

FIGURE 2

Flow Chart of bisulfite-conversion and subsequent PCR amplification

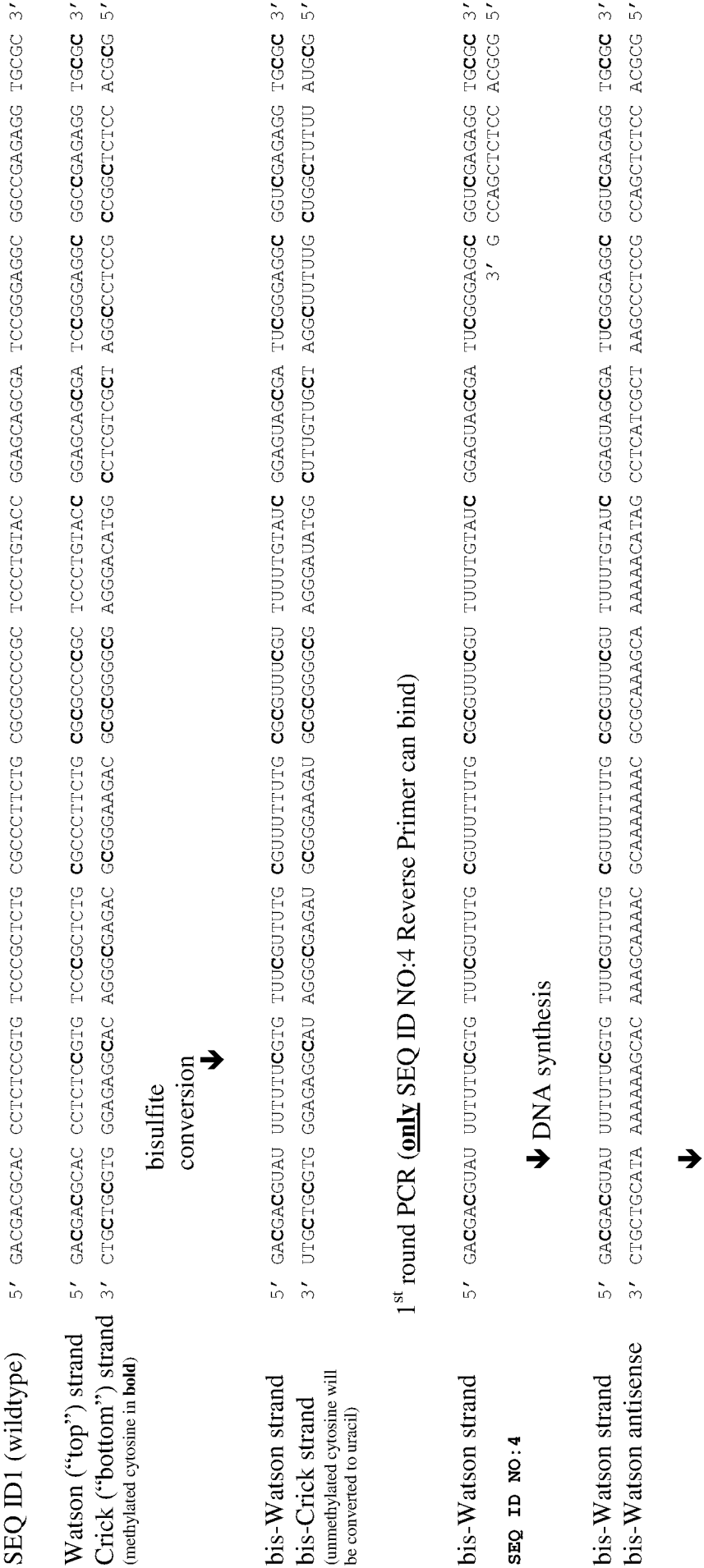


FIGURE 2 (continued)

2nd round PCR (SEQ ID3, 4, and 5-12 can bind depending on methylation status)

5' GACGAGGUAU UUTUTUCGTG TUUCGUTTG CGGUUTTGTG **CGCGUUUCGU** TUUUTCTAUC GGAGUAGCGA TUCGGAGGC GGUCAGAGG TGCGC 3'
3' G CCAGCTCTCC ACGCG 5'

SEQ ID NO: 4

5' GACGACGTAT TTTTTCGTG TTTC 3' **SEQ ID NO:3**
 3' CTGCTGCATA AAAAAAGCAC AAAGCAAAAC GCAAAAAAAC GCACAAATAG CCTCATCGT AAGCCCTCCG CCAGCTCTCC ACGCG 5'
 bis-Watson antisense

↓ DNA synthesis results in double-stranded DNA and fluorescence from degraded probe (eg SEQ ID NO:5)
The resulting top strand (bis-Watson sense strand) becomes SEQ ID NO:2.

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bis-Watson sense
 5' GACGACGAT TTTTTCGTG TTTCGTTTG CGTTTTTTG CGCGTTTCGT TTTTGTATC GGAGTAGCGA TTCCGGAGGC GTTCGAGAGG TGCGC 3'
 SEQ ID NO:2
 bis-Watson antisense
 3' CTGCTGCATA AAAAAAGCAC AAAGCAAAAC GCAAAAAAAC GCGCAAAAGCA AAAAACATAG CCTCATCGCT AAGCCCTCCG CCAGCTCTCC ACCGG 5'

Further rounds of PCR **↓** (SEQ ID NOs 3, 4, and 5-12 can bind depending on methylation status)