European Reference Genome Atlas

Pilot Project

Official Guidelines

Version 2

Last Updated: 05th September 2023

Authors: Pilot Executive Committee

(Ann M. Mc Cartney, Giulio Formenti, Alice Mouton)

Contents

Background

The European Reference Genome Atlas (ERGA, https://www.erga-biodiversity.eu/), the European hub of the Earth Biogenome Project (https://earthbiogenome.org/), is a moonshot proposal to generate high quality reference genomes for the entire eukaryotic Biodiversity across Europe. Threatened, endemic and iconic species as well as those important for agriculture, fisheries, pest control, and for the function and stability of ecosystems are considered by ERGA as critical biodiversity. We are a fast growing consortium with a member base of over 750 researchers across 38 countries (including all 27 EU member states and EU Associated Countries, as well as representatives of other countries within the European bioregion). To demonstrate the feasibility of such continent-wide collaboration, ERGA has launched a Pilot Project committed to diversity, equity and inclusion through synergistic collaborations between ERGA researchers to generate reference grade genomes that can support biological discovery for the broader scientific community. Please see our ERGA Code of Conduct.

1) ERGA Pilot Project Rules

Coordination

The ERGA Pilot project is coordinated by the ERGA executive board (ERGA Chair and vice-Chairs) through the ERGA Pilot executive committee (pilot@erga-biodiversity.eu). Participants should do their best to involve the ERGA Pilot executive committee in the decision-making process, keep the committee informed of the proceedings, and avoid bilateral agreements between parties.

Team member roles

1) Sample collector and/or sample provider
2) Taxonomy classification and/or vouchering (specimen and/or sample)
3) Sample ambassador[1]
4) Wet-lab processing including HMW DNA extraction, library preps, sequencing - hands-on person(s) and/or group/facility PI
5) Genome assembly pipeline development/optimization/correction/supervision (directly associated to the species) and/or availability of computational resources
6) Genome assembly and/or curation
7) Genome annotation and/or genome analysis

Nature of the collaboration

All team members are collaborators and co-authors in the release paper. Local community partners may be acknowledged or included as co-authors. This could be an ERGA general release paper, a genome note or a specific paper including genome analysis.

Designated PI(s)

1) Each genome has a designated Principal Investigator (PI) who is responsible to ensure the coordination of the project.
2) If a sample ambassador specifically requests to lead the species genome analysis, this person will become the PI for this species' genome.
3) Participants covering the costs for the genomes can also request to be PI or co-PIs, in which case the sample ambassador must be in agreement. In case the participants that are covering sequencing costs are not based in the same country as the sample origin, council members from the country of origin must agree to the request.
4) Other participants can also request to become PI or co-PIs. The sample ambassador, in agreement with other potential co-PIs, decides on the request. Co-PIs appoint a coordinator among them responsible for the project, along with organizing the handling of the samples, to ensure that proper documentation meeting the Nagoya Protocol and local regulations is provided.

Genomes without PIs

All genomes without a PI will be managed by the ERGA executive board. All team members will be co-authors and the genomes will be released in joint publications promoted by ERGA or as Genome Notes.

Sample ownership

All samples should be DNA barcoded according to best practices given the taxonomic group. The sample provider maintains full ownership of the sample(s). Whenever feasible, samples including any left-over of DNA/RNA or tissue, will be stored long-term for reproducibility in

[1] The sample ambassador coordinates and co-works with the team to organize the samples, permits, barcoding, storage up to shipment to laboratory, storage of vouchers. The sample ambassador should be based in the country of origin or have an established research project in the area of sampling that justifies the involvement in genome establishment for species from another country. If not based in the country, s/he must provide proof of compliance with CBD Nagoya protocol as well as permits for in-country sampling, sample import/export and handling. The sample ambassador can also act as sample provider.

biobanks designated in concert with the ERGA executive board. All biobanked samples will be publicly visible and access should be granted on request to the sample provider, or on terms agreed upon with the biobank.

Data availability and publication embargo

In accordance with the Nagoya Protocol and to align with both the FAIR and CARE data principles, all data, particularly raw reads, genome assemblies and associated metadata (including associated traditional knowledge), will be available through an ERGA temporary server space with access as agreed upon by all team members upon generation. The PIs, in agreement with the ERGA executive board, have the right to first presentation of the data and their analysis, including journal publications, pre-prints, public conference talks, and press releases. Embargoes cannot last for more than two years after data release. Data should be immediately deposited in the ENA public archive and must be made publicly available either upon publication or at the end of the embargo period. However, if an alternative means of access is preferred, please email the ERGA pilot executive committee.

Exceptions to the above rules

Exceptions to the above rules can be made in specific circumstances in agreement with the ERGA executive board and pilot executive committee (pilot@erga-biodiversity.eu).

2) ERGA Pilot Project Data Sharing Policy

ERGA Pilot Project Data Sharing Policy

Version 1

Date: 27th December 2021

Author: Ann Mc Cartney, Pilot Executive Committee, ERGA Chairs, ERGA Council, Pilot Project Sample Ambassadors

As a public service, and in accordance to the three pillars of the United Nations Convention of Biological Diversity and its Nagoya Protocol; the conservation, sustainable use, and fair and equitable sharing of benefits arising from biodiversity resources, the European Reference Genome Atlas (ERGA) Pilot Project is making both the raw data (ONT, PacBio HiFi, IsoSeq, HiC, PCR Free Illumina and RNASeq reads) and the corresponding genome assemblies and annotations for all species available to the ERGA community prior to scientific publication. To balance the importance to ERGA that all Pilot Project data be made openly available and accessible as soon as possible, while safeguarding the interests of our contributing ERGA Genome Teams, we are reserving a maximum of two years after data generation for ERGA scientists to publish on the genome sequencing and analysis without concerns about

preemption by other groups. The data will be made available on an ERGA cloud storage facility, and access to the cloud storage will require acceptance of this policy. Project teams may opt to make their data openly accessible and reusable in advance of formal publication. This option will be noted in the cloud storage metadata.

We strongly encourage researchers to contact us at pilot@erga-biodiversity.eu if there are any queries about referencing or publishing analysis based on pre-publication data obtained via our ERGA Nextcloud instance. **By accessing these data, you agree not to publish any articles containing analyses of genes or genomic data on a whole genome or chromosome scale prior to: 1) having obtained consent to do so; 2) publication, by the Genome Team's associated PI found in genome_teams.txt and/or collaborators of a comprehensive genome analysis**. Analyses include the use of the data in developing and improving genomics procedures, identification of complete (whole genome) sets of general, coding and noncoding genomic features, whole-genome- or chromosome-scale comparisons with other species and use of the assembly as a platform for population genomics analyses.

The embargo on publication of analyses by researchers outside of the corresponding Pilot Project Genome Team will extend until the publication of the sequencing project is accepted, but no longer than two years. After this, all publications or presentations using prepublication data accessed through the cloud repository should clearly acknowledge the contributing genome team and the ERGA Consortium for facilitating the genomic resources allowing this particular research to be carried out.

ERGA Pilot data in the cloud repository may be freely downloaded and used by all within ERGA who respect the restrictions in the previous paragraphs. The assembly and sequence data should not be redistributed or repackaged without permission from the Genome Team's associated PI. Exemptions previously mentioned relating to raw data and genome assembly and annotation pre-publication release must be agreed upon by the ERGA Pilot Executive Committee, are subject to all ERGA associated sequencing centre's rules and regulations and the terms agreed upon in the Material Transfer Agreement.

3) Sample Permits and Collection

By participating in the ERGA Pilot Project all Genome Teams have agreed to adhere to the *ERGA Sampling Code of Best Practice*. Each Genome Team must ensure that all necessary metadata is collected. To do this the Sample ambassador must first read the *ERGA Sample Collection Standard of Practice (SOP)* to inform the completion of our *ERGA Metadata Manifest*. For inclusion into the ERGA Pilot Project, to obtain an ERGA BioSample ID, and to ensure compliance with the UN Convention of Biological Diversity Art. 15 and the Nagoya Protocol Art.14, this manifest must be submitted to our metadata brokering service, *COPO*, for validation (https://copo-project.org/). Sample ambassadors should submit the manifest, by logging on to COPO using their ORCiD and clicking the "Submit Data" button. If it is the sample ambassadors' first time logging into COPO, they should email COPO at El.COPO@earlham.ac.uk to request access to the ERGA Pilot Group. Upon access, sample ambassadors can then submit their manifest. A short 10' video is available to aid sample ambassadors in the submission process (Note: do not click the "Demo User" button at the beginning of the process, instead click the "Submit Data" button).

In tandem, sample ambassadors should ensure that all associated permits and vouchers are uploaded into the ERGA Nextcloud Instance. As permits may have personally identifiable information, a directory has been created with 'Upload Only' permissions, with access granted only under the terms of the ERGA Privacy Policy. All sample ambassadors have been sent an email entitled "Giulio Formenti shared >>ERGA_SPECIMEN_ID<< with you" containing their unique upload only link to be used for Permit and Image deposition. If you have not received this email please contact the Pilot Executive Committee. Prior to uploading, permits/vouchers spanning multiple pages should be merged into a single pdf document, and each pdf should be renamed according to the following standard naming convention:

ERGA_SPECIMEN_ID-INDIGENOUS_RIGHTS.pdf

ERGA_SPECIMEN_ID-ETHICS.pdf

ERGA_SPECIMEN_ID-SAMPLING.pdf

ERGA_SPECIMEN_ID-NAGOYA.pdf

ERGA_SPECIMEN_ID-VOUCHER.pdf

Any additional permits you would like to share with us should also be merged into a single pdf document and be uploaded under the name:

ERGA_SPECIMEN_ID-MISC.pdf

Without a manifest, and the necessary permits/vouchers, species will not obtain an ERGA BioSample ID and will be rejected from entry into the ERGA Pilot Project

3.1 Biobanking & Vouchering Your Sample

After collection, and as stipulated in the rules of the Pilot (Section 1), all ERGA Pilot Species must have a sample deposited in a biobank as well as an associated voucher specimen. Preferably, sample ambassadors should place samples in Biobanks/Collections available within the country of sample collection. However, if such infrastructure is not available or if multiple depositions are intended for safety reasons, sample ambassadors should utilize an ERGA recognised Biobank or Collection. Biobanks storing ERGA samples (ideally at -80°C or below) should make these visible through the Global Genome Biodiversity Network, GGBN.org.

Biobanks provide long term standardized, documented, secure and sustainable storage of samples at ultra-low temperatures. This warrants reproducibility of results and facilitates future research on the species not only by the sample provider but by the biodiversity genomics community at large.

Sample Requirements: Samples for biobanking should follow standards of practice for molecular analysis preservation techniques (e.g. snap-frozen tissue; tissue in 96-100% ethanol, DESS, RNAlater; extracted buffered DNA, RNA, etc.; also ideally viable cells as best-possible resource). ERGA promotes the biobanking of samples that can be used for subsequent genomic and transcriptomic sequencing analyses, and therefore encourages the splitting of samples into separate aliquots (potentially deposited at different biobanks) that are fit for such purposes. For samples biobanked for the purposes of High Molecular Weight DNA extraction and HiC, the standard sample preservation recommended is ethanol, DESS, and AllProtect or viable cells whereas for RNAseq, samples preserved in RNA-later are sufficient.

Documentation and Permits: Prior to shipment, all samples should be taxonomically determined and have all of the necessary collection, export/import, and Access and Benefit-sharing permits in place. This information must accompany your sample on shipment.

Access to an ERGA associated Biobank: The LIB Biobank at Museum Koenig, Bonn, offers to centrally store any Pilot Project animal samples where national infrastructure is not available–or in addition to this.The LIB Biobank can store nucleotide, tissue, or viable cell samples in liquid nitrogen gas phase at -190°C, is run by dedicated staff and is equipped with a temperature monitoring system giving external alarms. It operates a cell lab able to produce cell cultures from viable samples transferred non-frozen (ideally cooled) in suitable media. HMW samples can be transferred frozen, in dry shippers / vapor shippers, or on dry ice. Extracted DNA is stable enough for shipment at higher temperatures. Samples are made visible via the international biodiversity biobanking portal GGBN.org. Contact the LIB Biobank under j.astrin@leibniz-lib.de or biobank@leibniz-lib.de.

Each biobanked sample should be referenced to a voucher specimen (for morphological examination) deposited in a public collection. The specimen vouchering process is an

indispensable first step to ensure the legal collection of accurate biological data, accurate taxonomic identification, and the replicability of genetic studies [ref: doi: 10.7554/eLife.68264].

Sample Requirements: For morphological vouchering, samples can be preserved in ethanol, but are often dried specimens. Alternatively whole organisms can be submitted if small enough. Herbarium vouchers should follow the standards for the respective organism group, display all organs (including below-ground parts, reproductive structures and diaspores) wherever possible and be accompanied by an appropriately stored silica-dried tissue sample, ideally from the same individual. High-quality, informative photographs can complement the voucher specimen or, if there is no other way, function as e-vouchers. An extensive list of registered collections can be found on the GBIF website (https://www.gbif.org/grscicoll/collection/search). Documented Collections being used for the Pilot are listed in **Annex Table 1**.

Documentation and Permits: All samples must have all of the necessary collection and Access and Benefit-sharing permits in place. This information, as well as the voucher specimen data required by the respective collection, must accompany your sample on shipment.

4) Sequencing Requirements for Reference Genome Establishment and Annotation

Through grass roots efforts of ERGA members and the generosity of sequencing centres and providers, the Pilot Project has managed to secure funding to support species from each ERGA associated country - >90 species in total. The goal of the Pilot Project is to achieve the genome assembly standards determined by Earth BioGenome Project (https://www.earthbiogenome.org/assembly-standards) and further to provide a high quality annotation for each genome assembly. In order to achieve this the Pilot Project requires the synergistic generation and use of long read sequence data (PacBio and ONT), scaffolding data, (HiC or OmniC) and annotation data (RNAseq/IsoSeq) (Figure 1).

Figure 1: Pilot Project Expected Data Types

4.1) ERGA Pilot Sequencing Centre Sample Requirements

ERGA has many sequencing centres throughout Europe (Figure 2) actively participating in the Pilot Project. Each sequencing centre has specific sample preparation requirements that Genome Teams are expected to follow prior to sample shipment. It is important that these sample requirements are adhered to, to minimise sample waste and to reduce costs associated with resampling. Please see **Annex Table 2** for a comprehensive list of sample requirements for each sequencing centre, and contact points for further questions.

Figure 2: Geographical Distribution of ERGA Pilot Project Sequencing Centres

4.2) Target Data Types and Coverage

We are asking each Genome Team to provide the following data types/volumes as a minimum:

- 30X Pacbio or 60X ONT,
- HiC scaffolding 25X per haplotype
- RNAseq:
    - These species have two alternative tissue [tissue-type/stage/condition types available and we make requests to accommodate both:
        - 1) ~5 tissues available: we request a total of 100M reads (tissues combined).
        - 2) tissues are pooled: we request 30M reads.

- PCR-free Illumina reads for polishing [Optional for PacBio, strongly advised for ONT]: 25X per haplotype (50X for diploid).

5) Data Generation & Long Term Storage

All sequencing centres are expected to notify Genome Teams upon raw data generation. After approval, all raw data should then be transferred to our ERGA data repository. Genome Teams uploading data should ensure they are strictly adhering to the *ERGA Nextcloud Upload/Download Guidelines* (https://ergapilot.bsc.es/index.php/apps/files/?dir=/ERGAPilot&fileid=619).

ERGA does not have a standardized pipeline for genome assembly, however we have best practice pipelines available to support ERGA members in their creation of a high-quality reference genome using ONT or PacBio data. As soon as genomes have been assembled by the Genome Team, standard ERGA QC metrics should be calculated and presented at our ERGA SAC Meeting. To schedule your presentation please email assembly@erga-biodiversity.eu. Following presentation, all genomes should be uploaded to our ERGA repository and the first 50 genomes submitted will undergo internal curation by the Wellcome Sanger Institute. Please email grit-jira@sanger.ac.uk, to instigate the curation process. Attached with this email, to facilitate the curation process, all sample ambassadors should also upload a file containing all of the correct metadata in yaml format, this should follow a standard yaml template. Note: All uploaded data should follow our folder structure system (See Figure 2). For access to our long term storage repository please email pilot@erga-biodiversity.eu. If your team does not submit in time for Sanger curation, all curations should be conducted internally by the genome team. After internal curation, these assemblies ought to be reviewed by two other genome teams participating in the Pilot Project. Please contact the Pilot Executive committee if you would like contact information for other participating genome teams.

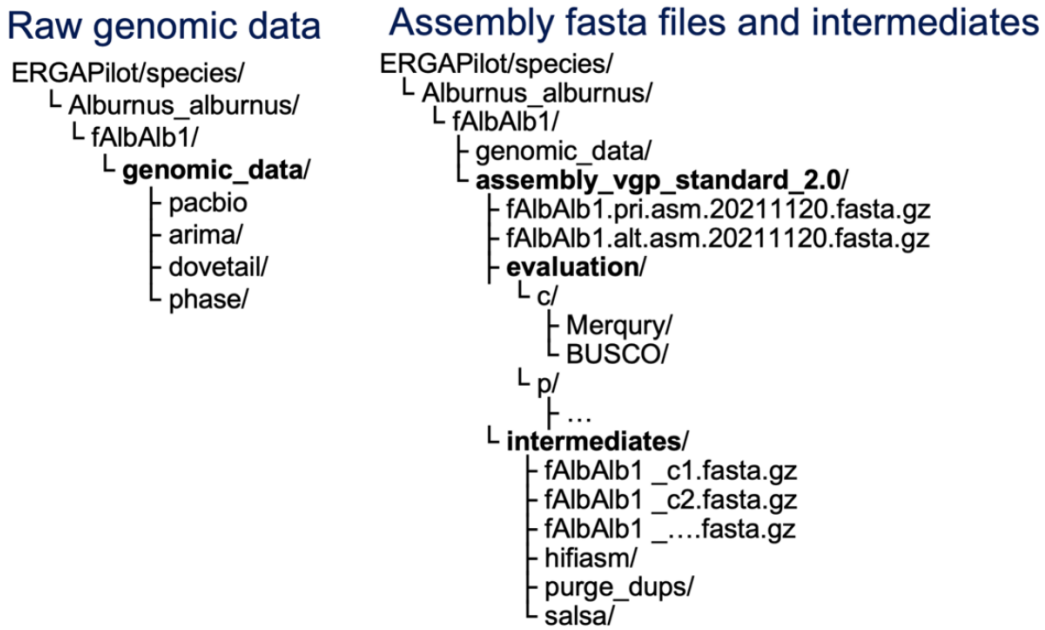


Figure 2: ERGA Data Repository Folder Structure

After curation, and under the guidance of the associated Genome Teams, the data will be brokered by COPO to the European Nucleotide Archive (ENA). The data will be associated with a newly generated ENA Bioproject ID, and the latter will be associated with the ENA umbrella Bioproject for the ERGA Pilot Project (https://www.ebi.ac.uk/ena/browser/view/PRJEB47820). This is linked to the broader ERGA umbrella BioProject (https://www.ebi.ac.uk/ena/browser/view/PRJEB43510), which is linked to the EBP BioProject, and with National/Regional/Affiliated BioProjects (Figure 3). Please follow the instructions on how to create an umbrella study here (https://ena-docs.readthedocs.io/en/latest/faq/umbrella.html). ERGA Pilot Project also have issued guidelines to facilitate Bioproject linking in the ENA and ENA's general documentation can be found here https://ena-docs.readthedocs.io/en/latest/. Note that the association with the ERGA umbrella BioProject will have to be performed by ERGA admins. Please reach out to them once you have completed the previous steps.

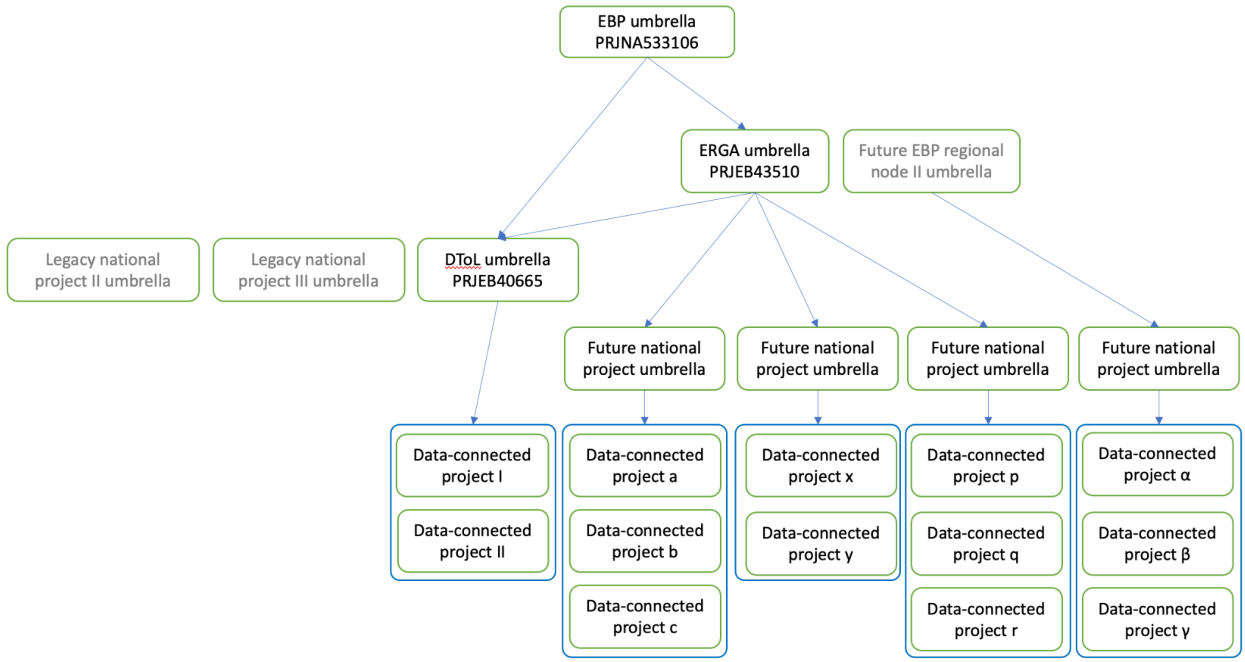
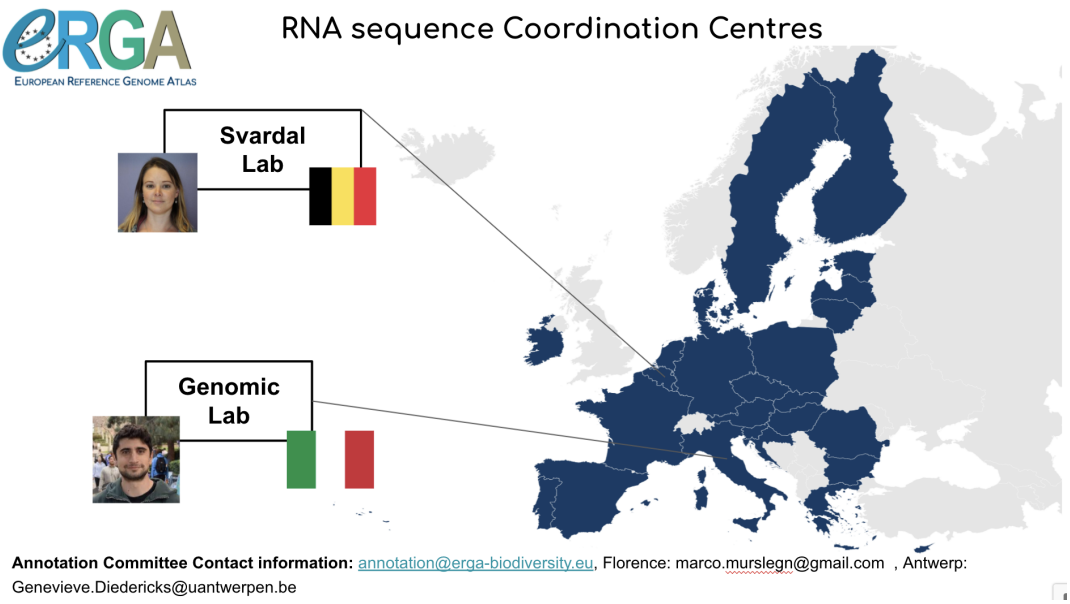


Figure 3: ERGA BioProject configuration in ENA.

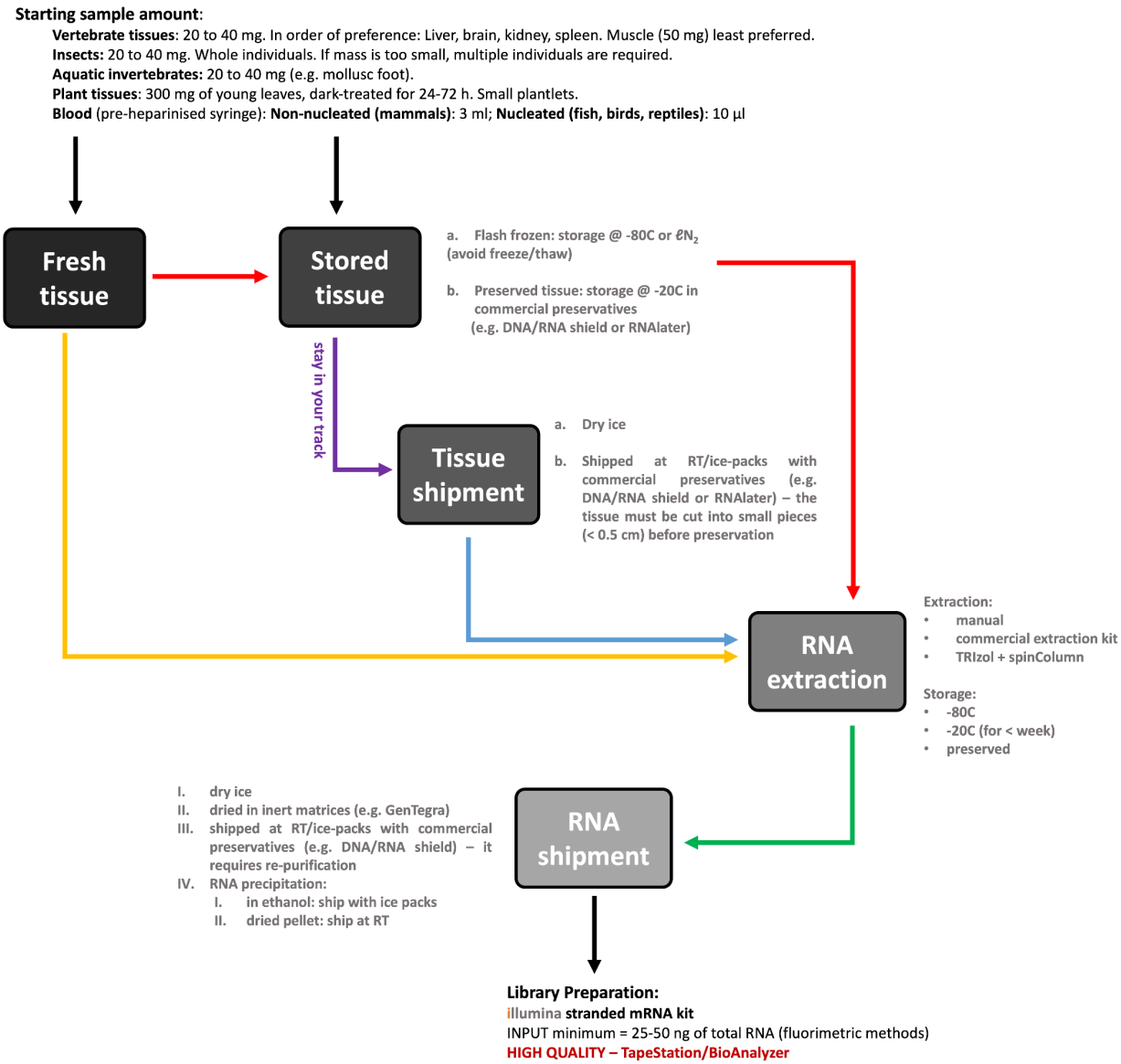
6) ERGA Supported Samples: Shipping Requirements

6.1) RNAseq Coordination Hub Locations



Annotation Committee Contact information: annotation@erga-biodiversity.eu, Florence: marco.murslegn@gmail.com , Antwerp: Genevieve.Diedericks@uantwerpen.be

6.2) RNAseq Sample Requirements



6.3) Shipping Details

FLORENCE:

Att. Claudio Ciofi
Department of Biology
University of Florence
Via Madonna del Piano 6
50019 Sesto Fiorentino (FI)
ITALY
Cell: +39 338 9151260

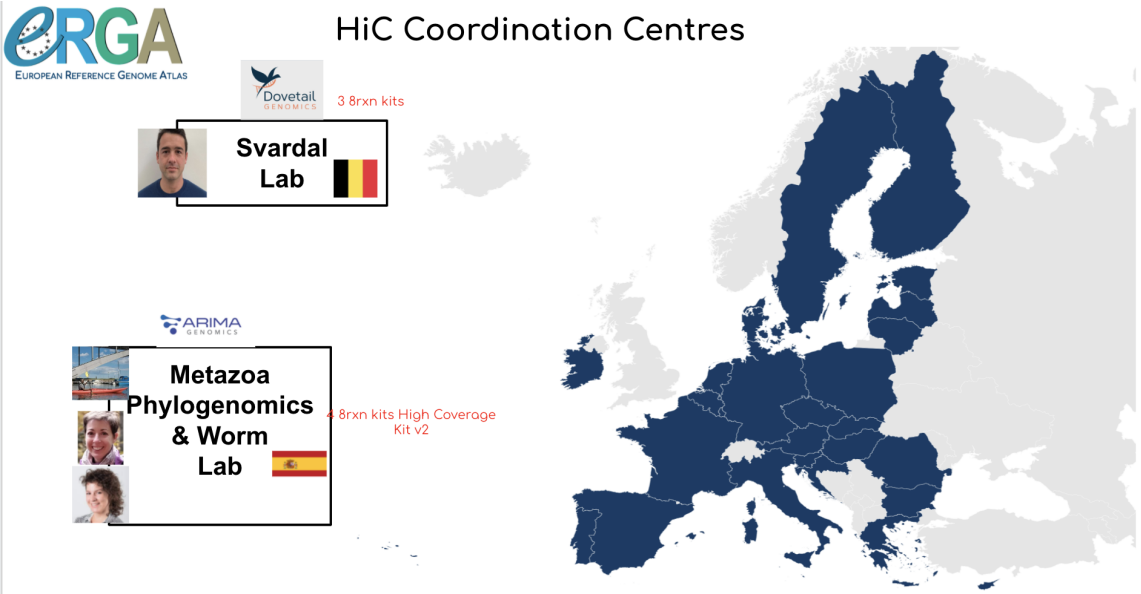
claudio.ciofi@unifi.it

Please email Marco and Claudio prior to shipment: claudio.ciofi@unifi.it,

marco.murslegn@gmail.com

ANTWERP:
University of Antwerp,
t.a.v. Genevieve Diedericks
Department of Biology
Campus Drie Eiken
Building D, Room D.D.144
Universiteitsplein 1
2610 Wilrijk
Belgium
Tel: +3249 3768 150
genevieve.diedericks@uantwerpen.be
Please email Genevieve prior to shipment: genevieve.diedericks@uantwerpen.be

6.4) HiC Coordination Hub Locations



6.5) HiC Sample Requirements

1. Label the top and side of the final sample tube with the sample ID, date of collection, and tissue mass.
2. Harvest fresh tissue using sterilized laboratory surgical tools.
3. Weigh the tissue and record the mass.

Arima- HiC Protocol:

- **Large animal tissues**: 50-200mg would be ideal (but less than 50mg can be used in low input protocol).
- **Small animal tissues** (whole specimens): they should occupy at least 50ul in volume. If less, please contact us before sending the samples (judit.salces@ibe.upf-csic.es).
- **Plant tissue**: 1-2 grams of young leaf tissue diced in 2cm pieces (healthy, no dead tissue, no stems).
- **Fungi**: Please, do contact Joseph Kirangwa (jkirangw@uni-koeln.de) for specific instructions.

Omni-C Protocol:

- **Birds, fish, and reptiles (nucleated blood cells)**: we prefer fresh blood samples (immediately snap-frozen and stored at -80°C as with other tissues), 10 µl volume (multiple replicates if possible).
- **Vertebrates in general**: spleen, liver, kidney, and brain are preferred tissues. The input amount is 25 mg (multiple replicates if possible).
    - Muscle is the least preferred tissue as it yields poorer results. If it is the only tissue available, at least 50 mg is required.
- **Small insects**: we may need to pool them to get sufficient yield. As such, multiple individuals are required (as many as feasible, considering the weight of a single individual).

4. Transfer the tissue to the bottom of a 1.5 mL conical tube/cryotube.
5. Snap-freeze the tissue by submerging the 1.5 mL conical tube/cryotube in liquid nitrogen for 30 seconds or until the tissue is fully frozen.
6. Store at -80°C until ready for shipment.
7. Ship samples on dry ice. Provide enough dry ice to last the entire duration of Transit (We highly suggest sending the samples on Mon/Tue to prevent transit during weekends). If possible, please send at least 2/3 replicates for crosslinking and sonication troubleshooting.

6.6) HiC Shipping Requirements

BARCELONA:
Judit Salces (lab P-51)
Instituto de Biologia Evolutiva (CSIC)
Passeig marítim de la Barceloneta 37-49
08003 Barcelona (Spain)

Please send an email once your sample has been shipped to judit.salces@ibe.upf-csic.es with the following information:

- Number of samples
- Tube IDs
- Tracking number of the parcel

- Expected day of arrival

ANTWERP:
University of Antwerp,
t.a.v. Henrique Leitao
Department of Biology
Campus Drie Eiken
Building D, Room D.D.144
Universiteitsplein 1
2610 Wilrijk
Belgium

7. Acknowledgements

We would like to acknowledge the contributions of all the ERGA Pilot Project participants, the ERGA Council, our HiC and RNAseq Coordination Hub Centres, and all ERGA Committees in the development of this guidance document. Further, we would like to thank LIB Biobank, and our sequencing centre partners (Annex: Table 2) for their support and assistance with text development. We would like to acknowledge access to the storage resources at Barcelona Supercomputing Center, which are partially funded from the European Union H2020-INFRAEOSC-2018-2020 programme through the DICE project (Grant Agreement no. 101017207). We would like to thank Alisha Ahamed, Josephine Burgin, Joana Paupério, Jeena Rajan and Guy Cochrane from the European Nucleotide Archive (ENA) for their support regarding data coordination and submission. Development of the pilot ERGA Data Portal (https://portal.erga-biodiversity.eu/) was funded by the European Molecular Biology Laboratory. Finally, we would like to acknowledge and thank all supplier partners that have kindly donated kits, reagents, and guidance to support species without funding to produce the generation of high-quality genomes and annotations. This support has been key to embedding a culture of diversity, equity, inclusion, and justice in the Pilot Project. Specifically we want to thank Dovetail Genomics, Part of Cantata Bio LLC, especially Mark Daly, Thomas Swale and Lily Shuie; Arima Genomics; PacBio; Integrated DNA Technologies (IDT); MagBio Genomics Europe GmbH; Zymo Research; Agilent Technologies; Fisher Scientific Spain; Illumina Inc.

Annex

Table 1: Collections in use by ERGA Pilot Project Sample Providers

Collections in Use
Bavarian Natural History Collections (SNSB), Germany
Natural History Museum, Hamburg, Germany
Bavarian State Collections of Paleontology and Geology,Germany
IPK Leibniz Institute, Germany
Herbarium Senckenbergianum, Germany

Botanischer Garten München-Nymphenburg -- Botanische Staatssammlung München, Germany
Zoological Research Museum Alexander Koenig, Bonn, Germany
Hungarian Natural History Museum, Hungary
Royal Belgian Museum of Natural Sciences, Brussels, Belgium
Muséum of zoologie and anthropology of the Université libre de Bruxelles (ULB), Belgium
Conservation Biology Research Lab Collection, University of Malta, Malta
Natural History Museum, Vienna, Austria
Institute of Microbiology, Prague, Czechia
University of Novi Sad, Serbia
Geneva Museum, Switzerland
Naturhistorisches Museum Basel, Switzerland
Musée cantonal de Zoologie, Lausanne, Switzerland
Muséum d'histoire naturelle de Genève, Switzerland
Natural History Museum Bern (NMBE), Switzerland
Herbarium Zürich, Switzerland
Herbarium Geneva, Switzerland
Museum of Natural History and Science, University of Lisbon, Portugal
Museum of Natural History and Science, University of Porto, Portugal
Museum of Lisbon, Portugal
DiSAA - Università degli Studi di Milano, Italy
MNHN, Paris, France
CGNRV, Paris, France
CRB (National Centre de Ressources Biologiques), France
Natural History Museum of Crete, University of Crete, Greece
Kew, UK
Natural History Museum of London, UK
Natural History Museum Sweden, Sweden
Natural History Museum, Denmark
Natural History Museum of Slovenia, Slovenia
Natural History Museum of Venice, Italy
Steinhardt Museum of Natural History, Israel
The National Natural History Collections, Hebrew University, Israel
CRBA (Centre de Recursos de biodiversitat Animal), Spain
Estación Biologica de Doñana, Spain
Entomoteca Dalberto Teixeira Pombo, University of Azores, Azores
National Museum of Natural History MNCN, Spain
Natural History Museum of the University of Oslo, Norway
University Museum of Bergen, Norway

National Museum of Natural History, Paris, France
Natural History Museum of Crete, Greece
Millennium seed bank, United Kingdom
Museu Nacional da História Natural e Ciência, Lisbon, Portugal
University of Warsaw, Poland
Natural History Museum of Venice, Italy
Università degli Studi di Bari “Aldo Moro”, Italy
Uppsala University, Uppsala, Sweden
Zoological Research Museum Alexander Koenig (ZFMK), Germany
University of Oulu, Oulu, Finland
Natural History Museum of the University of Oslo, Oslo, Norway
University of Zurich, Switzerland
Museum of Natural History and Science, U. Porto, Portugal, Porto.
CIBIO/BIOPOLIS, Vairão, Portugal
CRB Prunus, France
Botanical Institute Barcelona, Spain
Upper Austrian State Museum, Austria
Swedish Museum of Natural History, Sweden
University of Ioannina, Greece
Naturhistorisches Museum Bern, Switzerland
Royal Botanic Garden Edinburgh, Scotland

Table 2: Sequencing Centre Sample Requirements and Contact Points

	PacBio/ONT	HiC	IsoSeq & RNA	Shipping Requirements
Earlham Institute	**<u>PacBio HiFi</u>** **Recommended (per 3 SMRT cells required)** Input: gDNA Quantity: 23µg Concentration: 50ng/µl Volume: 50µl - 400µl. Quality: No more than 20% of molecules <40Kb in size. 260/280: 1.6-2.0. 260/230: 1.8-2.4. Should be supplied in a low salt buffer such as Qiagen Elution Buffer, 10 mM Tris-Cl, pH 8.5 or PacBio EB buffer. **Minimum (per 3 SMRT cells required)** Input: gDNA Quantity: 13µg Concentration: 50ng/µl Volume: 50µl - 400µl. Quality: No more than 20% of molecules <40Kb in size. 260/280: 1.6-2.0. 260/230: 1.8-2.4. Should be supplied in a low salt buffer such as Qiagen Elution Buffer, 10 mM Tris-Cl, pH 8.5 or PacBio EB buffer.	Protocol in development (Dovetail Omni-C protocol). All material flash-frozen.	**<u>Illumina NEBNext Ultra II DIrectional RNA-Seq (poly-A selection)</u>** **Recommended:** Input: Total RNA. Quantity: 2µg. Concentration: 50 - 100ng/µl. Volume: 20µl -50µl. Quality: RIN 7 Sample should contain no more than 15% of gDNA. Must be supplied in neutral buffer (e.g. 10 mM Tris-HCl, pH 8, or Low TE (10 mM Tris, pH 8, 0.1mM EDTA)), NOT water **Minimum:** Input: Total RNA. Quantity: 1µg. Concentration: 50 - 100ng/µl. Volume: 20µl -50µl. Quality: RIN 7 Sample should contain no more than 15% of gDNA. Must be supplied in neutral buffer (e.g. 10 mM Tris-HCl, pH 8, or Low TE (10 mM Tris, pH 8, 0.1mM EDTA)), NOT water. **<u>PacBio Iso-Seq</u>** **Recommended (per 2 SMRT cells required)** Input: Total RNA. Quantity: 1µg Concentration: ≥71ng/µl Volume: ≥17µl Quality: RIN ≥ 8. Sample should contain no more than 10% gDNA contamination. **Minimum (per 2 SMRT cells required):**	Contact: seanna.mctaggart@earlham.ac.uk

			Input: Total RNA. Quantity: 500ng Concentration: ≥71ng/µl Volume: ≥10µl Quality: RIN ≥ 8. Sample should contain no more than 10% gDNA contamination	
Wellcome Sanger Institute	Please contact the Sanger for more information.	Please contact the Sanger for more information.	Please contact the Sanger for more information.	**Shipment Address:** Tree of Life Sample Management Wellcome Sanger Institute Wellcome Genome Campus Hinxton Cambs CB10 1SA Please contact prior to sample submission and await approval before shipment. **Contact:** treeoflifesamples@sanger.ac.uk
GIGA-Genomics core facility	Provide HMW DNA. Size can be evaluated on a Fragment Analyser or an 0.8% agarose gel with a HMW ladder. Quantity (Qubit or picogreen) and determine purity with nanodrop (ratio 260/280 and 260/230). We will need 6 µg (or 100-200 fmol) of pure high molecular weight genomic DNA. Transport on dry ice.			Contact: wouter.coppieters@uliege.be
University of Florence	**TISSUE:** Collect at least 200 mg of tissue in a 1.5 screw-cap tube and flash freeze in either liquid nitrogen or at – 80 C. Ship on dry ice. **INSECTS:** Flash freeze in a screw-cap tube in either liquid nitrogen or at – 80 C. Ship on dry ice **NUCLEATED BLOOD:** Collect 200 ul blood into a 1.5 ml screw-cap tube containing 2 ul 0.5M EDTA dipotassium or tripotassium salt. Store at - 80 C and ship on dry ice. If EDTA is not available, prepare on ice two 1.5 ml screw cap tubes each with 1 ml 95%-100% molecular-grade ethanol. Aliquot 100ul of blood into each tube, store at – 80 C and ship on dry ice. **NON-NUCLEATED BLOOD:** Collect 2 ml blood into a screw-cap tube containing 20 ul 0.5M EDTA dipotassium or tripotassium salt. Store at - 80 C and ship on dry ice.		**IsoSeq:** Extract at least 500 ng RNA with a RIN > 8. Store RNA in a screw-cap 1.5 ml tube at – 80 C. Ship on dry ice. GenTegraRNA protection kit is currently being tested for shipping RNA at ambient temperature. **RNASeq:** Extract at least 500 ng RNA with a RIN > 8. Store RNA in a screw-cap 1.5 ml tube at – 80 C. Ship on dry ice. GenTegraRNA protection kit is currently being tested for	Ship on dry ice. GenTegraRNA protection kit is currently being tested for shipping RNA at ambient temperature. Contact: claudio.ciofi@unifi.it

	DNA: Extract 5 ug of DNA with a 260/280 nm ratio between 1.8 and 2.0 and a 260/230 nm ratio of approximately 2.0. Lower amount of DNA (> 20 ng) can be extracted for genomes of up to 500 Mb. Integrity of DNA will be measured in our lab via PFGE. Store DNA in a screw-cap 1.5 ml tube at – 80 C. Ship on dry ice.		shipping RNA at ambient temperature.	
CNAG	**ONT sequencing WGS** **Extracted HMW DNA:** ● Concentration: 50ng/ul (fluorometric) ● Quantity: 16ug ● Integrity: >48kb ● Purity: OD260/OD280 ratio 1.8 to 1.9., OD260/OD230 ratio 2.0-2.2. **DNA Storage:** 4ºC **DNA Shipping:** 4ºC **Animal tissues**: 10-15 vials with 50-80 mg of non-fatty soft tissue (internal organs). If the specimen is muscle tissue or the entire individual, please provide at least 200 mg/vial. **Mammalian cells**: 5 vials 1×10ˆ6 pelleted cells from cell culture or sorted cells **Non-nucleated blood**: Collect the whole blood into EDTA tubes and aliquot it in 500ul blood aliquots into 10-15 barcoded LVL tubes. If needed, EDTA 1,3ml tubes will be provided by the CNAG upon request (Sarstedt tubes; 1.3 ml micro specimen container, concentration 1,6 mg EDTA K3/ml blood). For the EDTA tube it is important to keep in mind that you must fill the tube with blood with the intended volume, otherwise the appropriate EDTA concentration may not be respected. **Nucleated blood**: 10-15 aliquots of 20 µl of nucleated blood, flash-frozen. Collect the whole blood into EDTA tubes and aliquot it in barcoded LVL tubes, see above procedure for non-nucleated blood. **Insects**: Due to the small size of insects, tissue isolation might be difficult. If this is the case, send the entire individual, further pooling of individuals might be necessary. To avoid keratinous and chitinous specimens, also a presence of organic acids (formic acid) or high amounts of pigments, we suggest to target colorless pupae or larvae or extraction of certain body segments that are low in contaminants. **Plants and filamentous fungi**: 10-15 4ml LVL vials with 2-5 g of young, etiolated, fresh leaves or non-fibrous tissue. **Specimen Storage:** **Solid tissues**: Snap-frozen in liquid nitrogen shall be stored on -80ºC or in liquid nitrogen. We prefer to avoid: tissue preserved in ethanol or in RNAlater. **Blood**: Snap-frozen in liquid nitrogen or dry ice. If not possible,	**Omni-C** **Input: cells** **Animal tissues**: 5 vials with 50 mg of non-fatty soft tissue (internal organs). If the sample is muscle tissue, please provide at least 200 mg. **Non-Mammalian tissues**: 5 vials with 50 mg of tissue. **Plant tissues**: 5 vials with 300 mg of young leaves or non-fibrous tissue **Mammalian cells**: 5 vials 1×10ˆ6 pelleted cells from cell culture or sorted cells **Non-nucleated blood (mammals):** If stored in EDTA, Heparin, 1-7ml of blood, as available in function of the size of the animal. If flash-frozen, buffy coat from 1-7ml of blood. **Nucleated blood**: 5 aliquots of 20 µl of blood if nucleated red cells (birds, reptiles, fishes), flash-frozen. Do not store in any buffers or collect in EDTA tubes and aliquot into provided barcoded tubes. **Storage:** **Tissues**: Snap-frozen in liquid nitrogen (liqN2). Not accepted: preserved in ethanol or in RNAlater. **Blood**: Snap-frozen in liquid nitrogen or dry ice, or for max 48hrs in EDTA, Heparin tubes. Not accepted: lysis buffers, detergents or ethanol to preserve the blood. **Cells**: Snap-frozen pellet in liquid nitrogen. If the cells come from cell culture, wash with 1X PBS 3 times after trypsinization, pellet by centrifugation, remove PBS and freeze in liquid nitrogen. ● **Shipping**: LN2	**RNASeq** **Input: extracted total RNA** ● Concentration: 50ng/ul ● Quantity: 1ug ● RIN: >8 **Storage**: -80ºC or LN2 **Shipping:** on dry ice in cryo-tubes	Contact: projectmanager@cnag.crg.eu

	blood in EDTA can be stored up to 48h at 4ºC prior to freezing. Aliquot the specimen before freezing to avoid freeze-thaw cycles. Not accepted: blood specimen in any lysis buffers. We prefer to avoid: to preserve the blood in ethanol. **Cultured cells**: Snap-frozen pellet in liquid nitrogen. If the cells come from cell culture, wash with 1X PBS 3 times after trypsinization, pellet by centrifugation, remove PBS and freeze in liquid nitrogen. Not accepted: cultured cells in any lysis buffers or glycerol cryopreserved. **Specimen Shipping**: on dry ice.			
Norwegian Sequencing Centre	HMW gDNA: amount depends on genome size, at least 10 µg DNA for genomes up to 2 Gb. More information: https://www.sequencing.uio.no/pacbio-services/dna-requirements/	Flash frozen for different sample types: https://www.sequencing.uio.no/pacbio-services/dna-sequencing/hi-c-sample-requirements/	Extracted total RNA, 20 µl normalized to 43 ng/µl.	All samples: frozen on dry ice only. More information: https://www.sequencing.uio.no/pacbio-submission/ Contact: cees-drift@sequencing.uio.no
Genoscope	If HMW DNA is to be extracted at Genoscope, the protocol and specific requirements will depend on the species and genome size. Some examples: **PLANTS:** Multiple screw-cap tubes each with 1 g of snap frozen dark-treated fresh young leaves. Ship on dry ice. **ALGAE**: 1 g of culture/tissue snap frozen on liquid nitrogen. Ship on dry ice. **NUCLEATED BLOOD** (e.g.: reptiles, amphibians, birds): Collect 250 ul blood into a screw-cap tube containing preferentially EDTA dipotassium. Ship on dry ice. **NON-NUCLEATED BLOOD** (e.g. mammals): Collect 2 ml of blood into a screw-cap tube containing preferentially EDTA dipotassium. Ship on dry ice. Blood samples should be frozen as quickly as possible after being drawn. Storage at 4 °C should be limited to 2 days or less to prevent sample degradation. Blood samples should be aliquoted to avoid repeated freeze-thaws. If sending extracted DNA - HMW-DNA: (UV/Qubit ratio <1.5, 260/280 = 1.8-2.0, 260/230 = 2.0 - 2.3)	For Dovetail Omni-C, the protocol and specific requirements will depend on the organism. Some examples: **MAMMALIAN CELLS:** aliquot 1x10^6 cells (wash in PBS, spin at 3000g for 5 min; remove supernatant, freeze at -80°C at least 30 min. Ship on dry ice. **MAMMALIAN TISSUE:** 20 mg of snap frozen tissue. Ship on dry ice. **NUCLEATED BLOOD:** 10 µL / tube containing EDTA (~1 x 10^5 cells). Ship on dry ice. **NON-NUCLEATED BLOOD:** 1 mL / tube of fresh blood sample (Dovetail recommendations although we haven't tested them yet). - **ANIMAL TISSUES** (other than insects & marine invertebrates): 20 mg of snap frozen tissue sample. Ship on dry ice. **-INSECTS AND MARINE INVERTEBRATES:** 20 mg of frozen tissue sample. Degutting adults prior to freezing is recommended.. Alternatively, they may be starved for a few days prior to freezing. Flash freeze the individuals in liquid nitrogen, either in bulk for inbred species or individually for outbred species, and store at -80°C prior to use. Ship on dry ice. **PLANTS:** 300 mg of snap frozen dark-treated	**RNASeq:** Extracted total RNA (RIN > 8) Minimum 200 ng (1 ug is recommended). Shipment on dry ice.	All samples shipped on dry ice. Contact: pcoutool@genoscope.cns.fr

		young leaves. Store at -80°C until use. Ship on dry ice.		
SciLifeLab	If for DNA extraction - specific requirements will apply.https://ngisweden.scilifelab.se/wp-content/uploads/2020/04/appendix_d_dnaextraction_material_guidelines_200415.pdf Amount of input tissue and DNA depends on the genome & body size.If sending extracted DNA - HMW-DNA, it must be ultrapure: (UV/Qubit ratio <1.5, 260/280 = 1.8-2.0, 260/230 = 2.0 - 2.3)	Depends on organism type, see https://ngisweden.scilifelab.se/methods/dovetail-omni-c/	**IsoSeq:** Extracted total RNA (RIN > 8) Minimum 500 ng total RNA. Minimum concentration 45 ng/ul, volume is not important. **RNASeq:** Extracted total RNA (RIN > 8) Minimum 200 ng (1 ug is recommended)	**All samples - solid frozen on dry ice only.** All extraction and sequencing projects must be registered via https://ngisweden.scilifelab.se/orders . **ALWAYS** contact us before shipment! We offer free, non-binding consultations for sample collection, DNA/RNA extractions, sequencing setup, etc. Contact: olga.pettersson@scilifelab.uu.se
Dresden MPI	Preferentially snap-frozen tissue or in suitable preservation solution (e.g. Allprotect, or EtOH for e.g. nucleated blood). For HMW gDNA: ultra-pure and long fragments. The SciLifeLab guide on tissue and DNA defines the requirements. In general, we offer extraction of HMW genomic DNA or consult on suitable extraction protocols. We will always do the final quality control of the sample.	Flash frozen tissue (or whole small organisms), preservation in Allprotect (Qiagen) or RNAlater is possible, ship always on dry ice, we follow the ARIMA HIC+ protocol.	**RNA:** Extracted RNA (RIN > 8) but also snap-frozen tissue or tissue in RNAlater works. Shipments on dry ice. **IsoSeq:** Extracted RNA (RIN >8, at least 300 ng total RNA) but also snap-frozen tissue or tissue in RNAlater works. Shipments on dry ice.	Tissues, HMW gDNA and RNA on dry ice, it is recommended to get in touch with us **before** sampling and shipping (dcgc-longread@groups.tu-dresden.de)
WGGC Cologne, Düsseldorf, Bonn			**RNASeq:** Extracted RNA	RNA: ship on dry ice; HMW DNA samples were also transported on dry ice Contact: nuernberg@uni-koeln.de
NGSP, University of Bern	**PacBio HiFi** We offer a HMW gDNA extraction service. Our exact protocol will depend on the species and the nature as well as quantity of available starting material. We have optimised our protocols for bacteria, various tissue types, insects, marine invertebrates, nucleated blood, non-nucleated blood and some plants. We are gaining experience but species that are more exotic will require a consultation prior to starting the project. It helps to send aliquots so that multiple protocols can be executed.	We offer a HiC service using Proximo Hi-C kits from Phase Genomics. To start such protocols, we need: **Human/Animal Cells:** 200K-2 M. Ship on dry ice **Human/Animal Blood:** 200-300 µL, Ship on dry ice **Human/Animal tissues:** 50-200 mg, Ship on	**IsoSeq:** Extracted total RNA (RIN/RQN ≥ 8, or ≥7 if plant material) Minimum 500 ng total RNA. Minimum concentration 45 ng/µL, volume is not important. **RNASeq:** Extracted total RNA (RIN ≥ 8 or ≥7 if plant material)	Please contact pamela.nicholson@unibe.ch For our exact shipping requirement and address. In general, original sample material should be shipped as specified/necessary. Already extracted and highly pure gDNA can be shipped at RT, cold, dry ice

	PLANTS: Multiple screw-cap tubes each with 1-10 g of snap frozen dark-treated fresh young leaves. Ship on dry ice. **NUCLEATED BLOOD:** Collect 10-300 µL blood into a screw-cap tube containing preferentially EDTA dipotassium. Ship on dry ice. **NON-NUCLEATED BLOOD:** Collect 1-2 mL of blood into a screw-cap tube containing preferentially EDTA dipotassium. Ship on dry ice. **MAMMALIAN CELLS:** aliquot 1x10^6 -1x10^7 cells (wash in PBS, spin at 3000g for 5 min; remove supernatant, freeze at -80°C at least 30 min. Ship on dry ice. **MAMMALIAN TISSUE:** 20-100 mg of snap frozen tissue sample aliquots. Ship on dry ice. **ANIMAL TISSUES** (not insects & marine invertebrates): 20-100 mg of snap frozen tissue sample aliquots (not liver). Ship on dry ice. **INSECTS/MARINE INVERTEBRATES/OTHER: please consult with us.** Blood samples should be frozen as quickly as possible after being drawn. Storage at 4 °C should be limited to 2 days or less to prevent sample degradation. Blood samples should be aliquoted to avoid repeated freeze-thaws.In general, fresh sample material that is not in any kind of preservation buffer or frozen tends to yield the best results. We tend to consult the following guides and repositories:1) https://extractdnaforpacbio.com/, 2) https://www.pacb.com/wp-content/uploads/Technical-Note-Preparing-DNA-for-PacBio-HiFi-Sequencing-Extraction-and-Quality-Control.pdf, 3) https://www.pacb.com/wp-content/uploads/Technical-note-Preparing-Samples-for-PacBio-Whole-Genome-Sequencing-for-de-novo-Assembly-collection-and-storage.pdf, and 4) Overview-Sequel-Systems-Application-Options-and-Sequencing-Recommendations.pdf (pacb.com) If you plan to extract your own HMW gDNA and wish to send it to the NGSP Bern, Switzerland, please carefully consider the sources of information above. If you would like to avoid futile sample shipments, please ensure that the gDNA is HMW (predominantly above 40KB) and ultra pure: (UV/Qubit ratio <1.5, 260/280 = 1.8-2.0, 260/230 = 1.8 - 2.3), prior to shipment by conducting fluorometry, spectrophotometry and basic electrophoresis. Upon arrival, we will perform a full gDNA QC.	dry ice ***Cells or blood samples are preferred.*** **Insects:** 50-200 mg, Ship on dry ice **Plant:** 0.2-1 g, Ship on dry ice ***Young non-woody tissues are preferred.*** NGS libraries are sequenced on an illumina sequencer at 2 x 150 bp < 0.4 Gbp > 100 million pairs 0.4 Gbp – 1.5 Gbp > 150 million pairs > 1.5 Gbp > 200 Million pairs https://phasegenomics.com/products/proximo/	Minimum 200 ng (1 µg is recommended, given the application) Minimum concentration 4 ng/uL, in 50 µL.	or in Biomatrica DNAstable tubes Already extracted total RNA should be shipped on dry ice or using a Tube Kit, for room temp preservation using Biomatrica RNAstable tubes

	Quantity Requirements (not including QC requirements):Standard HiFi library = min. 5 µg, Low input DNA HiFi library = min 300ng/600Mb, Ultra Low input HiFi library= min. 5-20 ng/500Mb			
Functional Genomic Centre Zurich	DNA must be of HMW,and ultra pure: (UV/Qubit ratio <1.5, 260/280 = 1.8-2.0, 260/230 = 1.8 - 2.3) **PacBio/ONT:** DNA must be of High-molecular-weight (HMW) and ultra pure: (UV/Qubit ratio <1.5, 260/280 = 1.8 - 2.0, 260/230 = 1.8 - 2.3). For standard PacBio HiFi Libraries, 90% or more of the DNA should be ≥ 10 kb, and 50% or more ≥ 30 kb, as measured on the Femto Pulse system. That corresponds to a genome quality number (GQN) of 9.0 or higher with 10 kb cutoff and 5.0 or higher with 30 kb cutoff. For specific input requirements please check the following webpages: https://fgcz-intranet.uzh.ch/tiki-index.php?page=PacBio_Sequel https://fgcz-intranet.uzh.ch/tiki-index.php?page=ONT+GridionX5 We currently do not offer DNA / RNA extraction services at the FGCZ.	**HiC:** HiC library preparation is not offered as a standard service at the FGCZ. We accept ready-made libraries for sequencing though.	**IsoSeq & RNA:** IsoSeq: Total RNA must have RIN > 8, minimum 300 ng total RNA. For Illumina RNA Seq please check the following webpage: https://fgcz-intranet.uzh.ch/FullServiceSequencing	**Shipping Requirements:** Ship on dry ice. Before shipping please get in touch with us and read our instructions: https://fgcz-intranet.uzh.ch/tiki-index.php?page=PacBio_Sequel **Contact**: sequencing@fgcz.ethz.ch simon.oliver.grueter@fgcz.ethz.ch
NCCT	**For PacBio or ONT DNA sequencing:** - Standard input: minimum 5 µg and ideally 15 µg HMW DNA (50% molecules >100kbp) - Ultra-low input: minimum 20 ng HMW DNA (50% molecules >100kbp) protocol for of up to 500 Mb Shipment on dry ice, on low retention plasticware, use plate for shipment >24 samples	**Genome conformation like HiC:** Ready to sequence library with fragment size 90% < 600 nt, concentration > 10 nM and volume > 15 µl Shipment on dry ice, on low retention plasticware, use plate for shipment >24 samples	**RNA sequencing on Illumina, PacBio or ONT:** Ideally >1 µg, volume >20µl (minimum 15µl) and concentration >50ng/µl(minimum 25ng/µl). When RIN can be calculated RIN > 8, or no sign of degradation. Shipment on dry ice, on low retention plasticware, use plate for shipment >24 samples	**Contact**: Nicolas.Casadei@med.uni-tuebingen.de
VIB/Antwerp	See requirements of SciLifeLab for extracts. Tissue snap frozen on liquid nitrogen from freshly dissected and stored in -80C.	**Omni-C**: Requirements as listed in section 6.5 of this document.	**RNA:** See requirements of SciLifeLab for extracts. Tissue snap frozen on liquid nitrogen from freshly dissected and stored in -80C.If that is not possible, tissue in RNA later.	All samples - solid frozen on dry ice **only**. Contact Henrique.Leitao@uantwerpen.be before shipping to get address and instructions.
GTF Lausanne	High molecular weight and ultrapure DNA is mandatory. For standard HiFi, general recommendation is 1.5 ug DNA per 2	We prepare libraries with the Proximo Hi-C kit from Phase Genomics (starting after the crosslinking step).	**IsoSeq** RNA must be high quality (RIN/RQN > 8.0).	See GTF Website for further information about the different applications.

	Gb haploid genome. Less good quality DNA requires more input material (because of stringent size selection). Small genome samples can be multiplexed in a single SMRT cell following the same input amount rule (considering the genome size is the sum of individual's genome sizes). Individual sample should be > 300 ng DNA. Lower input can be discussed but is a case per case decision. Ultra-low input (5 ng total) is possible but, contrary to standard HiFi workflow, it is based on a protocol involving PCR amplification. Quality control required: ● Size pattern with Fragment Analyzer (or equivalent). Agarose gel in not accurate enough. ● Nanodrop quantification with 230nm and 280nm ratios. ● Fluorometric method based DNA quantification (e.g. QubIT). Important for comparison with Nanodrop value. QC must be discussed with GTF before any shipment.	Input must be > 200'000 cells, > 20 mg tissue for insect samples, or > 200 mg for plant samples.	Official recommendation is 300 ng minimum. Lower input is possible but will require more PCR cycles for cDNA amplification. Multiplexing several RNA samples in a single SMRT cell is a standard workflow. **Short reads RNAseq** Any amount (down to single cell) and quality is possible. The most standard protocol involves mRNA selection which requires high quality RNA (RIN/RQN > 8.0) and input > 25 ng.	Please contact us as early as possible, ideally before Tissue/DNARNA extraction Please contact us for organizing sample shipment.
Università degli Studi di Bari "Aldo Moro"	**PacBio/ONT:** DNA must be of High-molecular-weight (HMW) and ultra pure: (UV/Qubit ratio <1.5, 260/280 = 1.8 - 2.0, 260/230 = 1.8 - 2.3). Amount of DNA depends on the genome size and the protocol to be used (please contact the center). Integrity of DNA will be measured in our lab via PFGE.			Ship on dry ice. **Shipment Address:** Carmela Gissi Dip Bioscienze, Biotecnologie e Ambiente Via Orabona 4 70125 Bari Italy **Contact:** carmela.gissi@uniba.it
Wageningen University & Research	**ONT sequencing WGS** **Extracted HMW DNA:** ● Concentration: >100ng/ul (Qubit BR) ● Quantity: >10ug ● Integrity: checked on 0.7% agarose gel and Tape Station. Minimum > 20 kb ● Purity: OD260/OD280 ratio 1.8 to 2.0., OD260/OD230 ratio 2.0-2.2. **DNA Storage:** 4ºC			Contact: marta.godia@wur.nl; richard.crooijmans@wur.nl

	DNA Shipping: 4ºC **Animal tissues**: 5-10 mg of non-fatty soft tissue (internal organs). If the specimen is muscle tissue or the entire individual, please provide at least 200 mg/vial. Immediately snap freeze. **Non-nucleated blood**: Collect 5ml whole blood into vacuum EDTA tubes and aliquot it in smaller blood aliquots. Immediately place in dry ice. **Nucleated blood**: 500 µl of nucleated blood, flash-frozen. Collect the whole blood into EDTA tubes. Immediately place in dry ice. **Tissue/blood Shipping:** dry ice			
Hungarian Centre for Genomics and Bioinformatics				**Shipping Address:** Szentágothai Research Centre University of Pécs 7624 Pécs, Ifjúság street 20 Hungary **Contact Point:** Name: Attila SAMU Email: samu@ibioscience.hu