Supplementary Figure S2: Overview of libary construction and sequencing protocols

Bacterial 16S rDNA

Target sequence ca. 400 bp
incl. V3/V4 regions

5'-NNNNNNNNNNNNNNNNNN-CCTACGGRAGGCAGCAG-XXXXXXXX-ATTAGATACCCYGGTAGTCC-NNNNNNNNNNNNNNNNNN-3'

5'-NNNNNNNNNNNNNNNNNN-GGACTACCRGGGTATCTAAT-XXXXXXXX-CTGCTGCCTYCCGTAGG-NNNNNNNNNNNNNNNNNN-3'

341F 806R

Conserved annealing sites

Library construction (PCR)

5'-NNNNNNNNNNNNNNNNNN-CCTACGGRAGGCAGCAG-XXXXXXXX-ATTAGATACCCYGGTAGTCC-NNNNNNNNNNNNNNNNNN-3'

Illumina adapter P5bis

5'-AATGATACGGCGACCACCGAGATCTACAC

linker

CTATGGTAATTCGT

annealing site

CCTACGGRAGGCAGCAG-->

5'-NNNNNNNNNNNNNNNNNN-GGACTACCRGGGTATCTAAT-XXXXXXXX-CTGCTGCCTYCCGTAGG-NNNNNNNNNNNNNNNNNN-3'

<--GGACTACCRGGGTATCTAAT

annealing site

GATNNNNNNNNNAGTCAGTCAGCC

index and linker

Illumina adapter P7

5'-CAAGCAGAAGACGGCATACGA

Final library

(P5bis)-341FcompV3

5'-AATGATACGGCGACCACCGAGATCTACACCTATGGTAATTCGTCCTACGGRAGGCAGCAG-3'..XXXXXX..5'-ATTAGATACCCYGGTAGTCCGGCTGACTGACTNNNNNNNNATCTCGTATGCCGTCTTCTGCTTG-3'

RC_806RcompNNN

(RC_P7)

5'-CAAGCAGAAGACGGCATACGAGATNNNNNNNNAGTCAGTCAGCCGGACTACCRGGGTATCTAAT-3'..XXXXXX..5'-CTGCTGCCTYCCGTAGGACGAATTACCATAGGTGTAGATCTCGGTGGTCGCCGTATCATT-3'

(RC_P5bis)

RC_341FcompV3

(P7)-806RcompNNN

Forward (RD1) Sequencing

341FSeqV3

5'-CTATGGTAATTCGTCCTACGGRAGGCAGCAG-3'-->

FLOW CELL

5'-CAAGCAGAAGACGGCATACGAGATNNNNNNNNAGTCAGTCAGCCGGACTACCRGGGTATCTAAT-3'..XXXXXX..5'-CTGCTGCCTYCCGTAGGACGAATTACCATAGGTGTAGATCTCGGTGGTCGCCGTATCATT-3'

(P7)

(RC_P5bis)

RC_341FcompV2

Index Sequencing

Index_seq_V3

5'ATTAGATACCCYGGTAGTCCGGCTGACTGACT-3'-->

FLOW CELL

5'-CAAGCAGAAGACGGCATACGAGATNNNNNNNNAGTCAGTCAGCCGGACTACCRGGGTATCTAAT-3'..XXXXXX..5'-CTGCTGCCTYCCGTAGGACGAATTACCATAGGTGTAGATCTCGGTGGTCGCCGTATCATT-3'

(P7)

Reverse (RD2) Sequencing

(P5bis)

5'-AATGATACGGCGACCACCGAGATCTACACCTATGGTAATTCGTCCTACGGRAGGCAGCAG-3'..XXXXXX..5'-ATTAGATACCCYGGTAGTCCGGCTGACTGACTNNNNNNNNATCTCGTATGCCGTCTTCTGCTTG-3'

FLOW CELL

<--5'-AGTCAGTCAGCCGGACTACCRGGGTATCTAAT-3'

806RseqV3