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1 ***dDocent*: a RADseq, variant-calling pipeline designed for population genomics of non-**  
2 **model organisms**

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**ABSTRACT**

Restriction-site associated DNA sequencing (RADseq) has become a powerful and useful approach for population genomics. Currently, no software exists that utilizes both paired-end reads from RADseq data to efficiently produce population-informative variant calls, especially for organisms with large effective population sizes and high levels of genetic polymorphism but for which no genomic resources exist. *dDocent* is an analysis pipeline with a user-friendly, command-line interface designed to process individually barcoded RADseq data (with double cut sites) into informative SNPs/INDELS for population-level analyses. The pipeline, written in BASH, uses data reduction techniques and other stand-alone software packages to perform quality trimming and adapter removal, *de novo* assembly of RAD loci, read mapping, SNP and INDEL calling, and baseline data filtering. Double-digest RAD data from population pairings of three different marine fishes were used to compare *dDocent* with *Stacks*, the first generally available, widely used pipeline for analysis of RADseq data. *dDocent* consistently identified more SNPs shared across greater numbers of individuals and with higher levels of coverage. This is most likely due to the fact that *dDocent* quality trims instead of filtering and incorporates both forward and reverse reads in assembly, mapping, and SNP calling, thus enabling use of reads with INDEL polymorphisms. The pipeline and a comprehensive user guide can be found at (<http://dDocent.wordpress.com>).

## INTRODUCTION

33

34 Next-generation sequencing (NGS) has transformed the field of genetics into genomics  
35 by providing DNA sequence data at an ever increasing rate and reduced cost (Mardis, 2008).  
36 The nascent field of population genomics relies on NGS coupled with laboratory methods to  
37 reproducibly reduce genome complexity to a few thousand loci. The most common approach,  
38 restriction-site associated DNA sequencing (RADseq), uses restriction endonucleases to  
39 randomly sample the genome at locations adjacent to restriction-enzyme recognition sites that,  
40 when coupled with Illumina sequencing, produces high coverage of homologous SNP (Single  
41 Nucleotide Polymorphism) loci. As such, RADseq provides a powerful approach for  
42 population level genomic studies (Ellegren, 2014; Narum et al., 2013; Rowe et al., 2011).

43 The original RADseq approach (Baird et al., 2008), and initial population genomic  
44 studies employing it (Hohenlohe et al., 2010), focused on SNP discovery and genotyping on  
45 the first (forward) read only. This is because the original RADseq method (Baird et al., 2008)  
46 utilized random shearing to produce RAD loci; paired-end reads were not of uniform length  
47 or coverage, making it problematic to find SNPs at high and uniform levels of coverage  
48 across a large proportion of individuals. As a result, the most comprehensive and widely used  
49 software package for analysis of RADseq data, *Stacks* (Catchen et al., 2013, 2011), provides  
50 SNP genotypes based only on first-read data. In contrast, RADseq approaches such as  
51 ddRAD (Peterson et al., 2012), 2bRAD (Wang et al., 2012), and ezRAD (Toonen et al., 2013)  
52 rely on restriction enzymes to define both ends of a RAD locus, largely producing RAD loci  
53 of fixed length (fRAD). Paired-end Illumina sequencing of fRAD fragments provides an  
54 opportunity to significantly expand the number of SNPs that can be genotyped from a single  
55 RADseq library.

56 Here, the variant-calling pipeline *dDocent* is introduced as a tool for generating  
57 population genomic data; a brief methodological outline of the analysis pipeline also is  
58 presented. *dDocent* is a wrapper script designed to take raw RADseq data and produce  
59 population informative SNP calls, taking full advantage of both paired-end reads. *dDocent* is  
60 configured for organisms with high levels of nucleotide and INDEL polymorphisms, such as  
61 found in many marine organisms (Guo et al., 2012;Keever et al., 2009;Sodergren et al.,  
62 2006;Waples, 1998;Ward et al., 1994). As input, *dDocent* takes paired FASTQ files for  
63 individuals and outputs raw SNP and INDEL calls as well as filtered SNP calls in VCF format.  
64 The pipeline and a comprehensive online manual can be found at  
65 (<http://dDocent.wordpress.com>). Finally, results of pipeline analyses, using both *dDocent* and  
66 *Stacks*, of populations of three species of marine fishes are provided to demonstrate the utility  
67 of *dDocent* compared to *Stacks*, the first and most comprehensive existing software package  
68 for RAD population genomics.

## 69 METHODS

### 70 *Implementation and basic usage*

71 The *dDocent* pipeline is written in BASH and will run using most Unix-like operating  
72 systems. *dDocent* is largely dependent on other bioinformatics software packages, taking  
73 advantage of programs designed specifically for each task of the analysis and ensuring that  
74 each modular component can be updated separately. Proper implementation depends on the  
75 correct installation of each third-party packages/tools. A full list of dependencies can be  
76 found in the user manual at (<http://ddocent.wordpress.com/ddocent-pipeline-user-guide/>) and  
77 a sample script to automatically download and install the packages in a Linux environment  
78 can be found at the *dDocent* repository (<https://github.com/jpuritz/dDocent>).

79 *dDocent* is run by simply switching to a directory containing the input data and starting  
80 the program. There is no configuration file; *dDocent* will proceed through a short series of  
81 command-line prompts, allowing the user to set up analysis parameters. After all required  
82 variables are configured, including an e-mail address for a completion notification, *dDocent*  
83 provides instructions on how to move the program to the background and run, undisturbed,  
84 until completion. The pipeline is designed to take advantage of multiple processing core  
85 machines and, whenever possible, processes should be invoked with multiple threads or  
86 occurrences. For most Linux distributions, the number of processing cores should be  
87 automatically detected. If *dDocent* cannot determine the number of processors, it will ask the  
88 user to input the value.

89 There are two distinct modules of *dDocent*: *dDocent.FB* and *dDocent.GATK*.  
90 *dDocent.FB* uses minimal, BAM-file preparation steps before calling SNPs and INDELS,  
91 simultaneously using FreeBayes (Garrison & Marth, 2012). *dDocent.GATK* uses GATK  
92 (McKenna et al., 2010) for INDEL realignment, SNP and INDEL genotyping (using  
93 HaplotypeCaller), and variant quality-score recalibration, largely following GATK Best  
94 Practices recommendations (Auwera & Carneiro, 2013;DePristo et al., 2011). The modules  
95 represent two different strategies for SNP/INDEL calling that are completely independent of  
96 one another. The remainder of this paper focuses on *dDocent.FB*; additional information on  
97 *dDocent.GATK* may be found in the user guide and results from *dDocent.GATK* can be  
98 found in Appendix S1.

#### 99 *Data input requirements*

100 *dDocent* requires demultiplexed forward and paired-end FASTQ files for every  
101 individual in the analysis. A simple naming convention (a single-word locality code/name

102 and a single-word sample identifier separated by an underscore) must be followed for every  
103 sample; examples are *LOCA\_IND01.F.fq* and *LOCA\_IND01.R.fq*. A sample script for using a  
104 text file with barcodes and sample names and *process\_radtags* from *Stacks* (Catchen et al.,  
105 2013) to properly demultiplex samples and put them in the proper *dDocent* naming  
106 convention can be found at the *dDocent* repository (<https://github.com/jpuritz/dDocent>).

#### 107 *Quality trimming*

108 After *dDocent* checks that it is recognizing the proper number of samples in the current  
109 directory, it asks the user if s/he wishes to proceed with quality trimming of sequence data. If  
110 directed, *dDocent* can use the program *Trim Galore!*  
111 ([http://www.bioinformatics.babraham.ac.uk/projects/trim\\_galore/](http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/)) to simultaneously remove  
112 Illumina adapter sequences and trim ends of reads of low quality. By default, *Trim Galore!*  
113 looks for double-digest RAD adapters (Peterson et al., 2012) and trims bases with quality  
114 scores less than Phred 10. Typically, quality trimming only needs to be performed once on  
115 data, so the option exists to skip this step in subsequent *dDocent* analyses.

#### 116 *De novo assembly*

117 Without reference material, population genomic analyses from RADseq depend on *de*  
118 *novo* assembly of a set of reference contigs. Inherently, not all RAD loci appear in all  
119 individuals due to stochastic processes inherent in library preparation and sequencing and to  
120 polymorphism in restriction-enzyme restriction sites (Catchen et al., 2011). Moreover,  
121 populations can contain large levels of within locus polymorphism, making generation of a  
122 reference sequence computationally difficult. *dDocent* minimizes the amount of data used for  
123 assembly by taking advantage of the fact that flRAD loci present in multiple individuals  
124 should have higher levels of exactly matching reads (forward and reverse) than loci that are

125 only present in a few individuals. Caution is advised for unique reads with low levels of  
126 coverage throughout the data set as they likely represent sequencing errors or polymorphisms  
127 that are shared only by a few individuals.

128 During assembly, paired-end reads are reverse complemented and concatenated to  
129 forward reads. Unique paired reads are identified and their occurrences are counted in the  
130 entire data set. These data are tabulated into the number of unique reads per levels of 1X to  
131 50X coverage; a graph is then generated and printed to the terminal. The distribution usually  
132 follows an asymptotic relationship (Figure 1), with a large proportion of reads only having  
133 one or two occurrences, meaning they likely will not be informative on a population scale.  
134 Highly polymorphic RAD loci still should have at least one allele present at the level of  
135 expected sequence coverage, so this can be used as a guide for informative data. The user  
136 chooses a cut-off level of coverage for reads to be used for assembly – note all reads are still  
137 used for subsequence steps of the pipeline.

138 After a cut-off level is chosen, remaining reads are returned in forward- and reverse-read  
139 files and then input directly into the RADseq assembly program *Rainbow* (Chong et al., 2012).  
140 The default parameters of *Rainbow* are used except that the maximum number of mismatches  
141 used in initial clustering should be changed from four to six. In short, *Rainbow* clusters  
142 forward reads based on similarity; clusters are then recursively divided, based on reverse  
143 reads, into groups representing single alleles. Reads in merged clusters are then assembled  
144 using a greedy algorithm (Pop & Salzberg, 2008). *dDocent* then selects the longest contig for  
145 each cluster as the representative reference sequence for that RAD locus. If the forward read  
146 does not overlap with the reverse read (almost always the case with fRAD), the forward read  
147 is concatenated to the reverse read with ten ‘N’ characters as padding. Finally, reference



148 sequences are clustered based on overall sequence similarity (chosen by user, 90% by default),  
149 using the program *CD-HIT* (Fu et al., 2012;Li & Godzik, 2006). This final cluster step  
150 reduces the data set further, based on overall sequence identity after assembly. Alternatively,  
151 *de novo* assembly can be skipped and the user can provide a FASTA file with reference  
152 sequences.

### 153 *Read mapping*

154 *dDocent* uses the MEM algorithm (Li, 2013) of *BWA* (Li & Durbin, 2009, 2010) to map  
155 quality-trimmed reads to the reference contigs. Users can deploy the default values of BWA  
156 or set an alternative value for each mapping parameter (match score, mismatch score, and  
157 gap-opening penalty). The default settings are meant for mapping reads to the human genome,  
158 so users are encouraged to experiment with mapping parameters. BWA output is ported to  
159 SAMtools (Li et al., 2009), saving disk space, and alignments are saved to the disk as binary  
160 alignment/Map (BAM). BAM files are then sorted and indexed.

### 161 *SNP and INDEL discovery and genotyping*

162 *dDocent* uses a two-step process to optimize the computationally intensive task of  
163 SNP/INDEL calling. First, quality-trimmed forward and reverse reads are reduced to unique  
164 reads. This data set is then mapped to all reference sequences using the previously entered  
165 mapping settings (see *Read Mapping* above). From this alignment, a set of intervals is created  
166 using BEDtools (Quinlan & Hall, 2010). The interval set saves computational time by  
167 directing the SNP-/INDEL-calling software to examine only reference sequences along contigs  
168 that have high quality mappings. Second, the interval list is then split into a single file for  
169 each processing core, allowing SNP/INDEL calling to be optimized with a scatter-gather  
170 technique. The program *FreeBayes* (Garrison & Marth, 2012) is then executed multiple times

171 simultaneously (one execution per processor and genomic interval). *FreeBayes* is a Bayesian-  
172 based, variant-detection software that uses assembled haplotype sequences to simultaneously  
173 call SNPs, INDELS, multi-nucleotide polymorphisms (MNPs), and complex events (e.g.,  
174 composite insertion and substitution events) from alignment files; *FreeBayes* has the added  
175 benefit for population genomics of using reads across multiple individuals to improve  
176 genotyping (Garrison & Marth, 2012). *FreeBayes* is run with minimal changes to the default  
177 parameters; minimum mapping quality score and base quality score are set to PHRED 10.  
178 After all executions of *FreeBayes* are completed, raw SNP/INDEL calls are concatenated into a  
179 single variant call file (VCF), using VCFtools (Danecek et al., 2011).

#### 180 *Variant Filtering*

181 Final SNP data-set requirements are likely to be highly dependent on specific goals and  
182 aims of individual projects. To that end, *dDocent* uses *VCFtools* (Danecek et al., 2011) to  
183 provide only basic level filtering, mostly for run diagnostic purposes. *dDocent* produces a  
184 final VCF file that contains all SNPs, INDELS, MNPs, and complex events that are called in  
185 90% of all individuals, with a minimum quality score of 30. Users are encouraged to use  
186 VCFtools and vcfliib (part of the *FreeBayes* package; <https://github.com/ekg/vcfliib>) to fully  
187 explore and filter data appropriately.

#### 188 *Comparison between dDocent and Stacks*

189 Two sample localities, each comprised of 20 individuals, were chosen randomly from  
190 unpublished RADseq data sets of three different, marine fish species: red snapper (*Lutjanus*  
191 *campechanus*), red drum (*Sciaenops ocellatus*), and silk snapper (*Lutjanus vivanus*). These  
192 three species are part of ongoing RADseq projects in our laboratory, and preliminary analyses  
193 indicated high levels of nucleotide polymorphisms across all populations. Double-digest

194 RAD libraries were prepared, generally following Peterson *et al.* (2012). Individual DNA  
195 extractions were digested with *EcoRI* and *MspI*. A barcoded adapter was ligated to the *EcoRI*  
196 site of each fragment and a generic adapter was ligated to the *MspI* site. Samples were then  
197 equimolarly pooled and size-selected between 350 and 400 bp, using a Qiagen Gel Extraction  
198 Kit. Final library enhancement was completed using 12 cycles of PCR, simultaneously  
199 enhancing properly ligated fragments and adding an Illumina Index for additional barcoding.  
200 Libraries were sequenced on three separate lanes of an Illumina HiSeq 2000 at the University  
201 of Texas Genomic Sequencing and Analysis Facility.

202 Demultiplexed individual reads were analyzed with *dDocent*, using three different levels  
203 of final reference contig clustering (90%, 96%, and 99% similarity) in an attempt to alter the  
204 most comparable analysis variable in *dDocent* to match analysis variables of *Stacks*. The  
205 coverage cut-off for assembly was 12 for red snapper, 13 for red drum, and nine for silk  
206 snapper. All *dDocent* runs used mapping variables of one, three, and five for match-score  
207 value, mismatch score, and gap-opening penalty, respectively. For comparisons, complex  
208 variants were decomposed into canonical SNP and INDEL representation from the raw VCF  
209 files, using *vcfallelicprimitives* from *vcflib* (<https://github.com/ekg/vcflib>).

210 For *Stacks*, reads were demultiplexed and cleaned using *process\_radtags*, removing reads  
211 with ‘N’ calls and low-quality base scores. Because *dDocent* inherently uses both reads for  
212 SNP/INDEL genotyping, forward reads and reverse reads were processed separately with  
213 *denovo\_map.pl* (*Stacks* version 1.08), using three different sets of parameters. The first set  
214 had a minimum depth of coverage of two to create a stack, a maximum distance of two  
215 between stacks, and a maximum distance of four between stacks from different individuals,  
216 with both the deleveraging algorithm and removal algorithms enabled. The second set had a

217 minimum depth of coverage of three to create a stack, a maximum distance of four between  
218 stacks, and a maximum distance of eight between stacks from different individuals, with both  
219 the deleveraging algorithm and removal algorithms enabled. The third set had a minimum  
220 depth of coverage of three to create a stack, a maximum distance of four between stacks, and  
221 a maximum distance of 10 between stacks from different individuals, with both the  
222 deleveraging algorithm and removal algorithms enabled. SNP calls were output in VCF  
223 format.

224 For both *dDocent* and *Stacks* runs, VCFtools was used to filter out INDELS and SNPs that  
225 had a minor allele count of less than five. SNP calls were then evaluated at different  
226 individual-coverage levels: the total number of SNPs; the number of SNPS called in 75%,  
227 90%, and 99% of individuals at 3X coverage; the number of SNPS called in 75% and 90% of  
228 individuals at 5X coverage; the number of SNPS called in 75% and 90% of individuals at 10X  
229 coverage; and the number of SNPS called in 75% and 90% of individuals at 20X coverage.  
230 Overall coverage levels for red snapper were lower and likely impacted by a few low-quality  
231 individuals; consequently, the number of 5X and 10X SNPs shared among 90% of individuals  
232 (after removing the bottom 10% of individuals in terms of coverage) were compared instead  
233 of SNP loci shared at 20X coverage. Results from two runs of *Stacks* (one using forward and  
234 one using reverse reads) were combined for comparison with *dDocent*, which inherently calls  
235 SNPs on both reads. All analyses and computations were performed on a 32-core Linux  
236 workstation with 128 GB of RAM.

## 237 **RESULTS AND DISCUSSION**

238 Results of SNP calling, including run times (in minutes) for each analysis (not including  
239 quality trimming), are presented in Table 1. Data from high coverage SNP calls, averaged

240 over all runs for each pipeline, are presented in Figure 1. While *Stacks* called a larger number  
241 of low coverage SNPs, limiting results to higher individual coverage and to higher individual  
242 call rates revealed that *dDocent* consistently called more high-quality SNPs. Run times were  
243 equivalent for both pipelines.

244 At almost all levels of coverage in three different data sets, *dDocent* called more SNPs  
245 across more individuals than *Stacks*. Two key differences between *dDocent* and *Stacks* likely  
246 contribute these discrepancies: (i) quality trimming instead of quality filtering, and (ii)  
247 simultaneous use of forward and reverse reads by *dDocent* in assembly, mapping, and  
248 genotyping, instead of clustering as employed by *Stacks*. As with any data analysis, quality of  
249 data output is directly linked to the quality of data input. Both *dDocent* and *Stacks* use  
250 procedures to ensure that only high-quality sequence data are retained; however, *Stacks*  
251 removes an entire read when a sliding window of bases drops below a preset quality score  
252 (PHRED 10, by default), while *dDocent* via *Trim Galore!* trims off low-quality bases,  
253 preserving high-quality bases of each read. Filtering instead of trimming results in fewer  
254 reads entering the *Stacks* analysis (between 65%-95% of the data compared to *dDocent*; data  
255 not shown), generating lower levels of coverage and fewer SNP calls than *dDocent*.

256 *dDocent* offers two advantages over *Stacks*: (i) it is specifically designed for paired-end  
257 data and utilizes both forward and reverse reads for *de novo* RAD loci assembly, read  
258 mapping, variant discovery, and genotyping; and (ii) it aligns reads to reference sequence  
259 instead of clustering by identity. Using both reads to cluster and assemble RAD loci helps to  
260 ensure that portions of the genome with complex mutational events, including INDELS or small  
261 repetitive regions, are properly assembled and clustered as homologous loci. Additionally,  
262 using *BWA* to map reads to reference loci enables *dDocent* to properly align reads with INDEL

263 polymorphisms, increasing coverage and subsequent variant discovery and genotyping.  
264 Clustering methods employed by *Stacks*, whether clustering alleles within an individual or  
265 clustering loci between individuals, effectively remove reads, alleles, and loci with INDEL  
266 polymorphisms because the associated frame shift effectively inflates the observed number of  
267 base-pair differences. For organisms with large effective population sizes and high levels of  
268 genetic diversity, such as many marine organisms (Waples, 1998; Ward et al., 1994),  
269 removing reads and loci with INDEL polymorphisms will result in a loss of shared loci and  
270 coverage.

## 271 CONCLUSION

272 *dDocent* is an open-source, freely available population genomics pipeline configured for  
273 species with high levels of nucleotide and INDEL polymorphisms, such as many marine  
274 organisms. The *dDocent* pipeline reports more SNPs shared across greater numbers of  
275 individuals and with higher levels of coverage than current alternatives. The pipeline and a  
276 comprehensive online manual can be found at (<http://dDocent.wordpress.com>) and  
277 (<https://github.com/jpuritz/dDocent>).

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- 289 Auwera G, Carneiro M. 2013. From FastQ Data to High-Confidence Variant Calls: The  
290 Genome Analysis Toolkit Best Practices Pipeline. *Current Protocols in Bioinformatics*:  
291 1–33.
- 292 Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, Selker EU, Cresko WA,  
293 Johnson EA. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD  
294 markers. *PLoS ONE* 3: e3376.
- 295 Catchen JM, Amores A, Hohenlohe P, Cresko W, Postlethwait JH. 2011. Stacks: building and  
296 genotyping Loci de novo from short-read sequences. *G3 (Bethesda, Md.)* 1: 171–182.
- 297 Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA. 2013. Stacks: an analysis tool  
298 set for population genomics. *Molecular ecology* 22: 3124–3140.
- 299 Chong Z, Ruan J, Wu C. 2012. Rainbow : an integrated tool for efficient clustering and  
300 assembling RAD-seq reads. : 1–6.
- 301 Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter  
302 G, Marth GT, Sherry ST, McVean G, Durbin R. 2011. The variant call format and  
303 VCFtools. *Bioinformatics (Oxford, England)* 27: 2156–2158.
- 304 DePristo MA, Banks E, Poplin R, Garimella K V, Maguire JR, Hartl C, Philippakis AA,  
305 Angel G del, Rivas MA, Hanna M, McKenna A, Fennell TJ, Kernysky AM, Sivachenko  
306 AY, Cibulskis K, Gabriel SB, Altshuler D, Daly MJ. 2011. A framework for variation  
307 discovery and genotyping using next-generation DNA sequencing data. *Nature genetics*  
308 43: 491–498.
- 309 Ellegren H. 2014. Genome sequencing and population genomics in non-model organisms.  
310 *Trends in ecology & evolution* 29: 51–63.
- 311 Fu L, Niu B, Zhu Z, Wu S, Li W. 2012. CD-HIT: accelerated for clustering the next-  
312 generation sequencing data. *Bioinformatics (Oxford, England)* 28: 3150–3152.
- 313 Garrison E, Marth G. 2012. Haplotype-based variant detection from short-read sequencing. :  
314 1–9.
- 315 Guo B, Zou M, Wagner A. 2012. Pervasive indels and their evolutionary dynamics after the  
316 fish-specific genome duplication. *Molecular biology and evolution* 29: 3005–3022.
- 317 Hohenlohe PA, Bassham S, Etter PD, Stiffler N, Johnson EA, Cresko WA. 2010. Population  
318 genomics of parallel adaptation in threespine stickleback using sequenced RAD tags.  
319 *PLoS genetics* 6: e1000862.



- 320 Keever CC, Sunday J, Puritz JB, Addison JA, Toonen RJ, Grosberg RK, Hart MW. 2009.  
321 Discordant distribution of populations and genetic variation in a sea star with high  
322 dispersal potential. *Evolution; international journal of organic evolution* 63: 3214–3227.
- 323 Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler  
324 transform. *Bioinformatics (Oxford, England)* 25: 1754–1760.
- 325 Li H, Durbin R. 2010. Fast and accurate long-read alignment with Burrows-Wheeler  
326 transform. *Bioinformatics (Oxford, England)* 26: 589–595.
- 327 Li W, Godzik A. 2006. Cd-hit: a fast program for clustering and comparing large sets of  
328 protein or nucleotide sequences. *Bioinformatics (Oxford, England)* 22: 1658–1659.
- 329 Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin  
330 R. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics (Oxford,  
331 England)* 25: 2078–2079.
- 332 Li H. 2013. Aligning sequence reads , clone sequences and assembly contigs with BWA-  
333 MEM. 00: 1–3.
- 334 Mardis ER. 2008. Next-generation DNA sequencing methods. *Annual review of genomics and  
335 human genetics* 9: 387–402.
- 336 McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytzky A, Garimella K,  
337 Altshuler D, Gabriel S, Daly M, DePristo MA. 2010. The Genome Analysis Toolkit: a  
338 MapReduce framework for analyzing next-generation DNA sequencing data. *Genome  
339 research* 20: 1297–1303.
- 340 Narum SR, Buerkle CA, Davey JW, Miller MR, Hohenlohe PA. 2013. Genotyping-by-  
341 sequencing in ecological and conservation genomics. *Molecular ecology* 22: 2841–2847.
- 342 Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. 2012. Double digest RADseq: an  
343 inexpensive method for de novo SNP discovery and genotyping in model and non-model  
344 species. *PloS one* 7: e37135.
- 345 Pop M, Salzberg S. 2008. Bioinformatics challenges of new sequencing technology. *Trends in  
346 Genetics* 24: 142–149.
- 347 Quinlan AR, Hall IM. 2010. BEDTools: a flexible suite of utilities for comparing genomic  
348 features. *Bioinformatics (Oxford, England)* 26: 841–842.
- 349 Rowe HC, Renaut S, Guggisberg A. 2011. RAD in the realm of next-generation sequencing  
350 technologies. *Molecular ecology* 20: 3499–3502.
- 351 Sodergren E, Weinstock GM, Davidson EH, Cameron RA, Gibbs RA, Angerer RC, Angerer  
352 LM, Arnone MI, Burgess DR, Burke RD, Coffman JA, Dean M, Elphick MR, Etensohn

- 353 CA, Foltz KR, Hamdoun A, Hynes RO, Klein WH, Marzluff W, et al. 2006. The genome  
354 of the sea urchin *Strongylocentrotus purpuratus*. *Science (New York, N.Y.)* 314: 941–952.
- 355 Toonen RJ, Puritz JB, Forsman ZH, Whitney JL, Fernandez-Silva I, Andrews KR, Bird CE.  
356 2013. ezRAD: a simplified method for genomic genotyping in non-model organisms.  
357 *PeerJ* 1: e203.
- 358 Wang S, Meyer E, McKay JK, Matz M V. 2012. 2b-RAD: a simple and flexible method for  
359 genome-wide genotyping. *Nature methods* 9: 808–810.
- 360 Waples RS. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in  
361 high gene flow species. *Journal of Heredity* 89: 438–450.
- 362 Ward RD, Woodwark M, Skibinski DOF. 1994. A comparison of genetic diversity levels in  
363 marine, freshwater, and anadromous fishes. *Journal of Fish Biology* 44: 213–232.
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365 Table 1. Results from individual runs of *dDocent* and *Stacks*. *dDocent* runs varied in the  
366 level of similarity used to cluster reference sequences: A (90%), B (96%), and C (99%). For  
367 *Stacks*, forward reads and reverse reads were separately processed with *denovo\_map.pl*  
368 (*Stacks* version 1.08), using three different sets of parameters: A, minimum depth of coverage  
369 of two to create a stack, a maximum distance of two between stacks, and a maximum distance  
370 of four between stacks from different individuals; B, minimum depth of coverage of three to  
371 create a stack, a maximum distance of four between stacks, and a maximum distance of eight  
372 between stacks from different individuals; and C, minimum depth of coverage of three to  
373 create a stack, a maximum distance of four between stacks, and a maximum distance of 10  
374 between stacks from different individuals. SNP calls were evaluated at different individual  
375 coverage levels: (i) total number of SNPs; (ii) number of SNPS called in 75%, 90%, and 99%  
376 at 3X coverage; (iii) number of SNPS called in 75% and 90% of individuals at 5X coverage;  
377 (iv) number of SNPS called in 75% and 90% of individuals at 10X coverage; and, (v) number  
378 of SNPS called in 75% and 90% of individuals at 20X coverage. Results from forward and  
379 reverse reads of *Stacks* were combined for comparison with *dDocent*, which inherently calls  
380 SNPs on both reads.  
381

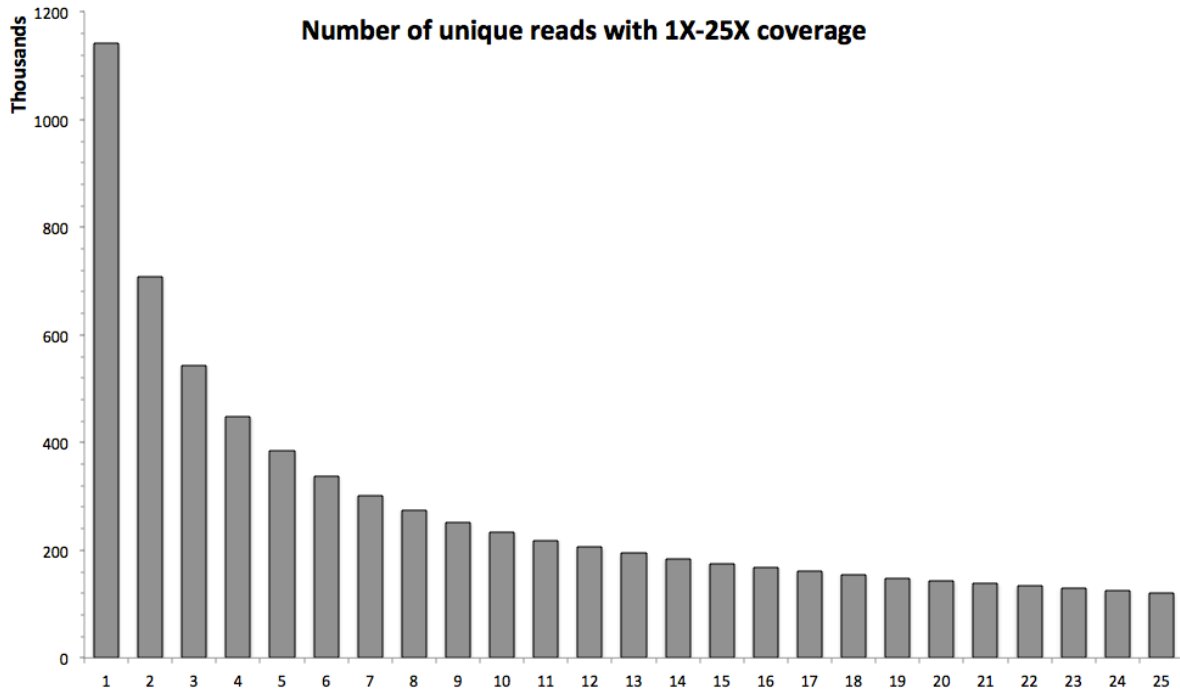
	<i>dDocent</i> A	<i>dDocent</i> B	<i>dDocent</i> C	<i>Stacks</i> A	<i>Stacks</i> B	<i>Stacks</i> C
	Red snapper					
Total 3X SNPS	30,130	30,043	29,907	28,817	33,479	34,459
75% 3X SNPs	12,507	12,249	12,012	4,150	5,735	5,728
90% 3X SNPs	5,368	5,187	5,039	675	987	983
99% 3X SNPs	52	25	5	0	0	0
75% 5X SNPs	8,144	7,946	7,793	2,632	4,351	4,324
90% 5X SNPs	2,775	2,696	2,606	179	579	574

75% 10X SNPs	4,151	4,017	3,914	783	1,618	1,579
90% 10X SNPS	785	729	682	7	48	47
90% IND 90% 5X	5,625	5,499	5,332	806	1,807	1,079
90% IND 90% 10x	2,403	2,298	2,196	129	441	434
Run time	59	58	57	70	47	53
Red drum						
Total 3X SNPS	27,263	27,329	27,295	45,792	50,821	52,366
75% 3X SNPs	23,339	23,328	23,226	24,134	28,991	28,981
90% 3X SNPs	20,764	20,704	20,586	13,439	17,946	17,874
99% 3X SNPs	7,121	7,022	6,937	828	1,264	1,259
75% 5X SNPs	20,015	20,009	19,946	21,021	26,526	26,464
90% 5X SNPs	16,739	16,680	16,588	10,494	15,282	15,207
75% 10X SNPs	16,078	16,042	15,970	12,928	17,018	16,983
90% 10X SNPS	10,988	10,942	10,842	4,159	6,734	6,705
75% 20X SNPs	7,975	7,933	7,824	2,276	3,538	3,516
90% 20X SNPs	3,534	3,512	3,455	243	1,974	1,961
Run time	55	55	53	58	55	65
Silk snapper						
Total 3X SNPS	35,763	35,645	35,509	48,742	55,505	58,352
75% 3X SNPs	17,518	17,244	16,992	7,596	9,705	9,696
90% 3X SNPs	8,586	8,353	8,157	2,007	3,439	3,433
99% 3X SNPs	2,552	2,380	2,276	132	527	523
75% 5X SNPs	10,775	10,547	10,385	4,789	7,290	7,274
90% 5X SNPs	4,936	4,725	4,606	1,225	2,573	2,570
75% 10X SNPs	5,252	5,018	4,876	2,094	3,547	3,546
90% 10X SNPS	2,191	2,058	1,938	489	1,224	1,223
75% 20X SNPs	2,220	2,098	1,984	703	1,415	1,411
90% 20X SNPs	801	721	675	136	417	418
Run time	98	100	60	93	89	204

382

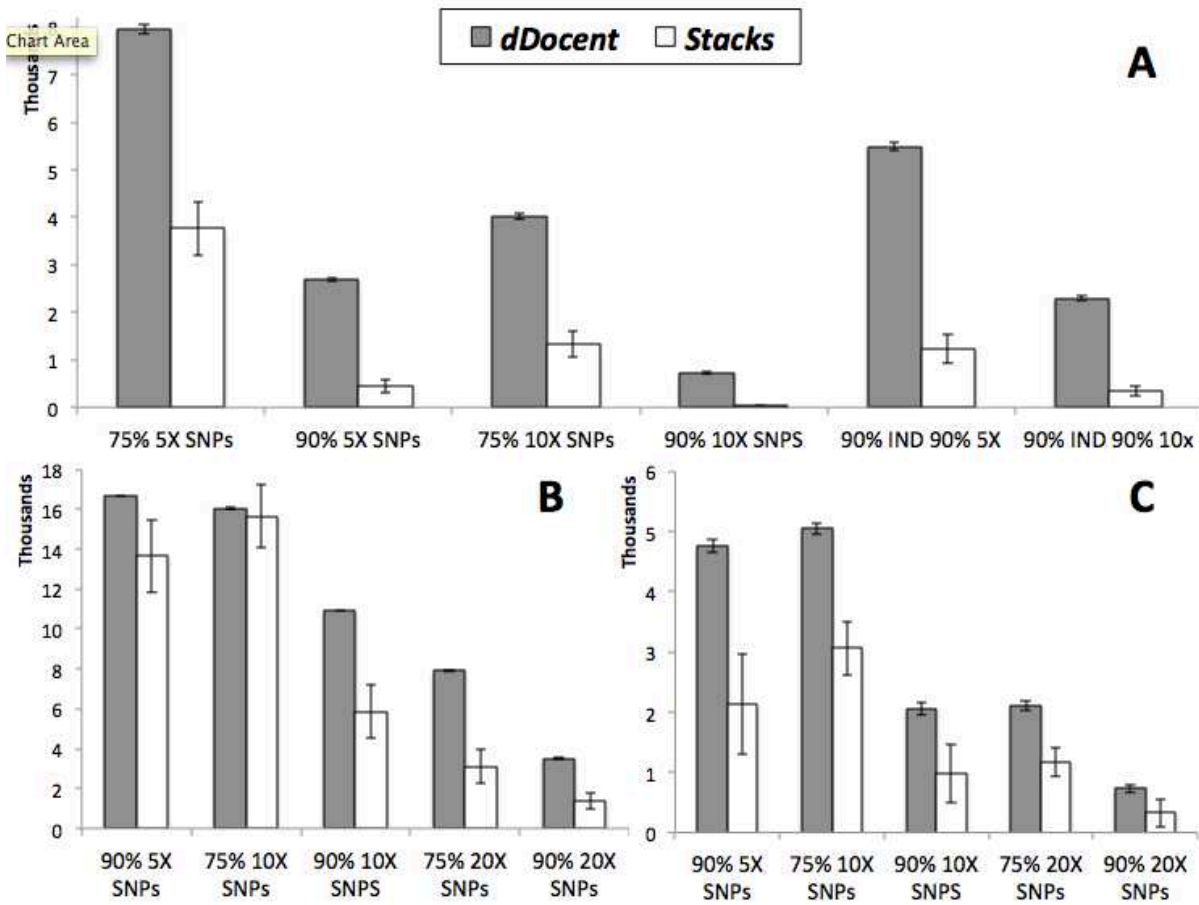
383

384 Figure 1. Levels of coverage for each unique read in the red snapper data set. The horizontal  
385 axis represents the minimal level of coverage and the vertical axis represents the number of  
386 unique paired reads in thousands.



387  
388

389 Figure 2. SNP results averaged across the three different run parameters for *dDocent* and  
 390 *Stacks*. (A) Red snapper, (B) Red drum, (C) Silk snapper (see Methods or Table 1 for SNP  
 391 categories description). Error bars represent standard error.



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