EMbaRC

European Consortium of Microbial Resource Centres

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Dissemination level: PU

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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Document properties	
Project	EMbaRC
Workpackage	JRA1
Deliverable	D14.14 formerly: D.JRA1.2.1
Title	Report on the dissemination of information and access to common database from current DNA banks
Version number	V1.0
Authors	Amparo Ruvira & David Ruiz Arahal (UVEG-CECT)
Abstract	Due to the growing demand of purified genomic and plasmid 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
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***
12.12.2011	0.1	Amparo Ruvira (UVEG-CECT)	First draft
27.02.2012	0.2	David Ruiz Arahal (UVEG-CECT)	General correction
05.03.2012	0.3	A. Ruvira & D.R. Arahal (UVEG-CECT), Jindrich Peiren & Wouter De Schamphelaire (UGent)	Conclusions and several corrections added

Contents

Abbreviation key

BRC	Biological Resource Centre
DNA	Deoxyribonucleic acid
EMBL	European Molecular Biology Laboratory
FTA	Fast technology for analysis of nucleic acids
JRA	Joint research activity
mRNA	Messenger ribonucleic acid
PCR	Polymerase Chain Reaction
rRNA	Ribosomal ribonucleic acid
RT	Room Temperature

1 Background and Objectives

Due to growing demand to the Culture Collections for purified genomic DNA from laboratories that are lacking skills in microbiology and/or molecular biology or lacking the necessary installations for strains manipulation, the creation of a European DNA Bank is a justified need. Thus, the aim of task JRA1.2 was to establish such a European microbial DNA Bank Network accessible via a central Web portal, in order to unify DNA bank material and databases of partners and to provide DNA samples of complementary collections. The European DNA bank provides both genomic DNA and individual genes cloned in expression plasmids or in general transfer vectors and covers (Bacteria, Archaea, fungi, yeast and protists, gDNA and cDNA libraries).

The network is intended to enhance taxonomic, systematic, genetic, conservation and evolutionary studies, and for uses in the validation, verification and authentication of techniques or procedures. Moreover, it is proposed as a tool for encouraging scientists to deposit into the European microbial DNA Bank samples of the genomic or plasmid DNA from which sequence information was previously deposited in international databases as Genbank and EMBL, in order to create added value through cooperation and cumulative characterization of data.

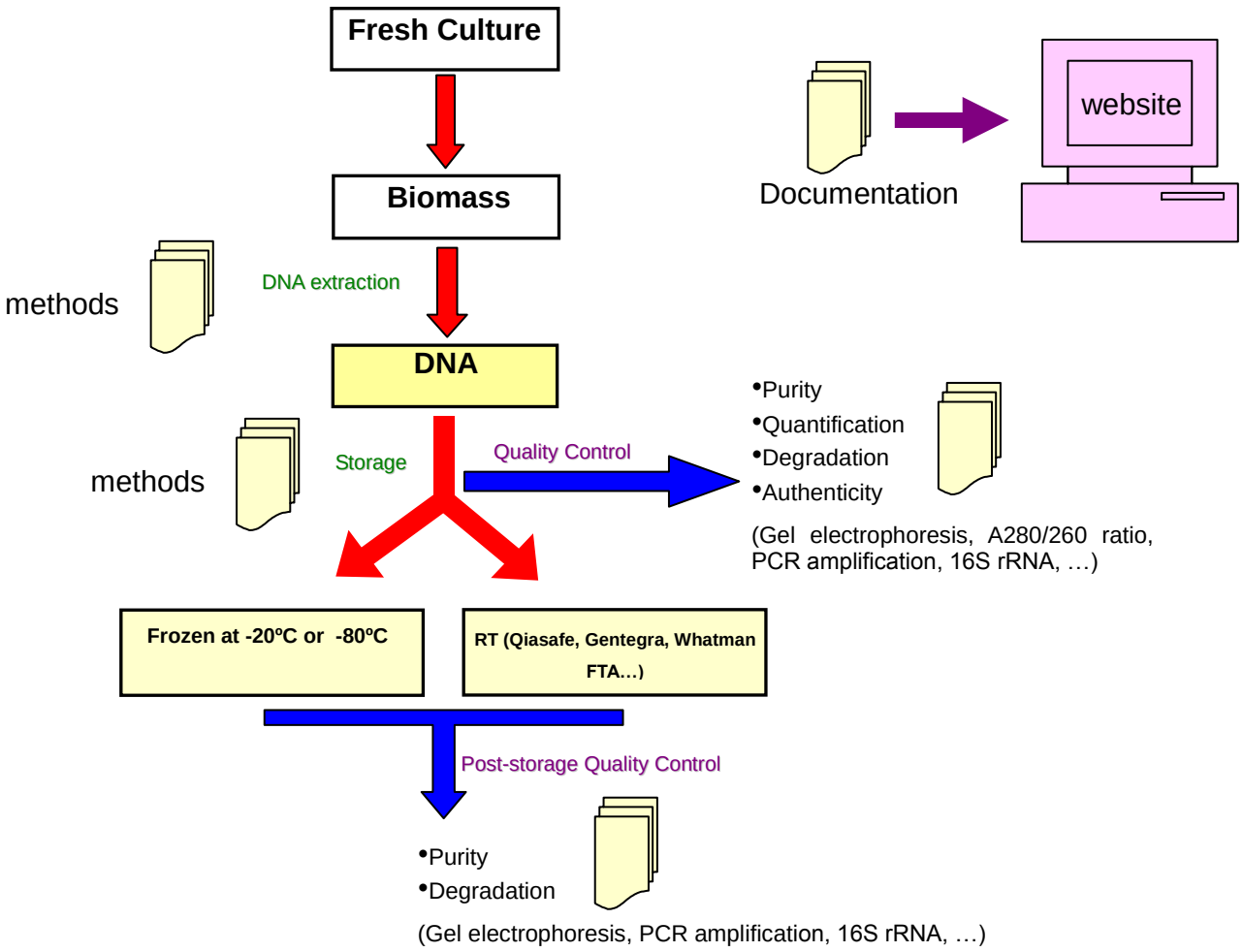
Focus is being placed on DNA subjected to gene sequence or complete genome analysis but also to samples from reference strains and other material of economic value, even on DNA derived from organisms that are fastidious or preservation recalcitrant, or from strains for which supply as viable cells is subjected to legal or administrative restrictions (like class III pathogens). Cloned into plasmids, important genes of those pathogens become easily accessible.

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 extraction, quality control and storage DNA protocols 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.

2 Procedures

The following work flow schematizes the principal steps and the control points needed in the DNA Bank generation process.



2.1 Documentation

Using the same template, each partner has documented the procedures applied in the different steps:

1. Extraction of genomic DNA and plasmid DNA
2. Quality control
3. Conservation method

Some of them are attached to this report to serve as example and the whole set has been up-loaded to the collaborative platform.

3 Central web portal

A web portal for The European microbial DNA Bank has been designed in-house. It is a user friendly site with valuable information on:

- Welcome, News and tools are displayed at the home page.
- Procedures: A list of pdf files about genomic and plasmid DNA extraction, quality control and storage procedures applied by each partner, as mentioned above.
- Availability: Lists of DNA holdings available for each partner.
- Order DNA: gives information on how to proceed to place an order.

Currently it is being evaluated by the partners. Once finalised it will be announced to users and stakeholders and linked (back and forward) to EMbaRC and partner's web sites. Snapshots are included in the annex.

Conclusion

This is the very first time a web site has been developed to integrate information about DNA banks from such a large number of European microbial Culture Collections. Initiatives existed had either a narrower geographical distribution (Gemeinholzer et al., 2011) or a different scope. An effort has been made to meet the user's needs and although it is clear that there is room for many improvements, this task can be considered a major innovative achievement in Biological Resourcing for the microbial domain.

References

Gemeinholzer B, Dröge G, Zetzsche H, Haszprunar G, Klenk H-P, Güntsch A, Berendsohn WG, Wägele J-W. Biopreservation and Biobanking. March 2011, 9(1): 51-55.

Annexes

JRA 1.2 DNA BANK

DNA EXTRACTION

Microbial Resource Center	WRITER	CHECKED BY	APPROVED BY
CECT-UVEG	M. A. Ruvira PhD student	D. R. Arahal Head of Research	E. Garay Director

Subject:
This document explains in details the process for genomic DNA preparation with REALPURE spin kit.
Application field:
Applicable for Gram positive and Gram negative bacteria.

1. PRINCIPLE:

To get a genomic DNA suspension using a physicochemical process.

2. PRODUCTS:

Product name	Reference	Provider	Preservation temperature
Real Pure Spin Kit	RBMEGS02 250 Preps.	Durviz	Room temperature
Tissue Lysis Buffer	E22	Durviz	Room temperature
Lysis/Binding Buffer	REA01	Durviz	Room temperature
Proteinase K	REA02	Durviz	-20 °C
Desinhibition Buffer	REA03	Durviz	Room temperature
Wash Buffer	REA04	Durviz	Room temperature
Elution Buffer	REA05	Durviz	Room temperature
REAL Spin Columns	RSC01	Durviz	Room temperature
Collection Tubes	R30	Durviz	Room temperature
Nuclease-free water	RBMEGS02	Durviz	Room temperature
Ethanol absolute	361086.1611	Panreac	Room temperature
Isopropanol	361090.1612	Panreac	+4 °C
Lysozime	L6876-5G	Sigma	-20°C

3. MATERIAL:

- Thermoblock (Labnet)
- Vortex
- Bench centrifuge (5810R, Eppendorf)
- P20 manual, P200 manual, P1000 manual (Gilson)
- 20 µl/ 200 µl /1000 µl tips
- Microtubes of 1.5 ml (Eppendorf)

4. PROCESS:

- Prepare identified microtubes for each strain.
- Centrifuge 1-1.5ml of bacteria (10^9 cells), or place 10-20 mg of biological material into a tube.
- Resuspend the pellet in 180 µl of Tissue Lysis Buffer and then add 20 µl Proteinase K. Shake with vortex and incubate at 55°C until the lysis is completed. For Gram + bacteria the treatment is different: incubate with 200 µl of PBS with 20 mg/ml of lysozyme during 30 minutes at 37°C, then add 20 µl Proteinase K and incubate at 55°C until the lysis is completed.

1/2

DNA EXTRACTION

- Add 200 µl of Lysis/Binding Buffer. Shake with vortex. Incubate at 70°C for 10 minutes.
- Add 100 µl of Isopropanol. Mix well.
- Pipette the lysate into reservoir of a combined MicroSpin Column _collection tube assembly.
- Centrifuge at 8000 rpm for 60 seconds. Remove the collection tube.
- Place the MicroSpin column in a new collection tube and add 500 µl Desinhibition Buffer to the reservoir.
- Centrifuge at 10000-12000 rpm for 60 seconds. Remove all the liquid.
- Add 500 µl of Wash Buffer into reservoir of MicroSpin column.
- Centrifuge at 12000 rpm for 60 seconds. Remove the liquid.
- 2° Wash. Add 500 µl of Wash Buffer into reservoir of MicroSpin column
- Centrifuge at 14000 rpm for 60 seconds. Remove the liquid.
- Centrifuge at maximum speed for 90 to remove the residual ethanol.
- Remove the collection tube and place the MicroSpin column in a 1.5 ml microtube. Add 100-200 µl of Elution Buffer (preheated at 70°C) into reservoir of MicroSpin column. Incubate for 1 minute.
- Centrifuge at maximum speed for 60 seconds. The microtube contains now the genomic DNA

- Check DNA purity and quality by using spectrophotometer and gel agarose electrophoresis. And store at -80°C and at room temperature (GenTegra).

DNA QUALITY CONTROL

Microbial Resource Center	WRITER	CHECKED BY	APPROVED BY
CECT-UVEG	M. A. Ruvira PhD student	D. R. Arahal Head of Research	E. Garay Director

Subject:
This document explains in details the process for assessing the quality control of DNA.
Application field:
Genomic DNA

1. PRINCIPLE:

To check genomic DNA for purity, integrity and quantity.

2. PRODUCTS:

Product name	Reference	Provider	Preservation temperature
Agarose D-1 low EEO	8008	Pronadisa	Room Temperature
TBE Buffer 5x	EO-861	National diagnostics	Room Temperature
Ethidium Bromide	15006-10	MoBio	Room Temperature
1Kb DNA Ladder	SM1331	Fermentas	+4 °C
6X Orange DNA loading	R0631	Fermentas	+4 °C

3. MATERIAL:

- P20 manual, P200 manual (Gilson).
- 20 µl /200 µl Tips.
- Microtubes of 1.5 ml (Eppendorf).
- Image Capture (Gel printer Plus).
- DNA Electrophoresis System, 14 well-combs, gel tray (LabNet).
- Spectrophotometer (Pharmacia Biotec).
- Quartz cuvette.

4. PROCESS:

1. **DNA electrophoresis:**
   - 0.8% agarosa gel. Weight out 0.4 g of agarose into a flask and add 50 ml of TBE 0.5%.
   - Heat solution in a microwave until agarose is completely dissolved.
   - Allow to cool. Place the appropriate comb in gel tray and pour the agarose.
   - Allow the agarose gel to solidify.
   - Place in electrophoresis system and cover with TBE 0.5% buffer.
   - Mix 2 µl of loading buffer 6X with 5 µl of sample (DNA extraction).
   - Load DNA samples and ladder.
   - Run at 70V for 1h.
   - Place agarose gel into the ethidium bromide (0.5 µg/ml) bath for 25 minutes.
   - Visualize DNA bands using gel imaging system and take a photo.
2. **DNA measurement:**
   - Dilute DNA 1/10 or 1/50.
   - Place DNA dissolution into the quartz cuvette and introduce it into the spectrophotometer.
   - Measure: A_{260}, A_{280}, A_{230}, A_{320}. Ratio A_{260}/A_{280}. Ratio A_{260}/A_{230}. Concentration (ng/ml).

DNA STORAGE

Microbial Resource Center	WRITER	CHECKED BY	APPROVED BY
CECT-UVEG	M. A. Ruvira PhD student	D. R. Arahal Head of Research	E. Garay Director

Subject:
This document explains in details the process for storage of DNA.
Application field:
Genomic DNA of prokaryotes.

1. PRINCIPLE:

To storage genomic DNA.

2. PRODUCTS:

Product name	Reference	Provider	Preservation temperature
GenTegra DNA 0.3 ml Tubes	GTD1001	GENVAULT	Room temperature

3. MATERIAL:

- P20 manual, P200 manual (Gilson).
- 20 µl/200 µl tips.
- Microtubes of 1.5 ml (Eppendorf).

4. PROCESS:

1. **DNA storage at -80°C:**

   - Aliquot DNA into microtubes :
     - N° aliquots: 4
     - Amount: > 500 ng

2. **Dry DNA storage, GenTegra Kit:**

   - Aliquot DNA into GenTegra microtubes:
     - N° 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 (21-25°C):
   - DNA recovery:
     - Apply a 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

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European Microbial DNA Bank Network

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News

About us - 15/12/2011

The European Microbial DNA Bank Network is a portal made by EMbaRC -the European Consortium of Microbial Resource Centres.

Read more

Procedures released for DNA extraction, storage and quality control - 13/03/2012

Several EMbaRC partners have released their internal procedures for DNA extraction, storage and quality control.

Read more

All the news

Welcome to the European microbial DNA Bank network

One of the objectives of EMbaRC project was to establish an European microbial DNA Bank Network in which DNA bank material and partners' databases were linked and made accessible via a central Web portal, providing DNA samples from complementary collections. The network is intended to serve the interests of recipients and donors of DNA samples to create added value through cooperation and cumulative characterization of genomic data. Emphasis is placed on DNA subjected to complete genome analysis but also to samples from reference strains, other material of economic value, organisms that are preservation recalcitrant or from strains for which supply as viable cells is subject to legal or administrative restrictions.

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- CRBIP
- DSMZ
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- BCCM
- MUM
- CBS

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European Microbial DNA Bank Network

Procedures

DNA extraction, storage and quality control

EMbaRC Biological Resource Centres of micro-organisms are glad to provide their internal procedures for DNA extraction, storage and quality control.

Procedures available for download.

Biological Resource Centre		Main resources preserved	Country	Procedure for DNA extraction	Procedure for DNA storage	Procedure for Quality control
Cirm	CIRM-BIA	Food bacteria	France			
	CIRM-BP	Pathogenic bacteria		[PDF]	[PDF]	[PDF]
	CIRM-CF	Filamentous fungi				
	CIRM-L	Yeasts		[PDF]	[PDF]	[PDF]
Institut Pasteur	CRBIP	Bacteria	France	[PDF]	[PDF]	[PDF]
DSMZ	DSMZ	Bacteria and Archaea	Germany			
www.cabi.org	CABI	Filamentous fungi	United Kingdom			
CECT	CECT	Bacteria, Filamentous fungi and Yeasts	Spain	[PDF]	[PDF]	[PDF]
BCCM	BCCM/LMG	Bacteria	Belgium	[PDF]	[PDF]	[PDF]
	BCCM/LMBP	Plasmids		[PDF]	[PDF]	[PDF]
	BCCM/MUCL	Filamentous fungi				
mum	MUM	Filamentous fungi	Portugal			
CBS	CBS	Filamentous fungi and Yeasts	the Netherlands	[PDF 1] [PDF 2] [PDF 3] [PDF 4]	[PDF]	[PDF]

Four extraction methods are proposed by CBS:

- [1] DNA isolation with Bio 101® FastDNA Kit
- [2] DNA isolation with MoBio Microbial DNA Isolation Kit
- [3] DNA isolation with SDS and CTAB based lysis and chloroform purification
- [4] DNA isolation with SDS based lysis and phenol chloroform purification

Writing: EMbaRC
Creation date: 15 December 2011
Update: 13 March 2012

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DNA available from CRBIP

CRBIP maintains DNA from a selection of bacteria held in the CIP collection.

CRBIP maintains DNA from bacteria of which the complete genome sequence is published. The DNA is preserved in capsules using the DNAshell® technology.

Aliquotes of DNA are dispensed into glass inserts placed in oxidation-proof metal capsules (DNAshells), introduced into a confinement chamber, subjected to rigorous desiccation, and then packaged in the absence of factors likely to cause alterations, under a controlled, inert atmosphere. Then, each capsule is hermetically sealed with a laser to ensure that it is perfectly watertight. A code is engraved onto each capsule with a laser before the addition of the insert, to ensure permanent, unalterable labelling.

The list of strains available appears in the following catalogue:

CRBIP DNA catalogue
pdf - 22.84 kB

See also
CRBIP homepage

Writing: EMbaRC
Creation date: 13 March 2012
Update: 13 March 2012
Contact:
crbip@pasteur.fr

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Order DNA

Order DNA at EMbaRC Microbial Resource Centres

General information on DNA order applicable to all partners.

Writing: EMbaRC
Creation date: 15 December 2011
Update: 12 March 2012

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Significance of this deliverable

Direct accessibility via this new web portal to microbial DNA resources and associated protocols provided by expert BRCs is of high added value for users and fits their growing needs in genomics.