

DNA Assembly Applications on the GeneTheatre

SUMMARY

Tasks

- Automation of Gene Assembly related methods on the GeneTheatre
- Automated generation of PCR, Golden Gate and Gibson reactions ready for incubation/cycling

Solution

- An affordable liquid handling benchtop system with ready to use graphical user interface

The GeneTheatre has been designed to fulfil the demand for entry level, affordable and compact robotics within the liquid handling, life sciences and bioengineering communities.

While designed to be entry level, the GeneTheatre has 12 freely selectable deck positions capable of handling a variety of application based labware like PCR plates and third-party equipment such as shakers and temperature controlled blocks.

To demonstrate the capabilities of this platform the GeneTheatre has been used to automate the production of reactions for the current best of breed DNA production and assembly methods including PCR, Golden Gate and Gibson¹. These methods enable the automated generation of reactions for the assembly of DNA molecules in a simple yet reliable way.

The aforementioned assembly applications have been designed to be carried out using a standard set of adaptors and deck layout, which is well documented in the pictorial user guides accompanying the methods. Furthermore, the application workflows are embedded in a ready to use graphical user interface with user instructions, making it simple to use these applications on the GeneTheatre without specific training.

¹ Requires 8 channel pipette 0.5 – 25 µl



CyBio® APPLICATION STUDIO – READY TO USE GRAPHICAL USER INTERFACE

The CyBio® Application Studio was designed as a user-friendly software solution to allow the reliable execution of workflows by those without significant experience of automation.

The front end is an intuitive touch screen design which is equally useable via mouse and cursor. When launching an application, key information such as reaction volume or sample number can be indicated, avoiding the need for any programming by the user. In addition, the software supplies the user with any necessary information, including how to setup the GeneTheatre instrument including advice on labware and reagents through the use of message boxes and graphics. The reliable and flexible software package underlying the Application Studio enables intelligent error management and can be used to program the GeneTheatre to carry out other liquid handling tasks if desired.



Fig. 1: CyBio® Application Studio front end

APPLICATION EXAMPLE

PCR reactions, Gibson and Golden Gate assemblies are all based upon the biochemical activity of one or more enzymes which work in combination to generate or assemble fragments of DNA. The products that result from these reactions are based upon a small number of reaction specific components (primers, templates or DNA fragments), the combination of which is unique to any given reaction and a master mix of buffers and required materials. The combinations of these specific components can be fully specified by the user through the use of an information supply document called a worklist. To produce these reaction mixes, all the gene assembly applications for the GeneTheatre follow a two phase workflow where the specific components are first added to the appropriate wells in a plate before a master mix solution is generated (PCR only) or supplied and transferred to the reactions:

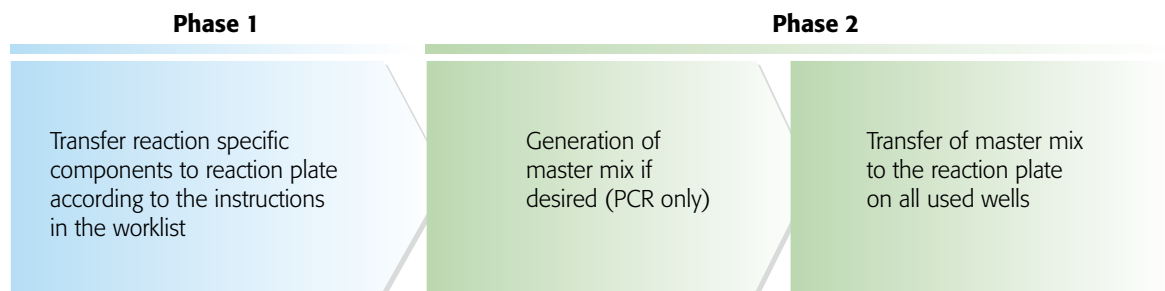


Fig. 2: General workflow for all gene assembly applications on the GeneTheatre

REQUIRED SYSTEM CONFIGURATION

Modules

Ordering information	Quantity	Operation	Order number
GeneTheatre BU	1	GeneTheatre BU incl. software, 2 x height adapter 40 mm and waste box I	844-00401-3

Pipetting heads

Ordering information	Quantity	Operation	Order number
1 channel pipette 0,5 – 25 µl or	1	1 or 8 channel pipetting tool for GeneTheatre with a volume range from 0.5 – 25 µl each	844-00410-0
8 channel pipette 0,5 – 25 µl	1		844-00413-0

Accessories

Ordering information	Quantity	Operation	Order number
Height adapter 40 mm	3	Height adapter 40 mm for GeneTheatre adapter and 50 µl/ 250 µl tip boxes as well as for the positioning of labware such as microplates and reservoirs	844-00445-0
Adapter standard 96 well	5	Adapter for 96 well microplates, autoclavable	844-00433-0
Adapter kombi, passive cooling	1	Adapter for 8 x 0.2 ml, 8 x 0.5 ml, 8 x 1.5/2.0 ml tubes with passive cooling function, including height adapter 40 mm	844-00437-0

Accessories

Ordering information	Quantity	Operation	Order number
4titude 96 well PCR plate	5	PCR plate for the handling of 96 samples	4ti-0740
CyBi®-TipBox 96/50 µl	5	50 µl tips, 96 tips/box, 10 boxes per case PCR-certified	OL3811-25-535-N

	1	2	3
A	Height adapter 40mm	Height adapter 40mm	Height adapter 40mm
B	Adapter standard 96 well	Adapter standard 96 well	Adapter standard 96 well
C	Height adapter 40mm	Adapter standard 96 well	Adapter standard 96 well
D	Waste Box I	Adapter kombi, passive cooling	Height adapter 40mm

Fig. 3: Required adapters and their positions for all DNA assembly based GeneTheatre applications



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