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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. This task aims at harmonizing and characterizing quality control protocols and establishing of guidelines and quality standards.
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Abbreviation key

BRC Biological Resource Centre

ng nanogram

μg microgram

μl microliter

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, 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 quality control validation, each partner has documented the procedures applied in their laboratories using a common template.

2 Quality control procedures

Due to the different nature and range of the microorganisms plus relevant experience in handling, each partner has developed its own quality control procedures.

2.1 CABI

The quality control validation is carried out by two methods:

A. Assessment of DNA purity and concentration

DNA extracted for fingerprinting may be assessed by undertaking DNA quantification using a spectrophotometer (e.g. GeneQuant Pro, GE Healthcare, UK), as follows:

- Reference blank containing DNA resuspension solution (e.g. TE or Qiagen buffer AE or ultrapure water) is used for calibration
- Aliquots (7 μ l) of DNA sample are added carefully (avoiding bubbles) to the ultralow volume cuvette and readings taken at A260, A280, A230, A320. Ratios A260/A280 and A260/A230 are calculated. Finally, DNA concentration is given in ng/ μ l.
- Ultralow volume cuvette is rinsed with ultrapure water and fresh sample applied

Alternatively, DNA samples may be assessed by gel electrophoresis in the following manner:

- Measure the required amount of 0.5 x TBE into the measuring cylinder, accordingly, 100ml for midi, gel.
- Pour the 0.5 x TBE buffer into the flask.
- Place the watchglass over the top of the flask and heat (carefully) in the microwave oven until agarose is dissolved and bubbling.
- Remove from the microwave oven using heat resistant gauntlet.
- Add a magnetic flea and place the conical flask on a magnetic stirrer. Add SafeView stain (5 μ l per 100ml) to gel.
- While the gel is cooling on the stirrer ensure that the gel tray is on a level surface using the spirit level. The mini gel trays need to be taped over at both ends using electrical tape before the gel is poured. Mini gel trays can fit into the gel tank horizontally so no tape is needed, for maxi gels, lubricate the rubber seal of the guards with Vaseline and slide the guards into place at either end.
- Place comb or combs in the appropriate places.
- When the gel has cooled sufficiently pour it into the gel tray. Push away any bubbles to the side using a pipette tip or wooden cocktail stick.
- When the gel has cooled and solidified fully, remove tape or the slot in guards and cover the gel in 0.5 x TBE containing SafeView Stain (5 μ l of stain per 100ml of buffer).
- Remove the combs.
- Load 10 μ l of size marker (DNA ladder) on each end of the row of wells in the gel.
- To prepare 100 bp DNA Ladder: Mix 50 μ l of stock solution to 950 μ l sterile water (1:20). Aliquot in to 50 μ l volumes.
- DNA samples are diluted by adding 1 μ l DNA to 9 μ l loading buffer in sample tubes
- Load contents of sample tube in the well.
- When finished loading replace the lid and connect the cables to appropriate sockets of the power pack. Ensure that that gel tray is fitted correctly; DNA migrates in the TBE buffer towards (+) electrode (anode).
- Stay clear from the tank. Switch the power pack on, press "Set" and by using the arrows set the voltage at appropriate constant voltage (i.e. 75V). Press "Set" again to set current (I) at 500mA, press "Set" again to set time at 1h. To start the electrophoresis press "run".
- At end of 1h, stop the electrophoresis by pressing "Set" and then switch the power pack off.
- Disconnect the connectors and remove lid.

- Place the gel in the centre of the UV transilluminator in the U:Genius unit.
- Follow instructions on Quick guide sheets attached to side of U:Genius for image examination, manipulation and capture.
- Click on the “Save” icon, then “change the file name” enter the file name in the format DDMMYPCR, save as Tiff file.
- Switch off the U:GENIUS unit when session is finished.
- Spray the sample tray and all worktops with laboratory disinfectant/DNA inactivation solution and wipe them down with paper towel.
- Make appropriate notes in the Laboratory Notebooks in accordance with their specific instructions. Transfer gel image to own PC by memory stick provided. Print out image on U:Genius printer and note the order of the samples.

B. DNA Fingerprinting

DNA fingerprinting is a powerful method for molecular typing and genetic characterization of a variety of microorganisms including fungi. In the case of filamentous fungi, no single DNA fingerprinting technique has evolved as a preferred method. Despite the fact that each method has its own set of assets and limitations an optimal DNA fingerprinting technique has been applied to most fungi. Process:

- Wear gloves to prevent cross-contamination and for your personal safety interests.
- Take water, PCR buffer 10×Taq (Qiagen Ltd., Crawley, UK), primer* (concentrated 100 pmoles/μl stock), dNTPs (100 mM stock, Promega Ltd., Southampton, UK), from the freezer to thaw in the fridge. Keep *Taq* DNA-polymerase (Qiagen Ltd, Crawley, UK) in the freezer.

*choice of primers determined by the researcher but most commonly ISSR/SSR/VNTR or other repeat unit.

- Clean the PCR setup designated worktop area by spraying with laboratory disinfectant/DNA inactivation solution and wiping down with paper towels.
- Pulse spin reagents and DNA extract to ensure that all liquid resides at the bottom of the tube by using microcentrifuge.
- Prepare equal number of PCR tubes as the number of samples to be amplified and add 3 more for positive and negative controls and Master Mix. Place them on a separate pink rack and clearly label the tubes with corresponding numbers or names and primer set used.
- To perform ISSR-PCR add the following to each PCR tube:

	1 @ 20 µl
H2O	15.5 µl
PCR buffer 10xTaq	2.0 µl
dNTPs	0.8 µl
Primer	0.5 µl
Taq DNA-polymerase	0.2 µl
Template	1.0 µl

- Primers for ISSR-A program: TGT, ACA, UBC888, UBC889, UBC890, UBC891.

Step 1: 2 min at 94 °C followed by 35 cycles of:

Step 2: 1 min at 94 °C,

Step 3: 1 min at 46 °C,

Step 4: 2 min at 72 °C,

Followed by 10 min at 72 °C, and holding step at 10 °C.

- Primers for ISSR-C program: CCA, CGA.

Step 1: 2 min at 94 °C followed by 35 cycles of:

Step 2: 1 min at 94 °C,

Step 3: 1 min at 53 °C,

Step 4: 2 min at 72 °C,

Followed by 10 min at 72 °C, and holding step at 10 °C.

- To perform VNTR-PCR add the following to each PCR tubes:

	1 @ 20 µl
H2O	18.875 µl
PCR buffer 10xTaq	2.5 µl
DNTPs	2.0 µl
Primer	0.5 µl
Taq DNA-polymerase	0.125 µl
Template	1.0 µl

- Primers for COLAP4 program: MR, RY, GACA.

Step 1: 5 min at 95 °C followed by 39 cycles of:

Step 2: 1 min at 95 °C,

Step 3: 1 min at 45 °C,

Step 4: 2 min at 72 °C,

Followed by 5.0 min at 72 °C, and holding step at 10 °C.

Table of Primers used:

Primer name	Primer sequence
ACA	5'-BDBACAACAACAACA-3'
TGT	5'-VHVTGTTGTTGTTGTTGT-3'
CCA	5'-DDBCCACCACCACCACCA-3'
CGA	5'-DHBCGACGACGACGACGA-3'
UBC888	5'-BDBCACACACACACACA-3'
UBC889	5'-DBDACACACACACACAC-3'
UBC890	5'-VHVGTGTGTGTGTGTGT-3'
UBC891	5'-HVHTGTGTGTGTGTGTG-3'
MR	5'-GAGGGTGGCGTTCT-3'
RY	5'-CAGCAGCAGCAGCAG-3'
GACA	5'-GACAGACAGACAGACA-3'

C. Gel electrophoresis

In order to assess PCR success and quality, samples must be subjected to gel electrophoresis, staining and UV visualization. Process:

- Measure out appropriate amount of agarose powder by using plastic weighing boat and the appropriate balance, accordingly 1.5g for midi gel (i.e. 1.5% w/v) in 100 ml TBE. Discard weighing boat after use.
- Measure the required amount of 0.5 x TBE into the measuring cylinder, accordingly, 100ml for midi, gel.
- Pour the 0.5 x TBE buffer into the flask.
- Place the watchglass over the top of the flask and heat (carefully) in the microwave oven until agarose is dissolved and bubbling.
- Remove from the microwave oven using heat resistant gauntlet.
- Add a magnetic flea and place the conical flask on a magnetic stirrer. Add SafeView stain (5µl per 100ml) to gel.
- While the gel is cooling on the stirrer ensure that the gel tray is on a level surface using the spirit level. The mini gel trays need to be taped over at both ends using electrical tape before the gel is poured. Mini gel trays can fit into the gel tank horizontally so no tape is

needed, for maxi gels, lubricate the rubber seal of the guards with Vaseline and slide the guards into place at either end.

- Place comb or combs in the appropriate places.
- When the gel has cooled sufficiently pour it into the gel tray. Push away any bubbles to the side using a pipette tip or wooden cocktail stick.
- When the gel has cooled and solidified fully, remove tape or the slot in guards and cover the gel in 0.5 x TBE containing SafeView Stain (5µl of stain per 100ml of buffer).
- Remove the combs.
- Load 10µl of size marker (DNA ladder) on each end of the row of wells in the gel.
- To prepare 100 bp DNA Ladder: Mix 50 µl of stock solution to 950 µl sterile water (1:20). Aliquot in to 50 µl volumes.
- Add 5 µl of loading buffer in to each PCR tube containing 20 µl or 25 µl of PCR product.
- Load 12.5µl of PCR product (see TP 05) in the well.
- When finished loading replace the lid and connect the cables to appropriate sockets of the power pack. Ensure that that gel tray is fitted correctly; DNA migrates in the TBE buffer towards (+) electrode (anode).
- Stay clear from the tank. Switch the power pack on, press “Set” and by using the arrows set the voltage at appropriate constant voltage (eg. 75V-110V). Press “Set” again to set current (I) at 500mA, press “Set” again to set time. To start the electrophoresis press “run”.
- When loading buffer has reached 2-3 cm from the bottom of the gel or the next row of wells stop the electrophoresis by pressing “Set” and then switch the power pack off.
- Disconnect the connectors and remove lid.
- Place the gel in the centre of the UV transilluminator in the U:Genius unit.
- Follow instructions on Quick guide sheets attached to side of U:Genius for image examination, manipulation and capture.
- Click on the “Save” icon, then “change the file name” enter the file name in the format DDMMYPCR, save as Tiff file.
- Switch off the U:GENIUS unit when session is finished.
- Spray the yellow tray and all worktops with laboratory disinfectant/DNA inactivation solution and wipe them down with paper towel.
- Make appropriate notes in the Laboratory Notebooks in accordance with their specific instructions. Transfer gel image to own PC by memory stick provided. Print out image on

U:Genius printer and note the order of the samples.

2.2 CBS-KNAW

The quality and quantity of extracts of filamentous fungi and yeast genomic DNA are verified using gel electrophoresis, the NanoDrop spectrophotometer method as well as validation of sequences of the internal transcribed spacer (ITS) domain of the ribosomal RNA gene (rRNA) and the D1/D2 domain of the 26S rRNA gene.

A. DNA electrophoresis:

- 0.8% agarose gel. Weigh out 0.4 g of agarose into a flask and add 50 ml of 1× TAE
- Heat solution in a microwave until agarose is completely dissolved.
- Allow to cool and mix 0.1 µg/ml ethidium bromide with dissolved agarose. Place the appropriate comb in gel tray and pour the agarose.
- Allow the agarose gel to solidify.
- Place in electrophoresis system and cover with 1× TAE.
- Mix 1 µl of loading buffer with 5 µl of DNA extract.
- Load DNA samples and DNA ladder.
- Run at 70 V for 1h.
- Visualize DNA bands using gel imaging system and take a photo.

B. DNA measurement:

- Use undiluted DNA.
- Place DNA sample onto the NanoDrop spectrophotometer.
- The NanoDrop calibration and measurements of the DNA are done according to the instruction manual of the equipment.
- Measure: A260, A280, A230, A320. Ratio A260/A280. Ratio A260/A230. Concentration (ng/µl).

C. Validation of D1/D2 and/or ITS sequences:

- Use undiluted or diluted (1/10 or 1/50) DNA in the PCR reactions, depending on the values obtained from the spectrophotometer and/or NanoDrop.
- Several PCR protocols have been published in order to optimize the results obtained from the different DNA regions for different fungal species. The standard primer pairs ITS5/ITS4 and LR0R/LR5 are used during PCR amplification.

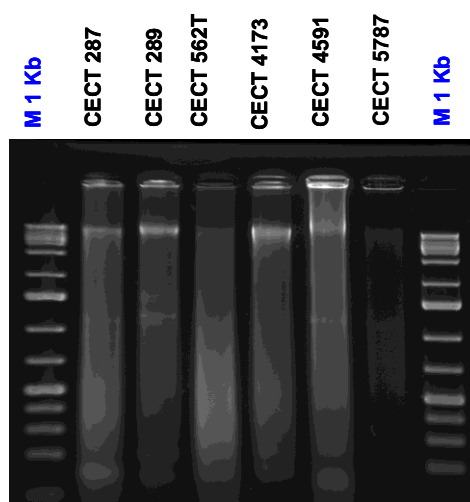
- The sequence reactions are done according to the instructions of the sequencing kit (ABI big dye terminator v. 3.1) and run on an ABI 3730XL DNA sequence analyser.
- The sequences obtained are validated by comparing it to already published sequences available in online sequence databases such as the CBS fungal and yeast database (<http://www.cbs.knaw.nl/collections/>) and GenBank (<http://www.ncbi.nlm.nih.gov/>).
- Sequences are also validated by comparing it to the unpublished sequence data available from or the depositor of the strain or various ongoing research projects within CBS.

2.3 UVEG-CECT

The following protocols are applied at CECT to check genomic DNA from prokaryotes for purity, integrity and quantity:

A. DNA electrophoresis:

- 0.8% agarose gel. Weigh out 0.4 g of agarose into a flask and add 50 ml of TBE (0.5x).
- 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 (0.5x) 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.



B. DNA measurement:

- Dilute DNA 1/10 or 1/50.
- Place DNA dissolution into the quartz cuvette and introduce it into the spectrophotometer.
- Measure: A260, A280, A230, A320. Ratio A260/A280. Ratio A260/A230. Concentration (ng/ml).

2.4 CRBIP

To check genomic DNA for purity, integrity and quantity, the protocol applied by CRIBP is the following:

A. DNA electrophoresis:

- 1% agarose gel. Weight out 0.3 g of agarose into a flask and add 30 ml of TBE 1%.
- Heat solution in a microwave until agarose is completely dissolved.
- Allow to cool.
- Add ethidium bromide and homogenise
- Place the appropriate comb in gel tray
- Allow the agarose gel to solidify.
- Place in electrophoresis system and cover with TBE 1% buffer.
- Mix 5 μ l of loading buffer with 5 μ l of sample (DNA extraction).
- Load DNA samples and ladder.
- Run at 90V for 1h.
- Visualize DNA bands using gel imaging system and take a photo.

B. DNA measurement:

- Place DNA into the spectrophotometer.
- Measure: A260, A280. Ratio A260/A280. Concentration (ng/ml).
- If necessary, dilute DNA 1/10 or 1/50 and make another measurement.

2.5 INRA

CIRM-Levures

The quality and quantity of extracts of yeast genomic DNA are verified using the following process:

DNA measurement:

The DNA amount is determined in ng/ μ l

- Purity is evaluated by the absorbance ratio 260nm/280nm of the solution using spectrophotometer Nanodrop® ND1000:

This ratio should be between 1.8 and 2.0 to confirm the DNA purity.

If the ratio is below 1.8 there may be a contamination with proteins and if ratio is above 2.0, there may be contamination with RNA.

- Concentration using absorbance at 260nm

According to the result, the DNA solution may be diluted with sterile dH₂O and purity will be measured again

- Concentration using agarose gel migration
 - Prepare 0.8% agarose gel. Weight out 0.8g of agarose into a flask and add 100ml of TBE 0.5X
 - Heat solution in a microwave until agarose is completely dissolved. Add 2 μ l of ethidium bromide (0.5 μ g/ml) into the agarose solution. Homogenize solution
 - 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 0.5X buffer
 - Load DNA samples (5 μ l of DNA solution and 5 μ l of loading blue charge) with a track for 5 μ l of DNA ladder
 - Run at 100V for 1h30
 - Visualize DNA bands using gel imaging system and take a picture

The quality of DNA is determined by the presence of DNA and the absence of RNA. If RNA is present, the DNA solution is treated again with RNase A

The integrity of DNA is determined by the absence of DNA smear.

CIRM-BP

The quality and quantity of extracts of bacteria genomic DNA are verified using the following process:

A. Spectrophotometer

The DNA amount is determined without any dilution with the Spectrophotometer Nanodrop® ND1000 in ng/μl. This allows the determination of the absorption curve (Measures DNA, RNA (A260) and Protein (A280) concentrations) and the sample purity (A260nm/A280nm ratio).

- Clean the upper and lower optical surfaces of the microspectrophotometer sample retention system as follows. Pipet 1 to 2 μl of clean deionized water onto the lower optical surface. Close the lever arm and tap it a few times to bathe the upper optical surface. Lift the lever arm and wipe off both optical surfaces with a Kimwipe.

Wiping the pedestals between measurements is usually sufficient to prevent sample carryover and avoid residue build-up. A final cleaning of all surfaces with deionized water is recommended after each user's last measurement. After a large number of samples, the areas around the pedestals should be cleaned thoroughly to prevent previous samples from being wiped back onto the pedestals, affecting low-level measurements. It may be advisable to clean the measurement surfaces with 2 μl water (as above) after particularly high-concentration samples.

- Open the NanoDrop software and select the nucleic acids module
- Initialize the spectrophotometer by placing 1.5 μl clean water onto the lower optic surface, lowering the lever arm and selecting "initialize" in the NanoDrop software. Once initialization is complete (~10 sec), clean both optical surfaces with a Kimwipe.
- Perform a blank measurement by loading 1.5 μl deionized water or buffer and selecting "blank". Once the blank is complete, clean both optical surfaces with a Kimwipe.

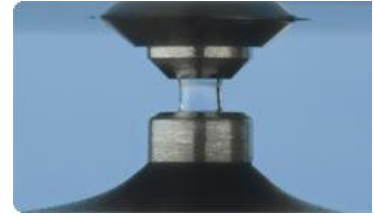
The water or buffer should always be measured to ensure that the instrument has been zeroed properly. The measurement of the water or buffer should be zero or very closed to zero.

- Measure the nucleic acid sample by loading 1.5 μl and selecting "measure". Once the blank is complete, clean both optical surfaces with a Kimwipe.

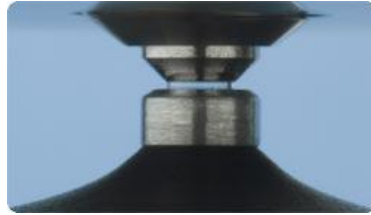
With the arm open, a sample is pipetted directly onto the pedestal.



After the arm is closed, a sample column is formed.



The pedestal then moves to automatically adjust for an optimal path length (0.05 mm - 1 mm).



When the measurement is complete, the surfaces are simply wiped with a lint-free lab wipe before going on to the next sample.



Check the wavelength of the peak in the spectra – this should be at 260 nm for DNA and RNA. The $A_{260\text{nm}}/A_{280\text{nm}}$ ratio should be between 1.8 and 2 to confirm the DNA purity. If this ratio is below 1.8 there is contamination with proteins and if this ratio is above 2, there may be contamination with RNA.

B. Electrophoresis

A high A_{260} reading does not always mean high genomic DNA yields. One of the main reasons for a high A_{260} reading that does not correlate to genomic DNA is the absorbance of UV due to highly degraded DNA or RNA. The DNA integrity should be assessed by 0.8% agarose gel electrophoresis to check that there is no smear on the gel due to DNA degradation.

- 0.8 % agarose gel. Weight out 0.8 g of agarose into a flask and add 100 ml of TBE 0.5x
- Heat solution in a microwave until agarose is completely dissolved. Add 2 μl ethidium bromide (0.5 $\mu\text{g}/\text{ml}$) into the agarose solution. Homogenize solution.
- 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 0.5x buffer.
- Mix 10 μl of loading buffer 6x with 2 μl of sample (DNA extraction).
- Load DNA samples and ladder.
- Run at 70 V for 1 h.

Visualize DNA bands using gel imaging system and take a photo.

To check that the sample is inhibitor free, the DNA is used in PCR reaction. A PCR with primers against 16S rDNA or genes like *oriC* or *rpoB* (enterobacteria) and *sodA* (staphylococci and streptococci) will also allow the DNA authentication by analyzing the sequences of the PCR

products. When possible, this could also be done with a PCR specific for the considered species.

2.6 BCCM

BCCM/LMBP

The quality control validation is carried out following two steps:

A. DNA measurement:

- Place 1 μ l of DNA dissolution onto the Nanodrop reader.
- Measure: A230, A260, A280, ratio A260/A230. Ratio A260/A280. Concentration (ng/ μ l).

B. Authenticity test:

- Restriction enzyme digestion
 - Add 1 μ g plasmid DNA, 10 u restriction enzyme (1 μ l) and 2 μ l of the appropriate buffer in a tube and add distilled water up to 20 μ l.
 - Place into a thermoblock at the appropriate temperature for 1 to 2 hours.
- Gel electrophoresis
 - Make a 1% agarose gel: weigh out 1 g of agarose into a flask and add 100 ml TAE buffer.
 - 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 TAE buffer.
 - Mix 4 μ l of 6x loading dye with 20 μ l of digested sample DNA.
 - Load DNA samples and Smart ladder.
 - Run at e.g. 100 V for 45 minutes*.

*: time depends on the set voltage (1-5 V/cm)

- Analysis of the restriction enzyme pattern
 - Place agarose gel into the ethidium bromide (300 μ l in 3 l bidi) bath for max. 15 to 30 minutes.
 - Rinse the gel in bidi.
 - Visualize the DNA bands using a gel imaging system and take a photo.

BCCM/LMG

Quality of extracted genomic DNA is evaluated spectrophotometrically by absorption measurements at different wavelengths and by agarose gel electrophoresis.

A. Purity control

- Dilute extracted gDNA 15 times in 0,1x TE buffer in a 96-well UV plate to a final volume of 120µl.
- Measure A260, A280, A234 with the spectrophotometer.

Remark: A320 measures background absorption and should therefore be used as background correction. Neither DNA nor proteins absorb at this wavelength. The A320 value should be subtracted from the other absorbance measurements. BCCM/LMG is considering the implementation of the A320 value.

- Purity is evaluated by two absorbance ratios:

$$\text{Purity A} = \frac{A_{260}}{A_{280}} \quad \text{Purity B} = \frac{A_{234}}{A_{260}}$$

- DNA is considered pure when Purity A has a value between 1.7 and 2.0 and Purity B has a value less than 0.85.
- Concentration of measured DNA can be calculated by following formula:

DNA concentration (µg/ml) = A260 x 50 x dilution factor

Remark: DNA concentration could be overestimated by the presence of RNA, which also absorbs at A260.

B. Agarose gel electrophoresis

- Post staining method
 - Prepare 1% agarose solution:
 - a. weigh 2.5 g agarose and transfer into a glass bottle.
 - b. Add 250 ml of 1xTBE.
 - Heat the solution in a microwave oven until the agarose is completely dissolved.
 - Place the appropriate comb in a gel tray and pour the agarose solution in the tray.
 - Allow the agarose solution to solidify.
 - Place the tray in the electrophoresis tank and cover completely with 1xTBE buffer.
 - Mix 1 µl of loading dye with 5 µl of sample (DNA).
 - Load DNA samples and ladder (5 µl).

- Run at 75V for 45 min.
- Place agarose gel into a 1.5 l Sybr Safe bath (dilution 10000x in 1x TBE).
- Visualize DNA bands by using the gel documentation system and capture the image.
- Pre staining method
 - Follow steps 1-2 of §2.1.
 - Add 1.5 µl sybr Safe in 30 ml agarose suspension.
 - Follow steps 3-8 of §2.1.
 - Visualize DNA bands by using the gel documentation system and capture the image.

2.7 MUM

To check genomic DNA for purity, integrity and quantity, the following protocol is applied at MUM:

A. Nanodrop measurement:

- Place 3 µl of extracted DNA onto the Nanodrop reader.
- Measure: A230, A260, A280, ratio A260/A230. Ratio A260/A280 and DNA Concentration.

B. Gel electrophoresis:

- Make a 0.9% agarose gel in TAE buffer (0.04 M Tris-base, 1.14% (v/v) glacial acetic acid, 1 mM EDTA).
- Mix 1 µl of 6x loading dye (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol) with 5 µL of genomic DNA.
- Load DNA samples and Quick-load 2-Log DNA Ladder on the agarose gel.
- Run the gel at 100 V for 30 minutes.

Conclusion

Several protocols are applied for quality control validation and all of them are valid for the development of the European microbial DNA Bank Network.

Significance of this deliverable

Extraction of DNA from microbial resources may cause DNA damage. Checking DNA quality after extraction is thus an essential step before long-term storage. Each partner described precisely the quality control procedures used, in terms of DNA concentration and purity.

This constitutes a very operational basis for each BRC willing to join the European microbial DNA bank network.