Test Instructions

Fast DNA Amplification based assay for the in vitro typing of a bacterial strain from pure cultured bacteria to support for infection control.
For Research Use Only.

Catalogue Number: ICT 18000, 24 tests

Store at -20°C upon receipt

Contents

1. Intended use

icTyping is intended to be used for typing of pure cultures of bacteria to the strain level for real-time infection control for research use only. Strains can be typed, based on restriction sites distributed throughout their genome. The intended user will be a specialized molecular diagnostic laboratory. The test will be carried out by trained laboratory personnel. No special training will be required for professional routine diagnostic laboratories performing molecular diagnostic tests.

2. Product Description

icTyping is a genotyping method based on strain specific genetic restriction sites distributed throughout the genome of bacteria. With the icTyping assay, lysed bacteria are digested and adaptors are ligated with a species specific restriction/ligation mix, followed by a selective PCR amplification step.

Reference strains are provided to control for technical errors. A DNA marker is added as a length reference to determine lengths of the PCR amplicons.

The digested and ligated bacterial DNA is amplified with fluorescently labeled adaptor specific primers, selectively targeting restriction fragments.

icTyping is provided with MM Lysisbuffer for bacterial lysis of pure cultured bacteria. MM Lysisbuffer provides a very rapid (5 minutes) lysis method for cultured bacteria yielding DNA suitable for icTyping and other downstream applications e.g. standard PCR (ESBL-PCR, 16S sequencing etc).

Validated Species:

icTyping Product no: ICT 18000
*Staphylococcus aureus**

* For use to other bacteria than validated, please contact inBiome B.V. for further instructions.

PCR amplification can be carried out with GeneAmp® PCR system 9700 version 3.11 (Applied Biosystems) and TAdvanced Thermal Cycler (Biometra). For separation and detection of the PCR amplicons an ABI Genetic Analyzer 3130XL or ABI Genetic Analyser 3500 is needed. For use of other machines, please contact inBiome B.V. for further instructions.

In the laboratory is strongly recommended:

Amplification area: Dedicated area for amplification. All materials (equipment, supplies, protection, gloves, etc.) have to be dedicated to this area. Materials from this area, may not be moved to the Pre-Amplification Area, and may not be moved to the Specimen Preparation Area.

3. icTyping kit components

MM Lysisbuffer (ICT 18004)
3 vials with a white colour insert, labelled “MM Lysisbuffer” containing 1760 µl Lysisbuffer.

RL mix (ICT 18003)
3 vials with a yellow colour insert, labelled “RL mix” containing 88 µl Restriction Ligation mix.
This mixture contains enzymes to restrict and to ligate adapters to the bacterial DNA.

PCR mix (ICT 18001)
3 vials with a blue colour insert, labelled “PCR mix” containing 88 µl PCR mix. This mixture contains all ingredients for PCR amplification including DNA polymerase.

Positive Control (ICT 18006)
3 vials with a red colour insert, labelled “Positive Control” containing 44 µl Positive Control.
The positive control contains bacterial DNA of *Staphylococcus aureus (MRSA)*.

eMix600L (ICT18005)
3 vials with a black colour insert, labelled “eMix600L” containing 176 µl eMix600L.

Note: Use all components of the same kit lot number.

4. Storage

- icTyping is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact inBiome B.V.
- All components should be stored at -20°C upon arrival.
- Repeated thawing and freezing of all icTyping kit components (more than two times) should be avoided, as this might affect the performance of the assay.
- Refreeze within half an hour after thawing.
- All components are temperature sensitive.
- It is necessary to keep kit reagents at 2 – 8 °C (on ice or in cooling block) when in use.
- Protect all components from light.
- Alteration in the physical appearance of test kit materials may indicate instability or deterioration. Expiry dates shown on component labels indicate the date beyond which components should not be used.

5. Warnings and Precautions

CAUTION: Handle cultured bacteria as Biohazardous material.
Handle samples as if capable of transmitting an infectious agent.

All samples should be regarded as infectious. These samples should be handled at the Biosafety Level 2 as recommended for any potentially infectious specimen in the Centre for Disease Control/National Institutes of Health Manual "Biosafety in Microbiological and Biomedical Laboratories," 1984.

A uni-directional workflow must be adhered to in the laboratory with different areas for sample preparation, pre-amplification and post-amplification.

Use sterile, DNase-RNase free, aerosol resistant pipette tips and wear protective gloves. Protect kit contents or generated PCR product from direct sunlight.

For handling eMix600L (ICT 18005) work in a well-ventilated area, wear safety goggles with side protection and protective gloves which meet the specification of standard norm EC directive 89/686 / EEC and the resultant standard EN374. Disposal considerations: Do not let product enter drains. Keep away from surface and ground water. Dispose of contents/containers must be in accordance with local, regional, national and international regulations.

NOTE: eMix600L (ICT 18005) contains Formamide. See MSDS and handle product accordingly!

Classification eMix600L (ICT 18005) according to regulation (EC) No 1272/2008 (CLP)

Section	Hazard Class	Category	Hazard class and category	Hazard statement code	Hazard statement
3.6	Carcinogenicity	2	Carc. 2	H351	Suspected of causing cancer
3.7	Reproductive toxicity	1B	Repr. 1B	H360F	May damage fertility, may damage the unborn child
3.9	Specific target organ toxicity, repeated exposure	2	STOT RE 2	H373	May cause damage to organs (blood, cardiovascular system) through prolonged or repeated exposure

6. Procedure icTyping

All procedures must be performed in the Amplification Preparation Area. Use aerosol barrier tips during the whole test procedure. Thaw only the components that are going to be used and keep them at 0-8°C (on ice or a cooling block). Mix and spin down briefly (3 seconds) before use. When handling eMix600L take precautionary measures into account (Section 5).

6.1 Bacterial Lysis

Required reagens

- MM Lysisbuffer (ICT 18004)

Required equipment

- Microcentrifuge (17,900 x g)
- 1 µL inoculation loops
- Sterile tubes

Procedure bacterial lysis

1. Fill out the required number of sterile tubes with 200 µl MML buffer.
2. Take a ¼ loop full of one single colony from an agar plate with a sterile 1 µL inoculation loop and suspend in the prepared tube.
3. Vortex during 10 seconds and spin down for 3 minutes at 17,900 x g.
4. Carefully take 50 µL from the supernatant/free bacterial DNA without disturbing the pellet and place it in a new sterile vial and discard the tube with pellet.
5. Proceed with Restriction Ligation or freeze (-20°C) for further applications.

6.2 Restriction Ligation

Required reagens

- RL mix (ICT 18003)
- Positive Control (ICT 18006)

Required equipment

- 37°C incubator
- Sterile tubes
- Nuclease free water

1. Prepare the required number of reaction tubes for the number of DNA samples to be restricted and ligated, plus an extra tube for Positive Control.
2. Thaw, vortex for 10 seconds and spin down briefly the RL mix and all DNA extracts.
3. Add 10 µL of RL mix and 10 µL DNA of each sample to a sterile vial.
4. Vortex, spin down and incubate the reaction tubes to the 37°C incubater for 30 minutes.
5. Dilute the RL reaction 20x by adding 380 µL nuclease free water to every reaction tube.
6. Proceed with the PCR or store the restricted/ligated DNA at 2-8˚C.

6.3. PCR

Required reagens

- PCR mix (ICT 18001)

Required equipment

- PCR machine
- PCR strips/plates

1. Prepare the required number of reaction tubes for the number of digested/ligated DNA samples to be amplified.

2. Thaw, vortex and spin down the PCR mix and add 10 µL of PCR mix per PCR reaction tube.

3. Vortex and spin down all digested/ligated and diluted DNA extracts. Add 10 µl DNA of each sample to the PCR reaction tube containing the PCR mix and mix by pipetting up and down.

4. Spin down for 30 seconds at approximately 1000 rpm and load the reaction tubes/plate into the PCR instrument and program the PCR system as listed below

5. The PCR products can be stored at 2-8°C

PCR Protocol*	
12 cycles	20 sec 94˚C
	20 sec 65˚C, reduce 0,7˚C per cycle
	30 sec 72˚C
23 cycles	20 sec 94˚C
	20 sec 56˚C
	30 sec 72˚C
Cooldown	∞ 4˚C

*For detailed instructions on how to use the PCR machine refer to the PCR machine manual.

6.4 Capillary Electrophoresis

Required reagens

- eMix600L (ICT 18005)

Required equipment

- ABI 3130XL / ABI3500

1. Thaw and vortex eMix600L

2. For every sample, add to one well of the ABI plate 20 µL of eMix600L and 5uL of PCR product.

3. Spin down the plate for 30 seconds at approximately 1000 rpm.

4. Heat the plate in the PCR machine at 94ºC for 3 minutes followed by a snap cooling on ice for 3 minutes. Use the appropriate cover for the plate so no evaporation can occur.

5. Replace the plate-cover with ABI septa. load the plate in the ABI machine and start the run.

6. The ABI plate can be stored at 2-8°C. The settings for ABI 3130/3500 is described in the tables below. For other instrument protocols than ABI 3130/3500 please contact inBiome B.V.

Instrument protocol ABI 3500				
Application type:	Capillary Length	Polymer	Dye Set	Advanced Options
Fragment	50 cm	POP7	G5	unchanged from default settings

Instrument Protocol Properties								
Run Module	Oven Temperature	Run Time	Run voltage	PreRun Time	PreRun Voltage	Injection Time	Injection Voltage	Data Delay
FragmentAnalysis50_POP7	60°C	1800 sec	15 kVolts	180 sec	15 kVolt	12 sec	1.6 kVolts	200 sec

Dye Set protocol ABI 3500				
Dye Set Name	Chemistry Matrix	Dye Selection	Reduced Selection	Calibration Peak Order
G5	Standard	Blue Green Yellow Red Orange	Blue Green Yellow Red Orange	Blue=5, Green=4, Yellow=3, Red=2, Orange=1

Parameters	
Matrix Condition Number Upper Limit	13.5
Locate Start Point	After Scan 1000, Before Scan 5000, Limit Scans To 3250, Sensitivity 0.4, Minimum Quality Score 0.95

Sizecalling protocol ABI 3500	GS600LIZ(60-600)+Normalization
	SizeCaller v1.1.0

Analysis Settings											
Analysis Range	Sizing Range	Size Calling Method	Primer Peak	Minimum Peak Height	Use Smoothing	Use Baselining (Baseline window (Pts))	Minimum Peak Half Width	Peak Window Size	Polynomial Degree	Slope Treshold Peak Start	Slope Treshold Peak End
Full	Full	Local Southern	Present	All colors 175	None	51	2	15	3	0.0	0.0

QC Settings				
Fail if Value	Suspect Range	Pass if Value	Assume Linearity	Pull Up
< 0.25	0.25-0.75	≥ 0.75	0 bp To 800 bp	Actuate Pull-Up flag if Pull-Up Ratio ≤0.05 and Pull-Up Scan≤ 1

Instrument protocol ABI 3130 / 3130XL SETTING A												
Application type:	Capillary Length	Polymer	Dye Set	Advanced Options								
GeneMapper_Generic	50 cm	POP7	G5	unchanged from default settings								
Instrument Protocol Properties												
Run Module	Oven Temperature	Run Time	Run voltage	PreRun Time	PreRun Voltage	Injection Time	Injection Voltage	Data Delay	PolyFill Vol	Current Stability	Voltage no of Steps	Voltage Step Interval
FragmentAnalysis50_POP7	60°C	5200 sec	6 kVolts	180 sec	15 kVolt	45 sec	1.6 kVolts	1000 sec	7300 steps	5.0 uAmps	40 nk	15 sec

Instrument protocol ABI 3130 / 3130XL SETTING B												
Application type:	Capillary Length	Polymer	Dye Set	Advanced Options								
GeneMapper_Generic	36 cm	POP7	G5	unchanged from default settings								
Instrument Protocol Properties												
Run Module	Oven Temperature	Run Time	Run voltage	PreRun Time	PreRun Voltage	Injection Time	Injection Voltage	Data Delay	PolyFill Vol	Current Stability	Voltage no of Steps	Voltage Step Interval
FragmentAnalysis50_POP7	60°C	5200 sec	4 kVolts	180 sec	15 kVolt	45 sec	1.6 kVolts	1000 sec	7300 steps	5.0 uAmps	40 nk	15 sec

Dye Set protocol ABI 3130 / 3130XL				
Dye Set Name	Chemistry Matrix	Dye Selection	Reduced Selection	Calibration Peak Order
G5	Standard	Blue Green Yellow Red Orange	Blue Green Yellow Red Orange	Blue=5, Green=4, Yellow=3, Red=2, Orange=1

File name convention

It is advised to use the following naming convention:
INSTITUTION_DEPARTMENT_ICtyping_SAMPLENAME.fsa*

*In the Software Service it is not allowed to use other special characters, besides "_".

Beware: File names must be made anonymously before uploading to the Software service, so they are not traceable to individuals.

6.5 Analyzing data

The icTyping products have been built together with the inBiome B.V. Software Service. For optimal results we strongly recommended to use this **free** software service.
The software handles all steps from raw data processing to peak calling and species/strain identification. Fragments are assigned to bacterial species according to our database and translation algorithm. Visualizations of profiles and interpretations of positive control are also available for users.

Analyzing data should be performed according to the instructions provided in Interpretation of results (Section 7) and the inBiome B.V. icTyping Software Service Manual.
When users are ready to work with the inBiome B.V. software service, then username and password should be requested via info@inbiome.com 10 working days before first usage of one of the products.

6.6 Procedural notes

1. Be extremely careful when handling materials to prevent contamination. Always mix and spin down reagents and samples before opening. In case of any suspicion of contamination, discard the materials.

2. Careful analytical techniques and strict adherence to the directions in the test instructions are essential to obtain reliable results.

3. Samples with equivocal results must be verified by repeat assays or isolation.

4. Do not pool reagents from different lots.

5. If the kit is damaged upon receipt, please contact your local distributor and/or inBiome B.V.

7. Interpretation of results

The following dyes are used for the different targets:

Target	Dye
Restriction specific Adaptor	FAM
Marker	LIZ

Marker peaks are found at: 20, 40, 60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440, 460, 480, 500, 514, 520, 540, 560, 580 and 600 nc.

Quality control
In each kit one positive control for each PCR run is provided. Additional controls may be analysed in addition to those provided. Established statistical methods for analysing control values and trends should be employed. When a profile of a species falls under a set quality limit, the software will label that sample as failed.

If the controls do not comply with the established limits and repetition excludes a technical issue, check the following areas:

1. Expiration date on reagent package and prepared reagents
2. Temperature of the reagents
3. Settings PCR System
4. Settings ABI System
5. Contamination

If controls are still invalid, please contact inBiome B.V. or your local distributor. **Note:** following criteria are obtained with inBiome B.V. software service.

8. Limitations of the procedure

1. Use only cultured micro-organisms as described in the product description (Section 2) or inBiome B.V. icTyping Software Service Manual. Other bacterial species have not been validated and may result into false positive results.

2. Wrongly use of the product, transport and storage may affect the outcome of the result (causing a false positive or a false negative result).

3. The user should have a formal training in PCR techniques or have gained appropriate experience in the field of PCR techniques.

4. Good laboratory practices and strict adherence to these Test Instructions are indispensable to avoid contamination during the process.

9. Summary Assay Specifications

Assay Protocol	
Sample pretreatment	**NA**
Reagent pretreatment	**NA**
Type of sample	**Cultured Bacteria**
Sample size limitations (w/o dead volume)	**NA**
Assay format	**GeneAmp® PCR system 9700 version 3.11, TAdvanced Thermal Cycler (Biometra), ABI 3130XL and ABI3500**
Automatic/Manual Dilution	**RL product 1:20**
Total assay time	**3.5 hr**
Reagents, Calibrators and Controls	
Tests per kit	**24**
MM Lysisbuffer / RL mix / PCR mix / Positive Control / eMix600L	
A. Matrix	**Liquid frozen**
B. Physical form	**Liquid frozen**
C. Stability (shelf life)*	**12 months**
D. Number of freeze/thaw cycles	**2**
E. Stability after defrosting (2-8°C)	**30 minutes between every freeze/thaw step**
Shipping conditions	**On dry ice**
Storage conditions	**-20°C**

Legal/Regulatory	
Intended use	**icTyping is intended to be used for typing of pure cultures of bacteria to the strain level for real-time infection control for research use only. Strains can be typed, based on restriction sites distributed throughout their genome. The intended user will be a specialized molecular diagnostic laboratory. The test will be carried out by trained laboratory personnel. No special training will be required for professional routine diagnostic laboratories performing molecular diagnostic tests.**

10. References

P.H.M. Savelkoul et al. (2009). Amplified Fragment Length Polymorphism Analysis; Methods Mol Biol;551:89-104.

List of symbols as used in labelling

	Manufacturer		Contains sufficient for <n> tests
	Catalogue number		Consult Instructions for Use
	Batch code (Lot)		Contents
	Use by date		Danger. May damage fertility or the unborn child. Use personal protective equipment as required. If exposed or concerned: Get medical attention/advice. (Read MSDS)
	Temperature limitation		

List of Abbreviations

ABI	**ABI Genetic Analyser**
DNA	**Desoxyribonucleic acid**
DNAse	**Desoxyribonuclease**
MSDS	**Material Safety Data Sheet**
NA	**Not Applicable**
PC	**Positive Control**
PCR	**Polymerase Chain Reaction**
RL	**Restriction Ligation**

WARRANTY

This product is warranted to perform as described in its labelling and in the inBiome B.V. literature when used in accordance with all instructions. inBiome B.V. DISCLAIMS ANY IMPLIED WARRANTY OF SPACE MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall inBiome B.V. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser.

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TECHNICAL ASSISTANCE

For additional information, please visit www.inbiome.com

For technical assistance please refer to the Catalogue Number: ICT 18000

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