Title

Efficient genotyping with backwards compatibility: converting a legacy microsatellite panel for muskellunge (*Esox masquinongy*) to genotyping-by-sequencing chemistry

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Abstract (250 word limit)
Microsatellites have been a staple of population genetics research for over three decades, and many large datasets have been generated with these markers. For example, microsatellites have been used to conduct genetic monitoring and construct large multigeneration pedigrees as well as genotype thousands of individuals from a given species to create high-resolution baselines of spatial genetic structure. However, the capillary electrophoresis (CE) approach used to genotype microsatellites is inefficient compared to newer genotyping-by-sequencing (GBS) approaches, and researchers have begun transitioning away from CE. Backward compatibility between GBS and CE would facilitate a seamless transition to a more efficient chemistry, while ensuring that research based on CE panels could continue. Here, we explore the feasibility of converting a legacy panel of 15 microsatellites developed for muskellunge (*Esox masquinongy*) from CE to GBS chemistry. Muskellunge are an important sportfish in the Great Lakes region, and the existing microsatellite panel has been used to genotype thousands of samples to develop a region-wide baseline of genetic structure. We successfully converted all 15 microsatellites to GBS chemistry. The GBS chemistry produced high genotyping rates (98%) and had high concordance with CE microsatellite genotypes (99%). Conversion to GBS required redesign of some primers to shorten amplicon length and adjust melting temperature, optimization of primer concentrations, and comparisons with CE genotypes to optimize genotyping parameters; however, none of these steps were especially onerous. Our results demonstrate that it is highly feasible to convert legacy CE panels to GBS, ensuring the seamless continuation of important, often long-term research.

Introduction

Microsatellites provide high power for resolving patterns of population structure and relatedness and have been a mainstay of population genetic studies for over three decades. Genotyping of microsatellites was initially conducted by visualization using polyacrylamide gel electrophoresis, which was then supplanted by more efficient capillary electrophoresis (CE). While genotyping using CE is generally effective and consistent within laboratories, reproducibility among labs can be problematic (Seeb et al. 2007). Additionally, microsatellites contain significant amounts of variation within single loci and exhibit a tendency to “stutter” due to replication slippage during PCR (Hossienzadeh-Colagar et al. 2016). These characteristics can make genotyping challenging, and scoring microsatellites by hand is a skill dwindling among younger researchers. Finally, genotyping microsatellites using CE is labor intensive, and datasets containing hundreds to thousands of individuals genotyped at tens of markers can take months to generate. For these reasons, many laboratories have started to move away from microsatellites in favor of single-nucleotide polymorphisms (SNPs), which can be efficiently genotyped using high-throughput sequencing technologies (Meek and Larson 2019).

Microsatellites are nonetheless still quite useful for applications like parentage, wherein each microsatellite locus provides significantly more power for discrimination among putative parents than less variable genetic markers like SNPs (Fernández et al. 2013). Moreover, large legacy genetic datasets in fields like fisheries management have been built using microsatellite genotypes, and the potential for the discontinuity or, worse yet, discard of these older datasets in light of NGS would be regretful, despite bringing an otherwise welcome technological advancement. For example, standardized sets of microsatellites have been used to genotype tens of thousands of brook trout (*Salvelinus fontinalis)* (Kazyak et al. 2018) and thousands of

93 muskellunge (*Esox masquinongy*) (Turnquist et al. 2017), providing valuable genetic baselines
94 that would take hundreds of thousands or even millions of dollars to replicate. Moreover, genetic
95 monitoring studies often use microsatellites (e.g., Duong et al. 2013), and re-genotyping historic
96 samples would be costly or even impossible, particularly if DNA or samples have degraded over
97 time. Thus, it is extremely important to identify a method that can efficiently genotype
98 microsatellites for comparison with and extension of existing datasets.
99
100 One potential approach that can be used to genotype microsatellites with current high-throughput
101 sequencing technology is genotyping-by-sequencing (GBS). This approach, which is cheaper and
102 more efficient than CE (Meek and Larson 2019), involves sequencing amplicons containing
103 microsatellites on a high-throughput sequencer followed by genotyping using automated
104 software that tabulates allele-specific reads (Zhan et al. 2017). Large GBS microsatellite panels
105 containing more than 100 loci have been developed in multiple salmonids and are currently
106 being genotyped to inform fisheries management and conservation (Bradbury et al. 2018; Layton
107 et al. 2020; Lehnert et al. 2020). However, attempts to integrate legacy microsatellites into these
108 panels have largely been unsuccessful (Bradbury et al. 2018). Reasons for this poor performance
109 include size and read length restrictions caused by finite sequencing length. Additionally,
110 microsatellites that have been used in legacy datasets are often imperfect, as they were developed
111 when the cost to discover a panel of markers was much higher, and thus, poorer performing
112 markers were retained. For example, a number of these markers contain constant bands or
113 present with atypical patterning. Microsatellites also tend to exhibit some level of stuttering,
114 which can make scoring genotypes difficult with both CE and GBS technologies. Recently,
115 Donaldson et al. (2020) explored the feasibility of converting a legacy microsatellite panel to
116 GBS chemistry and found that robustness varied substantially across markers and that DNA
117 quality and quantity significantly impacted results. Donaldson et al. (2020) recommended
118 focusing on tetranucleotide repeats or transitioning to SNPs for low quality DNA applications.
119 However, previous studies have not attempted to optimize the legacy microsatellite assays
120 themselves. It is therefore unclear whether more marginally performing markers can be
121 successfully converted to GBS technology with additional effort.
122
123 Here, we converted an CE-optimized panel of 15 microsatellite loci used for stock discrimination
124 and parentage analysis of muskellunge (*Esox masquinongy*) across the Midwest (Kapuscinski et
125 al. 2013; Miller et al. 2009; Wilson et al. 2016) into a GBS format. Muskellunge are an apex
126 predator and are a highly sought-after sportfish due to their trophy potential. Large stocking
127 programs exist for muskellunge, and multiple genetic studies have been conducted on this
128 species, often with the goal of defining management units and informing stocking programs
129 (Jennings et al. 2010; Miller et al. 2012). Laboratories conducting genetic research on
130 muskellunge have generally used the same microsatellite panel, facilitating comparisons among
131 regions to define large-scale patterns of population structure (e.g., Turnquist et al. 2017). In total,
132 thousands of muskellunge have been genotyped using this 15-microsatellite panel, representing a
133 valuable legacy dataset that can be used for population assignment and analysis of population
134 structure.
135
136 Our study is the first to our knowledge that has attempted to optimize legacy microsatellites for
137 GBS, and our results demonstrate that conversion to GBS may often require marker-specific
138 manipulation, including primer re-design, altering PCR reaction conditions, and manipulating

139 primer concentrations to achieve even sequencing coverage. However, our results indicate that,
140 with relatively minimal effort, it is likely possible to convert legacy microsatellite panels to GBS
141 chemistry. Our results also provide a workflow for researchers to transfer legacy microsatellite
142 panels to current technology, facilitating substantial increases in efficiency without sacrificing
143 backwards compatibility. This workflow will allow conservation geneticists to not only continue
144 to use existing marker panels but also build on legacy datasets, ensuring that important long-term
145 research is not interrupted or jeopardized by changing technologies.
146
147 **Methods**
148
149 *Samples*
150 Subsamples of 96 fin clip tissues were sourced from nine collections of muskellunge
151 opportunistically sampled by the Wisconsin DNR for traditional CE fragment analysis. Samples
152 included Lakes Monona (n = 27), Wissota (n = 16), Whitefish (n = 1), Sissabagama (n = 2),
153 Blaisdell (n = 5), Black Dan (n = 12), and Grindstone (n = 2); the Tiger Cat Flowage (n = 5); and
154 the Detroit River (n = 26). Genomic DNA was extracted using a DNeasy-96 Blood & Tissue Kit
155 (Qiagen, Watham, MA), with minor modification, and stored at -20 °C until use.
156
157 *Traditional electrophoretic fragment analysis*
158 Fifteen muskellunge microsatellite loci were PCR amplified on the 96 gDNAs in five
159 multiplexes (three loci per multiplex) according to (Sloss et al. 2008). Fragment analysis was
160 conducted on a 3730*xl* DNA Analyzer and GeneScan Analysis Software (Applied Biosystems,
161 Foster City, CA). Electrophoretic mobility was scored using Genemapper v4.0 (Applied
162 Biosystems, Foster City, CA). Loci and/or multiplexes that failed initially were PCR amplified
163 and reanalyzed.
164
165 *Microsatellite primer redesign for genotyping-by-sequencing*
166 The FASTA files associated with the microsatellite primer pair sequences described in (Sloss et
167 al. 2008) were queried from the NCBI Nucleotide database using BLAST (Altschul et al. 1990)
168 and imported into Geneious Prime v2019.1.3 (Biomatters, Inc., San Diego, CA). The original
169 primer sequences were realigned to the converted FASTA sequences for easier visualization
170 using Primer3 (Untergasser et al. 2012) as implemented through Geneious Prime. Select
171 individual primers or primer pairs were then redesigned to enable a more uniform amplicon size
172 across loci and to normalize to a target initial annealing temperature of 57 °C in the PCR
173 multiplex. Redesigned amplicons had an average size of 160 bp (σ = 21.4 bp, range = 135 bp to
174 199 bp), while original primers had an average size of 198 bp (σ = 48.6 bp, range 135 bp to 264
175 bp) (Table 1). Seven of 30 total primers, including one primer pair, required redesign (A5
176 forward and reverse, B120 reverse, C1 reverse, D5 forward, D114 reverse, and D116 reverse).
177 Forward primers were tagged on the 5' end with the Small RNA Sequencing Primer (5'-
178 CGACAGGTTCAGAGTTCTACAGTCCGACGATC-3') and reverse primers were tagged on
179 the 5' end with the Multiplexing Read 2 Sequencing Primer (5'-
180 GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3') for subsequent barcoding with 6 bp
181 i5 and i7 indexes in preparation for Illumina sequencing.
182
183 *Amplicon sequencing*

184 We followed an amplicon sequencing (specifically, GT-seq) workflow as per Campbell et al.
185 (2015) and Bootsma et al. (2020), including a multiplex PCR with all 30 primers, barcoding
186 PCR, normalization, pooling, size-selection and purification, and quality control (visualization
187 and dsDNA quantification). The final libraries were submitted to the University of Wisconsin-
188 Madison Biotechnology Center (UWBC) DNA Sequencing Facility for sequencing on a MiSeq
189 PE 150 micro flow cell (Illumina, Inc., San Diego, CA). Ideally, each primer pair would generate
190 equal numbers (approximately 7%) of total reads, and two rounds of primer pooling were
191 conducted toward balancing sequencing output among loci. The first round of testing was
192 conducted on 48 individuals and equalized input concentrations across all primers to 0.25 μM M
193 (0.5 μM M per pair). During the second round, primer pairs were coarsely re-pooled according to
194 their relative read counts generated during the first round. For example, concentrations of primer
195 pairs generating <1% of total reads were adjusted to 1.0 μM, whereas pairs generating nearly
196 15% or more were dropped to ≤0.3 μM, in the final primer pool. All 96 individuals described
197 above were genotyped in the second round.
198
199 *Genotyping-by-Sequencing and comparison to CE genotyping*
200 We used the program FLASH (Magoč and Salzberg 2011) with a maximum overlap (-M) of 150
201 bp to join R1 and R2 reads from the PE data. We then used the program MEGASAT (Zhan et al.
202 2017) with a mismatch parameter of 2 and minimum depth parameter of 10 to call microsatellite
203 genotypes from GBS data. The primer file for MEGASAT was constructed by visually
204 examining sequence data from a few individuals to identify the flanking sequence and
205 microsatellite motif. MEGASAT is a simple program with a graphical user interface that
206 facilitates rapid microsatellite genotyping from GBS data (minutes for this dataset) and includes
207 a helpful utility (Mplot.r) to visualize results. We quantified discrepancy rates between CE and
208 GBS genotyping for each locus and visually examined GBS and CE plots to investigate potential
209 explanations for the discrepancies. Before quantifying discrepancies, we removed any individual
210 missing > 50% of genotypes with either chemistry. Alleles were standardized between
211 chemistries by subtracting the difference in length between the CE and GBS alleles. This
212 standardization works consistently because CE fragments will always be longer than GBS
213 fragments; CE measures the whole fragment whereas GBS data analyzed in MEGASAT only
214 measures a portion (repeat region and some flanking sequence).
215
216 For three loci, comparisons between CE and GBS data indicated that the MEGASAT genotyping
217 algorithm was not calling correct genotypes, and we tuned MEGASAT ratios for these loci to
218 improve genotyping concordance with the CE data. For locus A10 we increased the R4 and R5
219 values to 0.9 to prevent calling stutter bands in homozygote genotypes as heterozygotes. We also
220 increased the R4 and R5 for locus A11 to 0.99 to prevent the same issue. For locus D5, we
221 decreased the R4 value to 0.4 to ensure that the shorter allele in heterozygotes was called. Tuning
222 these ratios was facilitated by comparisons to the CE output, which provided information on
223 likely genotypes. However, visual examination of read depth graphs from MEGASAT can be
224 used to look for idiosyncrasies, such as stutter or failure to call both alleles in heterozygotes (e.g.,
225 due to an atypical presentation in which the shorter allele generates fewer reads than the longer
226 allele) even without accompanying CE data. It is important to note that tuning the MEGASAT
227 ratios based on small datasets may result in incorrect genotypes in the future, and therefore,
228 building a robust dataset of CE and GBS genotypes is necessary to ensure these ratios are tuned
229 correctly to maximize concordance.

230
231 **Results and Discussion**
232 Trial one of GBS optimization on 48 individuals produced 754,260 total reads, of which 699,267
233 (93%) contained primer sequences for target loci. On average, 14,568 reads were retained per
234 individual (σ = 11,301, range 0 to 25,950), and loci had an average coverage of 860 (σ =880,
235 range 3 to 2,969) (Table 2). Trial two of GBS optimization on 96 individuals produced 1,145,477
236 total reads, of which 1,043,851 (91%) contained primer sequences for target loci. On average,
237 10,873 reads were retained per individual (σ = 6,324, range 0 to 20,018). Sequencing in trial two
238 of GBS produced more even coverage across loci than trial one, with an average coverage of 667
239 (σ = 490, range 60 to 1,623). Most notably, the locus with the lowest coverage (B110 in both
240 trials) increased in coverage from 3 on average in trial one to 60 in trial two. An average
241 coverage of three is not enough to produce reliable genotypes, whereas a coverage of 60 should
242 facilitate reliable and accurate genotyping (Nielsen et al. 2011). We achieved a much higher
243 percentage of on-target reads compared to Donaldson et al. (2020) (> 90% versus ~75%) and did
244 not observe significant primer interhybridization in our samples. It is possible that either our
245 PCR was better optimized or that the higher quality DNA we used increased the efficiency of our
246 PCR reactions.
247
248 Two of the 15 loci that we analyzed (D126 and D12) did not produce genotypes that could be
249 scored with the CE approach, while all 15 loci produced usable data with GBS. These two loci
250 routinely fail to amplify or be unequivocally scored with CE and are generally removed from
251 muskellunge datasets (e.g., Turnquist et al. 2017). Although they worked in the GBS dataset, we
252 removed them to facilitate comparisons between CE and GBS. We also removed 10 individuals
253 in total with low genotyping rates (> 50% missing data), five that failed in both chemistries, one
254 that failed in CE only, and four that failed in GBS only. The fact that more individuals failed in
255 GBS only than in CE only suggests that GBS may be slightly less robust to low quality DNA
256 (also observed in Donaldson et al. 2020). However, optimization of the GBS protocol may
257 improve genotyping rates for low quality samples (e.g., Eriksson et al. 2020).
258
259 Levels of missing data across individuals were slightly higher for CE compared to GBS (4.9% on
260 average for CE, 1.7% for GBS; Table 2) suggesting that, while low quality individuals may not
261 amplify well with GBS, levels of missing data in GBS are lower overall than in CE. Missingness
262 at each locus ranged from 0 to 9% for GBS and 0 to 15% for CE, with no clear trends in
263 missingness across the two chemistries (i.e. loci with high missingness in GBS data did not
264 necessarily have high missingness in CE data). Missingness in the GBS data did not appear to be
265 associated with coverage, with the exception of locus B110, which had the lowest coverage and
266 the highest rate of missingness. It is clear that some loci have inherently higher levels of
267 missingness than others; these rates could potentially be decreased through (further) PCR
268 optimization to increase read depth for GBS or signal strength/clarity for CE. If a single
269 multiplex PCR containing all loci for GBS like we employed is not possible, separating out
270 poorer performing loci into smaller multiplexes and then pooling PCR products within
271 individuals before adapter ligation and sequencing may help to reduce variation in coverage
272 (Bootsma et al. 2020).
273
274 We found high concordance between genotypes generated with CE and GBS, with 1,031 out of
275 1,044 genotypes (99%) displaying the same call in both chemistries (Table 2). Observed

276 genotypes that differed between chemistries were split into three categories, heterozygote in
277 GBS data and homozygote in CE (the alternate never occurred), no overlapping alleles between
278 chemistries, and a match for allele one between chemistries but not for allele two (Table 2). A
279 match for allele one but not allele two was the most common error (10/13 errors). In these cases,
280 the smaller allele generally matched between chemistries, while the larger allele was often
281 shifted down in GBS by one repeat (e.g., 2 bp). We suspect that GBS reflected the true allele size
282 as it is derived from sequence rather than electrophoretic mobility, but which chemistry is correct
283 is irrelevant when attempting to standardize genotypes. Other discrepancies included no
284 overlapping alleles (2/13 discrepancies) and a heterozygote in GBS and homozygote in CE (1/13
285 discrepancies). A few themes arose when comparing plots of GBS reads and CE trace plots.
286 First, low read depth did cause some genotyping errors, such as the presence of a false second
287 allele resulting in a heterozygote call. Second, it appears that GBS is better at detecting the large
288 alleles in heterozygotes when the difference in size between alleles is large. Signal intensity
289 diminishes significantly for the large allele relative to the small allele in CE in these situations,
290 and the large allele can often be missed during visualization. Increasing the minimum read depth
291 required to call a genotype would help with reducing errors due to low depth, but there is a
292 tradeoff between accuracy and number of genotypes called. We suggest that applications that
293 require high accuracy, such as parentage analysis, increase read depth cutoffs, whereas lower
294 depth requirements are likely acceptable for less sensitive applications like describing population
295 structure. Failure to detect large alleles is a well-known problem in CE (Dewoody et al. 2006)
296 but may be partially mitigated by ensuring DNA quality is high.
297
298 Discrepancy rates varied substantially by locus, with seven loci displaying no discrepancies,
299 three loci displaying one discrepancy, one locus (D6) displaying two discrepancies, one locus
300 (D5) displaying three discrepancies, and one locus (A11) displaying five discrepancies
301 (discrepancy proportions found in Table 2). The overall number of discrepancies per locus did
302 not appear to be related to average depth. Instead, we hypothesize that the underlying chemistry
303 of each marker is largely responsible for discrepancy rates. For example, accurate detection of
304 the second allele in heterozygotes for locus A11 was variable for both CE and GBS chemistries,
305 indicating that this locus may produce somewhat unreliable results. However, most loci
306 displayed very high concordance, indicating that standardization between CE and GBS data is
307 quite feasible. Finally, whether a locus contained a di- or tetra-nucleotide repeat did not appear to
308 substantially impact genotyping error or discrepancy rates, indicating that both repeat types can
309 produce robust genotypes with CE and GBS. We suggest that researchers attempting to convert a
310 legacy CE panel to GBS re-genotype a large number of individuals (at least 1,000) covering the
311 full range of known alleles to obtain an accurate characterization of potential discrepancies
312 between markers. If consistent errors are found, they can be accounted for, even if the underlying
313 cause of the errors is unknown or cannot be addressed.
314
315 Our results provide encouraging evidence that moving away from CE chemistry does not mean
316 that legacy CE panels need to be discarded. Indeed, conversion to GBS of legacy microsatellite
317 panels represents an opportunity for continued application of these already vetted panels and
318 provides cohesion between old and new visualization technologies, while vastly increasing
319 throughput and decreasing effort. While standardization between the two chemistries will require
320 some effort, we show that this process is possible and not especially onerous. Many datasets
321 generated with CE have taken decades and millions of dollars to assemble (e.g., Duong et al.

322 2013), and re-genotyping them with a new chemistry (e.g. SNPs) is likely not feasible. The
323 ability to merge CE and GBS datasets facilitates backward compatibility, allowing researchers to
324 seamlessly continue important research that leverages these legacy datasets.
325
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333
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Tables

Table 1: Description of 15 muskellunge microsatellite loci (prefix *Ema*) genotyped in this study. Amplicons that were not redesigned for GBS have an "NA" in the redesigned column. T_m is the approximate melting temperature for the primer pair. Primer concentration is the proportion of a primer pair in the total mixture of all primers.

Locus	Original amplicon length (bp)	Redesigned amplicon length (bp)	T_m (°C; est.)	Primer concentration	Repeat motif	Forward primer 5'-3'	Reverse primer 5'-3'
A10	154	NA	55	0.06	TG	GCCAGATGTTCCTCTTCG	TGGTCCAGAAAGCGTTATG
A102	135	NA	57-60	0.06	TG	GGAACAGGTAGTGGGCAGAG	CTTGGTGTGGGGTTTTGTG
A104	168	NA	56-58	0.11	AC	TGCAGTCTGGAACGACATC	TGCTCACAGCAATCTCATG
A11	144	NA	55-58	0.03	AC	TACCGTCACACACAGATGC	TGGTTCTCAAACTTTTTACACC
A5	233	184	53-55	0.11	AC	GTTGTAAGAGCCAATTGGTG	TTGGTTCCATTTATTGCCATG
B110	183	NA	55-56	0.11	AC	TGCCCCGTATCTCTCAAC	GGGTCTGTGTGGAAATAAATG
B120	235	146	55-56	0.11	AC	TGTTCCTGAAAGAGTTTTGTTG	CATAATGTACGATTGTGGCG
C1	212	144	57	0.02	TCCA	CATTGTCTGCCTGAGGTATCT	GTTGTTTCCCAGAGCCATTC
D114	277	150	55-59	0.06	TAGA	TGATCCACAAACACCTGAGTAG	TTTCCGGAGCGCTCTCTC
D116	264	135	55	0.06	TCTA	GCAAAAGGACACAACACTG	AGAATACACATAGAAGGTTGTACA
D126	135	135	55-57	0.08	TAGA	CCAATCAGAATGTGGCATTT	CTGACCTTCAGGGTTCCTTT
D12	199	199	56-58	0.03	TGTC	CGTATGAACAGTAGGTTTTGTCTG	GATAGGCACAATCCACCATC
D4	182	NA	55-56	0.06	CTAT	TCCCTATCGTAAATTACACACG	CAGAATGTGGCATTTTTAACAG
D5	264	183	53-55	0.06	TAGA	AATGACTTGATTTGACACGT	TGGTTATCTGGCATCATTG
D6	163	NA	59	0.06	CTAT	TCACTCTCGCAATTTCTATCTG	GGGGACAGGTAATTTGTAACTG

Table 2: Information on sequencing and genotyping of 13 of 15 microsatellite loci included in this study (D126 and D12 were not included because they did not work consistently for CE). Abbreviations are as follows: average (Avg.), proportion (Prop.), allele (al.), homozygote (homo.), and heterozygote (het.). Coverage is the total number of retained reads for a given locus divided by the number of individuals genotyped. Trial 1 is the first trial before primer concentrations were normalized and trial 2 was conducted with normalized primer concentrations. GBS genotypes compared with CE were produced from trial 2. The proportion of sequencing is the proportion of sequences from a given locus found in that trial. Proportion concordance is the concordance between genotypes where both CE and GBS chemistries were not missing. If a genotype was missing, those proportions are reported in the proportion missing column. Proportion GBS heterozygote CE homozygote is the proportion of individuals where the genotype was a heterozygote with

GBS and a homozygote with CE. Proportion no allele overlap is the proportion of individuals where no alleles overlapped between chemistries. Proportion allele 1 match allele 2 different is the proportion of individuals where allele 1 (the shorter allele) matched between chemistries but allele 2 did not.

Locus	Avg. coverage trial 1	Prop. of sequencing trial 1	Avg. coverage trial 2	Prop. of sequencing trial 2	Prop. Concordance	Prop. Missing GBS	Prop. missing CE	Prop. GBS het. CE homo.	Prop. no allele overlap	Prop. al. 1 match al. 2 different
A10	736	0.07	409	0.04	1.000	0.023	0.023	0.000	0.000	0.000
A102	1987	0.18	744	0.08	0.988	0.000	0.023	0.012	0.000	0.000
A104	62	0.01	410	0.04	1.000	0.000	0.047	0.000	0.000	0.000
A11	24	0.00	172	0.02	0.937	0.047	0.035	0.000	0.013	0.051
A5	679	0.06	421	0.04	1.000	0.023	0.023	0.000	0.000	0.000
B110	3	0.00	60	0.01	0.987	0.093	0.035	0.000	0.013	0.000
B120	39	0.00	361	0.04	0.988	0.000	0.047	0.000	0.000	0.012
C1	2969	0.27	649	0.07	1.000	0.000	0.047	0.000	0.000	0.000
D114	1206	0.11	1623	0.17	1.000	0.000	0.105	0.000	0.000	0.000
D116	1307	0.12	1337	0.14	1.000	0.000	0.035	0.000	0.000	0.000
D4	617	0.06	397	0.04	1.000	0.035	0.035	0.000	0.000	0.000
D5	1217	0.11	885	0.09	0.964	0.000	0.035	0.000	0.000	0.036
D6	331	0.03	1373	0.14	0.973	0.000	0.151	0.000	0.000	0.027
Average	860	0.08	680	0.07	0.987	0.017	0.049	0.001	0.002	0.010
Standard deviation	880	0.08	490	0.05	0.019	0.028	0.037	0.003	0.005	0.017