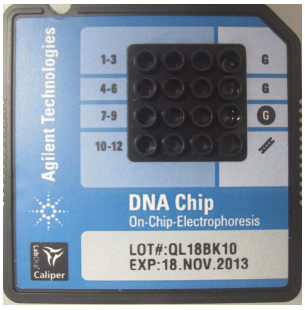
CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

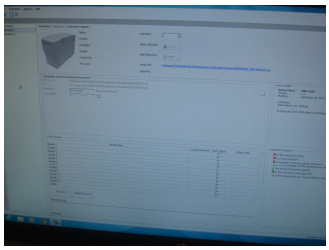
FACULTY OF TROPICAL AGRISCIENCES

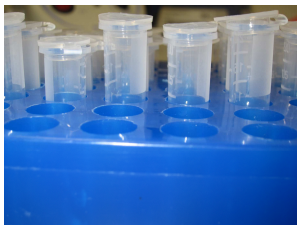
Department of Crop Sciences and Agroforestry

Laboratory of Molecular Biology

Book of practicals for work with Agilent 2100 Bioanalyzer- DNA1000 Assay Protocol







Prague

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Acknowledgments

This “**Book of practicals for work with Agilent 2100 Bioanalyzer - DNA 1000 Assay Protocol**” was financially supported and made in frame of the project of Fund of development of universities FRVŠ 1397/2013 “**Inovace vybavení laboratoří pro výuku aplikovaných rostlinných biotechnologií”**

CONTENT

1. Safety rules and regulations in Laboratory of molecular biology

- At the beginning of the work in the laboratory, students have to know laboratory safety rules and regulations in Laboratory of molecular biology.
- It is forbidden to eat, drink and smoke in the laboratory.
- Students have to wear laboratory coats, slippers and rubber gloves.
- Unauthorized experiments are strictly forbidden
- The laboratory must be kept clean and organized.
- Check the proper installation of the equipment. If there is any problem with equipment, do not use it and inform the supervisor.
- If a piece of equipment fails while being used, report it immediately to your laboratory assistant or tutor. Never try to fix the problem yourself because you could harm yourself or the others.
- Clean up your work area before leaving the laboratory.
- Turn off all electric devices before leaving the laboratory.
- Before leaving the laboratory, wash your hands.
- Ask the supervisor if you are in doubt.
- Read labels carefully.
- Never “smell” a solvent directly! Read label on the solvent bottle to identify its contents. Chemicals must never be tasted!
- Check where the laboratory fire extinguisher and wash station are located and how to use them.
- The staff and students are obliged to manipulate with poisonous, volatile and smelly substances exclusively in running hood.
- Always give the chemicals and reagents you used back to the place where you had taken them from.
- Special care should be taken while working with open fire, combustibles, corrosives and toxic substances.
- Always inform the teacher about any accident or injury and provide the first aid if necessary.
- The reagent solutions are always casted from the reagent bottle on the unlabeled side to avoid the damage of the label. Illegible inscription and incidental substitution linked with it can cause dangerous consequences.
- Concentrated acids, especially sulphuric acid, are diluted by infusion of acid into the water. Acid is infused in the thin stream to the solution which is mixed up by the glass stick throughout the whole dilution.
- Manipulation with irritating, smelling and toxic substances (i. e. chlorine, chloroform, carbon disulphide, etc.) and easily flammable substances (i. e. gasoline, acetone, etc.) is allowed only in well aired and functional hood.
- Throw toxic and nontoxic waste into the appropriate containers.
- Everybody who work in the laboratory, have to respect all the rules mentioned above and will inscribe their names into the presence book.

2. Agilent DNA 1000 kit Start Guide

Agilent DNA 1000 Kit

Check if the Agilent DNA 1000 content following reagements

DNA Chips DNA 1000
25 DNA Chips
1 Electrode Cleaner

Syringe Kit
1 Syringe

Reagents
(yellow) DNA 1000 Ladder
(green) DNA 1000 Markers 15/1500 bp (2 vials)
(blue) DNA Dye Concentrate (1 vial)
(red) DNA Gel Matrix (3 vials)
3 Spin Filters

Storage conditions

- Keep all reagents and reagents mixes refrigerated at 4°C when not in use to avoid poor results caused by reagement decomposition
- Protect dye and dye mixtures from light. Remove light covers only when pipetting. Dye decomposes when exposed to light

Equipment requiered for use of the Agient 2100 Bioanalyzer

- Chip priming station
- IKA vortex mixer

Additional Material Required

- Pipettes (10 µl, 100 µl and 1000 µl) with compatible tips
- 0.5 ml microcentrifuge tubes for sample preparation
- Microcentrifuge

Sample preparation

- For accurate determination of DNA concetration the total amount of DNA in samples must be between 0.5-50 ng/µl. If concentration is excessively high, dilute to 0.5-50 ng/µl.
- When analyzing restriction digest, add EDTA or heat inactivate the restriction enzyme according to the manufacturer instruction. Restriction endonucleases in combination with non-chelated metal ions may degrade internal DNA marker.

3. Setting up the Assay Equipment and Bioanalyzer

Before beginning the chip preparation protocol, ensure that the chip priming station and the bioanalyzer are set up and ready to use.

You have to

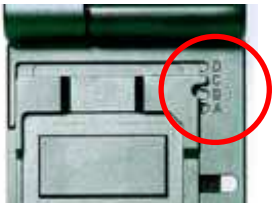
- replace the syringe at the chip priming station with each new DNA kit
- adjust the base plate of the chip priming station
- adjust the syringe clip at the chip priming station
- adjust the bioanalyzer's chip selector
- set up the vortex mixer
- finally make sure that you start the software before you load the chip.

NOTE: The Agilent DNA 1000 assay is a high sensitivity assay. Please read this guide carefully and follow all instructions to guarantee satisfactory results.

4. Setting up the Chip Priming Station

1 Replace the syringe:

a Unscrew the old syringe from the lid of the chip priming station.
b Release the old syringe from the clip. Discard the old syringe.
c Remove the plastic cap of the new syringe and insert it into the clip.
d Slide it into the hole of the luer lock adapter and screw it tightly to the chip priming station.



2 Adjust the base plate:

a Open the chip priming station by pulling the latch.
b Using a screwdriver, open the screw at the underside of the base plate.
c Lift the base plate and insert it again in **position C** Retighten the screw.

3 Adjust the syringe clip:

a Release the lever of the clip and slide it down to the **lowest** position.

NOTE: Replace the syringe with each new reagent kit.

5. Setting up the Bioanalyzer

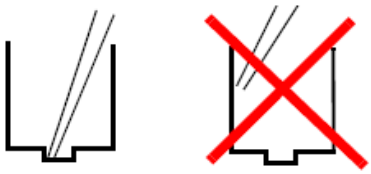
Adjust the chip selector:

1 Open the lid of the bioanalyzer and make sure that the electrode cartridge is inserted in the instrument. If not, open the latch, remove the pressure cartridge and insert the electrode cartridge.

2 Remove any remaining chip and adjust the chip selector to position (1).

6. Essential Measurement Practices

- Handle and store all reagents according to the instructions on the label of the individual box.
- Avoid sources of dust or other contaminants. Foreign matter in reagents and samples or in the wells of the chip will interfere with assay results.
- Keep all reagent and reagent mixes refrigerated at 4 °C when not in use.
- Allow all reagents and samples to equilibrate to room temperature for 30 minutes before use.
- Protect dye and dye mixtures from light. Remove light covers only when pipetting. The dye decomposes when exposed to light and this reduces the signal intensity.



- Always insert the pipette tip to the bottom of the well when dispensing the liquid. Placing the pipette at the edge of the well may lead to poor results.
- Use a new syringe and electrode cleaners with each new kit.
- Use loaded chips within 5 minutes after preparation. Reagents might evaporate, leading to poor results.
- Do not touch the Agilent 2100 bioanalyzer during analysis and never place it on a vibrating surface.

7. Agilent DNA 1000 Assay Protocol

Preparing the Gel-Dye Mix

1. Allow the DNA dye concentrate (blue 🔵) and DNA gel matrix (red 🔴) to equilibrate to room temperature for 30 minutes.

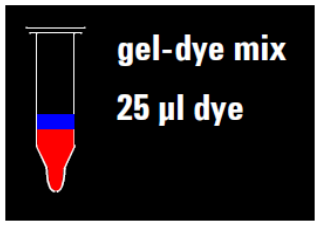
WARNING *Handling DMSO*

⇒Kit components contain DMSO. Because the dye binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care.

⇒Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples.

⇒Handle the DMSO stock solutions with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

2. Vortex the blue-capped DNA dye concentrate (blue 🔵) for 10 seconds and spin down. Make sure the DMSO is completely thawed.
3. Pipette 25 µl of the blue capped dye concentrate (blue 🔵) into a red-capped DNA gel matrix vial (red 🔴). Store the dye concentrate at 4 °C in the dark again.
4. Cap the tube, vortex for 10 seconds. Visually inspect proper mixing of gel and dye.
5. Transfer the gel-dye mix to the top receptacle of a spin filter.
6. Place the spin filter in a microcentrifuge and spin for 15 minutes at room temperature at 2240 g ± 20 % (for Eppendorf microcentrifuge, this corresponds to 6000 rpm).
7. Discard the filter according to good laboratory practices. Label the tube and include the date of preparation.

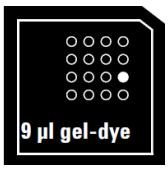


NOTE: **It is important that all the reagents have room temperature before starting the next step. Protect the dye concentrate from light.**

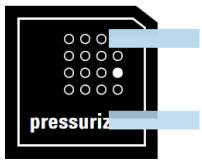
Always use the volumes indicated. Using different volumes in the same ratio will produce inaccurate results. The prepared gel-dye mix is sufficient for 10 chips. Use the gel-dye within 4 weeks of preparation.

Protect the gel-dye mix from light. Store the gel-dye mix at 4 °C when not in use for morethan 1 hour.

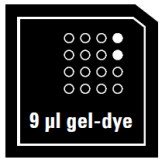
Loading the Gel-Dye Mix



1 Allow the gel-dye mix to equilibrate to room temperature for 30 minutes before use. Protect the gel-dye mix from light during this time.
2 Take a new DNA chip out of its sealed bag and place the chip on the chip priming station.
3 Pipette 9.0 µl of the gel-dye mix at the bottom of the well marked .
4 Set the timer to 60 seconds, make sure that the plunger is positioned at 1 ml and then close the chip priming station. The lock of the latch will click when the Priming Station is closed correctly.

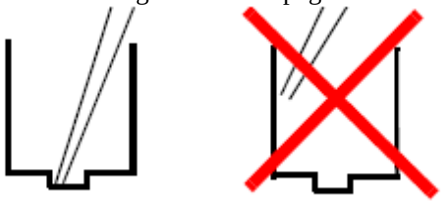


5 Press the plunger of the syringe down until it is held by the clip.
6 Wait for exactly 60 seconds and then release the plunger with the clip release mechanism.
7 Visually inspect that the plunger moves back at least to the 0.3 ml mark.
8 Wait for 5 seconds, then slowly pull back the plunger to the 1 ml position.



9 Open the chip priming station.
10 Pipette 9.0 µl of the gel-dye mix in each of the wells marked.

NOTE: Before loading the gel-dye mix, make sure that the base plate of the chip priming station is in position (C) and the adjustable clip is set to the lowest position. Refer to “Setting up the Chip Priming Station” on page 7 for details.



When pipetting the gel-dye mix, make sure not to draw up particles that may sit at the bottom of the gel-dye mix vial. Insert the tip of the pipette to the bottom of the chip well when dispensing. This prevents a large air bubble forming under the gel-dye mix. Placing the pipette at the edge of the well may lead to poor results.
Protect the gel-dye mix from light. Store the gel-dye mix at 4 °C when not in use for more than 1 hour.

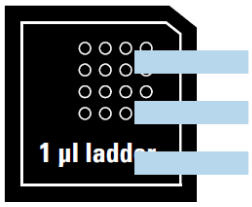
Loading the Marker

Pipette 5 µl of green-capped DNA marker (green🟢) into the well marked with the ladder symbol and into each of the 12 sample wells.

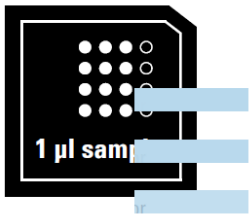
NOTE: Do not leave any wells empty, or the chip will not run properly. Add 5 µl of green-capped DNA marker (green □) plus 1 µl of deionized water to each unused sample well.

Loading the Ladder and the Samples

1 Pipette 1 µl of the yellow-capped DNA ladder (yellow ●) in the well marked with the ladder symbol .



2 In each of the 12 sample wells pipette 1 µl of sample (used wells) or 1 µl of deionized water (unused wells).



CAUTION *Wrong vortexing speed*

If vortexing speed is too high, liquid spill that disturbs the analysis may occur for samples generated with detergent containing PCR buffers.

⇒ Reduce vortexing speed to 2000 rpm!

NOTE: For optimal results, samples should be of pH 6 to 9 and should not have an ionic content greater than twice that of a typical PCR buffer.

3 Set the timer to 60 seconds.
4 Place the chip horizontally in the adapter of the IKA vortex mixer and make sure not to damage the buldge that fixes the chip during vortexing.
5 Vortex for 60 seconds at 2400 rpm.
6 Refer to the next topic on how to insert the chip in the Agilent 2100 bioanalyzer. Make sure that the run is started within 5 minutes.

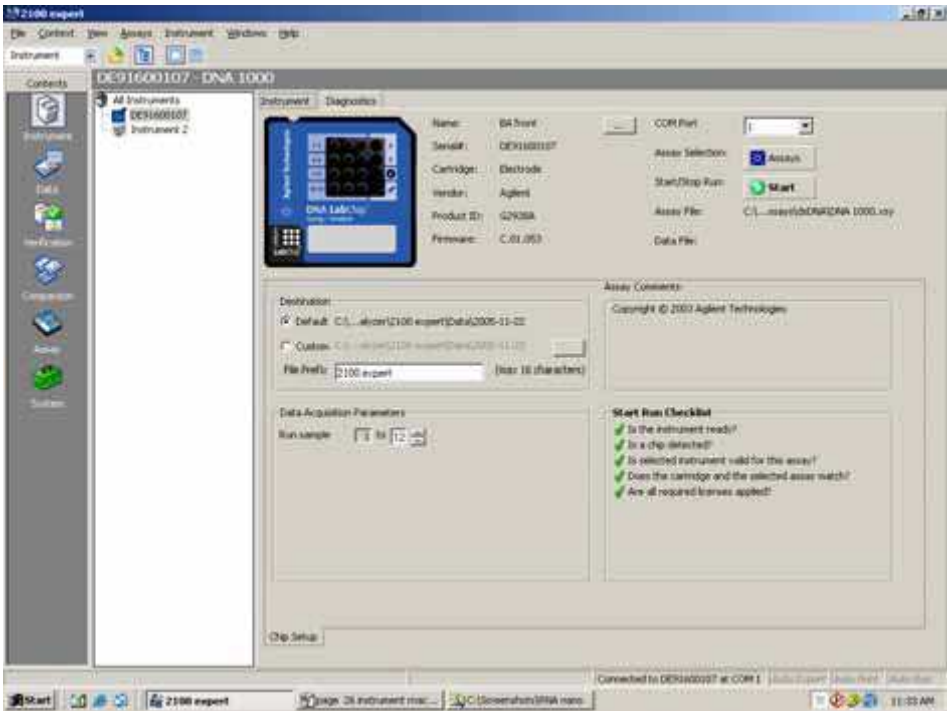
Inserting a Chip in the Agilent 2100 Bioanalyzer

1 Open the lid of the Agilent 2100 bioanalyzer.
2 Check that the electrode cartridge is inserted properly and the chip selector is in position (1). Refer to "Setting up the Bioanalyzer" on page 8 for details.

CAUTION Sensitive electrodes and liquid spills. Forced closing of the lid may damage the electrodes and dropping the lid may cause liquid spills resulting in bad results.

⇒ Do not use force to close the lid and do not drop the lid onto the inserted chip**.**

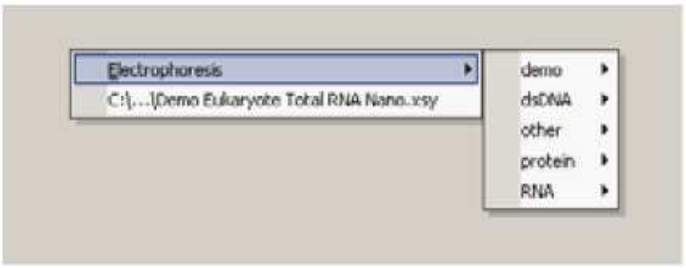
3 Place the chip carefully into the receptacle. The chip fits only one way.
4 Carefully close the lid. The electrodes in the cartridge fit into the wells of the chip.
5 The 2100 expert software screen shows that you have inserted a chip and closed the lid by displaying the chip icon at the top left of *Instrument* context.



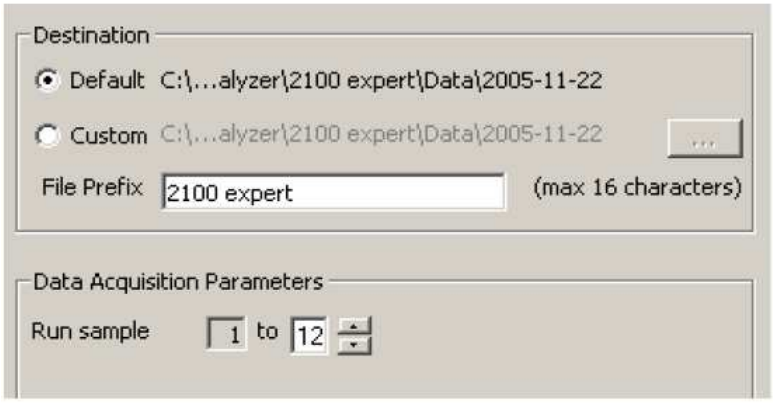
Starting the Chip Run

NOTE: Please note that the order of executing the chip run may change if the Agilent Security Pack software (only applicable for Agilent 2100 expert software Revision B.02.02 and higher) is installed. For more details please read the 'User's Guide' which is part of the Online Help of your 2100 expert software.

1In the *Instrument* context, select the appropriate assay from the Assay menu.

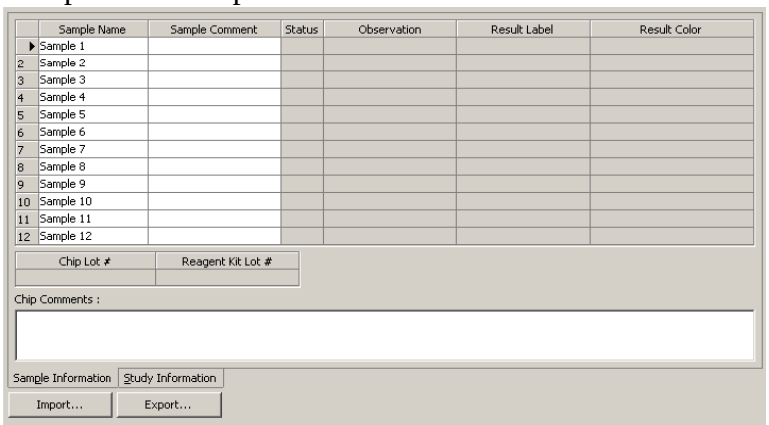


2Accept the current *File Prefix* or modify it. Data will be saved automatically to a file with a name using the prefix you have just entered. At this time you can also customize the file storage location and the number of samples that will be analyzed.



3 Click the *Start* button in the upper right of the window to start the chip run. The incoming raw signals are displayed in the *Instrument* context.

4 To enter sample information like sample names and comments, select the *Data File* link that is highlighted in blue or go to the *Assay* context and select the *Chip Summary* tab. Complete the sample name table.



5 Toreview the raw signal trace, return to the *Instrument* context.
6 After the chip run is finished, remove the chip from the receptacle of the bioanalyzer and dispose it according to good laboratory practices.

CAUTION *Contamination of electrodes*

Leaving the chip for a period longer than 1 hour (e.g. over night) in the bioanalyzer may cause contamination of the electrodes.

⇒ Immediately remove the chip after a run.

Cleaning Electrodes after a DNA 1000 Chip Run

When the assay is complete, *immediately* remove the used chip from the Agilent 2100 bioanalyzer and dispose it according to good laboratory practice. Then perform the following procedure to ensure that the electrodes are clean (i.e. no residues are left over from the previous assay).

CAUTION *Leak currents between electrodes*
Liquid spill might cause leak currents between the electrodes.

⇒ Never fill too much water in the electrode cleaner.

NOTE Use a new electrode cleaner with each new kit. After 5 assays, empty and refill the electrode cleaner.
After 25 assays, replace the used electrode cleaner by a new one.
When switching between different assays, a more thorough cleaning may be required.
Refer to the maintenance chapter on this CD Maintenance and Troubleshooting Guide for details which is part of the Online Help of the 2100 bioanalyzer software.

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1 Slowly fill one of the wells of the electrode cleaner with 350 µl deionized analysis-grade water.
2 Open the lid and place electrode cleaner in the Agilent 2100 bioanalyzer.
3 Close the lid and leave it closed for about 10 seconds.
4 Open the lid and remove the electrode cleaner.
5 Wait another 10 seconds to allow the water on the electrodes to evaporate before closing the lid.

NOTE After 5 assays, empty and refill the electrode cleaner.
After 25 assays, replace the used electrode cleaner by a new one.

NOTE When switching between different assays, a more thorough cleaning may be required.
Refer to the maintenance chapter on this CD Maintenance and Troubleshooting Guide for details which is part of the Online Help of the 2100 bioanalyzer software.

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8. Checking Your Agilent DNA 1000 Assay Results

DNA 1000 Ladder Well Results

To check the results of your run, select the Gel or Electropherogram tab in the *Data* context. The electropherogram of the ladder well window should resemble to those shown below.

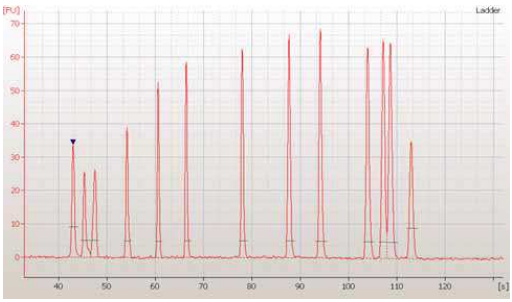

Figure 1 DNA 1000 ladder

Major features of a successful ladder run are:

• 13 peaks for DNA 1000 ladder
• All peaks are well resolved
• Flat baseline
• Correct identification of both markers

If the electropherogram of the ladder well window does not resemble the one shown above, refer to the *2100 Expert Maintenance and Troubleshooting Guide* for assistance.

DNA 1000 Sample Well Results

To review the results of a specific sample, select the sample name in the tree view and highlight the *Results* sub-tab. The electropherogram of the sample well window should resemble the one shown here.

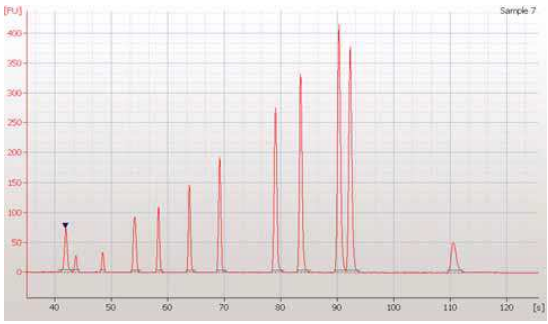

Figure 2 DNA peaks of a successful sample run

Major features for a successful DNA sample run are:

• All sample peaks appear between the lower and upper marker peaks. If some sample peaks are outside the marker bracket, adjust the upper or lower marker. Please refer to the 2100 Expert User's Guide or Online Help for details.
• Flat baseline
• Baseline readings at least 5 fluorescence units (see Zero Baseline in the User's guide or Online Help for details of how to see the baseline readings).
• Marker readings at least 3 fluorescence units higher than baseline readings.
• Both marker peaks well resolved from sample peaks (depends on sample).