

N5HCO0 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCT TAACTTCAGGGTGACCAAAAAATCA
N5HCO1 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCT TAACTTCAGGGTGACCAAAAAATCA
N5HCO2 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCT GA TAACTTCAGGGTGACCAAAAAATCA
N5HCO3 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCT TGC TAACTTCAGGGTGACCAAAAAATCA
N5HCO4 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCT ACGC TAACTTCAGGGTGACCAAAAAATCA
N5LCO0 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCT GGTCAACAAATCATAAAGATATTGG
N5LCO1 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCT A GGTCAACAAATCATAAAGATATTGG
N5LCO2 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCT TC GGTCAACAAATCATAAAGATATTGG
N5LCO3 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCT CAC GGTCAACAAATCATAAAGATATTGG
N5LCO4 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCT CTAC GGTCAACAAATCATAAAGATATTGG

flow cell bind N5

sequencing primer 1 bind

HCO / LCO (Folmer et al. 1994)

N = shift (Lundberg et al. 2013)

N7LCO0 CAAGCAGAAGACGGCATAACGAGAT CGGTCTCGGCATTCTGCTGAACCGCTCTTCCGATCT GGTCAACAAATCATAAAGATATTGG
N7LCO1 CAAGCAGAAGACGGCATAACGAGAT CGGTCTCGGCATTCTGCTGAACCGCTCTTCCGATCT A GGTCAACAAATCATAAAGATATTGG
N7LCO2 CAAGCAGAAGACGGCATAACGAGAT CGGTCTCGGCATTCTGCTGAACCGCTCTTCCGATCT TC GGTCAACAAATCATAAAGATATTGG
N7LCO3 CAAGCAGAAGACGGCATAACGAGAT CGGTCTCGGCATTCTGCTGAACCGCTCTTCCGATCT CAC GGTCAACAAATCATAAAGATATTGG
N7LCO4 CAAGCAGAAGACGGCATAACGAGAT CGGTCTCGGCATTCTGCTGAACCGCTCTTCCGATCT CTAC GGTCAACAAATCATAAAGATATTGG
N7HCO0 CAAGCAGAAGACGGCATAACGAGAT CGGTCTCGGCATTCTGCTGAACCGCTCTTCCGATCT TAACTTCAGGGTGACCAAAAAATCA
N7HCO1 CAAGCAGAAGACGGCATAACGAGAT CGGTCTCGGCATTCTGCTGAACCGCTCTTCCGATCT TAACTTCAGGGTGACCAAAAAATCA
N7HCO2 CAAGCAGAAGACGGCATAACGAGAT CGGTCTCGGCATTCTGCTGAACCGCTCTTCCGATCT GA TAACTTCAGGGTGACCAAAAAATCA
N7HCO3 CAAGCAGAAGACGGCATAACGAGAT CGGTCTCGGCATTCTGCTGAACCGCTCTTCCGATCT TGC TAACTTCAGGGTGACCAAAAAATCA
N7HCO4 CAAGCAGAAGACGGCATAACGAGAT CGGTCTCGGCATTCTGCTGAACCGCTCTTCCGATCT ACGC TAACTTCAGGGTGACCAAAAAATCA

flow cell bind N7

sequencing primer 2 bind

HCO / LCO (Folmer et al. 1994)

N = shift (Lundberg et al. 2013)

Figure S1. Fusion COI Primers developed in this study. The primers are based on the Folmer et al. (1994) primers. They include MiSeq flow cell and sequencing primer binding regions. All primers amplify an 832-bp fragment that can be sequenced directly after purification. Up to 10 libraries can be uniquely tagged and pooled in one MiSeq run with the tags consisting of the HCO or LCO primer + the primer shift up to 4 bp (always 4 bp in total). It is recommended that all 10 primer pairs are used in the following combination to maximise sequence diversity:

N5HCO0+N7LCO4, N5HCO1+N7LCO3, N5HCO2+N7LCO2, N5HCO3+N7LCO1, N5HCO4+N7LCO0,
N5LCO0+N7HCO4, N5LCO1+N7HCO3, N5LCO2+N7HCO2, N5LCO3+N7HCO1, N5LCO4+N7HCO0