The European Reference Genome Atlas Initiative: A Collaborative Effort to Advance Our Understanding of European Biodiversity

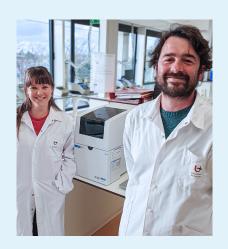
## A New Pan-European Consortium is Formed

Reference genomes are a powerful method to gain complete insight into the biology of unique species within our communities. They are critical for understanding the function of biodiversity in protecting threatened species, a concern of scientists worldwide. Considering this, researchers from across Europe decided to collaborate and form the European Reference Genome Atlas (ERGA) initiative. ERGA is a pan-European consortium of multidisciplinary scientists dedicated to generating a high-quality, complete genome for all European species, with 20 percent of those species being at risk of extinction.

In only two years, this group of like-minded scientists grew from approximately 60 members to over 600 members and counting, representing 234 research institutions. Establishing a fair and inclusive leadership hierarchy including a board of chairs, councils representing 38 European and associated countries, and 10 committees, ensured the continued growth and success of the initiative and its many dedicated members. Two researchers shared their knowledge and experiences throughout this ongoing endeavor: one of two Belgian council representatives and University of Liege researcher, Alice Mouton, Ph.D., along with University of Antwerp Postdoctoral Research Fellow Genevieve Diedericks, Ph.D.

# Pursuing Unified and Collaborative Reference Genomes for European Species

To demonstrate the feasibility of continent-wide collaboration, ERGA has launched a Pilot Project; established, funded, and driven entirely by its members. This project aims to build a pan-European genomics infrastructure that could support the inclusion and equal participation of each country at each step of the genome generation. According to Dr. Alice Mouton, co-leader of the pilot project with Dr. Ann Mc Cartney and Dr. Giulio Formenti, the challenge is to see if members can succeed in working together towards one common goal. "We asked the representative of each country to provide one species, so we could actually test this distributive model of reference genome establishment," she said.



Dr. Genevieve Diedericks and Henrique Leitão of the Svardal Lab, University of Antwerp, with the 4150 TapeStation system



To enable high-quality genomic results, the group implemented guidelines for species selection such as easy collection, small genomes, and possession of national or international permits. Today, there are more than 90 species in the project that conform to these guidelines. Furthering the collaborative nature of this project, several Sequencing Hubs and trans-border Genome Teams encourage equal participation for members at each step, from sample collection to sequencing and analysis. This multi-country support enables resource availability for every country involved while creating a feeling of partnership. "We have many sequencing centers and universities that contribute to the ERGA Pilot Project, either by contributing human resources to create the libraries or by providing free sequencing," Dr. Mouton stated.

The project also intends to test compliance with Earth BioGenome Project standards (https://www.earthbiogenome.org/), aiming for reference genomes with almost chromosome-level quality. This is accomplished through long-read sequencing, scaffolding, and annotation of data.

## **Overcoming Limitations Through Sample Quality Control**

Dr. Genevieve Diedericks is a member of the Svardal Lab at the University of Antwerp, one of three Sequencing Hubs where RNA sequencing and high throughput chromosome conformation capture (Hi-C) sequencing libraries are generated for scaffolding and annotating the genomes. During this project, she and her team discovered just how crucial quality control (QC) is for sample preparation and extraction processes, especially when faced with limited initial sample input and budgetary constraints.

Another challenge was limited resource availability. Therefore, guidelines were set to simplify the workflow and mitigate any potential library preparation failures. These guidelines included instructions for sample collection, storage, and shipment for enhanced sample preservation to avoid degradation and ensure ideal QC results. The Svardal Lab relied on two main methods for RNA and DNA homogenization, a simple and inexpensive needle and syringe method, and a second, more specialized bead bashing method requiring ceramic beads and specialized equipment. Once samples were completely homogenized, the extractions were performed per kit-specific protocols for each species type. A variety of species were analyzed, from fish and insects to various mammals and amphibians.

#### How is Sample Quality Tested?

"Before commencing with any library preparation, it is essential to complete a quality control step," Dr. Diedericks stated. This entails measuring RNA and DNA quantity using a fluorometric method and assessing the quality with an Agilent TapeStation system.

"We are fortunate enough to have access to a 4150 TapeStation system, and so we used the Agilent RNA ScreenTape assay to assess the degradation of

our RNA prior to continuing onto library prep," said Dr. Diedericks. She and her team noted the ease-of-use of the TapeStation system, including step-bystep loading instructions, run progress bar, and a specific post-run software for sample analysis. The software's color coordination of each sample's RNA integrity number equivalent (RIN<sup>e</sup>) helped the researchers interpret the results and determine which samples were most suitable to move onto library preparation. Their results showed two bands representing the 18S and 28S ribosomal banding patterns for each species in corresponding electrophoresis images. A higher RIN<sup>e</sup> value indicated more intact ribosomal gene fragments and provided a good indication of the efficacy of the overall RNA sample.

The DNA in Hi-C samples was also assessed for quantity and quality prior to library preparation. The Svardal Lab once again chose the Agilent TapeStation system to assess quality, through fragment size distribution and chromatin digestion efficiency (CDE) for the chosen extraction protocol. "We used the TapeStation system with a high-sensitivity DNA ScreenTape assay to generate our quality profiles," said Dr. Diedericks. Using assay-specific reagents and protocols, the flexible TapeStation system met their needs for both RNA and DNA sample quality control.

To produce high-quality libraries and optimal sequencing results it was important to focus on maximizing sample preparation and extraction, no matter the sequencing method or genetic starting material. Dr. Diedericks urged that "it is essential to do the intermittent QC steps as this will show you if you are on the right path-Even good, fresh samples could be problematic and difficult to work with." By focusing on sample quality assessment, her team identified ideal samples to produce strong sequencing results and advance the ERGA initiative's impactful studies.

# The Future of ERGA

Given current progress, members of ERGA are excited to continue working toward the atlas of reference genomes representing a diverse array of European species. Looking forward, members have pushed for funding initiatives to promote expansion and continue in the success seen thus far. Through these efforts, genomic biodiversity projects are being funded by Horizon Europe until early 2026, and formation of a partnership with Bioscan holds the ambitious intent to generate between 350 to 500 species in the reference genome atlas within the next three years. Even with these exciting new prospects, the original ERGA Pilot Project is continuing to grow and produce results. The hard work and dedication of the researchers, universities, vendors, and supporters made the project a reality, fostering an inclusive and collaborative understanding of biodiversity across Europe.

Read more about the ERGA initiative at: www.erga-biodiversity.eu/ and about the Biodiversity Genomics Europe (BGE) project at: https://biodiversitygenomics.eu/



Agilent 4150 TapeStation system

#### www.agilent.com/genomics/tapestation

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