# Agilent 5100 **Automated** Lab-on-a-Chip Platform

# DNA 1000HT<sup>4</sup> Quick Start Guide



# **Edition July 2004**



Agilent Technologies

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### **Software Revision**

This guide is valid for A.01.xx revisions of the Agilent 5100 Automated Lab-on-a-Chip Platform software, where xx refers to minor revisions of the software that do not affect the technical accuracy of this guide.

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Agilent 00000X Product Name Variable Manual Title Variable

# 5100 ALP DNA1000-HT- Quick Start Guide

This quickstart guide will introduce you to the basics of DNA analysis using the Agilent 5100 Automated Lab-on-a-chip Platform (ALP). It will give you a short overview on the hardware and software and will guide you through a standard process of analyzing your DNA samples.



1 5100 ALP DNA1000-HT- Quick Start Guide ALP instrument

# **ALP** instrument



# Log on to the 5100 ALP software

- **1** Switch on the ALP PC-Server and instrument and wait one minute until the system is initialized
- **2** Go to your desktop and double-click the following icon:



**3** The following login screen appears:

User Information User Name:	T
User Password:	
Database Information DB Alias:	<b>X</b>
Login Action	Login

**4** In order to get access to the software you need to provide login information. Type in your user name and user passwort and select the appropriate database alias from the drop down menu. Click on the Login button and the main screen of the software appears as follows:

#### 1 5100 ALP DNA1000-HT- Quick Start Guide

Log on to the 5100 ALP software

-											
Automate	d LabChip Plat	form - A.00.1251	154							_	
Application	Results Tools	View Help									
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100	Assay Name:										
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	Job Logbook	_	_	_	_	_	_	_	_	_	
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The 5100 ALP software is organized in contexts. The icons on the left column of the screen provide access to different contexts within the software:

#### **Instruments:**

This context is used to:

- connect to the instrument,
- start a job,
- display the incoming signal traces,
- check the instrument status.

### Jobs:

This context is used to set-up an experiment (job) on ALP. Each job is based on an assay, which defines the internal instrument control and setpoints for data aquisition. For example the DNA 1000 HT4 assay is used for the analysis of DNA samples. A job contains the following information, which must be entered before the execution of a job:

- assay, e.g. HT DNA 1000, which compromise set points for data aquisition and evaluation
- the number and type of well plates that are processed during the job
- storage position of the well plates inside the instrument
- barcode information of the well plates
- · the position of samples on each well plate and sample names
- optional: rules for result flagging
- required resources and instrument configuration

After job execution, it contains sample and ladder data that is stored in the database.

For the analysis of DNA samples on ALP, either a new job has to be defined or an already existing job in the database could be opened as a job copy.

After completing the analysis, the results can be reviewed in the Results context. For this the operator has to switch to the Results context.

**Results:** This context allows the user to get detailed information about the results of the analysis. The results context shows the acquired data both as electropherograms and gel like images, whereas the calculated values (e.g. concentration, fragment size, molarity) are listed in peak tables. The software provides several different way to filter and organize the data.

If not previously done within the Job Setup this context can also be used to define result flagging rules after the data has been aquired.

### NOTE

After login the appearance of the software depends on the last opened context.

1 5100 ALP DNA1000-HT- Quick Start Guide Connecting to the instrument

# **Connecting to the instrument**

### NOTE

Make sure that the instrument is switched on and initialized.

1 Click on Instruments to switch to the Instrument Context. The icon of the selected context will be highlighted on the Context menu of the 5100 ALP software:



2 In the software screen select the appropriate Instrument from the menu bar



**3** Select the *Instrument* Menu bar and click the *Connect Instrument* button or click on the Connect Instrument button in the menu bar to connect to the ALP instrument. When connection succeded, the button will change to *Disconnect Instrument*.



NOTE

After connection succeeded - the Real-time Signal Plot shows the online signal traces within the *Job*- and the *Instrument Status* tab.

1 5100 ALP DNA1000-HT- Quick Start Guide Creating a new Job

# **Creating a new Job**

Before starting the measurement of DNA samples several experiment parameters must be defined within the 5100 ALP Software. To provide all important information to the instrument a new job must be created within the *Jobs* context. During the run-time execution a job comprises the control of the process flow of sample and ladder runs as well as the resource management of reagent, chips and pipette tips.

1 Click on the *Jobs* icon to switch to the Jobs Context. The icon of the selected context will be highlighted on the Context menu of the 5100 ALP software will appear:

🛃 Automater	I LabChip Platform - A.00.1251154
Application	ob Iools <u>Vi</u> ew <u>H</u> elp
I 🗋 🚵 🕶 🛱	🔟 🗍 🖸 🚺 Jobs 🔹 🗐 🖉 😓
Jobs	
	Job Summary Plate Setup Result Flagging Job Properties
	Name: Comment:
Instruments	Created By:
	Created Date:
140×	Modified By:
Jobs	Modified Date:
<u> 201</u>	Document Flags:
	Import Information:
Assays	Job Specific
	Template:
	Assay Name:
Hesults	Assay Version:
5	Auto Report Settings:
	Job Logbook
nesources	Severity Time A Message

**2** To create a new job: Select the *Jobs* Menu bar. Select *New* and a new job will be opened.



**3** The Select Base Assay dialog box appears. For the measurement of DNA samples - Select the DNA 1000 Assay and click *Use Assay* to add the selected assay to the job. The name of the assay as well as the version will appear in the Job Summary tab

Creating a new Job

**4** Enter a new job name and a comment in the *Summary* tab.

🛃 Automated	d LabChip Platform - A	.00.1651208			
Job Tools	<u>View</u> <u>H</u> elp				
Jobs	- 🧐	😓 🚯 🚇 📄 🚵 • 🖕 🔊 🔒 📼			
Jobs				Untitled_7/	9/
-	Summary Plate Setup	Besult Flagging			
	Name:	Untitled_7/9/2004_10:05 AM	Comment:		1.0.1
	Created By:	RSROPER			
	Created At:	7/9/2004 10:05:45 AM			
	Modified By:	RSROPER			
	Modified At:	7/9/2004 10:05:45 AM			
<b>100</b>	Document Flags:	Archive			
	Import Information:	No Import Information			
	Job Specific				
	Template:				
	Assay Name:	M16_HT_DNA_1000_EAP_rev6		Change	
	Assay Version:	1.03		Upgrade	
	Auto Report:			Settings	
- 25	Job Logbook				
Resources	Type Time	🛆 Message			

- 5 To setup the microtiter plates that should be analyzed during the job switch to Plate Setup tab. The Microtiter plate setup will define:
- the number of samples and their position on the sample plate
- the sample plate type
- the location of the sample plates within the storage compartment (Depot).

Application	Job Tools View Help	i Jobs	-GMS				
Jobs					Un	titled_9/24/2003	3_1:56 PM 📝
	Job Summary Plate Setup	Result Flagging Job Properties					
Instruments Jobs Assays	Position Tune 1 /	incition I tabe! ter the currently selected one.	Barcode	Comment			Imported
Results	Plate Details Labet Type:	Barcode:	¥.	Comment			A. V
- 19	Plate Content			Sip	and Well Information		
	Categories: Sample Ledder (not selectable) Clear Mate Clear Pare Contents	1 2 3 4 5 0 A O O O O O O B O O O O O O E O O O O O O E O O O O O O			ample   Ladder	Z Comment	Sp. Color   Sample
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Start	🚮 🚑 🛛 🗷 screenshots.doo	- Micros	8	Rudi Salowsky - A	ssay Developer - db_mktg ष	Instrument Offline 9	24/2003 1:58:45 PM

The plate Setup tab appears as follows:

**6** Click on the red add-button **10** to add a sample plate to the plate list tab.



# NOTE

One line in the Plate setup table represents one sample plate.

**Creating a new Job** 

7 The *select plate type* dialog box appears. Select the sample plate type from the drop down menu.

Agilent - Select Plate Type	You are Please m compatib	about to insert the nake sure you sele ole with the requin	first plate for ct a plate type ements of the .	this job. that is assay		
	Туре:	96-Plate PCR Eppen	dorf twin	-		
	Comment:	1536-Plate				
		384-Plate				
		96-Plate				
Outertien		95-Plate AbGene 13	UU robotic			
Question		96-Plate PCR Eppen	idorf twin			
		96-Plate PCR WD14	5			
		dummy entry				
	,		<u>U</u> se Plate	<u>C</u> ancel		

- 8 Press Use Plate.
- **9** The selected sample plate appears in the *Plate Setup* tab.

Job <u>S</u> um	mary Plate Setu	p <u>R</u> esult Flag	jing		
🖬 🔛	🗖 🔘 🖸 🖸				
Position	Туре	Location	Label	Barcode	Comment
1	*96PCR_EPDF	B1	Plate 1		

- Summary Plate Setup Result Flagging • • • • Position Type Location Label Barcode Comment 1 96-Plate PCR E B1 PCR Test PCR Test Label: Barcode: Comment Location: Type: 96-Plate PCR Eppendorf twir 💌 **B1** -Samp Well Label Categories: Sample C Empty Wells
- 10 Insert a label for the selected plate in the plate details section of the Plate Setup tab .

**11** Select the location of the sample plate inside the Depot of the 5100 ALP instrument.

Plate Det	ails						
Label:	PCR Test	Barcode:				Comment	
Туре:	96-Plate PCR Eppendorf twir 💌	Location:		B1	-		
Plate Con	tent		B1				Sample T-
Categor	ies:	2 2 4	B2			1 1 3	Well L
💿 Samp		2 3 4	B4				
O Ladde							
C Empty	Wells						
Clea	r Plate Contents		C4				
			D3		C		
			D4		( and the second s		
		-	222	00	2-2-		

### CAUTION

Using a rack applicable for the storage of only two plates - the lowest position is labelled with B1 followed by B2 for the upper position. Avoid the selection of positions B3 and B4 while using a two plate rack.

12 To define the content of your sample plate drag the mouse over the wells of the microtiter plate on the screen. The selected wells appears green. By using a HT4-Sipper chip - always 4 samples will be processed in parallele and therefore 4 wells on the sample plate are always selected together. By selecting the wells the information will be directly submitted to the *Sample table* on the right side of the screen.



**13** Click the red add-button to add further sample plates to the *Plate Setup* tab.

The performance of the 5100 ALP instrument is optimized for the meassurement of  $4 \times 96$  well or  $1 \times 384$  well sample plates.

### Abbreviated Book Title Variable (Edit this)

NOTE

### NOTE

After defining 4 x 96 well plates - the Plate Setup tab should contain 4 lines representing all defined sample plates.

Jobs									Untitle	1_9/30/2003	9:54 A	.M 🔥
	Job Sum	mary 🛛 Plate Setup	<u>B</u> esult Flaggin	D.								
	Position	Type	Location	Label		Barcode	Co	mment			Im	ported
	1	"SEPCR_EPDF	B1	PCR1-96								×
	2	"S6PCR_EPDF	B2	PCR97-192								24
lahe.	3	"96PCR_EPDF	C1	PCR193-288								36
	4	"96PCR_EPDF	<b>III</b> C2	PCR289-384								×
49												
Accays	Plate Deta	ĸ	_	_		_				_		
Results	Label:	PCR289-384		Barcode:				Comment:				
	Туре	96PCR_EPDF	TWINT_SKIR	Location:		C2 - Cooled	٣					Ŧ
<u>99</u> 7	Plate Cont	ent							Sip and Well Information			
	Categori	es							Sample Ladder			
			1	2 3 4	5 6	7 8 9	10 11	12	Well Label	<ul> <li>Comment</li> </ul>	Sip Color	San A
	N Sam	ple							Al		1	Same
	Ladó	er [not selectable]	в 🗖		<b>O</b> O		00		81		1	Sam

14 Select Job>Save to save the newly created job to the database.

**15** Select Job>Close to close the newly created job in the *Jobs* context.

**16** To start a job on the 5100 ALP instrument switch to the *Instruments* context and open the job selected for analysis.

### NOTE

Before opening a job in the Instrument context - the job must be closed in the Job context.

### 1 5100 ALP DNA1000-HT- Quick Start Guide HT4 DNA Assay Protocol

# **HT4 DNA Assay Protocol**

# Reagent, supplies and equipment supplied by Agilent:

#### HT4-DNA 1000 reagent and supplies (order number xxx)

HT<sup>4</sup> DNA 1000 Reagents & Supplies

- +  $\mathrm{HT}^4$  DNA 1000 Storage buffer
- HT<sup>4</sup> DNA 1000 Marker
- +  $\mathrm{HT}^4$  DNA Gel Matrix
- +  $\mathrm{HT}^4$  DNA Dye
- +  $\,\mathrm{HT}^4\,\mathrm{DNA}$  focussing solution
- HT<sup>4</sup> DNA Ladder (10x)
- Reagent plate
- •

### HT<sup>4</sup> DNA Chip (order number xxx)

•  $\mathrm{HT}^4$  Sipper Chip

# additional material and equipment required:

- Pipettes (10 µl, 20 µl, 100 µl and 1000 µl) with compatible tips
- Deionized water
- · microtiter-plate-centrifuge
- · microtiter plate compatible PCR-machine with heatable lid
- · high pressure connection
- · microtiter plate sealer



Before the analysis of DNA samples, the chip will automatically be filled with reagents by the ALP system. To provide all reagents for the chip replenishment, a reagent plate has to be prepared by the user and inserted in

the dedicated depot position. One prepared column of the reagent plate

should contain reagents (gel, gel-dye and marker) sufficient for the analysis of 384 samples (e.g. 1x 384 well plate or 4x96 well plates).

For the analysis of more than 384 samples the number of columns filled with reagents must be calculated and adjusted.

### **Preparing the Gel-Dye Mix**

# WARNING

Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples. Kit components contain DMSO. No data is available addressing the mutagenecity or toxicity of the dye/DMSO reagent. Because the dye binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care. The DMSO stock solutions should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

1 Allow all reagent to equilibrate to room temperature for 30 minutes

### NOTE

It is important that all reagents have room temperature before starting the next step. Protect the dye concentrate from light.



**HT4 DNA Assay Protocol** 

- **2** In a microcentrifuge tube, pipette 280 µl of the DNA gel.
- **3** Vortex the DNA dye concentrate for 10 seconds and spin down.
- **4** Pipette 10.5 μl of the dye concentrate (colour) to the tube with 280 μl of the DNA gel.
- 5 Cap the tube, vortex for 10 seconds. Visually inspect proper mixing of gel and dye. Store the dye at 4°C in the dark again.
- **6** Transfer the gel-dye mix to the designated well position on the reagent plate (Refer to *Pipetting the Reagent Plate* on page 23)

**NOTE** Use the gel-dye within 1 week of preparation. Protect the gel dye from light-the dye will degrade when exposed to light and this reduces signal intensity. Store the gel-dye mix at 4°C when not in use for more than 1 hour.

### **Pipetting the Reagent Plate**

Always fill reagents in unused columns on a reagent plate.

1 Pipette 250  $\mu$ l of the gel in each of the gel wells A1, B1 and C1 on the reagent plate.



NOTE



**2** Transfer 250  $\mu$ l of the gel-dye mix to the well D1 on the reagent plate.

**3** Pipette 300  $\mu$ l of the marker (red) to the well G1 on the reagent plate.



### 1 5100 ALP DNA1000-HT- Quick Start Guide HT4 DNA Assay Protocol

**4** Pipette 250 μl of the focussing solution in each of the wells A12, B12, C12 and D12 on the reagent plate.



### NOTE

For the analysis of more than 384 samples the number of columns filled with reagents has to be adjusted (e.g. 3 reagent columns for 3 x 384- or 12 x 96 well sample plates). The position of the prepared columns on the reagent plate must be entered in the Start Job execution dialog before starting a job (Refer to *Start Job Execution Dialog* on page 36.).

- **5** Seal the reagent plate with aluminum foil on the Remp plate sealer at 170°C for 4 seconds.
- **6** Place the reagent plate in the designated rack located in position D of the right depot. Column 1 of the reagent plate must face the handle of the rack and the Chip Tub must be held by the clamps of the rack.
- 7 Insert the rack into the depot. The position of the reagent plate in the depot will be shown in the Instrument Status tab of the Instrument context



# **Preparing the Sample Plate**

The Agilent 5100 ALP system is able to analyze DNA samples prepared either on 96- or on a 384 well plate. DNA samples should be prepared according to the  $\rm HT^4$  DNA1000 assay specifications.

- **1** Confirm that your 96- or 384 well sample plate was specified by Agilent as appropriate for the ALP system
- **2** Pipette at least 20 µl of your samples on the designated wells of the sample plate

# NOTE The sample volume within each well should always exceed 25 μl. Lower sample volumes may cause impaired analyses

- **3** Seal the sample plate with aluminum foil on the Remp plate sealer for 4 seconds at 170°C.
- **4** Place the sample plate in the designated rack. Column 1 of the sample plate must face the handle of the rack and the plate must be held by the clamps of the rack.
- **5** Insert the rack into the designated position of the depot as defined before in the Job Set-up.



# Preparing the HT<sup>4</sup> DNA 1000 Chip

The DNA1000  $\mathrm{HT}^4$  Chip will be shipped ready-to-use in a specific box filled with storage buffer.

# **CAUTION** Always place the chip in the Chip Carrier or in the designated storage box. The chip contains 4 sippers on the underside of the chip. The sippers are fragile capillaries that can easily be damaged if not handled with care.

**1** Take the Chip Tub and the Chip Carrier out of the depot.

### NOTE

The Chip Carrier is the receptacle for the HT4DNA chip and is positioned on the Chip Tub.

- **2** Remove the Chip Carrier from the Chip Tub. Fill water in the water bath of the Chip Tub until the fins in tray well are covered with liquid.
- **3** Position the Chip Carrier on the Chip Tub.
- **4** Take the HT<sup>4</sup> DNA Chip out of the Storage box. Remove the liquid from the underside of the chip.
- **5** Clean the detection window of the chip by filling it with iso-propanol.
- **6** Carefully remove the iso-propanol with a pipette and cover the detection window with water. Carefully remove the water with a pipette and let the detection window dry up.
- 7 Insert the chip into the Chip Carrier. The sippers must be inserted in the cut-out of the Chip Carrier.

# CAUTION

The chip fits only one way. Do not force the chip insert. Otherwise capillaries may be damaged

- **8** Insert the Chip Tub with Chip Carrier and chip into the designated position of the rack. The electrodes of the Chip Carrier must face the handle of the rack and the Chip Tub must be held by the clamps of the rack.
- **9** Insert the rack into the depot. The position of the reagent plate in the depot will be shown in the Instrument Status tab of the Instrument context.



# **Preparing the Trough**

The trough is a storage device for locating the DNA Ladder in the instrument. Before starting a job, the trough has to be manually filled with DNA Ladder and inserted in the ALP 5100 instrument.

1 Dilute the 10 x DNA Ladder 1:10 to a final concentration of 1x DNA Ladder.

# CAUTION

Ladder and DNA samples should always have the same salt concentration. Adjust the salt concentration of the ladder to the salt concentration of the DNA sample by diluting the 10 x DNA Ladder with sample buffer. Sample buffers as PCR- and Restriction enzyme buffers are often 10 x concentrated

2 For the analysis of 384 DNA samples (1 x 384- or 4 x 96 well plate) - fill 4 x 25 µl of 1 x DNA Ladder in the trough wells starting with well positions D1, D3, D5, D7. The position D1 on the trough is marked by an arrow head and should be located down to the left.



### CAUTION

Avoid air bubbles on the bottom of the wells while pipetting ladder to the trough. Visible bubbles within the Ladder must be removed before starting the analysis.

# NOTE

For the analysis of a higher sample number - adjust the number of filled trough wells. Continue to fill wells with position D2, D4, D6, D8 of the trough and proceed in the filling procedure from the lower left to the upper right position of the trough.

**3** Insert the trough in a 384 well plate. Seal the trough with aluminum foil on a Remp-plate sealer for 6 sec at 170°C and cut off the extending foil. The sealed trough can either be used directly for analysis (refer to Starting a Job - page 37) or stored at 4°C for maximum 7 days.

# **Starting a Job**

	<b>1</b> Switch to the Instrument Context. The icon of the selected context will be highlighted on the Context menu of the 5100 ALP software.
NOTE	Before starting a job - the Magellan Software has to be connected with the ALP 5100 instrument.
	<b>2</b> Go to Instrument>OpenJob and select a job. Click the <i>Open Job</i> button to open the selected Job.
	<b>3</b> The Instrument status tab shows an overview of the different hotel compartments of the instrument.
NOTE	At this point of job execution the Required Hotel Loading information shows the required position of chips, reagents and samples as defined by the selected Job.



# 1 5100 ALP DNA1000-HT- Quick Start Guide

**Starting a Job** 

# NOTE

The Job Status Tab provides information on the number and type of the sample plates as defined by the selected Job



**4** Insert Chips, Reagent plates and sample plates as indicated by the *Required Hotel Loading* information within the *Instrument Status* tab.

# CAUTION

Column 1 (including well A1) of both the reagent plate and the sample plates as well as the electrodes of the Chip must face the handle of their designated racks. Wrong Chip position impairs the instrument processes and may damage the chip. Wrong reagent- and sample plate positions will lead to inappropriate results and may destroy the chip.

**5** Close all Hotel doors.

# NOTE

All instrument doors must be closed before starting a job. Executing a job with opened doors will lead to an instrument error

- **6** Click on Instrument>Start Job or on the *Execute Job* button on the menu bar to execute the selected job
- 7 The start Job Execution dialog appears.

# **Start Job Execution dialog**

### CAUTION

The Start Job execution dialog leads through a process of successive steps required to finalize the instument preparation and to check the availability of all resources needed for a successful job execution. All topics have to be carefully checked by the operator before proceeding with the final job execution.



1 Click on *Next* to proceed with the Job Start execution dialog.

### NOTE

Before varifying the loading of the depot, check the number of the column of the reagent plate prepared for chip replenishment.

### 1 5100 ALP DNA1000-HT- Quick Start Guide

**Starting a Job** 

- Start Job Execution Sample Plates Please make sure to insert the sample plates into the hotel as shown below. Job Options Verify Hotel Loading The plate(s) need to be verified. Inserted the plates(s) and push the button. Sample Plates **Required Hotel Loading** Chips Reagent Plates Trough 2 Pipette Tips Plate 1 ? EPDFTWINT\_SKIRT\* 2 Bottles Check Resources в C. D A Sample plate(s) need to be verified 2 🎒 < Back Next > Cancel Finish
- **2** Click on *Verify Hotel Loading* and the instrument will check the status of the hotel loading.

# NOTE

The instrument may need up to 5 minutes to verify the hotel loading. The subsequent opening of Hotel doors and exchange of plates will require a repeat of the hotel loading verfication.

5100 ALP DNA1000-HT- Quick Start Guide 1 Starting a Job

Start Job Execution				
Agilent Technologies	Verify Hotel Loading			
	Please wait until the requ	ired objects in the hotel are verified	I.	
	This will still take approxin	nately 319 seconds.		
	Cancel Verification			
Sample Plates			atal Landing	
Chips	(	Required Ho	oter Loading	1
Reagent Plates				
	2			
Pipette Tips				3
Bottles	Chip ? 4-sipper DNA chip	Plate 1 ? EPDFTWINT_SKIRT*		Reagent Plate ? Reagent start col: 1
Check Resources	1 Possible sips: 0			Ladder start col: 1
Ŭ				Respects
	-			
	A	в		U
				1
2			Cancel < Back	Next > Finish

**3** After completion of the Hotel Loading verification - click *Next* to proceed with the Job start execution dialog. The Job Start Execution dialog provides information on the identified chip.

### 1 5100 ALP DNA1000-HT- Quick Start Guide

**Starting a Job** 

Start Job Execution						
G Agilent Technologies	Chips Please make sure to insert 1 chi	p into the hotel rack A as sl	nown below.			r
Job Options	chip into the depot at position A Required chip type: Required chip lifetime (sips);	4-sipper DNA chip 24				
Depot Loading	Depot loca	tion A2 (upper)		ļ.		
Chips Reagent Plates						
Trough						
Pipette Tips Bottles	4 sipper DNA chip Depot loca	tion A1 (lower)				
Check Resources	A1					
	4-sipper DNA chip			]	/erify Depot Load	ding
			⊆ancel	< Back	Next >	Finish

- 4 Click Next to proceed with the Job Start execution dialog.
- **5** Check the origin of your reagent plate. The first unused column filled with reagent has to be defined

# NOTE

The reagent plate may contain reagent for several different jobs. The instrument needs the information on the position of prepared and unused reagent columns. The number of the first unused column on the reagent plate provides enough information to proceed.

**6** To define the column of prepared reagent selected for instrument preparation by inserting the number of used columns in the *Already Used* drop down menu.



# CAUTION

Inserting the number of a used or unprepared reagent plate column may damage the chip.

7 Click next to proceed with the Job Start execution dialog.

### 1 5100 ALP DNA1000-HT- Quick Start Guide

**Starting a Job** 



8 Click on Access Trough to insert a prepared Trough in the Trough Support.

# NOTE

Wait until the plate handler was positioned below the right backside door and the instrument was inactive for at least 10 seconds.

**9** Manually open the right door. Press the clip on the back of the Trough Support to remove it from the plate handler.



# **NOTE** Remove used Ladder Troughs from the Trough Support. Do not use Ladder Troughs twice.

### **10** Insert the trough into the Trough Support as follows:



# NOTE

The arrow head on trough must be located at the lower left corner of the trough support. The trough fits only one way.

- **11** Insert the Trough Support with trough in the designated position of the plate handler. The lock of the latch will click when Trough Support is inserted correctly.
- **12** Manually close the right door.
- 13 Click next to proceed with the Job Start execution dialog.

### 1 5100 ALP DNA1000-HT- Quick Start Guide

**Starting a Job** 

Start Job Execution	Pipette Tips Please insert the pipette tips into the tip container as shown below Number Of Required Tips 4
Job Options Sample Plates Chips Reagent Plates Trough Pipette Tips Bottles Check Resources	Access Tips
0 5	Cancel < Back Next > Finish

- **14** Click on Access Tips to insert new tips into the tip container. The left door will be automatically opened by the instrument.
- **15** Press both indentations of the Tip Container to take the Tip Container out of the instrument.

# **NOTE** Remove all tips that were left in the instrument. Always start filling of the tip container with new tips from position A1.

- **16** The number and position of tips that have to be inserted is displayed on the screen of the Start Execution dialog. Position A1 is at the lower left corner of the displayed Tip Container.
- **17** Insert new tips and plug the tip container into the receptacle of the plate handler.
- **18** Click on *Close Left Door* button.
- **19** Click *Next* to proceed with the Job Start execution dialog.

5100 ALP DNA1000-HT- Quick Start Guide 1 Starting a Job

Start Job Execution	
Addent Technologies	Bottles         Flease         fill in sufficient amount of TE Buffer         fill in sufficient amount of weter         empty waste bottle         Access Bottles         TE         TE         Buffer         Water         waste
2 5	Cancel < Back Next > Finish

20 Click Access Bottles to unlock the front cover of the instrument and open it.



**24** Carefully close the front cover of the instrument.

### 1 5100 ALP DNA1000-HT- Quick Start Guide

**Starting a Job** 

NOTE

Orange light must be turned off.

25 Click Next to proceed with the Job Start execution dialog.



**26** The appearing diagram ilustrates the calculated schedule for the current job. Click *Start Job* to execute the current job.

5100 ALP DNA1000-HT- Quick Start Guide 1 Job Execution

# **Job Execution**

During job execution information is available on three levels of the software to check the status of the current job.

### **Job Summary**

The Job Summary tab provides a general overview on Job name, - Creator, Creation date and assay name. All major events generated by the instrument are registered in the *Job Logbook* that will be consecutively filled during analysis.

🚮 Automate	ed LabChip P	latform - /	4.00.1251169			
Application	Instrument	Control	<u>T</u> ools <u>V</u> iew <u>H</u> elp			
Instruments		• 🔟 🎖	y 😓 🛛 🔂 • 🛍 🕻	PR09	- 🍡 💽 🆢	
	Job Summ	iary Jo <u>b</u> St	atus Ingtrument Status			
	Name		Ed. Joh#1 11/12/200	13 14-12 (2)	Comment	
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	Created Da	to:	11/12/2002 10:02:42	AM		
	Madica d Da		DCDODED	AM		
laha	Modified By	e.	RSRUPER			
	Modified Da	ate:	11/13/2003 10:17:14	AM		
	Document F	Flags:	cxDocumentFlags			
	Import Infor	mation:	No Import Information			
	Job Specific					
	Template:					
<u>462</u>	Assay Nam	e:	M12 DNA 1000 root	Rins F PUse		
	Assay Versi	ion:	? .01			
<b>1</b> .	Auto Repor	t Settings:	cxReportSettings			
	Job Loaboo	k	-			
	Severity	Time		∆ Message		
	•	11/13/2	003 10:17:18 AM	Begin job acquisiti	on for batch Ed_Job#1_11/12/20	003_14:12 (2).
	Q	11/13/2	003 10:17:19 AM	No chip loaded		
	9	11/13/2	003 10:23:02 AM	Chip loaded - Part	Nr: G3010-67005 SN: DS279021	4314 Type: "DNA_S4_V1" Sipcount: 274 Date of last use: 10/20/2003 6:07:36 PM
	9	11/13/2	003 10:27:14 AM	Chip unloaded - P	artNr: G3010-67005 SN: DS2790	214314 Type: *DNA_S4_V1* Sipcount: 274 Date of last use: 10/20/2003 6:07:36 PM
		11/13/2	003 10:45:17 AM	Chip loaded - Part	Nr: G3010-67005 SN: DS279021-	4314 Type: "DNA_S4_V1" Sipcount: 274 Date of last use: 10/20/2003 6:07:36 PM
	2	11/13/2	003 10:50:31 AM	Chip unloaded - P	artNr: G3010-67005 SN: DS2790	214314 Type: *DNA_S4_V1* Sipcount: 274 Date of last use: 10/20/2003 6:07:36 PM
		11/13/2	003 10:51:32 AM	Chip replenishmen	t started	
	1 X .	11/13/2	003 10:52:28 AM	Chip loaded - Parti	Nr: 63010-67005 SN: 05279021-	4314 Type: "UNA_54_V1" Sipcount: 274 Date of last use: TU/20/2003 6:07:36 PM
	1 X .	11/13/2	003 10:53:59 AM	Chip unloaded - P	artnr: G3010-67005 SN: D52790.	214314 Type: "DINA_54_V1" Sipcount: 274 Diate of last use: T0/20/2003 6:07:36 PM
	1 Å	11/13/2	003 11.13.06 AM	Chip roalenishmen	NI. 03010-07003 SN. 03273021- Finished	4314 Type: DNA_34_VT_Stpcount. 274 Date of last use. T0/20/2003 6.07.36 PM
	1 Å	11/13/2	003 11:43:35 AM	Crip repienisninen Rogin plato apguir	utimisneu ution for plato Diato 1	-
	1 ă	11/13/2	003 1143.30 AM	End plate acquisit	ion for plate Plate 1	
	i č	11/13/2	003 1:20:25 PM	Chin unloaded - P	artNr: G3010-67005 SN: DS2790	214314 Tune: *DN&: S4: V1* Sincount: 298 Date of last use: 11/13/2003 1:05:37 PM
	i č	11/13/2	003 1:38:27 PM	Chip loaded - Part	Nr: 63010-67005 SN: DS279021-	4314 Tune: "DNA: S4 V1" Sincount: 298 Date of last use: 11/13/2003 1:05:37 PM
	l ő	11/13/2	003 1:43:41 PM	Chip unloaded - P	artNr: G3010-67005 SN: DS2790;	214314 Tune: *DNA_S4_V1* Sincount: 298 Date of last use: 11/13/2003 1:05:37 PM
	i ii	11/13/2	003 1-45-07 PM	Chin loaded - Part	Nr. 63010-67005 SN: DS279021	4314 Tune: *DNA_S4_V1* Sincount: 298 Date of last use: 11/13/2003 1:05:37 PM
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Start	🖸 In 🔯	IL:\	Ad   🔛 Fr   🖾 C:	🥌 Ma 🌺 Cli	₩ Pix   ₩Cli   @ LE	Desktop » 🛃 📢 💓 🗖 🕼 🔲 🛄 🖉 🚺 💠 2:33 PM

#### **Job Status**

The *Job Status* tab is divided into several information panels. Information on the selected sample plates of the current job is displayed on the top of the tab. The Job Logbook appears in the middle of the screen next to the status display. The Real-time signal plot in the lower part of the screen displays the major incoming data acquired by the instrument.

1 Rinse Chip/Chip replenishment

🛃 Automated	d LabChip Platform - A.00.125I169				
Application I	Instrument Control Tools View Help				
	PROS	<b>- *</b>		Ed Job#1	11/12/2003 14:12 (2)
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	Executing Job				
Instruments	Position Type Location Label	Barcode	Comment		
	D 1 Seper EPDF B1 Plate 1				
Jobs					
<u> </u>	Prepare Job	Sin Well Label	Comment		▼
		_			
Assays					
Results		11/13/2003 10:17:1	Message 5 AM The job execution started by r	sroper on instrument PR09	
		11/13/2003 10:17:1 11/13/2003 10:17:1	8 AM Begin job acquisition for batch 9 AM No chip loaded	Ed_Job#1_11/12/2003_14:1	12 (2).
Resources		11/13/2003 10:23:0	2 AM Chip loaded - PartNr: G3010-6	7005 SN: DS2790214314 Ty	pe: "DNA_S4_V1" Sipcount: 274 Date of I
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	0		+		
	.10 8 10	12 14	16 18	20	22 24 min
Start	Ninhov . Oluváutho Madohe Fr. MFramel		Magallan & Ciphoar	eckton » 📽 🕼 🏭 🖉	

**2** Sample analysis

During data acquisition the analyzed plate is shown on the status display indicating the wells on the plate that are currently processed. The name and position of the analyzed samples are displayed on the top of the logbook.

🋃 Automate	d LabChip Plat	form - A.00.1	251169									_8>
Application	Instrument ⊆o	ntrol <u>T</u> ools	View He	lp .								
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49	Process Plate	e - Sample A	nalysis		Sip Well	Label		Comment				Category
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					11/13/20	03 10:45:17 AM	Chip loaded	- PartNr: G30	10-67005 SN: [	)S2790214314 Ty	pe: *DNA_S4_V1* Si	pcount: 274 Date of
					11/13/20	03 10:50:31 AM 03 10:51:32 AM	I Chip unload	led - PartNr: G	3010-67005 SN H	I: DS2790214314	Type: "DNA_S4_V1"	Sipcount: 274 Date
nesources					11/13/20	03 10:52:28 AM	Chip loaded	I - PartNr: G30	- 10-67005 SN: [	) S2790214314 Ty	pe: "DNA_S4_V1" Si	pcount: 274 Date of
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					11/13/20	03 11:43:35 AM	Chip replen	ishment finishe	id			poor a crist of a coord
		Plate 'Plate 1'	from Location	n B1	11/13/20	03 11:43:36 AM	Begin plate	acquisition for	plate Plate 1.			
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Start	OIn 💽 L:	Ad	Fr	🖾 C: 🔍 🌄 M	la 🎠 Cli	Pix 🌟 🤇	l		Desktop *	S4:€) ■ (	@₩,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	12:51 PM

### **3** Job finished

The *Job Status* tab of a finished Job contains the complete Logbook as well as the Real-time Signal Plot.



#### **Instrument Status**

The Instrument Status tab displays the current Depot loading of the instrument as well as the Real-time signal plot. The status bar coloured in blue as well as the coloured rectangle in the upper right corner (green = active instrument status/red = error status) provide information on the current instrument state.

🛃 Automated	LabChip Platform - A.00.125I169								_ 🗆 ×
Application Instruments	strument Control Tools View	Help		- 🍡 🙆 😹					
Instrumer	nts - PR09 - Executing	Job				Ed Job#1	11/12/2	003 14:12	2 (2) 🌻
	Job Summary Job Status Instrume	nt Status						_	
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Resources	Access Tips	A	B		C	Respects D		Exchange C	artridge
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	Real-time Signal Plot Adjust 🔺 🗸 🌗 Zoomout	Freeze 🐼 🛃	-	Configuration.	••				
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### 1 5100 ALP DNA1000-HT- Quick Start Guide Finalizing a job

# **Finalizing a job**

**1** Close job in the instrument context

NOTE After execution of the job the chip will be stored in the chip environment of the instrument.

#### Instrument clean up

1 Select Control Menu Bar click on *Chip and Basin Handling>Unload Chip from Chip Environment* to transfer the chip from the chip environment to the designated rack in the instrument depot.



- 2 Open the front door of the depot and take out the rack with the chip.
- **3** Carefully insert the chip into the Chip box and store it at 4°C.
- **4** Empty the waste bottle.
- 5 Remove the sample and reagent plates from the depot and store them at  $4^\circ\mathrm{C}$
- **6** Empty the tip container.

# **Result Review**

- 1 Click on the *Results* button on the Context toolbar to switch to the Result context. The Result context allows the analysis and review of the acquired data.
- **2** Go to *Results* and click on *Open...* or directly click on the *Open Job* button in the menu bar.



**3** Select a Job in the database and click on *Open Job*.

### 1 5100 ALP DNA1000-HT- Quick Start Guide

**Result Review** 



### NOTE

The Result context of the 5100 ALP software provides multifacetted options for analyzing, arranging and reviewing the acquired data. This guide will focus on the Graph and the Gel tab of the Details tab allowing to review the data either as an electropherogram or as gel-like image.

# **Details Tab**

The Details tab is divided into two different information panels: Sample table and Sample Details.



### 1. Sample Table

The Sample Table allows the grouping of all sample measurements within the selected job and provides information on Sample Category, Samples Name, Analysis State, etc.

<u>S</u> (	ummary	<u>O</u> vervie	w De	etails Result	Elagging			
Sa	mple Ta	ible						
<b>%</b> —			Group	by Plate	🔹 🥝 💟 Show Samples Only	• 🥥 💠 🗕		
		Resu	Categ	Well	Sample Name	Comment	Stal	<b>.</b>
	-	Al	S	E3	Unknown		1	
		All	S	F3	Unknown		1	
		Al	S	F3	Unknown		1	
	-	All	S	G3	Unknown		1	
		Al	S	G3	Unknown		1	
>	<b>~</b>	AI	S	H3	Unknown		1	
		AI	S	H3	Unknown		1	
		All	S	A4	Unknown		1	
		AI	S	A4	Unknown		1	
		All	S	B4	Unknown		1	

### 2. Sample Datails

The Sample Details tab contains the Gel-, Graph-, and Peak Table tab providing all sample related informations. All sample related informations are shown on the lower left part of the display, whereas the Assay Setpoint Parameters are shown on the right as a flip chart.

# The Graph Tab

Details		
Graph	Gel	Peak Table

The Graph Tab displays the acquired data as electropherograms and optional in the Peak Table the calculated values (fragment concentration, fragment size, molarity, etc.).

Click on sample row in the Sample Table to select a sample. The selected sample will be highlighted and displayed as an electropherogram in the Graph View



Further results are available in the a) Peak Table tab, b) Sample Information tab, and c) DA Log tab.

#### 1 5100 ALP DNA1000-HT- Quick Start Guide Besult Review

#### Peak table tab

The peak table tab contains peak related values (e.g. sample Size, Concentration, Molarity) calculated by the software.

	ID	△ Size [bp]	Co	ncentration	Molarity	Observation
ΞΟ	hannel :	LM				
	(	1				Lower Marker
ΞΟ	hannel :	S/UM				
		1	905	14.0	23.4	
		2	1500	5.0	5.1	Upper Marker
Pez	ak Table	Sample Inform	ation DALog			

#### Sample Information tab

The Sample Information tab provide general informations on the analysis of each sample



### DA Log tab

If data analysis of a focussed sample failed, the DA Log provides a short description of the failure.



1 5100 ALP DNA1000-HT- Quick Start Guide Result Review

# The Gel Tab

Details		
Graph	Gel	Peak Table

The Gel Tab contains the Gel-like image and the a) Peak Table tab, b) Sample Information tab, and c) DA Log tab as in the Graph Tab.

Graph Gel P	eak lable	🛲 🦳 rw Siow	Apply Expos	ure 😪						
1 E3	1 G3 1 H3	3 1 D4 1 E4 1	.E4 1F4	1 F4	1 G4	1 H4	1 B5	1 A5	1 A5	1
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w/ell:	D5 (34)	Number of Peaks	< 1	Jampics						
	20(04)	Total Concentrati	on: 15.4							
							B	eset	Ap	ply

# **Reviewing Results in the Gel Tab**

1 Check the samples in the Sample Table to show them in the Gel View.

NOTE

Only samples checked with a check mark on the left hand side of the Sample Table will be displayed in a gel-like image.

- 0	- 8-		Group by Place	- 🕑 🔛	Driow Dampies Only		
		Resu	Catec Well /	Sample Name		Comment	
	<b>V</b>	All	S E4	Unknown			
	•	All	S E4	Unknown			
	<b>v</b>	All	S F4	Unknown			
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a p	ls Gel	Peak Ta	ble <b>f</b> er (	LM 5/UM Apply E	xposure 🍃	1H4 185 1A5 1A5 1 Show Standard •	
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	ls n Gel 1	Peak Ta	3 1H3 1	LM S/UM Apply E	xposure 😓 LF4 1F4 1G4	1 H4 1 B5 1 A5 1 A5 1 Show Standard   Apply baseline removal Apply smoothing	V
	ls I Gel	Peak Ta	3 A3	Unknown LM S/UM Apply E D4 1E4 1E4 1	xposure 😓 LF4 1F4 1G4	1 H4     1 B5     1 A5     1 A5     1       Image: Standard Image:	(Sample)t 0.
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	Is 1 1 1	Peak Ta	5 AJ	LM         S/LM         Apply E           24         1 E4         1 E4         1	xposure S	1 H4     1 B5     1 A5     1 A5     1       Apply baseline renoval     Apply baseline renoval       Apply smoothing       Height threshold (Abs)       Area threshold (Sample       Width threshold (Sample	(Sample/) (Upper M 0. (Upper f 0.
		Peak Ta	25 AJ	LM S/LM Apply E 04 1E4 1E4 1 Concentration	xposure IF4 1F4 1G4 Molarity	1 H4       1 B5       1 A5       1         Apply baseline removal       Apply baseline removal         Apply anothing       Height threshold (Abs)         Area threshold (Sample         Width threshold (Sample         Observation	(Sample/I, 0, / /Upper M 0, / /Upper f 0, 3
	Is Gel 1 ID nannel	Peak Ta E3 1 A Si I: S/UM	s AJ	LM S/UM Apply E 24 1 E4 1 E4 2 Concentration	xposure IF4 IF4 IG4	1 H4       1 B5       1 A5       1 A5       1         Apply baseline removal Apply smoothing       Height threshold (Abs).       Area threshold (Sample Width threshold (Sample Width threshold (Sample Diservation	(Sample/I (Sample/I /Upper M e/Upper f 0.
		Peak Ta E3 1 A Si I: S/UM 1	2 (bp)	LM         S/LM         Apply E           94         1 E4         1 E4         1           1         E4         1 E4         1           Concentration         5         14.7	xposure IF4 1F4 1G4 Molanity 24.6	1 H4     1 B5     1 A5     1 A5     1       Apply baseline removal Apply smoothing     Apply smoothing     Height threshold (Abs)       Height threshold (Sample       Width threshold (Sample       Ibservation	(Sample/t Upper M 0.

**2** Double-click on a specific lane in the Gel-like image directly navigates to the associated electropherogram in the Graph Tab.

1 5100 ALP DNA1000-HT- Quick Start Guide

**Result Review** 

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