

1 Salas-Lizana and Oono's design

These are the sequences of the adapters reported by [1].

MseI-P1

*TACTGTCTCTTATACGAGAACAA
 |||||
 GTCCTCGTGGGCTCGGAGATGTTAAGAGACAG

EcoRI-P2[illegible]

Ligated fragment

[illegible]

Ligated fragment with amplification primers

The sequences of the Illumina amplification primers is taken from document # 1000000002694 v14 (page 4; July 2020). Note that the primer with *i7* identification will only hybridize after successful extension from *i5* primer.

AATGATACGGCGACCACGAGATCTACAC[15]TCGTCGGCAGCGTC
GTCGGCAGCGTCAGATGTGTATAAGAGACAGCAATTCNNNNNNNNNNTTACTGTCTTTATACGAGAACAA
|||||
AATGATACGGCGACCACGAGATCTACAC[15]TCGTCGGCAGCGTC

2 *SphI* and *HindIII* combination

I tentatively choose this combination of results on the grounds of the analysis in folder ‘2020-12-14’. If the *Coregonus* sp. ‘balchen’ reference genome was accurate, we would obtain about 59000 fragments in the size range 250-650, with the two ends cut by different enzymes. *SphI* recognizes the sequence GCATGC and produces CATG-3’ overhangs. And *HindIII* recognizes AAGCTT and produces 5’-AGCT overhangs. Below I show a possible design for the universal adapters. Note that there are two adapters, each made of two strands, and in principle I should not matter which one is specific for which enzyme.

HindIII-P1 adaptor

*AGCTCTGTCTCTTATACGAGAACAA
 |||||
 GTCTCGTGGGCTCGGAGATGTGTATTAAGAGACAG

SphI-P2 adaptor

GTCGGCAGCGTCAGATGTGTATAAGAGACAGCCATG
|||||||
AAGCACTTAACTTTTGTAAGCATGCATTGCTGGAGGAAGA

*GCTGCTCTCTTATATACACATCTGAGCGGCCGCCCCGACGA

Ligated fragment

Note that once the adapters are ligated to the genomic fragment (blue), the restriction sites are not available any more, and the fragment will not be digested again.

[illegible]

Ligated fragment with amplification primers

AATGATACGGCGACCAACCGAGATCTACAC[15]TCGTCGGCAGCGTC
 GTCGGCAGCGTCAGATGTGTATAAGAGACAGCCATGCNNNNNNNNNAAGCTCTGTCTTTATACGAGAACA
 |||||
 |||||
 GTCGTCGTGGGCTCGGAGATGTGTATGAAGAGACAGAGCTTNNNNNNNNNGCATGTCGCTGTCTTATATACAGCATCTCGTGCAGCGTCGCGACGA
 CAGAGACAGAGAGAGGCGCATACAGAGAT[17]GTCGTCGTGGGCTCGG

Amplified fragment

[illegible]

References

- [1] R. Salas-Lizana and R. Oono, “Double-digest radseq loci using standard illumina indexes improve deep and shallow phylogenetic resolution of lophodermium, a widespread fungal endophyte of pine needles,” *Ecology and Evolution*, vol. 8, no. 13, pp. 6638–6651, 2018.