De novo assembly of the Brown trout (Salmo trutta m. fario) brain and muscle transcriptome: transcript annotation, tissue differential expression profile and SNP discovery

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Abstract
Objectives: The Brown trout is a salmonid species with a high commercial value in Europe. Life history and spawning behaviour include resident (Salmo trutta m. fario) and migratory (Salmo trutta m. trutta) ecotypes. The main objective is to apply RNA-seq technology in order to obtain a reference transcriptome of two key tissues, brain and muscle, of the riverine trout Salmo trutta m. fario. Having a reference transcriptome of the resident form will complement genomic resources of salmonid species.

Data description: We generate two cDNA libraries from pooled RNA samples, isolated from muscle and brain tissues of adult individuals of Salmo trutta m. fario, which were sequenced by Illumina technology. Raw reads were subjected to de-novo transcriptome assembly using Trinity, and coding regions were predicted by TransDecoder. A final set of 35,049 non-redundant ORF unigenes were annotated. Tissue differential expression analysis was evaluated by Cuffdiff. A False Discovery Rate (FDR) ≤ 0.01 was considered for significant differential expression, allowing to identify key differentially expressed unigenes. Finally, we have identified SNP variants that will be useful tools for population genomic studies.

Keywords: Rnaseq, De novo transcriptome, Brain & muscle transcriptome, SNP discovery, Salmo trutta m. fario

Objective
Brown trout (Salmo trutta) has been extensively studied by its commercial and biological importance. From the sixty-six species in this family, S. trutta is a species native to Europe with a wide distribution area that includes Atlantic and Mediterranean European basins, as well as northern Africa and western Asia basins [1, 2]. The species has been introduced in North and South America and Australia by its commercial exploitation for sport fishing, as well as farmed for food and game fish, extending their actual geographical distribution as discontinuous populations on all continents except Antarctica [3].

Life history traits of Brown trout populations include resident forms such as riverine (S. trutta m. fario) and migratory forms such as anadromous (S. trutta m. trutta) ecotype [4, 5]. Anadromous and non-anadromous forms coexist in the same river being apparently genetically indistinguishable [6, 7]. An extended literature on Brown trout research has been produced that includes physiological, ecological and genetic aspects [8–10]. As a contribution to this global effort, here we provide a comprehensive transcriptome data set derived from brain and muscle tissues of Salmo trutta.

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m. fario ecotype by using RNA-seq technology. We also evaluated differential transcript expression among these two tissues identifying key differentially expressed unigenes. Finally, we applied an in-silico pipeline that allow us to discover SNP variants useful for population genomic studies. The generated data could provide new valuable genomic resources for population genetic and genomic studies that can help to answer opened questions about the live history traits of riverine S. trutta m. fario as well as differences among S. trutta ecotypes.

Data description
Salmo trutta m. fario. brain and muscle tissues were collected from 25 wild type individuals (15 females) captured at the Falmisell river (Lleida, Catalonia). RNA pools from brain (10.2 µg) and muscle (11.4 µg) tissues were obtained with equimolar concentration from each subject. The TruSeq™ RNA sample Prep Kit (Illumina, Madrid, Spain) was used to build cDNA libraries according to manufacturer instructions (Table 1, Data file 1). FASTQ sequence reads were assembled using Trinity [11] run on the paired end sequences with the

| Table 1 Overview of data files/data sets |
|-----------------------------------------|
| Label         | Name of data file/data set               | File types (file extension) | Data repository and identifier (DOI or accession number) |
|---------------|------------------------------------------|------------------------------|-----------------------------------------------------------|
| Data file 1   | Methodology description                   | Document file (.docx)       | Figshare https://doi.org/10.6084/m9.figshare.12902474.v1 |
| Data file 2   | Descriptive statistics of assembly-sequencing | Document file (.docx)       | Figshare https://doi.org/10.6084/m9.figshare.12902474.v1 |
| Data file 3   | FigS1 Size_distribution                   | Image file (.jpg)           | Figshare https://doi.org/10.6084/m9.figshare.12902405.v2 |
| Data file 4   | FigS2 GeneOntology                       | Image file (.jpg)           | Figshare https://doi.org/10.6084/m9.figshare.12902405.v2 |
| Data file 5   | FigS3 Differential_expression            | Image file (.jpg)           | Figshare https://doi.org/10.6084/m9.figshare.12902405.v2 |
| Data file 6   | Raw RNA-seq. Reads Brain tissue          | Fastq files (.fastq)        | NCBI Sequence Read Archive                               |
| Data file 7   | Raw RNA-seq. Reads Muscle tissue         | Fastq files (.fastq)        | NCBI Sequence Read Archive                               |
| Data file 8   | Trinity144                                | Fasta file (.fasta)         | Figshare https://doi.org/10.6084/m9.figshare.7326464     |
| Data file 9   | Predicted non-redundant Open Reading Frames (ORFs) | Fasta file (.fasta)         | NCBI GenBank https://identifiers.org/ncbi/insdc:GHGR0000000.1 |
| Data file 10  | Megablast hit alignment of non-redundant ORF unigenes to reference nucleotide databases | Spreadsheet (.xlsx)        | Figshare https://doi.org/10.6084/m9.figshare.7712708.v4   |
| Data file 11  | Blastx homology search of non-redundant ORF unigenes to reference protein databases | Spreadsheet (.xlsx)        | Figshare https://doi.org/10.6084/m9.figshare.7712708.v4   |
| Data file 12  | Krona_pie_chart_on_Non_redundant_ORF_to_NCBI_nt_and_maREF_seq_2018_HTML.html | HTML file (.html)          | Figshare https://doi.org/10.6084/m9.figshare.7712708.v4   |
| Data file 13  | Protein family (Pfam) assignment to non-redundant ORF unigenes | Spreadsheet (.xlsx)        | Figshare https://doi.org/10.6084/m9.figshare.12905777.v2   |
| Data file 14  | GOslim annotation of non-redundant ORF unigenes sequences | Spreadsheet (.xlsx)        | Figshare https://doi.org/10.6084/m9.figshare.12905777.v2   |
| Data file 15  | KEGG pathway annotation of non-redundant ORF unigenes sequences | Spreadsheet (.xlsx)        | Figshare https://doi.org/10.6084/m9.figshare.12905777.v2   |
| Data file 16  | Raw_Cufflinks_Brain_transcript_expression | Cufflinks output file (.txt)| Figshare https://doi.org/10.6084/m9.figshare.12905747.v1   |
| Data file 17  | Raw_Cufflinks_Muscle_transcript_expression | Cufflinks output file (.txt)| Figshare https://doi.org/10.6084/m9.figshare.12905747.v1   |
| Data file 18  | Raw_Cuffdiff_Brain_Muscle_transcript_differential_expression_testing | Cuffdiff output file (.txt)| Figshare https://doi.org/10.6084/m9.figshare.12905747.v1   |
| Data file 19  | Differential expressed non-redundant ORF unigenes at FDR_0.01 | Spreadsheet (.xlsx)         | Figshare https://doi.org/10.6084/m9.figshare.12905747.v1   |
| Data file 20  | Salmo trutta m. Fario—mapped SNP_to_ORF | Varian Call Format file (.vcf)| Figshare https://doi.org/10.6084/m9.figshare.12905831.v1   |
| Data file 21  | SNP context sequence                     | Spreadsheet (.xlsx)        | Figshare https://doi.org/10.6084/m9.figshare.12905831.v1   |
fixed default k-mer size of 25 and minimum contig length of 200. Descriptive statistics of assembly and sequencing is found at Table 1 (Data file 2 and Data file 3). Among the 144,984 contigs predicted by Trinity (Table 1, Data file 4 and Data file 8), we identify protein coding regions using TransDecoder package [11]. We retained the longest ORF predicted for each contig sequence with a minimum of 100 amino acids long. Transcript redundancy was further reduced by CD-hit [12], obtaining a final set of 35,189 non-redundant ORF unigenes as best cluster representatives (Table 1, Data file 5). Size distribution for clustered ORF unigenes is presented in Table 1 (Data file 3). This final set was characterized by homology search to nucleotide and protein databases (Table 1, Data file 10 and Data file 11). Taxonomic representation showed the top hits for a large fraction of unigenes (≈88%) to Neopterigii taxon, with 66% of unigenes assigned to family Salmonidae (Salvelius sp. (1%), Onchorinchus sp. (14%) and Salmo sp. (51%) (Table 1, Data file 12). A total of 4337 protein motif were assigned to 23,616 ORF unigenes, being the RNA recognition motif (6.4%), Immunoglobulin domain (4.8%), Tetratricopeptide repeat (4.8%) and Protein kinase domain (3.4%) the most prevalent (Table 1, Data file 13).

Similarity search by Blast2GO renders a total of 28,132 (80%) unigenes with GO annotation. GO terms were then simplified using a generic GOSlim vocabulary [13] (Table 1, Data file 14). The ten top GO terms among the Cellular Component (18,071, 64%), Molecular Function (20,691, 74%) and Biological Process (23,954, 85%) ontology at level 2 are shown in Table 1 (Data file 4). Mapping unigenes to the reference canonical pathways in the KEGG database, yields a total of 13,957 (39.8%) ORF unigenes assigned to 3421 KEGG terms (KO) defining a total of 386 pathways (Table 1, Data file 15).

Tissue specific transcriptome expression analysis was performed by normalization of raw reads (FPKM, fragments per kilobase of exon per million fragments) obtained from both tissues (Table 1, Data file 16 and Data file 17). Analysis reveals 1172 ORF unigenes expressed only in muscle, 8595 expressed only in brain and 12,072 expressed in both tissues (Table 1, Data file 5, FigS3). Differentially expressed unigenes at FDR < 0.01 and best homologous sequences are shown at Table 1 (Data file 18 and Data file 19).

Finally, we have identified 73,237 putative SNPs (Table 1, Data file 20) and extracted 150 bp sequence context to each SNP as a source for the design of PCR primers useful for genotyping protocols (Table 1, Data file 21).

Limitations
The use of pooled RNA samples does not allow us to detect sex or individual specific transcript expression profiles as well as limit our capability to detect transcripts expressed at low level in a specific individual. In addition, pooled samples avoid us to resolve SNP frequency distribution, being this parameter indirectly estimated according to the observed SNP sequence coverage in the pooled sample.

Abbreviations
BAM: Binary Sequence Alignment/Map; BLAST: Basic local alignment search tool; bp: base pair; CDS: Coding sequence; FPKM: Fragments Per Kilobase of exon model per Million mapped reads; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; ORF: Open reading frame; Pfam: Protein families database; FDR: False Discovery Rate; SAMtools: Sequence Alignment/Map tools; SNP: Single nucleotide polymorphism.

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Authors’ contributions
JF, NO and AP designed the study; NO, MP-P and MF captured animals and processed samples. NO, MP-P and JLR, carried out lab work and assisted with data analysis. JF obtained the founding, perform data analysis and drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The data described in this Data note can be freely and openly available on Figshare (https://doi.org/10.6084/m9.figshare.12902474.v1; https://doi.org/10.6084/m9.figshare.12902405.v2; https://doi.org/10.6084/m9.figshare.7325464.v1; https://doi.org/10.6084/m9.figshare.7712708.v4; https://doi.org/10.6084/m9.figshare.12905777.v2; https://doi.org/10.6084/m9.figshare.12905747.v1; https://doi.org/10.6084/m9.figshare.12905831.v1). Assembly of non-redundant ORF unigene sequences are available from NCBI transcriptome shotgun assembly (TSA) database (https://identifiers.org/ncbi/ncbd//GHGRR000000001); Raw sequence reads are available from the NCBI sequence read archive (SRA) database (https://identifiers.org/ncbi/sra/SPR151838). Please see Table 1 and references list [14–22] for details and links to the data.

Ethics approval and consent to participate
Permissions for electrofishing and capture of S. trutta m. fario individuals, was approved by the competent authorities: Departament de Medi Ambient i Habitatge de la Generalitat de Catalunya (current Departament d’Agricultura, Ramadera, Pesca, Alimentacio i Medi Natural) (SF/602) of the regional authorities of Catalonia.

Consent for publication
Not applicable.

Competing interest
The authors did not report any competing interests.

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