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PU	**Public**	**X**
PP	Restricted to **other programme participants** (including the Commission)	
RE	Restricted to a **group defined by the Consortium** (including the Commission)	

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Title	Common optimised methodology for the storage of DNA
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Authors	Amparo Ruvira & David Ruiz Arahal (UVEG-CECT)
Abstract	The first level in the development of the European microbial DNA Bank Network is based on the harmonization and characterization of extraction, quality control and storage DNA protocols to optimize the highly elaborated process of DNA banking. The aim of this task is harmonization and characterization of storage DNA protocols.
Validation process	Document prepared by UVEG-CECT in collaboration with all task contributors and submitted to the Executive Committee for agreement.

Revision table			
Date	***Version***	***Revised by***	***Main changes***
10.09.2012	0.1	A. Ruvira (UVEG-CECT)	First draft
25.09.2012	0.2	D.R. Arahal (UVEG-CECT)	Minor corrections
27.09.2012	0.3	A. Buddie (CABI)	Completion of Eucaryotic parts and general corrections

Contents

Abbreviation key

BRC	Biological Resource Centre
ng	nanogram
μg	microgram

1 Background and Objectives

Due to the growing demand of purified genomic DNA from culture collections, the objective of task JRA1.2 was to establish a European network of DNA banks from microorganisms, accessible through a website.

The development of the European microbial DNA Bank Network accessible via a central Web portal required two levels of work:

- First Level: Based on the harmonization and characterization of protocols for DNA extraction, sample quality control and storage of DNA to optimize the highly elaborated process of DNA banking.
- Second Level: Based on the creation of a complete database to be included in the Web portal.

For the harmonization of storage protocols, each partner has documented the procedures applied in their laboratories using a common template.

2 Storage procedures

Due to the different nature and range of microorganisms together with relevant experience in handling, each partner has developed its own storage procedure while being aware of other's options.

2.1 CABI

For filamentous fungi, yeasts, Chromists, bacteria and nematodes, DNA is extracted and stored in one of two ways:

- Commercial kits (e.g. Plant DNeasy® DNA extraction kit)
- As recommended by the protocol, before cell lysis homogenise visible tissue clumps by using a sterile micropestle followed by brief vortexing. Incubate cell lysis solution at 65˚C for two hours. Elute DNA in 50-100 µl buffer (buffer AE for Qiagen Plant DNeasy kits).
- Store DNA at -20°C.
- Proprietary DNA release solution (MicroLYSIS-Plus®)
- Manufacturer's instructions were followed, except that after lysis microcentrifuge tubes containing 20 µl of lysis solution were spun briefly and supernatant was collected into fresh

sterile 0.5 ml microcentrifuge tubes after the lysis process.

- Store DNA at -20°C.

2.2 CBS-KNAW

CBS-KNAW storage protocol is applied for genomic DNA of filamentous fungi and yeast. After checking the genomic DNA that is extracted from living strains in the CBS Collection of Fungi on quality and identity, approved DNA extracts are stored at low temperature to guarantee optimal stability and protection from contamination. Two kinds of procedures are used:

A. DNA cryogenic storage (average temperature below –175ºC)

- Number of aliquots: 1
- Amount: varies between 20 ng/µl – 120 ng/µl with volumes from 20 µl – 100 µl per tube.

B. DNA storage at average temperature of –80ºC

- Number of aliquots: 1
- Amount: varies between 20 ng/µl – 120 ng/µl with volumes from 20 µl – 100 µl per tube.

For both types of storage the same microtubes and labels are used. The quality and quantity of DNA from the different DNA extraction protocols used can vary quite a lot. Also, the quantity of DNA harvested from fungi varies between taxa and is dependent on factors such as hyphal wall structure and the strain's capacity to grow.

2.3 UVEG-CECT

For storage of genomic DNA at CECT, two different processes are applied in parallel:

A. DNA storage at -80ºC:

- Aliquot DNA into microtubes:

  Nº aliquots: 4

  Amount: > 500 ng

B. Dry DNA storage, GenTegra Kit:

- Aliquot DNA into GenTegra microtubes:

  Nº aliquots: 4

  Volume: 20-250 µl

Amount: 0.05-25 µg

- Dry DNA according the methods:

  FastDryer: 16 hours

  SpeedVac: 1-4 hours

- Store at room temperature (21-25ºC)
- DNA recovery:

  Apply appropriate volume of molecular biology-grade water: 35-250 µl (≤200 ng/µl)

  Incubate at room temperature for 15 minutes.

  Cap tubes and vortex for 1 minute

2.4 CRBIP

The storage process used at CRBIP for prokaryotic microorganisms has three parts:

A. Send DNA to Imagene corp. for DNA distribution in to capsules and then encapsulation with the CRBIP code engraved onto each capsule to ensure permanent labelling.

- Number of aliquots: generally 10 (but can be less or more according to needs)
- Amount: 1 µg / capsule

B. Long-term storage of the capsules at room temperature (21-25ºC)

C. DNA recovery from capsule

Opening the capsule

To recover the DNA sample from a mini capsule, use the shell opener specially designed for this purpose (one *shellOpener* can open up to15-20 *minicapsules*).

Place the mini capsule in the grey capsule holding base. The 2-D Data-matrix code must face down.

Cover the capsule-holding base with the red lid.

While holding the grey base, turn the lid clockwise until it stops.

Recover the opened mini capsule and proceed to rehydration.

Rehydration

The dried DNA sample is recovered simply by adding molecular biology-grade water.

DNA recovery volumes shall be calculated from the quantity divided by the target concentration.

The volume should fall within 20 and 200 µl (Recovering the sample with less than 20 µl may lower the yield of recovery).

After 5 to 10 minutes of rehydration, DNA solutions can be used for further analysis. It is recommended to use the samples quickly to limit evaporation (for small volume samples mostly). Short-term storage can be performed at +4°C.

2.5 INRA

CIRM-Levures

For genomic DNA of yeasts, the following storage process is applied:

DNA solutions in microtubes may be stored temporarily at 4°C during the quality assessment. Subsequently, if assessment was positive, DNA samples should be stored at -20°C

The DNA solution is aliquoted with 5 µg of genomic DNA per tube

CIRM-BP

For genomic DNA of prokaryotes, the following storage process is applied:

Store temporarily DNA solution in microtubes at 4°C during the quality assessment. After that, and if assessment was positive, store DNA samples at -20°C

For a short time (1-2 years), DNA could be store at +4°C, but for a long time (> 2 years) it is better to store DNA at -20°C, avoiding temperature variations.

2.6 BCCM

BCCM/LMBP

Plasmids are preserved in two formats: as plasmid-carrying cultures and as isolated plasmid DNA. The processes for long-term preservation, storage and recovery of the isolated plasmid DNA are described afterward:

A. DNA storage at -20 ºC:

<u>Preservation</u>

- Aliquot DNA into labelled microtubes: minimum 5 µg/tube.
- Add 2.5 to 3 volumes 100% ethanol and minimum 1/10 volume 3M sodium acetate (pH 5.2) to 1 volume DNA suspension.

<u>Storage</u>

- Freeze precipitated DNA at -20°C.

Recovery

- Centrifuge for 15 minutes at 13000 rpm.
- Remove the supernatant.
- Wash the pellet with 1 ml 70% ethanol.
- Centrifuge for 10 minutes at 13000 rpm.
- Remove the supernatant and dry the pellet for 15 minutes at 37°C.
- Resuspend the DNA pellet in 50 µl TE-buffer.

B. DNA storage at room temperature:

Preservation

- Aliquot DNA into labelled microtubes: minimum 5 µg/tube.
- Dry DNA using a SpeedVac during 1 to 4 hours

Storage

- Store the evaporated DNA at room temperature.

Recovery

- Resuspend the evaporated DNA in 50 µl TE-buffer.

BCCM/LMG

Bacterial genomic DNA, suspended in 0.1x TE buffer, is immobilized by freezing below its eutectic point. Process:

- Store extracted bacterial genomic DNA at -20°C.
- To use stored DNA, thaw the suspension completely at room temperature.
- Thawed DNA can be refrozen at -20°C whilst maintaining sample integrity and protecting against possible contamination.

2.7 MUM

For filamentous fungi, storage of genomic DNA is realized in ultrapure water at -20 °C.

- Aliquot/ dilute the extracted genomic DNA of fungi to a final concentration of 50 ng/µL.
- Store DNA at -20°C.

Conclusion

Several protocols are applied for storage genomic DNA and all of them are valid for the development of the European microbial DNA Bank Network.

Significance of this deliverable

Long term storage of high quality DNA is a key for the development of the European microbial DNA bank network.

This deliverable highlights and summarises in precise protocols different options to preserve isolated microbial DNA,

either dry, freezed or encapsulated, depending on the species and on the BRC facilities and strategy.