



The
Fleming
Fund



Guidance

Sample shipment to SeqAfrica partners for Whole Genome Sequencing (WGS)

For WGS clients

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Summary

This guide describes sample preparations and shipping guidelines for clients receiving Whole Genome Sequencing services by SeqAfrica. It covers recommendations and guidance on shipping bacterial isolates or extracted DNA to the sequencing centres for short-read sequencing.

How to perform DNA extraction is not included in this guide.



Preparations

Culturing

Make sure you are sending a pure culture without contaminants, or DNA extraction from a pure culture:

1. From a primary culture, select **one single isolated colony** to prepare a subculture.
2. Inspect the subculture carefully to ensure purity.
3. If the culture is not pure, prepare a new subculture.

If you are extracting DNA, we recommend the use of cultures that are not older than 24 hrs, as secondary metabolites in the bacteria builds up (due to lack of nutrients) which can interfere with the downstream analysis.

How to avoid environmental contaminants in your DNA extractions

- Make sure you are working on a clean surface, for example in a Laminar Air Flow (LAF)-bench or other sterile environment.
- Wear gloves.
- Keep petri dishes closed and upside down when not interacting with them.
- Visually inspect culture media in petri dishes for growth of contaminants.

Labelling

Label the sample tubes or vials clearly. This will help you stay organized and help you and the staff at the sequencing facility to match metadata with the correct sample. If label stickers are not available in your lab you can mark tubes or vials with a permanent marker. It is good practise to label both the sides and the lid of tubes.

Documentation requirements

Contact the appropriate local authorities to investigate what documentation your shipment require. It might depend on which country you are shipping to. Below are some examples of what they *could* be.

- Import permit (provided by the sequencing centre)
- Airway bill
- Customs invoice
- Packing list
- Donation letter
- Other paperwork or documentation as per shipping requirements of the shipping or recipient country.

Option A

Shipping bacterial isolates

Material needed for packaging and shipping isolates



Transport media

- Amies agar with charcoal:
Transport swabs, usually used in a clinical setting to sample directly from the patient. They work equally well when used for cultured bacteria, and these ones contain charcoal in the agar. The charcoal makes the environment suitable for both aerobic and anaerobic species, including *Campylobacter spp.*

The bacteria on the coal swabs prefer to be stored refrigerated (approx. 3 °C) until dispatch. However, the swabs can be stored at 3 – 25 °C.

- Semi-Solid/Solid agar transport media:
 - Dorset Egg transport media.
 - Trypticase Soy Agar (TSA) Stabs.
 - Amies Semi-Solid Agar Transport Medium.

When bacterial cultures are transported on/in one of the above listed transport media, the shipment can go at ambient temperature. However, if available to you it is good to include a few ice packs/bricks to keep the package cool.

- Brain Heart Infusion Broth with 20% glycerol:
Brain Heart Broth is a nutrient broth culture medium, containing brain and heart extracts, used in the isolation, cultivation and maintenance of fastidious and non-fastidious microorganisms, including aerobic and anaerobic bacteria. Glycerol acts as a cryopreservative to stabilize isolates in the frozen state.

Be aware that any transport medium using broth with glycerol requires the shipment to be shipped with dry ice. This adds weight and considerable cost to the shipment. Additionally, should your package be delayed during transport there is a risk that the cold chain is not maintained and the cultured are non-viable upon arrival to recipient sequencing centre.

Pros and cons of shipping isolates over DNA

- The sequencing facility can propagate your isolates, ensure a pure culture is produced and re-extract DNA, if necessary, without you needing to ship isolates again.
- Isolates must be shipped according to UN3373 standards, as “Biological samples, Category B”. This requires additional packing material.
- Depending on your transport media, your shipment might require refrigeration which is usually more expensive.

NOTE

! Be aware that based on the sequencing centres expertise and access to specific culture media, some of the pathogens you wish to have sequenced might have to be shipped as DNA extractions instead of bacterial culture. Please contact the SeqAfrica management team at seqafrica@food.dtu.dk if you are in doubt or have questions.

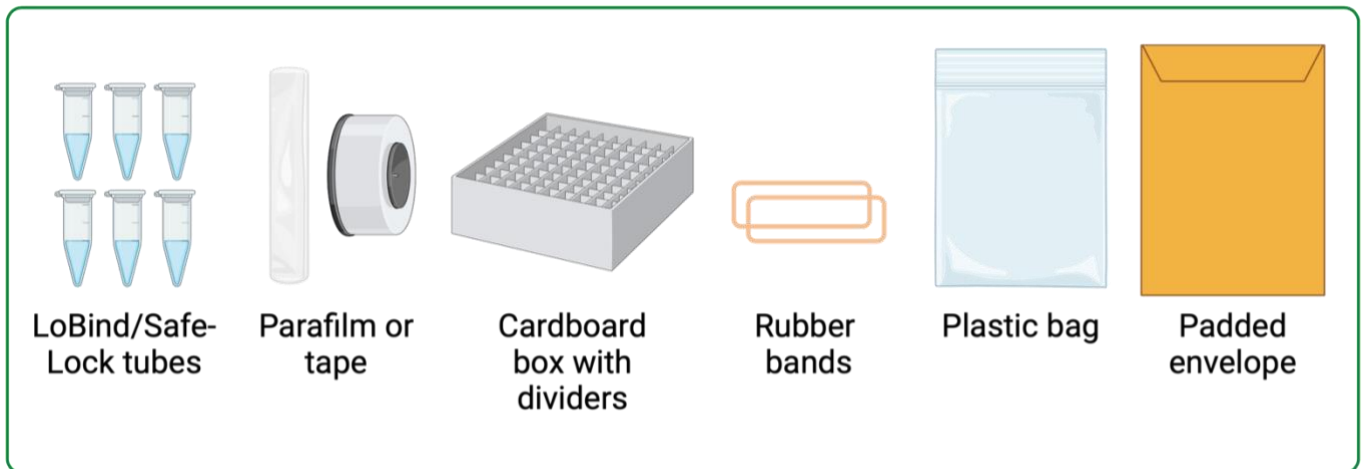
Packing your isolates

- Pack any bacterial isolates in Semi-Solid Transport media, like coal swabs, in an absorbing material.
- Pile the bacterial isolates inside a Biosafety bag and close it shut with the zip-lock. Take out as much air as possible.
- Put Biosafety bag into a box, for example a Bioshipper box. Make sure the box is not too big enabling your isolates to fall around during transit.
- Close the box and seal thoroughly with tape.
 - If using a Bioshipper box: close down the front flap of the box, then the side flaps, and lastly, the lid.

Option B

**Shipping
extracted
genomic DNA**

Material needed for packaging and shipping DNA



Appropriate transport tubes

When stored in standard tubes, a large portion of the extracted DNA may bind to the inside surface of the tubes. **LoBind tubes** have a hydrophilic surface (non-silicone) which minimizes surface binding of the DNA.

When shipping DNA via Air Freight the changes in atmospheric pressure might cause tubes with regular lids to pop open spilling your DNA sample. Using **a tube with Safe-Lock (PCR grade)** prevents unintentional lid opening. Ensure that any Safe-Lock tube you use also has a non-silicone surface like LoBind tubes. If Safe-Lock tubes are not accessible to you, be meticulous and seal close the lids using parafilm or tape.

Pros and cons of shipping DNA over isolates

- DNA is not hazardous and does not need to meet any specific standards for shipping. Nevertheless, we wish to take good care of it to protect its quality.
- If the DNA quality of your samples is insufficient for WGS, you will need to re-extract and re-ship samples thus increasing your costs.

Examples of appropriate DNA extraction kits

For WGS to be successful, it is paramount to have good quality DNA as the starting point. Below is a list of recommended manufacturers of appropriate DNA extraction kits:

- Qiagen (for example Dneasy Blood & Tissue kit)
- ThermoFisher/Invitrogen (for example Easy-DNA gDNA Purification Kit)
- Zymo
- Promega

Quality to control of DNA extraction

- Check your **DNA purity** prior to shipping (use for example [Nanodrop](#) or [Bioanalyzer](#)):
 - Measure UV 260/280 absorbance ratio values of the DNA samples to confirm that they are in the interval 1.8 – 2.0.

NOTE! If absorbance ratio values are outside the interval, the DNA should be re-extracted.

- Check your **DNA concentration** prior to shipping (use for example Qubit fluorometer and the dsDNA reagent kits).
- Send 50 µl DNA per sample.

NOTE! If the DNA concentration is < 6 ng/µl, send at least 80 µl.

- Store extracted DNA at 4 °C (preferred) or at room temperature until dispatch. Do not freeze.

Packing your DNA

- Seal the LoBind tubes containing your DNA with parafilm.
- Place the LoBind or Safe-Lock tubes containing your DNA in a cardboard box with dividers.
 - If you do not have access to a compartmentalised box, make sure the tubes are packed tightly in for example plastic or bubble wrap to avoid tubes falling around during transport.
- Place rubber bands around the cardboard box.
- Put cardboard box into a plastic bag and close it shut with the zip-lock. Take out as much air as possible.
- Put the plastic bag into a protected/padded envelope and close the envelope.