

## Supplementary Methods

### Reduced B12 uptake and increased gastrointestinal formate drive archaeome-mediated breath methane emission in humans

**Short title: B12 shortage, fibre and formate drive breath methane emission**

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#### Key Resources Table

| <u>REAGENT or RESOURCE</u>                       | <u>SOURCE</u>                 | <u>IDENTIFIER</u>     |
|--------------------------------------------------|-------------------------------|-----------------------|
| <u>Dietary information</u>                       |                               |                       |
| German Food Frequency Questionnaire (FFG)        | [1]                           | Robert Koch Institute |
| <u>Methane measurement</u>                       |                               |                       |
| GastroCH <sub>4</sub> ECK breath bags            | Bedfont Scientific Ltd, UK    | #GASTROCHECK-BAG-XL   |
| GastroCH <sub>4</sub> ECK Gastrolyzer            | Bedfont Scientific Ltd, UK    | NA.                   |
| <u>Commercial Kit</u>                            |                               |                       |
| PowerSoil® DNA Isolation Kit                     | QIAGEN, USA                   | #12888-100            |
| <u>PMA treatment</u>                             |                               |                       |
| PMA solution (20 mM)                             | VWR                           | #40019                |
| PMA-Lite™ LED Photolysis Device                  | Biotum                        | NA                    |
| <u>Cell disruption and DNA conc. measurement</u> |                               |                       |
| MagNaLyser                                       | MagNaLyser                    | NA                    |
| Qubit fluorometer 2.0                            | Thermo Fisher Scientific, USA | NA                    |
| Qubit dsDNA HS Assay Kit                         | Thermo Fisher Scientific, USA | #Q32854               |

|                                                                                                                         |                                 |                                       |
|-------------------------------------------------------------------------------------------------------------------------|---------------------------------|---------------------------------------|
| <b><u>PCR Reagents</u></b>                                                                                              |                                 |                                       |
| TAKARA Ex Taq® buffer with MgCl <sub>2</sub> (10x)                                                                      | Takara Bio Inc.                 | #RR001A                               |
| dNTP mix (200 µM)                                                                                                       | Takara Bio Inc.                 | #RR001A                               |
| TAKARA Ex Taq® Polymerase (0.5 U)                                                                                       | Takara Bio Inc.                 | #RR001A                               |
| PCR grade water                                                                                                         | Jena Bioscience, Germany        | #PCR-258S                             |
| <b><u>Oligonucleotides</u></b>                                                                                          |                                 |                                       |
| Universal forward primer: Illu-515FB<br>TCGTCGGCAGCGTCAGATGTGTATAAG<br>AGACAGGTG<br><b>YCAGCMGCCGCGGTAA</b>             | [2]                             | Eurofins Genomics AT GmbH,<br>Austria |
| Universal reverse primer: Illu-806RB<br>GTCTCGTGGGCTCGGAGATGTGTATAA<br>GAGACAGGG<br><b>ACTACNVGGGTWTCTAAT</b>           | [2]                             | Eurofins Genomics AT GmbH,<br>Austria |
| Archaeal forward primer (nested<br>PCR1): 344F<br>ACGGGGYGCAGCAGGCGCGA                                                  | [2]                             | Eurofins Genomics AT GmbH,<br>Austria |
| Archaeal reverse primer (nested<br>PCR1): 1041R<br>ACGGGGYGCAGCAGGCGCGA                                                 | [2]                             | Eurofins Genomics AT GmbH,<br>Austria |
| Archaeal forward primer (nested<br>PCR2): 519F<br>TCGTCGGCAGCGTCAGATGTGTATAAG<br>AGACAGCA<br><b>GCMGCCGCGGTAA</b>       | [2]                             | Eurofins Genomics AT GmbH,<br>Austria |
| Archaeal reverse primer (nested<br>PCR2): 806R<br>GTCTCGTGGGCTCGGAGATGTGTATAA<br>GAGACAGGG<br><b>ACTACVSGGGTATCTAAT</b> | [2]                             | Eurofins Genomics AT GmbH,<br>Austria |
| Bacterial primer (qPCR): 331F<br>TCCTACGGGAGGCAGCAGT                                                                    | [3]                             | Eurofins Genomics AT GmbH,<br>Austria |
| Bacterial primer (qPCR): 797R<br>GGACTACCAGGGTATCTAATCCTGTT                                                             | [3]                             | Eurofins Genomics AT GmbH,<br>Austria |
| mcrA primer (qPCR): M1F<br>GCAATGCAAATTGGTATGTC                                                                         | Modified from this paper<br>[4] | Eurofins Genomics AT GmbH,<br>Austria |
| mcrA primer (qPCR): M1R<br>TCATTGCGTAGTTAGGRTAGT                                                                        | Modified from this paper<br>[4] | Eurofins Genomics AT GmbH,<br>Austria |

|                                                  |                                      |                                                                                                                                         |
|--------------------------------------------------|--------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| <b><u>Illumina sequencing</u></b>                |                                      |                                                                                                                                         |
| Illumina MiSeq sequencing platform               | Illumina, Eindhoven, the Netherlands | Core Facility for Molecular Biology (Center for Medical Research, Graz)<br>[5]                                                          |
| Nextera XT Library construction kit              | Illumina, Eindhoven, the Netherlands | Macrogen (Seoul, South Korea)                                                                                                           |
| Illumina HiSeq technique                         | Illumina, Eindhoven, the Netherlands | Macrogen (Seoul, South Korea)                                                                                                           |
| <b><u>Software and Algorithms</u></b>            |                                      |                                                                                                                                         |
| QIIME2 version 2020.6                            | [6]                                  | <a href="https://qiime2.org">https://qiime2.org</a>                                                                                     |
| SILVA version 128-132                            | [7]                                  |                                                                                                                                         |
| R version 3.5.1<br>R Studio version 1.2.1335     | [8]                                  | <a href="https://www.r-project.org">https://www.r-project.org</a>                                                                       |
| decontam version 1.1.0                           | [9]                                  | <a href="https://github.com/benjineb/decontam">https://github.com/benjineb/decontam</a>                                                 |
| vegan version 2.5-5                              | [10]                                 |                                                                                                                                         |
| Calypso                                          | [11]                                 | <a href="http://cgenome.net:8080/calypso-8.84/faces/uploadFiles.xhtml">http://cgenome.net:8080/calypso-8.84/faces/uploadFiles.xhtml</a> |
| Galaxy set-up                                    | [12]                                 | provided by the Core Facility Computational Biology at the Medical University of Graz                                                   |
| LEfSe                                            | [13]                                 | <a href="http://huttenhower.sph.harvard.edu/galaxy/">http://huttenhower.sph.harvard.edu/galaxy/</a>                                     |
| Krona chart                                      | [14]                                 | Krona chart excel template                                                                                                              |
| nut.s - nutritional software version 1.32.79     | [15]                                 | <a href="http://www.nutritional-software.at">www.nutritional-software.at</a>                                                            |
| MG-Rast platform (server running version 4.0.3.) | [16]                                 | <a href="https://www.mg-rast.org/">https://www.mg-rast.org/</a>                                                                         |
| IBM SPSS Amos version 26                         | IBM                                  | <a href="http://www.ibm.com/analytics/us/en/technology/spss/">http://www.ibm.com/analytics/us/en/technology/spss/</a>                   |
| fastqc v0.11.8                                   | [17]                                 | <a href="http://www.bioinformatics.brahaam.ac.uk/projects/fastqc/">http://www.bioinformatics.brahaam.ac.uk/projects/fastqc/</a>         |
| trimmomatic v0.38                                | [18]                                 | <a href="http://www.usadellab.org/c">http://www.usadellab.org/c</a>                                                                     |

|                                                                      |                                   |                                                                                                                                                                         |
|----------------------------------------------------------------------|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                      |                                   | ms/?page=trimmomatic                                                                                                                                                    |
| bowtie2 v2.3.5                                                       | [19]                              | <a href="http://bowtie-bio.sourceforge.net/bowtie2/index.shtml">http://bowtie-bio.sourceforge.net/bowtie2/index.shtml</a>                                               |
| samtools v1.9                                                        | [20]                              | <a href="http://www.htslib.org/">http://www.htslib.org/</a>                                                                                                             |
| bedtools v2.29.0                                                     | [21]                              | <a href="https://bedtools.readthedocs.io/en/latest/">https://bedtools.readthedocs.io/en/latest/</a>                                                                     |
| diamond v0.9.25                                                      | [22]                              | <a href="http://www.diamondsearch.org/index.php">http://www.diamondsearch.org/index.php</a>                                                                             |
| MEGAN v6.20.13                                                       | [23]                              | <a href="https://software-ab.informatik.uni-tuebingen.de/download/megan6/welcome.html">https://software-ab.informatik.uni-tuebingen.de/download/megan6/welcome.html</a> |
| Megahit v1.1.3                                                       | [24]                              | <a href="https://github.com/voutcn/megahit">https://github.com/voutcn/megahit</a>                                                                                       |
| MaxBin v2.2.4                                                        | [25]                              | <a href="https://sourceforge.net/projects/maxbin2/">https://sourceforge.net/projects/maxbin2/</a>                                                                       |
| dRep v2.0.5                                                          | [26]                              | <a href="https://github.com/MrOlm/drep">https://github.com/MrOlm/drep</a>                                                                                               |
| GTDBtk v1.2.0                                                        | [27]                              | <a href="https://github.com/Ecogenomics/GTDBtk">https://github.com/Ecogenomics/GTDBtk</a>                                                                               |
| MaGe                                                                 | [28]                              | <a href="https://mage.genoscope.cns.fr/microscope/home/index.php">https://mage.genoscope.cns.fr/microscope/home/index.php</a>                                           |
| iRep v1.1.9                                                          | [29]                              | <a href="https://github.com/christopherbrown/iRep">https://github.com/christopherbrown/iRep</a>                                                                         |
| q2-sample-classifier-plugin                                          | [30]                              | <a href="https://docs.qiime2.org/2020.11/tutorials/sample-classifier/">https://docs.qiime2.org/2020.11/tutorials/sample-classifier/</a>                                 |
| q2-micom plugin v.0.8.0                                              | [31]                              | <a href="https://github.com/micom-dev/q2-micom">https://github.com/micom-dev/q2-micom</a>                                                                               |
| AGORA genus model database v1.03                                     | [32]                              | <a href="https://www.vmh.life/#downloadview">https://www.vmh.life/#downloadview</a>                                                                                     |
| <b><u>Deposited Data</u></b>                                         |                                   |                                                                                                                                                                         |
| Raw data (amplicon, metagenome)                                      | European Nucleotide Archive (ENA) | PRJEB41867                                                                                                                                                              |
| Supplementary Dataset (Sequencing data after decontam and removal of | Mendeley data [33]                | <a href="http://dx.doi.org/10.17632/hjj3tx7n84.1">http://dx.doi.org/10.17632/hjj3tx7n84.1</a>                                                                           |

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|---------------------------------------------------------------------------------|--|--|
| features with zero and one reads)<br>Supplementary Figures, Tables and<br>Items |  |  |
|---------------------------------------------------------------------------------|--|--|

### PCR conditions (amplicon):

|                             | universal   | Archaea        |               |
|-----------------------------|-------------|----------------|---------------|
|                             |             | (nested PCR 1) | (nested PCR2) |
| <b>Initial denaturation</b> | 94°C, 3′    | 95°C, 5′       | 95°C, 5′      |
| <b>Denaturation</b>         | 94°C, 45″   | 94°C, 30″      | 95°C, 40″     |
| <b>Annealing</b>            | 50°C, 60″   | 56°C, 45″      | 63°C, 2′      |
| <b>Elongation</b>           | 72°C, 1′30″ | 72°C, 1′       | 72°C, 1′      |
| <b>Final elongation</b>     | 72°C, 10′   | 72°C, 10′      | 72°C, 10′     |
| <b>No. of cycles</b>        | <b>35</b>   | <b>25</b>      | <b>30</b>     |

### PCR reagents and conditions (qPCR):

| component                 | final conc.     |
|---------------------------|-----------------|
| SsoAdvance Universal SYBR | 1X              |
| Forward primer A806F      | 300 nM          |
| Reverse primer A958R      | 300 nM          |
| Template - archaea        | 1µl             |
| Water                     | fill up to 10µl |
| Final Volume: 10µl        |                 |

|                             | bacteria       | methanogens    |
|-----------------------------|----------------|----------------|
| <b>Initial denaturation</b> | 95°C, 15′      | 94°C, 3′       |
| <b>Denaturation</b>         | 94°C, 15″      | 94°C, 45″      |
| <b>Annealing</b>            | 54°C, 30″      | 56°C, 45″      |
| <b>Elongation</b>           | 72°C, 40″      | 72°C, 30′      |
| <b>No. of cycles</b>        | <b>40</b>      | <b>40</b>      |
| <b>Melting curve</b>        | <b>60-95°C</b> | <b>60-95°C</b> |

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