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Manual

E. coli Genotyping Kit

Array Hybridisation Kit to detect important virulence genes of *Escherichia coli*

Kit order number: 205400050

50 reactions (ArrayTube format)

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

CONTENT

BACKGROUND

The ALERE ***E. coli*** **Genotyping Kit** allows a quick and simple method to detect important virulence genes of *Escherichia coli* (*E. coli*).

RNA-free, unfragmented genomic DNA of a pure and monoclonal *E. coli* 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 124 probes for different genetic markers plus controls. All of them are spotted in two spots each. The array contains markers for:

- family, genus and species identification
- toxins (i.e. shiga toxin)
- fimbrial associated proteins (i.e. bacterial adhesins)
- secretion systems (i.e. type III secretion system)
- serine protease autotransporters (SPATE)
- staining controls using biotinylated control spots.

GENERAL INSTRUCTIONS FOR USE

Intended Use

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

This assay allows genotypic characterisation of important virulence genes of *E. coli.* It cannot be used for other bacteria than *E. coli*.

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:

Email: cct.home@clondiag.com

Phone: +49 (0) 36 41 3111-155

Fax: + 49 (0) 36 41 3111-120

For up-to-date information regarding the kit, please visit our website at

http://alere-technologies.com/en/products/lab-solutions/e-coli/e-coli-genotyping-kit.html

Safety Precautions

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

Material Safety Data Sheets (MSDS)

According to OSHA 29CFR1910.1200, Commonwealth of Australia [NOHSC: 1005, 1008(1999)] and the latest regulations (EC) 1272/2008 (CLP) and 1907/2006 (REACH), the enclosed reagents do not require a Material Safety Data Sheet (MSDS), **except Hybridisation Buffer C1. The MSDS can be downloaded via our website from any lab solutions product page (e.g. http://alere-technologies.com/en/products/lab-solutions.html).** All other reagents 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. 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). If liquid is spilled, clean with a 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

DEVICES, SOFTWARE AND REAGENTS

- ArrayMate Reader (to be ordered separately, for details see below)
- Alternatively, Reader ATR03 (to be ordered separately, for details see below)
- Iconoclust software (provided with the reader)
- Report Generator (optional)

Whilst the ***E. coli* Genotyping** assay runs both on the ArrayMate Reader and on the ATR03 reader, respectively, this manual describes the reading of processed AT on the ArrayMate reader only. If you want to use ATR03, please refer to the latest version of the ATR03 manual or contact us.

Assay specific software (plug-in) is delivered with the reader or can be downloaded from our website, where it will occasionally be updated.

The ArrayMate Reader by default has all software on board. However, the ***E. coli* Genotyping** assay specific package might be missing, e.g., if you obtained the device for the use with another assay. Then you may need to install it separately. It will be provided upon kit order, and can also be downloaded from our website as discussed above. No issues regarding compatibility of software have been observed with the ArrayMate device.

The ATR03 reader requires several pieces of software to be installed on an external PC (please refer to the latest version of the ATR03 manual for details). The ***E. coli* Genotyping** assay specific software is not compatible with iconoclust versions older than version 4.4.

Software Installation

For analysis of the final image of the DNA microarray on the ArrayMate specific software plug-in 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.

Assay Plugin and SDK for the ArrayMate

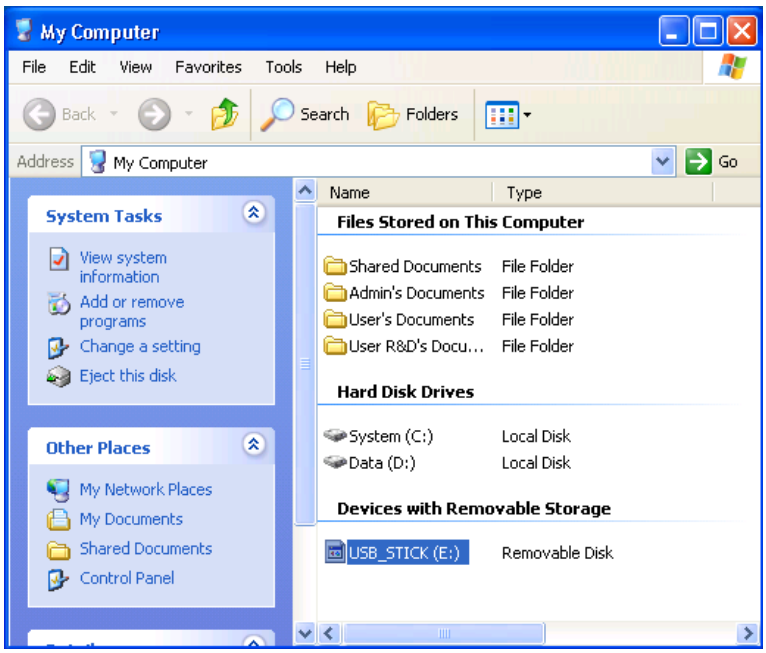
The following instruction describes the installation of the AssayPlugin and ArrayMate installation software (SDK).

1. Download the AssayPlugin **and** the ArrayMate SDK from http://alere-technologies.com/en/products/lab-solutions/e-coli/e-coli-genotyping-kit.html

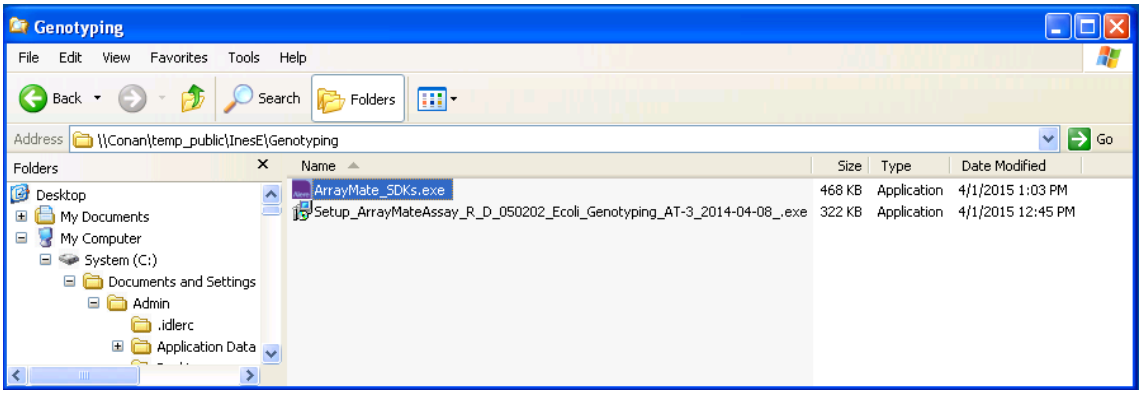
2. Copy downloaded files (Plugin and SDK setup) to an USB Memory Stick and connect it to the ArrayMate.

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** (default password: abcde).

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 ArrayTube rack with an unprocessed ArrayTube into the ArrayMate than press **Next.**

   After the Experiment Run the ArrayMate automatically enters the Archive mode and displays the results of the last experiment.
3. 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	050202	X	X	X
01-B	-	2	050202	X	X	X
01-C	-	3	050202	X	X	X
01-D	-	4	050202	X	X	X
01-E	-	5	050202	X	X	X
01-F	-	6	050202	X	X	X

Kit Components, Storage and Stability

All reagents are provided in surplus (see below). If necessary, all components may be ordered separately; please refer to the catalogue reference numbers (Cat#) at the end of this manual. 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 packaging. All components have been stability tested for short term shipment (<1 week) at ambient temperature (< 37°C). The assay components with limited stability are D1 and C3. The other kit components have proven to be stable six months post expiry.

Cell Lysis (optional)

- **A1**: Lysis Buffer (Cat# 245101000)
  Store at 18°C to 28°C (ambient temperature). Surplus: 200 %.
- **A2**: Lysis Enhancer (lyophilised, Cat# 245102000)
  Store at 18°C to 28°C (ambient temperature). Centrifuge A2 tubes shortly prior to opening. Add 200 µl Buffer A1 to Lysis Enhancer before use. Mix well and store for less than 1 week at 2°C to 8°C. Sufficient for 96 isolations.

DNA Labelling and Amplification

- **B1^{EC}**: Labelling Buffer, Store at 2°C to 8°C. Surplus: 45 %.
- **B2**: Labelling Enzyme, Store at 2°C to 8°C. Surplus: 300 %.

Hybridisation and Detection

- ArrayTubes (10 x 5 samples), protected against light and sealed under inert gas. Store at 18°C to 28°C. After opening, tubes are to be used within two weeks. Close unused ArrayTubes, protect them against humidity and dust and store in the dark. Avoid ANY touching or scratching the microarray on the bottom of the vial.

Please note: *Do not store or handle unused wells above 60 % relative humidity since this may irreversibly corrode the spots.*

- **C1**: Hybridisation Buffer. Store at 18°C to 28°C. Protect against sunlight. Surplus: 100 %.
- **C2**: Washing Buffer 1. Store at 18°C to 28°C. Surplus: 140 %.
- **C3**: 100x HRP Conjugate. Store at 2°C to 8°C. Surplus: 300 %.
- **C4**: Conjugate Buffer. Store at 18°C to 28°C. Surplus: 500 %
- **C5**: Washing Buffer 2. Store at 18°C to 28°C. Surplus: 140 %.
- **D1**: Horseradish Peroxidase Substrate. Store at 2°C to 8°C. Protect against direct sunlight. Surplus: 200 %.
- **Optional: CM^{EDL}**: Reference DNA from *E. coli* EDL933 (GenBank accession number NC_002655.2), c_{DNA} = 0.1-0.4 µg/µl. Store at 2°C to 8°C. Sufficient for 5-6 tests.

Components required but not provided

- Growth media for the cultivation of *E. coli*. The test should be performed with colonies harvested from 2xTY or Columbia blood agar. Liquid media should not been used because contaminations or mixed cultures cannot be ruled out easily.
- Equipment and consumables needed for the cultivation of *E. coli* (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# 11796828001).

Please note: *The DNA specimen needs to be free of RNA. Recommendation: a pre-treatment using the cell lysis components A1/A2 (see below) or a standard RNase A treatment during DNA preparation.*

- 1x PBS
- RNAse A (we recommend Qiagen's RNase A solution, 100 mg/ml, Qiagen Cat# 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
  Please note: *We recommend the Eppendorf's Thermomixer Comfort equipped with a heating block for 1.5ml tubes.*
- Pipettes: suitable for 1 µl-5 µl volumes, 90 µl, 100 µl, 200 µl, 1000 µl
- Multichannel Pipettes for 100-200 µl
- Sterile reaction vials suitable for PCR (VWR Cat# 732-0098)
- Ultrapure (PCR grade) water
- Pasteur pipettes (VWR Cat# 612-2856)

PROTOCOLS

Culturing and Harvesting Bacterial Cells

***E. coli* 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 *E. coli* on 2xTY or Columbia blood agar (overnight at 37°C or 48 h at room temperature). Make sure that you have a pure, monoclonal culture of *E. coli*. 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 ***E. coli* Genotyping Kit** is 0.5-2 µg (c_{DNA} = 0.1-0.4 µg/µl) 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 *E. coli* cells using bead beaters, ultrasonication or aggressive chemicals such as in alkaline lysis protocols. Most performance problems with the ***E. coli* Genotyping Kit** 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.

DNA Extraction via Spin Columns (e.g. Qiagen DNeasy Blood&Tissue)

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

Loop empty Loop full

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

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

Optional cell lysis with A1/A2 reagent (instead of 1xPBS):

- *Centrifuge A2 tube shortly, open it, add 0.2 ml of Lysis Buffer A1 to Lysis Enhancer A2 and dissolve.*
- *Add an inoculating loop full of monoclonal colony material of the E. coli isolate to this A1/A2 reagent and vortex thoroughly.*
- *Incubate the colony material of the E. coli isolate in A1/A2 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 that is as follows:
- Add 20 µl proteinase K (Qiagen Kit, or equivalent) and add 200 µl buffer AL (Qiagen Kit).
- Vortex briefly or shake vigorously.
- Incubate for 30-60 min at 56°C and 550 rpm in the thermoshaker.
- **important**: If A1/A2 reagent not used, add now 4 µl RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature before continuing.
- Add 200 µl 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 µl 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.

DNA Extraction by Heat Lysis

*Please note: Only a **fresh** overnight culture can be used. After DNA extraction by heating the linear amplification must be done **immediately**. Storage of extracted DNA is not recommended.*

- Add a 1 µl inoculating loop (Please Note: do not use too much culture material, see Figure below) of a monoclonal *E. coli* isolate to 50 µl PCR-grade distilled water and vortex thoroughly.
- Incubate at 99°C, 15 min at 550 rpm in a thermoshaker.
- Centrifuge for 5 min at 13,600 rpm at room temperature.
- Carefully pipette 25 µl supernatant into a new 1.5 ml tube and discard the old tube with the pellet.
- Add 0.25 µl RNase A (not provided / see above) with a stock concentration of 1 mg/ml.
- Incubate at 37°C, 5 min at 550 rpm in a thermoshaker.
- Use 5 µl of this DNA suspension for the linear amplification and internal Biotin labelling process.

Linear Amplification and Internal Biotin Labelling

Please keep in mind the limited surplus of reagents whilst pipetting. The surplus of $B1^{EC}$ labelling reagent is 45 %.

- Prepare a Master Mix by combining 4.9 µl of $B1^{EC}$ labelling reagent and 0.1 µl of B2 (DNA polymerase) per sample.
- Add 5 µl of *E. coli* DNA (c_{DNA} = 0.1-0.4 µg/µl) prepared as described above to 5 µl of the Master Mix ($B1^{EC}$/B2). Do not forget to label the vial!
- Perform amplification in a pre-programmed thermocycler (e.g., Eppendorf Mastercycler gradient with heated lid) according to following protocol:

<table>
<tr><td colspan="2">Pre-heat cover/lid to 105°C</td></tr>
<tr><td>300 sec at 96°C</td><td></td></tr>
<tr><td rowspan="3">50 cycles with</td><td>20 sec at 50°C</td></tr>
<tr><td>40 sec at 72°C</td></tr>
<tr><td>60 sec at 96°C</td></tr>
<tr><td colspan="2">Cool down to 4°C, hold</td></tr>
</table>

- 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 and the control DNA (CM) supplied with the kit.

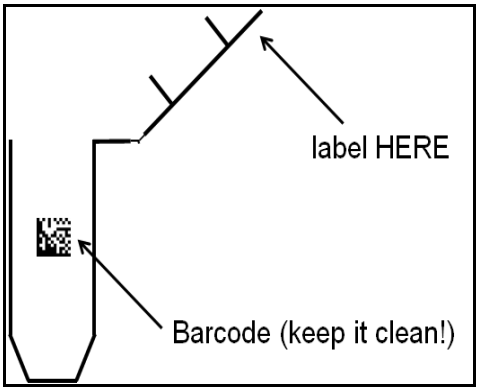
Hybridisation

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, only use C2 Washing Buffer!*

Always label your ArrayTubes 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.



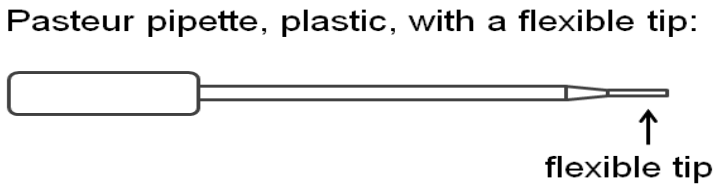
Avoid contact of data matrix barcode with organic solvents! The ArrayMate needs the information encoded in the data matrix to perform the assay and the analysis afterwards.

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

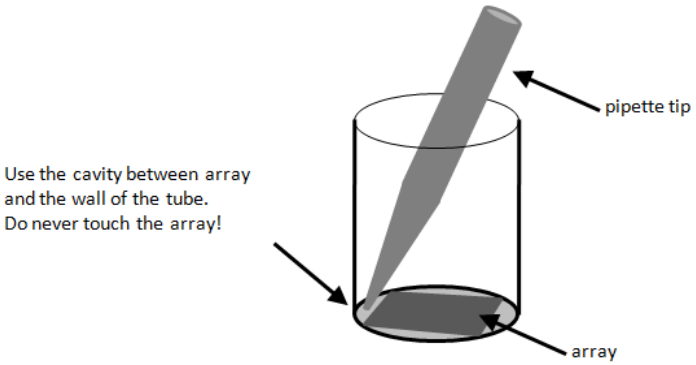
General Remarks - Handling of Liquids

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. Fine tipped soft, disposable Pasteur pipettes are suited best (such as VWR Cat# 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 be centrifuged (1 min, 13,000 rpm) 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, do **not** remove reagent D1 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 ArrayTubes and Eppendorf's Thermomixer Comfort, equipped with a heating block for 1.5 ml Eppendorf tubes. Thus we recommend the use of either device. 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 the control DNA supplied with the kit.

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 ArrayTube from the bag (open the bag at its predetermined breaking point).
- Add 500 µl of ultrapure water to each tube.
- Incubate in the thermoshaker at 55°C, 550 rpm for 2 minutes.
- Remove and discard the water WITHOUT TOUCHING THE ARRAY SURFACE.
- Add 500 µl buffer C1 to each tube.
- Incubate in the thermoshaker at 55°C, 550 rpm for 4 minutes.
- 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 ArrayTube (avoid extensive foaming).
- Incubate in the thermoshaker at 55°C, 550 rpm for 60 minutes.

Note: Meanwhile, login to the ArrayMate device and prepare your worklist (see section "worklist" S. 21)

Dilute Streptavidin-Horseradish-Peroxidase (C3, C4) – HRP-conjugate

- Combine reagent C3 (Streptavidin-Horseradish-Peroxidase) and Buffer C4 in a ratio of 1:100, the mixture is stable for 1 day at room temperature; C3 is delivered with a surplus of 300 %, C4 is delivered with a surplus of 500 %.

Pipetting scheme: ArrayTubes (AT)

	1 AT	2-3 ATs	4-6 ATs	7-10 ATs	11-15 ATs
C3	1.5 µl	3.5 µl	7 µl	11 µl	16 µl
C4	150 µl	350 µl	700 µl	1100 µl	1600 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°C to 25°C until use.

Washing after hybridisation

- Please keep in mind the limited surplus of C2 (140 %).
- Remove the ArrayTubes from the thermoshaker.
- Set the thermoshaker to 30°C for the following steps.
- Carefully open the tubes and remove the hybridisation mixture as completely as possible (without touching the array surface).
- 1st Washing step after hybridisation: add 500 µl of buffer C2 and incubate in the thermoshaker at 30°C, 550 rpm for 5 min, remove and discard the washing solution.
- 2nd Washing step after hybridisation: repeat 1st Washing step.

Please note: A carryover of more than 1 % of buffer C1 to the next step will denature the HRP.

Addition of HRP-conjugate

Please note: *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 ArrayTubes! Please keep in mind the limited surplus of C3 (300 %).*

- Add 100 µl of prepared C3/C4 mixture to each tube.
- Incubate in the thermoshaker at 30°C, 550 rpm for 10 minutes.
- Remove and discard C3/C4 mixture completely.

Washing step after binding of conjugate addition of HRP-conjugate

- Please keep in mind the limited surplus of C5 (140 %).
- 1st Washing step after conjugation: add 500 µl of buffer C5, incubate in the thermoshaker at 30°C, 550 rpm for 1 minute, remove and discard the washing solution.
- 2nd Washing step after conjugation: Repeat 1st Washing step.

Please 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

Please note: *Do **not move** ArrayTubes during staining. The reagent D1 contains a substrate for Horseradish Peroxidase. Please keep in mind the limited surplus of D1 (200 %).*

- Set the thermoshaker to 30°C for the following steps.
- Add 100 µl pre-warmed reagent D1 to each well (supernatant of centrifuged D1 without precipitate).
- Incubate at 25°C **WITHOUT** agitation for 10 min.
- Read out **WITHOUT** removing D1 (removal of D1 would leave air bubbles within the tubes).

Please note: *The ArrayTubes as used in this kit do have a different geometry than the 8-well ArrayStrips that are used in other kits. Therefore, unlike the directive for ArrayStrips, D1 is NOT to be removed from the ArrayTubes before reading.*

Check immediately all images for cleanliness (i.e., absence of dust particles, residual liquids) and for good focus. Dust particles and residual fluids inside the vial can be removed by cautiously washing twice with 200 µl PCR-grade distilled water. If necessary, scan and process again.

Data Analysis

Starting the ArrayMate Reader

- We recommend starting the ArrayMate Reader after starting the hybridisation; this allows the convenience of starting the device and importing 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-in as ***R&D User*** (Research and Development User) for full access to test specific software (default password: abcde). If you log-in as **User**, you will obtain only raw values, but neither positives/negatives interpretation nor strain assignment. The **Administrator** log-in (default password: 12345) will allow the installation of a new assay specific plug-in, which can be downloaded at http://alere-technologies.com.
- The user interface will be loaded, ArrayMate performs internal testing. This will require slightly 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.

Worklist

A **Worklist** file allows an identifier, such as a laboratory or sample number, to be linked to the respective array position on 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 that have headers written in the first line. The following headers are obligatory (in this order): position / sampleID / assayID (Table 1).
- Positions are consecutively numbered from 1 to a maximum of 6. Do not leave empty lines in the worklist. If you use EXCEL, position numbers should be entered into column A.
- Sample IDs are strain/sample/laboratory numbers such as exported from your LIMS (or assigned in any different way). Patients' names should not be used as sample IDs.
- The Assay ID allows the system to identify the current test and to correctly use information on layout, spot number, and identity etc. The ***E. coli*** **Genotyping Kit** has the Assay ID: **050202**.

Please note: When entering assay IDs manually, make sure to enter the correct number as entering wrong numbers could lead to errors or loss of data.

- We recommend using a printout of the worklist as a 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.
- You may use the software tool **Worklist Generator** to create a worklist easily.

  http://alere-technologies.com/en/products/lab-solutions/software-tools/worklist-generator.html

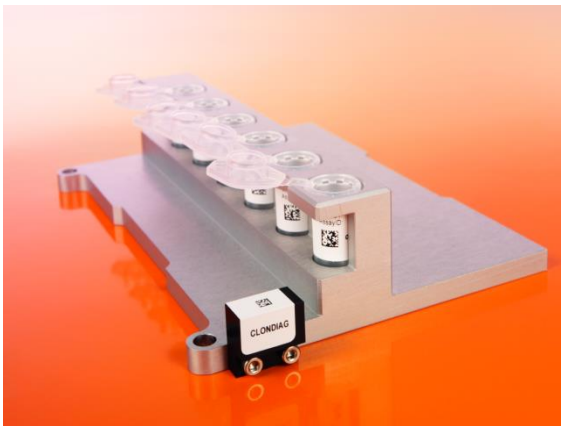
  Table 1: Example worklist. *Please note: Table header must be written **exactly** as shown.*

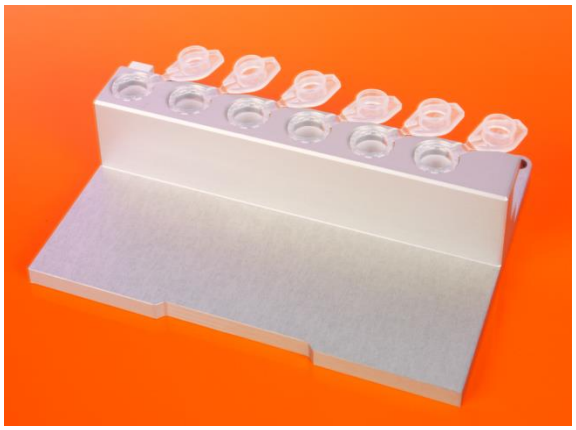
position	sampleID	assayID
1	EDL933_ATCC700927_260315	050202
2	CFT073_260315	050202
3	018_UI_007807_MK_260315	050202
4	084_BIK_014064_260315	050202
5	038_UI_007961_260315	050202
6	EPEC E2348.69_260315	050202

Data Acquisition in the ArrayMate Reader

- Insert your memory stick containing the worklist into any of the USB ports down to the right hand side of the ArrayMate.
- Press [...]; a folder selection dialogue will open.
- Select your worklist (path: “My Computer/Removable Disk”).
- Open your selected worklist by pressing **Enter** or **Open**.
- Press  (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 **OK**; the worklist window will close.

- Leave the memory stick in the ArrayMate if you intend to export ***E. coli* Genotyping** Test Reports afterwards (check the memory stick for computer viruses and malware using an appropriate program on a regular basis).
- Press **Next** (at the bottom right on the screen; reader is opening).
- Carefully insert the appropriate metallic adapter/frame into the ArrayMate. Do not apply strong force. Assure proper fit, otherwise the images may be out of focus.
- After having inserted the adapter, carefully insert the Array Tubes into the adapter.
- ArrayTubes need to be open with tube-lid connections placed into appropriate notches:





- **Assure proper fit**, otherwise the images may be out of focus.
- Barcodes on ArrayTubes and holder must be clean.
- Press **Next** (at the bottom right on the screen; reader closes, analysis program starts, it takes about 2-10 min, depending on the number of ArrayTubes; the 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 **Next** (at the bottom right on the screen; reader is opening).
- Remove the adapter with the ArrayTubes.

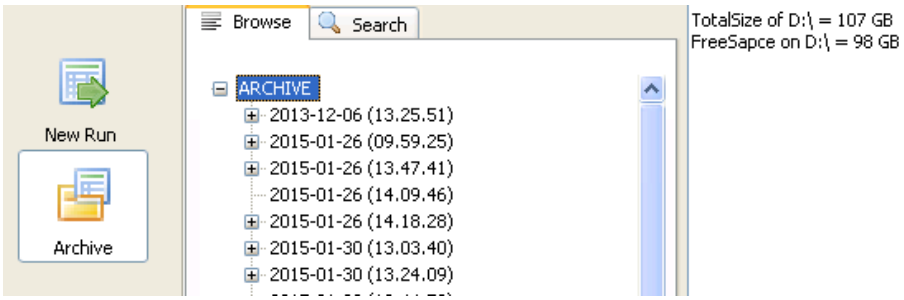
- Press **Next** (at the bottom right on the screen; reader is closing).

Results

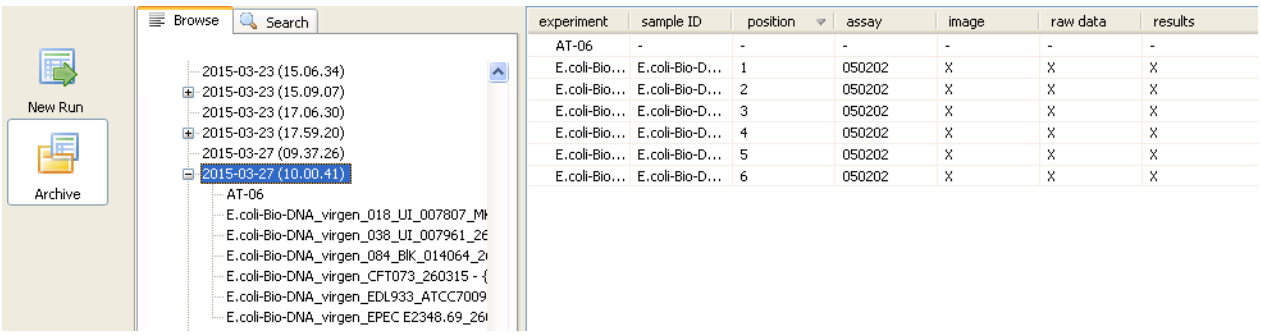
On the left-hand side of the screen you will see a list showing all runs stored on the ArrayMate´s hard disk. A run contains the results from all arrays analysed together within one frame. If this list is not displayed:

- Press **Archive** (left hand side) and activate the flag **Browse** (at the top left).
- The runs are organised like folders in **Windows Explorer**, and named **by default** according to the date of acquisition.

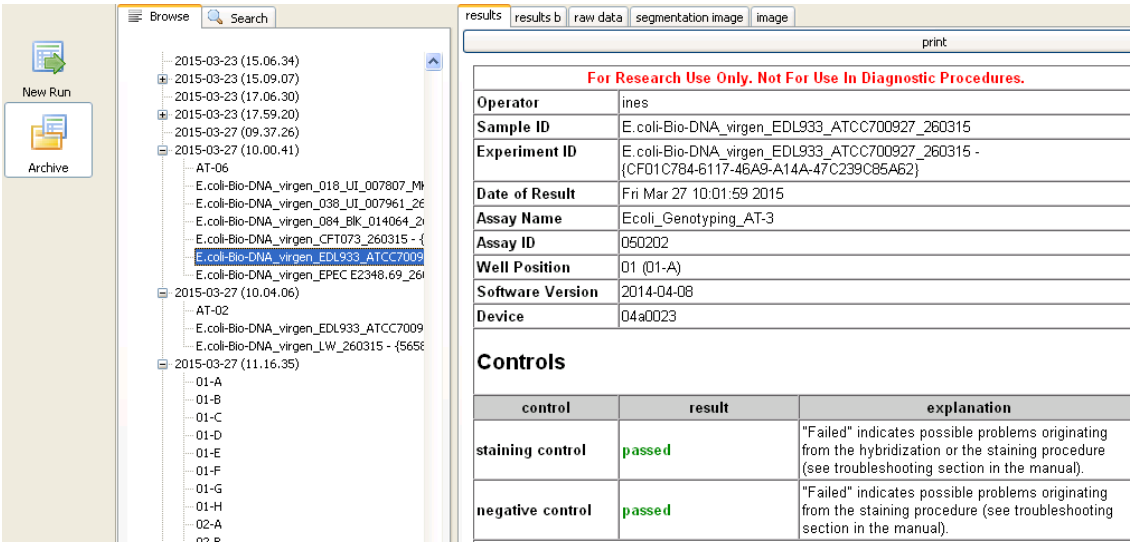
Example: there are several readings in the archive, by default they are named by date and time of day of creation (which you may have changed, see section “setup of the ArrayMate reader”):



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.

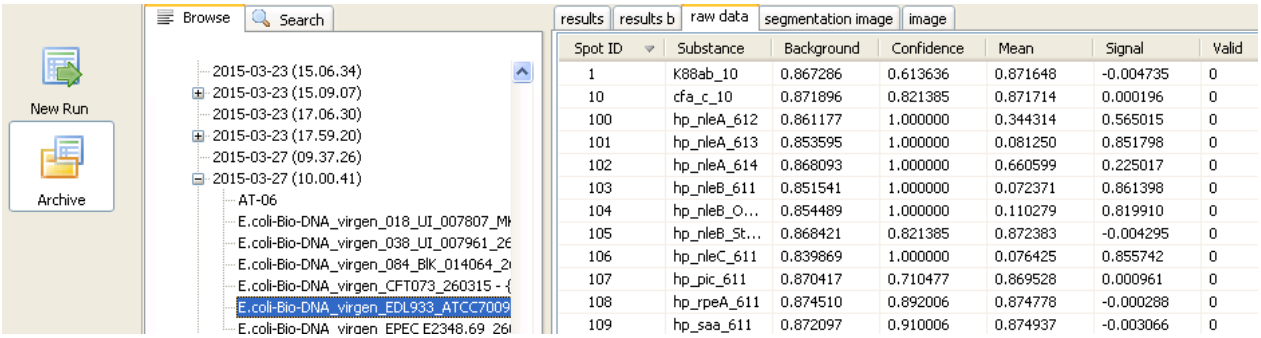


Activate the tab **results** (top left) and click onto the position of an individual experiment the report of this particular array will appear on the right side of the window:



*Please Note: the flag **results b** is not active with this assay.*

Activate the tab **raw data** (top left) and the raw signal results of this particular array will appear on the right side of the window:

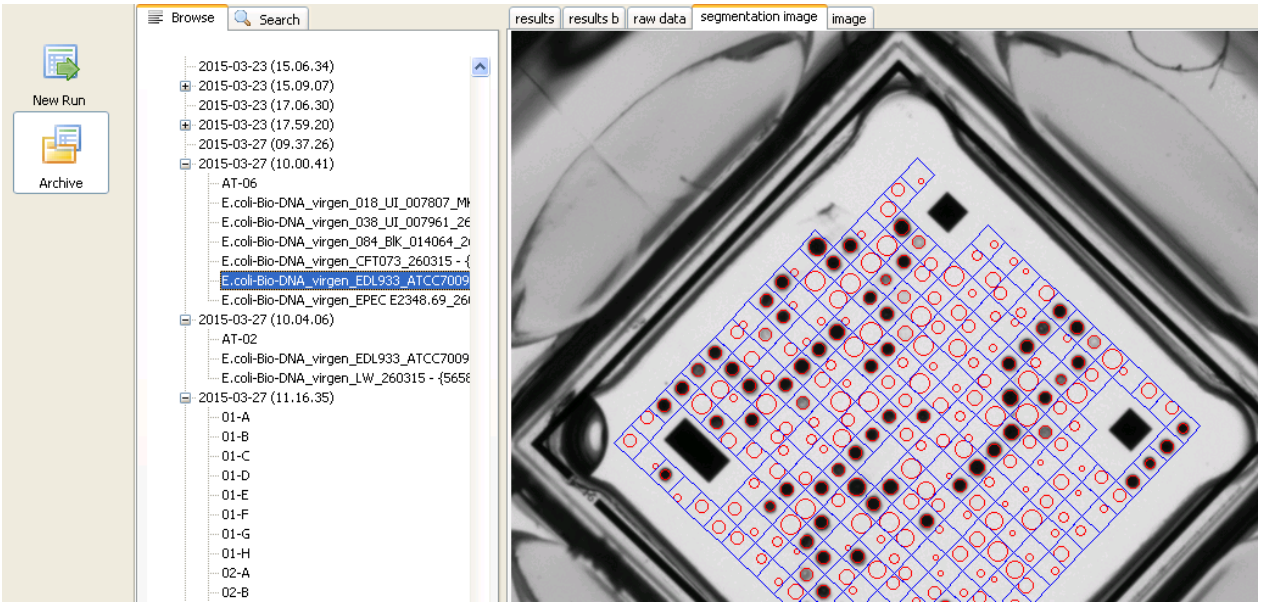


Interpretation of the "raw data" list:

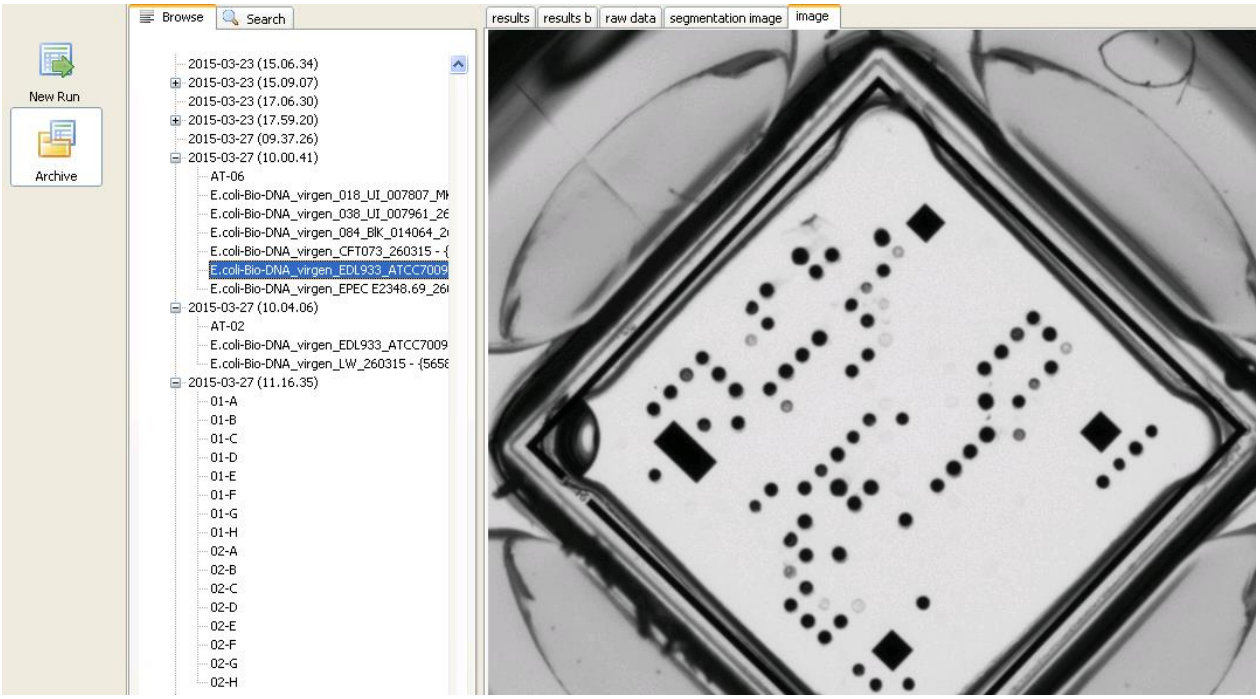
- Spot ID: numerical identifier of the spot on the array
- Substance: name of the DNA probe
- Confidence: an intrinsic estimate of spot confidence based on size and shape of that particular spot, where 1 = high confidence and 0 = no confidence
- Signal: spot signal intensity (grey scale value), where 1 = black and 0 = white
- Valid: 0 = valid; 1 = invalid (confidence below 0.75)
- Background: luminous intensity of the background, where 1 = maximum brightness and 0 = maximum darkness
- Mean: luminous intensity of the signal (spot), where 1 = maximum brightness and 0 = maximum darkness

Please note: The correlation between mean, background and signal is roughly: 1-mean/background, however, there are some correction factors that depend on the statistics of pixel distribution.

Activate the tab **segmentation image** and the analysed picture of this particular array will appear on the right side of the window:



Activate the tab **image** and the picture of this particular array will appear on the right side of the window:



Export of Test Results

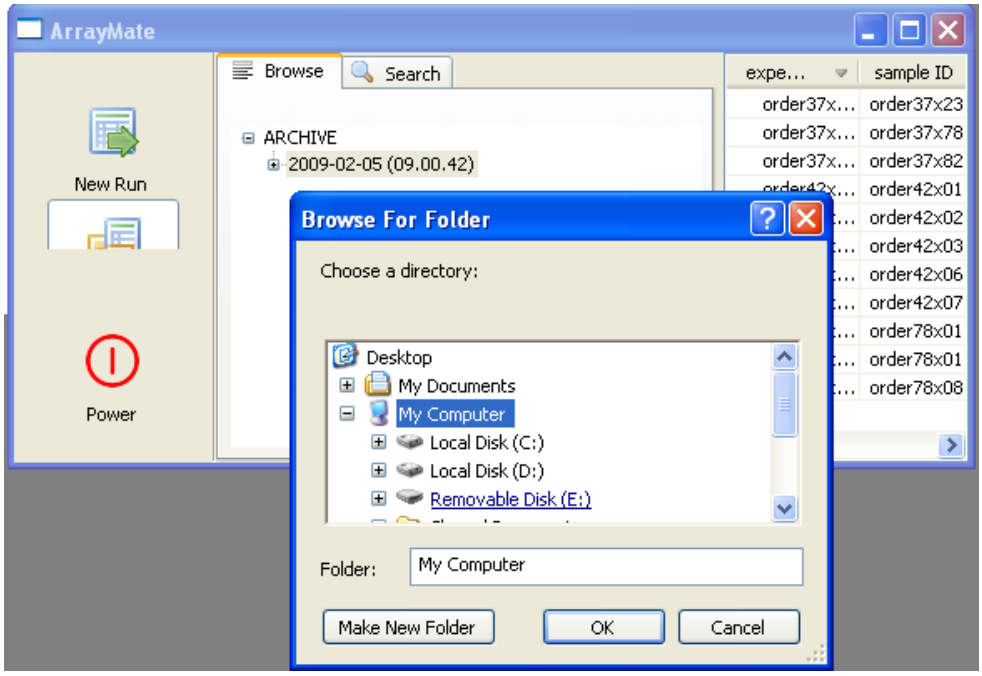
The generated result files in an html format will show information of all target genes. Possible invalid controls that might display in this report will be explained below (see Troubleshooting).

Other files that are generated and that can be exported include:

- A text file (*.txt) with the raw measurements (raw data),
- An image file (*.bmp) with the actual photo of the array,
- A second image file (*.png) in which the coordinate grid is superimposed and the recognised spots are circled (segmentation image), and
- A XML file (*.xml) that contains the same information like the html result sheets for future export into databases and for using the **Result Collector** tool.

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

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



- Click **My Computer**, then **Removable Disk** and choose the folder where to save or click **Make New Folder** (on the bottom, a new folder icon appears).
- Rename the new folder (e.g. with the experiment name or date).
- Click **Ok** (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 **Power** (at the bottom left on the screen): 
- Switch off the screen. There is no need to physically switch off the ArrayMate Reader.

TROUBLESHOOTING

In case of trouble always make sure that reagents are within the recommended shelf-life and stored under appropriate conditions.

Should you encounter a problem, we will always be happy to support you. Please e-mail to cct.home@clondiag.com and include a description of the problem as well as the array images (*.bmp files) in question.

Staining Control

A staining control is included to check whether possible problems originate from the hybridisation or the staining procedure. If the staining control has "Failed" proceed as follows:

Horseradish peroxidase conjugate may have degraded during storage. Add 1 µl mixture 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 with buffer C2 of the wells to remove all of buffer C1 prior to adding horseradish peroxidase conjugate.

If the staining control has "Passed", refer to the following hints.

Image Quality

In case of poor image quality we recommend to re-check DNA quantity and quality first by loading leftover DNA on an agarose gel.

In order to determine whether any problems originated from the DNA preparation, you might perform an experiment with the Control Material CM^{EDL}. This is genomic DNA from *E. coli* EDL933 (GenBank accession number NC_002655.2). It is be provided free of charge upon request. If the control experiment yields a valid result and a correct identification of probes *gapA, ihfA* and *gad,* there was probably an issue with DNA preparation. If the control experiment also fails, an error affecting later steps or a degradation of reagents from later steps is likely.

See also Appendix 2 – Images for troubleshooting (p. 35 and 36).

DNA Quality

The amount of DNA is crucial because of the linear kinetics of amplification (see Introduction). 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. A_{260} readings will cover RNA and other contaminants as well. Therefore pure DNA preparations without RNA contaminations are a prerequisite for proper DNA concentration measurement. RNAse treatment prior to A_{260} 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 for this reason not be prepared by disrupting bacterial cells using bead beaters, ultrasonication or aggressive chemicals such as in alkaline lysis protocols. We made good experiences with the manual QIAGEN DNeasy kit and the automated device EZ1.

DNA must be free of any traces of ethanol, as ethanol strongly influences the amplification. It is possible to heat the sample prior to adding it to the labelling mix (5-10 minutes at 70°C). Some problems with samples from the Qiagen EZ1 device for example were resolved after heating the samples (see above).

Physical Damage to 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.

Ambiguous Results

Apart from a "positive" or "negative" result for the individual markers on the ***E. coli* Genotyping** Test Report, the result can also be "ambiguous".

In cases affecting virulence factors, no definitive answer with regard to this specific marker can be given. This can be caused by poor sample quality, poor signal quality and, especially in some resistance-associated genes by the presence of plasmids in low copy numbers.

Allelic variants of some markers differ only in single or few nucleotides. This can cause the effect that the actual allele yields a positive signal while other, mismatching probes give ambiguous rather than negative results.

ADDITIONAL INFORMATION

Warranty

Alere guarantees the performance as described in this manual. Usage of the Assay 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, 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 reasons other than misuse, contact Alere for replacement or refund. Terms and conditions apply.

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

Disclaimer

This system is for research use only.

We do not accept any liability for damages caused by misuse.

Furthermore, we do not accept any liability for damages caused by inappropriate use of the device as a personal computer, for instance related to the use of additional software, to network connections, or to a breach of privacy related to the storage of confidential information (such as names of patients) on its hard disk and/or to the use of external storage devices that might be contaminated with spyware.

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 may be ordered separately:

component	name	amount	cat#	storage
A1	Lysis Buffer	30 ml	245101000	18-28°C
A2	Lysis Enhancer	96 units	245102000	18-28°C
B1^{EC}	Labelling Buffer	360 µl	245403000	2-8°C
B2	Labelling Enzyme	20 µl	245104000	2-8°C
C1	Hybridisation Buffer	30 ml	245105000	18-28°C
C2	Washing Buffer 1	120 ml	245106000	18-28°C
C3	HRP Conjugate 100x	200 µl	245107000	2-8°C
C4	Conjugate Buffer	30 ml	245108000	18-28°C
C5	Washing Buffer 2	120 ml	245109000	18-28°C
D1	HRP Substrate	15 ml	245110000	2-8°C
CM^{EDL}	Control Material *E. coli* EDL933 DNA	30 µl	on request	2-8°C
ArrayTubes	Identibac Ec 03	5 ATs	201009288	15-28°C

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

Legal Manufacturer

Alere Technologies GmbH

Loebstedter Str. 103-105

07749 Jena, Germany

Contact

If you require any further information on this product please contact cct.home@clondiag.com

LITERATURE

1. **Development of a miniaturised microarray-based assay for the rapid identification of antimicrobial resistance genes in Gram-negative bacteria.** Batchelor M, Hopkins KL, Liebana E, Slickers P, Ehricht R, Mafura M, Aarestrup F, Mevius D, Clifton-Hadley FA, Woodward MJ, Davies RH, Threlfall EJ, Anjum MF; Int J Antimicrob Agents. 2008 Feb 1.
2. **Identifying antimicrobial resistance genes of human clinical relevance within *Salmonella* isolated from food animals in Great Britain.** Muna F. Anjum, Suman Choudhary, Victoria Morrison, Lucy C. Snow, Muriel Mafura, Peter Slickers, Ralf Ehricht and Martin J. Woodward; J. Antimicrob. Chemother. (2011)

For further literature please refer to:

http://alere-technologies.com/en/science-technologies/publications/

UPDATES & SOFTWARE

Notifications on database/software updates and freeware tools can be found at:

http://alere-technologies.com/en/products/lab-solutions/e-coli/e-coli-genotyping-kit.html

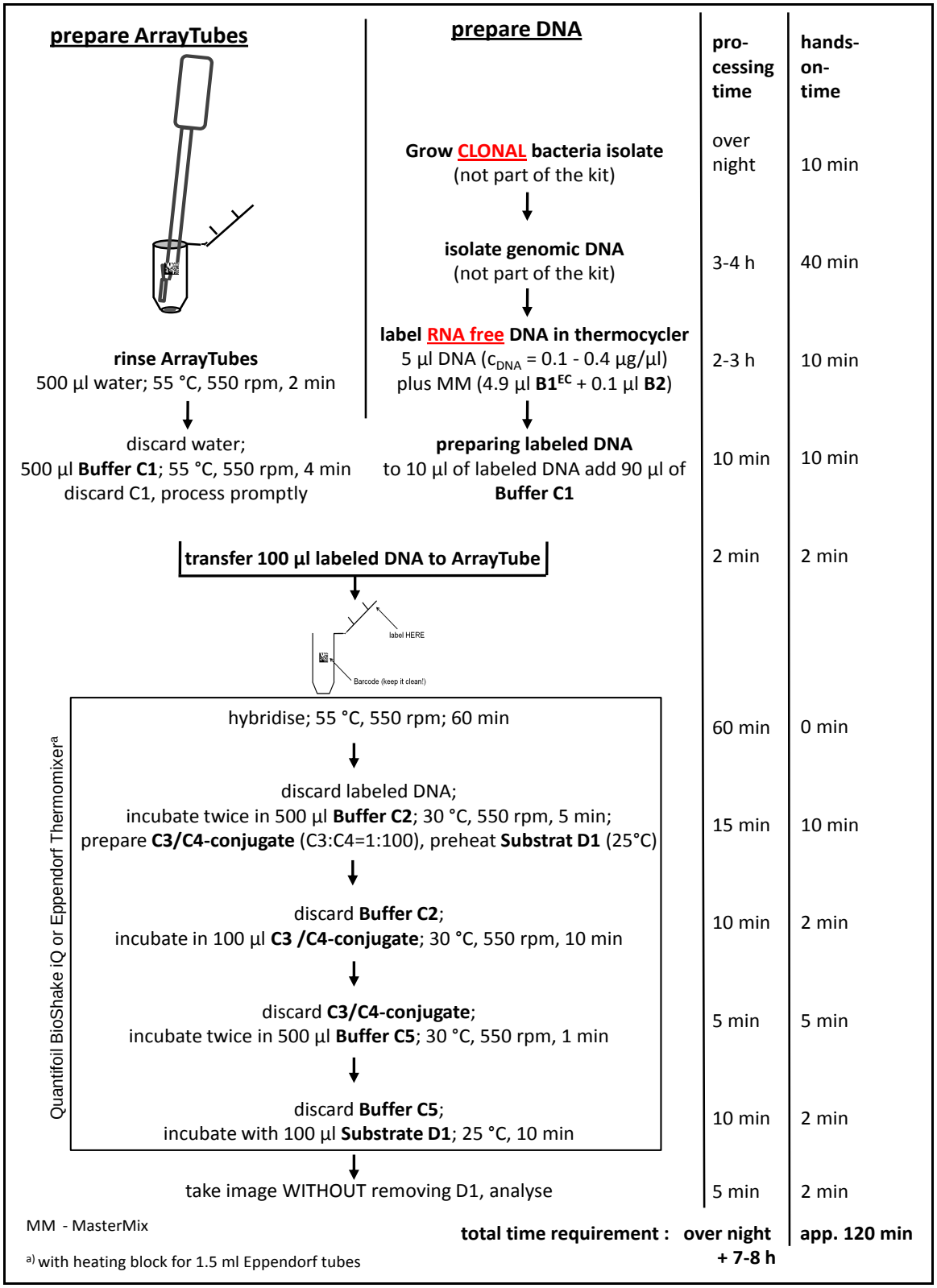
http://alere-technologies.com/en/products/lab-solutions/software-tools.html

and/or http://alere-technologies.com/en/news.html

Currently available freeware programs are:

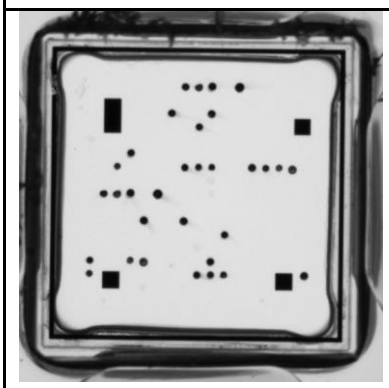
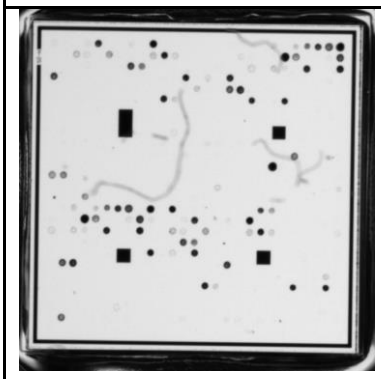
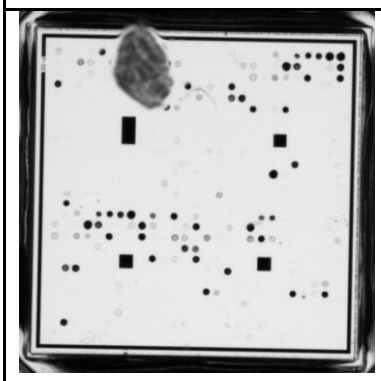
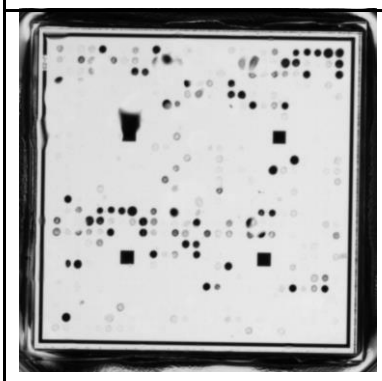
- Alere **Result Collector** for the conversion of multiple *result.xml files from the ArrayMate into spreadsheet tables. This should make it easier to compare isolates or to determine relative abundances of genes or strains etc.
- Alere **Worklist Generator** is a tool which helps you to create a well formatted worklist for the Arraymate.
- Alere **Report Generator** is a software tool to create reports using the assay software normally used and installed on the ArrayMate. It uses an image taken by the ArrayMate or a txt file (raw signal data file) and generates a report from the raw signal data.

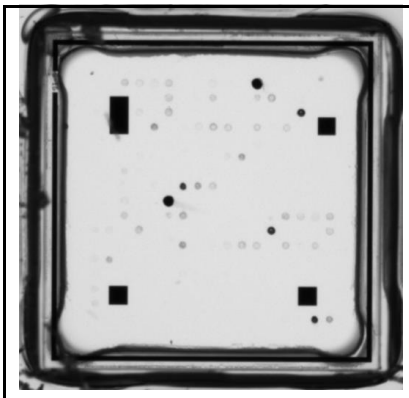
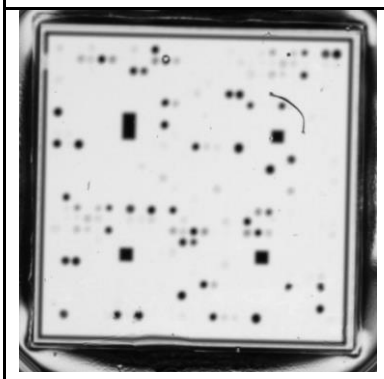
APPENDIX 1 - FLOW CHART



The figure on this page summarises the test procedure. However, please refer to the text section of this manual at any step of the test protocol for further important details.

APPENDIX 2 – IMAGES FOR TROUBLESHOOTING

Image	Comment	Handling
	Valid experiment.	Valid results, no error messages.
	The bottom of the AT is contaminated with dust particles.	Please clean the bottom of the well, scan and process again.
	The microarray surface is contaminated with dust particles.	If the microarray surface is contaminated with particles, wash the microarray with double distilled water (pipetting water carefully up and down, remove), scan and process again.
	The bottom of the AT is contaminated with a liquid (e.g. buffer).	Please clean the bottom surface with a cleanroom wipe, scan and process again.

	Signal intensity is too low.	This could be due to low DNA concentration, fragmented DNA, ethanol trace contaminations in DNA sample or expired reagents. The experiment should be repeated with a new DNA preparation. If this also fails, try an experiment with EDL933 control DNA (CM, available on request).
	Chip was not in focus during image acquisition.	Repeat image acquisition after fitting the ArrayTube in the frame.

APPENDIX 3 - GENE LIST

Accosiated with	Genes	Probes	Description
Family, Genus and Species Genes	gad	gad_10	glutamate decarboxylase (AE014075.1)
Family, Genus and Species Genes	gapA	prob_gapA_611	glyceraldehyde 3-phosphate dehydrogenase A (CP000468.1)
Family, Genus and Species Genes	ihfA	prob_ihfA_611	integration host factor subunit alpha (U00096.3)
Family, Genus and Species Genes	ipaH9.8	ipaH9.8_20	invasion plasmid antigen (AF047365.1 [160:1797])
Toxin	astA	astA_consens_10	heat stable enterotoxin (consensus sequence)
Toxin	cba	cba_10	colicin B activity protein (M16816.1)
Toxin	ccl	ccl_10	colicin activity protein (AF540491.1)
Toxin	cdtB	cdtB_40, cdtB_50, cdtB_60	cytolethal distending toxin subunit B (AJ508930.1)
Toxin	celB	celb_10	colicin lysis protein (X03632.1)
Toxin	cma	cma_20	colicin M activity protein (CP000971.1)
Toxin	cnf1	cnf1_20	cytotoxic necrotizing factor type 1 (CP000243.1)
Toxin	hlyA	hlyA_20	hemolysin A (AB011549.2)
Toxin	hlyE	hlyE_10	hemolysin E (AF052225.1)
Toxin	ipaD	ipaD_10	IpaD - invasin (CP000035.1)
Toxin	ltcA	ltcA_20	heat labile enterotoxin subunit A (AB011677.1)
Toxin	mchB	mchB_10	microcin H47 activity protein (AJ515252.1)
Toxin	mchC	mchC_20	member of the microcin operon (AJ515252.1)
Toxin	mchF	mchF_10	putative microcin L transport protein (AJ515252.1)
Toxin	mcmA	mcmA_10	microcin M truncated protein (AJ515252.1)
Toxin	pet	pet_20	enterotoxin (SPATE) (AF056581.1)
Toxin	sat	hp_sat_611	Sat serine protease (SPATE) (AJ586888.1)
Toxin	senB	senB_20	enterotoxin (Z54195.1)
Toxin	sta1	sta1_110	heat stable enterotoxin I (AJ555214.1)
Toxin	sta2	sta2_210	heat stable enterotoxin II (CP000795.1)
Toxin	stb	stb_10	heat stable enterotoxin Stb - enterotoxin B (M35729.1)
Toxin	subA	hp_subA_611	subtilase cytotoxin, subunit A (AF399919.3)
Toxin	toxB	hp_toxB_611, hp_toxB_612, hp_toxB_613	cytotoxin B (AB011549.2)
Toxin	virF	virF_20	transcriptional regulator - required for transcription of virB and icsA (AF348706.1)
Shiga Toxin	stx1	stx1A_10	shiga toxin 1
Shiga Toxin	stx2b	hp_stxB2_612	shiga toxin 2 variant b (AB012101.1)
Shiga Toxin	stx2e	hp_stxA2_616	shiga toxin 2 variant e (X81415.1)
Shiga Toxin	stx2f	hp_stxA2_611, hp_stxA2_613	shiga toxin 2 variant f (AJ270998.1)
Shiga Toxin	stx2g	hp_stxA2_617	shiga toxin 2 variant g (AJ966782.1)
Shiga Toxin	stx2a,c,d	hp_stxA2_614, hp_stxA2_618, hp_stxB2_614, stx2A_10	shiga toxin 2 variant a, shiga toxin 2 variant c or shiga toxin 2 variant d (X61283.1)

Accosiated with	Genes	Probes	Description
adhesins	eae_consensus	eae_consensus_10, eae_consensus_20, eae_consensus_30, eae_consensus_40	an outer membrane protein important for the attachment to host cells; pathogenesis factor (M58154.1)
adhesins	efa1	hp_efa1_611	lymphocyte inhibitory factor A - adherence factor (AF159462.2)
adhesins	espB_O157	espB_O157_20	EspB - protein (type III secretion system; O157:H7) (BA000007.2)
adhesins	espB_O26	espB_O26_40	EspB - protein (type III secretion system, 26:H- and O15:H-) (AJ287768.1)
adhesins	fasA	fasA_10	adhesin - fimbrial major subunit (M35257.1)
adhesins	fedA	fedA_10	adhesin - fimbrial major subunit (M61713.1)
adhesins	fedF	fedF_10	adhesin - fimbrial protein (Z26520.1)
adhesins	fim41a	fim41a_10	adhesin - fimbrial protein (X14354.1)
adhesins	iha	hp_iha_611	adherence-conferring protein (BA000007.2)
adhesins	nfaE	nfaE_10	chaperone protein - required for the expression of aggregative adherence fimbria II (S61968.1)
adhesins	saa	hp_saa_611	STEC autoagglutinating adhesin (AF399919.3)
fimbrae	bfpA	bfpA_10	BfpA protein - essential for apoptosis signalling (AB024946.1)
fimbrae	cfaC	cfa_c_10	outer membrane usher protein (M55661.1)
fimbrae	cofA	cofA_10	major pilin subunit - CFA/III pilin (D37957.1)
fimbrae	f17-A	f17-A_40, f17-A_50, f17-A_60	major fimbrial subunit protein (L77091.1)
fimbrae	f17-G	f17-G_20	major fimbrial subunit protein (pilin G) (L43372.1)
fimbrae	fanA	fanA_10	regulatory protein (X05797.1)
fimbrae	K88ab	K88ab_10	major subunit of K88 fimbriae (V00292.1)
fimbrae	lngA	lngA_20	longus pilus structural subunit (EF595770.1)
fimbrae	lpfA	hp_lpfA_611	major fimbrial subunit (AY057066.1)
fimbrae	perA	perA_10, perA_20	transcriptional activator (AF255772.1)
fimbrae	prfB	prfB_30	major pilu subunit operon regulatory protein (X76613.1)
fimbrae	sfaS	sfaS_10	adhesin - minor Shigella fimbriae subunit (X16664.4)
secretion systems	cif	hp_cif_611	cell cycle inhibiting factor (type III secretion system) (AY128535.1)
secretion systems	espA	hp_espA_O103H2_611, hp_espA_O119H6_611, hp_espA_O127H7_611, hp_espA_O157H11_611, hp_espA_O49H12_611, hp_espA_O55H7_611, hp_espA_O8_611	EspA - protein (type III secretion system) (AF054421.1)
secretion systems	espA_C_rodentium	hp_espA_Crod_611	EspA - protein (type III secretion system), associated with Citrobacter rodentium (AF311901.1)
secretion systems	espC	hp_espC_611	EspC - extracellular serine protease (type III secretion system) (AF297061.1)
secretion systems	espF	hp_espF_611, hp_espF_612	EspF - effector protein (type III secretion system) (AE005174.2)
secretion systems	espF_C_rodentium	hp_espF_Crod_611	EspF - effector protein (type III secretion system) (AF311901.1)
secretion systems	espF_O103H2	hp_espF_O103H2_611, hp_espF_O103H2_612	EspF - effector protein (type III secretion system) (AJ277443.1)
secretion systems	espI	hp_espI_611	EspI - non-LEE encoded effector protein (LEE - EPEC Locus of Enterocyte Effacement) (AJ278144.1)
secretion systems	espJ	hp_espJ_611, hp_espJ_612	EspJ - non-LEE encoded effector protein (LEE - EPEC Locus of Enterocyte Effacement) (AE005174.2)
secretion systems	etpD	hp_etpD_611	EtpD - type II secretion pathway related protein (AF074613.1)
secretion systems	nleA	hp_nleA_611, hp_nleA_612, hp_nleA_613, hp_nleA_614	NleA - non-LEE-encoded effector protein A (type III secretion system) (AM421997.1)
secretion systems	nleB	hp_nleB_611	NleB - non-LEE-encoded effector protein B (type III secretion system (BA000007.2)
secretion systems	nleB O157:H7	hp_nleB_O157H7_611	NleB - non-LEE-encoded effector protein B (type III secretion system), associated with serotype O157:H7 (BA000007.2)
secretion systems	nleB Salmonella	hp_nleB_Styp_611	NleB - non-LEE-encoded effector protein B (type III secretion system), associated with Salmonella enterica (AE008894.1)
secretion systems	nleC	hp_nleC_611	NleC - non-LEE-encoded effector protein C (type III secretion system) (AY485823.1)
secretion systems	tccP	hp_tccP_611, hp_tccP_612	Tir - cytoskeleton coupling protein (type III secretion system) (AB275113.1)
SPATE (serin protease autotransporters)	eaaA	hp_eaaA_611	EaaA - serine protease autotransporter of Enterobacteriaceae (SPATE)(AF151674.1)

Accosiated with	Genes	Probes	Description
SPATE (serin protease autotransporters)	eatA	hp_eatA_611	EatA - serine protease autotransporter of Enterobacteriaceae (SPATE) (AY163491.2)
SPATE (serin protease autotransporters)	epeA	hp_epeA_611	EpeA - serine protease autotransporter of Enterobacteriaceae (SPATE) (AY258503.2)
SPATE (serin protease autotransporters)	espP	hp_espP_611	EspP - serine protease autotransporter of Enterobacteriaceae (SPATE) (AF074613.1)
SPATE (serin protease autotransporters)	pic	hp_pic_611	Pic - serin protease autotransporter of Enterobacteriaceae (SPATE) (U35656.1)
SPATE (serin protease autotransporters)	rpeA	hp_rpeA_611	RpeA - serin protease autotransporter of Enterobacteriaceae (SPATE) (AY552473.1)
SPATE (serin protease autotransporters)	sepA	hp_sepA_611	SepA - serine protease autotransporter of Enterobacteriaceae (SPATE) (AY604009.1)
SPATE (serin protease autotransporters)	sigA	hp_sigA_611	SigA - serine protease autotransporter of Enterobacteriaceae (SPATE) (AF200692.2)
SPATE (serin protease autotransporters)	tsh	hp_tsh_611	Tsh - hemoglobin-binding protease (SPATE) (AJ223631.1)
SPATE (serin protease autotransporters)	vat	hp_vat_611	Vat - haemoglobin protease (SPATE) (AF242872.1)
Miscellaneous	ireA	ireA_20	siderophore receptor - iron-regulated outer membrane protein (AF320691.1)
Miscellaneous	iroN	iroN_10	outer membrane siderophore receptor (AF449498.2)
Miscellaneous	iss	iss_10	increased serum survival (AF042279.1)
Miscellaneous	katP	hp_katP_611	peroxidase and catalase (AB011549.2)
Miscellaneous	tir	hp_tir_4051.6_611, hp_tir_MPEC_611, hp_tir_O103H2_611, hp_tir_O111_611, hp_tir_O157H45_611, hp_tir_O157H7_611, hp_tir_NTH19_611	translocated intimin receptor (consensus)