**16S primers for amplicon generation and sequencing**

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| **LinkerPrimerSequence**  **(QW: HMP1 454 reverse)** | **ReversePrimer**  **(QW: HMP1 454 forward)** | **Region** |
| ATTACCGCGGCTGCTGG | AGAGTTTGATYMTGGCTCAG | V1V3 (27F & 534R) |
| CCGTCAATTCMTTTRAGT | CCTACGGGAGGCAGCAG | V3V5 (341F & 926R) |
| TACGGYTACCTTGTTAYGACTT | AACGCGAAGAACCTTAC | V6V9 (968F & 1492R) |
| **LinkerPrimerSequence**  **(QW: Illumina forward)** | **ReversePrimer**  **(QW: Illumina reverse)** | **Region** |
| GTGCCAGCMGCCGCGGTAA | GGACTACHVGGGTWTCTAAT | V4 (515F & 806R) |

**16S rRNA gene template (Escherichia coli str. K-12 substr. MG1655)**

**Turquoise – V1V3 primers; Red – V3V5 primers; Wave underline – V4 primers; Pink – V6V9 primers)**

AAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGTAACAGGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAGCTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAATGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTTGTTGGTGGGGTAACGGCTCACCAAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAGTCGTAACAAGGTAACCGTAGGGGAACCTGCGGTTGGATCACCTCCTTA

**Observation note:** For public available 16S databases such as Greengenes, SILVA, RDP etc., one important source for building those DBs is from Bacterial 16S Ribosomal RNA RefSeq Targeted Loci Project (PRJNA33175); however, those 16S rRNA RefSeq sequences are deposited by world-wide researchers, who used universal primers (27F and 1492R) often for generating almost full-length of 16S, but they may or may not remove primer sequences before depositing to NCBI. Thus, using primer sequences of 27F and 1492R for looking at the 16S V1 and V9 regions will become challenge. The program Cutadapt may also have difficulty to look for V1V3 and V6V9 regions.