309 standard settings. However, we use our own version of the SimulationRunner.m.
310 MGGRunner.m
311 ([github.com/GriersonMarucciLab/Analysis_Code_for_Mycoplasma_genitalium_whole-cell_mod](https://github.com/GriersonMarucciLab/Analysis_Code_for_Mycoplasma_genitalium_whole-cell_model)
312 el) is designed for use with supercomputers that start hundreds of simulations simultaneously. It
313 artificially increments the starting time-date value for each simulation, as this value is
314 subsequently used to create the initial conditions of the simulation. Our research copy of the
315 whole-cell model was downloaded 10th January 2017.

316

317 ***M. genitalium in-silico* Environmental Conditions**

318 *M. genitalium* is grown *in-vivo* on SP4 media. The *in-silico* media composition is based on the
319 experimentally characterized composition, with additional essential molecules added
320 (nucleobases, gases, polyamines, vitamins, and ions) in reported amounts to support *in-silico*
321 cellular growth. Additionally, the *M. genitalium* whole-cell model represents 10 external stimuli
322 including temperature, several types of radiation, and three stress conditions. For more
323 information see Karr *et al.* Supplementary Tables S3F, S3H, S3R (Karr et al., 2012).

324

325 **Equipment**

326 For the *M. genitalium* whole-cell model we used the University of Bristol Advanced Computing
327 Research Centres's BlueGem, a 900-core supercomputer, which uses the Slurm queuing
328 system, to run whole-cell model simulations.

329 We used a standard office desktop computer, with 8GB of ram, to write new code, and interact
330 with the supercomputer. We used the following GUI software on Windows 7: Notepad++ for
331 code editing, Putty (ssh software) for terminal access to the supercomputer, FileZilla (ftp
332 software) to move files in bulk to and from the supercomputer, and PyCharm (IDE software) as
333 an inbuilt desktop terminal and for python debugging. The command line software used
334 included: VIM for code editing, and SSH, Rsync, and Bash for communication and file transfer
335 with the supercomputers.

336

337 **Data Format**

338 For the *M. genitalium* whole-cell model the majority of output files are state-NNN.mat files,
339 which are logs of the simulation split into 100-second segments. The data within a
340 state-NNN.mat file is organised into the 16 cellular variables. These are typically arranged as
341 3-dimensional matrices or time series, which are flattened to conduct analysis. The other file
342 types contain summaries of data spanning the simulation. Each gene manipulated simulation
343 can consist of up to 500 files requiring between 0.4GB and 0.9GB. Each simulation takes 5 to
344 12 hours to complete in real time, 7 - 13.89 hours in simulated time.

345

346 **Data Analysis Process**

347 For the *M. genitalium* whole-cell model, the raw data is automatically processed as the
348 simulation ends. runGraphs.m carries out the initial analysis, while compareGraphs.m overlays
349 the output on collated graphs of 200 unmodified *M. genitalium* simulations. Both outputs are
350 saved as MATLAB .fig and .pdfs, though the .pdf files were the sole files analysed. The raw
351 .mat files were stored in case of further investigation.

352

353 The GO biological process terms used for further analysis were downloaded from Uniprot
 354 (Apweiler et al., 2004) (strain ATCC 33530/NCTC 10195), processed by a created script
 355 (github.com/squishybinary/Gene_Ontology_Comparison_for_Mycoplasma_genitalium_whole-ce
 356 ll_model) in combination with lists of genes, organised manually into tables of GO terms that
 357 were unaffected, reduced, or removed entirely by gene deletions, and then analysed.

358

359 **Modelling Scripts**

360 There are six scripts used to run the *M. genitalium* whole-cell model. Three are the experimental
 361 files created with each new experiment (the bash script, gene list, experiment list), and three are
 362 stored within the whole-cell model and are updated only upon improvement (MGGrunner.m,
 363 runGraphs.m, and compareGraphs.m). The bash script is a list of commands for the
 364 supercomputer(s) to carry out. Each bash script determines how many simulations to run,
 365 where to store the output, and where to store the results of the analysis. The gene list is a text
 366 file containing rows of gene codes (in the format 'MG_XXX',). Each row corresponds to a single
 367 simulation and determines which genes that simulation should knockout. The experiment list is
 368 a text file containing rows of simulation names. Each row corresponds to a single simulation
 369 and determines the final location of the simulation output and analysis results.

370

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375

376 **Competing Interests**

377 The authors declare no competing interests.

378

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388 **Author Contributions**

389 C.G., L.M., O.C., J.R-G were involved in ideation. O.C. conducted simulations comparing the
390 Stanford and Rockville 2 sets, a prototype version of the research presented here. J.R-G.
391 collated, simulated, analysed and repaired the nine sets, and wrote the paper and
392 supplementary data 1 - 16, 26 - 29. J.R. analysed the nine sets and wrote supplementary data
393 17 - 25. C.G. and L.M supervised the project. C.G., L.M., J.R. edited the paper.

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395 References

- 396 Apweiler, R., Bairoch, A., Wu, C. H., Barker, W. C., Boeckmann, B., Ferro, S., Gasteiger, E.,
397 Huang, H., Lopez, R., Magrane, M., Martin, M. J., Natale, D. A., O'Donovan, C., Redaschi,
398 N., & Yeh, L.-S. L. (2004). UniProt: the Universal Protein knowledgebase. *Nucleic Acids*
399 *Research*, 32(Database issue), D115–D119.
- 400 Benders, G. A., Noskov, V. N., Denisova, E. A., Lartigue, C., Gibson, D. G., Assad-Garcia, N.,
401 Chuang, R. Y., Carrera, W., Moodie, M., Algire, M. A., Phan, Q., Alperovich, N., Vashee, S.,
402 Merryman, C., Venter, J. C., Smith, H. O., Glass, J. I., & Hutchison, C. A. (2010). Cloning
403 whole bacterial genomes in yeast. *Nucleic Acids Research*, 38(8), 2558–2569.
- 404 du Plessis, D. J. F., Nouwen, N., & Driessen, A. J. M. (2011). The Sec translocase. *Biochimica*
405 *et Biophysica Acta*, 1808(3), 851–865.
- 406 Dzieciol, A. J., & Mann, S. (2012). Designs for life: protocell models in the laboratory. *Chemical*
407 *Society Reviews*, 41(1), 79–85.
- 408 Fleischmann, R. D., Adams, M. D., White, O., Clayton, R. A., Kirkness, E. F., Kerlavage, A. R.,
409 Bult, C. J., Tomb, J. F., Dougherty, B. A., & Merrick, J. M. (1995). Whole-genome random
410 sequencing and assembly of *Haemophilus influenzae* Rd. *Science*, 269(5223), 496–512.
- 411 Forster, A. C., & Church, G. M. (2006). Towards synthesis of a minimal cell. *Molecular Systems*
412 *Biology*, 2. <https://doi.org/10.1038/msb4100090>
- 413 Fraser, C. M., Gocayne, J. D., White, O., Adams, M. D., Clayton, R. A., Fleischmann, R. D.,
414 Bult, C. J., Kerlavage, A. R., Sutton, G., Kelley, J. M., Fritchman, J. L., Weidman, J. F.,
415 Small, K. V., Sandusky, M., Fuhrmann, J., Nguyen, D., Utterback, T. R., Saudek, D. M.,
416 Phillips, C. A., ... Venter, J. C. (1995). The Minimal Gene Complement of
417 *Mycoplasma-genitalium*. *Science*, 270(5235), 397–403.
- 418 Fredens, J., Wang, K., de la Torre, D., Funke, L. F. H., Robertson, W. E., Christova, Y., Chia, T.,
419 Schmied, W. H., Dunkelmann, D. L., Beránek, V., Uttamapinant, C., Llamazares, A. G.,

420 Elliott, T. S., & Chin, J. W. (2019). Total synthesis of *Escherichia coli* with a recoded
421 genome. *Nature*, 569(7757), 514–518.

422 Gibson, D. G., Benders, G. A., Andrews-Pfannkoch, C., Denisova, E. A., Baden-Tillson, H.,
423 Zaveri, J., Stockwell, T. B., Brownley, A., Thomas, D. W., Algire, M. A., Merryman, C.,
424 Young, L., Noskov, V. N., Glass, J. I., Venter, J. C., Hutchison, C. A., & Smith, H. O. (2008).
425 Complete chemical synthesis, assembly, and cloning of a *Mycoplasma genitalium* genome.
426 *Science*, 319(5867), 1215–1220.

427 Gibson, D. G., Benders, G. A., Axelrod, K. C., Zaveri, J., Algire, M. A., Moodie, M., Montague,
428 M. G., Venter, J. C., Smith, H. O., & Hutchison, C. A. (2008). One-step assembly in yeast
429 of 25 overlapping DNA fragments to form a complete synthetic *Mycoplasma genitalium*
430 genome. *Proceedings of the National Academy of Sciences of the United States of*
431 *America*, 105(51), 20404–20409.

432 Gibson, D. G., Glass, J. I., Lartigue, C., Noskov, V. N., Chuang, R. Y., Algire, M. A., Benders, G.
433 A., Montague, M. G., Ma, L., Moodie, M. M., Merryman, C., Vashee, S., Krishnakumar, R.,
434 Assad-Garcia, N., Andrews-Pfannkoch, C., Denisova, E. A., Young, L., Qi, Z. Q.,
435 Segall-Shapiro, T. H., ... Venter, J. C. (2010). Creation of a Bacterial Cell Controlled by a
436 Chemically Synthesized Genome. *Science*, 329(5987), 52–56.

437 Gil, R. (2015). The Minimal Gene-Set Machinery. In R. A. Meyers (Ed.), *Synthetic Biology* (pp.
438 443–478). John Wiley & Sons.

439 Gil, R., Silva, F. J., Pereto, J., & Moya, A. (2004). Determination of the core of a minimal
440 bacterial gene set. *Microbiology and Molecular Biology Reviews: MMBR*, 68(3), 518 – +.

441 Glass, J. I., Assad-Garcia, N., Alperovich, N., Yooseph, S., Lewis, M. R., Maruf, M., Hutchison,
442 C. A., Smith, H. O., & Venter, J. C. (2006). Essential genes of a minimal bacterium.
443 *Proceedings of the National Academy of Sciences of the United States of America*, 103(2),
444 425–430.

445 Glass, J. I., Merryman, C., Wise, K. S., Hutchison, C. A., 3rd, & Smith, H. O. (2017). Minimal
 446 Cells-Real and Imagined. *Cold Spring Harbor Perspectives in Biology*.
 447 <https://doi.org/10.1101/cshperspect.a023861>

448 Huang, C. H., Hsiang, T., & Trevors, J. T. (2013). Comparative bacterial genomics: defining the
 449 minimal core genome. *Antonie Van Leeuwenhoek International Journal of General and*
 450 *Molecular Microbiology*, 103(2), 385–398.

451 Hutchison, C. A., Chuang, R. Y., Noskov, V. N., Assad-Garcia, N., Deerinck, T. J., Ellisman, M.
 452 H., Gill, J., Kannan, K., Karas, B. J., Ma, L., Pelletier, J. F., Qi, Z. Q., Richter, R. A.,
 453 Strychalski, E. A., Sun, L. J., Suzuki, Y., Tsvetanova, B., Wise, K. S., Smith, H. O., ...
 454 Venter, J. C. (2016). Design and synthesis of a minimal bacterial genome. *Science*,
 455 351(6280), 1414–U73.

456 Hutchison, C. A., Peterson, S. N., Gill, S. R., Cline, R. T., White, O., Fraser, C. M., Smith, H. O.,
 457 & Venter, J. C. (1999). Global transposon mutagenesis and a minimal mycoplasma
 458 genome. *Science*, 286(5447), 2165–2169.

459 Jiang, W. Y., Bikard, D., Cox, D., Zhang, F., & Marraffini, L. A. (2013). RNA-guided editing of
 460 bacterial genomes using CRISPR-Cas systems. *Nature Biotechnology*, 31(3), 233–239.

461 Juhas, M., Eberl, L., & Glass, J. I. (2011). Essence of life: essential genes of minimal genomes.
 462 *Trends in Cell Biology*, 21(10), 562–568.

463 Kanehisa, M., & Goto, S. (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic*
 464 *Acids Research*, 28(1), 27–30.

465 Karas, B. J., Jablanovic, J., Sun, L. J., Ma, L., Goldgof, G. M., Stam, J., Ramon, A., Manary,
 466 M. J., Winzeler, E. A., Venter, J. C., Weyman, P. D., Gibson, D. G., Glass, J. I., Hutchison,
 467 C. A., Smith, H. O., & Suzuki, Y. (2013). Direct transfer of whole genomes from bacteria to
 468 yeast. *Nature Methods*, 10(5), 410 – +.

469 Karr, J. R., Sanghvi, J. C., Macklin, D. N., Gutschow, M. V., Jacobs, J. M., Bolival, B., Jr,

470 Assad-Garcia, N., Glass, J. I., & Covert, M. W. (2012). A whole-cell computational model
471 predicts phenotype from genotype. *Cell*, 150(2), 389–401.

472 Kazakiewicz, D., Karr, J. R., Langner, K. M., & Plewczynski, D. (2015). A combined systems
473 and structural modeling approach repositions antibiotics for *Mycoplasma genitalium*.
474 *Computational Biology and Chemistry*, 59 Pt B, 91–97.

475 Koonin, E. V. (2003). Comparative genomics, minimal gene-sets and the last universal common
476 ancestor. *Nature Reviews. Microbiology*, 1(2), 127–136.

477 Lagesen, K., Ussery, D. W., & Wassenaar, T. M. (2010). Genome update: the 1000th genome -
478 a cautionary tale. *Microbiology-Sgm*, 156, 603–608.

479 Macklin, D. N., Ahn-Horst, T. A., Choi, H., Ruggero, N. A., Carrera, J., Mason, J. C., Sun, G.,
480 Agmon, E., DeFelice, M. M., Maayan, I., Lane, K., Spangler, R. K., Gillies, T. E., Paull, M.
481 L., Akhter, S., Bray, S. R., Weaver, D. S., Keseler, I. M., Karp, P. D., ... Covert, M. W.
482 (2020). Simultaneous cross-evaluation of heterogeneous *E. coli* datasets via mechanistic
483 simulation. *Science*, 369(6502). <https://doi.org/10.1126/science.aav3751>

484 Mushegian, A. R., & Koonin, E. V. (1996). A minimal gene set for cellular life derived by
485 comparison of complete bacterial genomes. *Proceedings of the National Academy of*
486 *Sciences of the United States of America*, 93(19), 10268–10273.

487 Purcell, O., Jain, B., Karr, J. R., Covert, M. W., & Lu, T. K. (2013). Towards a whole-cell
488 modeling approach for synthetic biology. *Chaos*, 23(2), 025112.

489 Rancati, G., Moffat, J., Typas, A., & Pavelka, N. (2018). Emerging and evolving concepts in
490 gene essentiality. *Nature Reviews. Genetics*, 19(1), 34–49.

491 Rees-Garbutt, J., Chalkley, O., Landon, S., Purcell, O., Marucci, L., & Grierson, C. (2020).
492 Designing minimal genomes using whole-cell models. *Nature Communications*, 11(1), 836.

493 Reich, K. A. (2000). The search for essential genes. *Research in Microbiology*, 151(5), 319–324.

494 Sanghvi, J. C., Regot, S., Carrasco, S., Karr, J. R., Gutschow, M. V., Bolival, B., Jr, & Covert,

495 M. W. (2013). Accelerated discovery via a whole-cell model. *Nature Methods*, 10(12),
496 1192–1195.

497 Shuler, M. L., Foley, P., & Atlas, J. (2012). Modeling a Minimal Cell. In A. Navid (Ed.), *Microbial*
498 *Systems Biology* (Vol. 881, pp. 573–610). Humana Press.

499 Tomita, M., Hashimoto, K., Takahashi, K., Shimizu, T. S., Matsuzaki, Y., Miyoshi, F., Saito, K.,
500 Tanida, S., Yugi, K., Venter, J. C., & Hutchison, C. A. (1999). E-CELL: software
501 environment for whole-cell simulation. *Bioinformatics* , 15(1), 72–84.

502 Tsarnopoulos, I., Gourgues, G., Blanchard, A., Vashee, S., Jores, J., Lartigue, C., &
503 Sirand-Pugnet, P. (2016). In-Yeast Engineering of a Bacterial Genome Using CRISPR/Cas9.
504 *ACS Synthetic Biology*, 5(1), 104–109.

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