Blunt-end ligation with PCR addition of indexes

NTUni_p5 and NTUni_p7 (these are the Nextera transposes sequences, so their matching sequencing primers will be in the Illumina sequencing kits):

TCGTCGGCACGGTCAGATGTGTATAAGAGACAG

CAGAGCACCCCAGCCCTACACATATTCTCTGTC

(so this can't be an A-tailed - the extra "A" isn't in the sequencing primer and would cause base diversity issues during sequencing)

Now add the metagenomics primers (Hyb_F01_i5 with "ATCACG" and Hyb_R21_i7 with "CGAAAC" (rev comp) in this example) which get incorporated AFTER CYCLE 1:

Cycle 1 - only Hyb_R21_i7 hybridizes and extends to form the reverse compliment of the top strand:

TCGTCGGCACGGTCAGATGTGTATAAGAGACAG

CTGTCTCTTATACACATCTCCGAGCCCACGAGAC

CAGAGCACCCCAGCCCTACACATATTCTCTGTC

GACAGAAGATATGTGTAGACTCGACGCTGGGCTGCT

GGCTCGGGTGCTCTG CAAAGC TAGAGCATACGGCAGAAGACGAAC <- reverse primer binds in cycle 1

Cycle 2 - the newly-formed top strand now has the compliment to the forward primer which can now prime:

AATGATACGGCCGACCACCGAGATCTACAC ATCACG TCGTCGGCACGGTCAGATGTGTATAAGAGACAG

TCGTCGGCACGGTCAGATGTGTATAAGAGACAG

CTGTCTCTTATACACATCTCCGAGCCCACGAGAC

CAGAGCACCCCAGCCCTACACATATTCTCTGTC

GACAGAAGATATGTGTAGACTCGACGCTGGGCTGCT

GGCTCGGGTGCTCTG CAAAGC TAGAGCATACGGCAGAAGACGAAC <- made in cycle 1

this is all happening upside-down to the other strand in cycle 1