



## **S. aureus Genotyping Kit**

Array Hybridisation Kit for DNA-based detection of resistance genes and pathogenicity markers of *Staphylococcus aureus* and assignment of unknown *S. aureus* isolates to known strains.

**For Investigational Use Only. Not Intended for Use in Clinical Diagnostics.**

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## BACKGROUND

The IDENTIBAC *S. aureus* Genotyping Kit allows DNA-based detection of resistance genes and pathogenicity markers of *Staphylococcus aureus* and assignment of unknown *S. aureus* isolates to known strains.

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TEST PRINCIPLE. Clonal *S. aureus* DNA is amplified approximately 40-fold and labelled with biotin-dUTP adopting a so called linear PCR protocol. In linear PCR only one primer is used instead of a primer pair, producing single stranded reaction products only, therefore limiting the degree of amplification (limited risk of cross-contamination). Labelled single stranded DNA is transferred and hybridised to microarrays with 333 probes representing different genetic markers and a staining control probe (single redundant each). Spot recognition as well as geometric layout is performed automatically based on scans of the arrays and advanced image processing. The target set consists of a variety of species markers, toxin-, virulence- and antibiotic resistance genes, MSCRAMMS, enzymes and other types of markers.

THE RESULTING typing data provide information regarding genetic predisposition for antibiotic resistance and production of virulence factors of the analysed *S. aureus* isolates, respectively. Further, they provide information regarding strain assignment.

THE ASSAY IS DESIGNED to be performed by personnel trained in microbiological and molecular biological laboratory methods.

## GENERAL INSTRUCTIONS FOR USE

### Intended Use

***For Investigational Use Only. Not Intended for Use in Clinical Diagnostics.***

The kit is intended for use by personnel that are well-trained in molecular biology. Preparation of DNA from *S. aureus* colonies (clones) requires expertise in microbiology and the local regulations for handling of potentially infectious organisms are to be obeyed.

### 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 unless otherwise specified.

### Technical Support

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

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or visit our website: [www.identibac.com](http://www.identibac.com)

### Safety Precautions

DNA preparation from *S. aureus* must be performed under the biosafety conditions as defined by your local authorities for work with *S. aureus*. In most countries, Biosafety Level 2 is obligatory for this kind of work. *S. aureus* DNA may be processed in laboratories below this biosafety level, if potential contamination by living *S. aureus* bacteria can be definitively eliminated.

Always wear protective clothes as regulated for laboratory work by your local authorities.

### Material Safety Data Sheets (MSDS)

Per 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 buffers or solutions are spilled, clean with 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

## KIT COMPONENTS, STORAGE AND STABILITY

**Please keep in mind the limited surplus of each reagent when pipetting.** Arrays, A2 and B1<sup>ST</sup>, respectively, are by far the most expensive and hence the limiting components of the kit.

All components may be ordered separately; please refer to the order numbers at the end of this manual. For pricing please contact your local representative or our customer service, respectively.

### Cell Lysis

- **A1:** Lysis Buffer. Store at 18-28°C (ambient temperature). **Surplus: 50%.**
- **A2:** Lysis Enhancer (lyophilized). Store at 18-28°C (ambient temperature).  
Add 0.2 mL Buffer A1 to Lysis Enhancer before use. Mix well and store for less than 1 week at 2-8°C. **Sufficient for 96 isolations.**

### DNA labelling and amplification

- **B1<sup>ST</sup>**: Labelling Buffer. Store at 2-8°C. **Surplus: 25%**.
- **B2**: Labelling Enzyme. Store at 2-8°C. **Surplus: 50%**.

### Hybridisation and Detection

- **ArrayStrips** (12 x 8 samples), protected against light and sealed under inert gas. Store at 18°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. Avoid ANY touching or scratching the microarray on the bottom of the well.  
CAUTION: Do not store or handle unused wells above 60% relative humidity since this may irreversibly corrode the spots.
- **CapStrips** (24 strips)
- **C1**: Hybridisation Buffer. Store at 18-28 °C, protect against sunlight. **Surplus: 100%**.
- **C2**: Washing Buffer 1. Store at 18 °C - 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°C to 28°C, protect against direct sunlight.  
**Surplus: 200%**
- **C5**: Washing Buffer 2. Store at 18°C to 28°C, protect against direct sunlight.  
**Surplus: 200%**.
- **D1**: Horseradish Peroxidase Substrate. Store at 2-8°C, protect against direct sunlight.  
**Surplus: 50%**.
- **CM**: Control Material, Reference DNA preparation of *S. aureus* strain N315 (GenBank Entry BA000018), 0.1 µg/µL. S Store at 2-8 °C. **Sufficient for 5-6 tests.**

**Expiry date** is to 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 kit components with limiting stability are D1 and C3, respectively. The other components have proven to be stable even six months after the kit expiry date has passed.

**Components required but not provided**

- DNA preparation kit. The assay has been tested with the DNeasy Blood & Tissue Kit from Qiagen, cat# 69504. Please note: DNA isolation from *S. aureus* requires a pretreatment with the Cell Lysis component outlined above.

**Instrumentation provided by Alere (to be ordered separately)**

- ArrayMate Reader

**Materials required but not provided by Alere**

- Equipment, growth media and consumables needed for the cultivation of *S. aureus*
- Equipment needed for DNA isolation, e.g. pipettes, centrifuge, thermoshaker or robot
- Photometer for quality control of DNA.
- Equipment for DNA gel electrophoresis for quality control of DNA.
- Thermocycler
- Thermoshaker: we strongly recommend the BioShake iQ from Quantifoil Instruments (<http://www.qinstruments.com/>) equipped with a specialised platform for the warming of Alere's ArrayStrips  
Alternatively, you may use Eppendorf's Thermomixer Comfort, equipped with Quantifoil's ArrayStrip adapter for EP Thermomixer, order number: 312-001.
- Pipettes: suitable for 1µL-5µL volumes, 90µL, 100µL, 200µL, 1000µL
- Multichannel Pipettes for 100-200 µL
- Reagent tubes suitable for PCR
- Ultrapure water

## SOFTWARE AND READER

**Please keep in mind that you need to install a kit specific software on your reader!**

Image analysis for any IDENTIBAC test kit and on any IDENTIBAC reader is performed by the iconoclust software that has been delivered along with the reader. In addition, some test kit specific information tied to some reader specific information needs to be installed separately. This information (like spot names, marker names, location of the spots on the array, size of the image taken by the reader's specific camera) is provided by the specific iconoclust package.

**Whenever you want to work with a kit OR a kit version you have never worked before, please install the appropriate iconoclust package first!**

The appropriate package you may download from our website [www.identibac.com](http://www.identibac.com) and install it on your reader according to the instructions you will find on this website.

**Please note:**

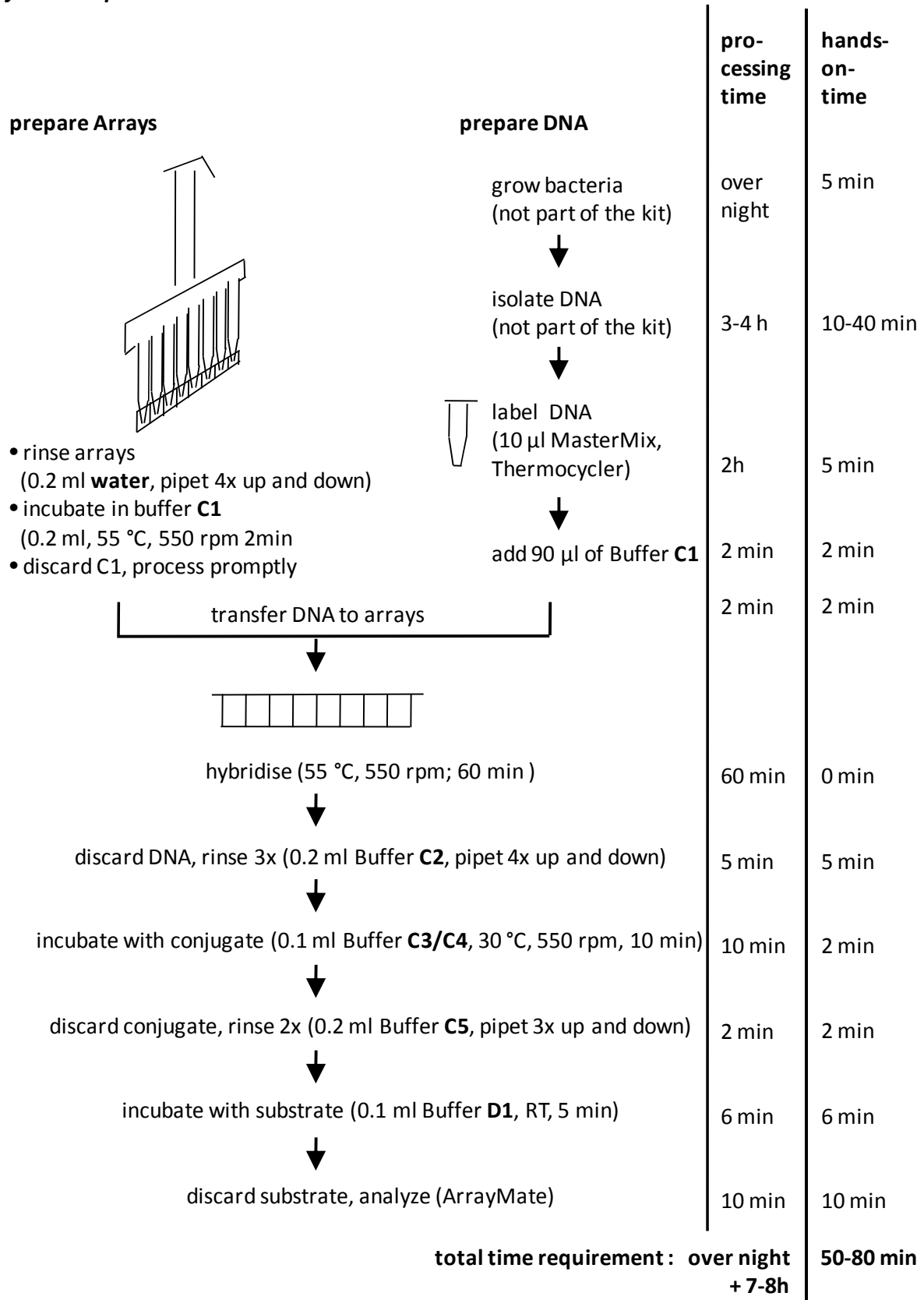
The *S. aureus* Genotyping kit may be used on the ArrayMate reader only. ATR03 is not suitable for reading of ArrayStrips. The **ArrayMate reader** by default has all software on board including the *S. aureus* Genotyping kit specific software package. Unlike ATR03, ArrayMate is not linked to problems with compatibility of software.

In case you have IDENTIBAC test kits running in your laboratory on the ATR03 reader you may transfer them to ArrayMate. However, you need to download and to install the ArrayMate specific packages for these kits first. You should NOT use a "package" for ArrayMate that has been designed for ATR03 and vice versa.

In case of any questions please contact your local distributor or Alere's technical service, respectively.

## PROTOCOL

The graphic on this page summarises the test procedure but due to limited space it is missing important details. Therefore it is crucial to refer to the text section of this manual at any step of the test protocol.



## 1. *S. aureus* DNA

The required specimen for the *S. aureus* Genotyping assay is 0.5-2 µg of intact DNA from a single clone of *S. aureus*. The DNA specimen needs to be virtually free of RNA as determined by agarose gel electrophoresis.

Please note: This assay requires much more DNA than common PCR applications. Most performance problems with the *S. aureus* Genotyping kit are due to insufficient amounts of DNA. We therefore strongly recommend to follow the protocol outlined below. Kit component A2 contains enzymes for *S. aureus* cell wall lysis and RNase.

From several DNA isolation methods tested so far, only the DNA extraction Kits from Qiagen in combination with the A1/A2 Cell Lysis component of the *S. aureus* Genotyping kit yielded sufficient DNA. The Cell Lysis component is required for *S. aureus* cell-wall digestion and for RNA digestion. It facilitates preparation of sufficient amounts of pure DNA.

**CAUTION:** *S. aureus* is a potential pathogen. All procedures prior to complete death of the bacteria need to be performed by properly trained staff in a biosafety Level 2 facility.

### Harvest of bacteria

From a bacterial clone grow enough bacteria to fill an inoculating loop (diameter: 1 mm) as pictured below.

- Add 0.2 mL of Lysis Buffer A1 to Lysis Enhancer A2 (tiny, colourless pellet), dissolve.
- Add an inoculating loop of a *S. aureus* isolate to this A1/A2 reagent.

loop empty



loop full



**It is important to harvest enough bacteria; this is a prerequisite for extraction of sufficient DNA.**

**Take an inoculating loop of 1mm diameter filled with bacteria as shown in the left picture.**

### Extraction of DNA

- Incubate for 30-60 min at 37°C and 550 rpm in the thermoshaker.

- Proceed with the DNA preparation protocol of the DNA preparation kit.

For the Qiagen DNeasy Blood&Tissue Kit:

- Add 25 µL proteinase K (Qiagen Kit)
- Add 200 µL buffer AL (Qiagen Kit)
- Vortex shortly or shake vigorously
- Incubate for 30-60 min at 56°C and 550 rpm in the thermoshaker
- From the Qiagen kit manual follow Qiagen protocol “Purification of Total DNA from Animal Blood or Cells” (next step: add 200 µL of Ethanol).

### **IMPORTANT BEFORE CONTINUING:**

- Check for DNA integrity and absence of RNA (agarose gel). In case, remove RNA using RNase (not provided outside component A2). Measure DNA concentration (A260 method).

## **2. Worklist**

For the regular “USER” mode of the ArrayMate it is obligatory to up-load a worklist onto the Reader before an analysis can be performed. For future data mining we recommend the use of a worklist also for the “R&D” mode. The worklist may be exported from a LIMS or designed in EXCEL or any other appropriate software. The final format must be a wordpad (\*.txt)-format that can be imported into the test specific ArrayMate software (see below). For setting up a worklist:

- Create a list with at least three columns.
- The columns must have headers written into the first line.
- Each header MUST be one word (different words may be linked by “\_”).
- Don’t use special characters like : ; ( / \ etc.
- The following headers are obligatory (in this order): position                      sampleID                      assayID

where:

- position = position in a 96 well format i.e. 1 = A1, 8=H1, 9=A2 etc.

List position numbers of wells that are to be analysed in a continuous fashion;

DO NOT leave empty lines in the worklist;

If you use EXCEL: You need to type the position numbers into column A (Note: the raw

numbers of the EXCEL spread template sheet CAN NOT be used to indicate well position).

- sampleID = your sample ID as exported from your LIMS (or assigned in any different way)
- assayID = 10248 (ID number of the S. aureus Genotyping assay). DO NOT use a different assay ID since this could lead to loss of data.

- You may add further columns and headers at your convenience (Note: information from these columns will NOT appear on the result screens or the Test Report).

• Example:

position	sampleID	assayID	comment
1	12345	10248	Dr. Jones
2	12346	10248	Dr. Miller
3	12347	10248	Dr. Yale
4	12348	10248	Dr. Palmer
5	12349	10248	Dr. Jones
6	12350	10248	Dr. Jones
7	12351	10248	Dr. Chapman
8	12352	10248	Dr. Scott
9	12353	10248	control
17	12354	10248	internal test

	1	2	3	4
A	1	9	17	
B	2			
C	3			
D	4			
E	5			
F	6			
G	7			
H	8			

these are the corresponding positions in the 96 well format

- We recommend using a printout of the worklist as guidance for pipetting.
- Always convert your file into a wordpad (.txt) format (tabstop-delimited version!)
- Safe the wordpad (\*.txt) file of the worklist onto the memory stick provided along with the ArrayMate.
- To avoid confusion make sure that earlier versions of the worklist have been deleted from the memory stick.

### **3. Linear amplification and biotin labelling**

Please keep in mind the limited surplus of reagents whilst pipetting. The surplus of B1<sup>ST</sup> is 25%.

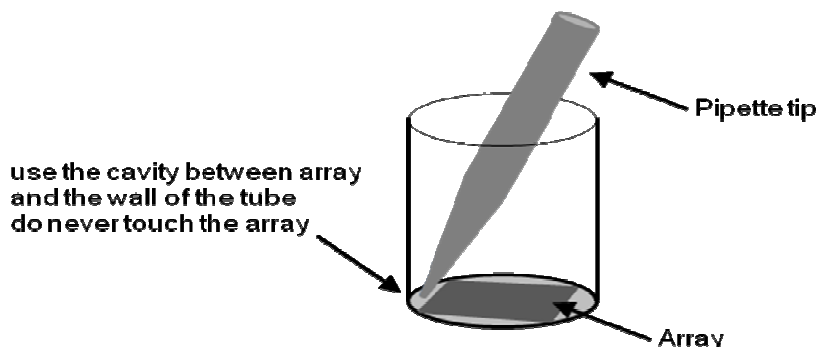
- Suspend 0.5-1.5 microgram of clonal and intact *S. aureus* DNA in 5 µL of ultrapure water in a reagent tube suitable for PCR (concentrate DNA if necessary by evaporation in a vacuum centrifuge or by precipitation). Do not forget to label the vial!
- Prepare a Master Mix by combining 4.9 µL of B1<sup>ST</sup> (2x Labelling Buffer) and 0.1 µL of B2 (DNA Polymerase) per specimen.
- Add suspended DNA to a 5 µL aliquot of the MasterMix
- Perform the following thermocycler protocol (required time: approximately 2 hours)

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

### **4. Hybridisation**

#### **General**

- Unused wells should be capped and during the whole procedure. The strips may be processed up to three times without loss of quality of the unused arrays.
- We recommend the use of a multichannel pipette and reagent reservoirs. Please keep in mind the limited surplus of C1 (100%).
- Never touch the Array surface. Remove liquid with a pipette: 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.



- Avoid any complete drying of the array surface during processing (i.e. do not allow it to stay without liquid for more than two minutes)
- We strongly recommend that the liquid is removed by multichannel pipettes instead of inverting the wells and flicking them out.
- 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.
- Avoid touching the bottom of the microarray strip and keep it clean
- Never rinse the wells with distilled water after hybridisation
- The correct temperature within the vessels is essential; therefore always use the appropriate equipment for heating. We strongly recommend the BioShake iQ from Quantifoil Instruments (<http://www.qinstruments.com/>) equipped with a specialised platform for the warming of Alere's ArrayStrips

#### Preparation of the hybridisation mixture

- Pre-heat the thermoshaker to 55 °C
- Add 90 µL of buffer C1 to each labelling product, mix gently (vigorous mixing results in foaming) and put aside.

#### Pre-washing of the arrays (2 washing steps)

- Remove the ArrayStrip from the bag (open the bag at its predetermined breaking point)
- Insert the ArrayStrip(s) into the white frame. **Assure the correct orientation (data matrix barcode close to row A) and proper fit.**
- 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 "Kit components, storage and stability/Hybridisation and Detection")
- Add 200 µL of ultrapure water to each well
- Mix carefully with a pipette (4x up and down) **WITHOUT TOUCHING THE ARRAY SURFACE**

- Remove and discard the water
- Add 200 µL buffer C1 to each well
- Incubate in the thermoshaker at 55 °C, 550 rpm for 2 minutes (covering the wells during this step is not required)
- Remove and discard buffer C1
- Proceed promptly (hybridisation mixtures must be ready when buffer C1 is removed)

### Hybridisation

- Transfer each hybridisation mixture (100 µL) to a prepared well on the ArrayStrip (avoid extensive foaming)
- Cap the wells
- Incubate for one hour at 55°C and 550 rpm on a thermoshaker

## **5. Prepare reagents for detection and staining**

### **Dilute Streptavidin-Horseradish-Peroxidase (C3, C4)**

- Combine Reagent C3 (Streptavidin-Horseradish-Peroxidase) : Buffer C4 = 1 : 100 => C3/C4  
The mixture is stable for 1 day at room temperature; C3 is delivered with a surplus of 100%, C4 is delivered with a surplus of 200%.
- 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
<b>C3</b>	1.5 µL	3.5 µL	7 µL	11 µL	16 µL	21 µL	32 µL	42 µL
<b>C4</b>	150 µL	350 µL	700 µL	1100 µL	1600 µL	2100 µL	3200 µL	4200 µL

- put aside at room temperature until use


### **Pre-warm the staining reagent D1**

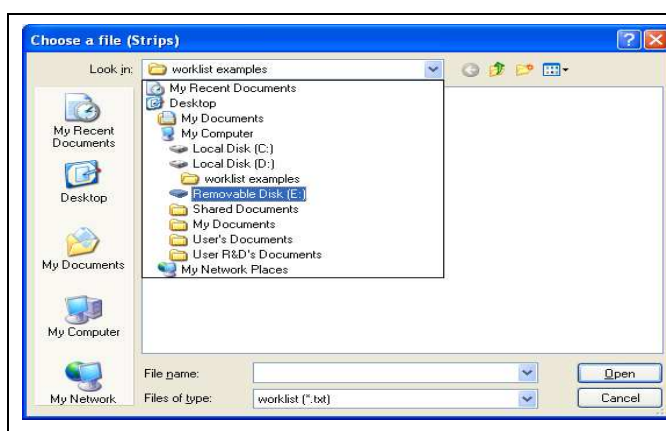
- Transfer enough reagent D1 into a separate vessel (e.g. a clean and sterile centrifuge tube), 100 µL for each well and a surplus of not more than 20%
- put aside at 20 to 25°C until use.

**Please note: cold D1 may yield in weak signals. Centrifuge D1 immediately prior to staining (see below), otherwise clots within D1 may contaminate your array.**

## 6. Setup of the ArrayMate Reader


We recommend to set up the ArrayMate Reader after having started the hybridisation; this allows you a restart without time pressure in case of any software problems. 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).
- Login as **“User”** (no password is required).  
(The user interface is loaded, ArrayMate performs internal testing for < 1 minute)
- Click on the icon “New Run” (left upper edge of the screen; a suggestion for a run name for the new run appears in the top line of the screen).
- You may now modify or change the experiment name at your convenience.
- Type in your operator ID.
- If desired, you may enter a comment into the “memo” field.
- Insert your memory stick containing the worklist (use any of the USB ports down to the right side of the ArrayMate).
- Press the button:  (a folder selection dialog opens)
- Select your worklist (path: “My Computer/Removable Disk”)
- Open your selected worklist with “Enter” or the button “Open”



**Caution:** Make sure to choose the correct worklist!

**Caution:** By default an example file for a worklist is selected. This file is only for training purposes! Do NOT use it for your experiments!

- Press the button:  (your imported worklist opens in a separate window). Check for correctness. If the new window is empty or the worklist is not the desired one, repeat the import.
- Press the button “ok” (worklist-window is closing).

- Leave the memory stick attached to the ArrayMate if you intend to export S. aureus Genotyping Test Reports afterwards.

## **7. Detection**

### **Washing after hybridisation (3 washing steps).**

- Please keep in mind the limited surplus of C2 (100%).
- Remove the strips from the thermoshaker
- Set the thermoshaker to 30°C for the HRP-conjugate step (cooling will take some time and may be supported with a cooling element)
- Carefully open the wells and remove the hybridisation mixture as completely as possible (without touching the array surface)
- Washing step after hybridisation:  
add 200 µL of buffer C2  
Mix carefully with a pipette (4x up and down) **WITHOUT TOUCHING THE ARRAY SURFACE**  
remove and discard the washing solution  
repeat the washing twice.

**NOTE:** a carryover of more than 1% of buffer C1 to the next step will denature the HRP

### **Addition of HRP-conjugate**

- NOTE:**
- the used wells do not need to be capped any more
  - Reagent C3 contains Streptavidin-Horseradish Peroxidase (HRP) that would denature and **lose its activity at 55°C. Do NEVER incubate above 30°C. Make sure that the thermoshaker has cooled down before mounting the ArrayStrips!**
  - Please keep in mind the limited surplus of C3 (100%).
- Combine Reagent C3 (HRP): Buffer C4 = 1 : 100 => C3/C4 (see section 5)
    - the mixture is stable for 1 day at room temperature
  - Add 100µL of C3/C4 to each well
  - Incubate for 10 minutes at 30°C and 550 rpm on a thermomixer
  - Remove and discard C3/C4 completely

**Washing step after binding of conjugate Addition of HRP-conjugate (2x)**

- add 200 µL of buffer C5  
Mix carefully with a pipette (4x up and down) WITHOUT TOUCHING THE ARRAY SURFACE  
remove and discard the washing solution
- Repeat this step once.

**NOTE:** a carryover of more than 0.5% of C3/C4 into the following staining reagent will create black particles which in the worst case may mimic signals (hybridised spots). On the same time, real signals may appear pale due to competition of soluble Horseradish Peroxidase with the DNA-bound enzyme for substrate molecules.

**Staining of bound HRP-conjugate**

- NOTE:**
- a carryover of more than 0.5% of C3/C4 will cause unwanted effects (see above)
  - wells do not need to be capped
  - do not move ArrayStrips during staining
  - Reagent D1 contains a substrate for Horseradish Peroxidase
  - Please keep in mind the limited surplus of D1 (50%).
- D1 needs to be pre-warmed and centrifuged, see section 5
  - Add 100µL of reagent D1 to each well (supernatant of centrifuged D1 without precipitate)
  - Incubate at room temperature WITHOUT agitation for 5 min
  - Remove and discard reagent D1 as completely as possible and analyse immediately (the dye precipitate is unstable and fades slowly; read out immediately but at maximum within 20 minutes).
  - **CAUTION:** the strips **MUST** be clean underneath the arrays and there **MUST NOT** be air bubbles or remains of liquid in the wells. Strips may be cleaned with lint-free wipes, bubbles may be removed by adding and removing D1 again.

## 8. Data Acquisition in the ArrayMate Reader

### The reading process

The setup of the reader is briefly described in the section “Setup of the ArrayMate Reader”.


- Press the button “next” (bottom/right on the screen; reader is opening).
- Carefully insert the appropriate metallic adapter (if there is more than one) 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 inserted strips. Strips are inserted in accordance to the worklist.*

- **CAUTION:** the strips **MUST** be clean and free from air bubbles and liquid!
- Barcodes on strips and holder must be clean.
- There must be no lids on the wells that are to be analysed (unused wells remain capped, however).
- Press the button “Next” (bottom/right on the screen; reader is closing, analysis program starts, it takes ca. 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 is opening)
- Remove the white frame with the ArrayStrip(s).

- Press the button “Next” (bottom/right on the screen; reader is closing).

## Results

- On the left hand of the screen you will see a list showing all readings stored on the ArrayMate. A reading contains the results from all arrays analysed together in one frame.

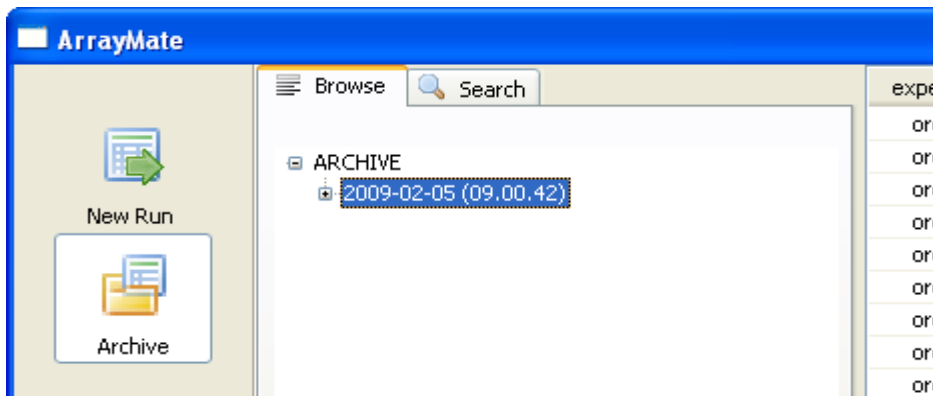
If this list is not visible:

- press the button “Archive” (left hand)
- activate the Flag “Browse” (top left) .

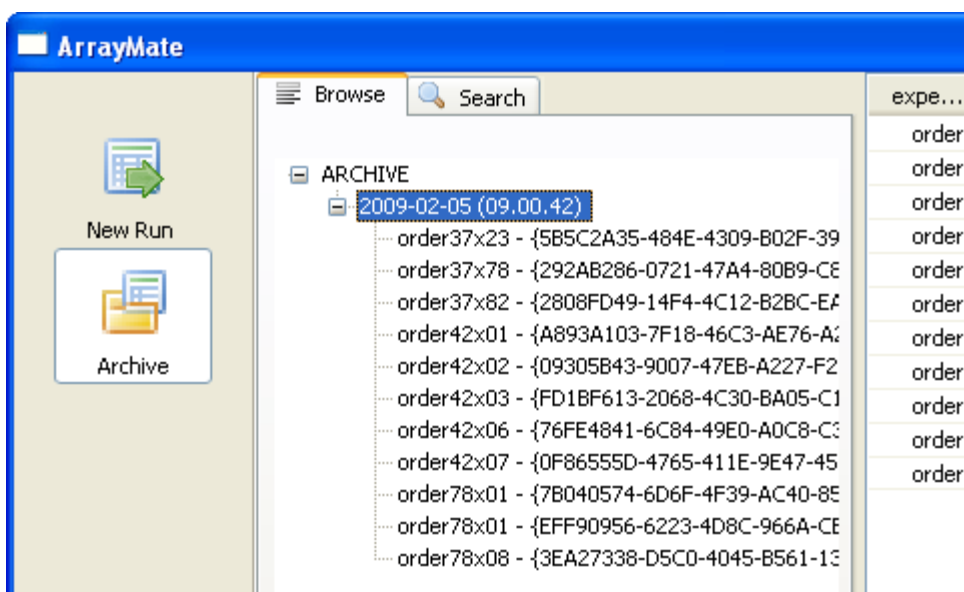
- Please note:

- the readings are organised like folders in “Windows”.

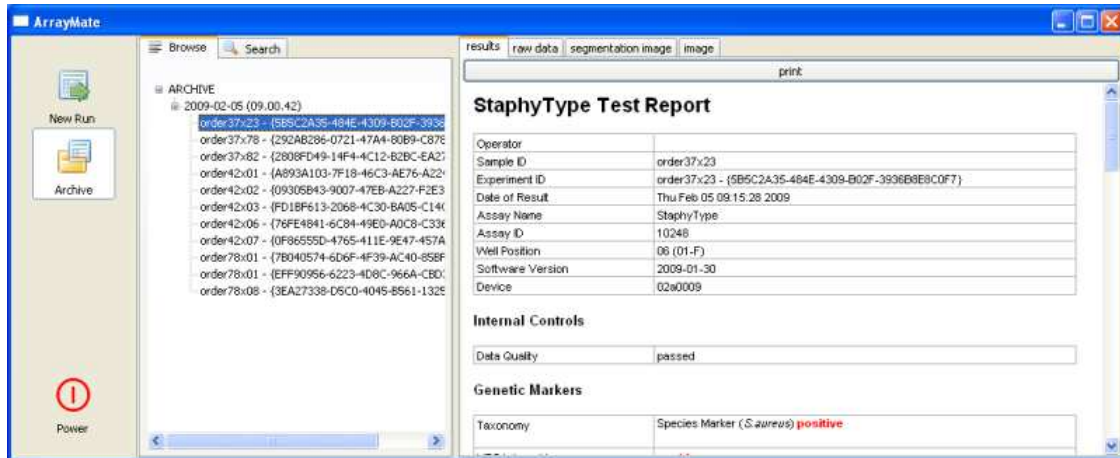
Example: there is one reading in the archive:



If you click on the plus symbol of the reading, the folder opens and you will see a list of the individual arrays (in alphabetical order according to Sample ID).

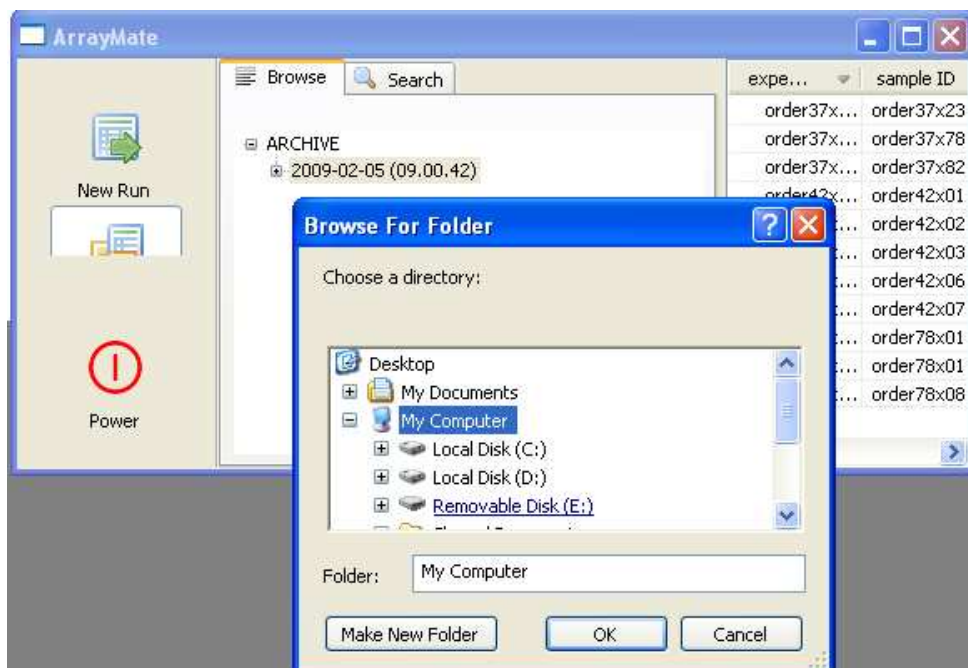


- Click on a Sample ID and the S. aureus Genotyping Test Report for this array is shown in the window on the right:



### Export of S. aureus Genotyping Test Reports


- **Note: only complete readings can be exported. The export of individual S. aureus Genotyping Test Reports is not possible**
- Right-Click on the desired reading (a menu appears with the option “Export Run Reports”)
- Right-Click on “Export RunReports” (a file browser opens)



- Click on “My Computer”, then on “Removable Disk” and choose an appropriate storage place

- Click on the button “Make New Folder” (on the bottom; a new folder icon appears)
- Rename the new folder(e.g. with the experiment name)
- Click on the ”Ok” button (data are exported now 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**

Click on the “Power”-button (left/down on the screen): 

Switch off the Screen. There is no need to physically switch off the ArrayMate.

**In case of any hang-up:** restart the computer by pressing the on/off button (bottom/left on the ArrayMate reader face).

## TROUBLESHOOTING

### Data Quality

In case of poor data quality we recommend to re-check DNA quantity and quality first 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 are a prerequisite for proper DNA concentration measurement. RNase treatment prior to A260 reading therefore is necessary (component A2 contains RNase).
- DNA must be unfragmented, 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 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.

### Staining Control

In case that Data Quality failed, the result of the Staining Control is displayed. If the Staining Control has “Passed”, refer to the troubleshooting guide above. If the Staining Control has “Failed” proceed as follows:

- Horseradish Peroxidase Conjugate may have degraded during storage. Add 1µL buffer C3/C4 to 9 µL D1 (substrate). If the solution turns green within 3-5 seconds, the Horseradish Peroxidase still has sufficient enzymatic activity.
- 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.

### **Physical damage of the array**

Scratching of the array surface with a pipette tip can lead to the damage of array spots that prohibits the acquisition of a valid signal. In this case the respective marker is not assigned as “negative”, but instead the message “none” appears next to the marker name. We recommend to review Section 4 for General Precautions in array handling.

### **Ambiguous Results**

Besides a “positive” or “negative” result for the individual markers on the *S. aureus* Genotyping Test Report, the result can also be “ambiguous”. In this case no definitive answer with regard to this specific marker can be given.

### **Report unavailable**

If the ArrayMate indicates that no report is available for an array (or multiple arrays on one strip), please check that the strip was positioned properly into the frame. If no obvious reason for the fault can be discovered, please contact the technical service.

### **Strain assignment**

In case the system was not able to assign an isolate to the predefined strains, it probably belonged to a rare or atypical strain. Please contact the technical service.

If no error was found with the sample or the reagent, we recommend repeating the entire analysis (starting with the bacterial colony) with a subset of the troublesome samples. Should the problem persist, please contact the technical service.

## LITERATURE

- A Field Guide to Pandemic, Epidemic and Sporadic Clones of Methicillin-Resistant *Staphylococcus aureus* (REVIEW)  
Stefan Monecke, Geoffrey Coombs, Anna C. Shore, David C. Coleman, Patrick Akpaka, Michael Borg, Henry Chow, Margaret Ip, Lutz Jatzwauk, Daniel Jonas, Kristina Kadlec, Angela Kearns, Frederic Laurent, Frances G. O'Brien, Julie Pearson, Antje Ruppelt, Stefan Schwarz, Elizabeth Scicluna, Peter Slickers, Hui-Leen Tan, Stefan Weber, Ralf Ehricht; PLoS ONE; April 2011; Volume 6; Issue 4; e17936
- Differentiation of CC59 Community-Associated Methicillin-Resistant *Staphylococcus aureus* in Western Australia. Coombs GW, Monecke S, Ehricht R, Slickers P, Pearson JC, Tan HL, Christiansen KJ, O'Brien FG. *Antimicrob Agents Chemother.* 2010 Mar 8.
- Molecular fingerprinting of *Staphylococcus aureus* from bone and joint infections. Luedicke C, Slickers P, Ehricht R, Monecke S. *Eur J Clin Microbiol Infect Dis.* 2010 Feb 26
- Characterization of methicillin-resistant *Staphylococcus aureus* ST398 from cases of bovine mastitis. Feßler A, Scott C, Kadlec K, Ehricht R, Monecke S, Schwarz S. *J Antimicrob Chemother.* 2010 Feb 17.
- Diversity of antimicrobial resistance pheno- and genotypes of methicillin-resistant *Staphylococcus aureus* ST398 from diseased swine. Kristina Kadlec, Ralf Ehricht, Stefan Monecke, Ulrike Steinacker, Heike Kaspar, Joachim Mankertz, Stefan Schwarz; *J Antimicrobial Chemotherapy*, 2009.
- Intra-strain variability of methicillin-resistant *Staphylococcus aureus* strains ST228-MRSA-I and ST5-MRSA-II. S. Monecke & R. Ehricht & P. Slickers & N. Wiese & D. Jonas; *Eur J Clin Microbiol Infect Dis* 2009.
- Assignment of *Staphylococcus aureus* isolates to clonal complexes based on microarray analysis and pattern recognition. Monecke S, Slickers P, Ehricht R; *FEMS Immunol Med Microbiol.* 2008 May 27.
- DNA microarray-based genotyping of methicillin-resistant *Staphylococcus aureus* strains from Eastern Saxony. Monecke S et al; *Clin Microbiol Infect.* 2008 Mar 27.
- Cost analysis of a hospital-wide selective screening programme for methicillin-resistant *Staphylococcus aureus* (MRSA) carriers in the context of diagnosis related groups (DRG) payment. M. H. Wernitz et al; *Clin Microbiol Infect* 2005; 11: 466–471.

## 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 temperatures in the laboratory between 18°C and 28°C. Kit components comprise the Arrays and their caps, the Lysis Enhancer, 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 ([www.identibac.com](http://www.identibac.com)).

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

### Quality Control

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

### List of components for separate order

If required, these reagents for the *S. aureus* Genotyping Kit may be ordered separately:

component	name	category	amount	order no	storage
<b>A1</b>	Lysis Buffer	buffer	30 mL	245101000	18-28 °C
<b>A2</b>	Lysis Enhancer	lyophilised enzymes	96 units	245102000	18-28 °C
<b>B1<sup>ST</sup></b>	MasterMix <i>S. aureus</i>	buffered reagents	700 µL	245103000	2-8 °C
<b>B2</b>	Labelling Enzyme	buffered enzyme	20 µL	245104000	2-8 °C
<b>C1</b>	Hybridisation Buffer	buffered reagents	30 mL	245105000	18-28 °C
<b>C2</b>	Washing Buffer 1	buffer	120 mL	245106000	18-28 °C
<b>C3</b>	HRP Conjugate 100x	buffered enzyme	200 µL	245107000	2-8 °C
<b>C4</b>	Conjugate Buffer	buffered reagents	30 mL	245108000	18-28 °C
<b>C5</b>	Washing Buffer 2	buffer	120 mL	245109000	18-28 °C
<b>D1</b>	HRP Substrate	buffered reagents	15 mL	245110000	2-8 °C
<b>CM</b>	Control Material	<i>S. aureus</i> DNA 0.1mg/mL	30 µL	245111000	2-8 °C
<b>ArrayStrips</b>	AS <i>S. aureus</i>	contained microarrays	12 St	240008566	15-28 °C
<b>StripCaps</b>	StripCaps	plasticware	24 St	245112000	18-28 °C

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

**LEGAL MANUFACTURER**

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