ddRADseq library design

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This is based on [1]. *SphI* recognizes the sequence GCATGC and produces CATG-3' overhangs. And *HindIII* recognizes AAGCTT and produces 5'-AGCT overhangs. Below I show the design for the adapters.

HindIII-P1 adaptor

```
*AGCTCTGTCTCTTATACGAGAACAA
     ||||||||||||
     GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG
```

SphI-P2 adaptor

```
  GTCGGCAGCGTCAGATGTGTATAAGAGACAGCCATG
  ||||||||||||||||||||||||||||||||||
GAGCTGTCTCTTATACACATCTGACGCTGCCGACGA*
```

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 would not be digested again.

```
  GTCGGCAGCGTCAGATGTGTATAAGAGACAGCCATGCNNNNNNNNNNNNAAGCTCTGTCTCTTATACGAGAACAA
  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GAGCTGTCTCTTATACACATCTGACGCTGCCGACGAGCTTNNNNNNNNNNNNGCATGGCTGTCTCTTATACACATCTGACGCTGCCGACGA
```

Ligated fragment with amplification primers

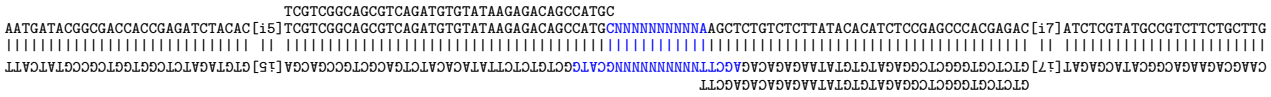
```
AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTC
                              GTCGGCAGCGTCAGATGTGTATAAGAGACAGCCATGCNNNNNNNNNNNNAAGCTCTGTCTCTTATACGAGAACAA
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
                            GAGCTGTCTCTTATACACATCTGACGCTGCCGACGAGCTTNNNNNNNNNNNNGCATGGCTGTCTCTTATACACATCTGACGCTGCCGACGA
                                                                                                    GTCTCGTGGGCTCGG
                                                                                                    CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGG
```

Amplified fragment

Below, the positions marked as *[i5]* and *[i7]* correspond to the 10-bases long indices. In all, the adapters and amplification primers will represent either 141 or 149 additional bases (145 on average), depending on whether we count overhangs or not as part of the length of the double stranded DNA fragment. Thus, aiming at an original size range of 250-650 means we should select amplified fragments in the range 395-795, or 400 to 800.

Sequencing primers

The sequence of the sequencing primers only need to be edited to match the restriction site.



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.