



Pseudomonas aeruginosa

Genotyping Kit 2

Array Hybridisation Kit for epidemiological typing of *Pseudomonas aeruginosa* isolates.

Kit order number: 245600096

96 reactions (ArrayStrip format)

For Research Use Only. Not for Use in Diagnostic Procedures.

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BACKGROUND

The ALERE *P. aeruginosa* Genotyping Kit 2 is a quick and simple method for the DNA-based assignment of unknown *P. aeruginosa* isolates to phylogenetic lineages.

RNA-free, unfragmented genomic DNA from pure and monoclonal *P. aeruginosa* colony material is amplified approximately 50-fold and internally labelled with biotin-11-dUTP using a linear amplification protocol. In contrast to standard PCR, a multiplex primer extension reaction is performed with two nested primers per target in each cycle. Two-versus-one primer for each target increase and synchronize the yield of biotin labelled single stranded (ss) DNA product for all markers. This allows a simultaneous sequence specific labelling and amplification of an essentially unlimited number of targets. However, sensitivity is lower than in a standard PCR (whereas contamination with amplicons is nearly impossible) and for that reason the method is restricted to clonal colony material and cannot be performed on samples such as swabs or other patient samples. Resulting biotin labelled ssDNA is transferred and hybridised to DNA oligonucleotide microarrays with 99 probes for different genetic markers plus controls. All of them are printed in five spots each.

The array contains three different kinds of markers and each marker is represented by one or more probes:

- 16 markers for kinship analysis (represented in total by 36 different probes)
- 39 markers of the so called accessory genome (represented in total by 39 different probes) which may be used for the analysis of microevolutionary events
- 24 probes for internal control of the analytic process. These probes test for proper DNA preparation (using conserved genes of *P. aeruginosa*), PCR (using a synthetic template), hybridisation (using control spots with biotinylated antisense partners) and staining reaction (using biotinylated control spots), respectively.

Oligonucleotide probes are directly spotted onto the array, primers and control oligonucleotides are present in the MasterMix B1^{P2}.

The resulting typing data provide information for kinship analysis. Strain assignment using this information was described in detail by Wiehlmann et al. 2007, PNAS vol. 104 no. 19, p. 8101–8106.

GENERAL INSTRUCTIONS FOR USE

Intended Use

For Research Use Only. Not for Use in Diagnostic Procedures.

This assay allows genotypic characterisation of *P. aeruginosa* isolates for research and epidemiological applications. It cannot be used for other bacteria than *P. aeruginosa*.

Specifications

Upon receipt the assay components need to be stored at different temperatures as specified on the package insert. The assay is to be performed at an ambient temperature of 18°C to 28°C.

Technical Support

If you require any further information on this product please contact:

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cct.home@clondiag.com

Safety Precautions

- The assay is intended for use by personnel that are trained in microbiological and molecular methods. Preparation of DNA from pure *P. aeruginosa* colonies (clones) requires expertise in microbiology and the local regulations for handling of pathogenic microorganisms (biosafety level 2) are to be obeyed.
- Isolated, cell-free *P. aeruginosa* DNA may be processed without further biosafety precautions, although contamination with *P. aeruginosa* or other bacteria needs to be ruled out.
- Always wear protective clothes as required for laboratory work by your local regulations.

Material Safety Data Sheets (MSDS)

According to OSHA 29CFR1910.1200, Commonwealth of Australia [NOHSC: 1005, 1008(1999)] and the latest amendments to the European Union Directives 67/548/EC and 1999/45/EC, the enclosed reagents do not require a Material Safety Data Sheet (MSDS). They do not contain more than 1% of a component classified as hazardous and do not contain more than 0.1 % of a component classified as carcinogenic. MSDS therefore are not provided. Nevertheless, the buffers may cause irritation if they come into contact with eyes or skin, and may cause harm if swallowed. The regular precautions associated with laboratory work should be obeyed (e.g., wear protective goggles, gloves and lab coat and avoid contact with the reagents). In case, any liquids are spilled, clean with disinfectant and/or laboratory detergent and water.

Alere assumes no liability for damage resulting from handling or contact with these products. If you have any questions please contact our Technical Support (see above).

Shipping Precautions

RID/ADR: "Kein Gefahrgut"/ No dangerous goods

IMDG: No dangerous goods

IATA: No dangerous goods

REAGENTS AND DEVICES

Assay Components, Storage and Stability

All reagents are provided in a certain surplus amount (see below). In case of need, all components may also be ordered separately; please refer to the order numbers at the end of this user guide. For pricing please contact your local representative or our customer service, respectively.

The expiry date can be found on each bottle and on the outer package. All components have been tested for stability for short term shipment (<1 week) at ambient temperature (< 37 °C). The assay components with a rather limited stability are D1 and C3. The other components have proven to be stable for up to six months after the assay expiry date has passed.

DNA Labelling and Amplification

- **B1^{P2}**: 2x Labelling Buffer.
Store at 2-8 °C. **Surplus: 25%**.
- **B2**: Labelling Enzyme.
Store at 2-8 °C. **Surplus: 50%**.

Hybridisation and Detection

- **ArrayStrips P.aeruginosa-4** (12 x 8 samples); protected against light and sealed under inert gas. Store at 15°C to 28°C. After opening to be used within two weeks. Close the unused wells with caps, protect them against humidity and dust and store them at a dark place.

Please note: Avoid any touching or scratching of the surface of the microarray at the bottom of the well. Do not store or handle unused wells at an air humidity of more than 60% since this may irreversibly corrode the spots.

- **ArrayStrip Cover**(24 strips)
- **C1**: Hybridisation Buffer.

Store at 18-28 °C, protect against sunlight. **Surplus: 100%.**

- **C2:** Washing Buffer 1.

Store at 18-28 °C, protect against direct sunlight. **Surplus: 100%.**

- **C3:** HRP Conjugate 100x.

Store at 2-8 °C, protect against direct sunlight. **Surplus: 100%.**

- **C4:** Conjugate Buffer. Store at 18-28 °C, protect against direct sunlight. **Surplus: 200%**
- **C5:** Washing Buffer 2. Store at 18-28 °C, protect against direct sunlight. **Surplus: 200%.**
- **D1:** Horseradish Peroxidase Substrate. Store at 2-8 °C, protect against direct sunlight. **Surplus: 50%.**

Instrumentation & Software

- ArrayMate Reader (to be ordered separately, for details see below)

The *P. aeruginosa* Genotyping assay may be used on the ArrayMate reader only. The alternative devices ATR01/03 are not suitable for reading ArrayStrip based assays. In case of any questions please contact us.

- Iconoclust software (provided with the reader)
- Test specific software plug-in (can be downloaded from Alere/CLONDIAG's website, check periodically for updates, for details see below). Information (such as spot names, marker names, location of the spots on the array, size of the image taken by the reader's specific camera) is delivered with the reader or can be downloaded from our website. These test-specific plug-ins will occasionally be updated. Please check the NEWS section of our website www.alere-technologies.com. Support is available under cct.home@clondiag.com.

Components required but not provided

- Growth media for the cultivation of *P. aeruginosa*

The test should be performed with colonies harvested from Columbia blood agar. Other nutrient-rich media (e.g. 2xTY or LB) may also suffice, but have not systematically been tested. Liquid media should not be used because contaminations or mixed cultures cannot easily be ruled out.

- Equipment and consumables needed for the cultivation of *P. aeruginosa* (incubator, inoculation loops, Petri dishes)
- DNA preparation kits:

The assay has been tested with the DNeasy Blood & Tissue Kit from Qiagen (cat# 69504) and High Pure DNA Isolations Kit from Roche (Cat. No. 11796828001).

Please note: The DNA specimen needs to be free of RNA. Recommendation: a pre-treatment with RNase A while DNA preparation.

- 1x PBS
- RNase A (we recommend Qiagen's RNase A solution, 100 mg/ml, Qiagen order# 19101)
- Equipment needed for DNA isolation, e.g. pipettes, centrifuge, thermoshaker or automated device (see above)
- Photometer (OD 260 nm) for measuring the concentration of DNA
- Equipment for non denaturing agarose DNA gel electrophoresis for quality control of DNA
- Thermocycler for PCR
- Thermoshaker
We strongly recommend the BioShake iQ by Quantifoil Instruments (<http://www.qinstruments.com/>) equipped with a customised heating block designed to fit ArrayStrips. Alternatively, you may use Eppendorf's Thermomixer Comfort, equipped a heating block for microtitre plates.
- Pipettes: suitable for 1µL-5µL volumes, 90µL, 100µL, 200µL, 1000µL
- Multichannel Pipettes for 100-200 µL
- Reaction vials suitable for PCR
- Ultrapure (PCR grade) water

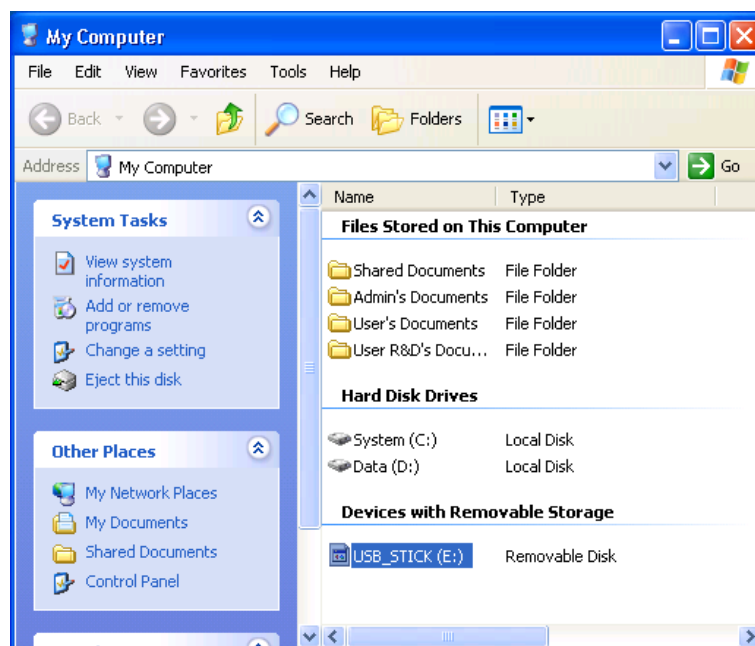
SOFTWARE INSTALLATION

For analysis of the final image of the DNA microarray on the ArrayMate a specific software plugin is required. This software plugin can be downloaded from our website www.alere-technologies.com under “Downloads” (plug-ins). Please install it on your reader according to the following instructions. Further analysis can be made after transferring the data from the ArrayMate to your computer by using the “Experiment String Collector” and the “Excel-Based Data Mining Tool” (see page 29).

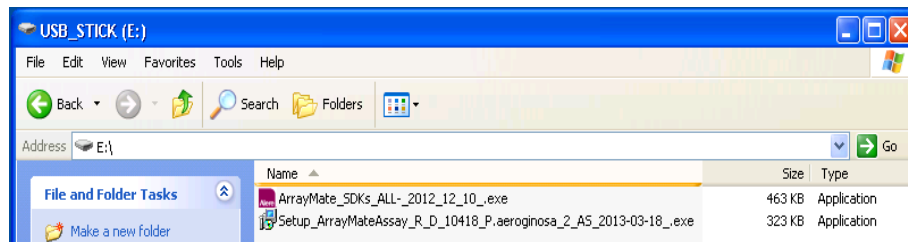
Assay Plugin and SDK for the ArrayMate

The following instruction describes the installation of the AssayPlugin and ArrayMate installation software (SDK).

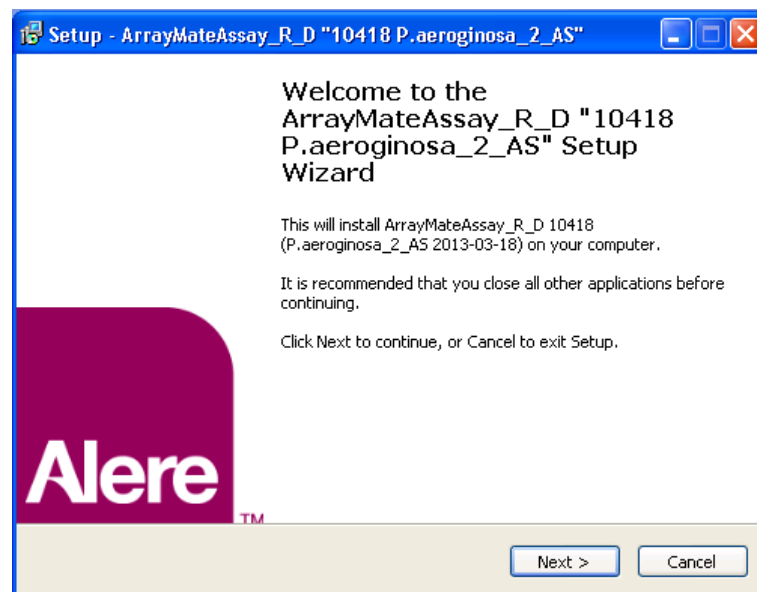
1. Download the AssayPlugin **and** the ArrayMate SDK from www.alere-technologies.com.
2. Copy downloaded files (Plugin and SDK setup) to an USB Memory Stick and connect it to the ArrayMate
3. Log on as user **admin** to the ArrayMate (default password: 12345)
4. Open the **Windows Explorer** and navigate to the downloaded **setup files**.



5. Start installation: double click on setup file of the AssayPlugin



6. The welcome screen of the setup appears

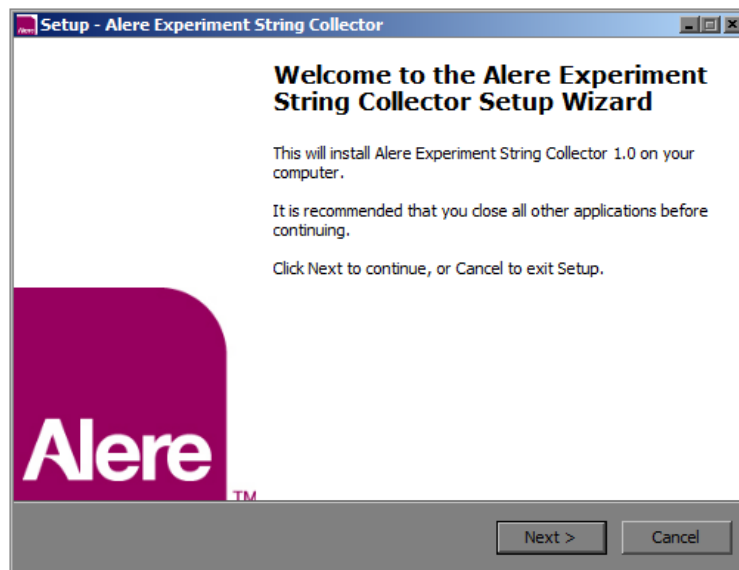


7. Follow the instructions and press **Finish** to complete the installation
8. Repeat this process for the **SDK** Setup
9. Log off and log in again as **User R&D** (password: abcde)

Experiment String Collector

The Experiment String Collector is a tool to collect the main experiment information from a set of experiments from the ArrayMate. The use of this tool is described below.

1. Download the Experiment String Collector from www.alere-technologies.com to your computer
2. Start the Setup executable (a welcome screen will appear)



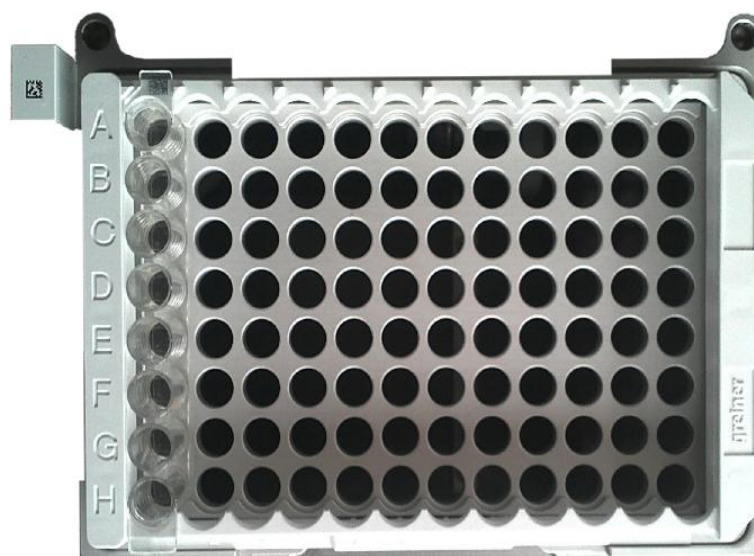
3. Follow the instructions and press **Finish** to complete. The software will start automatically after the installation.

Please Note: The Experiment String Collector is designed to work on Windows 7.

Test the AssayPlugin

The Software installation can be tested with the unprocessed Array by the following steps:

1. Log on to the ArrayMate in **User R&D Mode** (default password: abcde) and start a **New Run**
2. Choose automatic detection in **Experiment Infos** and press **Next**. Place the strip rack with the unprocessed ArrayStrip into the ArrayMate than press **Next**



3. After the Experiment Run the ArrayMate automatically enters the Archive mode and displays the results of the last experiment.
4. Each cell of the columns **image**, **raw data** and **results** must contain an "X". Otherwise, please retry the installation process of the AssayPlugin and the installation software (SDK).

expe...	sample ID	position	assay	image	raw data	results
01-A	-	1	10418	X	X	X
01-B	-	2	10418	X	X	X
01-C	-	3	10418	X	X	X
01-D	-	4	10418	X	X	X
01-E	-	5	10418	X	X	X
01-F	-	6	10418	X	X	X
01-G	-	7	10418	X	X	X
01-H	-	8	10418	X	X	X

PROTOCOL

Please note: All protocol steps described here in detail are also available as a short flow chart protocol (see Appendix 1).

Culturing and Harvesting Bacterial Cells

***P. aeruginosa* strains are potential pathogens. All procedures for cultivation of the bacterium and DNA preparation need to be performed by properly trained staff in a biosafety level 2 facility.**

Grow *P. aeruginosa* on Columbia blood agar (overnight at 37°C or 48 hrs at room temperature). Make sure that you have a pure, monoclonal culture of *P. aeruginosa*. Contamination with other bacteria, especially with other non-fermenting Gram-negative rods needs to be strictly avoided. If necessary, sub-clone, and incubate again.

Extraction of DNA

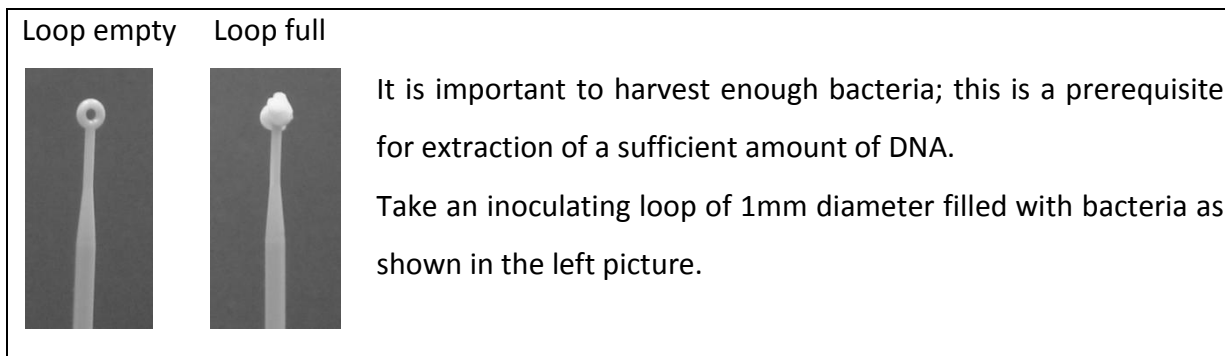
The required sample type for the *P. aeruginosa* genotyping assay is 0.5-2 µg ($C_{DNA} = 0.1-0.4 \mu\text{g}/\mu\text{l}$) of intact genomic DNA from a single clone. The DNA specimen needs to be free of RNA and it should not be fragmented. This can be determined by agarose gel electrophoresis.

DNA should not be prepared by disrupting *P. aeruginosa* cells using bead beaters, ultrasonication or aggressive chemicals such as in alkaline lysis protocols. Most performance problems with the *P. aeruginosa* genotyping assay are due to insufficient amounts or quality of DNA preparation. We therefore strongly recommend following the protocols outlined below.

The use of automated systems for DNA preparation (EZ1, Qiacube, Magnapure etc.) has not yet been systematically evaluated with this assay. While there are positive experiences with some of our other assays, we recommend testing some known strains for evaluation prior to routine use of these systems. Lysis steps and addition of RNase should be performed as described below before loading the samples in an automated system for DNA preparation.

Via Spin Columns (e.g. Qiagen DNeasy Blood&Tissue)

- Add an inoculating loop full of monoclonal colony material of the *P. aeruginosa* isolate to 0.2 ml 1xPBS and vortex thoroughly.



- Proceed with the DNA preparation protocol of the DNA preparation kit. For the Qiagen DNeasy Blood&Tissue Kit that is as follows:
- Add 20 μ L proteinase K (Qiagen Kit, or equivalent) and add 200 μ L buffer AL (Qiagen Kit)
- Vortex shortly or shake vigorously.
- Incubate for 30-60 min at 56°C and 550 rpm in the thermoshaker.
- Add 4 μ l RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature before continuing
- Add 200 μ l ethanol (96-100 %)
- Vortex the sample and centrifuge shortly.
- Transfer the complete content of the tube (including any precipitate) into a spin column that is placed in a 2 ml collection tube.
- Centrifuge at room temperature, time and speed need to be determined depending on viscosity of the sample and type of centrifuge used (e.g., 1 min at 8000 rpm). All liquid should be collected in the collection tube afterwards.
- Discard collection tube with liquids.
- Place the spin column in a new 2 ml collection tube (provided with the Qiagen kit).
- Add 500 μ l Buffer AW1.

- Centrifuge at room temperature (e.g., 1 min at 8000 rpm).
- Discard collection tube with liquids.
- Place the spin column in a new 2 ml collection tube (provided with the Qiagen kit).
- Add 500 µl Buffer AW2.
- Centrifuge at room temperature, the membrane of the spin column should be dry, and all liquid should be in the collection tube (e.g., 3 min at 14000 rpm).
- Discard collection tube with liquids.
- Place the spin column in a clean 1.5 ml tube (not provided with the Qiagen kit).
- Add 50 µl Buffer AE (or PCR grade distilled water) directly onto the membrane of the spin column.
- Incubate at room temperature for 5 min to elute DNA.
- Centrifuge (e.g., 1 min at 8000 rpm).
- Optional: add another 50 µl Buffer AE (or PCR grade distilled water) directly onto the membrane, incubate at room temperature for 1-5 min and centrifuge again.
- Discard the spin column.

Please note : Ethanol from Washing Buffers strongly inhibits the enzymes used in the assay. Such contamination might occur during elution of prepared DNA by drops adhering to the funnel of the spin columns. Thus these funnels should be gently touched and tried with sterile filter paper or wipes prior to the elution step. Alternatively, prepared DNA can shortly be heated to evaporate ethanol (e.g., 10 min at 70 °C with open lid).

- Check for DNA integrity and absence of RNA (e.g., agarose gel). If necessary, you might perform another digestion step with additional RNase A (not provided). Measure DNA concentration (A_{260} method), it shouldn't be less than 0.1 µg/µl. The concentration might be increased by heating and evaporation of water, or by using a speed vac centrifuge.

Linear Amplification and Internal Biotin Labelling

Please keep in mind the limited surplus of reagents whilst pipetting. The surplus of B1^{P2} labelling reagent is 25 %.

- Prepare a Master Mix by combining 4.9 µL of B1^{P2} labelling reagent and 0.1 µL of B2 (DNA polymerase) per sample.
- Add 5 µl of *P. aeruginosa* DNA ($C_{DNA} = 0.1-0.4 \mu\text{g}/\mu\text{l}$) prepared as described above to 5 µl of the Master Mix (B1^{P2}/B2). Do not forget to label the vial!
- Perform amplification in a pre-programmed thermocycler (e.g., Eppendorf Mastercycler gradient with heated lid, VWR, cat No. 460-0108) according to following protocol:

Pre-heat cover/lid to 105°C	
300 sec at 96°C	
50 cycles with	20 sec at 62°C
	40 sec at 72°C
	60 sec at 96°C
Cool down to 4°C, hold	

- The amplification products can be stored frozen until usage

Please note: When using another device, some adaptations might be necessary. Before starting routine use, please test the protocol with a few known reference strains.

Hybridisation

General Remarks - Handling of Arrays

- *Never touch the array surface!*
- *Avoid complete drying of the array surface during processing!*
- *Do not allow it to stay without liquid for more than two minutes!*
- *Never rinse the wells with distilled water after the hybridisation step, use only C2 Washing Buffer!*

Unused wells should be capped during the whole procedure. The strips may be processed up to three times without a loss of quality of properly capped unused arrays. Close all wells that will not be used with a cap and leave it there until you use these wells (for storage conditions after use: see section “Assay components, storage and stability/Hybridisation and Detection”).

Always label your array strips with a laboratory marker at the recommended position. Never label them on the bottom or across the data matrix barcode! This may cause an error.



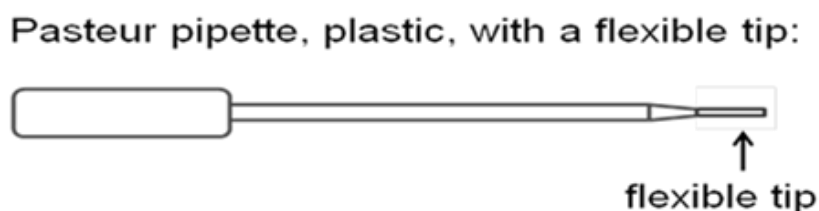
Avoid contact of data matrix barcode with organic solvents! The ArrayMate needs the information encoded in the data matrix to perform the assay and the analysis afterwards.

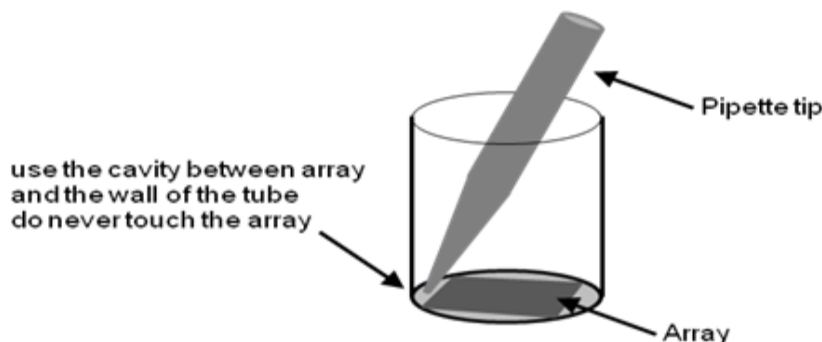
Avoid touching the bottom of the microarray strip and keep it clean.

General Remarks - Handling of Liquids

We recommend the use of a multichannel pipette and reagent reservoirs. Please keep in mind the limited surplus of C1 (100 %).

We strongly recommend that the liquid is removed by pipetting rather than by inverting the strips and flicking the liquids out. Fine tipped soft, disposable Pasteur pipettes are suited best (such as BRANDT / Cat. No. 612-2856). Always place the pipette tip at the cavity between the array and the wall of the reagent well. If you touch the array surface, probes may be scratched off and this may cause an error.





General Remarks – the Substrate (Precipitating Dye) D1

An appropriate amount of substrate (precipitating dye) should be filled into an Eppendorf tube and taken out of the refrigerator when starting the procedure allowing it to pre-warm to room temperature / 25 °C. Cold D1 may yield weak signals. D1 should shortly be centrifuged prior to use to remove bubbles as well as possible precipitates.

Triggered by peroxidase, in case of positive reactions, the dye precipitates but it is not covalently bound. The precipitate can be dissolved by vigorous shaking. Thus the arrays must not be shaken, dropped or moved abruptly during the staining procedure and afterwards.

After completion of staining, remove and discard reagent D1 as completely as possible and scan immediately. The dye precipitate fades slowly in presence of liquids.

General Remarks - Thermoshakers

The correct temperature within the vessels is essential; therefore always use the appropriate equipment for heating. Because of a possibly inhomogeneous distribution of the temperature within the heating block and because of possible differences between displayed and actual temperatures, the use of different brands of thermoshakers might affect test performance. We tested the assay with BioShake iQ by Quantifoil Instruments (<http://www.qinstruments.com/>) equipped with a customised heating block designed to fit ArrayStrips and Eppendorf's Thermomixer Comfort, equipped with a heating block for microtitre plates. When using other devices, some modifications to the protocol might be necessary. Before starting routine use, please test the protocol with a few known reference strains or with the control DNA (*P. aeruginosa* PAO1).

Protocol for Quantifoil's BioShake iQ and Eppendorf's Thermomixer Comfort with Microtitre Plate Adapter

- Switch on the thermoshaker and let it pre-heat to 60 °C.
- Remove the ArrayStrip(s) from the pouch.
- Insert the ArrayStrip(s) into the white frame. Assure the correct orientation (data matrix barcode close to row A) and proper fit.
- Pre-wash the array in two steps:
 - First, PCR-grade distilled water, 200 µl per well, just pipette 4x up and down, remove and discard.
 - Second, C1 Hybridisation Buffer, 200 µl per well, at 60 °C, 2 min and 550 rpm in the thermoshaker.
- Add 90 µL of buffer C1 to each tube with (10 µL) labelled amplification product, mix gently.
- Remove the buffer from the well with the array and add the mixture of C1 and labelled amplification product.
- Incubate at 60 °C, 60 min and 550 rpm in the thermoshaker.
- Meanwhile, prepare conjugate: for each experiment add 1 µl conjugate 100x HRP to 100 µl C4 Conjugation Buffer. This mixture is stable for around one working day at room temperature; C3 is delivered with a surplus of 100%, C4 is delivered with a surplus of 200%.

Suggested pipetting scheme:

	1 well	2-3 wells	4-6 wells	7-10 wells	11-15 wells	16-20 wells	21-30 wells	31-40 wells
C3	1.5 µl	3.5 µl	7 µl	11 µl	16 µl	21 µl	32 µl	42 µl
C4	150 µl	350 µl	700 µl	1100 µl	1600 µl	2100 µl	3200 µl	4200 µl

- Remove liquid from the well and add 200 µl C2 Washing Buffer, discard C2.
- Repeat this step twice.

- The thermoshaker needs some minutes to cool down from 60 °C to 30 °C. To speed up use the cooled cover (for Quantifoil's BioShake iQ, can be ordered from Quantifoil) or an ice pack (Eppendorf's Thermomixer Comfort).
- After removal of C2 Washing Buffer add 100 µl diluted conjugate to each well, incubate at 30 °C, 10 min and 550 rpm in the thermoshaker.
- Remove liquid and wash with 200 µl C5 Washing Buffer, pipette 4x up and down, remove and discard.
- Repeat this step.
- Add 100 µl of D1 substrate (precipitating dye, at 25 °C, see above) per well.
- Incubate at 30 °C, 5 min in the thermoshaker, *but do not shake!*
- Remove liquid completely.
- The bottom of the ArrayStrips may be cautiously be cleaned with wipes, bubbles may be removed by removing and adding D1.
- Scan and process (see below).

Data Analysis

Starting the ArrayMate Reader

We recommend starting the ArrayMate Reader after having started the hybridisation; this allows you to conveniently start the device and to import the worklist file (see below)

Please note that this is a short instruction only. For more detailed information please refer to the ArrayMate User Manual.

- Switch on the ArrayMate (main switch on the rear below the electric cable plug, operating switch on the bottom/left corner of the front side).
- Switch on the screen (switch right hand below the screen).

- Log on as **R&D User** (Research and Development User) for full access to test specific software (a default password will be provided together with the ArrayMate device). If you log on as **User**, you will obtain only raw values, but no interpretation as positives/negatives and no strain assignment. **Administrator** log-in will allow manipulation of file folders and software; and this should be done only upon direct advice of Alere's IT team.
- The user interface will be loaded, ArrayMate performs internal testing. This will require less than a minute.
- Click on the icon **New Run** (left upper edge of the screen). A suggestion for a run name / folder name for the new run appears in the top line of the screen. You may modify or change the experiment name at your convenience.
- Type in your operator ID (**optional**).
- You may enter a comment into the **memo** field (**optional**).

Worklist

A **Worklist** file allows linking an identifier such as a laboratory/sample number to a position of an array within the ArrayStrip. For privacy reasons, arrays should not be identified by patient names. Worklists can be generated using spreadsheet software such as EXCEL (see below) but must be saved in the *.txt file format that can be imported into the test specific ArrayMate software. Do not use special characters (such as : ; ()[] / \ ä ü etc.).

- Create a list with at least three columns with obligatory headers in the order: position / sample ID / assay ID (Table 1).
- Positions are continuously numbered from 1 to a maximum of 96. Position 1 would correspond to A1, 8 to H1, 9 to A2 and 96 to H12 (Table 2). Do not leave empty lines in the worklist. If you use EXCEL, position numbers should be typed into column A.
- Sample ID is strain / sample / laboratory number such as exported from your LIMS (or assigned in any different way). Patient name should not be used as Sample ID.
- The Assay ID enables the system to identify the current test and to correctly use information on layout, spot number, and identity etc.. *P. aeruginosa* Genotyping 2 Assay has the Assay ID: 10418. *Assay ID numbers must not be confused as this could lead to errors or loss of data.*
- You may add further columns and headers with notes and comments at your convenience. Information from these columns will not appear on the result screen or in the Test Report.
- We recommend using a printout of the worklist as template for pipetting.
- Save the worklist as tab separated *.txt file on the memory stick provided together with the ArrayMate.
- To avoid confusion, make sure that worklists are named unambiguously or that worklists from earlier experiments are deleted.



Table 1: Example worklist:

Position	sampleID	assayID	comment
1	2013-12345	10418	
2	2013-12346	10418	
3	2013-12347	10418	
4	2013-12348	10418	
5	2013-12349	10418	
6	2013-12350	10418	
7	987654	10418	<i>Isolate referred from Dr. J. Doe.</i>
8	<i>P. aeruginosa</i> PAO1	10418	<i>Control strain</i>

Table 2: Positions in the 96 well format

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
H	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

Data Acquisition in the ArrayMate Reader

- Insert your memory stick containing the worklist into any of the USB ports down to the right side of the ArrayMate.
- Press the button ; a folder selection dialog will open.
- Select your worklist (path: “My Computer/Removable Disk”).
- Open your selected worklist with **Enter** or the button **Open**.
- Press the button  (your imported worklist opens in a separate window). Proofread. If the new window is empty, or if it was the wrong worklist, repeat the import.
- Press the button **OK**; the worklist window will close.

- Leave the memory stick attached to the ArrayMate if you intend to export Test Reports afterwards.
- Press the button **Next** (bottom / right on the screen; reader opens).
- Carefully insert the appropriate metallic adapter / frame into the ArrayMate. Do not apply any strong force. Assure proper fit, otherwise the images may be out of focus.
- Carefully insert the white frame with the array strips into the metallic adapter. Assure the correct orientation (Position A1 in the frame next to the data matrix barcode on the adapter) and proper fit, otherwise the images may be out of focus.



ArrayStrip frame with Strips
inserted in accordance with the
worklist.

Please note: ArrayStrips must be clean. They should not contain any liquids during analysis. Barcodes must be clean. There must be no ArrayStrip Covers on the wells to be analysed (however, unused wells should remain covered).

- Press the button **Next** (bottom / right on the screen; reader closes, analysis program starts, reading takes 2-10 min dependent on the number of strips; reader takes images, and automatically analyses the data). The progress of the reading is indicated by the following symbols:

photographed:



in analysis:



ready:



- The reader indicates the end of the entire process with an acoustic signal (beep).
- Press the button **Next** (bottom / right on the screen; reader opens).
- Remove the white frame with the ArrayStrip(s).
- Press the button **Next** (bottom / right on the screen; reader closes).

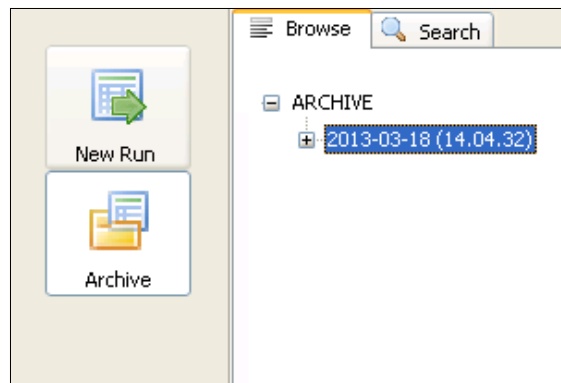
Results

On the left hand side of the screen you will see a list showing all runs stored on the ArrayMate's hard disk. A run contains the results from all arrays analysed together within one frame. If this list is not displayed:

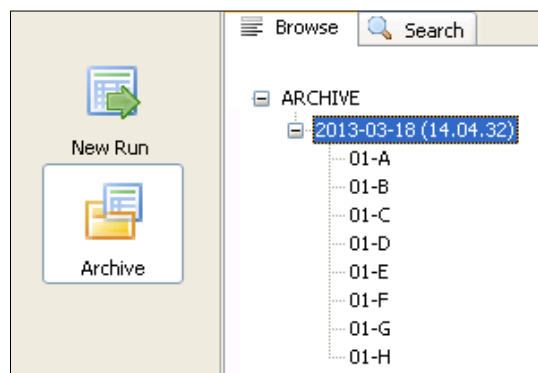
- Press the button **Archive** (left hand) and activate the flag **Browse** (top left).

The runs are organised like folders in "Windows Explorer", and named by default according to the date of acquisition.

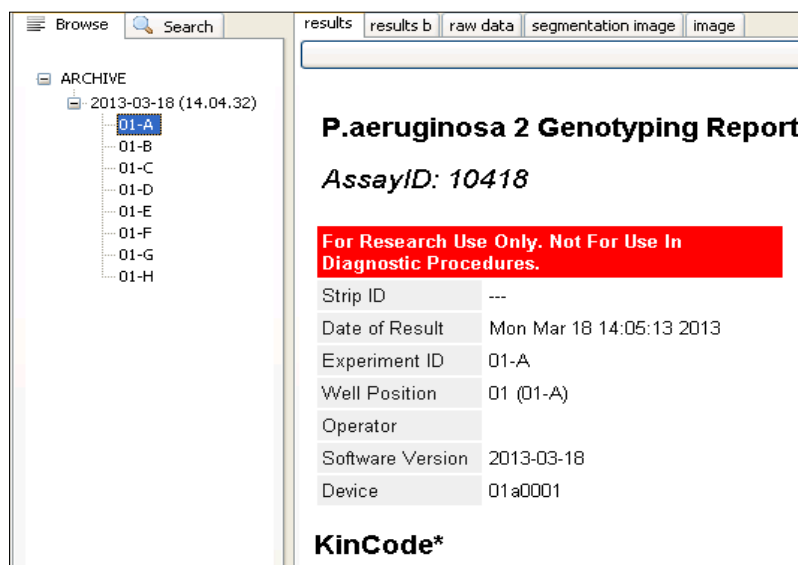
Example: There is one experiment run in this archive:



If you click on the plus symbol left on the run name, the folder opens and you will see a list of the individual arrays ordered by Sample ID.



Click on a Sample ID and the *P.aeruginosa* 2 Genotyping Report for this array is shown in the window on the right:



P.aeruginosa 2 Genotyping Report
AssayID: 10418

For Research Use Only. Not For Use In Diagnostic Procedures.

Strip ID	---
Date of Result	Mon Mar 18 14:05:13 2013
Experiment ID	01-A
Well Position	01 (01-A)
Operator	
Software Version	2013-03-18
Device	01a0001

KinCode*

Export of Test Reports

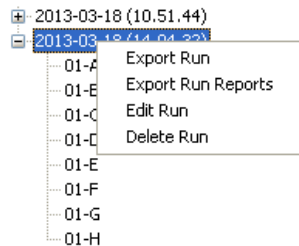
Two result files in html-format will be generated. The shorter report gives a summary on genotyping information. A longer html result sheet (“result_B.res.html”) provides information on all probes. Possible error messages in these reports are explained below (see Troubleshooting).

Other files that are generated and can be exported include

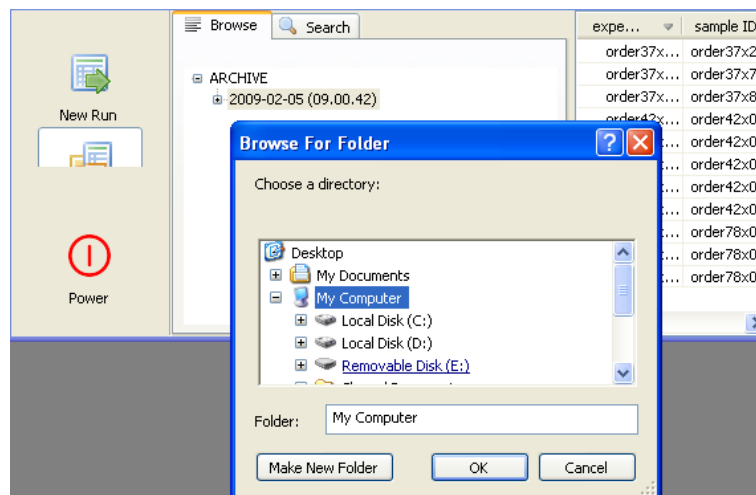
- A *.csv file containing the experimental result as a string which can be imported into an Excel-based list (for data mining; see chapter 3 below),
- A *.txt file with the raw measurements,
- An image file (*.bmp) showing the actual photo of the array,
- A second image file (*.png) in which the coordinate grid is superimposed and the recognised spots are circled, and
- A *.xml file providing the same information as the html result sheet for future export into databases etc.,
- A *.log file containing specific data of the evaluation of spots and substances,
- An *.out file containing output log data which help our service to trace image evaluation errors.


Please note: Only complete runs can be exported. The export of individual Test Reports is not possible.

- Right-click on the selected run (a menu appears with the option **Export Run**).



- Right-click on **Export Run** (a file browser opens).



- Click on **My Computer**, subsequently on **Removable Disk**, and choose the folder to save or click on the button **Make New Folder** (on the bottom; a new folder icon appears).
- Rename the new folder (e.g. with the experiment run name or date).
- Click on the **OK** button (data are exported into the new folder on your memory stick).
- Do NOT remove the memory stick as long as the hourglass symbol is visible.
- Switch off the device by clicking on the **Power**-button (left / down on the screen): 
- Switch off the Screen. There is no need to physically switch off the ArrayMate.

Contents of the Test Report

The ArrayMate creates a test report that lists all markers analysed along with an individual test result for each marker. In addition, the test report provides a rating of the data quality along with identification of a possibly erroneous step of the experimental process.

The html file (*.html) may be viewed with any browser software (e.g. Internet Explorer, Firefox etc.) and may be printed or converted into a *.pdf file (for details please refer to the ArrayMate's user manual). The contents of the test report are described in more detail in the following section.

Kinship Analysis: Markers of the Core Genome ("KinCode" / "SNP Pattern")

Sixteen different markers of the core genome were selected for genetic identification of *P. aeruginosa* isolates. In the test report, these markers are named "SNP Pattern" and are formatted as a string of 16 digits. Each digit may adopt the value "0", "1", "2", "X", respectively. Strings containing "0" and "1" digits are used for deriving the four-digit alphanumeric "KinCode" which contains all information of the 16 digit code, but is easier to comprehend. However, if the test result of some digit is unclear, it is rated "2" in the 16 digit string. If the test result of a marker is invalid, it is rated "X" in the 16 digit string, and then the four-digit KinCode remains incomplete. In these cases, the respective alphanumeric digit of the KinCode is replaced by "X". For each complete KinCode, background information regarding the frequency of the respective code is given, based on a data base established by L. Wiehlmann (unpublished).

SNP pattern and KinCode were described by Wiehlmann et al. 2007 (see literature).

Marker Controls

These controls are important for the rating of data quality. Furthermore, in case of handling mistakes they provide important information regarding the experimental step that may have failed.

- If the Staining Control failed (and along with it all other controls) this indicates failure of the Horseradish Peroxidase-mediated conversion of D1, that means a failure of one of the steps after hybridisation.
- If all controls (including the Hybridisation Control) failed, but **not** the Staining Control, this indicates a failure of the Hybridisation step.
- If the Labelling, Genus/Species and DNA Stringency Control failed, but not the Hybridisation Control and the Staining Control, this indicates a failure of PCR.
- If only the Genus/Species and DNA Stringency Control failed, this indicates poor quality of DNA.
- Failure of Negative Controls indicates a high nonspecific signal background.

Please refer to the troubleshooting section below for further information.

Plausibility Controls

Some combinations of markers in nature are impossible to occur for biological reasons. The software checks for those combinations and returns a warning if they are diagnosed. In these cases the test result is implausible. Please refer to the troubleshooting section below for further information.

Accessory Genome and Microevolution (“Features of the Isolate”)

In addition to the markers that characterise a clone, additional markers of the accessory genome were added to the array. Originally (Wiehlmann et al. 2007), 41 markers of the accessory genome had been selected, but meanwhile two of them (TB-C47-1 and TB-C47-2) have been removed because of faulty results.

The remaining 39 markers are listed in the test report as “Features of the Isolate” and they may be exported along with the KinCode as a 39 digit code in the “0”, “1”, “2” or “X” format as described above. This information may be used to characterise microevolutionary events within a clone that is characterised by a uniform core genome pattern. The markers of the accessory genome have been described in detail by Wiehlmann et al. 2007 (see literature).

Recommendation for a Project Specific Database

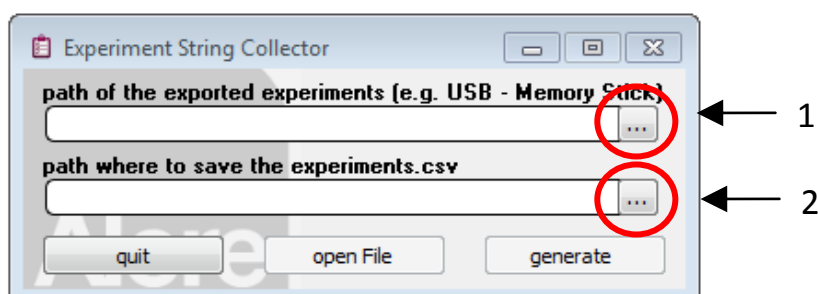
The CSV file (*.csv), which contains the experimental result as a string. For comparison of the results and for data mining each of these strings may be imported into an “Excel Based Data Mining Tool” (see 3.1) as suggested by Wiehlmann et al. (2007). Alere Technologies provides a drawing of this Excel sheet that has been developed from the sheet suggested by Wiehlmann et al. Further, Alere Technologies provides a tool (the “Experiment String Collector” see 3.2) that enables the user to collect the strings from all *.csv files within one folder in one single step. Both, the Excel datasheet and the string collector, are described in the following section.

String Collector

The tool called “Experiment String Collector” enables the user to collect all data (strings) from *.csv files found within one folder into one output *.csv file (CommaSeparatedValue files being generic files for export into spreadsheet programs).

As outlined above and in the ArrayMate manual, the results of each experimental run can be exported from the ArrayMate to an USB stick into a folder. In order to collect all data strings found within this folder into a single *.csv file, proceed as follows:

- Download the string collector from our website www.alere-technologies.com and install it on your office computer according to the instructions you will find in the Software and Reader section of this manual.
- Open the string collector by double clicking its icon on your desktop; the main window appears:



- Select the path of the source folder (the folder of the ArrayMate Data export) by clicking

into area **1** (indicated by the first red circle on the image above)

- Select the path of the target folder (the folder to save the *.csv file containing the collected data strings) by clicking into area **2** (indicated by the second red circle on the image above)
- Single click the **generate** button; all *.csv files in the source folder will be scanned, and the content will be collected within one *.csv file named Experiments_YYYY_MM_DD-HH_MM_SS.csv
- Click on the button **open File**; the Experiments.csv file created opens in the notepad text editor
- The content of the new *.csv file may now be transferred into an Excel-based Data Mining Tool by copy / paste.

Excel-Based Data Mining Tool

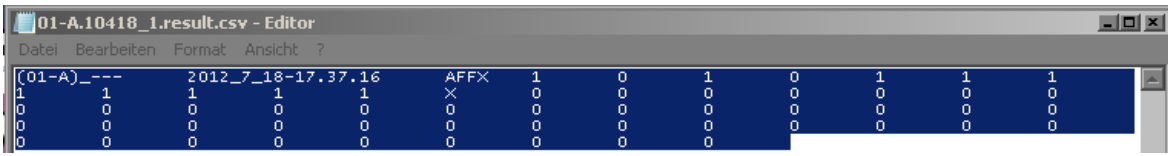
An Excel-Based Data Mining tool was developed together with the group of Lutz Wiehlmann. The template for a user specific data sheet can be downloaded from our website: www.alere-technologies.com.

The Excel sheet draft is separated into two areas:

- The first area (columns A to M) is meant for entering data like source of the specimen manually.
- The second area (columns N to BS) is meant for pasting experiment results provided by the ArrayMate in the *.csv files in a copy / paste manner. These data consist of the Identifier of the result, the date of test, the KinCode and the strings for SNP pattern and Features of the Isolate as a 16 + 39 digit string that may be used for Kinship Analysis.

In order to copy the data from an individual *.csv file into the data mining list proceed as follows:

- Open the *.csv-file of the experiment with any editor program (Editor, Notepad, etc.), select and copy “all”



- paste the string into the data mining list in the “Pseudomonas test results” table, column N “Identifier of result”

N	D	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN	BO	BP	BQ	BR	BS									
Area for pasting test results																																																																		
Identifier of result	Date of test	Heat Code																																																																
			oriC; PAOI non-PAO	opdL; PAOI non-PAO	alkB2; PAOI non-PAO	catS-1; PAOI non-PAO	catS-2; PAOI non-PAO	opdI; PAOI non-PAO	ampC-1; PAOI non-PAO	ampC-3; PAOI non-PAO	ampC-4; PAOI non-PAO	ampC-5; PAOI non-PAO	ampC-6; PAOI non-PAO	ampC-7; PAOI non-PAO	hliCaffiCb	hliCa-SMP; PAOI non-PAO	eneS	ipwA type I	ipwA type IIa	ipwA type IIb	ipwA type III	ipwB	LES	PAO636	PAO722	PAO728	PAO185	PAO221	PAO395	hls-island	orfA	orf1	orfJ	orfM	RF-1753	acetyltransferase	pkl-1	pkl-3	PAP1-1 pH chaperone	PAP1-1 luminal binding pr	pKLC conserved hypothe	pKLC adhesin	pKLC fatty acid synthase	PAGH-213-4	PAGH-213-5	PAGH-213-6	C-45	C-46	C-47	PAGH-2	PAGH-213-1	PAGH-3-1	PAGH-3-4	idMidProp- island 1	idMidProp- island 2	PAGH-1										
01-A12692509104	2011_12_7-15.52.4	0100	1	0	0	0	1	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
01-G12692509104	2011_12_7-16.3.5	0900	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

- Enter the appropriate additional data into columns A to M manually

In case it is intended to enter the result of more than one array into the database we recommend the use of the String Collector (see below).

TROUBLESHOOTING

For training and qualification purposes we recommend our Control-L Kit (Alere order number 205500010). In case of doubt, this kit is the perfect tool to make sure that your laboratory processes are working correctly. It may also be used for the functional testing of individual kit components.

Poor DNA Quality: Only the DNA Extraction Control failed. In these cases we recommend to re-check DNA quantity and quality by loading leftover DNA on an agarose gel.

- The amount of DNA is crucial. A260 readings will cover RNA and other contaminants as well. Therefore pure DNA preparation without bulk amounts of RNA is prerequisite for proper DNA concentration measurement. RNase treatment prior to A260 reading therefore is necessary (component A2 contains RNase).

- Some *P. aeruginosa* colonies protect themselves with thick layers of slime. It may help to wash the bacterial pellet 3x in 1 ml of 5 mM EDTA, pH 7.0 (re-suspend and centrifuge at 3,000 × g for 6 min at room temperature, discard the supernatant).
- DNA must not be fragmented, as fragmentation reduces the efficiency of amplification and labelling due to the distance between primer and probe binding sites.
- DNA should be free of RNA, as free RNA reduces the efficiency of amplification and labelling by effectively removing primer from the reaction mix due to competitive hybridisation.
- DNA must be free of any traces of ethanol, as ethanol strongly influences the amplification. This is an important issue as, e.g., in the top of QIAGEN tubes a drop of ethanol-containing washing buffer might get trapped. The tubes really need to be centrifuged on high speed. Alternatively, it is possible to heat the sample prior to adding it to the labelling mix (some 5 minutes at 70 °C). Some problems with samples from the Qiagen EZ1 device for example were resolved after heating the samples.
- All reagents for DNA extraction need to be within the recommended shelf-life and stored in the appropriate way.

We have good experience with the manual QIAGEN DNeasy Kit and the automated device EZ1. Contrarily, alkaline lysis, mechanical bead-beater extraction or ultrasonication in our hands resulted in poor DNA recovery, poor DNA quality or both.

An alternative method that is fast and simple but that did work in the hands of several customers was described by Wiehlmann et al. 2007 (see literature)

PCR failed: Labelling and DNA Extraction Controls failed, but not the Hybridisation Control and the Staining Control. In this case, please carefully review the PCR process for correctness. In some cases, inhibitors of PCR are isolated along with DNA. In these cases a rerun of DNA purification may help.

Hybridisation failed: All controls (including the Hybridisation Control) failed, but not the Staining Control. In this case please carefully review the hybridisation step. Was the buffer (C1) used correct? Was the hybridisation temperature used correctly?

Staining failed: All controls failed. This indicates a failure of one of the steps after hybridisation. The most likely reasons are:

- The component D1 was too cold when administered. Please carefully follow the steps that are outlined in section 8.
- Horseradish Peroxidase Conjugate (C3) or its substrate D1 may have degraded during storage. If this may be an option, we recommend testing C3 and D1 with our Control-L kit.
- Enzymatic reaction is inhibited by carryover of buffer C1. Ensure proper washing of the wells to remove all of Buffer C1 prior to adding Horseradish Peroxidase Conjugate (C3/C4).
- Horseradish Peroxidase may have degraded because of an incubation temperature $\gg 30^{\circ}\text{C}$. Therefore, please review the incubation step with C3/C4 for correctness. Make sure that the shaker indeed has cooled down to 30°C before incubation.

Negative control failed. In this case a high nonspecific background may result in false-positive markers. In this case please carefully check the hybridisation step and the subsequent washing steps for correctness. Did you use the correct buffers? Did you incubate at the correct temperature? Is your thermoshaker working correctly i.e. at the temperature it is supposed to work? Alternatively, these options may be checked:

- The C3/C4 may not have been washed away correctly. In this case, the D1 turns dark green whilst incubating and this may result in nonspecific background.
- The array may have dried out during hybridisation if the cap had not been closed properly.

Plausibility controls failed. This may either indicate a failure of the test protocol; in this case the additional failure of other controls (see above) is expected. In any case we recommend to review the conditions of hybridisation (especially the **real** hybridisation temperature) and the washing steps after hybridisation.

Alternatively, failure of the plausibility controls may indicate the mixture of different *P. aeruginosa* clones; in this case we recommend subcloning and repeat of the experiment.

Physical damage to the array. Scratching of the array surface with a pipette tip can lead to damage of an array spot that prohibits the acquisition of a valid signal. In this case the respective spot is set invalid (=“1”), the corresponding spot may be positive and valid (=“0”). We recommend reviewing section 4 for general precautions in array handling.

LITERATURE

- Wiehlmann et al. 2004, JOURNAL OF BACTERIOLOGY Vol. 186, No. 13, p. 4228–4237.
- Wiehlmann et al. 2007, PNAS vol. 104 no. 19, p. 8101–8106.

ADDITIONAL INFORMATION

Warranty

Alere guarantees the performance as described in this manual. Usage of the Kit was successfully tested at ambient temperatures up to 37 °C, a guarantee is limited to ambient temperature in the laboratory between 18 °C and 28 °C. Kit components comprise the ArrayStrips, the reagents for DNA labelling and for detection of labelled DNA products on the array, the ArrayMate reader and its software. In case one of these components fails within the expiry date due to other reason than misuse, contact Alere for replacement or refund. Terms and conditions apply.

If you have any problem or question, please contact our technical service.

Quality Control

Each batch is stringently tested with the use of standard DNA preparations for good performance and correctness of results.

List of Components for Separate Order

If required, these reagents for the *P. aeruginosa* Genotyping Kit 2 may be ordered separately:

component	name	category	amount	order no	storage
B1 ^{P2}	Labelling buffer	Buffered reagents	700 µl	245103002	2-8 °C
B2	Labelling Enzyme	Buffered enzyme	20 µl	245104000	2-8 °C
C1	Hybridisation buffer	Buffered reagents	30 ml	245105000	18-28 °C
C2	Washing buffer 1	Buffer	120 ml	245106000	18-28 °C
C3	HRP Conjugate 100x	Buffered enzyme	200 µl	245107000	2-8 °C
C4	Conjugate buffer	Buffered reagents	30 ml	245108000	18-28 °C
C5	Washing buffer 2	Buffer	120 ml	245109000	18-28 °C
D1	HRP Substrate	Buffered reagents	15 ml	245110000	2-8 °C
ArrayStrips	AS <i>P.aeruginosa-4</i>	microarrays	12 ST	240009724	15-28 °C
ArrayStrip Cover	ArrayStrip Cover	plasticware	24 ST	245112000	-

For pricing, please contact your local representative or our customer service, respectively.

www.clondiag.com

www.alere.com



LEGAL MANUFACTURER

Alere Technologies GmbH

Loebstedter Str. 103-105

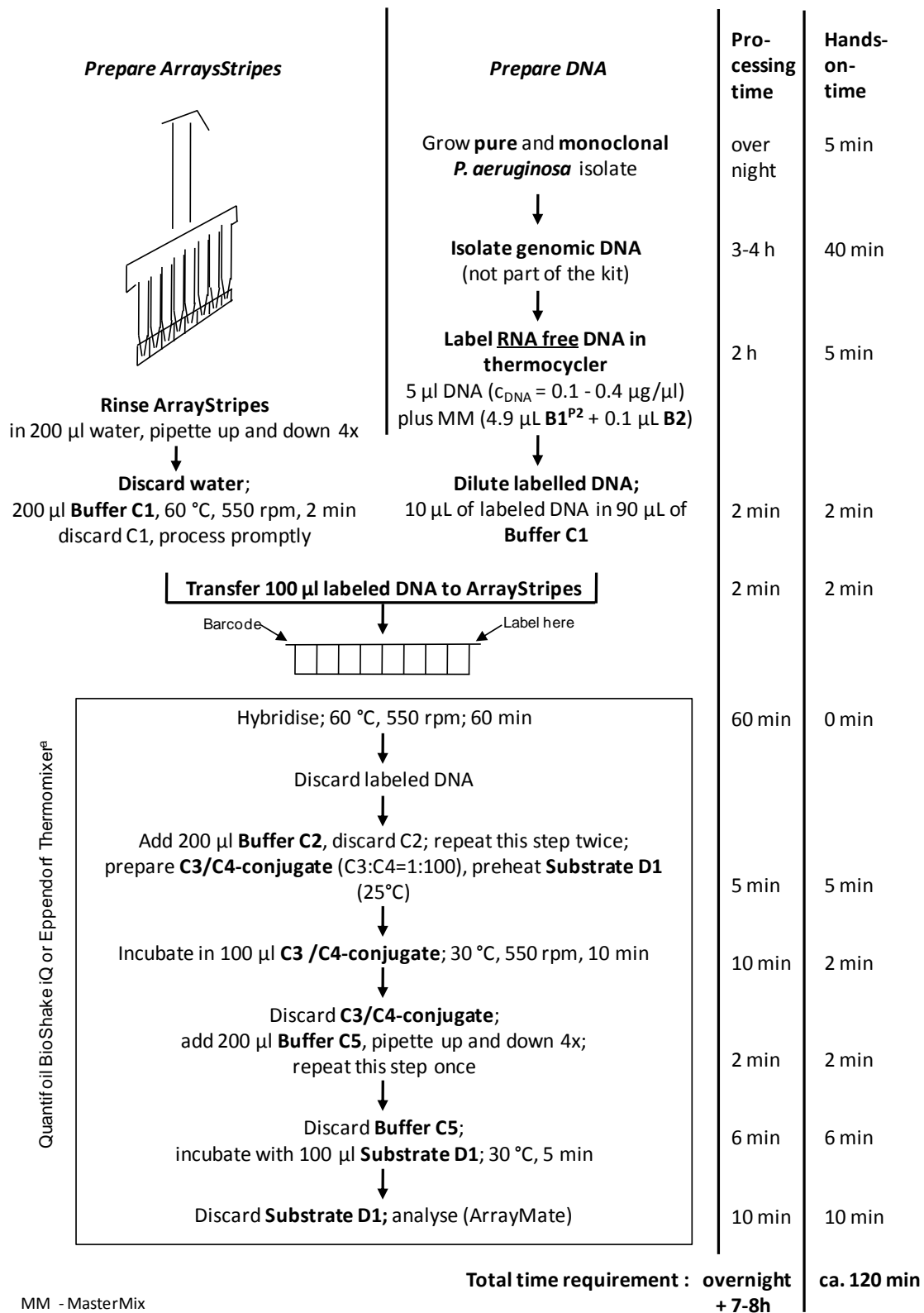
07749 Jena, Germany

CONTACT

info@identibac.com

cct.home@clondiag.com

APPENDIX 1 – Flow Chart



MM - MasterMix

^{a)} with heating block for microtitre plates

APPENDIX 2 – Probes and Primers for KinCode Genotyping and the Accessory Genome

Probes	Reference	Probes	Antisense primer 1	Antisense primer 2
oriC PAO	PAO1- Sequence, Stover et al. (updated 2006)	GAAGCCCAGCAATTGCGTGT TTC	AGCCTCGACACCGGTT CTCG	ACCATCTCGTTTCATCC CCAGG
oriC non-PAO	UCBPP-PA14, complete genome, Lee et al. (2006)	GAAGCCCAGCAACTGCGTGT TTC		
oprL (1) PAO	PAO1- Sequence, Stover et al. (updated 2006)	GGTGTGCAGGGTGTTCGCG CGG	TTCTGAGCCCAGGACT GCTCG	TGCAGCGCAGGTTCT GAGCC
oprL (1) non-PAO	UCBPP-PA14, complete genome, Lee et al. (2006)	GGTGTGCAGGGCGTTTCGCG CGG		
oprL (2) PAO	PAO1- Sequence, Stover et al. (updated 2006)	GTGCTGCAGGGTGTTCGCG G		
oprL (2) non-PAO	UCBPP-PA14, complete genome, Lee et al. (2006)	GCTGCAGGGCGTTTCGCGCG		
fliCa (1) PAK	PAK, Totten and Lory (1990), flagellin type a2, Giske et al. (2006)	CAAGATCGCCGACGCGGTCA AC	AGCTGATGGTATCGCC GTCCG	CTAGTGATCGCACCGG AGCC
fliCa (1) non-PAK	ATCC15691, Spangenberg et al. (1998), flagellin type a1, Giske et al. (2006)	CAAGATCGCCGCTGCGGTCA AC		
fliCa (2) PAK	PAK, Totten and Lory (1990), flagellin type a2, Giske et al. (2006)	CAAGATCGCCGACGCGGTCA ACGAC		
fliCa (2) non-PAK	ATCC15691, Spangenberg et al. (1998), flagellin type a1, Giske et al. (2006)	CAAGATCGCCGCTGCGGTCA ACGAC		
alkB2 PAO	PAO1- Sequence, Stover et al. (updated 2006)	CCTCGCCCTGTTCACCCCG TCTGG	TTCTCGCCGGCATAG TAGGC	
alkB2 non-PAO	ATCC 15691, Morales et al. (2004)	CTCGCCCTGTTCACCCCGCT CTGG		
citS-1 PAO	PAO1- Sequence, Stover et al. (updated 2006)	TCGAGCAACTGGCAGAGAAA TCCG	GCAGGTAGCAGGTTTC CAGG	AACTGTTCCTTCTGCG CGCGG
citS-1 non-PAO	UCBPP-PA14, complete genome, Lee et al. (2006)	CGAGCAACTGGCGGAGAAAT CCG		
citS-2 PAO	PAO1- Sequence, Stover et al. (updated 2006)	GCGGAAAATTCCTGCACAT GATGTT	TGATCGGCTTGGTCTC GCAGG	GCTGATCGGCTTGGTC TCGC
citS-2 non-PAO	Kiewitz and Tummler. J Bacteriol. (2000) 182:3125-35.	GCGGAAAATTCCTCCACAT GATGTT		
oprI (1) PAO	PAO1- Sequence, Stover et al. (updated 2006)	AGCTCAGCAGACTGCTGACG AGG	GCTGGCTTTTCCAGC ATGCG	TTGCGGCTGGCTTTTT CCAGC
oprI (1) non-PAO	UCBPP-PA14, complete genome, Lee et al. (2006)	AGCTCAGCAGACCCTGACG AG		
oprI (2) PAO	PAO1- Sequence, Stover et al. (updated 2006)	GCTCAGCAGACTGCTGACGA GGCTAACG		
oprI (2) non-PAO	UCBPP-PA14, complete genome, Lee et al. (2006)	GCTCAGCAGACCCTGACGA GGCTAAC		
ampC-1 PAO	PAO1- Sequence, Stover et al. (updated 2006)	ACGGCCGCGGGTGACGCC	CGCATCTTGTCTGGG TCAGG	TCGTGAGGCGCATCT TGTCC
ampC-1 non-PAO	De Champs et al. (2002), Kiewitz and Tummler. J Bacteriol. (2000) 182:3125-35.	ACGGCCGCGAGGTGACGCC G		
ampC-3 PAO	PAO1- Sequence, Stover et al. (updated 2006)	CGACCTACGCGCCGGGCGAG	GGCGAGATAGCCGAA CAGGC	CACTTGCTGCTCCATG AGCC
ampC-3 non-PAO	De Champs et al. (2002), Kiewitz and Tummler. J Bacteriol. (2000) 182:3125-35.	CGACCTATGCGCCGGGCGAG C		
ampC-4 PAO	PAO1- Sequence, Stover et al. (updated 2006)	CGTTCGAACGGCTCATGGAG CAG	ACGTGAGGTTGGTCT GTTCCG	GTAGCCTTCGGCATCC AGCG
ampC-4 non-PAO	De Champs et al. (2002), Kiewitz and Tummler. J Bacteriol. (2000) 182:3125-35.	CGTTCGAACGACTCATGGAG CAGC		
ampC-5 PAO	PAO1- Sequence, Stover et al. (updated 2006)	TGGAGCAGCAAGTGTTCGCC GC	amplified with primers of ampC-4	
ampC-5 non-PAO	De Champs et al. (2002), Kiewitz and Tummler. J Bacteriol. (2000) 182:3125-35.	TGGAGCAGCAACTGTTCGCC GC		
ampC-6 PAO	PAO1- Sequence, Stover et al. (updated 2006)	GAACAAGACCGGTTCCACCA ACGG	TCGGCATTGGGATAGT TGCGG	
ampC-6 non-PAO	UCBPP-PA14, complete genome, Lee et al. (2006)	AACAAGACCGGCTCCACCA CGG		
ampC-7 PAO	PAO1- Sequence, Stover et al. (updated 2006)	CGACCTGGGCCTGGTGATCC T	TTGGGATAGTTGCGGT TGCC	TGGCGTAGGCGATCTT CACCC
ampC-7 non-PAO	De Champs et al. (2002), Kiewitz and Tummler. J Bacteriol. (2000) 182:3125-35.	GCGACCTGGGACTGGTGATC CTGG		
fliC a	ATCC15691, Spangenberg et al. (1998)	GTCGCTGAACGGCACTACT TCA	CGATCGGATGTCGAC GGTGC	TGCCGATCGGATGTC GACG
fliC b	PAO1- Sequence, Stover et al. (updated 2006)	GCCGACCAACTGAACTCCAA CTCG	TGACGTTCTCGCCGGT AGCG	CAGTAGCGGTACCGGT CTGC
exoS	PAO1- Sequence, Stover et al. (updated 2006)	CAGCCAGTCAGGACGCGC A	CAGGGTCGCCAGCTC GCTCGCC	AGGGTCGCCAGCTCG CTCGC
exoU	UCBPP-PA14, complete genome, Lee et al. (2006)	CGCCAGTTTGAAGACGGAGT CACC	AGTGATCTGCCGCGG CCCTGCC	GTGATCTGCCGCGGCC CTGC
fpvA type I	PAO1- Sequence, Stover et al. (updated 2006)	CCTGAATCCGACCATTCGCG AGTC	CGTTCAGGTCGTAGAC CGCGC	GCGATACCAACTGTCC TGCGGC
fpvA type IIa	de Chial et al. (2003)	TCGGAGTACTCTACGAA GCAGC	TGCCGAAGGTGAATGG CTTGCC	CCTGATGGTCCGATCC CAGC

Probes	Reference	Probes	Antisense primer 1	Antisense primer 2
fpvA type IIb	Spencer et al. (2003)	CCAATCCCTATCGCTGGAACCGTACC	GCCGAGGGTCAAGAACCACTGG	TCTTGGCCAGTCATA CGCGC
fpvA type III	de Chial et al. (2003)	GCTCGGGACTCGCATTTCTGTC	TAACCCCAAGGCCCAT TGGAGG	GCCACCGCTTCGAAT AACCCC
fpvB	PAO1- Sequence, Stover et al. (updated 2006)	GCCTTATTGCTCGGTCTCTCCTCG	AATTGCTCGAGGGATG CGGC	GGTCGAAACGGATCGC CAGG
LES	LES400 (personal communication C. Winstanley)	TGCATAGGAGTCATGCCGACAGCA	GCCCCGCGTCATTTTC ACGTCC	AATGCTCTGGGCAACG AGCC
PA0636	PAO1- Sequence, Stover et al. (updated 2006)	GCCAAATGGGTACGAAAGCAACG	ATGCCATCGTTGAAGG CACCCG	TGCCATCGTTGAAGGC ACCG
PA0722	PAO1- Sequence, Stover et al. (updated 2006)	CGTGTGCGGAACCTCGCATGGC	TCTGGCGGAATCAGGT AGGCC	CTTCCGGGAGAAACC ACCG
PA0728	PAO1- Sequence, Stover et al. (updated 2006)	CTGGAGCCTCGAAAAGTGGCTC	AGCCAAGACGGTTGTT CGCGG	TCAATGACGCCGAGTT GCGCG
PA2185	PAO1- Sequence, Stover et al. (updated 2006)	ACGAGGGTGATGGCTGGGAATACG	CTCGGACAGGTTCAAG CTGG	GCCATTGCTGCAACA CTTCC
PA2221	PAO1- Sequence, Stover et al. (updated 2006)	CAGTTGTCGCCAGGTCTGGA GAATCC	TTCTTGGCCAGAGTT GGACC	AGCTTAAGGCCGTGGC ACTCG
PA3835	PAO1- Sequence, Stover et al. (updated 2006)	CACATCAATGTCAGCCCACG CCA	CCGGAGAATTCGCGTC CACC	TGCTGACGATGAAGCC CCAGC
fla-island	Arora et al. (2001)	ACCTGTGTCTCGTGGAGGGTATGTT	CCCCGTGTTCCGTAGA CTTGTC	
orfA	Arora et al. (2001)	CGCTGGAGGGTATGTTCCCGAAGG	GTTCCACAGGCGCTGC GGCGC	GTTCCACAGGCGCTGC GGCGC
orfI	Arora et al. (2001)	CCTGGACCTCTCCAAGGTTCCGCT	AAACTGCCCGGCCCCCATCC	GGAAAACTGCCCGC CCCCC
orfJ	Arora et al. (2001)	GCCATTCGACGACCAACAAGGC	ACGCTCGCAGCGCCT CACGCG	GGCCTGGCTGCGAAC GCTCGC
PA0980	PAO1- Sequence, Stover et al. (updated 2006)	CGGTATGAAGATGGGTGGTTGGTTCG	ACCTCCAGCACCAGACA CACC	ATCCGATCCACCTCCA CACC
XF1753	UCBPP-PA14, complete genome, Lee et al. (2006)	TGCGAGGACCAGAAACCTTGATGG	GCGCGCGTTGAGAA ACAGG	CGGAGTTGAAAAGCT GGCCC
acetyltransferase	UCBPP-PA14, complete genome, Lee et al. (2006)	CGAAGCGTAGGGTCTTCGTAGCC	ACGACGTCACCGTCGA GACCG	ACCGCTTTCTGGTGA GCTGG
pKL-1	Klockgether et al. (2004)	CACCATGCAAAATGCTCGATGACTGC	ATCTGAACCCAGGGGA TCCGC	CCCCGGAGTCATTGGT CTGG
pKL-3	Klockgether et al. (2004)	TCTGAAGTCCGGCTATCACCTGGA	GACCTACACTCCAACC GCTGG	TTCCCTTGTGCGCGAG AAGC
TB-C47-1	P.aeruginosa TB, pKLC102 related gene island integrated in tRNA(Lys) PA4541.1	GCAGGCGTCCAAGTTGGAGCTCTCC	GCCTGTTGACCCCTT TGACC	TACTCCTGCCTGTTGG ACCCC
TB-C47-2	P.aeruginosa TB, pKLC102 related gene island integrated in tRNA(Lys) PA4541.1	TCCAACAGGCAGGAGTACAGGGTG	TCTGTCAATCCCCTTT GGGG	AGCCCTTTTGTCAA TCCCC
PAPI-1 pili chaperone	UCBPP-PA14, complete genome, Lee et al. (2006)	GGAAACAACAGTGGGGCGTGAC	CGCTCAAGCGCTATCC CACC	CGCATCGGCCTGTAC AACG
PAPI-1 luminal binding protein	UCBPP-PA14, complete genome, Lee et al. (2006)	CCAGTTGGCACCACCATGCTTGC	CGGTAGAGAGCTGGG TTGGC	AACTGGAGCTAGGGC AGAGC
pKLC conserved hypothetical	Klockgether et al. (2004)	GCCTGCCTACTTGTCCCAA CGC	CTACCAGCTTGGGCG TAGC	AAGCGATAGCGTGCT CCTGC
pKLC adhesin	Klockgether et al. (2004)	GGCTGTATTGCCGCCATTC TCC	CCGGCTATATCCGGG CTACC	ATTGGCGTGCTGTTT ACGCCC
pKLC fatty acid synthase	Klockgether et al. (2004)	CGACAGACAGAAAGGGTTCTTGCGC	GGTGGCGTGGGTTTT TCTGC	AGGTCGTAGCGGAAG GTGGTGG
PAGI-2/3-4	Larbig et al. (2002)	GCGCCTTCTCCTCTTTCAGATGT	TGTCCCGGCTCAGTTC AACG	GCAACACCTTGGCGTT TGCC
PAGI-2/3-5	Larbig et al. (2002)	CAGTATGGTACGGACACGAA GCGC	TCAAGCTCGTTGTGGA CCGC	GTTACGACGGCGTGCT GTCCG
PAGI-2/3-6	Larbig et al. (2002)	CCATGGTCGGAACAGGCACG ATATGC	CAACACGGGACTGGC GATCC	TACATCATCCGCAACG CGCGC
C-45	Larbig et al. (2002)	CGAGGAGTTTTCGGACCCGCTTTGA	TCATCCAGCAAGCCAT TGCGC	TGGATCGCTTTCCGC CATCG
C-46	Larbig et al. (2002)	CGAAGTCTGAGGTGTGGACC CGC	CGCGGTGCTGGTTGC GCTGC	CGCTGGCAGTTCCGCT GGCC
C-47	Larbig et al. (2002)	CCACTCGATCATGTTGAGCATGGCTCC	TATTGACGACATCCG CGCGCC	CACCAAGAACC CGTGCTCG
PAGI-2	Larbig et al. (2002)	GCATCATTGCGCGTCACATC TGGT	ACGCAACGTATTCGGC GACCC	CGCAACGTATTCGGC ACCC
PAGI-2/3-1	Larbig et al. (2002)	GACCGCAAGCAGAAACGGCA TGC	GGTGCTCGACCCAAG CATCG	TCCTTGAAGTTCTTGG CGCGC
PAGI-3-1	Larbig et al. (2002)	CCCCTGCTCATAACCCGTT CCTG	GACGAATACCCAGTG CTTGG	GCAGACGAATACCCAG CTGCG
PAGI-3-8	Larbig et al. (2002)	GGTTAGTCCCTTCTGCCCGCATCG	ATCGTGGCAGGATGTC CACCG	TAGGCGGGCCTTTTGA AGGTGC
tRNA(Pro)- island 1	P.aeruginosa TB, gene island integrated into tRNA(Pro) PA2736.1	GTGTACCGCCCATGTCTAGCAGC	TCCACGCCGAGGGAC GTGCC	GCTCCACGCCGAGGG ACCTGCC
tRNA(Pro)- island 2	P.aeruginosa TB, gene island integrated into tRNA(Pro) PA2736.1	AGGCCATGGGCTAGCCGGATGC	AGGAGGCCGATGACA ACACCC	TGCCGATTCCATGCTC ACGCC
PAGI-1	Liang et al. (2001)	TTCTCGGTGTCGAGGGATTC TCGG	GCATTGCGCACGGAAG GAAGG	GAAGGCATCATGGCAT TCGCC