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TECHNICAL NOTE

Full length MHC IIβ exon 2 primers for salmonids: a new resource for next generation sequencing

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Abstract Major histocompatibility complex (MHC) genes are often implicated in disease resistance, sexual selection and local adaptation in salmonids, a highly studied and socio-economically important taxa. However, genotyping highly polymorphic genes is difficult, expensive, and prone to PCR and cloning artifacts that can result in false alleles. With the advent of next generation sequencing, it is possible to effectively "clone" PCR products in a massively parallel fashion with the use of individually-tagged fusion primers. Primers that amplify a gene of interest across a variety of taxa, a single set of 50–150 primers can facilitate cost efficient genotyping and become a common lab resource. Here we developed MHC IIβ exon 2 primers for full-length amplification, including an additional PBR region, and demonstrate effectiveness with representatives from five genera of salmonids. These primers may facilitate parallel next generation sequencing for efficient, cost effective, and accurate genotyping of this complex locus.

Keywords Major histocompatibility complex · MHC · Salmonids · Salmonidae · *Coregonus* · *Oncorhynchus* · *Salmo* · *Salvelinus* · *Thymallus* · 454 · Next generation sequencing · Pyrosequencing · Exon 2 · MHC IIβ · MHC 2 beta · MHC 2 · Amplicon sequencing · Fusion primer · Peptide binding region · PBR · PCR · MIDs

Major histocompatibility complex (MHC) genes have been extensively examined for their potentially important role across a broad spectrum of species (Bernatchez and Landry 2003; Sommer 2005; Piertney and Oliver 2006). In the highly studied and socio-economically important family of salmonids, MHC genes have been implicated in sexual selection (Piertney and Oliver 2006), parasite resistance (Dionne et al. 2009) and local adaptation (Bernatchez and Landry 2003; Evans and Neff 2009). The MHC IIβ exon 2 locus encodes the peptide-binding region (PBR) and represents the peptide specific binding groove, the functional element that results in the display of extracellular antigens in order to initiate a targeted immune response (Janeway and Travers 2005). Previous studies have focused on ~215 bp region of this exon that includes part of the peptide binding region. An additional PBR region of high polymorphism at the 5′end of exon 2 (Croisetiere et al. 2008) makes primer development difficult for the entire exon 2, especially for a single set of primers that amplify a broad spectrum of taxa.

With the state-of-the-art of next generation sequencing, there is a greater advantage for cross-species primers. Roche 454 pyrosequencing may genotype individuals in a massively parallel and efficient process, and PCR artifacts may be statistically removed from the dataset (Lenz & Becker 2008; Wegner 2009). However, there is a non-trivial initial cost for primers since each parallel reaction must be differentially tagged with a unique identification sequence. Fusion primers that amplify a gene of interest in a broad spectrum of taxa may become a lab resource for

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many projects. Here we present new primers for the purpose of amplifying the entire MHC IIβ exon 2 ($\sim$255 bp) and demonstrate utility in all representatives of five genera (*Coregonus*, *Oncorhynchus*, *Salmo, Salvelinus* and *Thymallus*) of salmonids. This opens the opportunity for a single set of tagged primers to accommodate next generation sequencing protocol for efficient, parallel, and accurate genotyping of this important gene for all species of salmonids.

Total DNA was extracted from about 20 mg of adipose, muscle, or fin tissue using either the salt-extraction protocol from (Aljanabi and Martinez 1997) of Qiagen DNeasy Kit for animal tissue (Qiagen, Valencia, California) for three individuals for each five salmonid genera. DNA concentrations (3–130 ng/μl) as well as purity were determined by spectrophotometry using a NanoDrop2000® (Thermo labsystems, Waltham, Massachusetts).

The *Coregonus clupeaformis* and *Salvelinus fontinalis* MHC IIβ sequences were obtained from a BAC library (HQ287750 Jeukens et al. 2011) and published sequences (Croisetiere et al. 2008). The forward SaCo_F, 5′-ATGTTTTCCTTTTAGATGGATATTTT-3′ and reverse SaCo_R, 5′- AGCCCTGCTCACCTGTCTTA -3′ primers were developed *in silico* by aligning full-length exon 2 alleles, from four genera of the salmonidea taxon (*Coregonus* (consensus), *Oncorhynchus* (U20943.1), *Salvelinus* (consensus), *Salmo* (X70167.1)) using CLC genomics workbench 3 (CLC bio, Aarhus, Denmark) and Primer 3

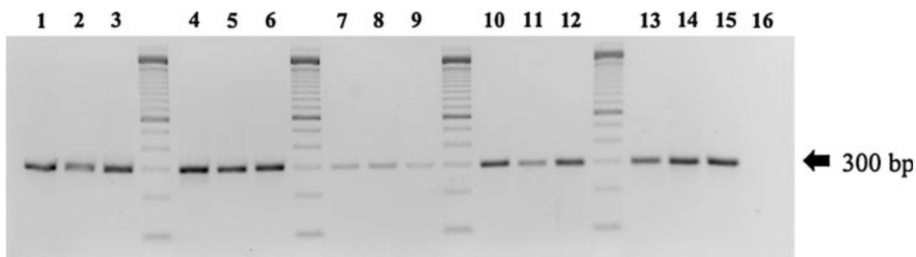

Fig. 1 2% agarose gel presenting exon 2 amplicon sizes for five genera of Salmonids with 100 bp scale. 1–3 *Oncorhynchus* sp., 4–6 *Thymallus* sp., 7–9 *Salmo* sp., 10–12 *Salvelinus* sp., 13–15 *Coregonus* sp., 16 negative

```
                                             20                                             40                                             60
                                              |                                              |                                              |
Oncorhynchus  --  ---  ---  ---  ---  ---  ---  ---  ---  NAT  CAN  AGG  NTG  GNA  CAG  TGC  CGA  TAC  TCC  TCA  AAG  GAC  CTG  C  43
  Salvelinus  --  ---  ---  ---  ---  ---  ---  ---  ---  ...  ...  ...  ...  ...  ...  ...  ...  .N.  ...  ...  ...  ...  ...  .  43
       Salmo  --  ---  ---  ---  ---  ---  ---  ---  ---  ..N  ...  NTN  ...  NGN  N..  ...  ...  .N.  ...  ...  ...  ...  ...  .  43
   Coregonus  --  ---  ---  ---  ---  ---  ---  ---  ---  ..N  ..G  NN.  G..  A.N  ...  ...  .N.  .N.  A..  ...  N..  ..N  ...  .  43
   Thymallus  --  ---  ---  ---  ---  ---  ---  ---  ---  ...  ..G  NN.  G..  N.N  ...  N.T  ...  ...  ...  ...  ...  ..A  ...  A  43
     SaCo_F   AT  GTT  TTC  CTT  TTA  GAT  GGA  TAT  TTT  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  -  26
     SaCo_R   --  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  -  -

                       80                                       100                                      120
                        |                                         |                                        |
Oncorhynchus  AT  GGT  GCA  GAG  TNT  ATA  GAC  TCT  TAN  NNN  TTC  AAT  NAG  GNN  GAA  TAN  NTC  AGA  TTC  NAC  AGC  ACT  GTG  G  112
  Salvelinus  ..  ...  AT.  ...  ..N  ...  ...  ...  ..T  GTT  ...  ...  ...  ..T  ...  N..  ...  ...  ...  A..  ...  ...  ...  .  112
       Salmo  .G  ...  AT.  ...  .TN  ...  N..  ...  ..T  GTT  ...  ...  ...  .CT  ...  N.T  ...  ...  ...  A..  ...  ...  ...  .  112
   Coregonus  ..  .N.  AT.  ...  ...  ...  ...  ...  ..T  G.T  ...  ..N  ...  ..T  ...  N.T  ...  ...  ...  A..  NN.  ...  ..A  .  112
   Thymallus  ..  .A.  AT.  ...  .T.  ...  ...  ...  ..T  GTT  ...  ...  ...  ..T  ...  N.T  A..  ...  ...  A..  ...  ...  ...  .  112
     SaCo_F   --  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  -  26
     SaCo_R   --  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  -  -

         140                                 160                                      180                                     200
          |                                   |                                        |                                       |
Oncorhynchus  GG  AAG  TAT  GTT  GGA  TAC  ACT  GAG  NNN  GGT  NTG  AAG  AAT  GCA  GAA  GCA  TGG  AAC  AAA  GGT  ---  TCT  GNG  C  178
  Salvelinus  ..  ...  .N.  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  .N.  .N.  ---  ...  .AN  .  178
       Salmo  ..  ...  .N.  ...  ...  ...  ...  ...  C..  ..A  G..  ...  ...  ...  ...  ..C  ...  ...  ...  ...  ---  C..  .A.  .  178
   Coregonus  .A  ...  .N.  ...  ...  ...  ...  ...  CAT  ..N  G..  ...  ...  ...  ...  ...  .NN  ...  .NN  .NN  GCN  ..G  N.N  .  181
   Thymallus  ..  ...  ...  ...  ...  ...  ...  ...  C..  ...  ..N  N.N  ...  ...  A.T  NN.  ...  ...  .NN  .A.  ACT  NNG  N.T  .  181
     SaCo_F   --  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  -  26
     SaCo_R   --  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  -  -

                            220                                      240                                      260
                             |                                        |                                        |
Oncorhynchus  TG  GCT  CNA  GAG  CTA  GGG  GAG  CTG  GAG  CGT  TNC  TGN  AAG  CNT  ANC  GCT  GCT  ANC  NAN  TAC  AGC  GCC  NTA  C  247
  Salvelinus  ..  ...  ...  ...  ...  ...  ...  ...  ...  ...  .T.  ...  ...  .A.  .A.  ...  ...  .A.  C.C  ...  ...  ...  G..  .  247
       Salmo  ..  ...  G..  ...  ...  ...  ...  ...  ...  ...  NT.  ..T  ...  .A.  .A.  ...  NN.  NT.  C.C  ...  N..  N..  A..  .  247
   Coregonus  ..  ...  N..  .N.  .N.  ...  NNN  ...  ...  N..  .A.  N..  ..N  N..  .A.  ..N  .N.  NT.  ..C  ...  ..T  N..  A.N  .  250
   Thymallus  ..  ...  .A.  ...  .A.  ...  N..  ...  ...  N..  .A.  ..C  ...  NA.  .A.  ...  AAA  N..  .NC  ...  NNT  .T.  G..  .  250
     SaCo_F   --  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  -  26
     SaCo_R   --  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  -  -

                   280                                300
                    |                                  |
Oncorhynchus  TG  GA-  ---  ---  ---  ---  ---  ---  -  251
  Salvelinus  ..  ..-  ---  ---  ---  ---  ---  ---  -  251
       Salmo  ..  ..-  ---  ---  ---  ---  ---  ---  -  251
   Coregonus  ..  ..-  ---  ---  ---  ---  ---  ---  -  254
   Thymallus  ..  ..-  ---  ---  ---  ---  ---  ---  -  254
     SaCo_F   --  ---  ---  ---  ---  ---  ---  ---  -  26
     SaCo_R   --  --T  AAG  ACA  GGT  GAG  CAG  GGC  T  20
```

Fig. 2 Exon 2 consensus sequences amplified from the five salmonid genera with codons represented

Plus on the web (Untergasser et al. 2007). Protocol for MHC IIβ primers was according to Qiagen HotStar Taq PCR Kit (Invitrogen, Carlsbad, California) with an annealing temperature of 55°C.

The visualization and quantification of amplicons was checked on a 2% UltraPure™ agarose gel (Invitrogen). All amplicons had the correct intended band size for all genera (Fig. 1). The relative weakness of the Atlantic salmon amplicons may be due to less initial template and/or presence of a SNP at the 3′ end of the reverse primer (data not shown).

Our goal was to amplify all loci of the MHC IIβ exon 2; not to determine number of loci in each species. In Atlantic and Pacific salmon, this is often, but not always, presented as a single locus (Miller and Withler 1996; Jacob et al. 2010). However, in other fish species, including threespine stickleback (*Gasterosteus aculeatus*), multiple transcribed loci are found within a single individual (Wegner et al. 2008). Since multiple loci amplify in lake whitefish (personal communication F.-O. Gagnon-Hébert), for these samples all three PCR products were cloned with the TOPO TA Cloning kit (Invitrogen) according to the manufacturer protocol, with up to fourteen clones sequenced per individual. For the other genera, direct sequencing was performed with known homozygous and heterozygous individuals (*Salvelinus* sp.) and unknown allelic composition in the three other genera. Amplicons were sequenced on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, California) in the IBIS genomic center at Laval University. Then, amplicons were aligned in CLC genomics workbench 3 to confirm sequence specificity of our primers (Fig. 2).

Here we presented new primers to amplify the entire MHC IIβ exon 2 gene for representatives of five genera of salmonids. Though the previous primers designed for Atlantic salmon have also been used in multiple salmonids (Miller and Withler 1996), a highly polymorphic portion of the PBR is excluded. Primers with cross species amplification utility of highly studied genes may easily be added to tagged fusion primers for massively parallel, efficient, accurate, and cost effective genotyping with a next generation sequencing protocol.

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