Data\_description (Word version)

The analyses of Fst used a spreadsheet containing variant calls and inferred genotypes for the sampled individuals that was provided by the company LGC Genomics GmbH, Ostendstraße 25, Berlin, Germany (www.lgcgroup.com/genomics).

The genotypes were obtained as follows.

**Sequencing:**

Sequencing amount: 200x average raw read coverage per SNP target and sample

Sequencing type: 75 bp single read (Illumina NextSeq 500 v2)

**Initial data pre-processing included the following steps:**

* Demultiplexing of all library groups using the Illumina bcl2fastq 2.17.1.14 software
* 1 or 2 mismatches or Ns were allowed in the barcode read when the barcode distances between all libraries on the lane allowed for it
* Clipping of sequencing adapter remnants from all reads
* Reads with final length < 65 bases were discarded
* Quality trimming of adapter clipped Illumina reads
* Reads containing Ns were removed
* Reads were trimmed at the 3'-end to obtain a minimum average Phred quality score of 30 over a window of ten nucleotides
* Reads with final length < 65 bases were discarded
* Creation of FastQC reports for all FASTQ files
* Generation of the spreadsheet “read\_counts.xlsx”, containing all read counts for all samples

**Genotyping:**

The software Freebayes v1.0.2-16 (<https://github.com/ekg/freebayes#readme>), with ploidy set to 2 was used. Parameters were

 --min-base-quality 20 --min-supporting-allele-qsum 10 --read-mismatch-limit 4 --min-coverage 4 --min-alternate-count 2 --report-genotype-likelihood-max --exclude-unobserved-genotypes --genotype-qualities --ploidy 2 --min-alternate-fraction 0.166666666667 --targets design-id\_analysis-targets.bed --report-monomorphic --no-mnps --no-complex --mismatch-base-quality-threshold 10 --fasta-reference design-id.fasta --bam /Mapping/Bowtie2-global/ design-id/Group\_Freebayes-ploidy2/Freebayes-ploidy2\_sorted.bam --targets /VariantAnalysis/Bowtie2-global\_design-id/Group\_Freebayes-ploidy2/Freebayes/Freebayes-ploidy2\_batch.bed --vcf /VariantAnalysis/Bowtie2-global\_ design-id/Group\_Freebayes-ploidy2/Freebayes/Freebayes-ploidy2\_batch\_raw.vcf

This is appropriate for all chromosomes of the guppy, because the chromosome carrying the sex-determining locus has diploid coverage in males as well as females, as the Y chromosome (defined as carrying the male-determining factor) is not genetically degenerated, and is not hemizygous in males (Bergero *et al.* 2019; Darolti *et al.* 2020).

**References**

Bergero, R., J. Gardner, B. Bader, L. Yong and D. Charlesworth, 2019 Exaggerated heterochiasmy in a fish with sex-linked male coloration polymorphisms. Proceedings of the National Academy of Sciences of the United States of America 116**:** 6924-6931.

Darolti, I., A. Wright and J. Mank, 2020 Guppy Y chromosome integrity maintained by incomplete recombination suppression. Genome Biology and Evolution**:** evaa099.