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Finding Nemo: Hybrid assembly with Oxford Nanopore and Illumina reads greatly improves the Clownfish (Amphiprion ocellaris) genome assembly

--Manuscript Draft--

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Abstract:	Background: Some of the most widely recognised coral-reef fishes are clownfish or anemonefishes, members of the family Pomacentridae (subfamily: Amphiprioninae). They are popular aquarium species due to their bright colours, adaptability to captivity and fascinating behavior. Their breeding biology (sequential hermaphrodites) and symbiotic mutualism with sea anemones have attracted much scientific interest. Moreover, there are some curious geographic-based phenotypes which warrant investigation. Leveraging on the advancement in Nanopore long read technology, we report the first hybrid assembly of the clown anemonefish (Amphiprion ocellaris) genome utilizing Illumina and Nanopore reads, further demonstrating the substantial impact of modest long read sequencing data sets on improving genome assembly statistics. Findings: We generated 43 Gb of short Illumina reads and 9 Gb of long Nanopore reads representing an approximate genome coverage of 54× and 11×, respectively, based on the range of estimated k-mer-predicted genome sizes of between 791 to 967 Mbp. The final assembled genome size is contained in 6,404 scaffolds with an accumulated length of 880 Mb (96.3% BUSCO-calculated genome completeness). Compared to the Illumina-only assembly, the hybrid approach generated 94% fewer scaffolds with 18-fold increase in N50 length (401 kb) and increased the genome completeness by an additional 16%. A total of 27,240 high quality protein-coding genes were predicted from the clown anemonefish, 26,211 (96%) of which were annotated functionally with information from either sequence homology or protein signature searches. Conclusions: We present the first genome of any anemonefish and demonstrate the value of low coverage (~11×) long Nanopore reads sequencing in improving both genome assembly contiguity and completeness. The near-complete assembly of the A. ocellaris genome will be an invaluable molecular resource for supporting a range of genetic, genomic and phylogenetic studies specifically for clownfish and more generally for other related fish species of the family Pomacentridae
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Response to Reviewers:	Reviewer #1 Comment 1: In general, given the prominence (with 'dramatically') of nanopore data in the title, I would like to encourage the authors to elaborate on this aspect of the study in the Conclusion section. For example, why did you use this particular strategy (MaSuRCA assembler), and what are its strengths and weaknesses? How does long-read coverage affect the assembly process (this study uses only three nanopore flowcells - would this be a recommended efficient strategy to 'fix' any Illumina-based assembly)? How far are we from non-hybrid nanopore-based assemblies? Response: We've added discussion on this matter in the Conclusion. We chose MaSuRCA due to its demonstrated accuracy in addition to its ability to better utilize Nanopore reads in its initial assembly step. Despite producing a highly contiguous genome assembly of the clown fish, MaSuRCA hybrid assembly is an extremely computationally expensive and time-consuming. We are hopeful that the cost of Nanopore sequencing will reduce further especially with the recent release of PromethION which may revolutionize routine long read sequencing. However, as of now, high coverage long read sequencing is still financial challenging for smaller research group despite its indubitable value in assembling challenging (repetitive and/or heterozygous) genome. "Hybrid assembly of Illumina and Nanopore reads is one of the new features of the MaSuRCA assembler version 3.2.2 that works by constructing long and accurate mega-reads from the combination of long and short read data. Although this is a relatively computationally-intensive strategy with long run-times, we observed substantial improvement in the genome statistics when compared to Illumina-only assembly. As Nanopore long technology becomes more mature, it is likely that future de novo genome assembly will shift towards high coverage long read-only assembly followed by genome polishing using Illumina reads." ---------- Comment 2: Finally, a very similar genome project manuscript was recently posted on BioRxiv: Anna Marcionetti et al., First draft genome assembly of an iconic clownfish species (Amphiprion frenatus), doi 10.1101/205443, 18 October 2017 The manuscripts do not cite each other, but arrive at similar genome assembly qualities using similar strategies. Response: Although the manuscript was posted on BioRxiv, the genome assembly itself is currently unavailable to the public and, according to the manuscript, will only be available in DRYAD repository (under 'Data Accessibility). So far, we were not able to locate the data on DRYAD. ---------- Comment 3: 1. Line 128: an upper limit of 1 Mbp reads probably did not exclude anything. What was the actual longest read length? Response: We had initially obtained reads up to 40Mbp base-called from an older version of Albacore. But after re-analysis with version 2.0.1 for this assembly, we had gotten far more sensible Nanopore read lengths and therefore did not apply any maximum length cutoff. We have removed the details on upper limit in the revised manuscript. The actual longest mappable read length for our Nanopore read dataset was 101,379bp, aligned to scaffold6249 (276,565bp) and contains genes with IDs: AMPOCE_00020294 and AMPOCE_00020295. ---------- Comment 4: 2. Line 131…/Supplemental Figure 1. Not all Illumina data were apparently used for the k-mer profile. Does this perhaps explain the considerable difference in estimated

genome size and assembled genome size? If not, is there another explanation? Also, the legend to the figure ('genome profiling') could be more informative (e.g. genome size estimate…)

Response:
Based on this reviewer comment, we re-ran GenomeScope using the k-mer profile from all Illumina reads, which estimated genome sizes of 806 to 812 Mbp with different k-mer sizes, not too different from the initial number. A separate independent analysis was performed with BBMap, which estimated a haploid genome size of 967Mb. Given this result, the assembled genome size is well within the range of genome size estimated based on different methods. We have added the results from the BBMap analysis in the manuscript at lines 139 to 140. Supplemental Figure 1 has also been improved.

Comment 5:
3. A (supplementary) table with sequencing statistics (yield for each type of data, incl. RNA-seq) would be appropriate.

Response:
Sequencing statistics has been summarized for each sample ID in the new Supplemental Table 1.

Reviewer #2
Comment 1:
Title:
The reference to Nemo 2.0 and the phrase "dramatically improves" led me to believe that this was the second version of an already existing genome assembly. However, I could not find any other Amphiprion ocellaris assemblies by googling, besides a bioRxiv preprint of Amphiprion frenatus (https://www.biorxiv.org/content/early/2017/10/18/205443). I am not sure if this warrants changing the title, but please be aware of it. Also, I dislike using "genome" to refer to the genome assembly. I don't think you actually improve the genome present in the species.

Response:
The “2.0” in the title was included due to this manuscript (10.1016/j.gene.2006.03.028) with a similar title “Finding Nemo” and was not due to the recent A. frenatus genome in bioRxiv (see also Response to Reviewer’s 1 Comment 2). However, given that our study is not a follow-up of the phylogenetic study reported by Santini and Polacco (2006), we agree that this can be confusing to readers and have removed “2.0” from the title. We have also added “assembly” into the title as per reviewer’s suggestion and slightly modified the title.

Comment 2:
Abstract:
Line 54: "93 % less scaffolds". This should be "fewer" if I'm not mistaken.

Response:
Replaced “less” with “fewer”.

Comment 3:
Lines 60-65: I prefer to see "genome assembly" instead of just "genome". I find it more accurately descriptive.

Response:
Edited title as per suggestion.

Comment 4:
Lines 120-125: The MaSuRCA quick start guide (ftp://ftp.genome.umd.edu/pub/MaSuRCA/MaSuRCA_QuickStartGuide.pdf) explicitly says that Illumina reads should not be pre-processed before providing them to MaSuRCA. It is not clear whether or not the "clean" reads were used in the assemblies. Were the "clean" reads used? Or were they only used for genome size estimation?

	Response: Since the raw reads obtained from the Illumina MiSeq already had adapter sequences trimmed off by default, reads used as input to MaSuRCA were only adapter-trimmed. However, no quality-trim/cleaning/error correction was performed on these reads since the program performs its own error correction steps. “Clean” reads which excluded bacteria/virus contaminants and mitochondrial origins were used mainly for genome size estimation to not underestimate the genome size when max kmer coverage is applied in GenomeScope. “Kraken-unclassified reads i.e. non-microbial/viral origin were aligned to the complete mitogenome of NTM A3764 (See “Mitogenome Assembly”) to exclude sequences of organellar origin. This results in a total of 42.35 Gb “clean” short reads.” ---------- Comment 5: Line 149: "10 iterations of Pilon". Did you actually see any improvements after this many iterations? How did you assess the improvements? Response: We have added details on the assemblies obtained after each pilon iteration as Supplemental Table 3. While the contiguity of the assembly (indicated by N50) is not improved much after each iteration, the number of gaps and ‘N’s are reduced and these numbers are observed to plateau at later iterations (i9 or i10). In addition, pilon reports the changes made in the assembly. Based on this output, the number of changes made decreases with each iteration and eventually almost plateaus as well (Supplemental Table 3). ---------- Comment 6: Line 155: Here you claim a "94 % decrease in the number of scaffolds", while you claim 93 % in the abstract. Which is correct? I guess both if you use different criteria for which scaffolds are included or not (>500 bp). Response: 94% decrease is correct - fixed this in abstract
Additional Information:	
Question	**Response**
Are you submitting this manuscript to a special series or article collection?	No
Experimental design and statistics Full details of the experimental design and statistical methods used should be given in the Methods section, as detailed in our Minimum Standards Reporting Checklist. Information essential to interpreting the data presented should be made available in the figure legends. Have you included all the information requested in your manuscript?	Yes
Resources A description of all resources used, including antibodies, cell lines, animals and software tools, with enough information to allow them to be uniquely	Yes

identified, should be included in the Methods section. Authors are strongly encouraged to cite Research Resource Identifiers (RRIDs) for antibodies, model organisms and tools, where possible. Have you included the information requested as detailed in our Minimum Standards Reporting Checklist?	
Availability of data and materials All datasets and code on which the conclusions of the paper rely must be either included in your submission or deposited in publicly available repositories (where available and ethically appropriate), referencing such data using a unique identifier in the references and in the “Availability of Data and Materials” section of your manuscript. Have you have met the above requirement as detailed in our Minimum Standards Reporting Checklist?	Yes

Finding Nemo: Hybrid assembly with Oxford Nanopore and Illumina reads greatly improves the Clownfish (*Amphiprion ocellaris*) genome assembly

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Abstract

Background: Some of the most widely recognised coral-reef fishes are clownfish or anemonefishes, members of the family Pomacentridae (subfamily: Amphiprioninae). They are popular aquarium species due to their bright colours, adaptability to captivity and fascinating behavior. Their breeding biology (sequential hermaphrodites) and symbiotic mutualism with sea anemones have attracted much scientific interest. Moreover, there are some curious geographic-based phenotypes which warrant investigation. Leveraging on the advancement in Nanopore long read technology, we report the first hybrid assembly of the clown anemonefish (*Amphiprion ocellaris*) genome utilizing Illumina and Nanopore reads, further demonstrating the substantial impact of modest long read sequencing data sets on improving genome assembly statistics.

Findings: We generated 43 Gb of short Illumina reads and 9 Gb of long Nanopore reads representing an approximate genome coverage of 54× and 11×, respectively, based on the range of estimated k-mer-predicted genome sizes of between 791 to 967 Mbp. The final assembled genome size is contained in 6,404 scaffolds with an accumulated length of 880 Mb (96.3% BUSCO-calculated genome completeness). Compared to the Illumina-only assembly, the hybrid approach generated 94% fewer scaffolds with 18-fold increase in N_{50} length (401 kb) and increased the genome completeness by an additional 16%. A total of 27,240 high quality protein-coding genes were predicted from the clown anemonefish, 26,211 (96%) of which were annotated functionally with information from either sequence homology or protein signature searches.

Conclusions: We present the first genome of any anemonefish and demonstrate the value of low coverage (~11×) long Nanopore reads sequencing in improving both genome assembly contiguity and completeness. The near-complete assembly of the *A. ocellaris* genome will be an invaluable molecular resource for supporting a range of genetic, genomic and phylogenetic studies specifically for clownfish and more generally for other related fish species of the family Pomacentridae.

Keywords: clownfish, long reads, genome, transcriptome, hybrid assembly

Data description

The clown anemonefish, *Amphiprion ocellaris* (NCBI Taxon ID: 80972, Fish Base ID:6509), is a well-known tropical marine fish species among the non-scientific community especially following the Pixar film "Finding Nemo" and its sequel "Finding Dory" [1]. The visual appeal of *A. ocellaris* due to its bright coloration and behaviour and ease of husbandry has maintained a strong global demand for this species in the marine aquarium trade driving a fine balance between positive environmental awareness versus sustainable ornamental use [1, 2]. Further, given high survival rates and ability to complete their lifecycle in captivity, captive-breeding programs to partially sustain their global trade have been successful [3]. For the scientific community, *A. ocellaris* or anemonefishes in general, are actively studied due to their intriguing reproductive strategy i.e. sequential hermaphroditism [4-7] and mutualistic relationships with sea anemones [8-12]. Phenotypic body-colour variation based on host-anemone use and geography also pose additional questions regarding adaptive genetic variation [13]

In recent years, concurrent with the advent of long read sequencing technologies [14], several studies have explored combining short but accurate Illumina reads with long but less accurate Nanopore/PacBio reads to obtain genome assemblies that are usually more contiguous with higher completeness than assemblies based on Illumina-only reads [15-19]. To further contribute to the evaluation of long read technology in fish genomics [15], we sequenced the whole genome of *A. ocellaris* using Oxford Nanopore and Illumina technologies and demonstrate that hybrid assembly of long and short reads greatly improved the quality of genome assembly.

Whole genome sequencing

Tissues for genome assembly and as reference material were sourced from the collection of the Museum and Art Gallery of the Northern Territory (NTM). The samples used for DNA extraction and subsequent whole genome sequencing were from freshly-vouchered captive bred *A. ocellaris* specimens representing a unique black and white colour phenotype found only in the Darwin Harbour region, Australia (NTM A3764, A4496, A4497).

Genomic DNA was extracted from multiple fin clip and muscle samples using E.Z.N.A.® Tissue DNA Kit (Omega Bio-tek, Norcross, GA). For Illumina library prep, approximately 1µg of gDNA from isolate A3764 was sheared to 300 bp using a Covaris Focused-ultrasonicator (Covaris, Woburn, MA) and subsequently processed using TruSeq DNA sample prep kit (Illumina, San Diego, CA) according to the manufacturer's instructions. Paired-end sequencing was performed on a single lane of HiSeq 2000 (Illumina, San Diego, CA) located at the Malaysian Genomics Resource Centre Berhad. Two additional libraries were constructed from specimen NTM A3764 and both libraries were sequenced on the MiSeq (2×300 bp setting) located at the Monash University Malaysia Genomics Facility.

To generate Oxford Nanopore long reads, approximately 5 µg of gDNA was extracted from isolates NTM A4496 and A4497, size-selected (8 – 30 kb) with a BluePippin (Sage Science, Beverly, MA) and processed using the Ligation sequencing 1D kit (Oxford Nanopore, UK) according to the manufacturer's instructions. Three libraries were prepared and sequenced on three different R9.4 flowcells using the MinION portable DNA sequencer (Oxford Nanopore, UK) for 48 hours.

Sequence read processing

Raw Illumina short reads were adapter-trimmed with Trimmomatic v.0.36 (*ILLUMINACLIP:2:30:10*, *MINLEN:100*) (Trimmomatic , RRID:SCR_011848)[20] followed by a screening for vectors and contaminants, using Kraken v.0.10.5 (Kraken, RRID:SCR_005484)[21] based on the MiniKraken DB. Kraken-unclassified reads i.e. non-microbial/viral origin were aligned to the complete mitogenome of NTM A3764 (See "Mitogenome Assembly") to exclude sequences of organellar origin. This results in a total of 42.35 Gb "clean" short reads. Nanopore reads were base-called from their raw FAST5 files using the Oxford Nanopore propriety base-caller, Albacore version 2.0.1. Applying a minimum length cut-off of 500 bp, this study produced a total of 8.95 Gbp in 895,672 Nanopore reads (N_{50}: 12.7 kb). A table with sequencing statistics is available as Supplemental Table 1.

Genome size estimation

K-mer counting with the "clean" Illumina reads was performed with Jellyfish v.2.2.6 (Jellyfish, RRID:SCR_005491)[22], generating k-mer frequency distributions of 17-,

21- and 25-mers. These histograms were processed by GenomeScope [23] that estimated a genome size of 791 to 794 Mbp with approximately 80% of unique content and a heterozygosity level of 0.6% (Supplemental Figure 1). Given that we had previously excluded adapters as well as sequences from contaminant or organellar sources, the max kmer coverage filter was not applied (*max kmer coverage: -1*). A separate estimation performed by BBMap [24] estimated a haploid genome size of 967 Mbp. The genome sizes estimated from both approaches are within the range of sizes listed for other *Amphiprion* species (792 Mb - 1.2 Gb) as reported on the Animal Genome Size Database (http://www.genomesize.com accessed on 11th November 2017)

Hybrid genome assembly

Short reads used for assemblies described in this study were only trimmed for adapters, but not for quality. Both short-read-only and hybrid *de novo* assemblies were performed with MaSuRCA v.3.2.2 (MaSuRCA, RRID:SCR_010691)[25]. During hybrid assembly, errors were encountered in the fragment correction step of the Celera Assembler (CA)(Celera assembler, RRID:SCR_010750). To overcome this, given that the CA assembler is no longer maintained, we disabled the *frgcorr* step based on one of the developer's recommendations and the hybrid assembly was subsequently improved with 10 iterations of Pilon v.1.22 (Pilon , RRID:SCR_014731)[26], using short reads to correct bases, fix mis-assemblies and fill assembly gaps. To assess the completeness of the genome, BUSCO v.3.0.2 (BUSCO , RRID:SCR_015008)[27] was used to locate the presence or absence of the Actinopterygii-specific set of 4,584 single copy orthologs (OrthoDB v9).

The short-read-only and hybrid assemblies yielded total assembly sizes of 851 Mb and 880Mb, respectively. Statistics for assemblies for each Pilon iteration are available in Supplemental Table 2. Inclusion of Nanopore long reads for hybrid assembly representing approximately 11× genome coverage led to a 94% decrease in the number of scaffolds (> 500 bp) from 106,526 to 6,404 scaffolds and an 18-fold increase in the scaffold N_{50} length from 21,802 bp to 401,715 bp (Table 1). In addition, the genome completeness was also substantially improved in the hybrid assembly, with BUSCO detecting complete sequences of 96.3% (4,417/4,584) of single-copy orthologs in the Actinopterygii-specific dataset.

Transcriptome sequencing and assembly

Total RNA extraction from RNAshield-preserved whole body and muscle tissues of isolate A4496 used Quick-RNA MicroPrep (Zymo Research Corpt, Irvine, CA) according to the manufacturer's protocols. After assessing total RNA intactness on the Tapestation2100 (Agilent), mRNA was enriched using NEBNext Poly(A) mRNA magnetic isolation kit (NEB, Ipwich, MA) and processed with NEBNext Ultra RNA library prep kit for Illumina (NEB, Ipwich, MA). Libraries from both whole body and muscle tissues were sequenced on a fraction of MiSeq V3 flowcell (1×150 bp). Single-end reads from both libraries in addition to two publicly available *A. ocellaris* transcriptome sequencing data (SRR5253145 and SRR5253146, Bioproject ID: PRJNA374650) were individually assembled using Scallop v0.10.2 [28] based on HiSat2 [29] alignment of RNA-sequencing reads to the newly generated *A. ocellaris* genome. The transcriptome assemblies were subsequently merged using the tr2aacds pipeline from the EvidentialGene [30] package and similarly assessed for completeness using BUSCO version 3 [27]. The final non-redundant transcriptome assembly, which was subsequently used to annotate the *A. ocellaris* genome, contains 25,264 contigs/isotigs (putative transcripts) with an accumulated length of 68.4 Mb and BUSCO-calculated completeness of 92.8% (Table 1).

Genome annotation

Protein-coding genes were predicted with the MAKER v.2.31.9 genome annotation pipeline (MAKER, RRID:SCR_005309)[31]. A total of three passes were run with MAKER2; the first pass was based on hints from the assembled transcripts as RNA-seq evidence (*est2genome*) and protein sequences from 11 fish species downloaded from Ensembl (Ensembl , RRID:SCR_002344)[32] (*protein2genome*), whereas the second and third passes included gene models trained from the first (and then, second) passes with *ab initio* gene predictors SNAP (SNAP , RRID:SCR_002127) [33] and Augustus (Augustus: Gene Prediction , RRID:SCR_008417)[34]. In the final set of genes predicted, sequences with Annotation Edit Distance (AED) values less than 0.5 were retained. A small AED value suggests a lesser degree of difference between the predicted protein and the evidences used in the prediction (i.e. fish proteins, transcripts). This resulted in a final set of 27,240 protein-coding genes with an average AED of 0.14 (Table 1). A BUSCO analysis on the completeness of the

predicted protein dataset detected the presence of 4,259 (92.9%) single-copy orthologs from the Actinopterygii-specific dataset.

Further, to infer putative function of these predicted proteins, NCBI's *blastp* v.2.6.0 (*-evalue 1e-10, -seg yes, -soft_masking true, -lcase_masking*) (BLASTP , RRID:SCR_001010)[35] was used to find homology to existing vertebrate sequences in the non-redundant (NR) database. Applying a hit fraction filter to include only hits with ≥70 % target length fraction, the remaining un-annotated sequences were subsequently aligned to all sequences in the NR database. With this method, 20,107 proteins (74%) were annotated with a putative function based on homology. Additionally, InterProScan v.5.26.65 (InterProScan , RRID:SCR_005829)[36] was used to examine protein domains, signatures and motifs present in the predicted protein sequences. This analysis detected domains, signatures or motifs for 26,211 proteins (96%). Overall, 96% of the predicted clown fish protein-coding genes were functionally annotated with information from at least one of the two approaches.

Mitogenome recovery via genome skimming

Genome skimming [37, 38] was performed on three additional *A. ocellaris* individuals from known localities (Supplemental Table 3). Mitogenome assembly was performed with MITObim version 1.9 (MITObim , RRID:SCR_015056)[39] using the complete mitogenome of *A. ocellaris* (GenBank: NC009065.1) as the bait for read mapping. The assembled mitogenomes were subsequently annotated with MitoAnnotator [40]. Consistent with original broodstock collection from northern Australia, the captive bred black and white *A. ocellaris* NTM A3764 exhibits strikingly high whole mitogenome nucleotide identity (99.98%) to sample NTM A3708 as a wild collection from Darwin Harbour, Australia. In addition, the overall high pair-wise nucleotide identity (> 98%) of NTM A3764 to newly generated and publicly available *A. ocellaris* whole mitogenomes further supports its morphological identification as *A. ocellaris* (Supplemental Table 3).

Identification of the *cyp19a1a* gene associated with sexual differentiation

The validated *cyp19a1a* enzyme of *Danio rerio* (Uniprot: O42145) was used as the query (E-value = 1e-10) for blastp search against the predicted *A. ocellaris* proteins. The top blast hit, AMPOCE_00012675-RA (71.5% protein identity to O42145), was searched (tblastn) against the NCBI TSA database (Taxon: *Amphirion*) and showed

strikingly high protein identity (99%) to a translated RNA transcript from *Amphiprion bicinctus* (c183337_g1_i2 : GDCV01327693) [5]. The *cyp19a1a* gene codes for a steroidogenic enzyme that converts androgens into estrogens [41] and was recently shown to be instrumental during sex change in *Amphiprion bicinctus* as evidenced by significant correlation and differential expression of this gene between male and mature females [5]. We also observed similar profile based on mapping of RNA reads from the publicly available male and female transcriptomes of *A. ocellaris* to the *cyp19a1a* gene region as visualized using Integrative Genomics Viewer [42] (Figure 2). The *A. ocellaris cyp19a1a* gene is located on a 419 kb scaffold and is spanned by multiple Minimap2-aligned Nanopore reads [43]. It is noteworthy that in the Illumina-only assembly, this gene is fragmented and located on 3 relatively short scaffolds (Figure 2).

Conclusion

We present the first clownfish genome co-assembled with high coverage Illumina short reads and low coverage (~11×) Nanopore long reads. Hybrid assembly of Illumina and Nanopore reads is one of the new features of the MaSuRCA assembler version 3.2.2 that works by constructing long and accurate mega-reads from the combination of long and short read data. Although this is a relatively computationally-intensive strategy with long run-times, we observed substantial improvement in the genome statistics when compared to Illumina-only assembly. As Nanopore long technology becomes more mature, it is likely that future de *novo* genome assembly will shift towards high coverage long read-only assembly followed by multiple iterations of genome polishing using Illumina reads.

Availability of supporting data

Data supporting the results of this article is available in the GigaDB repository [44]. Raw Illumina and Nanopore reads generated in this study are available in the Sequence Read Archive (SRP123679) whereas Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession NXFZ00000000, both under BioProject PRJNA407816.

Acknowledgements

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Competing interests

The authors declare that they have no competing interests

Figure Legends

Figure 1. The clown anemonefish (*Amphiprion ocellaris*): Photo by Michael P. Hammer

Figure 2. Mapping of MinION long reads, Illumina-assembled scaffolds and RNA-sequencing reads of male and female *A. ocellaris* to genomic region containing the *cyp19a1a* gene. Transcripts per million (TPM) values were calculated using Kallisto version 0.43.1 [45].

Supplemental Figure 1. Genome profiling of *A. ocellaris* based on Illumina short reads

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Table 1. Genome and Transcriptome statistics of the clownfish (*Amphiprion ocellaris*) genome

	Illumina (≥500bp)	**Illumina + Nanopore (≥500bp)**
Genome Assembly		
Contig statistics		
Number of contigs	133,997	7,810
Total contig size (bp)	851,389,851	880,159,068
Contig N_{50} size (bp)	15,458	323,678
Longest contig (bp)	204,209	2,051,878
Scaffold statistics		
Number of scaffolds	106,526	6,404
Total scaffold size (bp)	852,602,726	880,704,246
Scaffold N_{50} size (bp)	21,802	401,715
Longest scaffold (bp)	227,111	3,111,502
GC / AT / N (%)	39.6 / 60.2 / 0.14	39.4 / 60.5 / 0.06
BUSCO Genome Completeness		
Complete	3,691 (80.5%)	4,417 (96.3%)
Complete and single copy	3,600 (78.5%)	4,269 (93.1%)
Complete and duplicated	91 (2.0%)	148 (3.2%)
Fragmented	534 (11.6%)	63 (1.4%)
Missing	359 (7.9%)	104 (2.3%)
Transcriptome Assembly		
Number of contigs	25,364	
Total length (bp)	68,405,796	
Contig N_{50} size (bp)	3,670	
BUSCO completeness		
Complete	4,253 (92.8%)	
Complete and single-copy	4,128 (90.1%)	
Complete and duplicated	125 (2.7%)	
Fragmented	127 (2.8%)	
Missing	204 (4.4%)	
Genome Annotation		
Number of protein-coding genes	27,420	
Number of functionally-annotated proteins	26,211	

Mean protein length	514 aa
Longest protein	29,084 aa (titin protein)
Average number (length) of exon per gene	9 (355 bp)
Average number (length) of intron per gene	8 (1,532 bp)

Figure 1

Click here to download Figure Figure1.jpg

Figure 2

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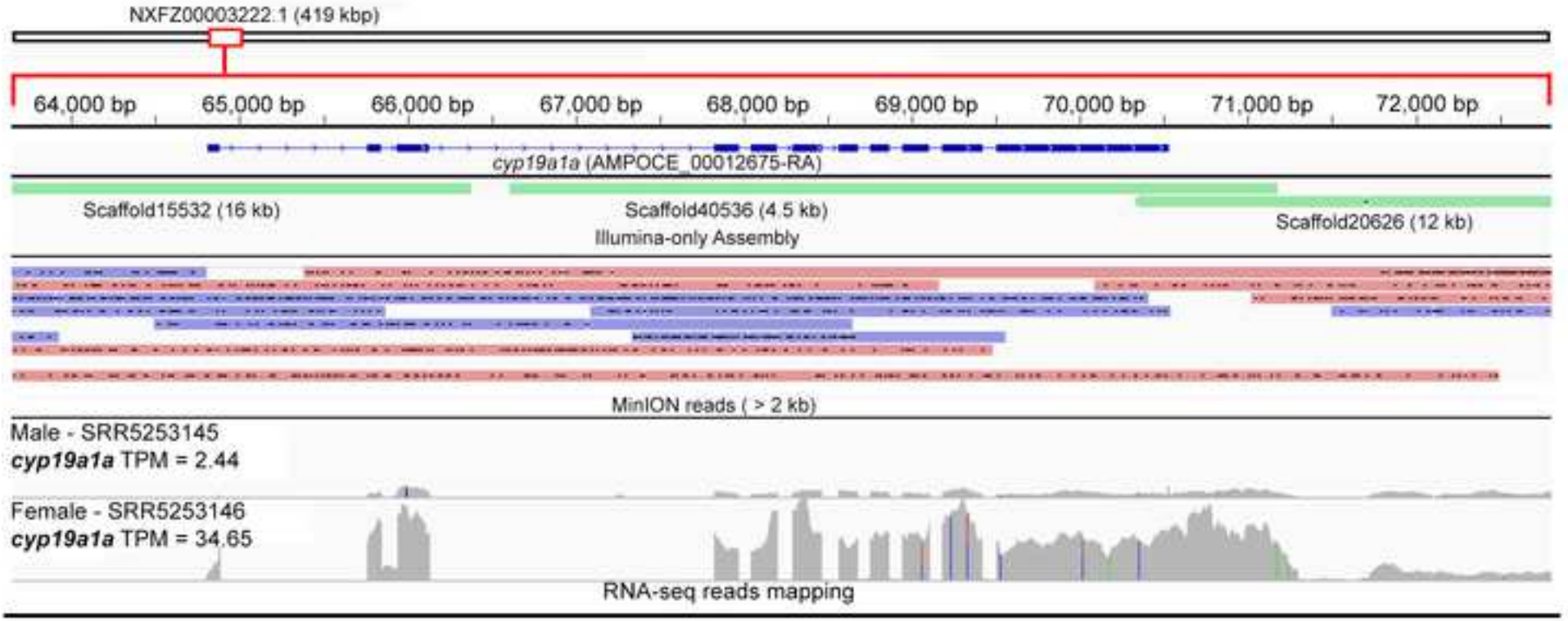

Supplementary Figure 1