Applied Biosystems

Getting Started Guide

For Research Use Only. Not for use in diagnostic procedures.

NOTICE TO PURCHASER

PLEASE REFER TO THE SNPlex™ GENOTYPING SYSTEM 48-PLEX USER GUIDE FOR LIMITED LICENSE OR DISCLAIMER INFORMATION.

Your installation and/or use of the Gemini Script Files ("Method") may affect the service coverage of your instrument under warranty or service contract. Prior to installing and/or using the Method, check the warranty or service coverage of your instrument, including limitations thereof, or check with your service provider. APPLIED BIOSYSTEMS MAKES NO WARRANTIES OF ANY KIND WHATSOEVER, EXPRESS OR IMPLIED, WITH RESPECT TO THE METHOD, INCLUDING BUT NOT LIMITED TO WARRANTIES OF FITNESS FOR A PARTICULAR PURPOSE OR MERCHANTABILITY OR THAT THE METHOD IS NON-INFRINGING. ALL OTHER WARRANTIES ARE EXPRESSLY DISCLAIMED. YOUR USE OF THE METHOD IS SOLELY AT YOUR OWN RISK, WITHOUT RECOURSE TO APPLIED BIOSYSTEMS.

TRADEMARKS:

Part Number 4360790 Rev. B
6/2005

Contents

Preface

How to Use This Guide

Purpose of This Guide

This guide provides a representative workflow using the SNPlex™ Genotyping System 48-plex with the TECAN Genesis RSP robot. It provides information to assist you in selecting equipment and setting up the laboratories and is intended to be used with the *SNPlex™ Genotyping System 48-plex User Guide* (PN 4360856).

Audience

This guide is written for principal investigators and laboratory staff who intend to use the SNPlex™ Genotyping System 48-plex with the TECAN Genesis RSP robot.

Assumptions

This guide assumes that you have read the *SNPlex™ Genotyping System 48-plex User Guide* and the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide* (PN 4363143) and that you have a working knowledge of the assays and methods used for the SNPlex Genotyping System 48-plex.

Text Conventions

- **Bold** indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow bracket (>) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File > Open > Spot Set**.

User Attention Words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: The size of the column affects the run time.

IMPORTANT! To verify your client connection to the database, you need a valid Oracle user ID and password.

Safety Alert Words

Safety alert words also appear in user documentation. For more information, see "Safety Alert Words" on page ix.

How to Obtain More Information

For more information about using the SNPlex Genotyping System 48-plex, refer to the:

- *SNPlex™ Genotyping System 48-plex User Guide* (PN 4360856)
- *SNPlex™ Genotyping System 48-plex Quick Reference Card* (PN 4360855)
- *SNPlex™ Genotyping System 48-plex Assay Design and Ordering Guide (PN 4357460)*
- *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide* (PN 4363143)
- *SNPlex™ Genotyping System 48-plex Automating PCR Using the Tomtec Quadra 3 Getting Started Guide (PN 4358100)*

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

How to Obtain Support

To contact Applied Biosystems Technical Support from North America by telephone, call **1.800.899.5858**.

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Safety and EMC Compliance Information

Safety Conventions Used in This Document

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word–**IMPORTANT, CAUTION, WARNING, DANGER**–implies a particular level of observation or action, as defined below:

Definitions

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

Chemical Hazard Warning

WARNING **CHEMICAL HAZARD.** Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page ix.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Safety

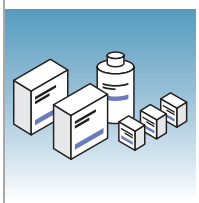
Chemical Waste Hazard

CAUTION **HAZARDOUS WASTE.** Refer to Material Safety Data Sheets (MSDSs) and local regulations for handling and disposal.

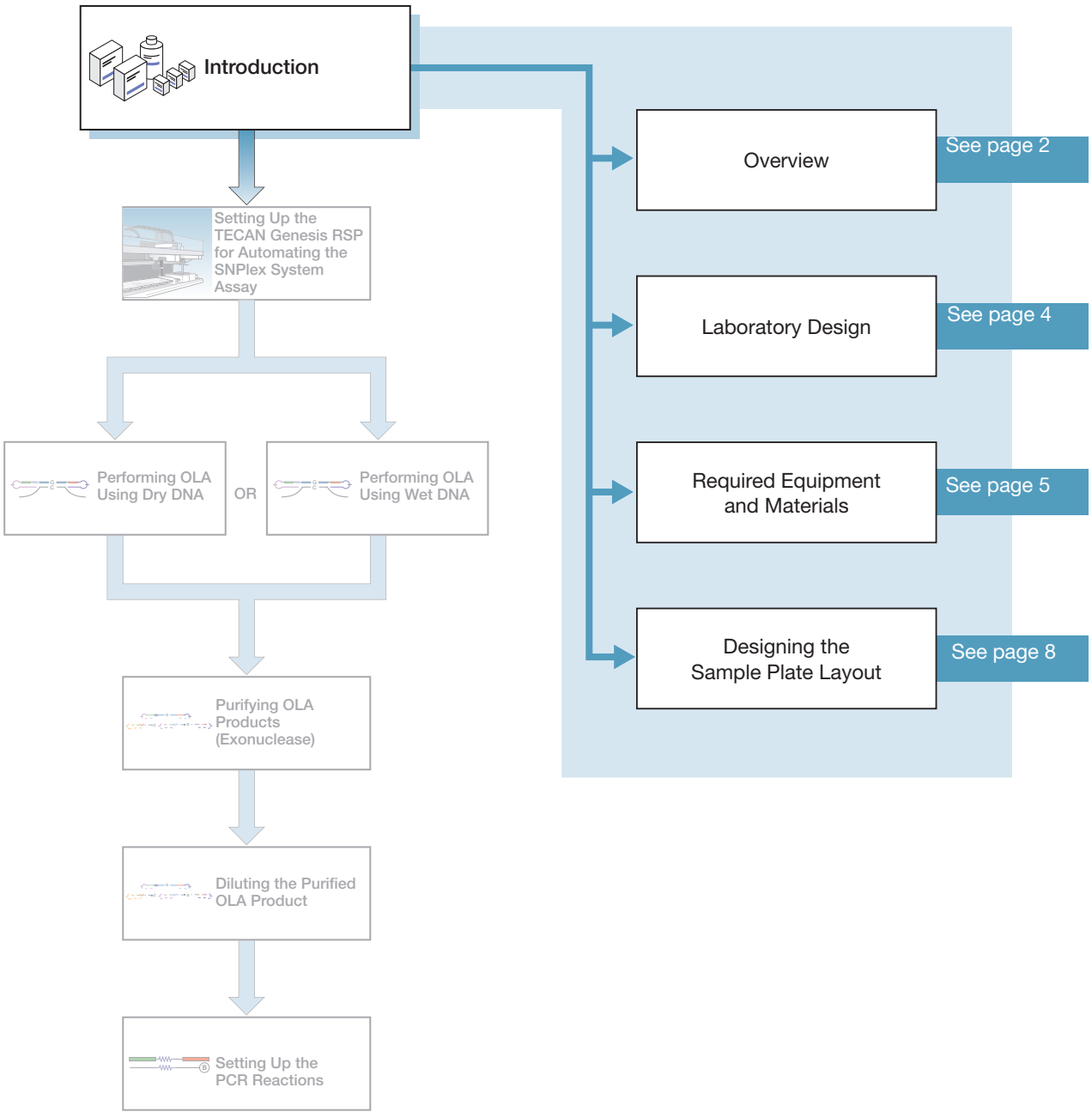
Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.



Introduction



Notes

Overview

The SNPlex™ System assay consists of several protocols which involve manipulating small volumes between 96- and 384-well plates. This guide contains the protocols required to complete the oligonucleotide ligation assay (OLA) portion of SNPlex System assays using the TECAN Genesis RSP.

About This Guide

The *SNPlex™ Genotyping System 48-plex Automating OLA Using the TECAN Genesis RSP Getting Started Guide* provides instructions for automating the OLA procedures using:

- The TECAN Genesis RSP Robot with the 8-channel liquid handling accessory (LiHa). For information about automating the OLA using other instruments, refer to the appropriate guide. (See "Documentation" on page 7.)
- The 384-well protocol using a batch of four SNPlex™ OLA reaction plates. For information about the 96-well protocol, refer to the *SNPlex™ Genotyping System 48-plex User Guide* (PN 4360856) for instructions.
- The Applied Biosystems 3730*xl* DNA Analyzer to collect data.
- GeneMapper® software v3.7 to analyze data.

Assumptions

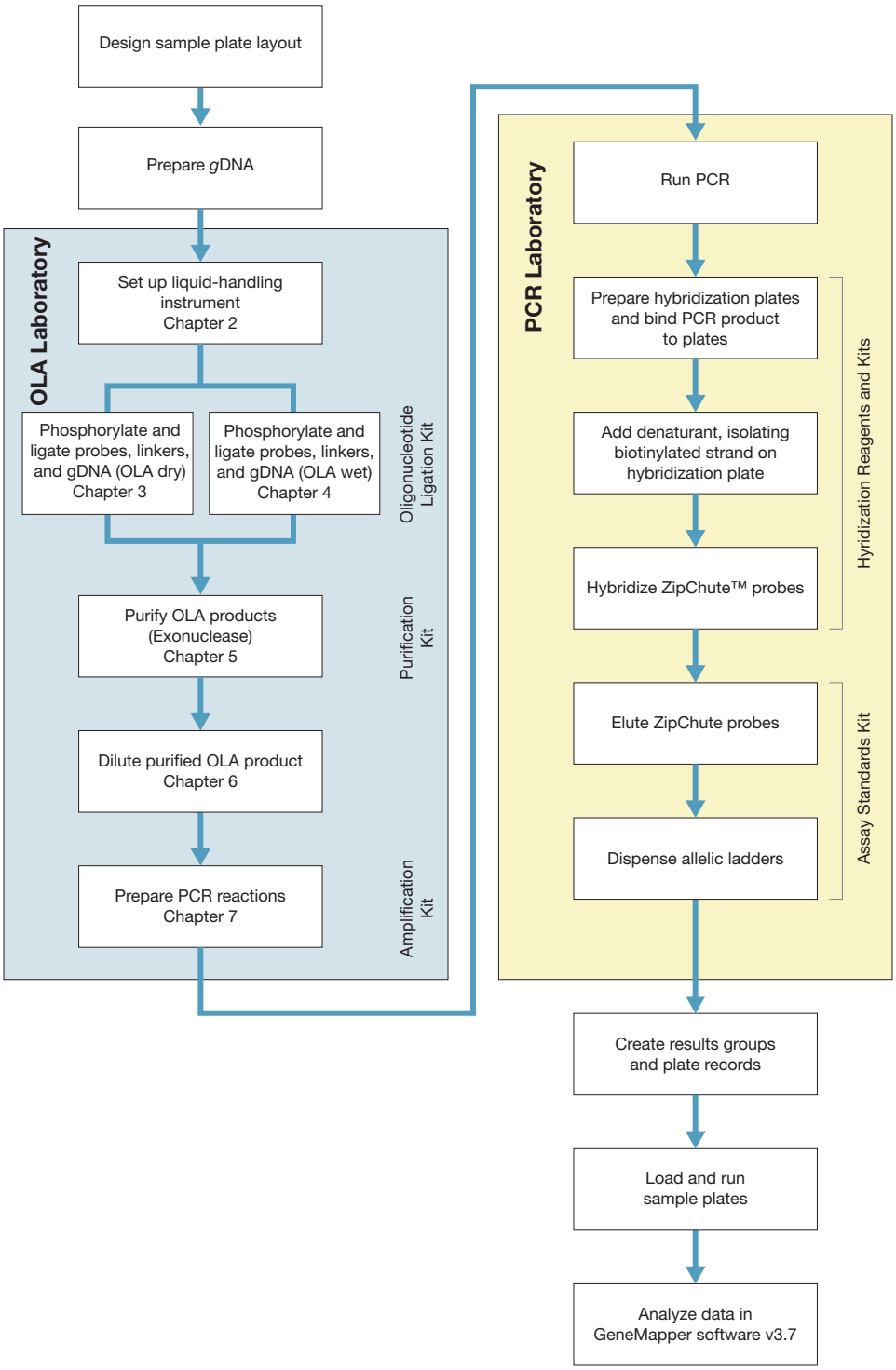
This guide assumes that you have read the *SNPlex™ Genotyping System 48-plex User Guide* (PN 4360856) and the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide* (PN 4363143) and that you have a working knowledge of the assays used for the SNPlex Genotyping System 48-plex.

This guide also assumes that you have a working knowledge of the operation of the TECAN Genesis RSP robot, including setup, configuration, and programming scripts.

Notes

SNPlex System Automation Workflow

The following diagram illustrates the SNPlex System workflow.

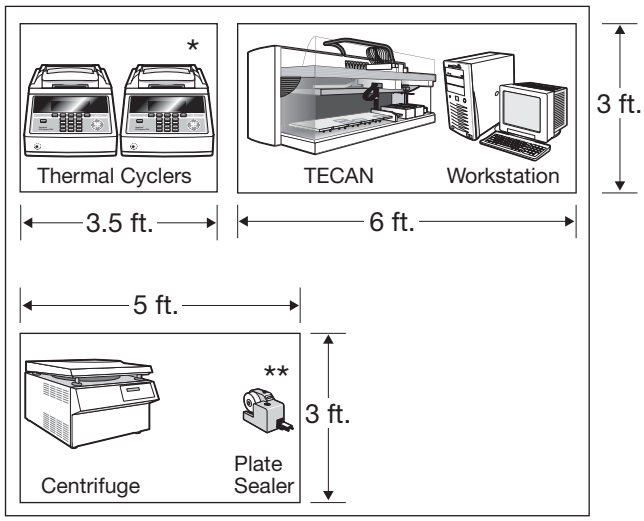


Notes

Laboratory Design

The protocols contained in this guide should be performed in an amplicon-free OLA lab, such as that shown in the following figure.

OLA Lab Layout



* This is a vertical shelf unit, depending on the number of shelves, can house 2 thermal cyclers per shelf.

** Optional

For additional information about laboratory design, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

Plate Sealing

A plate sealer is recommended but not required.

Applied Biosystems has found that certain adhesive plate covers negatively impact the performance of the SNPlex System assay. If you do not use a plate sealer, you may use one of the recommended plate covers listed in Table 1-3 on page 1-9 of the *SNPlex™ Genotyping System 48-plex User Guide*. If you use covers other than the recommended plate covers, test them using the SNPlex™ System Control Set (see Appendix A of the *SNPlex™ Genotyping System 48-plex User Guide*).

Notes

Required Equipment and Materials

Equipment

Item	Vendor	Part Number
GeneAmp® PCR System 9700, Dual 384-Well Sample Block Module	Applied Biosystems	N8050002
Sealer for microtiter plates. Recommend: • ALPS-300 Heat Sealer • Air compressor with clean air package • Easy Peel Foil Sealing Film for ALPS-300	ABGene	AB-0950 CMP-950 AB-3739
Centrifuge (equipped to accommodate reaction plates)	Major Laboratory Supplier (MLS)	–
TECAN Genesis Robotic Sample Processor (RSP) 150 or 200 System with single 8-Channel Liquid Handling (LiHa) Arm, low-volume option, TECAN Gemini Software, version 3.5, and:	Contact your TECAN Schweiz AG representative for information	
• 250-µL syringes • Resistant, adjustable, low-volume tips for 384-well plates • Microplate carriers, 3-position • Tip wash carrier • Adapters for 384-well reaction plates • Adapters for 96-well reaction plates • 100-mL Trough carrier, 3-position • 100-mL Trough insert	TECAN Schweiz AG	71-500 612-533 61-418 61-400 61-781 61-527 61-449 70-744

Consumables and Small Equipment

Item		Vendor	Part Number
15-mL centrifuge tubes, conical bottom		MLS	–
96-well deep-well plates		VWR	82006-448
Pipettors (2–20 µL, 10–200 µL, and 100–1000 µL)		MLS	–
Reaction Plates	ABI PRISM® 384-Well Clear Optical Reaction Plate, with Barcode, 50 plates	Applied Biosystems	4309849
	ABI PRISM® 384-Well Optical Reaction Plate with Barcode, 500 plates	Applied Biosystems	4326270
	MicroAmp® Optical 96-Well Reaction Plate	Applied Biosystems	N8010560

Notes

Item *(continued)*		Vendor	Part Number
Reaction Plate Covers • Heal seals and sealers	MicroAmp® Full 96-Well Plate Cover	Applied Biosystems	N8010550
	ABI PRISM™ Optical Cover Compression Pad	Applied Biosystems	4312639
	Easy-Peel 610 meter roll	ABGene	AB-3739
	Easy-Peel individual sheets	ABGene	AB-0745
	UNISEAL AL	Whatman	7704-0002
	Thermo-Sealer	ABGene	AB-0384
	Plate Sealer, ALPS 300™	ABGene	AB-0950
• Adhesive seals	384 Well Microplate Aluminum Sealing Tape	Corning	6569
	Adhesive PCR foil seal	ABGene	AB-0626
	SILVERseal	Greiner	676 090
Vortex		MLS	–

Kits and Reagents

Item	Vendor	Part Number
SNPlex System Kits Required for This Protocol		
SNPlex™ System Oligonucleotide Ligation Kit Includes • SNPlex™ OLA Master Mix • SNPlex™ Universal Linkers, 48-plex • SNPlex™ dATP, 100X	Applied Biosystems	4357460
SNPlex™ System Ligation Probes	Applied Biosystems	4346978
SNPlex™ System Purification Kit Includes • SNPlex™ Exonuclease Buffer, 10X • SNPlex™ Lamda Exonuclease • SNPlex™ Exonuclease I	Applied Biosystems	4349357
SNPlex™ System Amplification Kit Includes • SNPlex™ Amplification Master Mix, 2X • SNPlex™ Amplification Primers, 20X	Applied Biosystems	4349358
Other Reagents		
Nuclease-free water	Promega	P119C
Sterile 1X TE buffer (10 mM Tris-base, pH 8.0, and 1 mM Na_2EDTA)	Fluka	93283

Notes

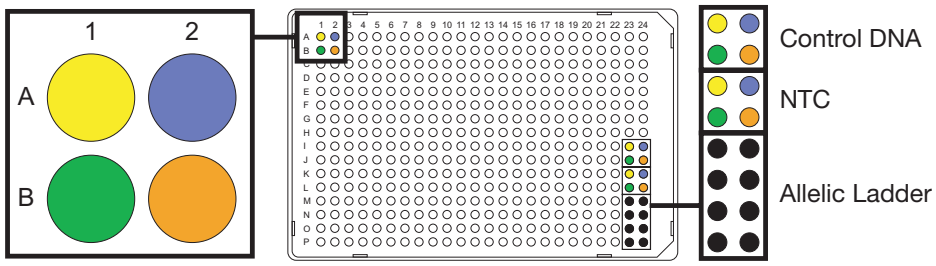
Documentation

Document Name	Part Number
SNPlex™ Genotyping System 48-plex Assay Ordering Guide	4357460
SNPlex™ Genotyping System 48-plex User Guide	4360856
SNPlex™ Genotyping System 48-plex Quick Reference Card	4360855
SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide	4363143
TECAN Gemini Software Manual, Version 3.5	TECAN Schweiz AG 391-201
TECAN Genesis Workstation Operating Manual	TECAN Schweiz AG 391-197
SNPlex™ Genotyping System 48-plex Automating PCR Using the Tomtec Quadra 3 Getting Started Guide	4358100

Notes

Designing the Sample Plate Layout

Each 384-well plate is divided into four quadrants, each with 96 wells. The convention used in this document is that the first of 96 wells in quadrant 1 is well A1, well B1 for quadrant 2, A2 for quadrant 3, and B2 for quadrant 4.



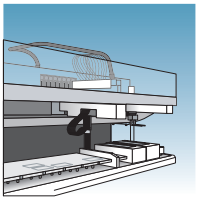
A1 = Quadrant 1 A2 = Quadrant 3
B1 = Quadrant 2 B2 = Quadrant 4

A number of plate layouts are possible, assuming that each batch consists of four 384-well plates [as illustrated in "Reaction Plate Layouts and the Associated Scripts for the OLA Protocol (Dry gDNA)" on page 21 and "Reaction Plate Layouts and the Associated Scripts for the OLA Protocol (Wet gDNA)" on page 39.]

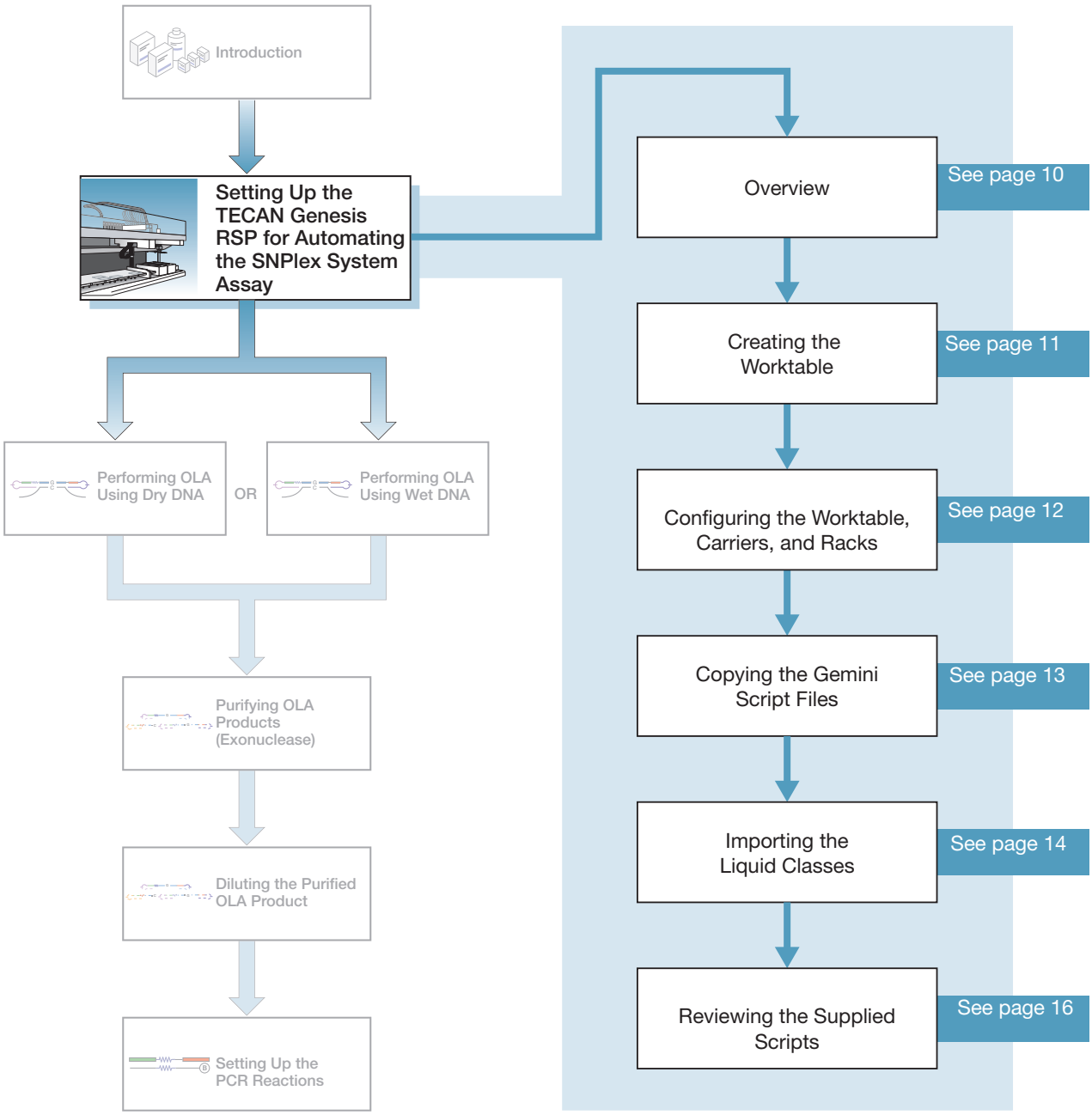
The layout of a sample plate must be coordinated with the structure and naming of Data Collection software run folders in order for GeneMapper® software to correctly analyze the data. Each run must include an allellic ladder, control DNA, and no template control (NTC).

For additional information about sample plate layout, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide* (PN 4363143). Note that different robotics manufacturers have differing conventions for the order and location of plate quadrants. Be cognizant of these differences when programming robotics.

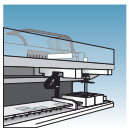
Notes



Setting Up the TECAN Genesis RSP for Automating the SNPlex System Assay



Notes



Overview

Definitions of Terms

Worktable — Work surface of the instrument where the carriers are placed for access by the Liquid Handling Arm.

Carrier — A unit placed on the worktable, it is identified by its position on the worktable.

Rack — A unit placed in a carrier. Racks include 96-well and 384-well plates and troughs.

About Script Files

Script files contain the instructions for a workflow on a robot. Script files are specific for a given robot and they can be read only by the software of that robot. For example, the four scripts provided for performing OLA using dry gDNA on the TECAN Genesis RSP can be read only by the TECAN Gemini software.

Applied Biosystems provides a total of 11 scripts for automating the OLA laboratory process:

- Eight scripts for the OLA setup, four for using dry gDNA and four for using wet DNA.
- One script for the exonuclease purification step
- One script for the OLA dilution step
- One script for the PCR setup step

These scripts are explained in succeeding chapters of this guide.

IMPORTANT! The sample plate layouts and scripts provided in this document are designed for experiments using batches of four 384-well plates.

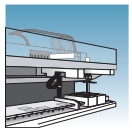
Summary

Setting up the TECAN Genesis RSP Robot for automating the OLA laboratory process involves five tasks:

- "Creating the Worktable" on page 11
- "Configuring the Worktable, Carriers, and Racks" on page 12
- "Copying the Gemini Script Files" on page 13
- "Importing the Liquid Classes" on page 14
- "Reviewing the Supplied Scripts" on page 16

If you are using the TECAN instrument exclusively for the SNPlex System assay (or if you are using the SNPlex System worktable for all other applications), setting up the instrument is a one-time process. If you are using the instrument with other worktables, you may need to repeat some steps in the setup process.

Notes



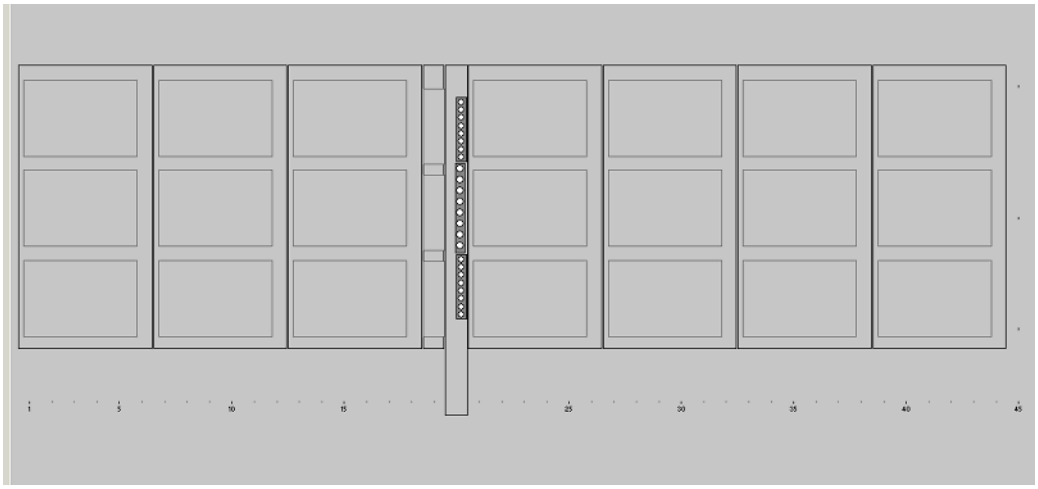
Creating the Worktable

A worktable setup indicates the position of carriers and racks on the instrument worktable.

Building the worktable includes both physically building the worktable on the instrument and modelling the physical worktable in the TECAN Gemini software.

IMPORTANT! Build the worktable before the Installation and Operation Qualifications (IQ/OQ) are performed.

The following illustration shows the positions on an empty worktable for the TECAN Genesis RSP:

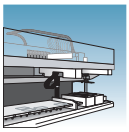


The TECAN worktable consists of:

- Up to seven 3-position microplate carriers, placed on worktable grid positions:
  - 1
  - 7
  - 13
  - 21
  - 27
  - 33
  - 39
- A 3-position 100-mL Trough carrier, placed on worktable grid position 19
- A tip wash station, placed on worktable grid position 20

Note: If you have a new TECAN Genesis RSP instrument, a TECAN representative must perform IQ/OQ, using the worktable that you just set up.

Notes



Configuring the Worktable, Carriers, and Racks

Before you can use the TECAN Genesis RSP instrument, you must calibrate the carriers and racks on the worktable. TECAN Genesis documentation refers to this process as "configuration."

During this process, coordinates of each position on the worktable are established, enabling the instrument to precisely move objects to and from various locations on the worktable.

IMPORTANT! Configuring the worktable, carriers, and racks ensures the correct transfer of materials, which is essential to proper script operation. Do not use the TECAN Genesis RSP instrument until you have completed the configuration process.

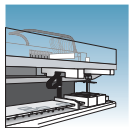
Refer to the TECAN Genesis RSP documentation for more information about this process.

Carriers and Racks

Configure specific carriers and racks to run the SNPlex System scripts. Use the names specified in the supplied script files and given below.

Carrier	Associated Racks
Microplate, 3 Pos., landscape	• PCR (384) in 61-781 Adapter • PCR (96) in 61-527 Adapter • Deep Well, landscape
Trough 100ml, 3 Pos.	Trough 100ml
Wash station	• Waste • Cleaner shallow, 8 Pos. • Cleaner deep, 8 Pos.

Notes



Copying the Gemini Script Files

Downloading the Zip Archive

1. Go to http://www.appliedbiosystems.com.
2. Click **Support** at the top of the page.
3. On the Support page, click **Software Downloads**.
4. From the Select Product Software menu, select **SNPlex Genotyping System**.
5. From the Software Type menu, select **Main Page**.
6. Download the appropriate zip archive for your robot.

Copying the Files

1. Power on the TECAN workstation and launch the TECAN Gemini software.
2. Select **File > Open**.
3. Browse to the TECAN script files in the downloaded zip archive and open a script file.
4. Select **File >Save** to save the file in an appropriate location on the workstation.
5. Repeat steps 3 and 4 until you have saved the following files:

1a_SNPLEX_OLA_PN_S4_D_LiHa.gem	1d_SNPLEX_OLA_P1_S16_W_LiHa.gem
1b_SNPLEX_OLA_P4_SN_D_LiHa.gem	2_SNPLEX_EXO_LiHa.gem
1c_SNPLEX_OLA_P16_S1_D_LiHa.gem	3_SNPLEX_EXO_DILUTION_LiHa.gem
1d_SNPLEX_OLA_P1_S16_D_LiHa.gem	4_SNPLEX_PCR.gem
1a_SNPLEX_OLA_PN_S4_W_LiHa.gem	Dispenses per Row CALC.gem (subroutine)
1b_SNPLEX_OLA_P4_SN_W_LiHa.gem	Rack-Well Offsets for 384 well plates.gem (subroutine)
1c_SNPLEX_OLA_P16_S1_W_LiHa.gem	

Note: You can also use Microsoft Windows® Explorer to copy the files from the location to which you downloaded them to an appropriate location on the TECAN Genesis RSP workstation.

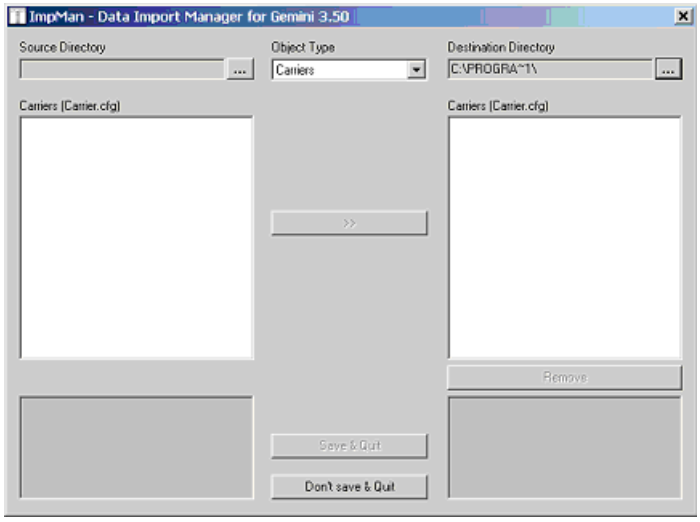
Notes

Importing the Liquid Classes

The liquid classes contain pipetting parameters for the use of the various SNPlex™ Genotyping System, 48-plex assay reagents.

IMPORTANT! If you use the TECAN Genesis RSP instrument for tasks other than automating the SNPlex System assay, back up your carrier, rack, and liquid class setting files before importing the SNPlex System workspace file. Parameters for the SNPlex System assay may be different than those for other configurations and these new parameters may overwrite existing parameters. Refer to the TECAN Gemini software documentation for information on backing up your files.

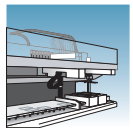
1. Quit the TECAN Gemini software.
2. Launch the ImpMan.exe software utility.



Note: The ImpMan.exe software utility is in the same folder as the TECAN Gemini application.

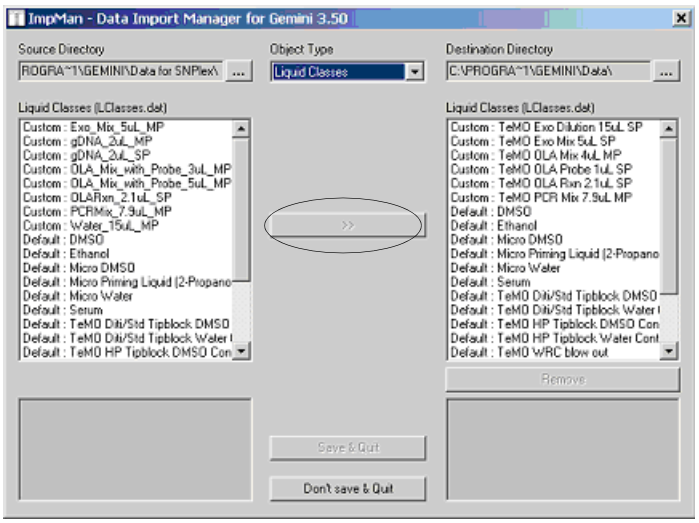
3. Under Object Type, select **Liquid Classes**.
4. Under Source Directory, navigate to the extracted folder for the files for the SNPlex OLA TECAN Genesis RSP and select the **Data_SNPLEX_OLA** folder.

Notes



5. Under Destination Directory, navigate to the Data folder currently in use by the TECAN Gemini software:

   C:\Program Files\Gemini\Data



6. In the Source Directory list, select a liquid class, then click >> to import it to the Destination Directory.

   Repeat this step until you have imported all eight of the following liquid classes:

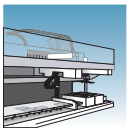
Custom: gDNA_2uL_MP	Custom: Exo_Mix_5uL_MP
Custom: gDNA_2uL_SP	Custom: PCRMix_7.9uL_MP
Custom: OLA_Mix_with_Probe_3uL_MP	Custom: OLARxn_2.1uL_SP
Custom: OLA_Mix_with_Probe_5uL_MP	Custom: Water_15uL_MP

7. Click **Save & Quit**.

8. Launch the TECAN Gemini software.

9. Select **Setup** > **Liquid Classes** and verify that all eight new liquid classes are listed.

Notes



Reviewing the Supplied Scripts

Verify the Script Conditions

Because the proper operation of a script depends on the presence of specific conditions (such as the number of 384-well reaction plates and the location of the subroutine files), you should verify that the scripts you copied have the specifications listed in the following table:

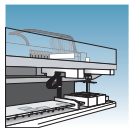
IMPORTANT! If the location of subroutine files is different than the default location in the supplied scripts, you must edit the location of the subroutine files:
In the TECAN Gemini software, open the script, select the appropriate **Sub-Routine** line in the script and navigate to the correct file system location for that script.

Script Name	Number of 384-well Reaction Plates	Number of 96-deep well Reagent Source Plates	Number of 96-well gDNA Source Plates	Number of 100 mL Troughs	Estimated Run Time (hr:min)
1a_SNPLEX_OLA_PN_S4_D_LiHa.gem	4	1	—	—	0:15
1b_SNPLEX_OLA_P4_SN_D_LiHa.gem	4	1	—	—	0:15
1c_SNPLEX_OLA_P16_S1_D_LiHa.gem	4	2	—	—	0:15
1d_SNPLEX_OLA_P1_S16_D_LiHa.gem	4	1	—	—	0:16
1a_SNPLEX_OLA_PN_S4_W_LiHa.gem	4	1	4	—	0:50
1b_SNPLEX_OLA_P4_SN_W_LiHa.gem	4	1	4	—	0:45
1c_SNPLEX_OLA_P16_S1_W_LiHa.gem	4	2	1	—	0:46
1d_SNPLEX_OLA_P1_S16_W_LiHa.gem	4	1	16	—	2:00
2_SNPLEX_EXO_LiHa.gem	4†	1	—	—	0:15
3_SNPLEX_EXO_DILUTION_LiHa.gem	4†	—	—	1	0:15
4_SNPLEX_PCR_LiHa.gem	4† + 4	1	—	—	1:35
Dispenses per Row CALC.gem (subroutine)	—	—	—	—	—
Rack-Well Offsets for 384 well plates.gem (subroutine)	—	—	—	—	—

† The reaction plates for these scripts are the output plates that are carried over from the previous method.

Note: Four scripts are used to complete the OLA process. Select one of the eight OLA setup scripts (that is, 1a_dry, 1a_wet, 1b_dry, 1b_wet, and so forth). The four OLA reaction plates are carried over into script 2 (Exo), then into script 3 (Dilution), and then into script 4 (PCR). In script 4, aliquots from the original four reaction plates are transferred into four new PCR reaction plates.

Notes



Verify the Labware

Scripts also require the use of specific labware in order to function properly. The following table lists the labware used to develop the TECAN Genesis RSP scripts.

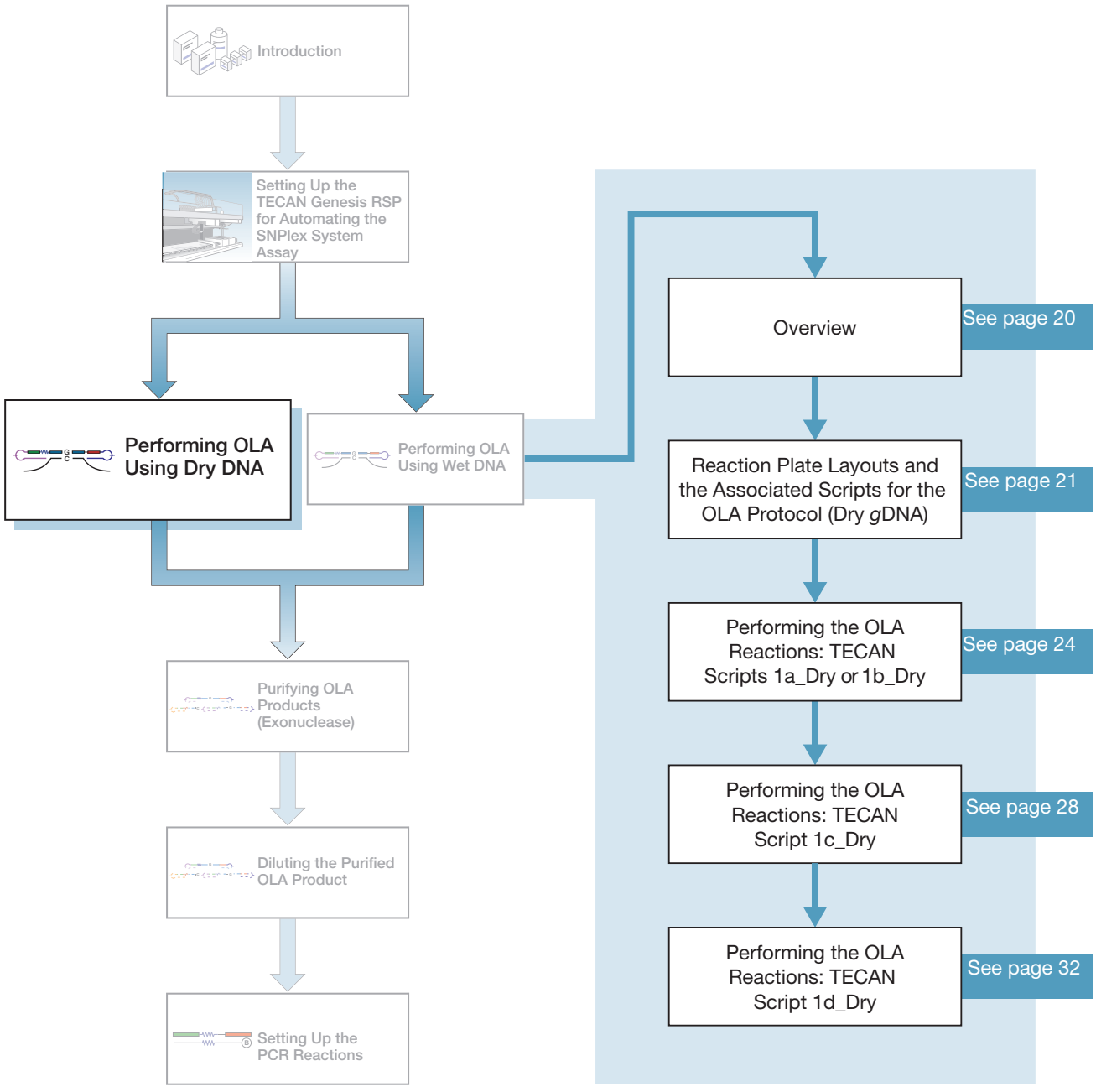
Description	Vendor and Part Number	TECAN Rack Name
ABI PRISM® 384-Well Clear Optical Reaction Plates, with Barcode and 61-781 Adapter	Applied Biosystems 4309849 TECAN 61-781	PCR (384) in 61-781 Adapter
MicroAmp® 96-Well Optical Reaction Plates and 61-527 Adapter	Applied Biosystems N8010560 TECAN 61-527	PCR (96) in 61-527 Adapter
96-deep well plate, square well, 2 mL per well	VWR 82006-448	Deep Well, Landscape
100-mL Trough insert	TECAN 70-744	Trough 100ml

Once the setup is complete, you can start using the TECAN Genesis RSP instrument for automating the OLA laboratory protocols of the SNPlex System assay, as described in the following chapters.

Notes

Notes

Performing OLA Using Dry gDNA



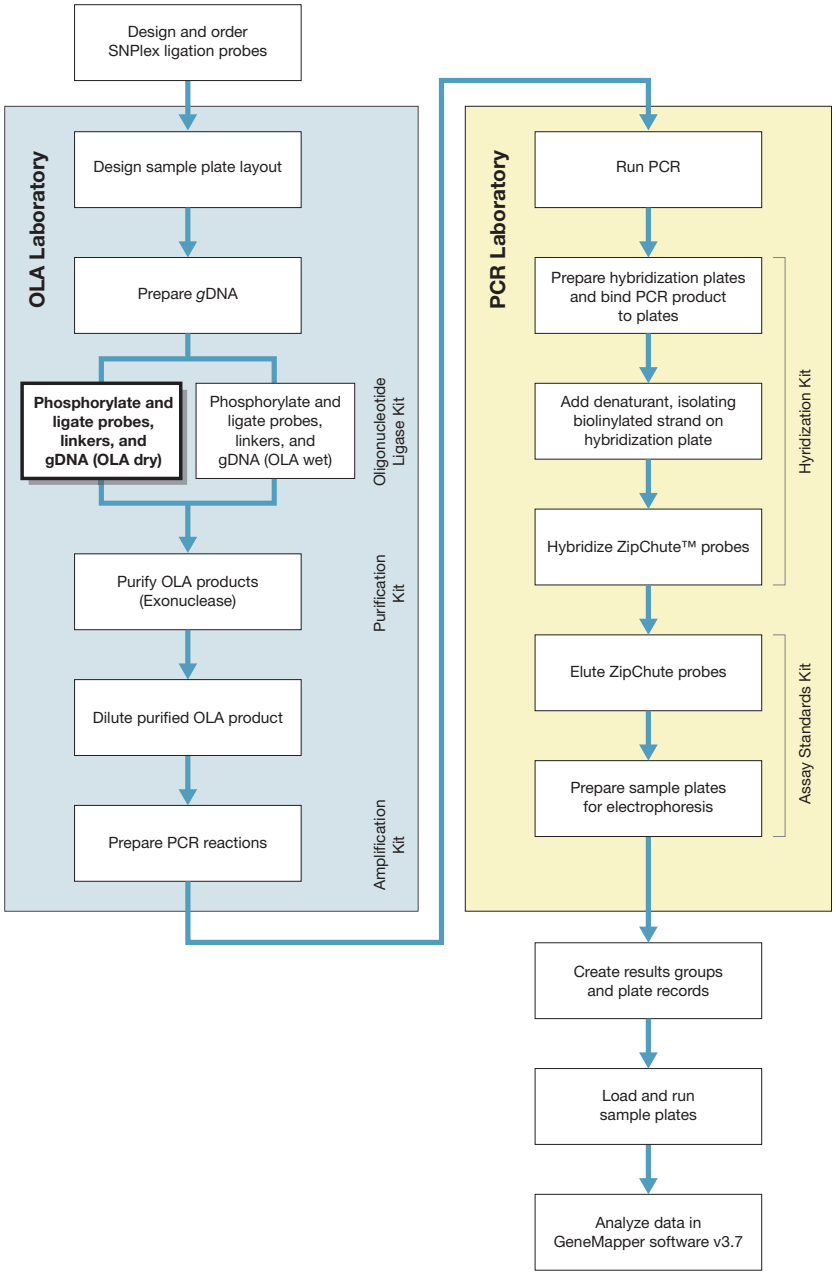
Notes

Overview

About This Chapter

This chapter provides information about automating the OLA protocol using the TECAN Genesis RSP with the 8-channel LiHa using fixed tips. The procedures in this chapter assume the use of a batch of four SNPlex™ OLA reaction plates, each containing 37 ng/well of dried, fragmented gDNA samples. If you are using wet gDNA, refer to Chapter 4, "Performing OLA Using Wet gDNA."

Where You Are In the SNPlex System Assay Workflow



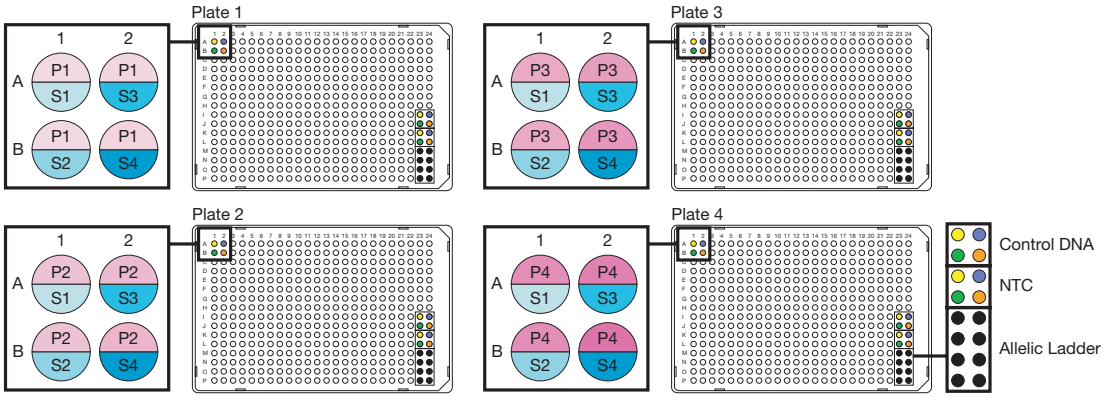
Notes

Reaction Plate Layouts and the Associated Scripts for the OLA Protocol (Dry gDNA)

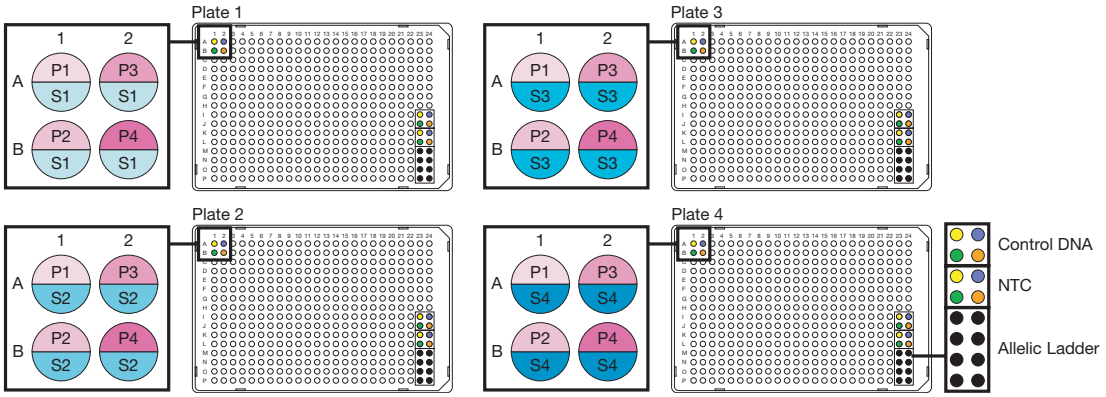
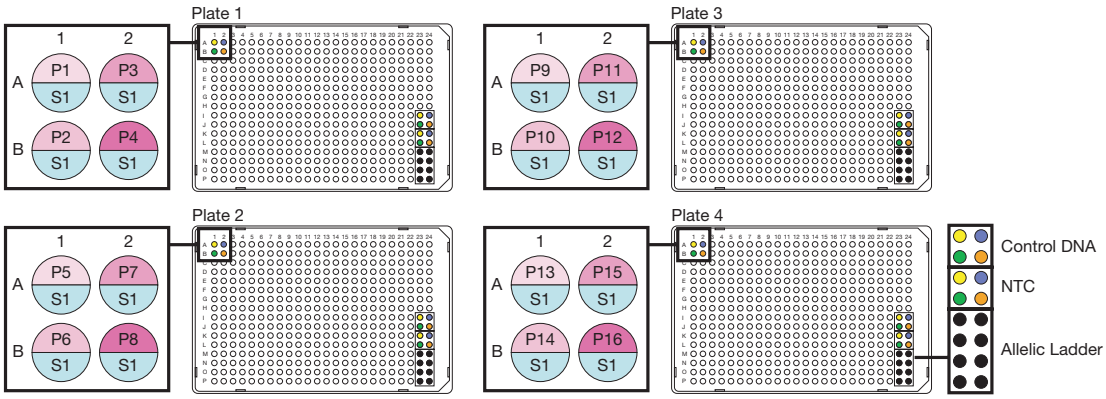
You can select from four TECAN Genesis RSP reaction plate layouts, depending on the number of samples and probe pools assayed in a single run. The following table gives the plate layouts and the associated script for each layout. The quadrant representation comes from the division of the 384-well reaction plates into four 96-well quadrants.

Although plate layouts are flexible, some layouts are more efficient with reagent usage than others. The layout that assays 1472 samples with a single probe pool is most efficient since reagent dead volume is limited to 8 wells (1 column) of the source container. The layout that assays 92 samples with 16 probe pools is the most inefficient since reagent dead volume is spread across 128 wells (16 columns) of two source containers. For optimal use of SNPlex System reagent kits, consider plate layouts, batch sizes, and "dried vs wet" gDNA setup.

Script Number†	Script File Name‡	Number of Probe Pools	Total Number of Samples§	Reference
1a_dry	1a_SNPLEX_OLA_PN_S4_D_LiHa.gem	4	368	"Performing the OLA Reactions: TECAN Scripts 1a_dry or 1b_dry" on page 24

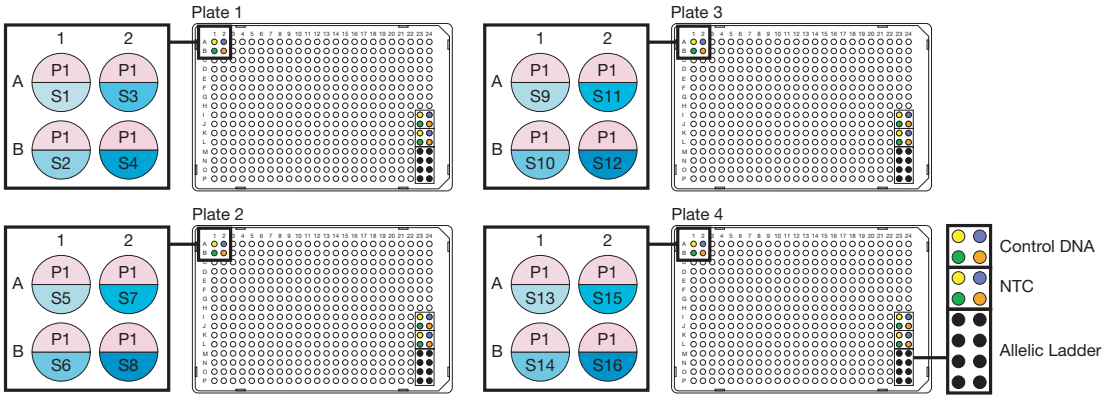


Notes

Script Number†	Script File Name‡	Number of Probe Pools	Total Number of Samples§	Reference
1b_dry	1b_SNPLEX_OLA_P4_SN_D_LiHa.gem	4	368	"Performing the OLA Reactions: TECAN Scripts 1a_dry or 1b_dry" on page 24
				
1c_dry	1c_SNPLEX_OLA_P16_S1_D_LiHa.gem	16	92	"Performing the OLA Reactions: TECAN Script 1c_dry" on page 28
				

Notes

Script Number†	Script File Name‡	Number of Probe Pools	Total Number of Samples§	Reference
1d_dry	1d_SNPLEX_OLA_P1_S16_D_LiHa.gem	1	1472	"Performing the OLA Reactions: TECAN Script 1d_dry" on page 32



† Script numbering reflects scripts provided for other SNPlex System assay protocols.
‡ P = Ligation Probe Pool number; S = Unique collection of 92 gDNA samples (4 positions of a 96-well quadrant are reserved for 1 control DNA, 1 NTC, and 2 allelic ladder wells).
§ Total number of samples in a batch containing four 384-well plates.

Notes

Performing the OLA Reactions: TECAN Scripts 1a_dry or 1b_dry

About These Scripts

Scripts 1a_dry or 1b_dry were developed for setups that have 368 gDNA samples and four probe pools.

These scripts use a single worktable setup. The difference between them is the pattern in which the instrument dispenses the reagents into the wells of the OLA reaction plates.

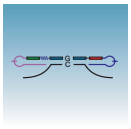
Preparing the Reagents

1. Thaw the SNPlex™ Oligonucleotide Ligation Kit components at room temperature.
2. Label a 96-deep well plate Assay Mix.
3. Label four 15-mL centrifuge tubes Mix 1 to Mix 4.
4. For each of the four SNPlex™ Ligation Probe Pools, prepare an Assay Mix in a 15-mL centrifuge tube and mix thoroughly.

	Manual	Automated		
		Automation Losses		Automation Totals
Assay Mix Component	**Volume (µL) for 1 Quadrant†**	**Source Plate Dead Volume (µL)‡**	**Transfer Loss Excess (µL)§**	**Total Volume (µL) for 4 Quadrants††**
Nuclease-free water	250.3	92.0	57.1	1150.0
SNPlex™ OLA Master Mix	272.0	100.0	62.0	1250.0
SNPlex™ Universal Linkers, 48-plex	5.4	2.0	1.2	25.0
SNPlex dATP	5.4	2.0	1.2	25.0
SNPlex Ligation Probe Pool (500 nM)	10.9	4.0	2.5	50.0
Total	544.0	200.0	124.0	2500.0

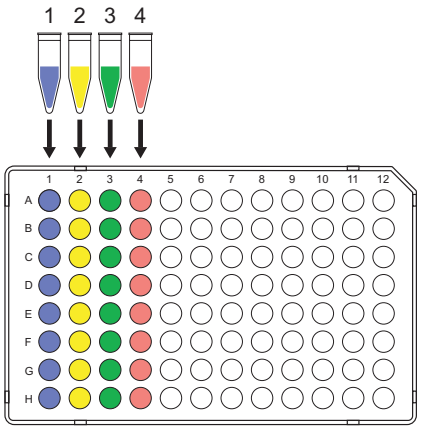
† (12 reactions per tip + excess volume) × 8 tips × volume for one reaction.
‡ 8 tips in source plate × (20 µL per tip + conditioning volume).
§ Transfer losses for transferring bulk mixture into 96-well source plate.
†† Use the volumes in this column to prepare the reagents. Refer to the *SNPlex™ Genotyping System 48-plex User Guide* for per reaction volumes. Volumes given in this table include allowances for dead volume and excess volume for pipetting losses. For more information about calculating dead volumes, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

Notes



5. Add 300 µL of Assay Mix 1 into each well of column 1 of the 96-well plate labeled Assay Mix, starting on the far left of the plate as shown in the plate diagram below. Add Assay Mix 2 to column 2, and so forth.

   Note: Do not add Assay Mix to columns 5 through 12; these remain empty.



6. Briefly centrifuge the plates to remove trapped air bubbles and collect contents at the bottom of each well.

7. Label four 384-well reaction plates containing dried gDNA samples OLA1 to OLA4 (see Script Number 1a_dry on page 21).

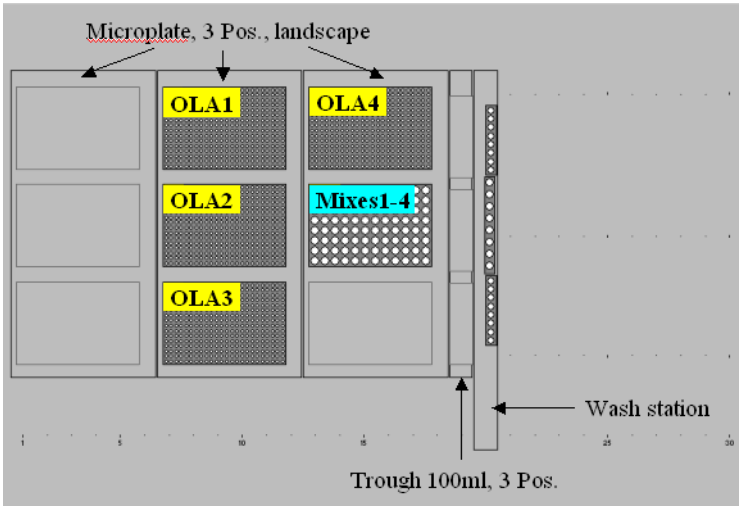
Running Scripts 1a_dry or 1b_dry

Scripts 1a_dry or 1b_dry transfer each of the four Assay Mixes to quadrants 1 to 4, respectively, of the four OLA reaction plates.

The scripts wash the fixed tips after multidispensing each Assay Mix to each quadrant. There are 12 dispenses of each Assay Mix per aspiration except for tips 7 and 8, which aspirate 11 dispenses per aspiration, keeping the last two wells in each quadrant empty.

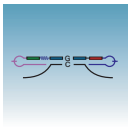
Notes

Scripts 1a_dry or 1b_dry use a single worktable setup:

Plate	Position	Worktable for Script 1a_dry or 1b_dry
Assay Mix Plate	Mixes1-4	
OLA reaction plates containing dried samples	OLA1 to OLA4	

1. In the TECAN Gemini software, select either script 1a_dry or script 1b_dry:
   - 1a_SNPLEX_OLA_PN_S4_D_LiHa.gem
   or
   - 1b_SNPLEX_OLA_P4_SN_D_LiHa.gem
2. Place the uncovered 96-well Assay Mix plate on the carrier in the position labeled Mixes1-4, as shown.
3. Place the uncovered 384-well dried gDNA sample plates in adapters and onto the carriers in positions OLA1 through OLA4, as shown.
4. Start the script.
5. When prompted to verify the worktable setup, verify the plate positions and click **OK** to begin dispensing.
6. When dispensing is complete, remove and discard the 96-well deep well Assay Mix plate.
7. Remove, seal, and briefly centrifuge the OLA reaction plates (OLA1 to OLA4), and proceed to"Thermal Cycling the OLA Reactions" on page 27.

Notes



Thermal Cycling the OLA Reactions

Thermal cycle the OLA plates using the following conditions:

Step	Step Type	Temperature (°C)	Time
1	HOLD	48	30 min
2	HOLD	90	20 min
3	25 cycles	94	15 sec
		60	30 sec
		51, 3% ramp	30 sec
4	HOLD	99	10 min
5	HOLD	4	∞

Next Steps

At this point, the OLA reaction is complete. Proceed to Chapter 5, "Purifying OLA Products (Exonuclease)."

Notes

Performing the OLA Reactions: TECAN Script 1c_dry

About This Script

TECAN Script 1c_dry was developed for setups that have 92 gDNA samples and 16 ligation probe pools.

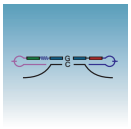
Preparing the Reagents

1. Thaw the SNPlex Oligonucleotide Ligation Kit components at room temperature.
2. Label two 96-deep well plates Assay Mix Plate 1 and Assay Mix Plate 2.
3. Label 16 15-mL centrifuge tubes Mix 1 to Mix 16.
4. For each of the 16 SNPlex Ligation Probe Pools, prepare an Assay Mix in a 15-mL centrifuge tube and mix thoroughly.

	Manual	Automated		
		Automation Losses		Automation Totals
Assay Mix Component	**Volume (µL) for 1 Quadrant†**	**Source Plate Dead Volume (µL)‡**	**Transfer Loss Excess (µL)§**	**Total Volume (µL) for 1 Quadrant††**
Nuclease-free water	250.3	92.0	57.1	399.2
SNPlex™ OLA Master Mix	272.0	100.0	62.0	434.0
SNPlex™ Universal Linkers, 48-plex	5.4	2.0	1.2	8.7
SNPlex™ dATP	5.4	2.0	1.2	8.7
SNPlex Ligation Probe Pool (500 nM)	10.9	4.0	2.5	17.4
Total	544.0	200.0	124.0	868.0

† (12 reactions per tip + excess volume) × 8 tips × volume for one reaction.
‡ 8 tips in source plate × (20 µL per tip + conditioning volume).
§ Transfer losses for transferring bulk mixture into 96-well source plate.
†† Use the volumes in this column to prepare the reagents. Refer to the *SNPlex™ Genotyping System 48-plex User Guide* for per reaction volumes. Volumes given in this table include allowances for dead volume and excess volume for pipetting losses. For more information about calculating dead volumes, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

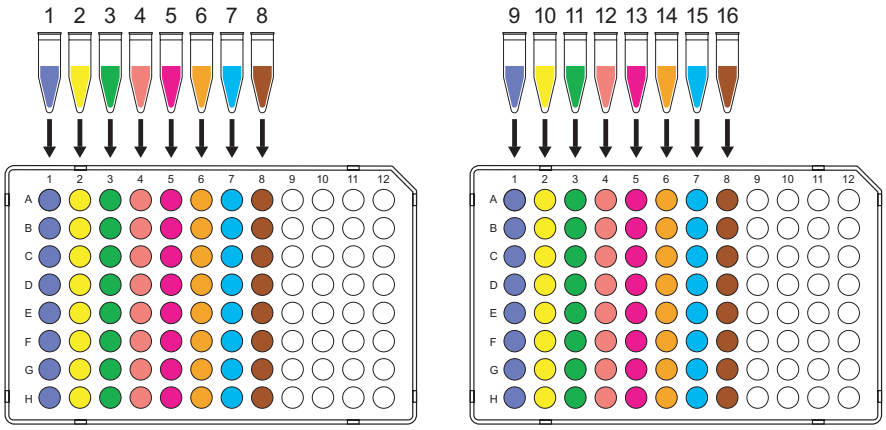
Notes



5. Add 95 µL of Assay Mix 1 into each well of column 1 in Assay Mix plate 1, starting on the far left of the plate as shown in the plate diagram below. Add Assay Mix 2 to column 2, and so forth until you have added Assay Mix 8 to column 8.

   Repeat the process for Assay Mix plate 2, manually pipetting Assay Mix 9 to column 1 in Assay Mix plate 2, Assay Mix 10 to column 2, and so forth until you have added Assay Mix 16 to column 8.

   Note: Do not add Assay Mix to columns 9 through 12 in either Assay Mix plate; these remain empty.



6. Briefly centrifuge the plates to remove trapped air bubbles and collect contents at the bottom of each well.

7. Label four 384-well reaction plates containing dried gDNA samples OLA1 to OLA4 (see Script Number 1c on page 22).

Running Script 1c_dry

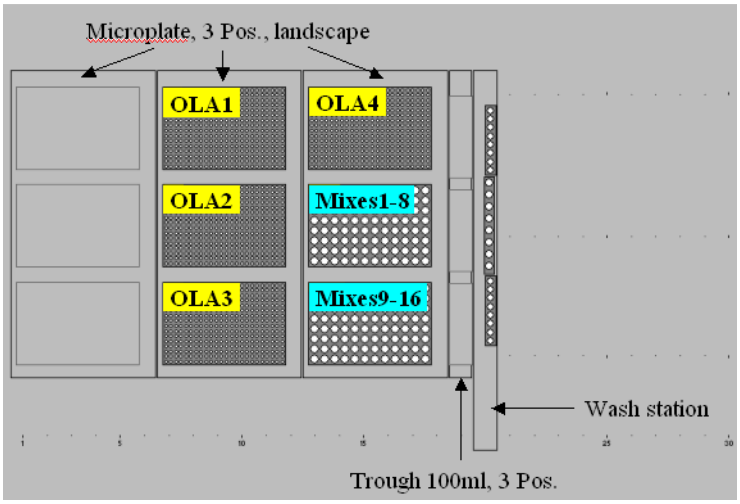
The script transfers the Assay Mixes:

- Assay Mixes 1 to 4 from Assay Mix plate 1 to quadrants 1 to 4 of plate OLA1
- Assay Mixes 5 to 8 from Assay Mix plate 1 to quadrants 1 to 4 of plate OLA2
- Assay Mixes 9 to 12 from Assay Mix plate 2 to quadrants 1 to 4 of plate OLA3
- Assay Mixes 13 to 16 from Assay Mix plate 2 to quadrants 1 to 4 of plate OLA4

The script washes the fixed tips after multidispensing each Assay Mix to each quadrant. There are 12 dispenses of each Assay Mix per aspiration except for tips 7 and 8, which aspirate 11 dispenses per aspiration, keeping the last two wells in each quadrant empty.

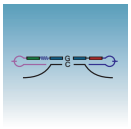
Notes

Script 1c_dry uses a single worktable setup:

Plate	Position	Worktable for Script 1c_dry
Assay Mix Plate 1	Mixes1-8	
Assay Mix Plate 2	Mixes9-16	
OLA reaction plates containing dried samples	OLA1 to OLA4	

1. Place the uncovered 96-well Assay Mix plate 1 onto the carrier in the position labeled Mixes1-8, as shown.
2. Place the uncovered 96-well Assay Mix plate 2 onto the carrier in the position labeled Mixes9-16, as shown.
3. Place the uncovered 384-well dried gDNA sample plates in adapters and onto the carriers in positions OLA1 through OLA4.
4. Start the script.
5. When prompted to verify the worktable setup, verify the plate positions and click **OK** to start dispensing.
6. When dispensing is complete, remove and discard the 96-well Assay Mix plates.
7. Remove, seal, and briefly centrifuge the OLA reaction plates (OLA1 to OLA4), and proceed to "Thermal Cycling the OLA Reactions" on page 31.

Notes



Thermal Cycling the OLA Reactions

Thermal cycle the OLA plates using the following conditions:

Step	Step Type	Temperature (°C)	Time
1	HOLD	48	30 min
2	HOLD	90	20 min
3	25 cycles	94	15 sec
		60	30 sec
		51, 3% ramp	30 sec
4	HOLD	99	10 min
5	HOLD	4	∞

Next Steps

At this point, the OLA reaction is complete. Proceed to Chapter 5, "Purifying OLA Products (Exonuclease)."

Notes

Performing the OLA Reactions: TECAN Script 1d_dry

About These Script 1d_dry

Script 1d_dry was developed for setups that have 1472 gDNA samples and 1 ligation probe pool. This script uses a single worktable setup.

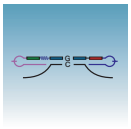
Preparing the Reagents

1. Thaw the SNPlex Oligonucleotide Ligation Kit components at room temperature.
2. Label one 96-deep well plate Assay Mix.
3. Label one 15-mL centrifuge tube Assay Mix.
4. Prepare an Assay Mix in a 15-mL centrifuge tube and mix thoroughly.

	Manual	Automated		
		Automation Losses		Automation Totals
Assay Mix Component	**Volume (µL) for 1 Quadrant†**	**Source Plate Dead Volume (µL)‡**	**Transfer Loss Excess (µL)§**	**Total Volume (µL) for 16 Quadrants††**
Nuclease-free water	250.3	92.0	57.1	4152.8
SNPlex™ OLA Master Mix	272.0	100.0	62.0	4514.0
SNPlex™ Universal Linkers, 48-plex	5.4	2.0	1.2	90.3
SNPlex™ dATP	5.4	2.0	1.2	90.3
SNPlex Ligation Probe Pool (500 nM)	10.9	4.0	2.5	180.6
Total	544.0	200.0	124.0	9028.0

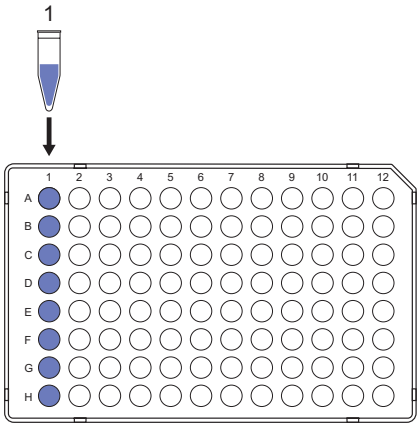
† (12 reactions per tip + excess volume) × 8 tips × volume for one reaction.
‡ 8 tips in source plate × (20 µL per tip + conditioning volume).
§ Transfer losses for transferring bulk mixture into 96-well source plate.
†† Use the volumes in this column to prepare the reagents. Refer to the *SNPlex™ Genotyping System 48-plex User Guide* for per reaction volumes. Volumes given in this table include allowances for dead volume and excess volume for pipetting losses. For more information about calculating dead volumes, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

Notes



5. Add 1115 µL of the Assay Mix into each well of column 1 in the Assay Mix plate, starting on the far left of the plate as shown in the plate diagram below.

   Note: Do not add Assay Mix to columns 2 through 12; these remain empty.



6. Briefly centrifuge the plate to remove trapped air bubbles and collect contents at the bottom of each well.

7. Label four 384-well reaction plates containing dried gDNA samples OLA1 to OLA4 (see "1d_dry" on page 23).

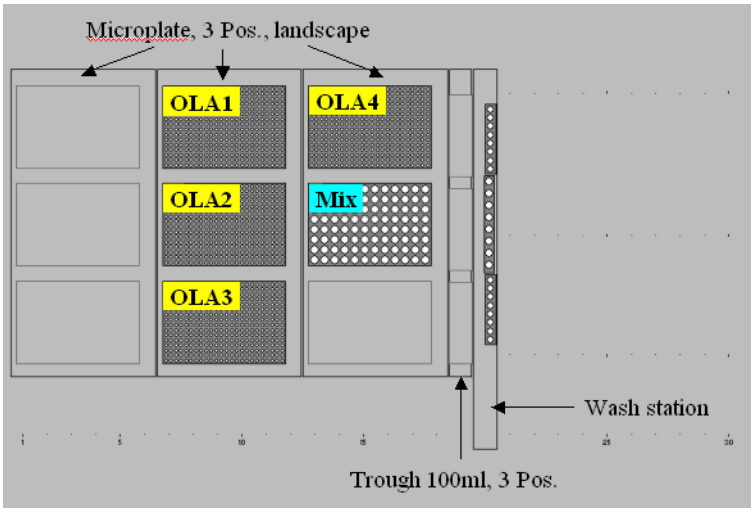
Running Script 1d_dry

The script transfers the Assay Mix to each quadrant of plates OLA1 to OLA4.

The script washes the fixed tips after multidispensing the Assay Mix to each quadrant. There are 12 dispenses of each Assay Mix per aspiration except for tips 7 and 8, which aspirate 11 dispenses per aspiration, keeping the last two wells in each quadrant empty.

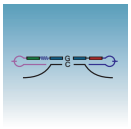
Notes

Script 1d_dry uses a single worktable setup:

Plate	Position	Worktable for Script 1d_dry
Assay Mix Plate	Mix	
OLA reaction plate containing dried samples	OLA1 to OLA4	

1. Place the uncovered 96-well Assay Mix plate onto the carrier in the position labeled Mix.
2. Place the uncovered 384-well dried gDNA sample plates in adapters and onto the carriers in positions OLA1 through OLA4.
3. Start the script.
4. When prompted to verify the worktable setup, verify the plate positions and click **OK** to start dispensing.
5. When dispensing is complete, remove and discard the 96-well Assay Mix plate.
6. Remove, seal, and briefly centrifuge the OLA reaction plates (OLA1 to OLA4), and proceed to "Thermal Cycling the OLA Reactions" on page 35.

Notes



Thermal Cycling the OLA Reactions

Thermal cycle the OLA plates using the following conditions:

Step	Step Type	Temperature (°C)	Time
1	HOLD	48	30 min
2	HOLD	90	20 min
3	25 cycles	94	15 sec
		60	30 sec
		51, 3% ramp	30 sec
4	HOLD	99	10 min
5	HOLD	4	∞

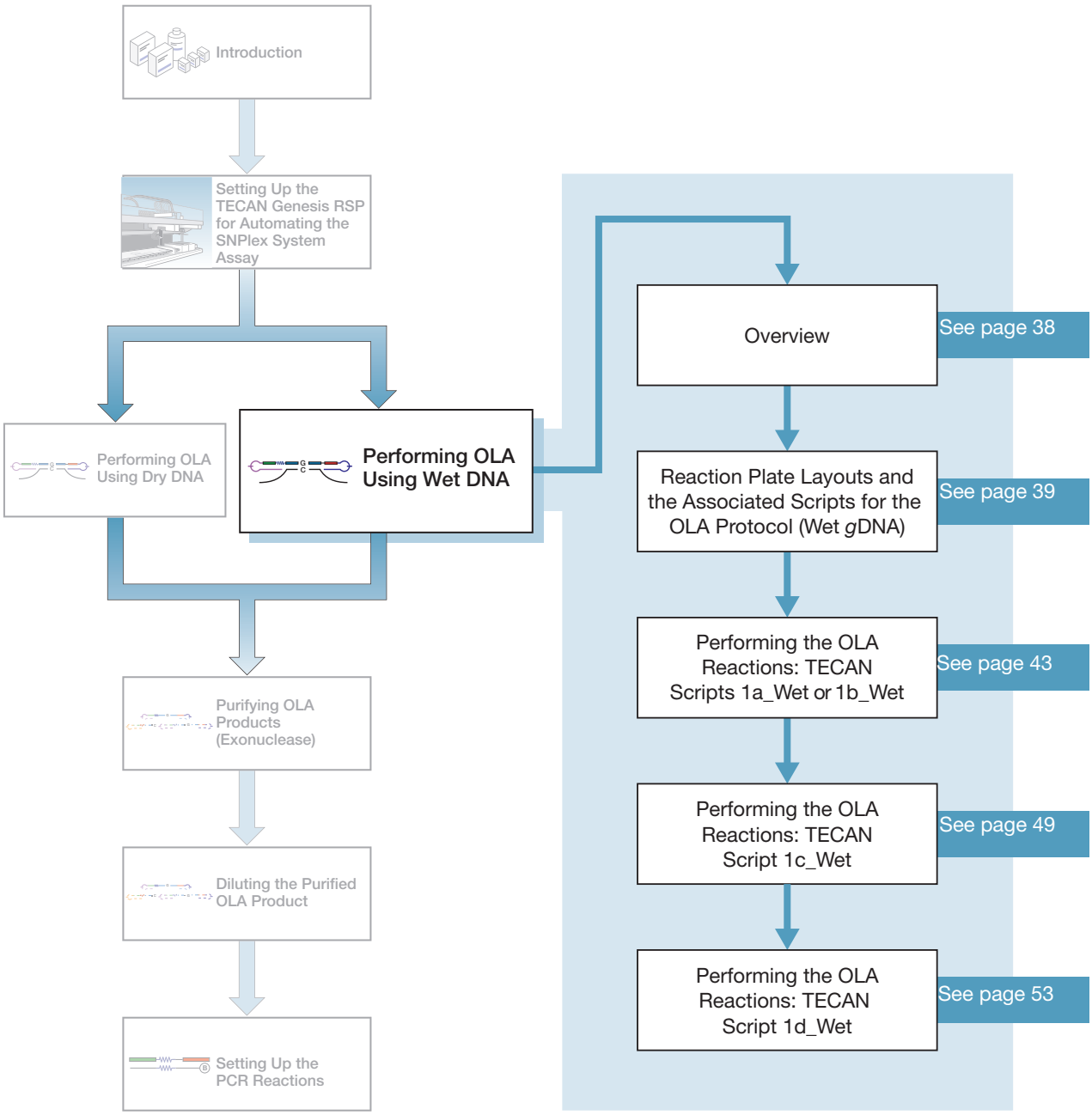
Next Steps

At this point, the OLA reaction is complete. Proceed to Chapter 5, "Purifying OLA Products (Exonuclease)."

Notes

Notes

Performing OLA Using Wet gDNA



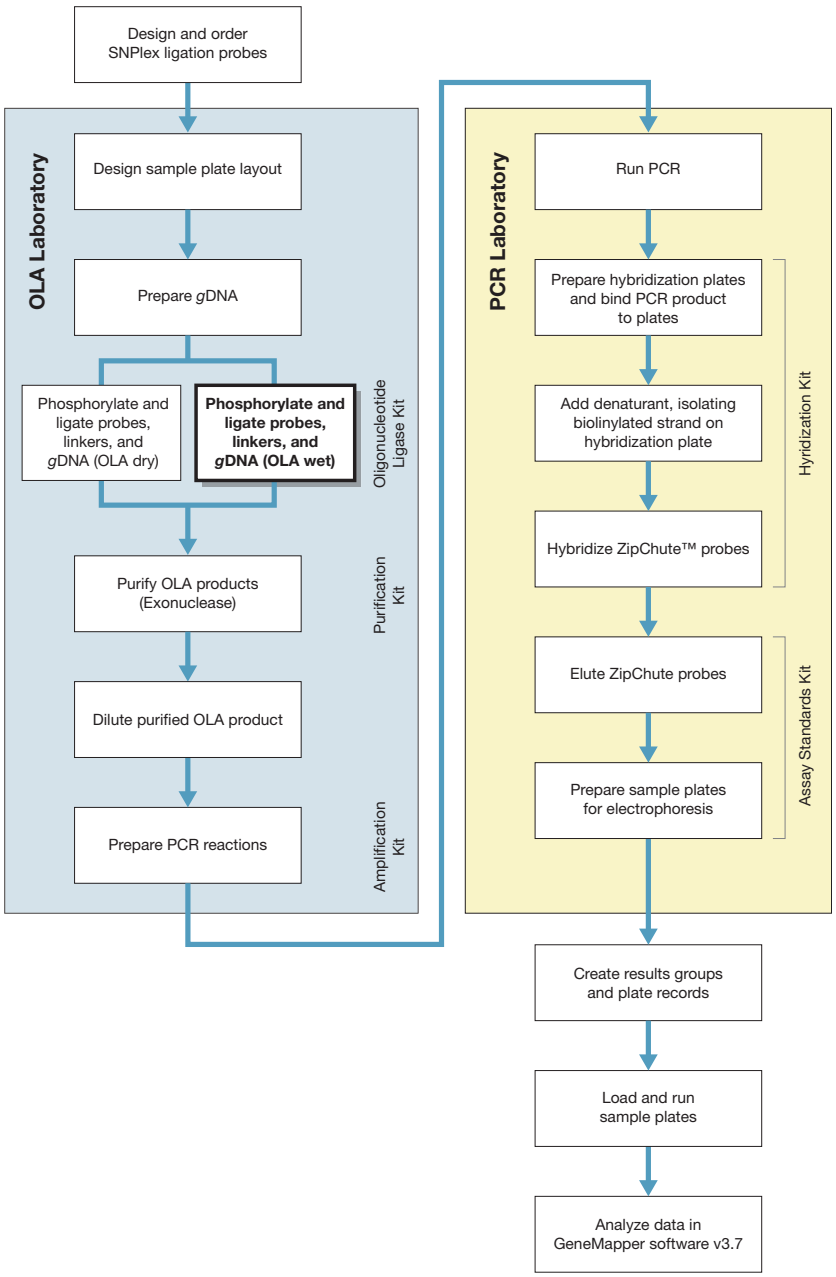
Notes

Overview

About This Chapter

This chapter provides information about automating the OLA protocol using the TECAN Genesis RSP Robot with the 8-channel LiHa. The procedures in this chapter assume the use of a batch of four SNPlex™ OLA reaction plates, and that gDNA samples have been quantified, fragmented, and diluted to a concentration of 18.5 ng/µL before beginning this procedure. If you are using dry gDNA, refer to Chapter 3, "Performing OLA Using Dry gDNA."

Where You Are In the SNPlex System Assay Workflow



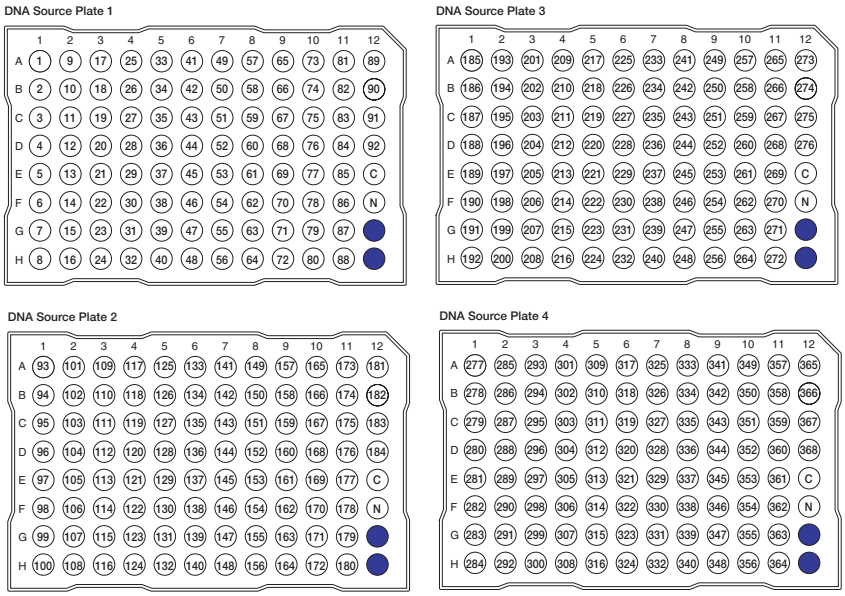
Notes

Reaction Plate Layouts and the Associated Scripts for the OLA Protocol (Wet gDNA)

Arraying gDNA in 96-Well Plates

Array your gDNA samples in MicroAmp® 96-Well Optical Reaction plates, 92 samples per plate. Reserve well E12 for the SNPlex™ kit control gDNA and well F12 for the No Template Control (NTC). Leave wells G12 and H12 empty – these wells are used as Allelic Ladder wells during analysis.

For example, you may array 368 gDNA samples among four 96-well plates as shown:



Volumes for Source Plates

The volumes of the gDNA samples you use depend on your total number of samples and the number of SNPlex™ Ligation Probe pools you are using. The volumes in the table below include dead volumes to be sure the robotic tips remain submerged during aspiration.

Script Number	Script File Name	Number of Probe Pools	Number of gDNA Source Plates	Total Number of gDNA Samples	Required Volume (µL) of gDNA Samples
1a_wet	1a_SNPLEX_OLA_PN_S4_W_LiHa.gem	4	4	368 (4 × 92)	20
1b_wet	1b_SNPLEX_OLA_P4_SN_W_LiHa.gem	4	4	368 (4 × 92)	20
1c_wet	1c_SNPLEX_OLA_P16_S1_W_LiHa.gem	16	1	92 (1 × 92)	60
1d_wet	1d_SNPLEX_OLA_P1_S16_W_LiHa.gem	1	16	1472 (16 × 92)	12

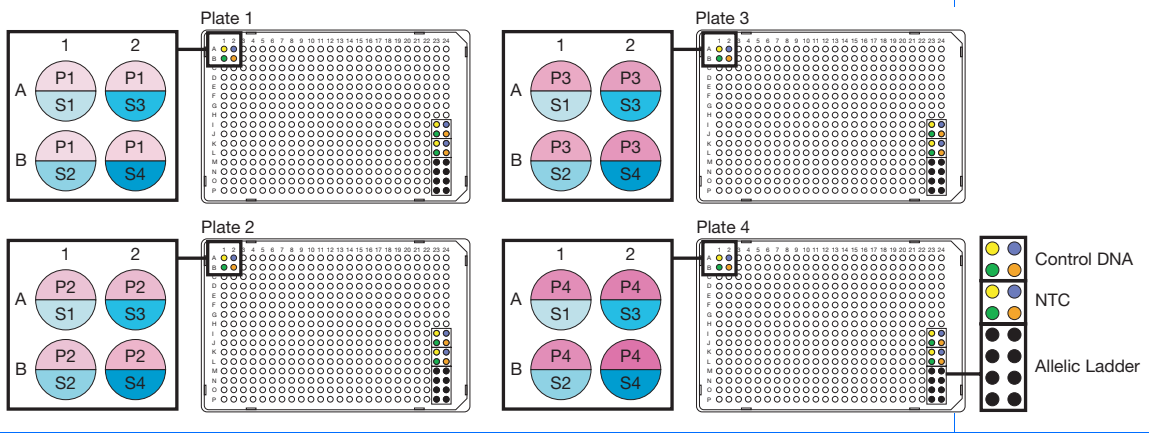
Notes

Reaction Plate Layouts

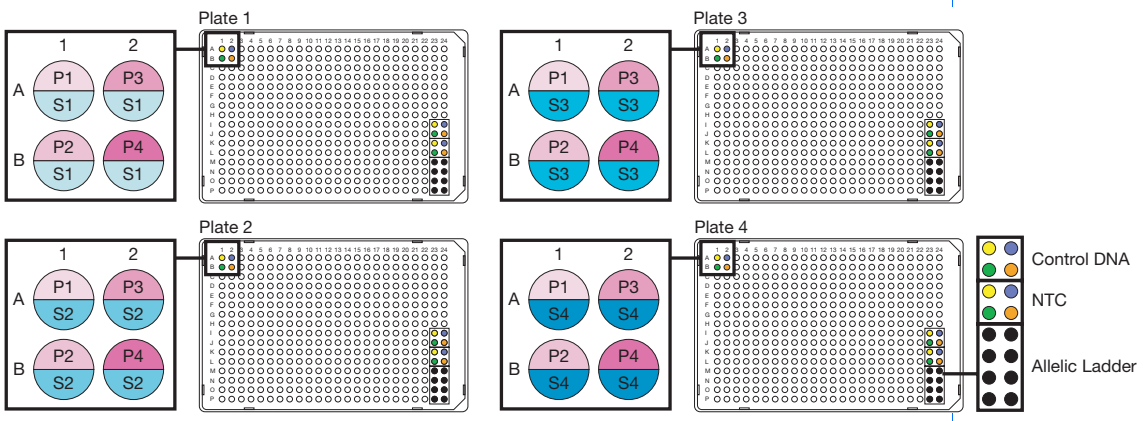
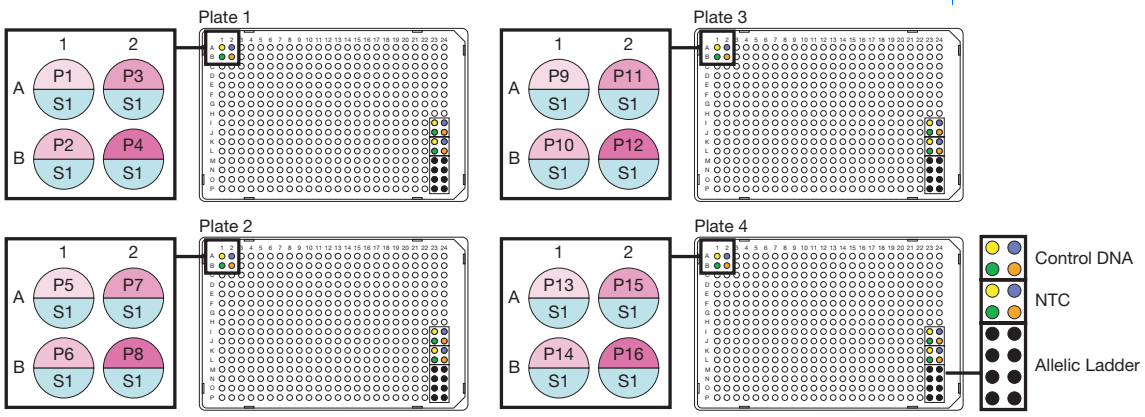
You can select from four TECAN Genesis RSP reaction plate layouts, depending on the number of samples and probe pools assayed in a single run. The following table gives the plate layouts and the associated script for each layout. The quadrant representation comes from the division of the 384-well reaction plates into four 96-well quadrants.

Although plate layouts are flexible, some layouts are more efficient with reagent usage than others. The layout that assays 1472 samples with a single probe pool is most efficient since reagent dead volume is limited to 8 wells (1 column) of the source container. The layout that assays 92 samples with 16 probe pools is the most inefficient since reagent dead volume is spread across 128 wells (16 columns) of two source containers. For optimal use of SNPlex System reagent kits, consider plate layouts, batch sizes, and "dried vs wet" gDNA setup.

Script Number†	Script File Name‡	Number of Probe Pools	Total Number of Samples§	Reference
1a_wet	1a_SNPLEX_OLA_PN_S4_W_LiHa.gem	4	368	"Performing the OLA Reactions: TECAN Scripts 1a_wet or 1b_wet" on page 43

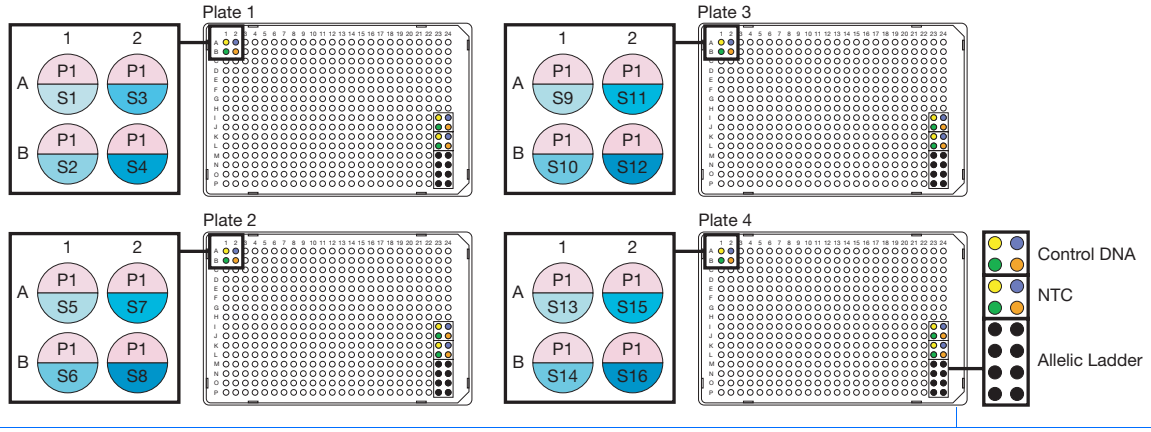


Notes

Script Number†	Script File Name‡	Number of Probe Pools	Total Number of Samples§	Reference
1b_wet	1b_SNPLEX_OLA_P4_SN_W_LiHa.gem	4	368	"Performing the OLA Reactions: TECAN Scripts 1a_wet or 1b_wet" on page 43
				
1c_wet	1c_SNPLEX_OLA_P16_S1_W_LiHa.gem	16	92	"Performing the OLA Reactions: TECAN Script 1c_wet" on page 49
				

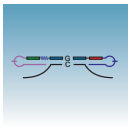
Notes

Script Number†	Script File Name‡	Number of Probe Pools	Total Number of Samples§	Reference
1d_wet	1d_SNPLEX_OLA_P1_S16_W_LiHa.gem	1	1472	"Performing the OLA Reactions: TECAN Script 1d_wet" on page 53



† Script numbering reflects scripts provided for other SNPlex System assay protocols.
‡ P = Ligation Probe Pool number; S = Unique collection of 92 gDNA samples (4 positions of a 96-well DNA source plate are reserved for 1 control DNA, 1 NTC, and 2 allelic ladder wells)
§ Total number of samples in a batch containing four 384-well plates.

Notes



Performing the OLA Reactions: TECAN Scripts 1a_wet or 1b_wet

About These Scripts

Scripts 1a_wet and 1b_wet were developed for setups that have 368 gDNA samples and four probe pools.

These scripts use a single worktable setup. The difference between the scripts is the pattern in which the instrument dispenses the reagents into the wells of the OLA reaction plates.

Preparing the Reagents

1. Thaw the SNPlex™ Oligonucleotide Ligation Kit components at room temperature.
2. Label a 96-deep well plate Assay Mix.
3. Label four 15-mL centrifuge tubes Mix 1 to Mix 4.
4. For each of the four SNPlex™ Ligation Probe Pools, prepare an Assay Mix in a 15-mL centrifuge tube and mix thoroughly.

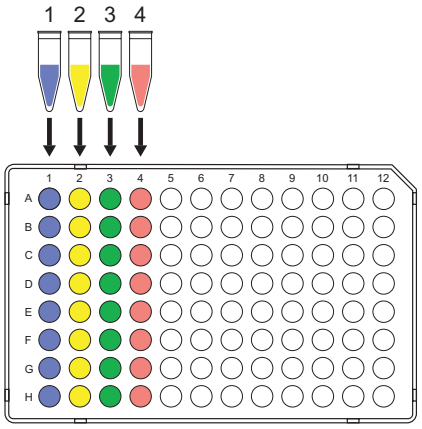
	Manual	Automated		
		Automation Losses		Automation Totals
Assay Mix Component	**Volume (µL) for 1 Quadrant†**	**Source Plate Dead Volume (µL)‡**	**Transfer Loss Excess (µL)§**	**Total Volume (µL) for 4 Quadrants††**
Nuclease-free water	33.6	28.3	12.2	165.0
SNPlex™ OLA Master Mix	280.0	153.3	101.7	1375.0
SNPlex™ Universal Linkers, 48-plex	5.6	3.1	2.0	27.5
SNPlex™ dATP	5.6	3.1	2.0	27.5
SNPlex Ligation Probe Pool (500 nM)	11.2	6.1	4.1	55.0
Total	336.0	184.0	122.0	1650.0

† (12 reactions per tip + excess volume) × 8 tips × volume for one reaction.
‡ 8 tips in source plate × (20 µL per tip + conditioning volume).
§ Transfer losses for transferring bulk mixture into 96-well source plate.
†† Use the volumes in this column to prepare the reagents. Refer to the *SNPlex™ Genotyping System 48-plex User Guide* for per reaction volumes. Volumes given in this table include allowances for dead volume and excess volume for pipetting losses. For more information about calculating dead volumes, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

Notes

5. Add 195 µL of Assay Mix 1 into each well of column 1 of the 96-well plate, starting on the far left of the plate as shown in the plate diagram below. Add Assay Mix 2 to column 2, and so forth.

   Note: Do not add Assay Mix to columns 5 through 12; these remain empty.



6. Briefly centrifuge the plates to remove trapped air bubbles and collect contents at the bottom of each well.

7. Label four 384-well reaction plates OLA1 to OLA4 (see Script Number 1a_wet on page 38).

Running Script 1a_wet

Script 1a_wet transfers the 94 samples in source plate:

- DNA1 to quadrant 1 of reaction plates OLA1 to OLA4
- DNA2 to quadrant 2 of OLA1 to OLA4
- DNA3 to quadrant 3 of OLA1 to OLA4
- DNA4 to quadrant 4 of OLA 1 to OLA4

The script dispenses four times for each sample aspiration and washes the fixed tips after multidispensing a group of eight samples (one sample per tip) into each set of quadrants.

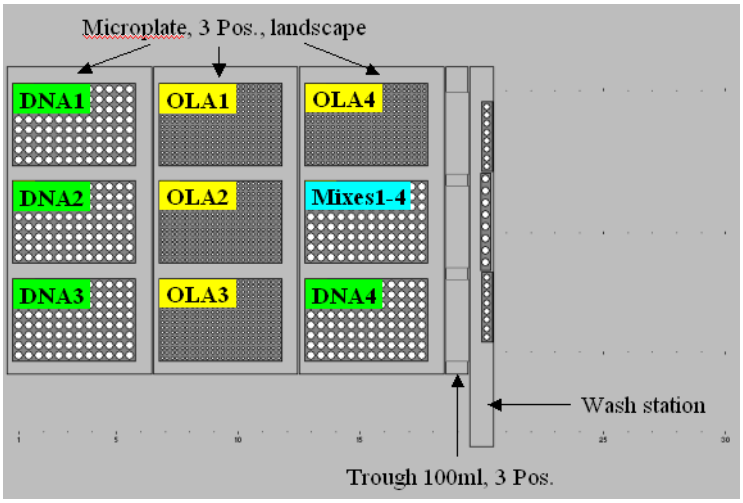
Once the samples have been transferred, the script transfers:

- Assay Mix 1 to OLA1
- Assay Mix 2 to OLA2
- Assay Mix 3 to OLA3
- Assay Mix 4 to OLA4

The script washes the fixed tips after multidispensing each Assay Mix to each quadrant. There are 12 dispenses of each Assay Mix per aspiration except for tips 7 and 8, which aspirate 11 dispenses per aspiration, keeping the last two wells in each quadrant empty.

Notes

Script 1a_wet uses a single worktable setup:

Plate	Position	Worktable for Script 1a_wet
Assay Mix Plate	Mixes1-4	
Empty 384-well reaction plates	OLA1 to OLA4	
DNA source plates	DNA1 to DNA4	

1. In the TECAN Gemini software, select script 1a_wet:
   1a_SNPLEX_OLA_PN_S4_W_LiHa.gem
2. Place the uncovered 96-well Assay Mix plate onto the carrier in the position labeled Mixes1-4, as shown.
3. Place the empty OLA reaction plates in adapters and onto the carriers in positions OLA1 through OLA4, as shown.
4. Place the 96-well DNA source plates in adapters and onto the carriers in positions DNA1 through DNA4, as shown.
5. Start the script.
6. When prompted to verify the worktable setup, verify the plate positions and click **OK** to begin dispensing.
7. When dispensing is complete, remove and discard the 96-well Assay Mix plate.
8. Remove, seal, and store (optional) the DNA source plates.
9. Remove, seal, and briefly centrifuge the OLA reaction plates (OLA1 to OLA4), and proceed to"Thermal Cycling the OLA Reactions" on page 46.

Notes

Thermal Cycling the OLA Reactions

Thermal cycle the OLA plates using the following conditions:

Step	Step Type	Temperature (°C)	Time
1	HOLD	48	30 min
2	HOLD	90	20 min
3	25 cycles	94	15 sec
		60	30 sec
		51, 3% ramp	30 sec
4	HOLD	99	10 min
5	HOLD	4	∞

Next Steps

At this point, the OLA reaction is complete. Proceed to Chapter 5, "Purifying OLA Products (Exonuclease)."

Notes

Running Script 1b_wet

Script 1b_wet transfers the 94 samples in source plate:

- DNA1 to all quadrants of reaction plate OLA1
- DNA2 to all quadrants of reaction plate OLA2
- DNA3 to all quadrants of reaction plate OLA3
- DNA4 to all quadrants of reaction plate OLA4

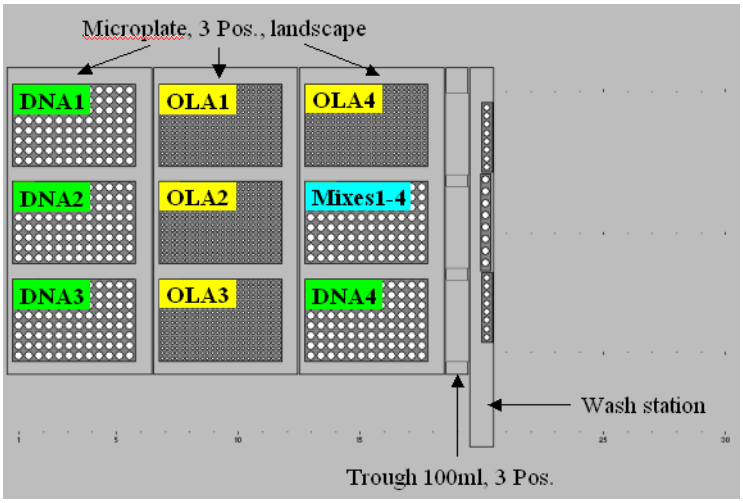
The script washes the fixed tips after multidispensing a group of eight samples (one sample per tip) into four quadrants. There are four dispenses per aspiration of each sample.

Once the samples have been transferred, the script transfers:

- Assay Mix 1 to quadrant 1 of all four OLA reagent plates
- Assay Mix 2 to quadrant 2 of all four OLA reagent plates
- Assay Mix 3 to quadrant 3 of all four OLA reagent plates
- Assay Mix 4 to quadrant 4 of all four OLA reagent plates

The script washes the fixed tips after multidispensing each Assay Mix to each quadrant. There are 12 dispenses of each Assay Mix per aspiration except for tips 7 and 8, which aspirate 11 dispenses per aspiration, keeping the last two wells in each quadrant empty.

Script 1b_wet uses a single worktable setup:

Plate	Position	Worktable for Script 1b_wet
Assay Mix Plate	Mixes1-4	
Empty 384-well reaction plates	OLA1 to OLA4	
DNA source plates	DNA1 to DNA4	

1. In the TECAN Gemini software, select script 1b_wet:
   1b_SNPLEX_OLA_P4_SN_W_LiHa.gem

2. Place the uncovered 96-well Assay Mix plate onto the carrier in the position labeled Mixes1-4, as shown.

Notes

3. Place the empty OLA reaction plates in adapters and onto the carriers in positions OLA1 through OLA4, as shown.
4. Place the 96-well DNA source plates in adapters and onto the carriers in positions DNA1 through DNA4, as shown.
5. Start the script.
6. When prompted to verify the worktable setup, verify the plate positions and click **OK** to begin dispensing.
7. When dispensing is complete, remove and discard the 96-well Assay Mix plate.
8. Remove, seal, and store (optional) the DNA source plates.
9. Remove, seal, and briefly centrifuge the OLA reaction plates (OLA1 to OLA4), then proceed to "Thermal Cycling the OLA Reactions".

Thermal Cycling the OLA Reactions

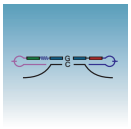
Thermal cycle the OLA plates using the following conditions:

Step	Step Type	Temperature (°C)	Time
1	HOLD	48	30 min
2	HOLD	90	20 min
3	25 cycles	94	15 sec
		60	30 sec
		51, 3% ramp	30 sec
4	HOLD	99	10 min
5	HOLD	4	∞

Next Steps

At this point, the OLA reaction is complete. Proceed to Chapter 5, "Purifying OLA Products (Exonuclease)."

Notes



Performing the OLA Reactions: TECAN Script 1c_wet

About This Script

TECAN Script 1c_wet was developed for setups that have 92 gDNA samples and 16 ligation probe pools.

Preparing the Reagents

1. Thaw the SNPlex Oligonucleotide Ligation Kit components at room temperature.
2. Label 2 96-deep well plates Assay Mix Plate 1 and Assay Mix Plate 2.
3. Label 16 15-mL centrifuge tubes Mix 1 to Mix 16.
4. For each of the 16 SNPlex Ligation Probe Pools, prepare an Assay Mix in a 15-mL centrifuge tube and mix thoroughly.

	Manual	Automated		
		Automation Losses		Automation Totals
Assay Mix Component	**Volume (µL) for 1 Quadrant†**	**Source Plate Dead Volume (µL)‡**	**Transfer Loss Excess (µL)§**	**Total Volume (µL) for 1 Quadrant††**
Nuclease-free water	33.6	18.3	12.2	64.2
SNPlex™ OLA Master Mix	280.0	153.3	101.7	535.0
SNPlex™ Universal Linkers, 48-plex	5.6	3.1	2.0	10.7
SNPlex™ dATP	5.6	3.1	2.0	10.7
SNPlex Ligation Probe Pool (500 nM)	11.2	6.1	4.1	21.4
Total	336.0	184.0	122.0	642.0

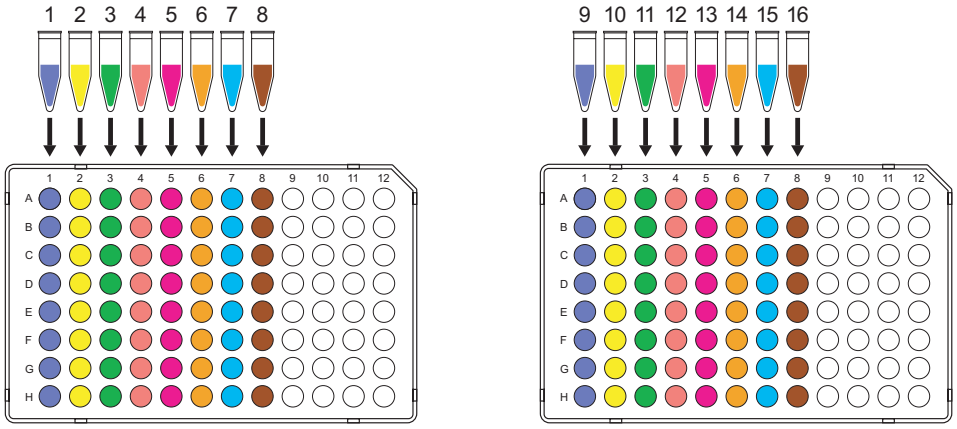
† (12 reactions per tip + excess volume) × 8 tips × volume for one reaction.
‡ 8 tips in source plate × (20 µL per tip + conditioning volume).
§ Transfer losses for transferring bulk mixture into 96-well source plate.
†† Use the volumes in this column to prepare the reagents. Refer to the *SNPlex™ Genotyping System 48-plex User Guide*) for per reaction volumes. Volumes given in this table include allowances for dead volume and excess volume for pipetting losses. For more information about calculating dead volumes, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

Notes

5. Add 65 µL of Assay Mix 1 into each well of column 1 in Assay Mix plate 1, starting on the far left of the plate as shown in the plate diagram below. Add Assay Mix 2 to column 2, and so forth until you have added Assay Mix 8 to column 8.

   Repeat the process for Assay Mix plate 2, manually pipetting Assay Mix 9 to column 1 in Assay Mix plate 2, Assay Mix 10 to column 2, and so forth until you have added Assay Mix 16 to column 8.

   Note: Do not add Assay Mix to columns 9 through 12; these columns remain empty.



6. Briefly centrifuge the plates to remove trapped air bubbles and collect contents at the bottom of each well.

7. Label 4 96-deep well plates OLA1 to OLA4.

Running Script 1c_wet

Script 1c_wet transfers the 94 samples in source plate DNA1 to all quadrants of all four OLA reaction plates.

The script washes the fixed tips after multidispensing a group of eight samples (one sample per tip) into four quadrants. There are four dispenses per aspiration of each sample.

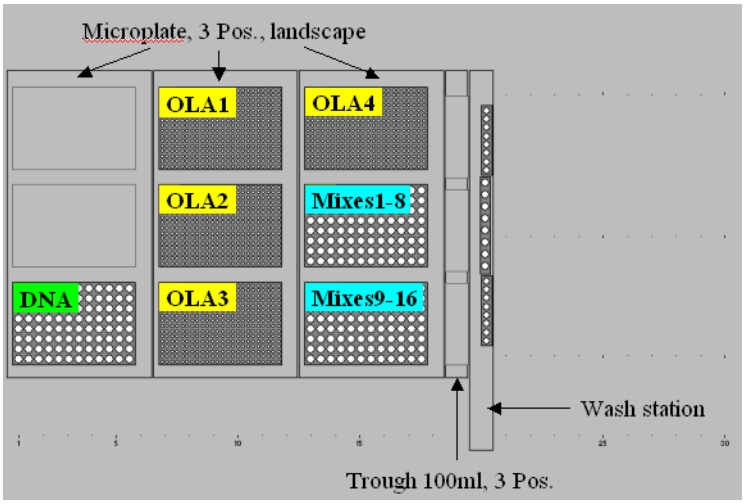
Once the samples have been transferred, the script transfers:

- Assay Mixes 1 to 4 from Assay Mix plate 1 to quadrants 1 to 4 of plate OLA1
- Assay Mixes 5 to 8 from Assay Mix plate 2 to quadrants 1 to 4 of plate OLA2
- Assay Mixes 9 to 12 from Assay Mix plate 3 to quadrants 1 to 4 of plate OLA3
- Assay Mixes 13 to 16 from Assay Mix plate 4 to quadrants 1 to 4 of plate OLA4

The script washes the fixed tips after multidispensing each Assay Mix to each quadrant. There are 12 dispenses of each Assay Mix per aspiration except for tips 7 and 8, which aspirate 11 dispenses per aspiration, keeping the last two wells in each quadrant empty.

Notes

Script 1c_wet uses a single worktable setup:

Plate	Position	Worktable for Script 1c_wet
Assay Mix Plate 1	Mixes1-8	
Assay Mix Plate 2	Mixes9-16	
Empty 384-well reaction plates	OLA1 to OLA4	
DNA source plate	DNA	

1. In the TECAN Gemini software, select script 1c_wet:
   1c_SNPLEX_OLA_P16_S1_W_LiHa.gem
2. Place the uncovered 96-well Assay Mix plate 1 onto the carrier in the position labeled Mixes1-8, as shown.
3. Place the uncovered 96-well Assay Mix plate 2 onto the carrier in the position labeled Mixes9-16, as shown.
4. Place the OLA reaction plates in adapters and onto the carriers in positions OLA1 through OLA4, as shown.
5. Place the empty 96-well DNA source plate in an adapter and onto the carrier in positions DNA, as shown.
6. Start the script.
7. When prompted to verify the worktable setup, verify the plate positions and click **OK** to start dispensing.
8. When dispensing is complete, remove and discard the 96-well Assay Mix plates.
9. Remove, seal, and store (optional) the DNA source plate.
10. Remove, seal, and briefly centrifuge the OLA reaction plates (OLA1 to OLA4), then proceed to "Thermal Cycling the OLA Reactions" on page 52.

Notes

Thermal Cycling the OLA Reactions

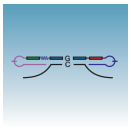
Thermal cycle the OLA plates using the following conditions:

Step	Step Type	Temperature (°C)	Time
1	HOLD	48	30 min
2	HOLD	90	20 min
3	25 cycles	94	15 sec
		60	30 sec
		51, 3% ramp	30 sec
4	HOLD	99	10 min
5	HOLD	4	∞

Next Steps

At this point, the OLA reaction is complete. Proceed to Chapter 5, "Purifying OLA Products (Exonuclease)."

Notes



Performing the OLA Reactions: TECAN Script 1d_wet

About This Script

Script 1d_wet was developed for setups that have 1472 gDNA samples and 1 ligation probe pool. This script uses a single worktable setup.

Preparing the Reagents

1. Thaw the SNPlex Oligonucleotide Ligation Kit components at room temperature.
2. Label one 96-deep well plates Assay Mix.
3. Label one 15-mL centrifuge tube Assay Mix.
4. Prepare an Assay Mix in a 15-mL centrifuge tube and mix thoroughly.

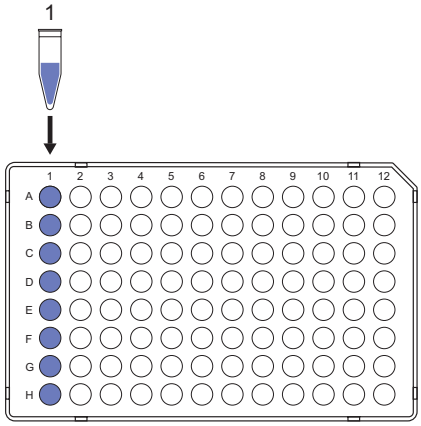
	Manual	Automated		
		Automation Losses		Automation Totals
Assay Mix Component	**Volume (µL) for 1 Quadrant†**	**Source Plate Dead Volume (µL)‡**	**Transfer Loss Excess (µL)§**	**Total Volume (µL) for 16 Quadrants††**
Nuclease-free water	33.6	18.3	12.2	568.2
SNPlex™ OLA Master Mix	280.0	153.3	101.7	4735.0
SNPlex™ Universal Linkers, 48-plex	5.6	3.1	2.0	94.7
SNPlex™ dATP	5.6	3.1	2.0	94.7
SNPlex Ligation Probe Pool (500 nM)	11.2	6.1	4.1	189.4
Total	336.0	184.0	122.0	5682.0

† (12 reactions per tip + excess volume) × volume for one reaction.
‡ 8 tips in source plate × (20 µL per tip + conditioning volume).
§ Transfer losses for transferring bulk mixture into 96-well source plate.
†† Use the volumes in this column to prepare the reagents. Refer to the *SNPlex™ Genotyping System 48-plex User Guide* for per reaction volumes. Volumes given in this table include allowances for dead volume and excess volume for pipetting losses. For more information about calculating dead volumes, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

Notes

5. Add 695 µL of the Assay Mix into each well of the first column of the Assay Mix plate, using only the far left column of the plate as shown in the plate diagram below.

   Note: Do not add Assay Mix to columns 2 through 12; these columns remain empty.



6. Briefly centrifuge the plate to remove trapped air bubbles and collect contents at the bottom of each well.

7. Label 384-well clear optical reaction plates OLA1 to OLA4.

Running Script 1d_wet

Script 1d_wet transfers:

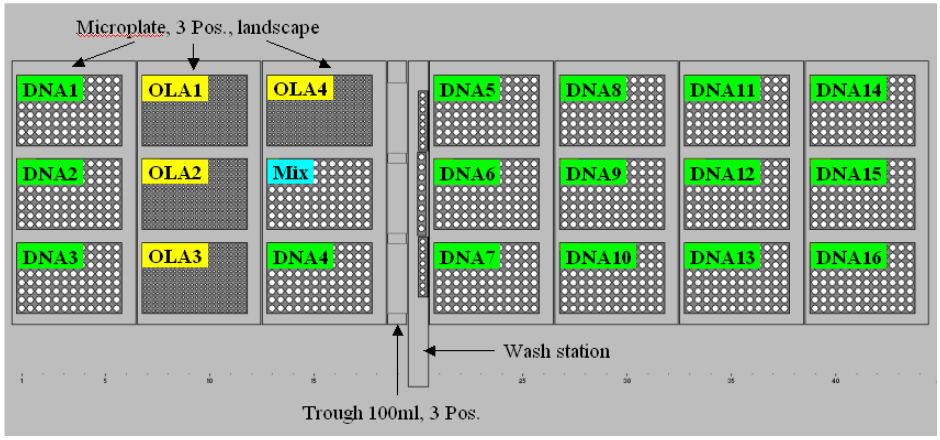
- DNA1 to DNA4 to quadrants 1 to 4 of plate OLA1
- DNA5 to DNA8 to quadrants 1 to 4 of plate OLA2
- DNA9 to DNA12 to quadrants 1 to 4 of plate OLA3
- DNA13 to DNA16 to quadrants 1 to 4 of plate OLA4

The script washes the fixed tips after single-dispensing a group of eight samples (one sample per tip).

Once the samples have been transferred, the script transfers Assay Mix to all quadrants of OLA1 to OLA4. It washes the fixed tips after multidispensing each assay mix to each quadrant. There are 12 dispenses of each Assay Mix per aspiration except for tips 7 and 8, which aspirate 11 dispenses per aspiration, keeping the last two wells in each quadrant empty.

Notes

Script 1d_wet uses a single worktable setup:

Plate	Position	Worktable for Script 1c_wet
Empty 384-well reaction plates	OLA1 to OLA4	
First four DNA source plates	DNA1 to DNA4	
Remaining DNA source plates	DNA5 to DNA16	
Assay Mix plate	Mix	

1. In the TECAN Gemini software, select script 1d_wet:
   1d_SNPLEX_OLA_P1_S16_W_LiHa.gem
2. Place the empty 384-well OLA reaction plates in adapters and onto the carriers in the positions labeled OLA1 through OLA4, as shown.
3. Place the first four 96-well DNA source plates in adapters and onto the carriers in positions DNA1 through DNA4, as shown.
4. Place the remaining 96-well DNA source plates in adapters and onto the carriers in positions DNA5 through DNA16, as shown.
5. Place the Assay Mix plate onto the carrier in position Mix, as shown.
6. Start the script.
7. When prompted to verify the worktable setup, verify the plate position and click **OK** to start dispensing.
8. When dispensing is complete, remove and discard the 96-well Assay Mix plate.
9. Remove, seal, and store (if necessary) the DNA source plates.
10. Remove, seal, and briefly centrifuge the OLA reaction plates (OLA1 to OLA4), then proceed to "Thermal Cycling the OLA Reactions" on page 56.

Notes

Thermal Cycling the OLA Reactions

Thermal cycle the OLA plates using the following conditions:

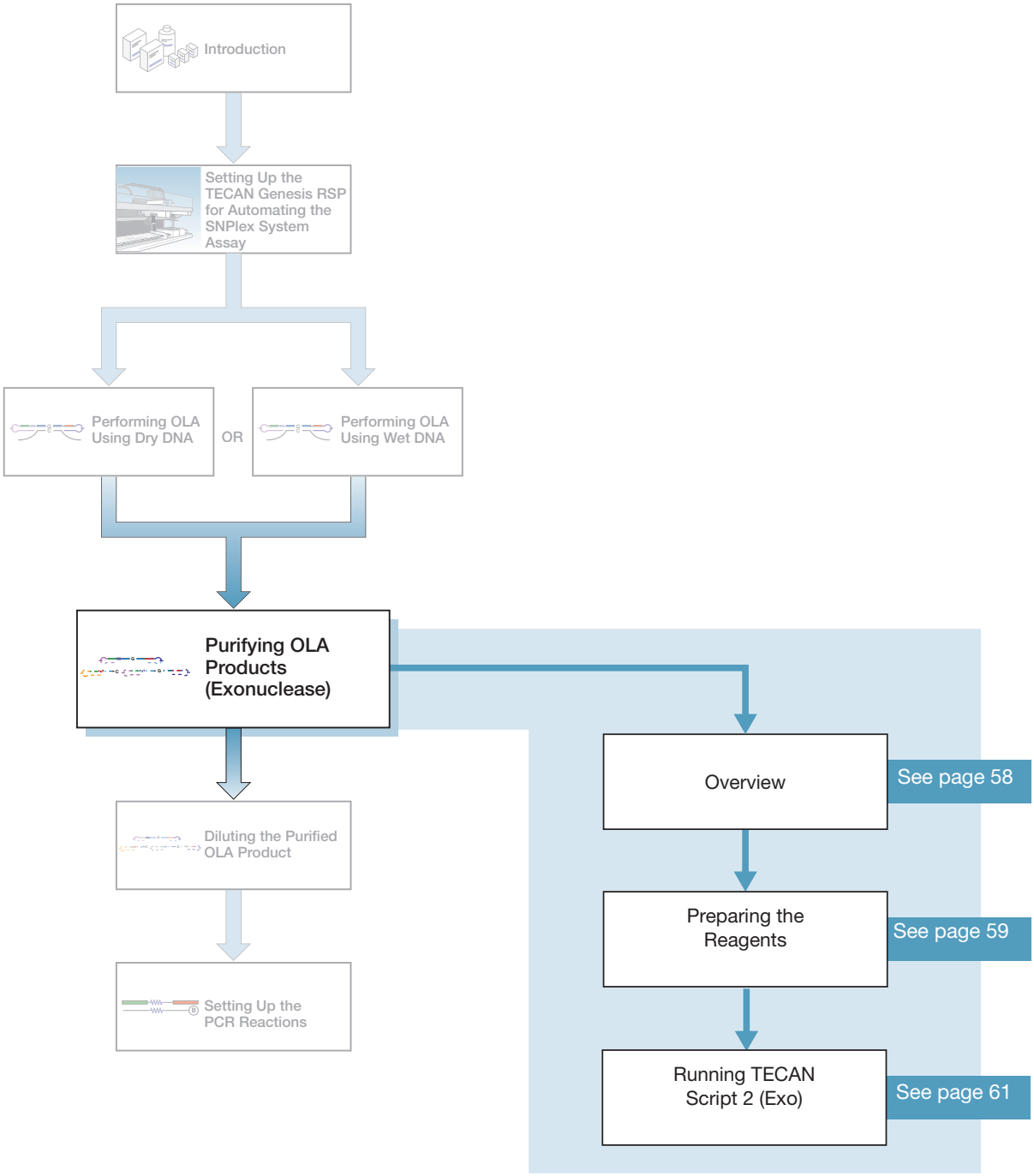
Step	Step Type	Temperature (°C)	Time
1	HOLD	48	30 min
2	HOLD	90	20 min
3	25 cycles	94	15 sec
		60	30 sec
		51, 3% ramp	30 sec
4	HOLD	99	10 min
5	HOLD	4	∞

Next Steps

At this point, the OLA reaction is complete. Proceed to Chapter 5, "Purifying OLA Products (Exonuclease)."

Notes

Purifying OLA Products (Exonuclease)



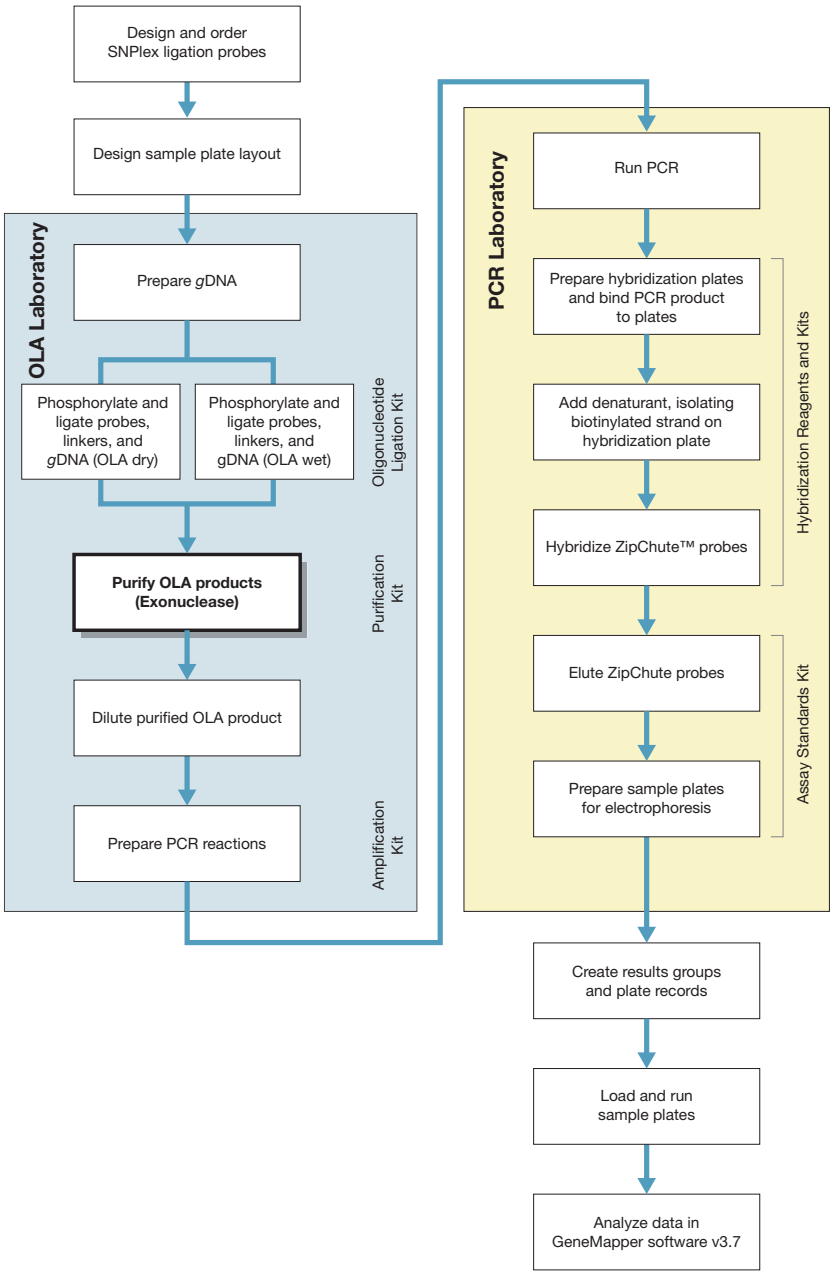
Notes

Overview

About This Chapter

This chapter provides information about automating the addition of Exonuclease mix to the OLA reactions using the TECAN Genesis RSP Robot with the 8-channel LiHa. The procedures in this chapter assume that you have completed the OLA preparation using either dry (Chapter 3) or wet (Chapter 4) gDNA .

Where You Are In the SNPlex System Assay Workflow



Notes

Preparing the Reagents

Preparing the Exonuclease Mix

1. Thaw the SNPlex™ System Purification Kit components at room temperature.
2. Label a 96-deep well plate Exo Mix.
3. Label a 15-mL centrifuge tube.
4. Combine the following volumes of reagents in a 15-mL centrifuge tube and mix thoroughly.

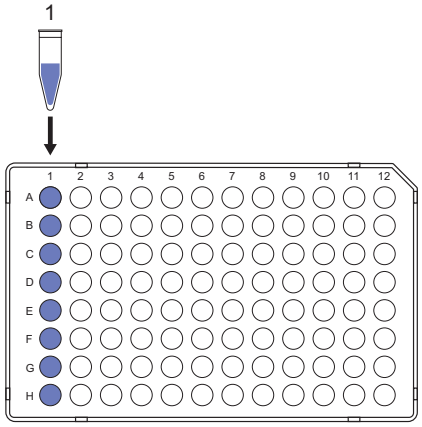
	Manual	Automated			
		Automation Losses		Automation Totals	
Exonuclease Mix Component	**Volume (µL) for 1 384-Well Plate†**	**Source Plate Dead Volume (µL)‡**	**Transfer Loss Excess (µL)§**	**Total Volume (µL) for 1 384-Well Plate**	**Total Volume (µL) for 4 384-Well Plates††**
Nuclease-free water	1747.2	168.0	130.2	2045.4	7287.0
SNPlex™ Exonuclease Buffer	208.0	20.0	15.5	243.5	867.5
SNPlex™ Lamda Exonuclease	83.2	8.0	6.2	97.4	347.0
SNPlex™ Exonuclease I	41.6	4.0	3.1	48.7	173.5
Total	2080	200	155	2435	8675

† (12 reactions per tip + excess volume) × 8 tips × 4 quadrants × volume for one reaction.
‡ 8 tips in source plate × (20 µL per tip + conditioning volume).
§ Pipetting losses for transferring bulk mixture into 96-well source plate.
†† Use the volumes in this column to prepare the reagents. Refer to the *SNPlex™ Genotyping System 48-plex User Guide* for per reaction volumes. Volumes given in this table include allowances for dead volume and excess volume for pipetting losses. For more information about calculating dead volumes, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

Notes

5. Add 1065 µL of Exonuclease mix into each well of column 1 of the 96-well deep well plate.

Note: Do not add Exonuclease mix to columns 2 through 12; these columns remain empty.



6. Briefly centrifuge the plates to remove trapped air bubbles and collect contents at the bottom of each well.

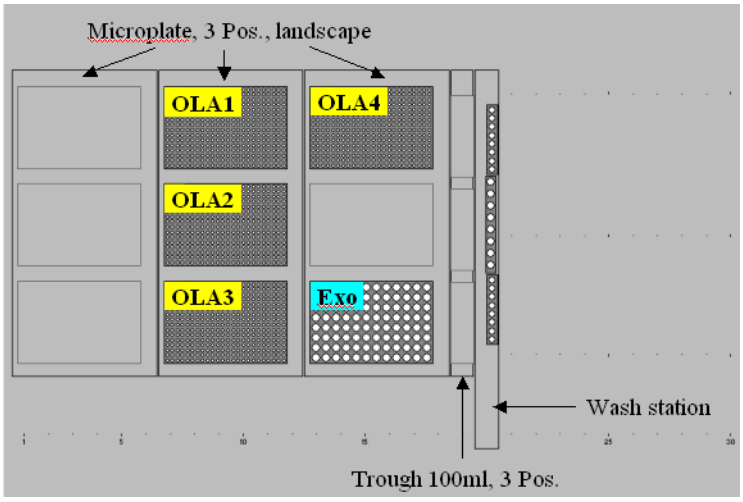
Notes

Running TECAN Script 2 (Exo)

Running the Script

Script 2 transfers the Exonuclease mix to the OLA reaction plates.

Script 2 uses a single worktable setup:

Plate	Position	Worktable for Script 2
Exonuclease plate	Exo	
gDNA sample plates	OLA1 to OLA4	

1. In the TECAN Gemini software, select script 2: **2_SNPLEX_EXO_LiHa.gem**
2. Place the uncovered 96-well Exonuclease mix plate in an adapter on carrier in the position labeled Exo, as shown.
3. Place the OLA reaction plates (from the OLA procedure you followed) into adapters and onto the carriers in positions OLA1 through OLA4, as shown.
4. Start the script.
5. When prompted to verify the worktable setup, verify the plate positions and click **OK** to begin dispensing.
6. When dispensing is complete, remove and discard the Exonuclease mix plate.
7. Remove, seal, and briefly centrifuge the OLA reaction plates (OLA1 to OLA4), then proceed to "Thermal Cycling the OLA Reaction Plates" on page 62.

Notes

Thermal Cycling the OLA Reaction Plates

Thermal cycle the OLA plates using the following conditions:

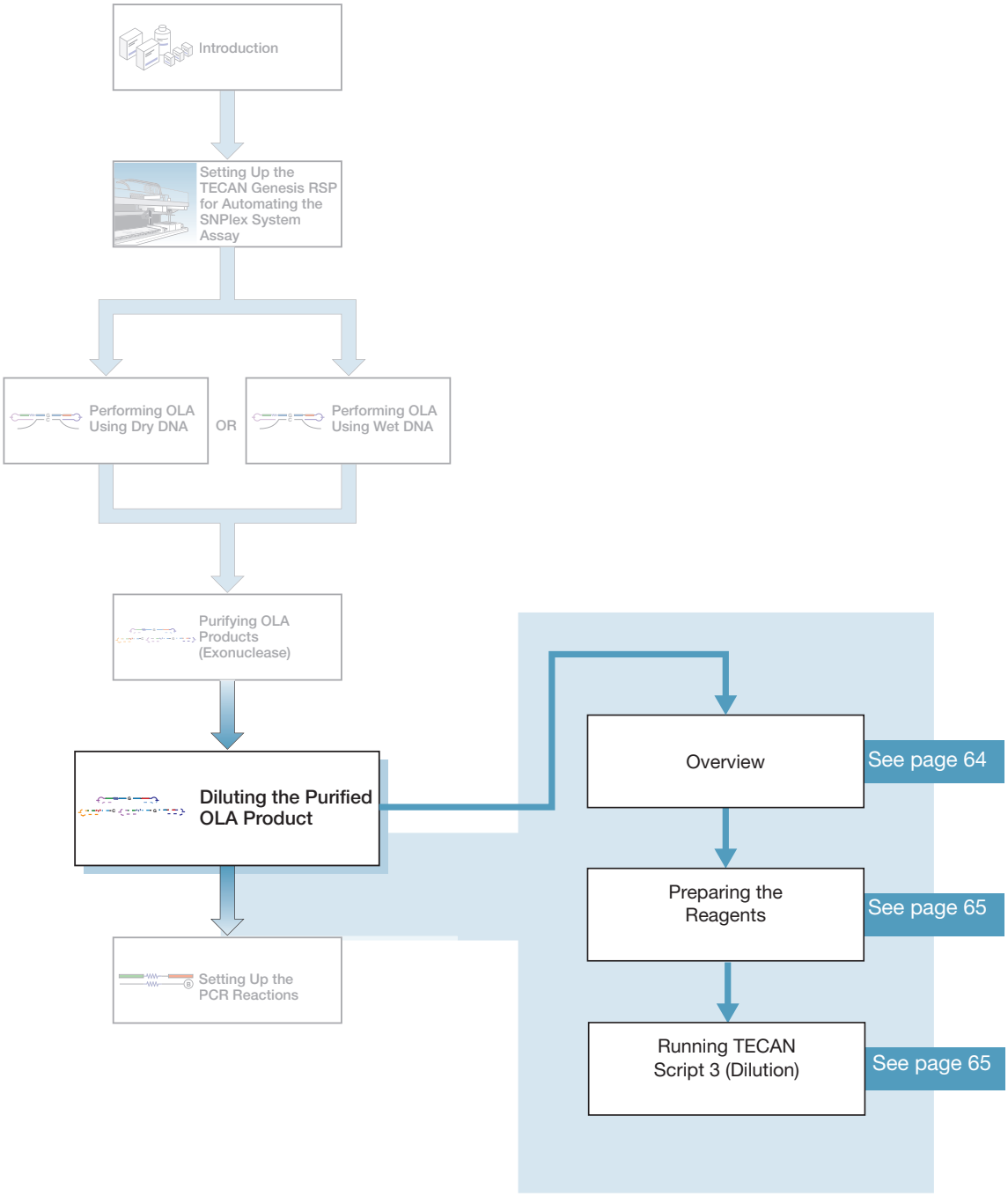
Step	Step Type	Temperature (°C)	Time
1	HOLD	37	90 min
2	HOLD	80	10 min
3	HOLD	4	∞

Next Steps

At this point, the Exonuclease reaction is complete. Proceed to Chapter 6, "Diluting the Purified OLA Product."

Notes

Diluting the Purified OLA Product



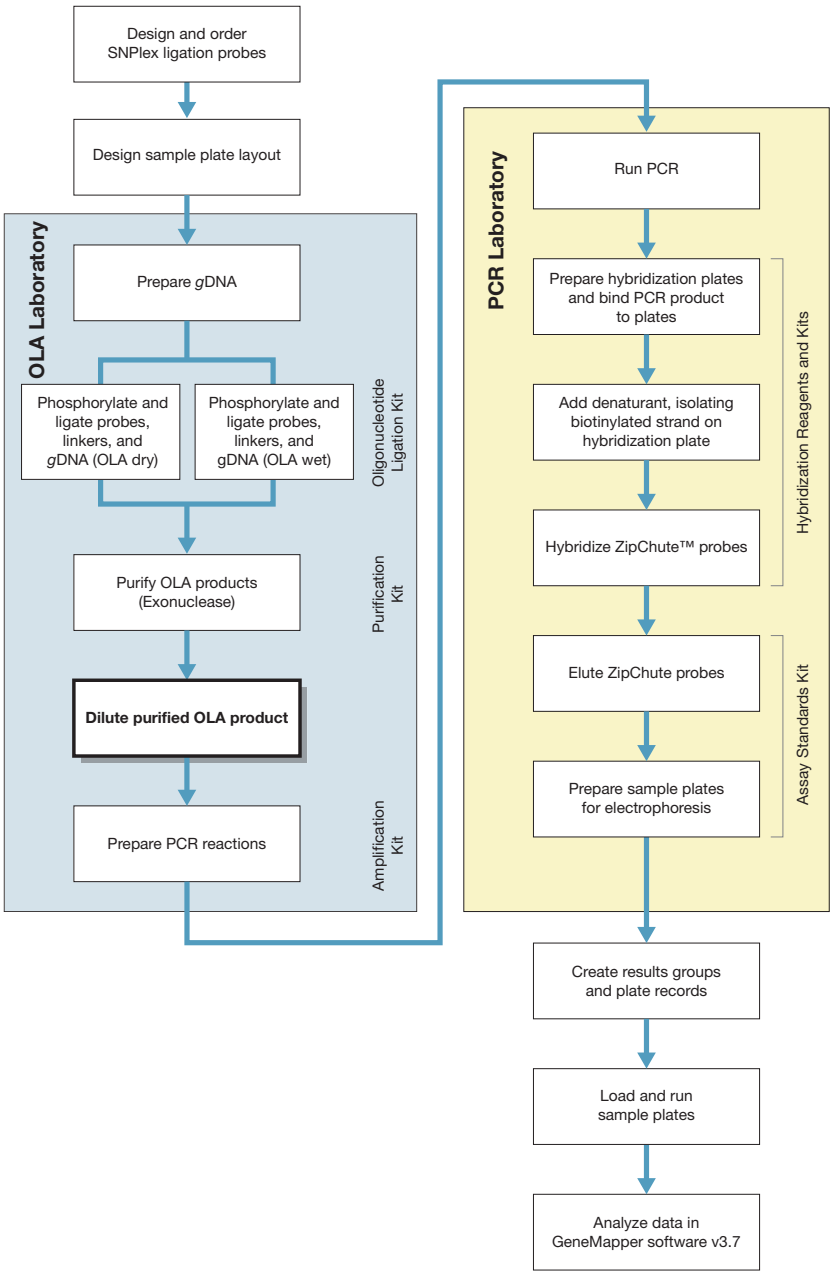
Notes

Overview

About This Chapter

This chapter provides information about automating the dilution of the OLA/Exonuclease mixture using the TECAN Genesis RSP Robot with the 8-channel LiHa. The procedures in this chapter assume that you have completed the OLA/Exonuclease procedure (Chapter 5).

Where You Are In the SNPlex System Assay Workflow



Notes

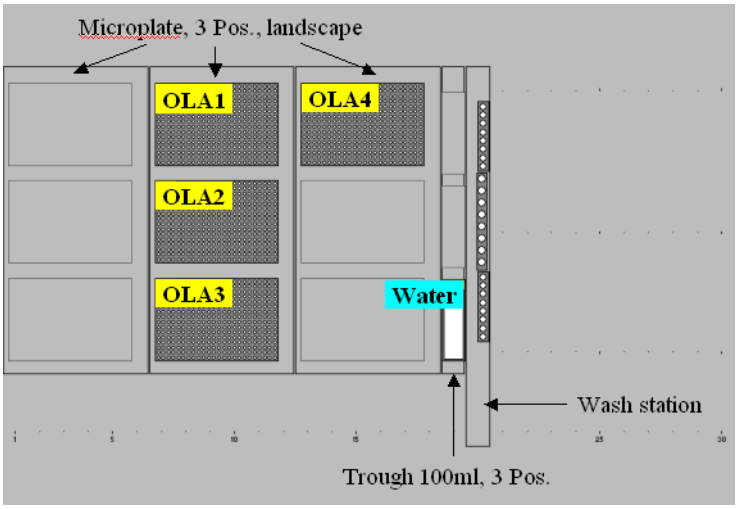
Preparing the Reagents

Fill a 100-mL trough with approximately 50 mL of nuclease-free water.

Running TECAN Script 3 (Dilution)

Running the Script

Script 3 transfers nuclease-free water to the OLA reaction plates. The script uses a single worktable setup:

Plate	Position	Worktable for Script 3 (Dilution)
Nuclease-free water trough	Water	
OLA/Exonuclease reaction plates	OLA1 to OLA4	

1. In the TECAN Gemini software, select script 3: **3_SNPLEX_EXO_DILUTION_LiHa.gem**
2. Place the nuclease-free water trough in the trough carrier in the position labeled Water, as shown.
3. Place the OLA reaction plates (from the Exonuclease procedure) into adapters and onto the carriers in positions OLA1 through OLA4, as shown.
4. Start the script.
5. When prompted to verify the worktable setup, verify the plate positions and click **OK** to begin dispensing.

Notes

6. When dispensing is complete, remove and discard the contents of the nuclease-free water trough.

7. Remove, seal, and briefly centrifuge the OLA reaction plates (OLA1 to OLA4).

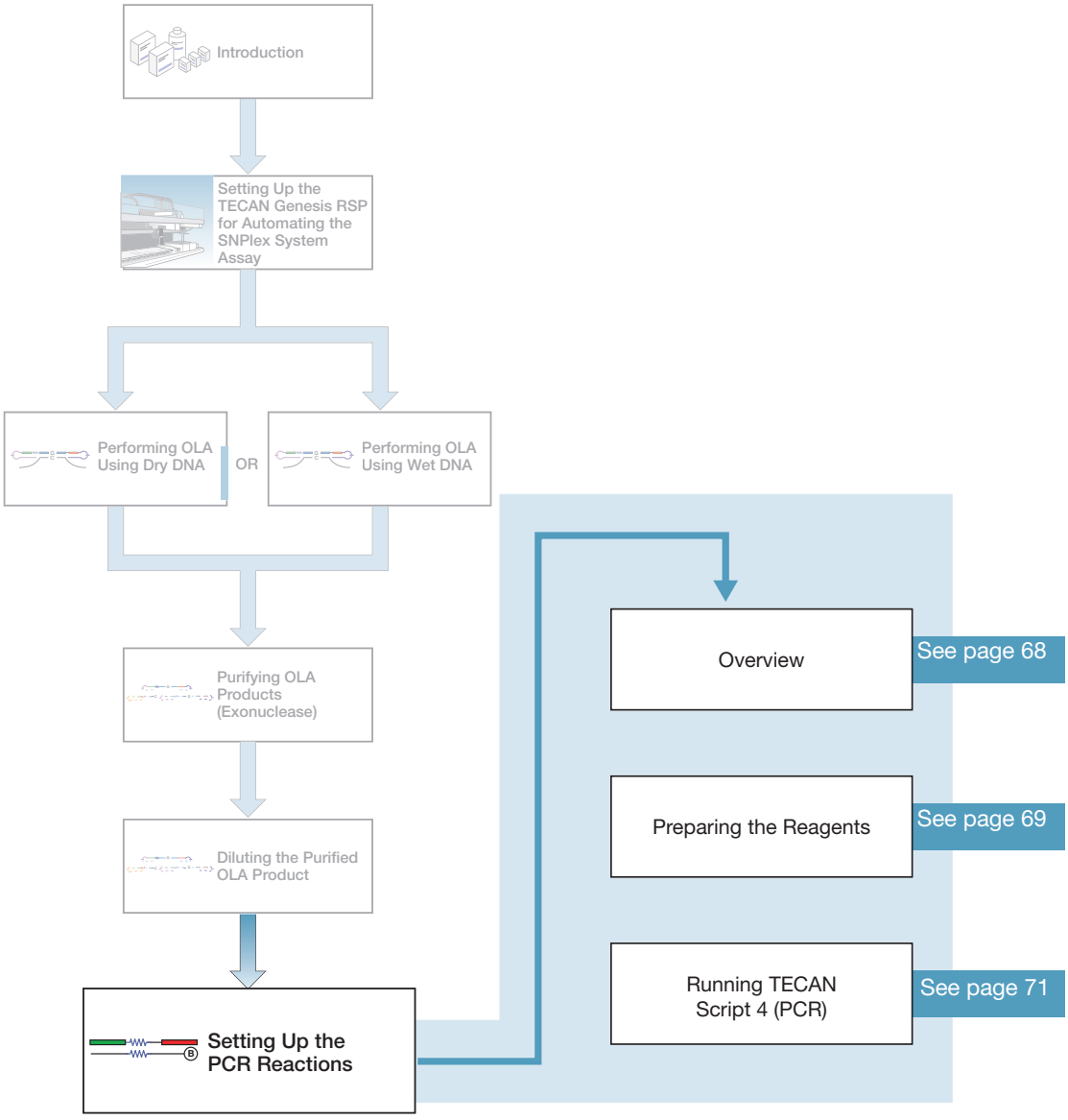
Note: If you are not immediately proceeding to Chapter 7, "Setting Up the PCR Reactions," seal the plates and store them at –20 °C.

Next Steps

At this point, the OLA/Exonuclease dilution is complete. Proceed to Chapter 7, "Setting Up the PCR Reactions."

Notes

Setting Up the PCR Reactions



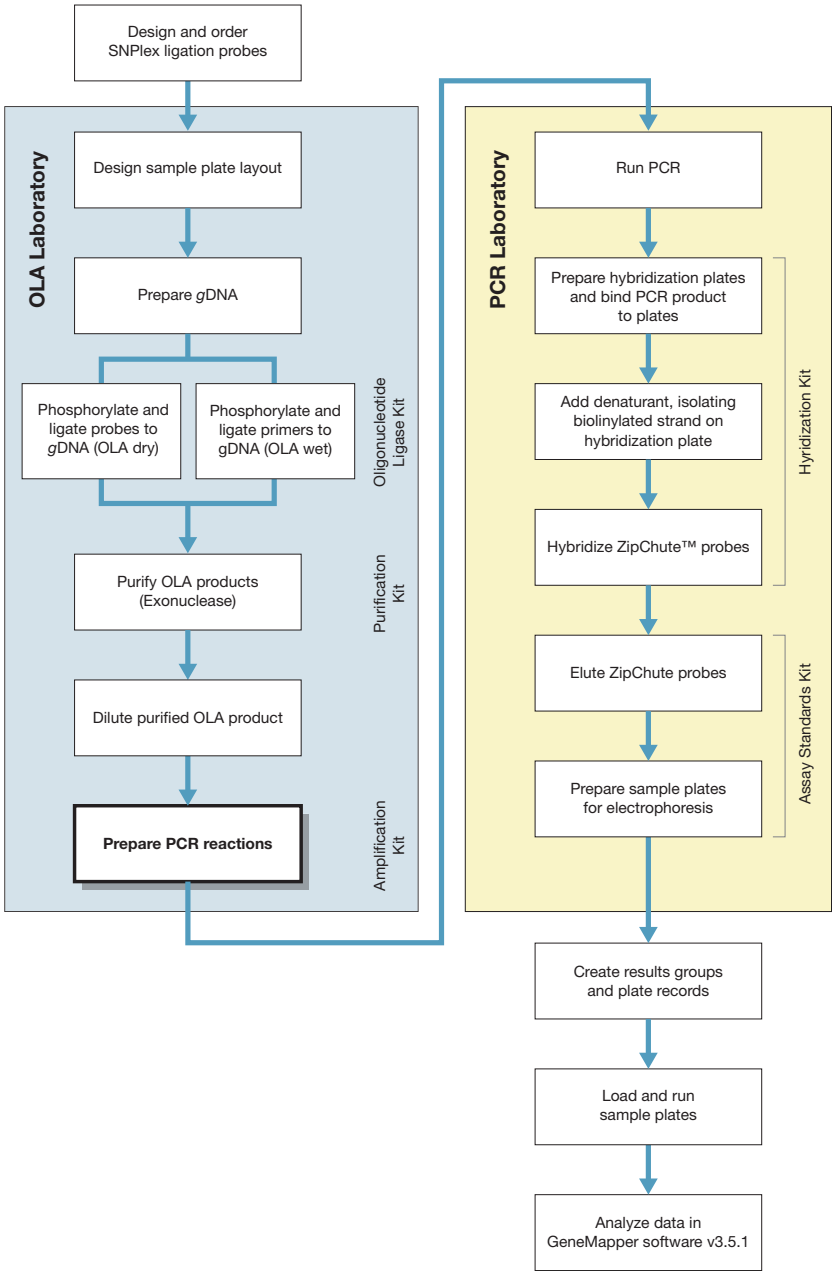
Notes

Overview

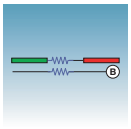
About This Chapter

This chapter provides information about automating the setup of the OLA reactions for transfer to the PCR laboratory using the TECAN Genesis RSP Robot with the 8-channel LiHa. The procedures in this chapter assume that you have completed the OLA/Exonuclease Dilution procedure (Chapter 6).

Where You Are In the SNPlex System Assay Workflow



Notes



Preparing the Reagents

Preparing the PCR Mix

1. Thaw the SNPlex™ System Amplification Kit components at room temperature.
2. Label a 96-deep well plate PCR Mix.
3. Label a 15-mL centrifuge tube.
4. Combine the following volumes of reagents in a 15-mL centrifuge tube and mix thoroughly.

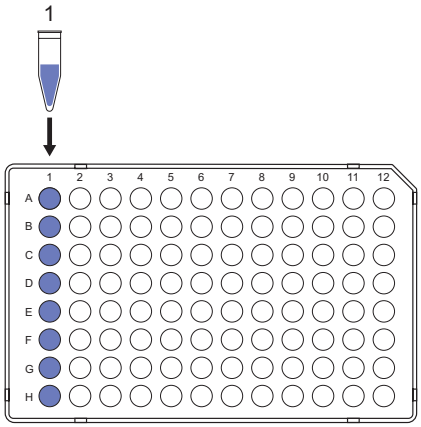
	Manual	Automated			
		Automation Losses		Automation Totals	
PCR Mix Component	**Volume (µL) for 1 384-Well Plate†**	**Source Plate Dead Volume (µL)‡**	**Transfer Loss Excess (µL)§**	**Total Volume (µL) for 1 384-Well Plate**	**Total Volume (µL) for 4 384-Well Plates††**
Nuclease-free water	1016.9	68.4	19.6	1104.9	4155.6
SNPlex™ Amplification Master Mix	2101.0	141.4	40.4	2282.8	8585.9
SNPlex™ Amplification Primers	210.1	14.1	4.0	228.3	858.6
Total	3328	224	64	3616	13600

† (12 reactions per tip + excess volume) × 8 tips × 4 quadrants × volume for one reaction.
‡ 8 tips in source plate × (20 µL per tip + conditioning volume).
§ Pipetting losses for transferring bulk mixture into 96-well source plate.
†† Use the volumes in this column to prepare the reagents. Refer to the *SNPlex™ Genotyping System 48-plex User Guide* for per reaction volumes. Volumes given in this table include allowances for dead volume and excess volume for pipetting losses. For more information about calculating dead volumes, refer to the *SNPlex™ Genotyping System 48-plex General Automation Getting Started Guide*.

Notes

5. Add 1695 µL of PCR Mix into each well of column 1 of the 96-well plate.

   Note: Do not add PCR mix to columns 2 columns 2 through 12; these remain empty.



6. Briefly centrifuge the plates to remove trapped air bubbles and collect contents at the bottom of each well.

7. Label four Label four 384-well clear optical reaction plates PCR1 to PCR4.

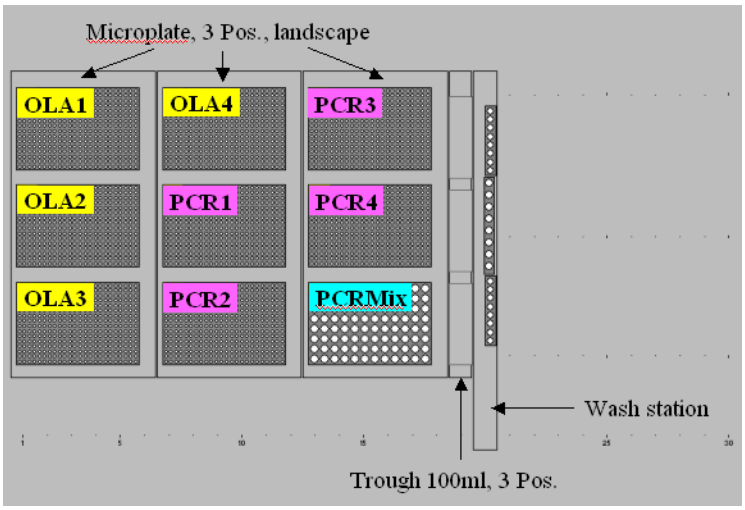
Notes

Running TECAN Script 4 (PCR)

Running the Script

Script 4 transfers the PCR mix to each OLA reaction plate, then transfers an aliquot of each OLA/Exonuclease reaction to its corresponding well in the PCR reaction plates. The script washes the tips after each transfer of the OLA/Exonuclease reactions.

Script 4 uses a single worktable setup:

Plate	Position	Worktable for Script 4 (PCR)
PCR Mix plate	PCRMix	
OLA/Exonuclease reaction plates	OLA1 to OLA4	
PCR reaction plates	PCR1 to PCR4	

1. In the TECAN Gemini software, select script 4: **4_SNPLEX_PCR.gem**.
2. Place the uncovered 96-well PCR Mix plate onto the carrier in the position labeled PCRMix, as shown.
3. Place the OLA reaction plates (from the OLA/Exonuclease dilution procedure) into adapters and onto the carriers in positions OLA1 through OLA4, as shown.
4. Place the empty PCR reaction plates into adapters and onto the carriers in positions PCR1 through PCR4, as shown.
5. Start the script.
6. When prompted to verify the worktable setup, verify the plate positions and click **OK** to begin dispensing.
7. When dispensing is complete, remove and discard the PCR mix plate.

Notes

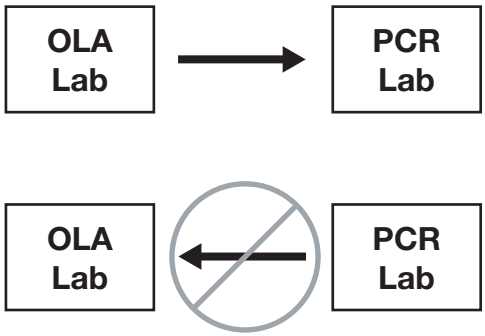
8. Remove, seal, and store the OLA reaction plates (OLA1 to OLA4) at –20 °C.

9. Remove, seal, and briefly centrifuge the PCR reaction plates.

Next Steps

At this point, the OLA Laboratory procedures are complete. Transfer the PCR reaction plates to the PCR laboratory.

IMPORTANT! Never move equipment, containers, or other items from the PCR Laboratory or data collection area into the OLA Laboratory.



Notes

Worldwide Sales and Support

Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches 150 countries on six continents. For sales office locations and technical support, please call our local office or refer to our Web site at **www.appliedbiosystems.com**.

Applera is committed to providing the world's leading technology and information for life scientists. Applera Corporation consists of the Applied Biosystems and Celera Genomics businesses.

Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

06/2005

Applied Biosystems

Part Number 4360790 Rev. B