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1       **Genomic analysis of a second rainbow trout line (Arlee) leads to**  
2               **an extended description of the IGH VDJ gene repertoire**

3  
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26 **Abstract**

27 High-throughput sequencing technologies brought a renewed interest for immune  
28 repertoires. Fish Ab and B cell repertoires are no exception, and their comprehensive  
29 analysis can both provide new insights into poorly understood immune mechanisms, and  
30 identify markers of protection after vaccination. However, the lack of genomic description  
31 and standardized nomenclature of IG genes hampers accurate annotation of Ig mRNA deep  
32 sequencing data. Complete genome sequences of Atlantic salmon and rainbow trout  
33 (Swanson line) recently allowed us to establish a comprehensive and coherent annotation of  
34 Salmonid IGH genes following IMGT standards. Here we analyzed the IGHV, D, and J genes  
35 from the newly released genome of a second rainbow trout line (Arlee). We confirmed the  
36 validity of salmonid IGHV subgroups, and extended the description of the rainbow trout IGH  
37 gene repertoire with novel sequences, while keeping nomenclature continuity. This work  
38 provides an important resource for annotation of high-throughput Ab repertoire sequencing  
39 data.

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## 44 Introduction

45 Immunoglobulins (IG) – also known as antibodies - are the antigen specific receptors  
46 expressed by B cells (Lefranc and Lefranc 2001, Lefranc and Lefranc 2020). **Germline IG loci**  
47 **contain non contiguous V,(D) and J genes that can be assembled to produce expressible**  
48 **genes encoding IG variable regions** ((Tonegawa 1983), reviewed in (Max and Fugmann  
49 2013)). During the B lymphocyte differentiation, IG loci are subjected to genomic  
50 rearrangements of variable (V), joining (J), diversity (D) genes, leading to the expression of a  
51 unique antigen receptor by each B lymphocyte. The IG heavy (IGH) chains present the  
52 highest diversity due to the inclusion of the D gene in a two-step recombination that  
53 produces the VH domain (V-D-J-REGION), while the L chains result from a V-J rearrangement  
54 that produces the VL domain (V-J-REGION). The V-(D)-J junction **recombination** process is  
55 not exact, and the deletion of nucleotides at the joint sites as well as the insertion of so-  
56 called palindromic (P) and non-templated (N) nucleotides by the enzyme **terminal**  
57 **deoxynucleotidyltransferase (TdT) encoded by the DNA nucleotidylexotransferase (DNNT)**  
58 **gene**, are commonly observed (Lefranc and Lefranc 2020). An IGH V-(D)-J rearrangement  
59 leads to an exon encoding an IG variable domain (V-DOMAIN) (Lefranc, Pommie et al. 2003).  
60 V-domains are beta-barrels, and their antigen binding site is made of three loops named  
61 complementarity determining region (CDR)1, 2 and 3 protruding at the top of the domain.  
62 The CDR3 is produced by the recombination of the IGH-V(D)J genes and is the most variable  
63 mainly due to imprecise recombination and addition/deletion of nucleotides at the D-J and  
64 V-(D)-J gene junctions (Wu and Kabat 1970, Lefranc and Lefranc 2001). CDR1 and CDR2 are  
65 encoded by IGHV genes; being structurally unconstrained loops, they are highly variable  
66 across these genes and also participate to the IGV domain variation. Thus, the diversity of IG

67 results from the association of heavy and light chains, from the combination of different V-  
68 (D)-J genes and from junctional mechanisms.

69

70 Teleost fish have typical B cells expressing a large diversity of IG (reviewed in (Fillatreau, Six  
71 et al. 2013)). As in other jawed vertebrates, fish B cell clonal responses are induced by  
72 infection or immunization, and antibodies (Abs) are critical for the protection induced by  
73 most vaccines. Fish B cells can express three IG heavy chain isotypes (reviewed in (Fillatreau,  
74 Six et al. 2013)):  $\mu$  and  $\delta$  (for IgM and IgD classes, respectively) which are conserved in all  
75 classes of vertebrates with jaws; and  $\tau$  (for IgT class), which is specific to fish and is  
76 specialized to mucosal defense (Salinas, Zhang et al. 2011). Isotypic commutation and switch  
77 recombination do not occur in fish, but the structure of the IGH locus and recombination  
78 mechanisms ensure an exclusive expression of either IgM/IgD or IgT within a recombination  
79 unit. IgM/D and IgT are not co-expressed by the same B cell (Hansen, Landis et al. 2005).  
80 Thus, they define two lineages of B cells expressing distinct repertoires built on the same  
81 pool of IGHV genes combined with different IGHD and IGHJ genes (Danilova, Bussmann et  
82 al. 2005, Hansen, Landis et al. 2005, Zhang, Salinas et al. 2010, Castro, Jouneau et al. 2013).

83

84 Many aspects of the biology of fish B cells remain poorly known. The location and  
85 mechanisms of fish B lymphopoiesis are still elusive (Zwollo 2011, Liu, Li et al. 2017); the  
86 equivalent of VPRED (V-preB) and IGLL5 (lambda-5) chains are unknown in these species,  
87 and the main stages of B cell development might be different in fish and mammals. The  
88 bases of allelic/loci exclusion are not understood. It is also important to note that the  
89 anatomy of lymphoid tissues is very different between fish and well-known mammalian  
90 models. For example, the lack of lymph nodes in fish raises the questions of where immune

91 responses are initiated. Detailed phenotypes and functional specialization of B cell subsets  
92 still have to be defined, and memory B cells in fish have not been accurately described  
93 (Yamaguchi, Quillet et al. 2018). The existence of somatic hypermutation of fish IG  
94 sequences is now well established (Yang, Waldbieser et al. 2006, Jiang, Weinstein et al.  
95 2011, Magor 2015), but its regulation remains poorly understood. Because IG are central to  
96 B cell biology, antibody repertoire analysis is essential for the characterization of these  
97 mechanisms and populations. Such approaches remain complex in the absence of a  
98 complete and comprehensive genomic description of IG loci in many fish species.

99

100 The development of high-throughput sequencing technologies during the last decade led to  
101 many studies of the clonal composition of B cell populations. Deep sequencing of human  
102 and mouse B cell repertoires has already revolutionized the field, shedding new light on  
103 ontogeny of B cells, their importance for autoimmunity and cancer, and their implication in  
104 the responses to infections or vaccines (reviewed in (Hou, Chen et al. 2016)). There is also a  
105 growing interest for B cell repertoires among fish immunologists, with connections to basic  
106 questions about immune mechanisms as well as to applied issues such as markers of  
107 protection after vaccination. Salmonids (family Salmonidae) have become important models  
108 for such studies (Castro, Navelsaker et al. 2017, Krasnov, Jorgensen et al. 2017) for several  
109 reasons: (1) their immune system and, more specifically, their antibody responses to  
110 pathogens have been well-studied (2) high-quality genome sequences are available for  
111 farmed and wild species including rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon  
112 (*Salmo salar*), brown trout (*Salmo trutta*), Coho salmon (*Oncorhynchus kisutch*) and chinook  
113 salmon (*Oncorhynchus tshawytscha*). A whole genome duplication (WGD) during the early  
114 evolution of the salmonids led to extraordinary complex repertoires of IGHV, D and J genes

115 clustered at two genomic locations, and of IGL genes belonging to four subtypes (Yasuike,  
116 Boer et al. 2010, Magadan, Krasnov et al. 2019, Rego, Hansen et al. 2020) (3) Atlantic  
117 salmon and rainbow trout are key species for fish farming globally, and their pathogens  
118 have been extensively investigated. IGH loci of salmonids have been studied for thirty years,  
119 but complete genome sequences recently allowed us to establish a comprehensive and  
120 coherent annotation for this family, based on Atlantic salmon (Yasuike, Boer et al. 2010) and  
121 rainbow trout. This led to an IMGT standardized nomenclature of IGH genes in these two  
122 species, taking into account the particularities of Salmonid loci, ie a large number of IGHV,  
123 IGHD and IGHJ genes, a distribution of these genes into two distinct locations on two  
124 chromosomes, and a common set of IGHV subgroups (Magadan, Krasnov et al. 2019). A  
125 consistent nomenclature of IGHV subgroups and IGHJ genes is particularly important for  
126 comparative studies of AIRRseq data (Adaptive Immune Receptor Repertoire sequencing) ie,  
127 RNA-seq sequencing data of IG transcripts expressed in multiple contexts across Salmonids.

128 However, the rainbow trout IGH repertoire defined in (Magadan, Krasnov et al. 2019) based  
129 on the genome sequence of the Swanson line (Omyk\_1.0) was not complete. Indeed, a  
130 number of rainbow trout IGH cDNA sequences present in the GenBank did not have any  
131 close counterpart in the Swanson genome assembly (hence, in the IMGT gene directory).  
132 When annotating our own deep sequencing datasets from isogenic lines (Quillet, Dorson et  
133 al. 2007) genetically rather distant from the Swanson line (Palti, Gao et al. 2014), we also  
134 realized that a number of IGHV genes were missing in the reference IMGT directories. In  
135 fact, such gaps in our annotation could be due to either genome assembly issues or real  
136 differences between the IGH loci of the Swanson line and those of other trout lines. For  
137 example, the PacBio long read sequencing technology used for the Arlee genome  
138 significantly improved the quality of the assembly, compared to the Swanson genome

139 (based on Illumina sequencing), and hence some differences in the annotation of IGHV  
140 genes between the Swanson and Arlee may be caused by this difference in the quality of the  
141 two genome assemblies rather than biological differences between the two rainbow trout  
142 lines.

143

144 In this work, we pursued our effort of annotation and nomenclature standardization using  
145 the newly released genome assembly from the Arlee line of rainbow trout  
146 (USDA\_OmykA\_1.1). Both the Swanson and Arlee are homozygous clonal YY male lines  
147 developed through androgenesis by the lab of Gary Thorgaard at Washington State  
148 University. The Swanson line was derived in 1991 from a semi-wild population from the  
149 Swanson River in the Kenai Peninsula of Alaska (Robison, Wheeler et al. 2001). The Arlee  
150 clonal line was derived from the Arlee strain, a domesticated hatchery strain that was used  
151 by the Montana Department of Fish, Wildlife and Parks (Ristow, Grabowski et al. 1998) and  
152 is thought to have originally been collected from Northern California like most farmed  
153 rainbow trout stocks that were imported to Europe (Gary Thorgaard, Personal  
154 Communication). In addition, fish from the Arlee line were found to have low nonspecific  
155 cellular cytotoxicity in the peripheral blood (Ristow, Grabowski et al. 1995). Here we aimed  
156 at extending the IMGT rainbow trout IGH annotation to a second line coming from a  
157 different region of North America. Testing the salmonid IGH classification and nomenclature  
158 established from the Swanson line on the IGH repertoire present in the Arlee line confirmed  
159 the validity of the IGHV subgroups, and provided a first picture of the variation of these  
160 genes between rainbow trout populations. This update was performed to take into account  
161 all new sequences, while keeping nomenclature continuity. **We thus** produced a non-  
162 redundant directory of IGHV sequences to help with annotation of IG repertoire in this

163 species. This resource will significantly extend the diversity of sequences available for the  
164 IMGT/HighV-QUEST (Alamyar, Giudicelli et al. 2010, Alamyar, Giudicelli et al. 2012, Li,  
165 Lefranc et al. 2013) or other annotation tools.

166

## 167 **Materials and methods**

### 168 *Gene annotation*

169 Chromosome 12 (CM023230.2) and 13 (CM023231.2) from the recently released rainbow  
170 trout genome assembly (USDA\_OmykA\_1.1) which was derived from the Arlee line were  
171 examined to locate IGH loci. IGHV, IGHD and IGHJ gene sequences were previously  
172 identified by Magadan *et al.* (Magadan, Krasnov et al. 2019) in the rainbow trout genome  
173 assembly (Omyk\_1.0) which was derived from the Swanson line (Pearse, Barson et al. 2019)  
174 and were used as queries to identify the chromosomal regions containing the IGH loci in the  
175 Arlee genome assembly. All these IGH genes identified in the Swanson genome assembly  
176 had been previously submitted to the Immunoglobulins (IG), T cell receptors (TR) and major  
177 histocompatibility (MH) Nomenclature Sub-Committee (IMGT-NC) of the International  
178 Union of Immunological Societies (IUIS) Nomenclature Committee. The obtained IMGT gene  
179 names had been included in the IMGT-NC Report #2019-10-0402 ([www.imgt.org](http://www.imgt.org)).

180

### 181 *Extension of the IMGT nomenclature to the Arlee line IGH repertoire*

182 IGHV sequences from the two lines were aligned pairwise, and clusters of sequences  $\geq 98\%$   
183 identical were identified. This first step produced a list of sequences for which a  
184 straightforward 1:1 correspondence exist between genes from each genetic background.

185 We then subjected the other IGHV sequences from the Arlee line to IMGT/V-QUEST  
186 [*Oncorhynchus mykiss* and *Salmo salar*; IGH] and IMGT/BlastSearch (IMGT/GENE-DB

187 nucleotide sequences (F+ORF+inframeP)) analysis. For functional genes or for  
188 ORF/pseudogenes with lower degree of mutation, IMGT/V-QUEST identified the subgroup  
189 to which assign the IGHV gene. When a higher mutation/indel number impaired the  
190 IMGT/V-QUEST analysis, subgroup assignment was operated from IMGT/BlastSearch results.  
191 All IGHV sequences from the Arlee line were assigned to an IGHV subgroup defined from the  
192 Atlantic salmon repertoire.

193

194 *Comparison of the IMGT gene sets from Swanson and Arlee lines with a collection of IGHV*  
195 *cDNA available in GenBank*

196 Rainbow trout IGHV cDNA sequences were collected from GenBank using Blastn with all  
197 sequences from Swanson and Arlee. cDNA sequences (301 bp upstream the YxC pattern  
198 corresponding to the end of the V gene) were clustered using VSEARCH (Rognes, Flouri et al.  
199 2016) (threshold 98% identity). An identity matrix was inferred from pairwise local sequence  
200 alignments computed with ClustalW.

201

## 202 **Results and discussion**

### 203 **1. Comparison of the structure of IGHV loci between Swanson and Arlee rainbow** 204 **trout lines**

205 The newly released genome assembly from the Arlee clonal line of rainbow trout  
206 (USDA\_OmykA\_1.1) resulted in elucidation of complex loci such as the IGH locus. In the  
207 Arlee genome assembly, the IGH genes are within two regions named IGHA and IGHB, that  
208 are located on chromosomes 13 (CM023231.2) and 12 (CM023230.2), respectively. This is  
209 similar to the rainbow trout reference genome Omyk\_1.0 (GCA\_002163495.1) (Pearse,  
210 Barson et al. 2019), which was obtained from the Swanson homozygous line (Magadan,

211 Krasnov et al. 2019). IGHA and IGHB loci span a total length of approximately 5 and 4.3 Mbp,  
212 respectively. Each locus comprises a number of IGHV genes upstream of a few D-J-C-clusters  
213 comprising IGHD and IGJ genes associated to an IGHC gene, either IGHT or, for the most 3'  
214 cluster, IGHM and IGHD) (see Figure 1, and figure S1 for comparison with the map of the  
215 IGH loci from Swanson).

216 The annotation of IGH loci in the rainbow trout reference genome Omyk\_1.0 had provided a  
217 first rainbow trout IMGT reference directory for IGH sequences  
218 (<http://www.imgt.org/vquest/refseqh.html>). In the Omyk\_1.0 genome (Swanson line), a  
219 total of 129 IGHV genes were identified, of which 57 were considered fully functional or  
220 with an open reading frame (ORF) without stop codon. The annotation of USDA\_OmykA\_1.1  
221 genome (Arlee line) resulted in a lower number of IGHV genes, but a higher number of  
222 functional genes: a total of 118 IGHV genes were identified, of which 68 seem to be  
223 functional or with ORF. The distribution of these IGHV genes on chromosomes is as follows:  
224 61 IGHV genes on chromosome 12, of which 31 are functional or with ORF, and 57 IGHV  
225 genes on chromosome 13, of which 37 are functional or with ORF.

226 A comparison of the genomic location of IGHV genes between Arlee (Figure 1) and Swanson  
227 (Figure S1) genome sequences reveals a substantial level of variation (see genes encircled in  
228 red and blue in Figure 1 and Figure S1, which are found in only one assembly) especially in  
229 the locus B on chromosome 12. However, the order of functional genes – as identified based  
230 on both location and sequence similarity - was globally conserved, allowing an upgrade of  
231 the nomenclature without major discontinuity.

232

233 **2. Creation of an extended nomenclature of rainbow trout IGHV genes**

234 We have previously established a standardized IMGT nomenclature of salmonid IGH genes  
235 based on genome assemblies for Atlantic salmon and the Swanson line of rainbow trout.  
236 Differences between the IGH loci of the Swanson and Arlee lines of rainbow trout led us to  
237 update this nomenclature for this species.

### 238 **2.1 Extending IGH nomenclature to the Arlee strain genome**

239 While it was important to maintain nomenclature continuity as much as possible, a gene  
240 name could be transferred only when there was convincing evidence of the gene  
241 equivalence. We therefore combined sequence similarity and positional information to  
242 extend the IMGT nomenclature to the repertoire of the Arlee line. Finding the best  
243 compromise can be challenging especially for IGHV genes in the large duplicated loci found  
244 in Salmonids. We therefore used at best the flexibility offered by the IMGT names. IGHV  
245 names are constituted as follows: first, IGHV, then the subgroup number, the letter D if the  
246 gene is located in the duplicated locus (IGH B, on chromosome 12), then a first dash and a  
247 number ( $N_1$ ) defining the gene rank in the locus of reference (for Salmonids, *Salmo salar*,  
248 see (Magadan, Krasnov et al. 2019)) and finally, when necessary, a second dash followed by  
249 a number( $N_1$ ) denoting the identity of a gene within a gene microcluster. For example, the  
250 name IGHV16D-69-4 denote a gene belonging to the subgroup 16, located in a microcluster  
251 at the reference rank 69 within locus IGH B; the final number “4” does not refer to a  
252 position information, but is only an ID usually based on the date of the gene description in  
253 the microcluster. While this name format may appear to be unusually complicated, it  
254 provides an exquisite flexibility to integrate additional genes, or describe gaps while keeping  
255 a nomenclature consistent across haplotypes or genomes updates.

256

### 257 **2.2 IGHV genes**

258 We first postulated that IGHV (V exon) nucleotide sequence of the same gene should  
259 generally not differ by more than 2% between the two lines. We therefore performed a  
260 clustering based on this criterion, and found a Swanson counterpart for 87 genes found in  
261 the Arlee line, out of 118. Overall, 73% of the Arlee IGHV genes, belonging to subgroups 1, 2,  
262 3, 4, 6, 8, 9, 10, 11, 12, 14, 15, and 16, could be easily matched to a unique counterpart in  
263 Swanson (*ie*, with a nucleotide sequence >98% similar). Within this subset, we could verify  
264 that all Arlee IGHV located within the locus A on the chromosome 13 (and, respectively,  
265 within the locus B on chromosome 12) matched Swanson genes from the corresponding  
266 locus.

267 The other Arlee IGHV sequences were then analyzed individually. IMGT/BlastSearch on  
268 IMGT/GENE-DB nucleotide sequences (F+ORF+inframeP) and IMGT/V-QUEST analysis on  
269 Atlantic Salmon and Swanson rainbow trout showed that all these sequences could be  
270 classified into one of the 16 IGHV subgroups identified in our previous work ([Table S1](#)).

271 These sequences were attributed novel names following the format described above, and  
272 extending the member list of each subgroup previously defined in (Magadan, Krasnov et al.  
273 2019). [Table S1](#) presents the IGHV repertoires from Swanson and Arlee lines and the  
274 updated gene nomenclature.

275 We did not detect obvious imbalance of variation between subgroups between Swanson  
276 and Arlee genomes. However, we found 3 functional IGHV7 and 3 IGHV14 in the Arlee line  
277 while in the Swanson assembly these subgroups count only pseudogenes (or ORF). In both  
278 lines, neither IGHV5 nor IGHV16 subgroup contains any functional gene.

279 Importantly, our data support the validity of the IGHV salmonid subgroups previously  
280 defined in (Magadan, Krasnov et al. 2019) based on *Salmo salar* and *Oncorhynchus mykiss*.

281 Future work will be necessary to determine if other *Oncorhynchus* (such as Coho, Chinook,

282 Sockeye salmon) or *Salmo* (such as the Brown trout) species possess IG genes that require  
283 the creation of additional subgroups.

284 Rainbow trout Swanson and Arlee lines come from distinct populations: the Swanson line is  
285 from Alaska, while the Arlee line is from the southern interior region (Montana). The  
286 differences between the subgroup content of these lines illustrate that IGHV sequences are  
287 subjected to relatively fast variation. Indeed, germline insertions and deletions involving  
288 genes from nearly every IGHV subgroup has been described in humans (Pramanik, Cui et al.  
289 2011, Watson and Breden 2012), suggesting that particular regions of IGH locus may be  
290 subject to frequent rearrangements. Significant difference between the levels of divergence  
291 might imply difference in selection pressure on regions of IGH locus and the functional  
292 importance of these IGHV genes. Thus, a wider dataset collecting the full IGHV repertoire of  
293 multiple lines would allow an in-depth analysis to investigate patterns of geographic  
294 variation. In particular, it would be interesting to test whether these variations may be  
295 adaptive, possibly under selection pressure exerted by pathogens.

296

### 297 **2.3 IGHD and IGHJ genes**

298 In fish, IGH rearrangements encoding the three immunoglobulin classes (IgM, IgD and IgT/Z)  
299 can use the same set of IGHV genes when expressed from a given IGH locus (i.e. IGHA or  
300 IGHB in Salmonids). In contrast, distinct IGHD-IGHJ gene clusters are associated either to  
301 IGHT or to IGHM-IGHD constant genes (Hansen, Landis et al. 2005). Our nomenclature of  
302 salmonid IGHD and IGHJ genes reflects this particular feature of fish IGH (Tables 2 and 3). D  
303 and J gene names are constituted as follows (Magadan, Krasnov et al. 2019): first, IGHD or  
304 IGHJ then a number X, followed, if the gene is part of DJC cluster encoding IgT, by "T" and a  
305 second number Y, then by "D" if the gene belongs to the duplicated locus (ie locus B), and

306 finally by a star (“\*”) and the allele number. X is the rank of the gene within a DJC cluster,  
307 and Y is the rank of the DJ cluster, both counted from 5’ to 3’ end of the locus. For example,  
308 IGHD1T2D\*01 would be the name of the first allele (\*01) of the first D gene (D1) within the  
309 second IGHD-IGHJ cluster encoding IgT, in the IGHB locus. Our annotation of the IGHD and J  
310 genes of the Arlee strain is largely based on the annotation established for the Swanson  
311 strain of rainbow trout and for the Atlantic salmon.

312 Based on the conserved pattern of their RS sequences, characterized by 12 bp spacer and  
313 typical heptamer and nonamer sequences (Ramsden, Baetz et al. 1994), 26 IGHD genes  
314 were annotated in the Arlee genome assembly, 14 on Chromosome 12 (locus IGHB) and 12  
315 on Chromosome 13 (locus IGHA) (Figure 1). The previous annotation in Swanson genome  
316 assembly described 19 IGHD genes on Chr 12, and 11 on Chr 13 (Magadan, Krasnov et al.  
317 2019) and Figure S1. Analysis of nucleotide similarity of Arlee IGHD coding regions  
318 indicated that most of them have identical counterparts in the same or different locus of  
319 this trout line (Figure 2). In general, they also present a counterpart in Swanson with 98-  
320 100% of nucleotide sequence identity. There are 5 exceptions in the IGHB locus:  
321 IGHD2T2D, IGHD1T3D, IGHD2T3D, IGHD3T3D and IGHD4T3D (see Table 1 and Figure S2A),  
322 either due to real differences between the IGHD genes of the Swanson and Arlee lines, or  
323 to assembly issues likely in Swanson. As previously described in Swanson genome  
324 assembly (Magadan, Krasnov et al. 2019), only one "long" D gene (37 bp) is found in Arlee  
325 rainbow trout genome assembly within the IGHA locus (Figure 2).

326 Twenty IGHJ genes were identified in Arlee genome assembly, 11 on Chromosome 12 (IGHB  
327 locus) and 9 on Chromosome 13 (IGHA locus). In the Swanson genome assembly 9 IGHJ  
328 genes were identified on Chr 12, and 10 on Chr 13 (Magadan, Krasnov et al. 2019). Two  
329 Arlee IGHJ genes (IGHJ1T2D and IGHJ2T2D) located on Chr12 are pseudogenes, while the

330 other IGHJ genes appear to be functional. They present a conserved 5'J-RS, with the  
331 nonamer and heptamer separated by 22/23bp. As described for IGHD genes, the functional  
332 Arlee IGHJ genes have similar counterparts in the same or different locus (Figure 3 and  
333 Figure S2B). They also have highly similar or identical counterpart in Swanson (with 98-  
334 100% of nucleotide sequence identity), with the exception of IGHJ pseudogenes,  
335 IGHJ1T3D, and IGHJ2T3D, all of them belonging to IGHB locus (Table 2).

336 It is important to note that the impact of sequencing and assembly errors is particularly  
337 problematic for the comparative annotation of short genes similar to each other and  
338 organized in multiple clusters as IGHD and IGHJ. Therefore, it will be important to integrate  
339 new information from genomic and transcriptome/repertoire data to reach a definitive  
340 consensus.

341

### 342 **3. Combined sequences from Swanson and Arlee lines cover most of the rainbow** 343 **trout repertoire of functional IGHV genes**

344 In salmonids, several research groups have started using repertoire sequencing to monitor  
345 IG responses to infection or vaccination (Castro, Jouneau et al. 2013, Krasnov, Jorgensen et  
346 al. 2017, Magadan, Jouneau et al. 2018, Magadan, Jouneau et al. 2019, Navelsaker,  
347 Magadan et al. 2019). However, the annotation of such data has been hampered by the lack  
348 of standardized sequence databases. The analysis of somatic hypermutations in IG  
349 sequences was especially problematic in absence of a genomic reference resource for IGH  
350 genes (Abos, Estensoro et al. 2018). Another issue was that original names did not reflect  
351 phylogenetic relationships between genes from rainbow trout and Atlantic salmon, making  
352 inter-specific comparison very complicated.

353 This encouraged us to use our IMGT standardized nomenclature and database of IGH genes  
354 from Atlantic salmon and rainbow trout, to construct an IMGT IGH gene directory for  
355 annotation of RepSeq data using IMGT/HighV-QUEST (Alamyar, Giudicelli et al. 2010,  
356 Alamyar, Giudicelli et al. 2012, Li, Lefranc et al. 2013). For rainbow trout, this directory  
357 comprises all IGHV genes detected in the genome of the Swanson line. **However, we**  
358 **realized that several IGHV genes in experimental datasets produced from French farmed**  
359 **trout were missing in the Swanson-based database (R Castro, personal communication; S**  
360 **Magadan and P Boudinot, unpublished data; these sequences were from the "Synthetic"**  
361 **strain maintained at INRA (D'Ambrosio, Phocas et al. 2019)).** These sequences were actually  
362 highly similar to genes found in the Arlee genome. We therefore believe that the  
363 annotation of the Arlee IGH loci presented in this work contributes to fill the gaps noted in  
364 the Swanson IGH repertoire.

365

366 To further test if the combination of Swanson and Arlee IGH loci provides a **broad**  
367 description of the IGHV repertoire of rainbow trout, we mined the GenBank for such  
368 sequences. We found a large collection of rainbow trout cDNA (835 entries) sequenced by  
369 Steve Kaattari and his colleagues, using fish from a Troutlodge stock (Brown, Kaattari et al.  
370 2006). This stock was presumably coming from Washington state, hence from a third trout  
371 population different from Swanson and Arlee. To compare this cDNA collection to the Arlee  
372 and Swanson repertoires, we built the complete similarity matrix based on pairwise  
373 alignment of all pairs of sequences, and selected all cDNA which were less than 98% similar  
374 to any Arlee or Swanson sequence. We thus identified 53 sequences (6% of the whole  
375 collection). These sequences clustered into 10 sets of sequences more than 98% similar to  
376 each other (Table 3). All these sequences were then analyzed using IMGT/V-QUEST and

377 IMGT/BlastSearch (IMGT/GENE-DB nucleotide sequences F+ORF+allP) to check how  
378 divergent they were from previously annotated germline gene sequences (Table 3).

379 Six clusters (1-6; 40 sequences) were 95%-98% similar to their best hit among Swanson or  
380 Arlee sequences, and two other clusters (7-8; 8 sequences) were 95-96% similar to  
381 sequences present in Arlee only. Differences between these sequences and their known  
382 best match do not show bias in the CDR1-IMGT and CDR2-IMGT (Figure S3). These  
383 observations are not in favor of somatic hypermutation, however we cannot exclude that  
384 this mechanism may explain (at least some of) these differences.

385

386 Two clusters were without  $\geq 95\%$  match either in Arlee or Swanson (clusters 9-10, see Table  
387 3). For cluster 9, differences between cDNA sequences and their best batch IGHV1-41\*01  
388 were distributed along the V domain (2 in CDR1, 4 in FR2, 3 in CDR2 and 3 in FR3, see Figure  
389 S3), while for cluster 10 they were rather concentrated in FR1 (10 positions in FR1, one in  
390 CDR1, and 2 in FR2, see Figure S3). These sequences may represent two novel IGHV genes,  
391 which were not present in the IGH loci of Arlee or Swanson. Overall, these observations  
392 indicate that the sequence database constructed from Swanson and Arlee genomes covers a  
393 large fraction of the entire rainbow trout repertoire of functional IGHV genes. Locally,  
394 populations certainly have additional variants but our data suggest that they likely not  
395 represent more than a small proportion of their functional genes. This point will be clarified  
396 by future analyses of Ig repertoire sequencing data from trout of various origins. There is  
397 apparently a substantial level of structural variation across trout populations, which affects  
398 the number and composition of the set of pseudogenes in the IGH loci. Future analyses will  
399 show if the locus B is particularly prone to such variations.

400

## 401 **Conclusions**

402 This work extended the IMGT classification and nomenclature of rainbow trout IGH genes  
403 from the Swanson line to the Arlee line. Our results confirmed the validity of the IGHV  
404 subgroups, which were all found in both lines, **and the global structure of both loci (ie, the**  
405 **order of IGHV functional genes)**. It also unveiled differences at the gene level, especially for  
406 IGHV **pseudogenes**. It is difficult at this stage to determine the respective contributions of  
407 the sequencing technology used and of true genetic divergence to these differences. A  
408 comparison of our extended IGH sequence database (Arlee+Swanson) with all cDNA  
409 sequences available in the GenBank strongly suggested that it **provides a broad description**  
410 **of the rainbow trout repertoire of functional genes**. Hence, this database provides a basis  
411 for robust annotation of repertoire sequencing (AIRR) datasets. Importantly, it makes it  
412 possible to get sequences annotated at the gene level rather than at the subgroup level,  
413 which will be crucial for polymorphism / hypermutation analyses. Future data combining  
414 genome and transcript sequencing will help to determine the respective contribution of  
415 polymorphism and somatic hypermutation in the variation of IGHV mRNA sequences across  
416 various fish stocks, organs, and immune status.

417

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429

### 430 **Figure legends.**

431 **Figure 1.** Rainbow trout (*Oncorhynchus mykiss*, line Arlee) IGH locus (A) on chromosome 13  
432 (**CM023231.2**) (IGHA) and (B) on chromosome 12 (**CM023230.2**)(IGHB). The orientation of  
433 both rainbow trout IGH loci are forward (FWD). IGHV genes are in blue, IGHD in black, IGHJ  
434 in red, and IGHC in green. Pseudogenes are in grey. The boxes representing the genes are  
435 not to scale. Exons are not shown. Distances are indicated in Mbp (million base pair) or in kb  
436 (kilobase). IGH gene names are according to IMGT nomenclature. The location of IGHC exon  
437 clusters is schematically indicated and is analyzed in details in Gao *et al.* (Gao, Magadan et  
438 al. 2020). This map can be compared with the map of IGH A and B loci based on the  
439 Swanson genome (see  
440 [http://www.imgt.org/IMGTrepertoire/index.php?section=LocusGenes&repertoire=locus&species=rainbow\\_trout&group=IGH](http://www.imgt.org/IMGTrepertoire/index.php?section=LocusGenes&repertoire=locus&species=rainbow_trout&group=IGH)).

442 **IGHV genes encircled in red dotted lines were not found in the Swanson assembly.**

443

444 **Figure 2.** Alignment of coding nucleotide IGHD sequences annotated from the Arlee  
445 (OmykA) genome assembly (USDA\_OmykA\_1.1). Sequences from the locus A (respectively  
446 B) are represented in blue (respectively in black).

447

448 **Figure 3.** Alignment of coding nucleotide IGJH sequences annotated from the Arlee (OmykA)449 genome assembly (USDA\_OmykA\_1.1). Pseudogene sequences are denoted by a ( $\psi$ ).

450

451

452 **Tables**453 **Table 1.** IGHD genes annotated in Arlee genome (USDA\_OmykA\_1.1) on chromosome 13

454 (CM023231.2) and chromosome 12 (CM023230.2) and the corresponding counterpart in

455 Swanson genome (Omyk\_1.0)

456

Locus	<i>O. mykiss Arlee</i>			<i>O. mykiss Swanson</i>	
	IGHD gene	Chromosomal position <sup>1</sup>		IGHD gene	IGHC associated gene
		Start	End		
IGHA (Chr 13)	IGHD1T1*01	51936009	51936021	IGHD1T1*01	IGHT
	IGHD2T1*01	51936280	51936294	IGHD2T1*01	
	IGHD3T1*01	51943169	51943181	IGHD3T1*01	
	IGHD1T2*02	51987496	51987532	IGHD1T2*01	
	IGHD3T2*01	51988273	51988285	IGHD3T2*01	
	IGHD2T2*01	51988548	51988562	IGHD2T2*01	IGHM-IGHD
	IGHD1*02	52049134	52049146	IGHD1*01	
	IGHD2*01	52049585	52049598	IGHD2*01	
	IGHD3*01	52050027	52050038	IGHD3*01	
	IGHD4*01	52050814	52050825	IGHD4*01	
	IGHD5*01	52051256	52051267	IGHD5*01	
IGHD6*01	52051711	52051722	IGHD6*01		
IGHB (Chr 12)	IGHD2T1D*01	84581653	84581667	IGHD2T1D*01	IGHTD
	IGHD3T1D*01	84588394	84588406	IGHD3T1D*01	
	IGHD4T1D*01	84589031	84589042	IGHD4T1D*01	
	IGHD2T2D*01	84644559	84644573		
	IGHD1T3D*01	84681104	84681116		
	IGHD2T3D*01	84681518	84681537		
	IGHD3T3D*01	84688239	84688251		
	IGHD4T3D*01	84688876	84688887		
	IGHD1D*02	84769576	84769588	IGHD1D*01	IGHMD-IGHDD
	IGHD2D*01	84770031	84770043	IGHD2D*01	
	IGHD3D*01	84770461	84770472	IGHD3D*01	
	IGHD4D*01	84771248	84771259	IGHD4D*01	
	IGHD5D*01	84771692	84771703	IGHD5D*01	
IGHD6D*01	84772148	84772159	IGHD6D*01		

457 <sup>1</sup> Chromosomal position of the coding region, without RS sequences.



459 **Table 2.** IGHJ genes annotated in Arlee genome (USDA\_OmykA\_1.1) on chromosome 13  
 460 (CM023231.2) and chromosome 12 (CM023230.2) and the corresponding counterpart in  
 461 Swanson genome (Omyk\_1.0)  
 462

Locus	<i>O. mykiss</i> Arlee			<i>O. mykiss</i> Swanson	
	IGHJ gene	Chromosomal position <sup>1</sup>		IGHJ gene	IGHC associated gene
		Start	End		
IGHA (Chr 13)	IGHJ1T1*01	51936627	51936674	IGHJ1T1*01	IGHT
	IGHJ2T1*01	51937103	51937154	IGHJ2T1*01	
	IGHJ1T2*01	51988942	51988989	IGHJ1T2*01	
	IGHJ2T2*02	51989455	51989506	IGHJ2T2*01	IGHM-IGHD
	IGHJ1*01	52065893	52065943	IGHJ1*01	
	IGHJ2*01	52066062	52066112	IGHJ2*01	
	IGHJ3*01	52066267	52066317	IGHJ3*01	
	IGHJ4*02	52066602	52066652	IGHJ4*01	
	IGHJ5*02	52066987	52067036	IGHJ5*01	
IGHB (Chr 12)	IGHJ1T1D*01	84582048	84582095	IGHJ1T1D*01	IGHTD
	IGHJ2T1D*01	84582556	84582607	IGHJ2T1D*01	
	IGHJ1T2D <sup>2</sup>	84645351	84645407		
	IGHJ2T2D <sup>2</sup>	84646319	84646392		
	IGHJ1T3D*01 <sup>3</sup>	84681919	84681966		IGHMD-IGHDD
	IGHJ2T3D*01 <sup>3</sup>	84682434	84682485		
	IGHJ3D*02	84789478	84789528	IGHJ3D*01	
	IGHJ4D*01	84789647	84789697	IGHJ4D*01	
	IGHJ5D*01	84789852	84789902	IGHJ5D*01	
	IGHJ6D*02	84790211	84790261	IGHJ6D*01	
	IGHJ7D*01	84790599	84790648	IGHJ7D*01	

463 <sup>1</sup> Chromosomal position of the coding region, without RS sequences. <sup>2</sup> Pseudogenes are highlighted in  
 464 grey. <sup>3</sup>Arlee IGHJ1T3D and IGHJ2T3D nucleotide sequences present 100% identity with Swanson  
 465 IGHJ1T1D\*01 and IGHJ2T1D\*01, respectively.  
 466

467 **Table 3.** Clusters of IGHV sequences from the cDNA collection with no match (>98%  
 468 similarity) in Swanson and/or Arlee genomes. When a match was found in Arlee only,  
 469 sequences are underlined and in italic.  
 470

	Cluster composition	IGHV subgroup	% of identity with the best match	Best match (swanson)
1	EF442475	8	96.81%	Swanson IGHV8-30*01 F, or Swanson IGHV8-40*01 F
2	EF438713.1; EF438707.1 EF438675.1; EF438627.1 EF438621.1; EF438612.1 EF438604.1; EF438574.1 EF438570.1; EF438569.1 EF438517.1; EF438516.1 EF438499.1	9	97.54%; 97.54% 97.19%; 97.19% 97.54%; 97.54% 97.54%; 97.54% 97.54%; 97.54% 97.54%; 97.54%	Swanson IGHV9-23*01 F
3	EF438527.1; EF438508.1; EF438505.1	9	96.49%; 96.14% 96.49%	Swanson IGHV9-15*01 F

4	EF438463.1; EF442512.1; EF442511.1.1	1	96.81%; 96.10% 96.81%	Swanson IGHV1-13*01 F
5	EF438762.1; EF438736.1; EF438439.1	8	95.39%; 95.74% 95.39%	Swanson IGHV8-11*01 P
6	EF467934.1; EF438660.1 EF438653.1; EF438651.1 EF438640.1; EF438563.1 EF438560.1; EF438525.1 EF438500.1; EF438491.1 EF438465.1; EF438450.1 EF438449.1; EF438421.1 EF438407.1; EF438399.1 EF438387.1	3	97.59%; 97.59% 97.94%; 97.94% 97.25%; 97.94% 97.94%; 97.59% 97.59%; 97.94% 97.94%; 97.94% 96.91%; 97.94% 97.59%; 97.94% 97.94%	Swanson IGHV3D-30*01 ORF
7	EF442660.1		91.40%	Swanson IGHV9D-2*01 F
8	EF442518.1; EF442482.1 EF442442.1; EF442438.1 EF442441.1; EF442440.1 EF442439.1	9	<u>95%</u> 87.72%; 87.72% 87.72%; 87.72% 86.67%; 87.37% 87.37%	<u>Arlee IGHV9-22-2</u> Swanson IGHV1-42*01 F
		1	<u>96%</u>	<u>Arlee IGHV1D-14-3 F</u>
9	EF438784.1; EF438709.1; EF438666.1; EF442641.1	4	93.06%; 93.06% 92.71%; 93.06%	Swanson IGHV1-41*01 P
10	EF442503.1	1	92.01%	Swanson IGHV1-18*01 F

471

472

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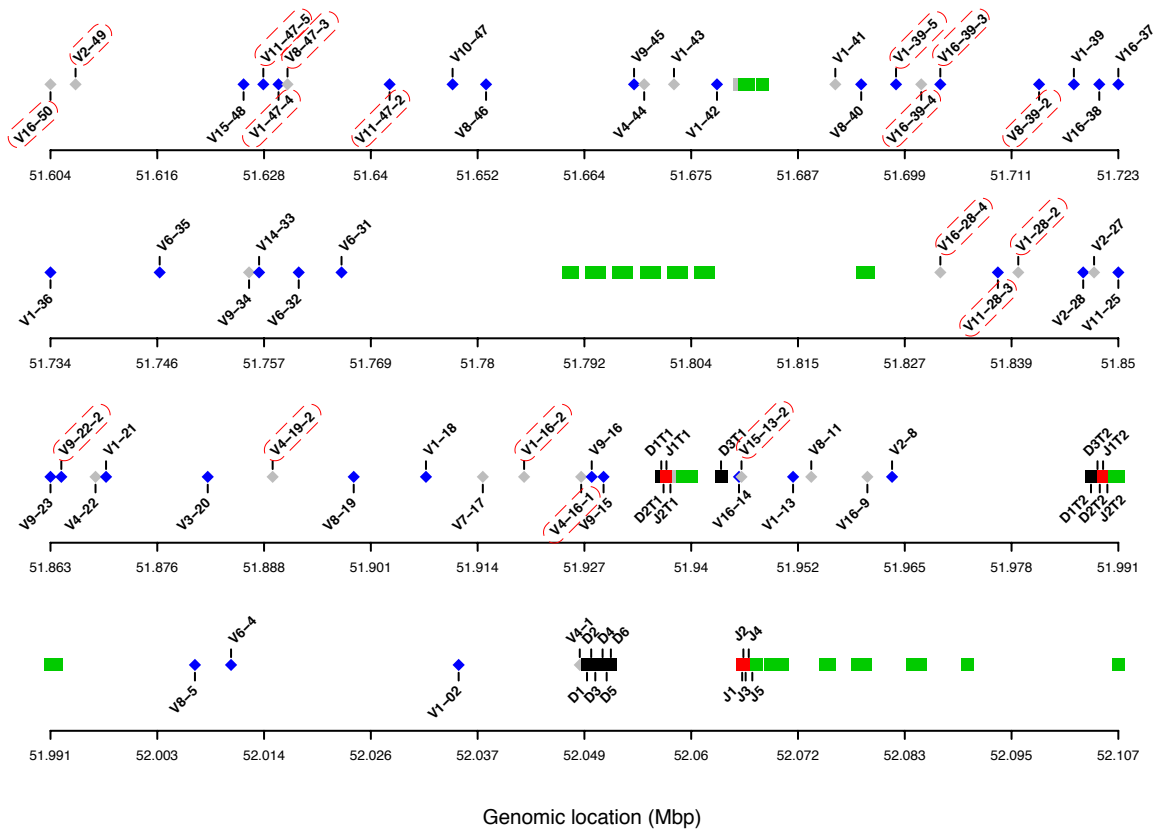
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611

Figure 1

Locus IGH A (chromosome 13)



Locus IGH B (chromosome 12)

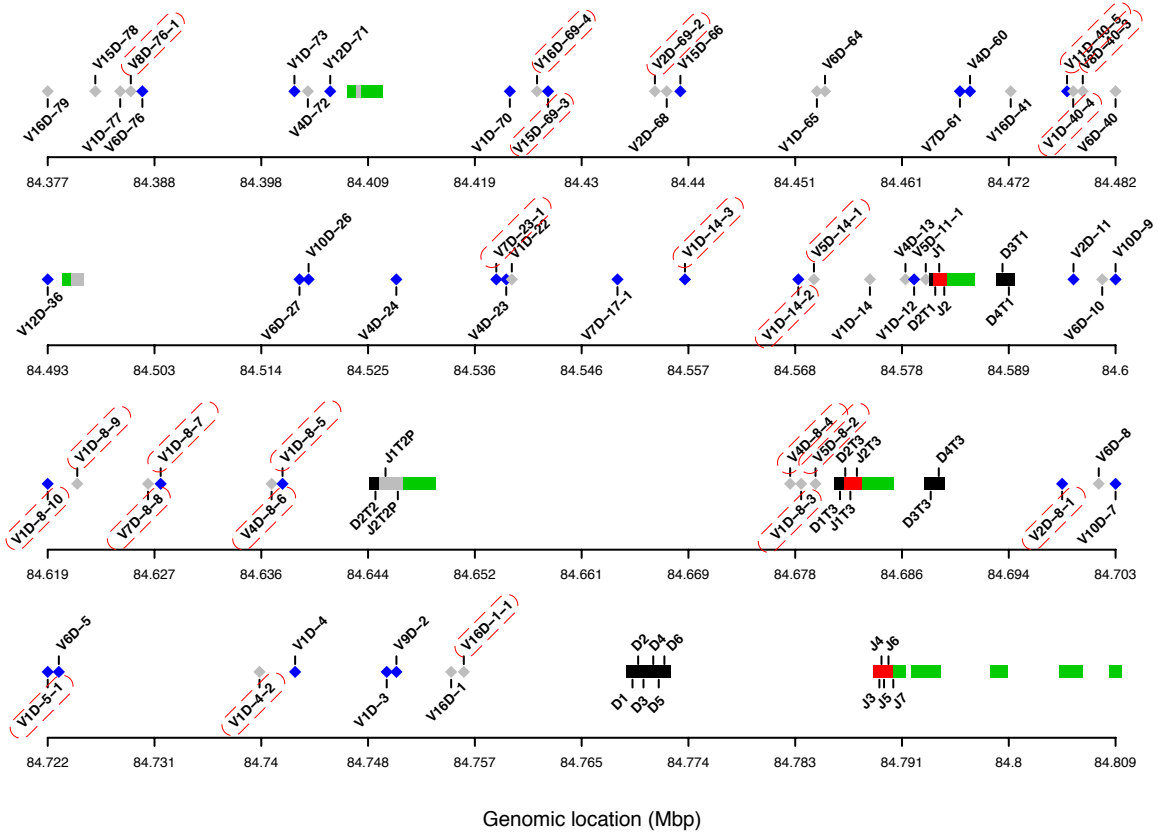


Figure 2

Chr12_IGHD2T1D	-----ATATGGGGTGGGGG-----
Chr13_IGHD2T2	-----ATATGGGGTGGGGT-----
Chr12_IGHD3T1D	--CCACATAGCGGGT-----
Chr12_IGHD3T3D	--CCACATAGCGGGT-----
Chr12_IGHD4T1D	-----TACGGGAATGGC-----
Chr12_IGHD4T3D	-----TACGGGAATGGC-----
Chr12_IGHD3D	-----TACGGGAATGGC-----
Chr13_IGHD1T1	---ACTATATGGGGC-----
Chr13_IGHD3T2	---ACTATATGGGGC-----
Chr12_IGHD1T3D	---ACTATATGGGGC-----
Chr13_IGHD1	ATAACAACGGGG-----
Chr12_IGHD1D	ATAACAACGGGG-----
Chr13_IGHD4	--CAGAATAACGGC-----
Chr12_IGHD4D	--CAGAATAACGGC-----
Chr13_IGHD5	-----TACACTGGGAGC-----
Chr12_IGHD5D	-----TACACTGGGAGC-----
Chr13_IGHD6	-----TATGGGGCAGC-----
Chr12_IGHD6D	-----TATGGGGCAGC-----
Chr12_IGHD2T3D	ATATGGGGTGGGCTGGGGG-----
Chr13_IGHD2T1	ATATGGGCTGGGGG-----
Chr13_IGHD2	--CCATATAGCGGGT-----
Chr13_IGHD3T1	--CCATATAGCAGGT-----
Chr12_IGHD2D	--TCATATAGCGGGT-----
Chr12_IGHD2T2D	---A-TACTGGCTGGGGG-----
Chr13_IGHD3	----TATGGGAGTGGC-----
Chr13_IGHD1T2	ACTATACAGTTACAGTTTGGGCTTCTTATTTGAGAGC

