## Supplementary information

# MIG-seq: an effective PCR-based method for genome-wide single-nucleotide polymorphism genotyping using the next-generation sequencing platform

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**Supplementary Table 1**. Sequences of MIG-seq primer set-2 for the 1st PCR

| Name | Sequences (5′–3′) |
|---|---|
| Forward primers: | (Tail + **anchor: CTG**) + SSR + **anchor** |
| $(CTG)_4$AC-f | CGCTCTTCCGATCT**CTG**CTGCTGCTGCTG**AC** |
| $(GCT)_4$TG-f | CGCTCTTCCGATCT**CTG**GCTGCTGCTGCT**TG** |
| $(GTA)_4$TG-f | CGCTCTTCCGATCT**CTG**GTAGTAGTAGTA**TG** |
| $(GTT)_4$AG-f | CGCTCTTCCGATCT**CTG**GTTGTTGTTGTT**AG** |
| Reverse primers: | (Tail + **anchor: GAC**) + SSR + **anchor** |
| $(CTG)_4$AC-r | TGCTCTTCCGATCT**GAC**CTGCTGCTGCTG**AC** |
| $(GCT)_4$TG-r | TGCTCTTCCGATCT**GAC**GCTGCTGCTGCT**TG** |
| $(GTA)_4$TG-r | TGCTCTTCCGATCT**GAC**GTAGTAGTAGTA**TG** |
| $(GTT)_4$AG-r | TGCTCTTCCGATCT**GAC**GTTGTTGTTGTT**AG** |

Underlined and boldface nucleotides denote tail and anchor sequences, respectively. The differences between forward and reverse primer sets lie only in their tail sequences. SSR; simple sequence repeat

**Supplementary Table 2**. Segregation of allelic variants, their goodness of fit to the expected 1:1 ratio and linkage groups for 43 putative SNP loci, in megagametophyte samples from a mother tree of *Picea abies*

| Locus | Genotype of the mother tree | Observed segregation in megagametophyte samples | | P value (Chi-square test) * $P < 0.05$ | | Linkage groups with complete linkage |
|---|---|---|---|---|---|---|
| | | Allele-1 | Allele-2 | | | |
| 15 | A / G | 6 | 10 | 0.317 | | |
| 40 | A / G | 11 | 5 | 0.134 | | A |
| 136 | G / T | 10 | 4 | 0.109 | | |
| 276 | C / T | 9 | 7 | 0.617 | | B |
| 277 | A / G | 5 | 11 | 0.134 | | |
| 343 | C / T | 9 | 7 | 0.617 | | |
| 353 | C / T | 7 | 8 | 0.796 | | |
| 416 | C / T | 7 | 8 | 0.796 | | |
| 433 | C / G | 6 | 10 | 0.317 | | |
| 437 | A / G | 6 | 10 | 0.317 | | |
| 560 | C / T | 7 | 9 | 0.617 | | |
| 666 | A / T | 7 | 9 | 0.617 | | |
| 718 | C / T | 10 | 6 | 0.317 | | |
| 756 | C / T | 12 | 4 | 0.046 | * | |
| 776 | A / G | 8 | 8 | 1.000 | | |
| 923 | A / C | 6 | 9 | 0.439 | | |
| 993 | G / T | 13 | 3 | 0.012 | * | C |
| 1005 | A / G | 9 | 6 | 0.439 | | |
| 1048 | A / G | 5 | 11 | 0.134 | | D |
| 1086 | C / T | 9 | 7 | 0.617 | | B |
| 1118 | A / C | 9 | 7 | 0.617 | | |
| 1311 | A / G | 8 | 8 | 1.000 | | |
| 1368 | A / G | 5 | 10 | 0.197 | | A |
| 1419 | A / C | 9 | 7 | 0.617 | | E |
| 1428 | C / T | 5 | 11 | 0.134 | | D |
| 1698 | A / C | 6 | 4 | 0.527 | | |
| 1750 | G / T | 8 | 3 | 0.132 | | C |
| 1768 | A / G | 12 | 4 | 0.046 | * | |
| 1771 | C / T | 8 | 8 | 1.000 | | |
| 1853 | C / T | 7 | 9 | 0.617 | | |
| 1856 | G / T | 4 | 6 | 0.527 | | |
| 2025 | C / T | 7 | 9 | 0.617 | | |
| 2054 | A / G | 9 | 7 | 0.617 | | E |
| 2074 | A / G | 10 | 6 | 0.317 | | |
| 2080 | A / G | 8 | 8 | 1.000 | | |
| 2107 | C / T | 7 | 9 | 0.617 | | F |
| 2113 | C / T | 7 | 9 | 0.617 | | F |
| 2136 | C / T | 9 | 7 | 0.617 | | |
| 2148 | A / C | 12 | 4 | 0.046 | * | |
| 2263 | C / G | 10 | 5 | 0.197 | | |
| 2315 | A / C | 7 | 9 | 0.617 | | |
| 2324 | G / T | 11 | 5 | 0.134 | | |
| 2402 | C / G | 11 | 5 | 0.134 | | |

**Supplementary Table 3**. Genotypes of seven microsatellite loci in 18 ramets (samples) of *Sasa palmata.* Three groups with identical genotypes (clones) are enclosed by a dotted line

| | Locus | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample ID | *Sasa718* | | *BWSS1* | | *BWSS3* | | *BWSS4* | | *BWSS5* | | *BWSS7* | | *BWSS8* | |
| Spa01 | 161 | 161 | 109 | 109 | 140 | 140 | 93 | 97 | 75 | 75 | 97 | 97 | 109 | 109 |
| Spa02 | 148 | 173 | 109 | 109 | 170 | 170 | 93 | 97 | 75 | 75 | 88 | 93 | 109 | 109 |
| Spa03 | 148 | 173 | 109 | 109 | 170 | 170 | 93 | 97 | 75 | 75 | 88 | 93 | 109 | 109 |
| Spa04 | 161 | 179 | 109 | 109 | 140 | 140 | 93 | 97 | 75 | 75 | 86 | 88 | 109 | 109 |
| Spa05 | 150 | 165 | 105 | 105 | 140 | 170 | 101 | 103 | 75 | 75 | 88 | 88 | 109 | 109 |
| Spa06 | 161 | 173 | 109 | 109 | 170 | 170 | 97 | 97 | 75 | 75 | 88 | 93 | 109 | 109 |
| Spa07 | 171 | 173 | 109 | 109 | 187 | 187 | 97 | 103 | 75 | 75 | 88 | 97 | 109 | 109 |
| Spa08 | 171 | 173 | 109 | 109 | 187 | 187 | 97 | 103 | 75 | 75 | 88 | 97 | 109 | 109 |
| Spa09 | 171 | 173 | 109 | 109 | 187 | 187 | 97 | 103 | 75 | 75 | 88 | 97 | 109 | 109 |
| Spa10 | 165 | 165 | 109 | 109 | 140 | 140 | 97 | 103 | 75 | 75 | 86 | 86 | 109 | 109 |
| Spa11 | 161 | 163 | 109 | 109 | 140 | 170 | 95 | 101 | 75 | 75 | 88 | 88 | 109 | 109 |
| Spa12 | 148 | 167 | 109 | 109 | 170 | 170 | 101 | 101 | 75 | 75 | 88 | 88 | 109 | 109 |
| Spa13 | 161 | 163 | 109 | 111 | 140 | 183 | 97 | 103 | 75 | 75 | 88 | 95 | 109 | 109 |
| Spa14 | 161 | 169 | 109 | 109 | 140 | 191 | 97 | 99 | 75 | 75 | 82 | 86 | 109 | 109 |
| Spa15 | 161 | 165 | 109 | 109 | 140 | 140 | 95 | 95 | 75 | 75 | 88 | 88 | 109 | 109 |
| Spa16 | 148 | 163 | 109 | 111 | 140 | 170 | 93 | 101 | 75 | 75 | 88 | 97 | 109 | 109 |
| Spa17 | 148 | 163 | 109 | 111 | 140 | 170 | 93 | 101 | 75 | 75 | 88 | 97 | 109 | 109 |
| Spa18 | 163 | 165 | 109 | 109 | 140 | 140 | 103 | 103 | 75 | 75 | 86 | 88 | 109 | 109 |

**Supplementary Table 4**. Description of DNA extraction for the eight species analyzed by MIG-seq

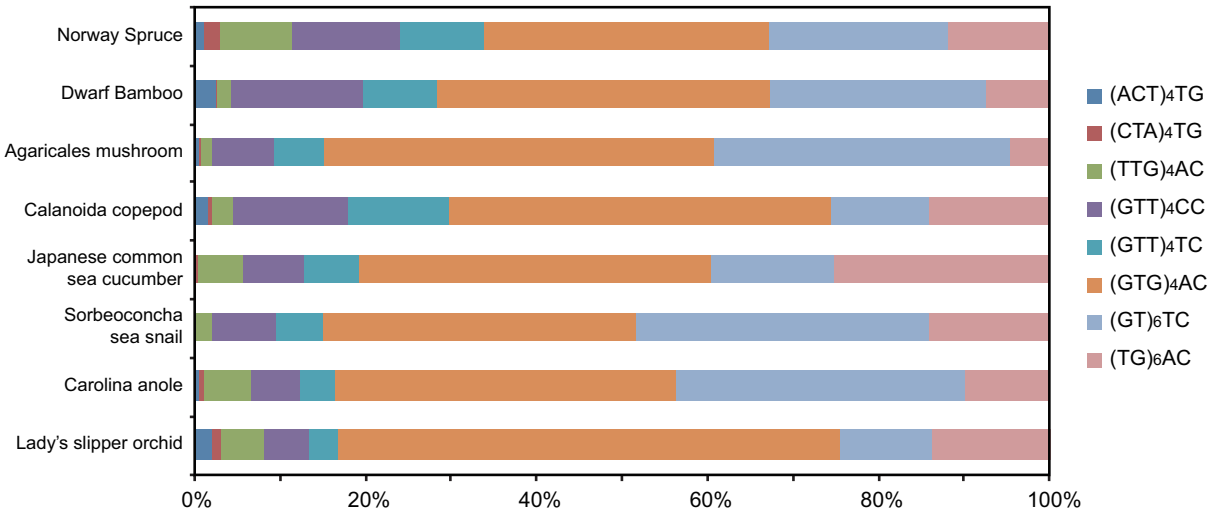
| Species | Method (kit) | Amount of tissue used for DNA extraction (approx. mg, fresh weight) | Amount of DNA used as PCR template (ng) |
|---|---|---|---|
| Norway spruce (*Picea abies*) | DNeasy Plant Mini Kit (Qiagen) | 0.01 (seed), 50 (leaf) | 10–50 |
| Dwarf bamboo (*Sasa palmata*) | CTAB method* | 50 | 50–100 |
| Nameko mushroom (*Pholiota microspora*) | Illustra Nucleon PhytoPure (GE Healthcare) | 50 | 10–100 |
| Calanoida copepod (*Eodiaptomus japonicus*) | GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich) | 0.006 (dry) | 2–3 |
| Japanese common sea cucumber (*Apostichopus japonicus*) | QuickGene DNA tissue kit S (Kurabo) | 5 | 10–20 |
| Predatory sea snail (*Laguncula pulchella*) | DNeasy Blood & Tissue Kit (Qiagen) | 0.6–2.1 | 10–20 |
| Carolina anole (*Anolis carolinensis*) | Wizard Genomic DNA Purification Kit (Promega) | 5 | 50–100 |
| Lady's-slipper orchid (*Cypripedium macranthos* var. *rebunense*) | DNeasy Plant Mini Kit (Qiagen) | 20–50 | 10–20 |

*Reference: Murray, M.G. & Thompson, W.F. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* **8**, 4321–4325 (1980).

**Supplementary Table 5**. Sequences of six-base indices in the reverse primer for the 2nd PCR of MIG-seq

| Index ID | Sequence | Index ID | Sequence | Index ID | Sequence | Index ID | Sequence |
|---|---|---|---|---|---|---|---|
| R001 | AACATA | R097 | AAAGTG | R193 | AACATT | R289 | AACGCC |
| R002 | AACTAG | R098 | AACCTG | R194 | AACTAA | R290 | AAGGAC |
| R003 | AAGACT | R099 | AATGTA | R195 | AAGCCA | R291 | AAGTGG |
| R004 | AAGTTG | R100 | ACACTG | R196 | AATTCT | R292 | ACAATT |
| R005 | AATTGT | R101 | ACCGAG | R197 | ACCACG | R293 | ACGAAT |
| R006 | ACCCGA | R102 | ACGACG | R198 | ACCTGC | R294 | ACTAGC |
| R007 | ACCTCA | R103 | ACGCTC | R199 | ACGACA | R295 | AGATCA |
| R008 | ACGGCT | R104 | ACGTGA | R200 | AGACCT | R296 | AGCCTG |
| R009 | ACGTAT | R105 | ACTATC | R201 | AGCCGA | R297 | AGGATG |
| R010 | ACTACT | R106 | ACTGAC | R202 | AGGACT | R298 | ATCATG |
| R011 | ACTCGT | R107 | ACTTAA | R203 | AGGCGC | R299 | ATGTTG |
| R012 | ACTGCG | R108 | AGATCT | R204 | ATGGAG | R300 | CAAGGA |
| R013 | AGAACG | R109 | AGATGC | R205 | CAACGT | R301 | CACCTT |
| R014 | AGAAGT | R110 | AGCAAT | R206 | CACCGG | R302 | CATTAG |
| R015 | AGAGTT | R111 | AGCGAC | R207 | CATGTA | R303 | CCATAG |
| R016 | AGCCTT | R112 | AGCTTG | R208 | CCACTG | R304 | CCGGAT |
| R017 | AGCTCC | R113 | AGTGAT | R209 | CCGATC | R305 | CCTCGA |
| R018 | AGTAGG | R114 | ATATAC | R210 | CGACCA | R306 | CGACTC |
| R019 | ATACCG | R115 | ATCACT | R211 | CGCTGA | R307 | CGAGCC |
| R020 | ATATGG | R116 | ATCCGG | R212 | CGGTAG | R308 | CGGACC |
| R021 | ATCATC | R117 | ATCGTG | R213 | CGTTAA | R309 | CGTGGT |
| R022 | ATCCTA | R118 | ATGCTT | R214 | CTACTT | R310 | CGTTGG |
| R023 | ATGCAC | R119 | ATGGTA | R215 | CTCTTG | R311 | CTGACG |
| R024 | ATGGCC | R120 | ATGTGC | R216 | CTTATG | R312 | CTTCAT |
| R025 | ATGTAA | R121 | ATTCTC | R217 | GAACAC | R313 | GACGAC |
| R026 | ATTAGC | R122 | ATTTCG | R218 | GACCAT | R314 | GAGTTC |
| R027 | ATTGCT | R123 | CAATTA | R219 | GAGCGA | R315 | GATCTC |
| R028 | CAAGCG | R124 | CACTCC | R220 | GATTCC | R316 | GCCTAG |
| R029 | CACGCA | R125 | CATAGG | R221 | GCCGGA | R317 | GCTCAA |
| R030 | CATACA | R126 | CATCTG | R222 | GCTAGG | R318 | GCTGTG |
| R031 | CATCAT | R127 | CATTGC | R223 | GCTGAT | R319 | GGACAT |
| R032 | CCACAT | R128 | CCAATG | R224 | GGAGTT | R320 | GGATAA |
| R033 | CCCAAT | R129 | CCACGA | R225 | GGTATC | R321 | GTAAGT |
| R034 | CCGAGT | R130 | CCGACA | R226 | GTAGAG | R322 | GTAGCA |
| R035 | CCGTTG | R131 | CCGCAC | R227 | GTGTAC | R323 | GTGTTA |
| R036 | CCTGGC | R132 | CCTACC | R228 | GTTCCA | R324 | TAACAA |
| R037 | CGAGGT | R133 | CGAAGG | R229 | GTTGGA | R325 | TACTCA |
| R038 | CGATTT | R134 | CGATAC | R230 | TACGTG | R326 | TAGCGG |
| R039 | CGCCCT | R135 | CGCAAC | R231 | TAGCCT | R327 | TAGTAT |
| R040 | CGCTTC | R136 | CGCGTT | R232 | TCAACG | R328 | TCATGC |
| R041 | CGGATT | R137 | CGGACG | R233 | TCGGCT | R329 | TCTACA |
| R042 | CGTTCC | R138 | CGTACT | R234 | TCTTGT | R330 | TGCCAA |
| R043 | CTATCT | R139 | CTACAA | R235 | TGATTG | R331 | TGCTGT |
| R044 | CTCGAC | R140 | CTATTC | R236 | TGCGCA | R332 | TGTAGA |
| R045 | CTGATC | R141 | CTCTCG | R237 | TGGCTC | R333 | TTACGC |
| R046 | CTGGAT | R142 | CTGCCA | R238 | TTAACC | R334 | TTAGCG |
| R047 | CTGTGA | R143 | CTGTAC | R239 | TTATGA | R335 | TTCAAT |
| R048 | CTTGAA | R144 | CTTACG | R240 | TTGAAC | R336 | TTGAGA |
| R049 | GAACTC | R145 | GAAATG | R241 | AAGGCG | R337 | AAGCAT |
| R050 | GAAGAT | R146 | GAATCG | R242 | AATACG | R338 | AATCGT |
| R051 | GAAGCC | R147 | GACTTG | R243 | AATGGC | R339 | AATGTT |
| R052 | GAATGT | R148 | GAGCCA | R244 | ACATCG | R340 | ACATTC |
| R053 | GACACA | R149 | GAGTGA | R245 | ACCGCT | R341 | ACCATA |
| R054 | GACCGA | R150 | GATCCT | R246 | ACTAAG | R342 | ACCTAT |
| R055 | GAGATA | R151 | GCAAGG | R247 | ACTCTG | R343 | ACGTGT |
| R056 | GAGTAT | R152 | GCCGGT | R248 | AGCACA | R344 | ACTGAA |
| R057 | GATAAT | R153 | GCGACT | R249 | AGCGGC | R345 | AGCATC |
| R058 | GATTAA | R154 | GCTCAT | R250 | ATAGTG | R346 | AGCTAC |

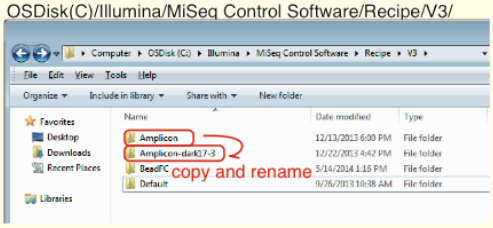
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| R059 | GCCATC | R155 | GCTTAC | R251 | ATGATA | R347 | AGGCTT |
| R060 | GCCTGC | R156 | GGACCA | R252 | ATTCCT | R348 | ATGCCG |
| R061 | GCTAGC | R157 | GGATAG | R253 | CAATCC | R349 | ATTGCC |
| R062 | GCTCTC | R158 | GGCACT | R254 | CACGAT | R350 | CAATGG |
| R063 | GCTTCT | R159 | GGCTAT | R255 | CATTGA | R351 | CATGAC |
| R064 | GGAGTG | R160 | GGGTGC | R256 | CCATGT | R352 | CCAAGC |
| R065 | GGATTC | R161 | GGTGAC | R257 | CCGTAA | R353 | CCGAAG |
| R066 | GGCCAC | R162 | GTACGA | R258 | CGAGAT | R354 | CCTAAT |
| R067 | GGCTGA | R163 | GTATAA | R259 | CGGCTA | R355 | CGAATA |
| R068 | GGTACA | R164 | GTGAAG | R260 | CGTATT | R356 | CTAAGG |
| R069 | GTAACC | R165 | GTGCCT | R261 | CTAACA | R357 | CTAGCT |
| R070 | GTAGCT | R166 | GTTCTA | R262 | CTCAAC | R358 | CTCGAA |
| R071 | GTATGC | R167 | TAAACG | R263 | CTGATT | R359 | CTGCGT |
| R072 | GTGATT | R168 | TAACTG | R264 | CTTCTA | R360 | CTTGCA |
| R073 | GTTAGT | R169 | TACAAG | R265 | GAAGGC | R361 | GACTCT |
| R074 | GTTGCA | R170 | TACTTA | R266 | GACGTA | R362 | GATACT |
| R075 | TAACGC | R171 | TAGGTA | R267 | GATAAC | R363 | GATTAT |
| R076 | TAATCC | R172 | TATCGT | R268 | GCACCG | R364 | GCAAGA |
| R077 | TACGAC | R173 | TATCTC | R269 | GCGGTA | R365 | GCATTA |
| R078 | TAGATC | R174 | TATGCG | R270 | GCTCGC | R366 | GCGTGA |
| R079 | TATACC | R175 | TCCATT | R271 | GCTTCA | R367 | GCTCTT |
| R080 | TATGGC | R176 | TCCTAC | R272 | GGCTCC | R368 | GGAACT |
| R081 | TCCCTA | R177 | TCGCAT | R273 | GTAATG | R369 | GGACGA |
| R082 | TCGAGG | R178 | TCGGAG | R274 | GTGCAA | R370 | GGCTTA |
| R083 | TCGCTG | R179 | TCGTAA | R275 | GTTACC | R371 | GTACTA |
| R084 | TCTATG | R180 | TCGTGC | R276 | GTTGCG | R372 | GTGGCC |
| R085 | TCTGGT | R181 | TCTCGC | R277 | TAATCT | R373 | GTTCAC |
| R086 | TCTTCG | R182 | TCTGTA | R278 | TACTGG | R374 | TACCTC |
| R087 | TGACAT | R183 | TGAAGC | R279 | TAGGTT | R375 | TAGAGT |
| R088 | TGAGTA | R184 | TGAGGG | R280 | TCCACC | R376 | TCCGCG |
| R089 | TGCACG | R185 | TGATGT | R281 | TCTCGG | R377 | TCTGAG |
| R090 | TGCTTT | R186 | TGCGTG | R282 | TGACGG | R378 | TGAATC |
| R091 | TGGTGA | R187 | TGGACC | R283 | TGCCGC | R379 | TGATAT |
| R092 | TTACAC | R188 | TGTGCT | R284 | TGCTTC | R380 | TGCGAG |
| R093 | TTCTCA | R189 | TTAGCA | R285 | TGTTCG | R381 | TGGAGC |
| R094 | TTGACT | R190 | TTCCGC | R286 | TTAGAC | R382 | TGTTGC |
| R095 | TTGCGT | R191 | TTCGTA | R287 | TTCGTT | R383 | TTCTCG |
| R096 | TTTACA | R192 | TTGCTA | R288 | TTGCTG | R384 | TTGGCA |




**Supplementary Figure 1**. Differences in the number of amplified reads among eight primers used for the 1st PCR

Copy and rename 'Amplicon' folder
in OSDisk(C)/Illumina/MiSeq Control Software/Recipe/V3/

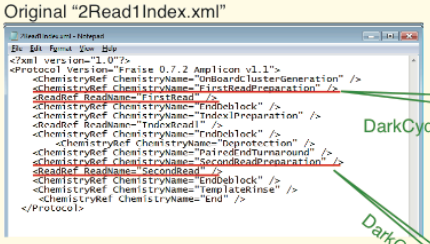
OSDisk(C)/Illumina/MiSeq Control Software/Recipe/V3/



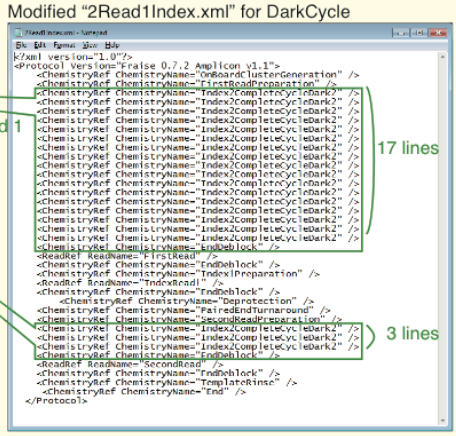

Modify the protocol of seqeunce reading program.

OSDisk(C)/Illumina/MiSeq Control Software/Recipe/V3/Amplicon-dark17-3/Protocol/**2Read1Index.xml**

Original "2Read1Index.xml"



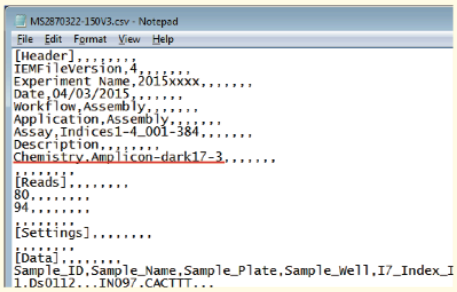
Modified "2Read1Index.xml" for DarkCycle




To skip the first 17 bases of Read 1,
Between
`<ChemistryRef ChemistryName="FirstReadPreparation" />`
and
`<ReadRef ReadName="FirstRead" />`,
insert the following line 17 times
`<ChemistryRef ChemistryName="Index2CompleteCycleDark2" />`
and the last part of insertion, insert the following line
`<ChemistryRef ChemistryName="EndDeblock" />`

To skip the first 3 bases of Read 2,
Between
`<ChemistryRef ChemistryName="SecondReadPreparation" />`
and
`<ReadRef ReadName="SecondRead" />`,
insert the following line 3 times
`<ChemistryRef ChemistryName="Index2CompleteCycleDark2" />`
and the last line of insertion, insert the following line
`<ChemistryRef ChemistryName="EndDeblock" />`

Creat a sample sheet as usual, then open it with text editor,
and change "Amplicon" to "Amplicon-dark17-3" on "Chemistry" line.

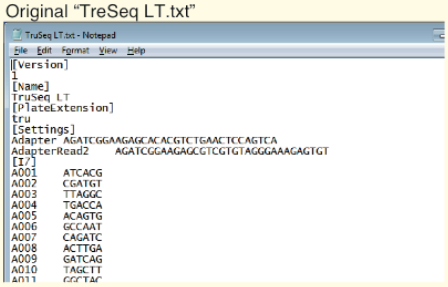



**Supplementary Figure 2**. Procedure for setting the DarkCycle in the MiSeq Control Software

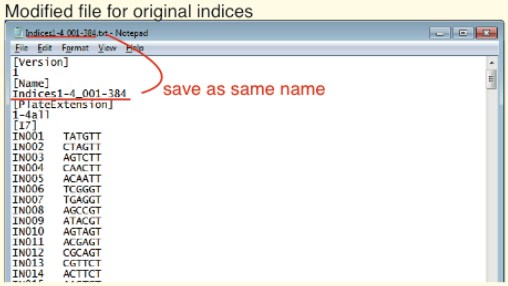
Edit “**SamplePrepkits**” and “**Applications**” folders in OSDisk(C)/Program Files (x86)/Illumina/Illumina Experimental Manager/

1. In “SamplePrepkits” folder, copy an existing file ( “TruSeq LT.txt” etc.).
   Change [Name] and [PlateExtension] column, and replace the index list of [P7].
   Save the file as the same name of [name] column.

Original “TreSeq LT.txt”




Modified file for original indices




Caution: the sequence of listed index must be the reverse complement of index sequence of primer oligo.

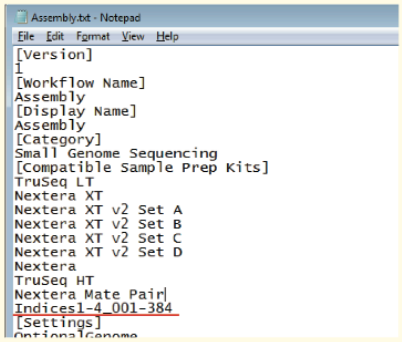
Example: primer sequence

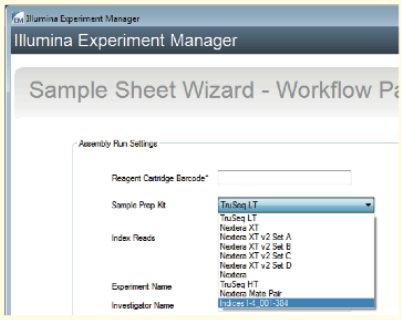
R001_AACATA:CAAGCAGAAGACGGCATACGAGATAACATAGTGACTGGAGTTCAGACGTGCGTGCTCTTCCGATCTGAC

Listed seqnece in index list TATGTT

2. Edit “Assembly.txt” in “Applications” folder.
   Add the modified sample prep kit to the list of
   [Compatible Sample Prep kits]

   The added sample prep kit will be fould in Illumina Experimental Manager

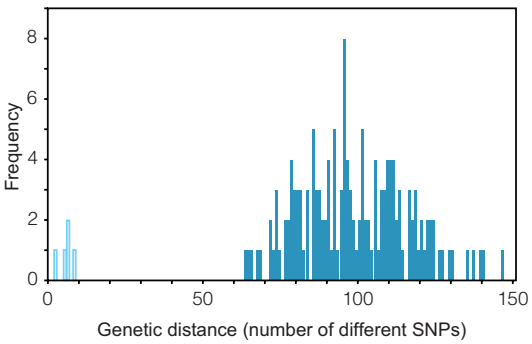



**Supplementary Figure 3**. Procedure for setting the original indices in the Illumina Experimental Manager in the program files of the MiSeq operation system

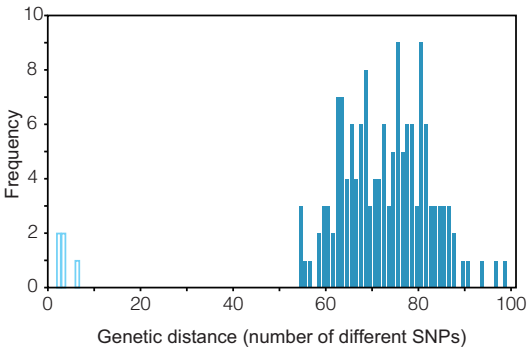
**a**

| | Spa01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spa02 | 137 | | | | | | | | | | | | | | | | |
| Spa03 | 110 | 5 | | | | | | | | | | | | | | | |
| Spa04 | 101 | 106 | 97 | | | | | | | | | | | | | | |
| Spa05 | 85 | 85 | 72 | 63 | | | | | | | | | | | | | |
| Spa06 | 94 | 101 | 96 | 85 | 71 | | | | | | | | | | | | |
| Spa07 | 113 | 118 | 96 | 88 | 95 | 110 | | | | | | | | | | | |
| Spa08 | 120 | 116 | 92 | 92 | 95 | 111 | 8 | | | | | | | | | | |
| Spa09 | 79 | 89 | 73 | 65 | 76 | 81 | 6 | 2 | | | | | | | | | |
| Spa10 | 114 | 107 | 98 | 80 | 76 | 79 | 111 | 123 | 93 | | | | | | | | |
| Spa11 | 130 | 117 | 97 | 97 | 101 | 112 | 124 | 122 | 92 | 117 | | | | | | | |
| Spa12 | 122 | 95 | 67 | 87 | 78 | 111 | 102 | 110 | 78 | 107 | 129 | | | | | | |
| Spa13 | 121 | 95 | 86 | 85 | 77 | 81 | 113 | 113 | 81 | 86 | 104 | 103 | | | | | |
| Spa14 | 123 | 107 | 83 | 116 | 90 | 105 | 139 | 135 | 95 | 146 | 118 | 112 | 108 | | | | |
| Spa15 | 95 | 90 | 73 | 78 | 64 | 87 | 101 | 103 | 77 | 85 | 109 | 98 | 96 | 110 | | | |
| Spa16 | 126 | 108 | 99 | 91 | 95 | 90 | 94 | 100 | 82 | 108 | 120 | 116 | 94 | 124 | 90 | | |
| Spa17 | 109 | 92 | 80 | 74 | 83 | 68 | 80 | 86 | 71 | 92 | 101 | 89 | 73 | 96 | 78 | 6 | |
| Spa18 | 140 | 118 | 87 | 109 | 88 | 105 | 95 | 105 | 79 | 111 | 109 | 119 | 105 | 127 | 100 | 102 | 83 |



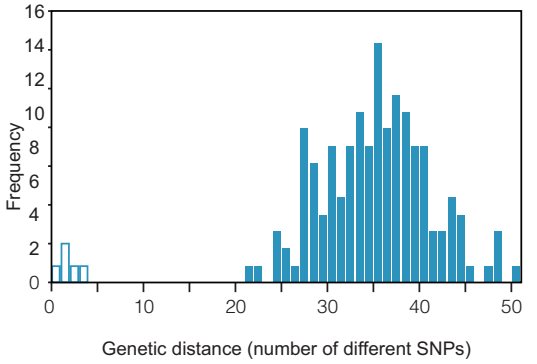

**b**

| | Spa01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spa02 | 78 | | | | | | | | | | | | | | | | |
| Spa03 | 70 | 3 | | | | | | | | | | | | | | | |
| Spa04 | 70 | 68 | 65 | | | | | | | | | | | | | | |
| Spa05 | 68 | 67 | 62 | 54 | | | | | | | | | | | | | |
| Spa06 | 68 | 77 | 71 | 67 | 59 | | | | | | | | | | | | |
| Spa07 | 76 | 76 | 75 | 73 | 72 | 81 | | | | | | | | | | | |
| Spa08 | 81 | 80 | 78 | 77 | 75 | 85 | 2 | | | | | | | | | | |
| Spa09 | 69 | 66 | 63 | 59 | 65 | 71 | 3 | 2 | | | | | | | | | |
| Spa10 | 68 | 80 | 78 | 64 | 63 | 75 | 83 | 87 | 82 | | | | | | | | |
| Spa11 | 69 | 72 | 68 | 74 | 75 | 80 | 82 | 87 | 76 | 75 | | | | | | | |
| Spa12 | 81 | 67 | 58 | 68 | 63 | 81 | 74 | 78 | 65 | 70 | 90 | | | | | | |
| Spa13 | 80 | 67 | 65 | 61 | 56 | 60 | 77 | 80 | 63 | 63 | 75 | 79 | | | | | |
| Spa14 | 73 | 73 | 63 | 80 | 68 | 79 | 85 | 93 | 83 | 98 | 74 | 84 | 81 | | | | |
| Spa15 | 66 | 55 | 54 | 62 | 58 | 62 | 72 | 80 | 68 | 64 | 72 | 69 | 62 | 75 | | | |
| Spa16 | 89 | 75 | 74 | 76 | 72 | 66 | 78 | 83 | 77 | 86 | 86 | 81 | 74 | 96 | 63 | | |
| Spa17 | 85 | 71 | 66 | 62 | 64 | 60 | 67 | 72 | 65 | 75 | 77 | 70 | 62 | 86 | 54 | 6 | |
| Spa18 | 84 | 64 | 60 | 82 | 78 | 80 | 67 | 76 | 71 | 84 | 79 | 77 | 62 | 80 | 61 | 65 | 59 |




**c**

| | Spa01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spa02 | 40 | | | | | | | | | | | | | | | | |
| Spa03 | 37 | 3 | | | | | | | | | | | | | | | |
| Spa04 | 30 | 33 | 33 | | | | | | | | | | | | | | |
| Spa05 | 32 | 35 | 32 | 30 | | | | | | | | | | | | | |
| Spa06 | 47 | 44 | 40 | 28 | 39 | | | | | | | | | | | | |
| Spa07 | 31 | 38 | 37 | 30 | 37 | 45 | | | | | | | | | | | |
| Spa08 | 35 | 41 | 39 | 36 | 40 | 50 | 0 | | | | | | | | | | |
| Spa09 | 30 | 39 | 37 | 29 | 37 | 43 | 1 | 1 | | | | | | | | | |
| Spa10 | 31 | 25 | 21 | 24 | 38 | 37 | 28 | 32 | 27 | | | | | | | | |
| Spa11 | 34 | 30 | 27 | 28 | 28 | 48 | 34 | 39 | 34 | 37 | | | | | | | |
| Spa12 | 34 | 27 | 27 | 32 | 36 | 39 | 35 | 42 | 38 | 28 | 35 | | | | | | |
| Spa13 | 44 | 29 | 27 | 39 | 36 | 43 | 42 | 44 | 43 | 32 | 44 | 41 | | | | | |
| Spa14 | 36 | 33 | 30 | 38 | 33 | 40 | 35 | 38 | 34 | 35 | 33 | 32 | 30 | | | | |
| Spa15 | 33 | 33 | 31 | 31 | 25 | 33 | 39 | 43 | 36 | 24 | 28 | 37 | 26 | 35 | | | |
| Spa16 | 48 | 38 | 35 | 34 | 35 | 40 | 36 | 41 | 35 | 35 | 43 | 32 | 38 | 35 | 36 | | |
| Spa17 | 48 | 36 | 35 | 35 | 38 | 40 | 36 | 40 | 33 | 33 | 42 | 31 | 38 | 37 | 37 | 2 | |
| Spa18 | 38 | 29 | 29 | 32 | 39 | 40 | 28 | 30 | 27 | 27 | 34 | 37 | 24 | 34 | 22 | 27 | 27 |




**Supplementary Figure 4**. Examples of clone identification by MIG-seq analysis based on different parameters (the minimum depth of coverage required to create a stack (m) = 5, 5 and 20; the minimum number of populations in a locus (r) = 0.5, 0.75 and 0.75 for **a**, **b** and **c**, respectively) used in the data analysis (see Figure 2 and Methods). Matrix of the differences between pairwise numbers of SNPs (left) and a histogram showing the frequency distribution of the differences between the pairwise numbers of SNPs (right) are presented.