The Earth BioGenome Project aims to produce reference genomes for all ~1.8 million known eukaryotic species over the next decade. Achieving this goal will require the current pace of reference genome production to increase by at least two orders of magnitude. Automation of the assembly process with a pipeline that is widely accessible to any research group will be required to achieve this speed-up. Enabling this goal requires sustained effort in three major areas: genome assembly optimization and best-practice development, computational infrastructure provisioning, and dissemination and training.

To optimize the assembly process and devise best practices, we combined the expertise of two projects—the Vertebrate Genomes Project (VGP) and the European Reference Genome Atlas (ERGA). The VGP is a collaborative effort to generate reference genomes for all ~70,000 vertebrate species. In the past 5 years, the VGP has released hundreds of new reference genomes supported by the development of automated assembly tools and workflows. The ERGA is a pan-European scientific initiative to generate reference genomes for all ~200,000 European eukaryote species, many of which are on the International Union for Conservation of Nature Red List of species at risk of extinction.

Advancing from the prior VGP work, originally on the DNAnexus platform (Supplementary Note, section 1.1), we developed a pipeline within the Galaxy ecosystem that combines Pacific Biosciences (PacBio) high-fidelity (HiFi) reads with long-distance information from Hi-C maps and/or optical maps to generate nearly complete assemblies (Supplementary Note, section 1.3). The pipeline further uses Hi-C or whole-genome sequence data from parents to produce chromosomal-level or whole-genome-level phased genomes, respectively. To streamline the assembly process and ensure quality, the pipeline includes extensive quality control (QC) functions at every step (Supplementary Fig. 1 and Supplementary Note, section 2.1). We suggest at least 30× PacBio HiFi coverage, and up to 60× coverage to accurately assemble highly repetitive regions, as well as 30× Hi-C coverage per haplotype. This is important to ensure a uniform read distribution during the random Poisson sampling process of whole-genome sequencing.

Galaxy allows users to execute complex workflows on thousands of datasets and terabytes of data either via a graphical user interface or programmatically via application programming interface (API) scripts. Major global Galaxy instances in the United States (https://usegalaxy.org), the European Union (http://usegalaxy.eu) and Australia (https://usegalaxy.org.au) are freely accessible to researchers worldwide and supported by public cloud infrastructures so that users are not required to install any tools or procure any infrastructure. Galaxy can also be installed locally to use existing high-performance

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**Fig. 1 | VGP–Galaxy assembly pipeline (version 2.1) consists of 10 workflows that can be combined into 8 analysis trajectories depending on the combination of input data.** A decision on whether to invoke workflow 6 is based on the analysis of QC output of workflows 3, 4 or 5 (see Supplementary Information for full explanation). Thicker lines connecting workflows 7, 8 and 9 reflect the fact that these workflows are invoked separately for each phased assembly (once for maternal and once for paternal).
computing (HPC) systems and configured to access heterogeneous, geographically distributed storage and computing resources.

The resulting VGP–Galaxy assembly pipeline is organized into 10 Galaxy workflows (Fig. 1; Supplementary Note, section 2.1) to account for different combinations of input data and stages of the assembly process. We systematically evaluated several scaffolding approaches, resulting in best-practice workflows using Hi-C and/or Bionano optical mapping data. We further implemented a dedicated mitogenome assembly pipeline to validate species identification and provide mitochondrial reference assemblies.
We also developed a decontamination workflow to remove exogenous sequences (e.g., viral and bacterial sequences), as well as mitochondrial artfacts that are often present in draft assemblies, as required for submission to public archives (Supplementary Note, section 2.2.4).

We first tested the automated workflows on the assembly of a reference genome of zebra finch (Taeniopygia guttata), for which a wide variety of genomic sequencing data types are available. This led to the development of three types of assembly trajectories (Fig. 1 and Supplementary Table 1): solo assembly (workflows 1, 3, 6 and 9; Fig. 1) using PacBio HiFi data for single individuals; Hi-C assembly (workflows 1, 4, 8 and 9) obtained by adding Hi-C data for phasing and scaffolding the contigs; and trio assembly (workflows 2, 5, 8 and 9) produced by using Illumina short-read data from parents for haplotype phasing (Fig. 1 and Supplementary Table 1).

To validate the pipeline, we used 51 vertebrate datasets for which PacBio HiFi and Hi-C data were available. We compared these assemblies against 19 previous PacBio continuous long-read–based genomes of similar size and complexity to confirm and extend the improvements to HiFi technology over continuous long-read methods reported previously (Fig. 2, Supplementary Table 5, Supplementary Fig. 6).

Given the improved haplotype resolution that resulted from adding Hi-C data, even for large (~4.3 Gbp), repeat-rich genomes, we recommend Hi-C HiFi phasing when parental data are not available. It is now possible to use well-tested kits as long as samples have been preserved properly (fresh frozen and without DNA and RNA preservatives that protect DNA but reduce protein crosslinks). For use with difficult-to-obtain samples, we have included pipeline options that do not require Hi-C data (Fig. 1).

Although all genome assemblies reported here are for vertebrates, the above principles and our pipeline can be applied to other animal, plant or fungal genomes by modifying a few parameters such as, for example, BUSCO clades necessary for accurate QC reporting (Supplementary Methods, section 3.3).

Our future work will focus on the continuous maintenance of the pipeline to improve its efficiency and scalability, automation of the curation process, incorporation of ultra-long-read data and development of effective genome annotation procedures.

To increase the robustness of the pipeline, we are developing additional workflows to take advantage of Oxford Nanopore Technologies (ONT) data, and particularly of ultra-long (UL) reads (>100 kb). These workflows use HiFi/UL hybrid assembly tools such as Verkko and the HiFi+UL version of HiFiasm, both of which we integrated into Galaxy. Each technology complements missing information from the other, with ONT reads being less accurate and HiFi reads being shorter and underperforming on certain genomic patterns, leading to sequencing bias that could affect specific taxa (Supplementary Fig. 14). This integration of complementary sequencing technologies will make our pipeline even more effective at generating complete and accurate reference genomes.

**Data availability**

The workflows, their description and instructions on how to use them can be found at https://galaxyproject.org/projects/vgp/workflows/. The requisite tools are installed on usegalaxy.org and usegalaxy.eu, and are in the process of being installed on usegalaxy.org.au. These genomes were supported by collaborators of the VGP and ERGA, and the QC analyses reported here to test the VGP Galaxy pipeline do not release those that are under specific embargo policies for genome-wide analyses (e.g., https://genomel10k.ucsc.edu/data-use-policies/). New genome assemblies are available in the GenomeArk repository: https://www.genomark.org/. After manual curation, the assemblies are submitted to the US National Center for Biotechnology Information (NCBI) under the BioProject Vertebrate Genome Project: https://www.ncbi.nlm.nih.gov/bioproject/489243.

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The authors declare no competing interests.

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