

**Figure S3:** Overview of used fusion sequencing primers. They contain standard illumina flow cell binds and primer binding sites as well as in line tags to distinguish between multiplexed samples. They can be used to amplify the target COI barcoding region and PCR products can directly be sequenced after PCR cleanup.

We recommend using the parallel sequencing strategy outlined in Elbrecht & Lesse 2015, maximising sequence diversity for sequencing and doubling the amount of samples which can be tagged (up to 288). See Figure S4 for ideal tagging combinations.

#### References:

Elbrecht V, Leese F (2015) Can DNA-Based Ecosystem Assessments Quantify Species Abundance? Testing Primer Bias and Biomass—Sequence Relationships with an Innovative Metabarcoding Protocol. PlosONE

A) P5 Primers

P5\_BF1\_0 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTACWGGWTGRACWGTNTAYCC

P5\_BF1\_1 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTCACWGGWTGRACWGTNTAYCC

P5\_BF1\_2 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTATACWGGWTGRACWGTNTAYCC

P5\_BF1\_3 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTTCGACWGGWTGRACWGTNTAYCC

P5\_BF1\_C AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTCAGACWGGWTGRACWGTNTAYCC

P5\_BF1\_4 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTGGTTACWGGWTGRACWGTNTAYCC

P5\_BF2\_0 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTGCHCCHGAYATRGCHTTYCC

P5\_BF2\_1 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTTGCHCCHGAYATRGCHTTYCC

P5\_BF2\_2 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTGAGCHCCHGAYATRGCHTTYCC

P5\_BF2\_B AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTATGCHCCHGAYATRGCHTTYCC

P5\_BF2\_3 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTAGTGCHCCHGAYATRGCHTTYCC

P5\_BF2\_4 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTCTTTGCHCCHGAYATRGCHTTYCC

P5\_BR1\_0 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTARYATDGTRATDGCHCCDGC

P5\_BR1\_1 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTTARYATDGTRATDGCHCCDGC

P5\_BR1\_2 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTCGARYATDGTRATDGCHCCDGC

P5\_BR1\_3 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTGTCARYATDGTRATDGCHCCDGC

P5\_BR1\_C AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTTGAARYATDGTRATDGCHCCDGC

P5\_BR1\_4 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTCCGGARYATDGTRATDGCHCCDGC

P5\_BR2\_0 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTTCDGGRTGNCCRAARAAYCA

P5\_BR2\_1 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTCTCDGGRTGNCCRAARAAYCA

P5\_BR2\_2 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTAATCDGGRTGNCCRAARAAYCA

P5\_BR2\_B AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTGCTCDGGRTGNCCRAARAAYCA

P5\_BR2\_3 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTTGGTCDGGRTGNCCRAARAAYCA

P5\_BR2\_4 AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGCTCTTCCGATCTGTAAATCDGGRTGNCCRAARAAYCA

flow cell bind P5

sequ primer 1 bind

N = shift (Lundberg et al. 2013)

## B) P7 Primers

P7\_BF1\_0 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTACWGGWTGRACWGTNTAYCC  
P7\_BF1\_1 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTCACWGGWTGRACWGTNTAYCC  
P7\_BF1\_2 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTATACWGGWTGRACWGTNTAYCC  
P7\_BF1\_3 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTTCGACWGGWTGRACWGTNTAYCC  
P7\_BF1\_C CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTCAGACWGGWTGRACWGTNTAYCC  
P7\_BF1\_4 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTGGTTACWGGWTGRACWGTNTAYCC  
P7\_BF2\_0 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTGCHCCHGAYATRGCHTTYCC  
P7\_BF2\_1 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTTGCHCCHGAYATRGCHTTYCC  
P7\_BF2\_2 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTGAGCHCCHGAYATRGCHTTYCC  
P7\_BF2\_B CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTATGCHCCHGAYATRGCHTTYCC  
P7\_BF2\_3 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTAGTGCHCCHGAYATRGCHTTYCC  
P7\_BF2\_4 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTCTTGCHCCHGAYATRGCHTTYCC  
P7\_BR1\_0 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTARYATDGTRATDGCHCCDGC  
P7\_BR1\_1 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTTARYATDGTRATDGCHCCDGC  
P7\_BR1\_2 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTCGARYATDGTRATDGCHCCDGC  
P7\_BR1\_3 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTGTCARYATDGTRATDGCHCCDGC  
P7\_BR1\_C CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTTGAARYATDGTRATDGCHCCDGC  
P7\_BR1\_4 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTCCGGARYATDGTRATDGCHCCDGC  
P7\_BR2\_0 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTTCDGGRTGNCCRAARAAYCA  
P7\_BR2\_1 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTCTCDGGRTGNCCRAARAAYCA  
P7\_BR2\_2 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTAATCDGGRTGNCCRAARAAYCA  
P7\_BR2\_B CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTGCTCDGGRTGNCCRAARAAYCA  
P7\_BR2\_3 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTTGGTCDGGRTGNCCRAARAAYCA  
P7\_BR2\_4 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCCTGCTGAACCGCTCTTCCGATCTGTAAATCDGGRTGNCCRAARAAYCA

flow cell bind P7

sequ primer 2 bind

**N** = shift (Lundberg et al. 2013)