
HLA Typing Using Olerup SSP[®] Kits and the QIAxcel[®] Advanced System

For analysis and typing of PCR products from different HLA loci using the QIAxcel Advanced and the Helmborg-SCORE software

Introduction

Human leukocyte antigens (HLA) are a group of proteins that are present on each cell surface and allow the immune system to recognize “self” from “foreign” (1). HLA antigens therefore play a fundamental role in organ transplantation as fully or nearly fully matched organs (e.g., kidneys) will result in a lower risk for rejection and in longer graft survival (2). HLA alleles have also been associated with a number of diseases, cancer and drug hypersensitivity (3).

HLA genes are found on the short arm of chromosome 6 and are highly polymorphic with paternal and maternal alleles being coexpressed in each individual. In 1992, Olerup SSP AB pioneered the field of molecular HLA typing with the introduction of its invention PCR-SSP (PCR amplification with sequence-specific primers) (4). This technology enabled for the first time determination of accurate HLA types at the DNA level within a few hours. The method is based on DNA amplification with up to 96 highly multiplexed primer mixes and results in a complex pattern of positive and negative reactions specific for the alleles present in the sample. The method usually requires the separation of the SSP products on a standard agarose gel with gel documentation and manual data entry into the typing software. The QIAxcel Advanced System enables automated and fast separation of PCR fragments, analysis and digital data transfer to the Helmborg-SCORE™ analysis software. The QIAxcel is a highly automated capillary electrophoresis system that provides reliable results with minimal hands-on time and no exposure to toxic gel stains like ethidium bromide. PCR products from different HLA loci can be analyzed and typed in a few minutes.

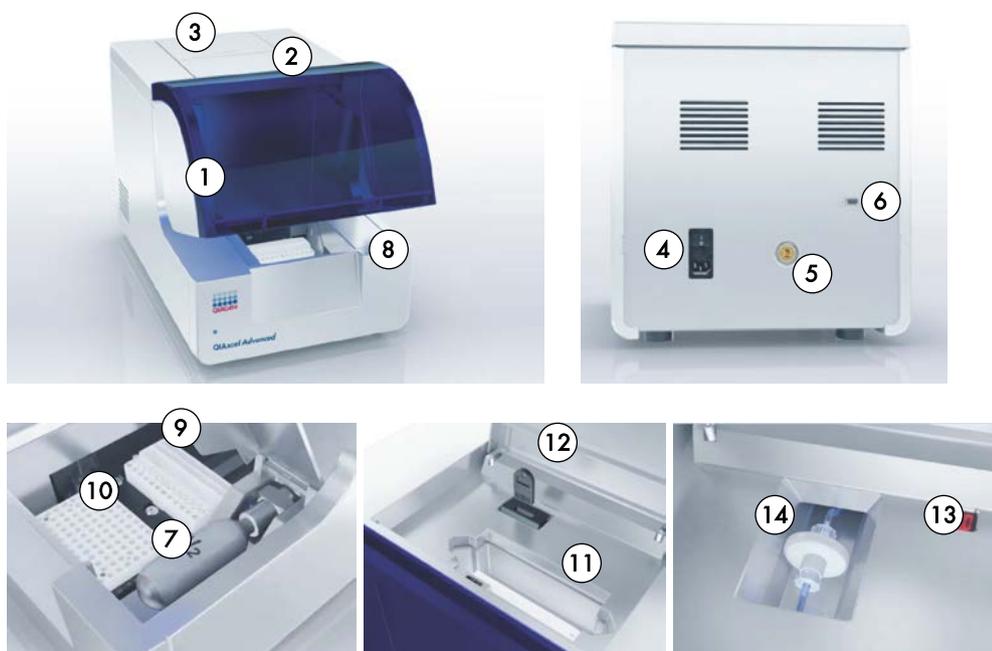
The following procedure is for Research Use Only. Not for use in diagnostic procedures.

QIAxcel Advanced System

The QIAxcel Advanced instrument is a capillary electrophoresis system used for the separation, detection and analysis of nucleic acids (DNA and RNA). Convenient, ready-to-use cartridges provide an array of 12 capillaries and a reservoir containing proprietary gel polymers mixed with the fluorescent dye. Automated sample loading and analysis limit error-prone manual steps, thereby ensuring reproducibility of measurements. As no hazardous compounds need to be

handled manually, the system affords both convenience and safety. Any type of 12-tube strip (e.g., QX 0.2 ml 12-Tube Strip (80), cat. no. 929703) or 96-well plate may be used with the QIAxcel instrument. Up to 96 samples per run are analyzed unattended in as little as 3 minutes per 12 samples or approximately 25 minutes per 96-well plate. Digital data collection and management of experiments ensure reproducibility, traceability and standardized results.

Features of the QIAxcel Advanced instrument



- | | | | |
|---|---|----|--------------------------|
| 1 | Sample door | 8 | N ₂ door |
| 2 | Cartridge door | 9 | Buffer tray |
| 3 | Service door | 10 | Sample plate holder |
| 4 | Power connection for AC Connection; On/off switch | 11 | Cartridge bay |
| 5 | Tube fitting for external N ₂ connection | 12 | Slot for smart key |
| 6 | Connection with the lap top (RS232 connection) | 13 | Digital pressure display |
| 7 | N ₂ cylinder | 14 | Purge filter |

Note: If using an external N₂ source, the output pressure must not exceed 75 psi. The QIAxcel Advanced instrument is equipped with an internal regulator that regulates the pressure generated by the external N₂ source to approximately 40 psi (37–45 psi), which is the instrument's operating pressure.

Materials needed

- QIAxcel DNA Fast Analysis Kit (3000) (Cat. no. 929008)

Note: The QIAxcel Gel Cartridge in the kit is reusable. One kit allows you to analyze up to 3000 samples without loss of performance.

- QX Nitrogen Cylinder (6) (Cat. no. 929705)

QIAxcel DNA Fast Analysis Kit contents

QIAxcel DNA Fast Analysis Kit	(3000)
Catalog no.	929008
Number of assays	12 x 250
QIAxcel DNA Fast Analysis Cartridge (with smart key)	1
QX FA Separation Buffer	40 ml
QX Wash Buffer	40 ml
QX Mineral Oil	50 ml
QX Intensity Calibration Marker	600 µl
QX 0.2 ml 12-Tube Strips	2
QX Colored 0.2 ml 12-Tube Strips	2
QX DNA Size Marker 50 bp – 1.5 kb	100 µl
QX Alignment Marker 15 bp/3 kb	1.5 ml
Handbook	1

Store the QIAxcel DNA Cartridge and the QX Intensity Calibration Marker, Alignment and DNA Size Marker at 4–8°C upon arrival. All other components can be stored at room temperature. Stock solutions of the QX DNA Size Marker and QX Alignment Marker (also available separately) should be stored long-term at –30°C to –15°C.

Prior to use, place the QIAxcel DNA Cartridge into the QIAxcel Advanced instrument in the “Park Position” with buffer in the buffer tray, and allow it to stand for at least 20 minutes. If the QIAxcel DNA Cartridge will be used again the next day, leave it in the instrument in the “Park Position.” To store the cartridge for 2 or more days, close the purge port with the purge port seal, return the cartridge to its blister package making sure to insert the capillary tips into the soft gel, and store it at 4–8°C in an upright position (see the orientation label on the blister package).

Note: Storing the QIAxcel DNA Cartridge below 2°C can severely damage the cartridge.

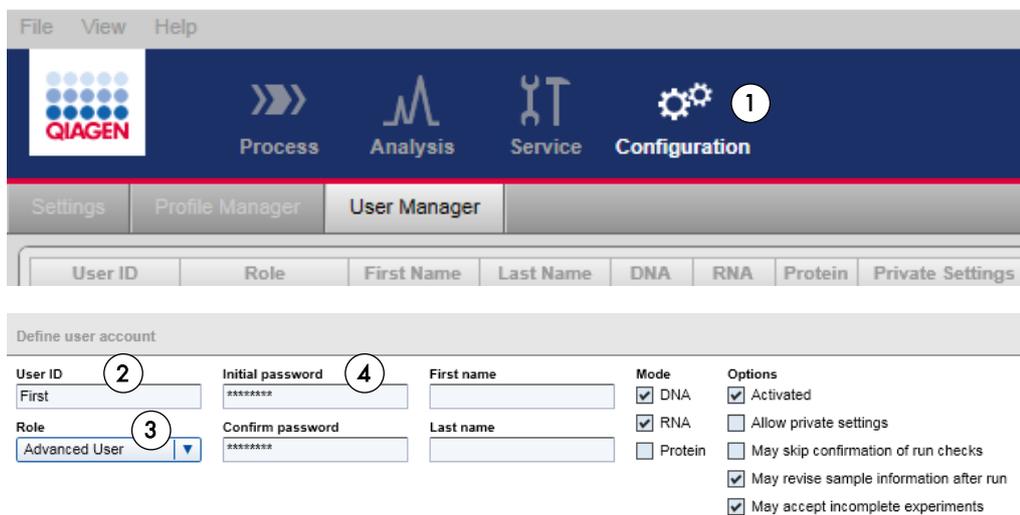
The QIAxcel DNA Fast Analysis Kit is not suited for concentration determination.

Software requirements

ScreenGel software version 1.1 or higher is required. After installing the software, an administrator should define and add new users.

1. Select **User Manager** in the **Configuration** environment (1).
2. For each new user, define **User ID** (2), **Role** (3) and **Password** (4).

Note: For laboratory managers, we recommend defining Role as **Advanced User**.



File View Help

QIAGEN

Process Analysis Service Configuration 1

Settings Profile Manager User Manager

User ID	Role	First Name	Last Name	DNA	RNA	Protein	Private Settings
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Define user account

User ID 2 First name Mode Options

First ***** 4

Role 3 Confirm password Last name

Advanced User *****

DNA Activated

RNA Allow private settings

Protein May skip confirmation of run checks

May revise sample information after run

May accept incomplete experiments

For automated analysis, save the Process Profile file **Olerup SSP HLA typing** (by copy/paste) in the **Process Profile** folder of the ScreenGel software by selecting **File** in the Main Menu, then **Open Data Directory** and **Application data**.

Procedures

Prepare and insert the buffer tray into the buffer tray holder

1. Before using the buffer tray (see “Features of the QIAxcel Advanced instrument”, page 2) for the first time, wash it with hot water and rinse it thoroughly with deionized water.
2. Fill the wash purge (WP) and wash idle (WI) positions of the buffer tray with 8 ml QX Wash Buffer each.
3. Fill the BUFFER position of the buffer tray with 18 ml QX Separation Buffer.
4. Carefully add 2 ml mineral oil to positions WP and WI each and 4 ml mineral oil to position BUFFER to prevent evaporation.

5. Click  in the **Status Information** panel of the ScreenGel software to move the buffer tray holder to the front of the instrument. Allow the buffer tray holder to reach its stop position.
6. Open the sample door and carefully place the filled buffer tray into the buffer tray holder. Ensure that the slots for the 12-tube strips face the front of the instrument.

Note: Be careful not to spill any solutions in the instrument or cause any cross-contamination between buffers loaded on the buffer tray. You may also fill the buffer tray after placing it in the instrument, using a pipet.

Prepare and load the alignment marker

1. Load 15 µl QX Alignment Marker 15 bp/3 kb into each tube of a 12-tube strip (e.g., QX 0.2 ml 12-Tube Strip).
2. Add 1 drop of mineral oil to each tube.
3. Place the strip into the MARKER1 position of the buffer tray.

Alignment markers are injected from the MARKER1 position of the buffer tray and co-migrate with the DNA samples for analysis.

Note: Prepared Alignment Marker can be used for up to 40 runs.

Load the cartridge into the instrument

1. Remove the QIAxcel DNA Cartridge from its packaging and carefully wipe off any soft gel debris from the capillary tips using a soft tissue.
2. Remove the purge cap seal from the back of the QIAxcel DNA Cartridge (**A**) and place the cartridge in the instrument. The cartridge description label should face the front of the instrument (**B**).
3. Insert the smart key into the smart key socket. It may be inserted in either direction (**C**).



4. Close the cartridge door.

Note: Prior to use, place the QIAxcel DNA Cartridge in the “Park Position” with buffer in the buffer tray and allow it to stand for at least 20 min.

Perform an intensity calibration for a new cartridge

Every QIAxcel DNA Cartridge requires an intensity calibration prior to the first run. The calibration is done only once for each cartridge and serves to normalize the intensity of each capillary, applying a correction factor in every subsequent run. This corrects for natural intensity reading variation between capillaries in a cartridge.

1. Load 15 µl QX Intensity Calibration Marker into each tube of a 12-tube strip (e.g., QX Color 0.2 ml 12-Tube Strip). Add a drop of mineral oil and insert the strip into the MARKER2 position of the buffer tray.
2. Click **Start calibration** under **Calibration** in the **Service** environment of the ScreenGel software.
3. Upon completion, calibration results are displayed next to the gel image or in the electropherogram view. The **Results Table** shows the area, calibration factor and result (“Pass” or “Fail”) for each channel.
4. Accept the calibration data.

Sample and DNA size marker preparation

The minimum volume required for analysis is 10 µl. Less than 0.1 µl of the sample is injected into the QIAxcel DNA Cartridge for analysis, and the remaining DNA can be kept for re-analysis or other downstream processes (e.g., Sanger sequencing and cloning).

Note: To prevent capillaries from drying out, fill all 12 positions in a row with either sample or 15 µl of QX Dilution buffer.

DNA size marker

For analysis of SSP tests, use the 50 bp – 1.5 kb DNA size marker at a concentration of 5 ng/µl that is included in the QIAxcel DNA Fast Analysis Kit and is ready to use. Before analysis of PCR samples (Olerup SSP products), run the DNA size marker, analyze it and save it as the Reference Marker Table to be used for the subsequent runs. Pipet 15 µl of the size marker in one tube of a 12-tube strip and fill each of the 11 empty tubes with the 15 µl of QX Dilution buffer. Check the corresponding option (1) in the **Marker Selection** dialog and enter the total concentration (2).

The screenshot shows the software interface with three main panels. On the left is a sidebar with 'Profile Definition' selected. The central panel is 'Marker Selection' with three radio button options: 'No Marker', 'Reference Marker Table', and 'Run size marker side by side with sample' (which is selected and circled with a '1'). Below these is a dropdown menu for 'Size Marker' set to '50 bp - 1.5 kb (5 ng per ul)' and a 'Save as ...' button. The right panel is 'Size Marker' with a 'Total conc.' input field set to '5 ng/ul' (circled with a '2'). Below this is a table with two columns: 'Size [bp]' and 'Conc. [ng/µl]'. The table contains five rows of data.

Size [bp]	Conc. [ng/µl]
15	--
50	0.625
100	0.625
200	0.625
300	0.625

Note: If running the size marker, define the position of the size marker by right-clicking the corresponding position in the **Sample Row Selection** panel (1).

The screenshot shows two panels. The left panel is 'Method Details' with a table containing method information. The right panel is 'Sample Row Selection' showing a grid of 12 columns and 5 rows (A-E). The first column is circled with a '1' and has a menu icon, indicating where to right-click to define the size marker position.

Method	Range	M	Inj. Time	RpR	Runs	Preview
DM80 v2.0	A	A1	8	1	1	*****

	1	2	3	4	5	6	7	8	9	10	11	12
A	☰	●	●	●	●	●	●	●	●	●	●	●
B	●	●	●	●	●	●	●	●	●	●	●	●
C	●	●	●	●	●	●	●	●	●	●	●	●
D	●	●	●	●	●	●	●	●	●	●	●	●
E	●	●	●	●	●	●	●	●	●	●	●	●

For subsequent runs with the same cartridge, a Reference Marker Table should be used instead of running the size marker in parallel with samples. For details, see "Preparing a Reference Marker Table", page 24.

Olerup SSP samples

Place the PCR samples into the sample plate holder (see “Features of the QIAxcel Advanced instrument”, page 2). Make sure to run and analyze the DNA size marker before analyzing the samples and use the protocol that includes the Reference Marker Table. The size marker must be run with the same cartridge and method as the samples.

Choose the Process profile **Olerup SSP with RMT**. It has the following parameters: Method DM80 v2.0; Reference Marker table (“DM80 v2.0_50bp-1.5kb”) Analysis and Export.

The screenshot displays the software interface for configuring a PCR run. The left sidebar contains navigation options: Profile Definition, Process Profile, Run Parameters, Analysis, Marker, Peak Calling, Report/Export, Start a Process, Sample Selection, Sample Information, and Run Check. The main window is divided into three tabs: Profile Definition, Plate Definition, and Method Details. The Method Details tab is active, showing a table with columns for Method, Range, M, Inj. Time, RpR, and Runs. The table contains one entry: DM80 v2.0, A - H, (blank), 8, 1, 8. Below the table are buttons for Insert, Add, and Delete. There are also checkboxes for 'Allow row deselection' and 'Allow marker definition', both of which are checked. At the bottom of the Method Details panel, there are input fields for Method (DM80 v2.0), Range (A - H), Marker (blank), Injection Time (8 sec), and Runs per Row (1). A note states: '* This method costs 1 regular run(s) decrement in the cartridge key.' There are OK and Cancel buttons at the bottom. The Sample Row Selection panel on the right shows a grid of 8 rows (A-H) and 12 columns (1-12). Each cell in the grid contains a blue dot, indicating that all positions are selected. An information icon and text below the grid state: 'Left-click to add or remove sample rows' and 'Right-click to define marker position'.

Method	Range	M	Inj. Time	RpR	Runs	Preview
DM80 v2.0	A - H		8	1	8	

The Analysis protocol is **Default Fast Analysis v2.0** with a Sample threshold of 0.14RFU and an Alignment marker threshold of 5 S/N.

Analysis Properties					
<input type="checkbox"/> Smear Analysis Profile					
Minimum Distance	<table border="1"> <thead> <tr> <th>Start</th> <th>Value</th> </tr> </thead> <tbody> <tr> <td>0.00 min</td> <td>0.25 sec</td> </tr> </tbody> </table>	Start	Value	0.00 min	0.25 sec
Start	Value				
0.00 min	0.25 sec				
Suspend Integration	<table border="1"> <thead> <tr> <th>Start</th> <th>End</th> </tr> </thead> <tbody> <tr> <td>0.00 min</td> <td>0.50 min</td> </tr> </tbody> </table>	Start	End	0.00 min	0.50 min
Start	End				
0.00 min	0.50 min				
Threshold	<table border="1"> <thead> <tr> <th>Start</th> <th>Value</th> </tr> </thead> <tbody> <tr> <td>0.00 min</td> <td>0.14 RFU</td> </tr> </tbody> </table>	Start	Value	0.00 min	0.14 RFU
Start	Value				
0.00 min	0.14 RFU				
Alignment Marker Threshold	5 S/N				

The Reference Marker Table is **DM80 v2.0_50bp-1500bp**.

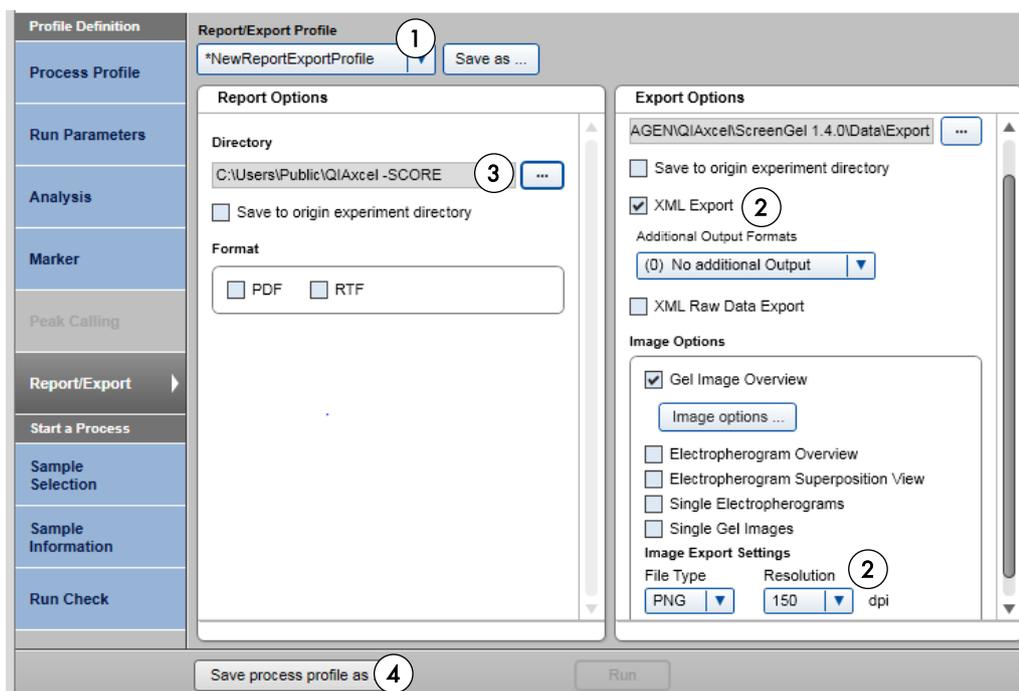
Marker Selection

- No Marker
- Reference Marker Table
- Run size marker side by side with sample

Reference Marker
 DM80 v20_50bp-1500bp

Export Settings

QIAxcel ScreenGel software and the SCORE software need to communicate: the sample information file prepared by SCORE software is imported by ScreenGel SW. After the sample analysis by ScreenGel software, results are exported to SCORE. Therefore, create a new folder with the name **QIAxcel-SCORE** under **C:\Users\Public** on the computer or on a network drive (the new export folder is needed because SCORE cannot communicate with the ScreenGel export default folders.) In ScreenGel, choose **NewReportExportProfile** (1). Select **XML Export** and PNG file format for the image export (2). Define the export directory (3) and choose the newly created folder in the selection window (3). Save the new process profile with the **Save process profile as...** button (4) for future use (e.g., with the name **Olerup SSP complete protocol**).



Helmborg-SCORE Software

The Helmborg-SCORE software is used to analyze the SSP amplification pattern of a sample and suggest the corresponding HLA genotype. As the first step, SCORE is used to generate a XML data file with information about the samples, typing kits and experiment name used. This file is then imported by the ScreenGel software (**Sample Information**) before the run. After the analysis of the PCR products, results are exported as a new XML file together with a PNG gel image. Both softwares should therefore be configured to have access to the same data import and export folder as explained in "Export Settings" on page 10. This folder should be created in a location with unrestricted user access (e.g., **C:\Users\Public** or a network drive) and should be saved with the ScreenGel and SCORE softwares for future experiments.

Details about configuration and operation of SCORE can be downloaded from www.olerup-ssp.com/score.

Configuration of Helmborg-SCORE software

1. Start the software and logon with your user name and password.
2. Choose **Configuration (1)** and **Gel configuration (2)** from the menu.



3. Define the gel configuration as in the screenshots below.

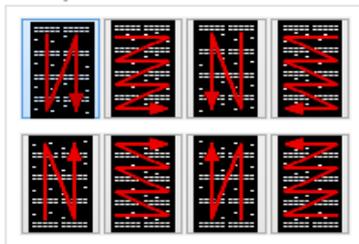
Gel type

16 wells per row 24 wells per row

Pipetting order



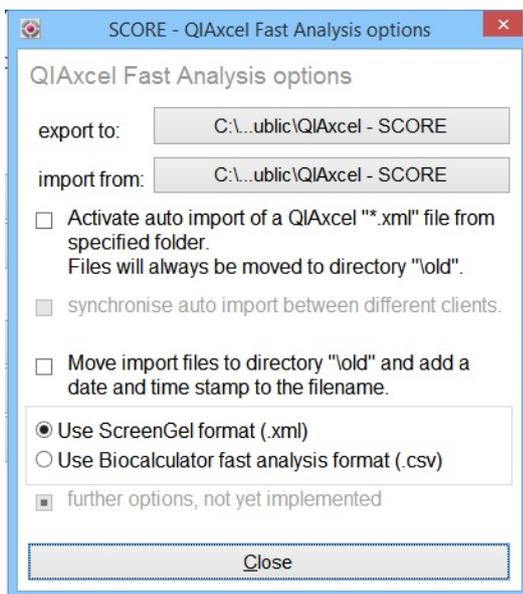
Strip order



4. Go to **Configuration** and select **QIAXcel options**.



5. Select the same data import and export directory as in the ScreenGel software (e.g., **C:\Users\Public\QIAXcel-SCORE**, see "Export Settings", page 10).

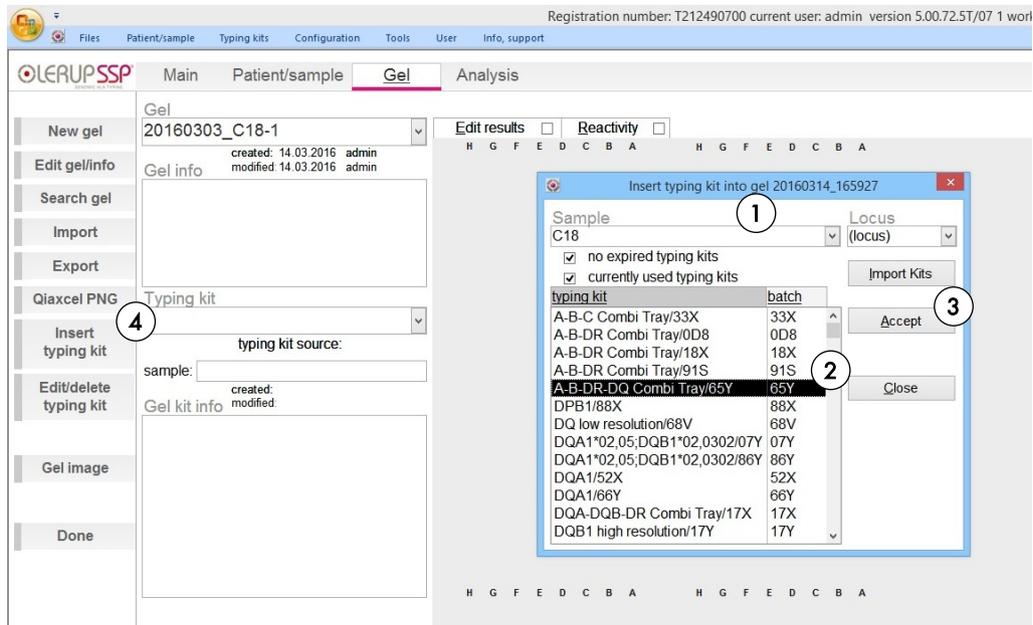


Prepare the experiment in the Helmsberg-SCORE software

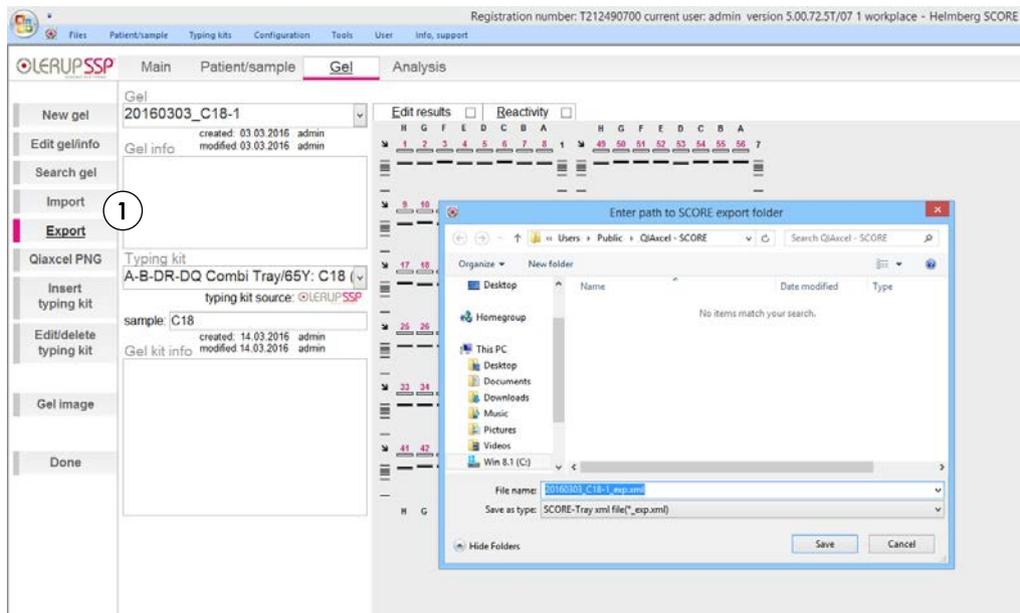
1. Select the **Patient/sample** tab (1) from the menu and enter the relevant data for each sample to be run on the QIAxcel. Please note that "Patient ID" (i.e., the sample ID) is the minimum information required. Click on **New Patient** (2) (i.e., new PCR sample) to enter the next data set if necessary or click on **Done** (3) after all information has been entered.

The screenshot shows the Helmsberg-SCORE software interface. The title bar indicates the registration number (T212490700), current user (admin), and version (5.00.72.5T/07 1 workplace). The main menu includes 'Main', 'Patient/sample' (highlighted with a circled '1'), 'Gel', 'Analysis', and 'Help'. On the left, a sidebar contains buttons for 'New Patient' (circled '2'), 'Search', 'Delete', 'ask before deleting', 'Archive', 'Export', 'New Sample', 'Search', 'Delete', and 'Done' (circled '3'). The main area is titled 'Patient and sample data input' and contains the following fields: 'Family name:' (text input), 'First name:' (text input), 'Birth:' (text input), 'Patient ID:' (text input with 'C18' entered), 'Source lab ID:' (text input), 'External ID:' (text input), 'Group:' (dropdown menu), and 'Ethnicity:' (dropdown menu with 'no data' selected). Below these fields are 'created:' and 'modified:' labels. A message box states: 'You first have to enter patient details before you can enter a sample. Enter either family name and first name or/and patient ID. However, if you just have a sample ID at hand, enter this number in the field "Patient ID". This ID will then automatically be propagated to the field sample ID.'

- In the **Insert typing kit** window (4), select the first sample to run from the sample drop down menu (1). Insert the typing kit (2) for this sample into the virtual gel and click **Accept** (3).
- If more than one sample is run, click on **Insert typing kit** (4). In the typing kit window choose your next sample via the drop down menu and select the corresponding typing kit. Repeat for all samples to be run together.



4. Click **Export (1)** to export these data to the **QIAxcel-SCORE** folder, as defined on page 12. The file will have the format *gel name_exp.xml*.



Perform the run on the QIAxcel

1. Switch on the QIAxcel instrument (see "Features of the QIAxcel Advanced instrument", page 2). Switch on the computer linked to the instrument and open the ScreenGel software.
2. Load the Alignment marker in the Marker 1 position in the buffer tray.
3. Load the Olerup SSP plate on the sample plate holder in the correct order (see "Features of the QIAxcel Advanced instrument", page 2). E.g. the first well of the first sample will be in the lower left corner of the sample tray holder (in position H1).

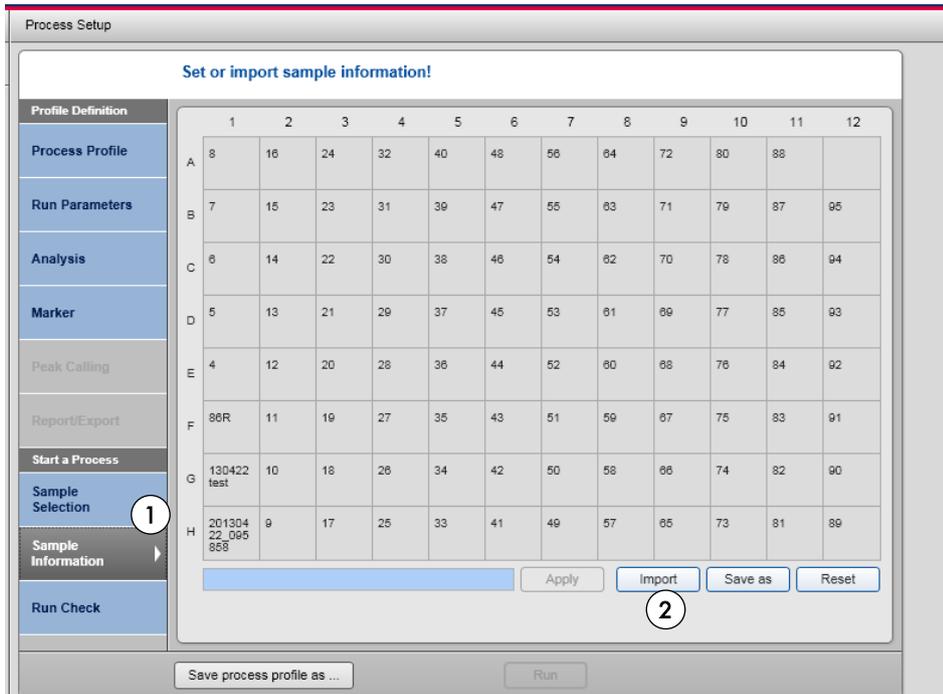
4. Select **Olerup SSP complete** from the **Process Profile** drop-down menu (1) with the Reference Marker Table created for the cartridge in use. For details, see “Preparing a Reference Marker Table”, page 24.

The screenshot shows a software interface with a left-hand navigation menu and a main configuration area. The navigation menu includes 'Profile Definition', 'Process Profile', 'Run Parameters', 'Analysis', 'Marker', 'Peak Calling', and 'Report/Export'. The 'Process Profile' section is active, showing a 'Mode' section with a 'Cartridge Type' dropdown set to 'DNA Fast Analysis'. Below this is a 'Process Profile' dropdown menu, which is highlighted with a circled '1' and contains the text 'Olerup SSP complete'. Underneath is a 'Profile' section with another 'Cartridge Type' dropdown set to 'DNA Fast Analysis'. At the bottom is an 'Included Steps' section with checkboxes for 'Run', 'Analysis', 'Peak Calling', and 'Report', all of which are checked.

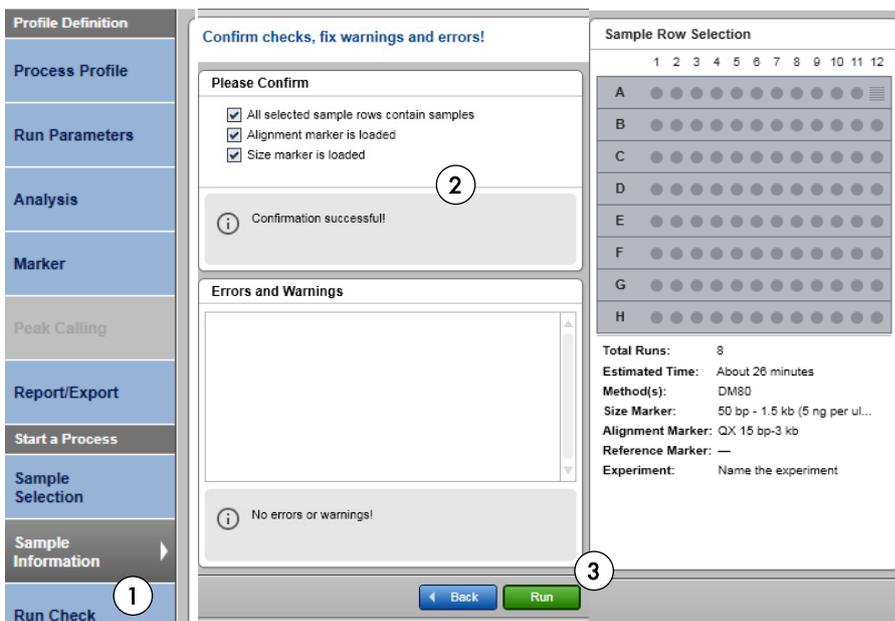
5. Open the **Sample Row Selection** dialog (1). Check that all rows are selected. You can deselect them with the left mouse click.

The screenshot shows a dialog box titled 'Sample Row Selection'. On the left, there are fields for 'Plate ID' (2016-03-06_19-24-27) and 'Experiment Directory' (\\QIAGEN\QIAxcel\ScreenGel 1.4.0\Data\Experiment\DN), along with a 'Provide Sample Information' checkbox. Below these is a 'Reference Marker' section with radio buttons for 'No Marker', 'Use selected marker table' (which is selected), and 'Run size marker side by side with sample'. On the right, there is a grid with columns numbered 1 through 12 and rows labeled A through F. All cells in the grid are filled with blue dots, indicating that all rows and columns are selected. A circled '1' is placed above column 5.

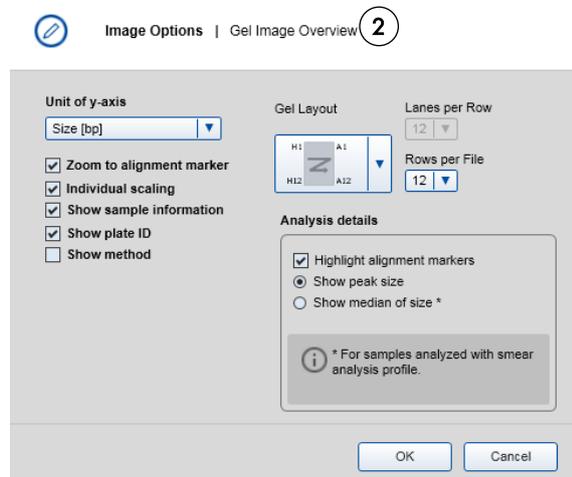
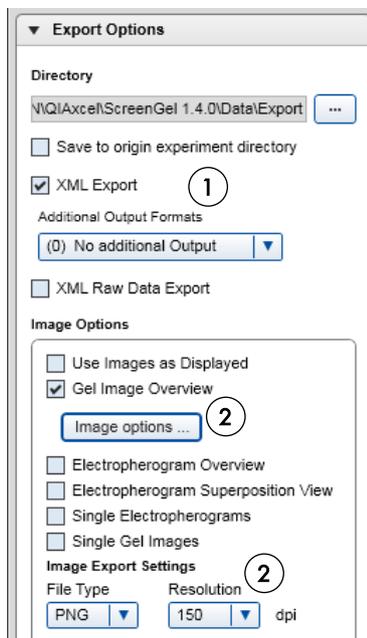
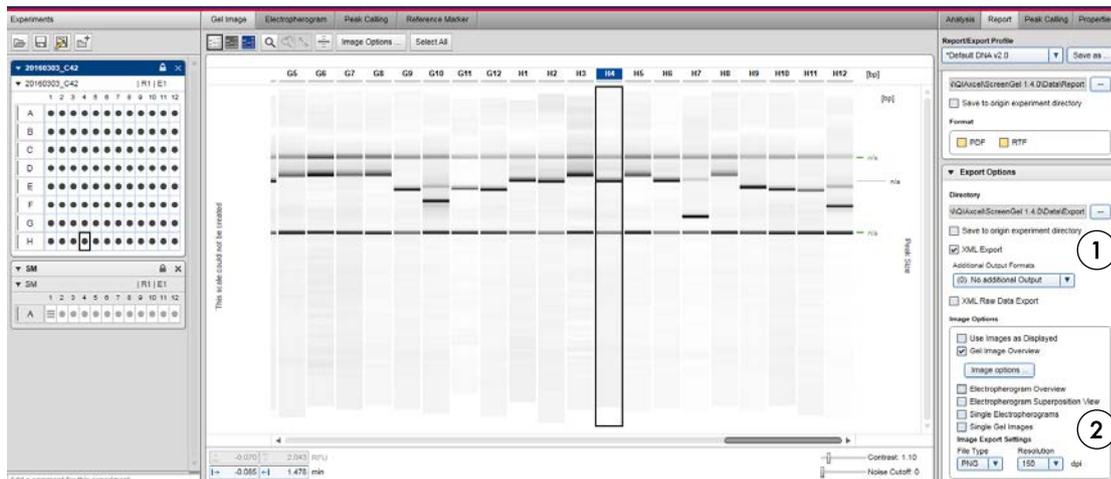
- Open the **Sample Information** dialog (1). Import (2) the SCORE export file from the folder **QIAxcel-SCORE**. The file name has the format *gel_name_exp.xml*.



- Open the **Run Check** dialog (1) and confirm that samples and markers are loaded correctly (2). Click **Run** to start the run (3).



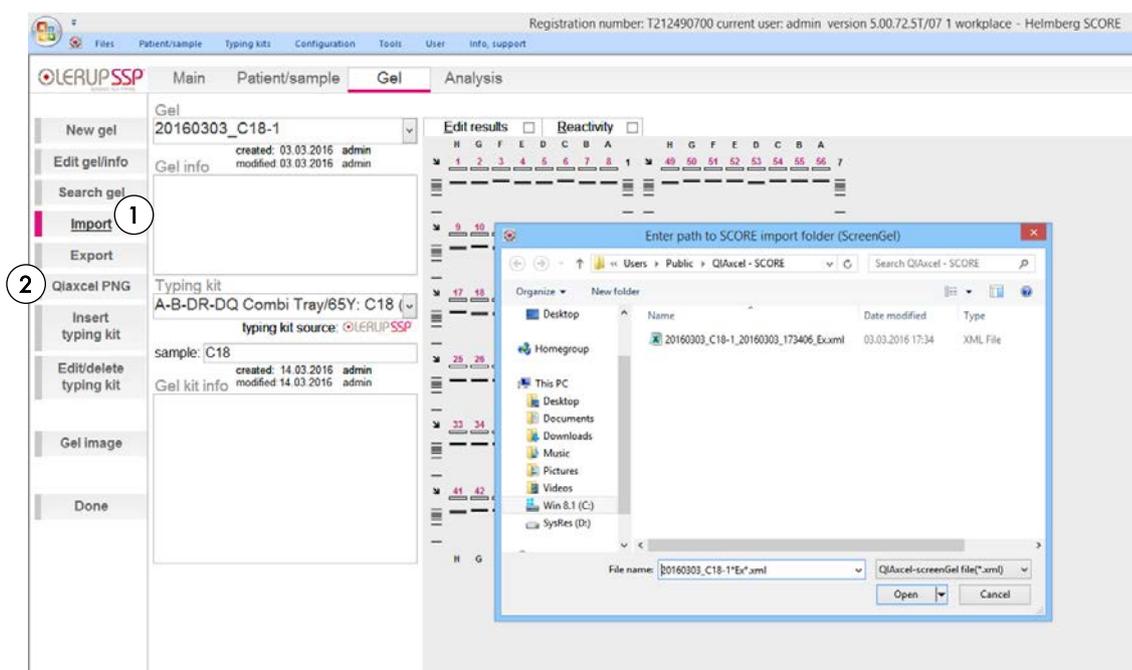
- After the QIAxcel run and data analysis, export the XML file with the sizes (1) and gel image as a PNG file (2) to the **QIAxcel-SCORE** folder and import these to the SCORE software.



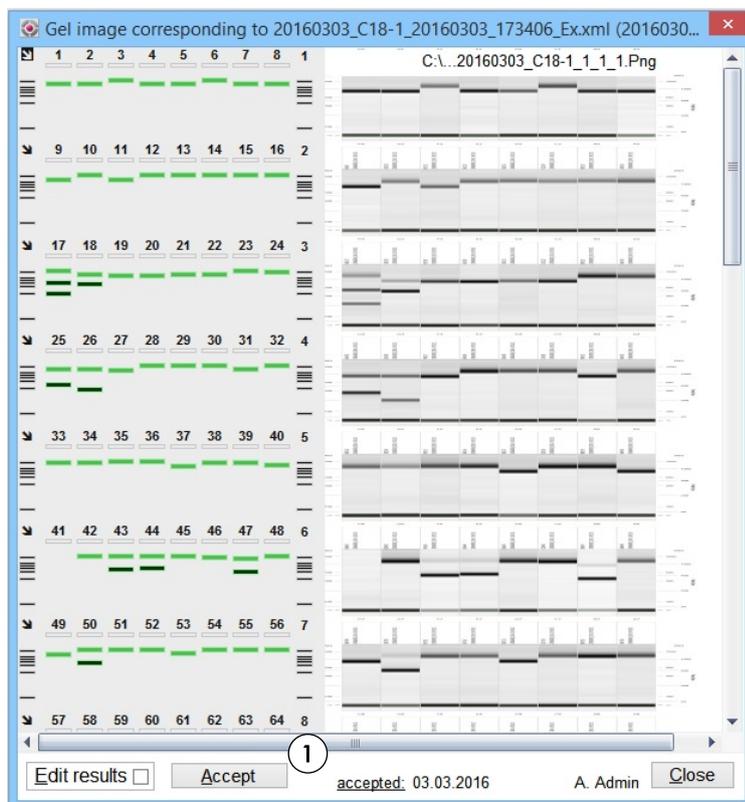
Import in Helmsberg-Score

1. Choose **Import** on the left side of the screen (1). SCORE will open the path to your default import folder as defined in "Export Settings", page 10. The correct import file name is determined automatically and only matching files will be displayed. Import the file matching the gel into SCORE.

Select the corresponding PNG image when prompted or use **QIaxcel PNG (2)** to search for the corresponding PNG image. Only matching files will be displayed.



2. Check for correct band calling in the gel image window. The displayed colors on the virtual gel are specific for QIAxcel data. Light green depicts a control band, dark green is used for positive bands of the correct size and orange bands indicate any band of unexpected size. Make edits if necessary and click **Accept (1)** to apply the changes. Note that any edits change the band color to black or pink to indicate manually entered data.



- Proceed by switching to **Analysis (1)** and print the typing report if needed. For details about analysis and report option, please refer to the SCORE Software User Manual.

Registration number: T212490700 current user: admin version 5.0

Files Patient/sample Typing kits Configuration Tools User Info, support

OLERUP SSP Main Patient/sample **Gel** SSP **Analysis** Typing kits SSP details

Gel 20160303_C18-1

created: 03.03.2016 admin
modified 03.03.2016 admin

Gel info

Search gel

Import

Export

Qiaxcel PNG

Typing kit A-B-DR-DQ Combi Tray/65Y: C18

Insert typing kit typing kit source: OLERUP SSP

sample: C18

Edit/delete typing kit created: 14.03.2016 admin
modified 21.03.2016 admin

Gel kit info

Gel image

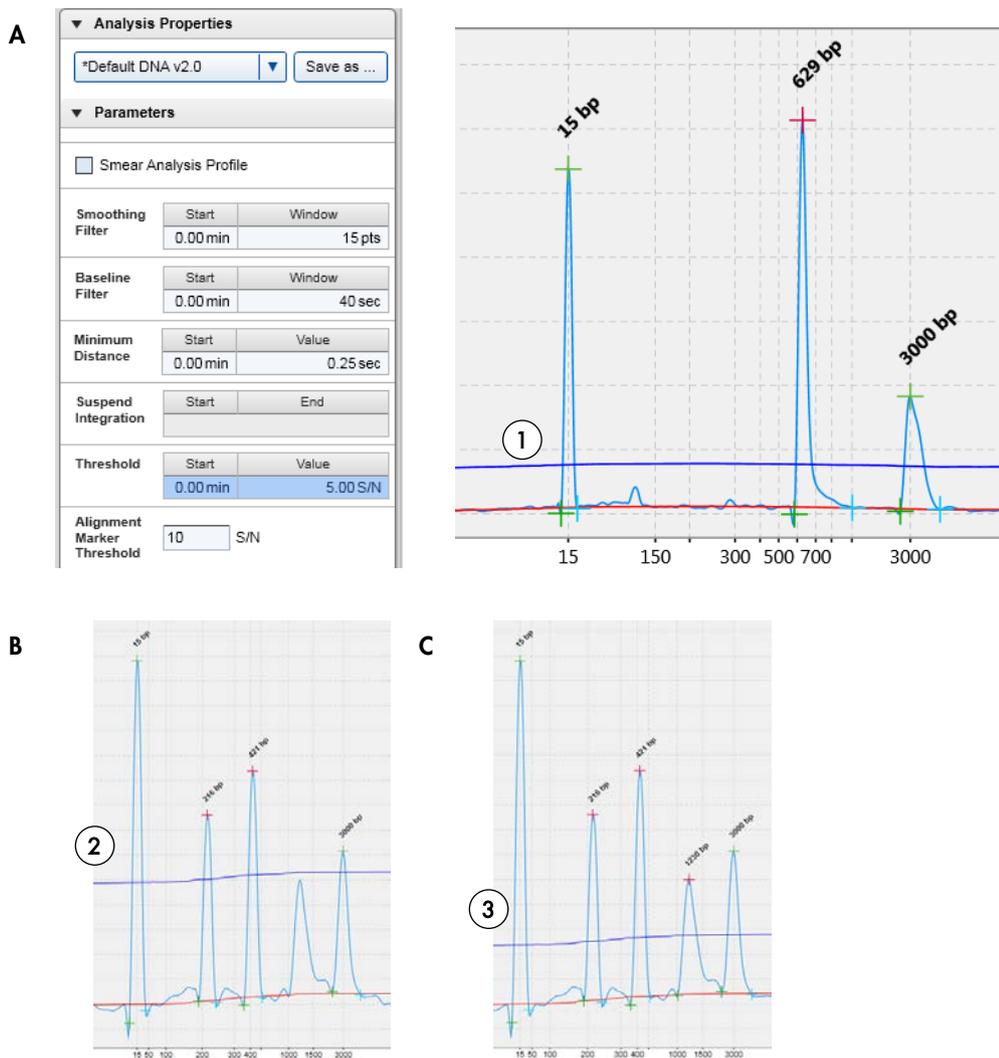
Done

Edit results Reactivity

Lane	Wells	Band Pattern
1	1-8	Band at 1, 2, 3, 4, 5, 6, 7, 8
2	9-16	Band at 9, 10, 11, 12, 13, 14, 15, 16
3	17-24	Band at 17, 18, 19, 20, 21, 22, 23, 24
4	25-32	Band at 25, 26, 27, 28, 29, 30, 31, 32
5	33-40	Band at 33, 34, 35, 36, 37, 38, 39, 40
6	41-48	Band at 41, 42, 43, 44, 45, 46, 47, 48
7	49-56	Band at 49, 50, 51, 52, 53, 54, 55, 56
8	57-64	Band at 57, 58, 59, 60, 61, 62, 63, 64
9	65-72	Band at 65, 66, 67, 68, 69, 70, 71, 72
10	73-80	Band at 73, 74, 75, 76, 77, 78, 79, 80
11	81-88	Band at 81, 82, 83, 84, 85, 86, 87, 88
12	89-96	Band at 89, 90, 91, 92, 93, 94, 95, 96

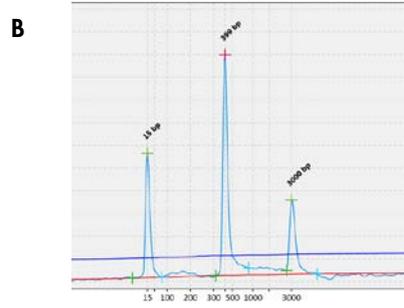
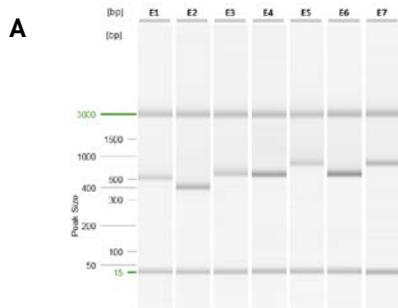
Analysis

A set threshold is applied for peak detection. Signals that exceed the threshold value, indicated with a blue line (1), are detected as peaks. The threshold is set to 0.14 RFU in the process profile **Olerup SSP complete**. This threshold can be increased (2) or decreased (3) manually under the **Analysis Properties (A)** if unspecific peaks appear and need to be excluded (B) or if weak bands appear below the threshold (C).



Report and Export

After the analysis, the ScreenGel software presents the results in a Report and/or Export file as a gel image (A), electropherogram (B) and tabulated data (C). Customize the Report or Export file to meet your requirements.



C

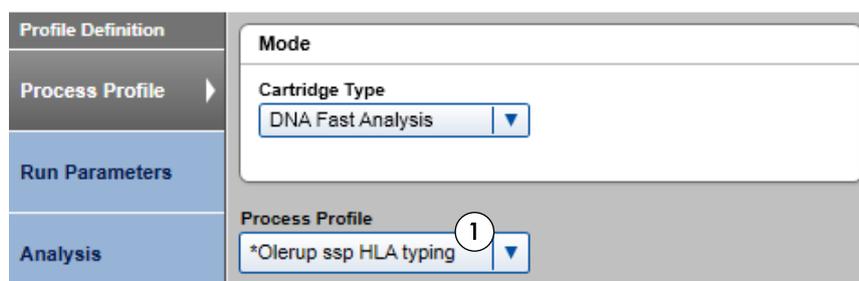
Sample Info: 17					Position: H3
#	Size [bp]	Conc. [ng/μl]	Mol. [nmol/l]	NA %	
1	15	n/a	n/a	n/a	
2	216	1.37	9.62	32.96	
3	421	1.63	5.86	37.18	
4	1230	1.34	1.65	29.86	
5	3000	n/a	n/a	n/a	

Preparing a Reference Marker Table

For subsequent runs with the same cartridge and the same protocol, a Reference Marker Table can be used instead of running a size marker in parallel with the samples.

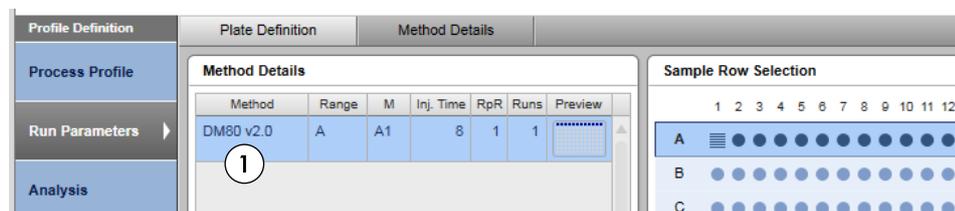
To prepare a Reference Marker Table, run the DNA size marker with the same protocol that will be used for the analysis of the samples.

1. Choose **Olerup SSP HLA typing** from the **Process Profile** drop-down menu.



The screenshot shows the software interface with the 'Process Profile' dropdown menu open. The selected option is '*Olerup ssp HLA typing', which is circled with a '1'. Other options visible include 'DNA Fast Analysis'.

2. Set up the run for the DNA size marker 50 bp – 1.5 kb with the **DM80 v2.0** method (1).

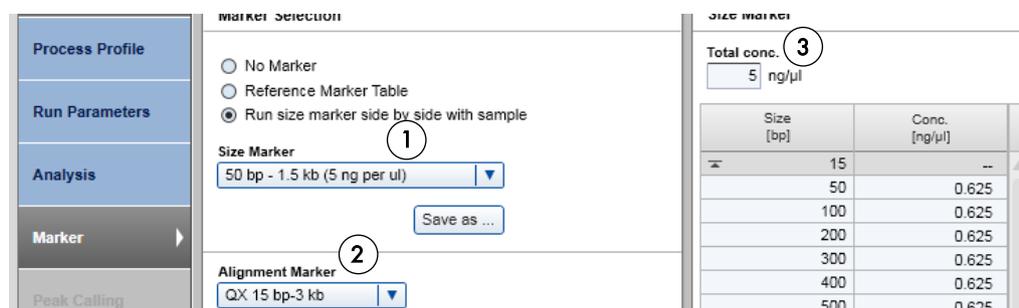


The screenshot shows the 'Method Details' table with the following data:

Method	Range	M	Inj. Time	RpR	Runs	Preview
DM80 v2.0	A	A1	8	1	1	

The 'DM80 v2.0' method is circled with a '1'. To the right, the 'Sample Row Selection' shows a grid of wells for rows A, B, and C, with wells 1-12 in each row.

3. Open the **Marker** dialog and select **Run size marker side by side with the sample**. Choose the corresponding size (1) and alignment marker (2) from the drop-down menus and make sure that the size marker concentration is set to 5 ng/μl (3).



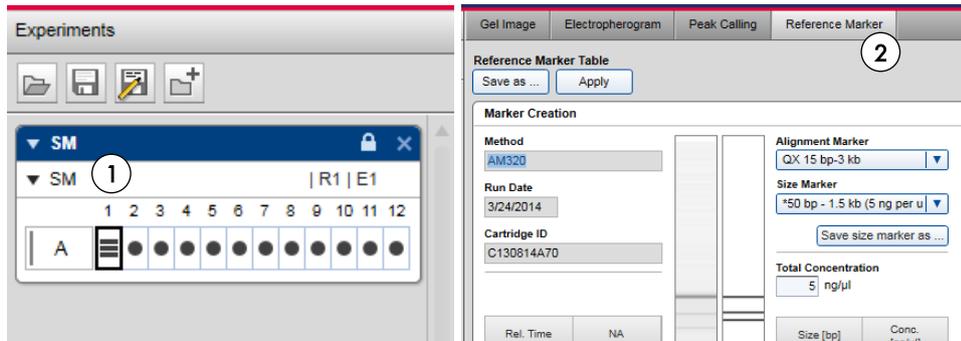
The screenshot shows the 'Marker Selection' dialog with the following settings:

- Run size marker side by side with sample
- Size Marker: 50 bp - 1.5 kb (5 ng per ul) (circled with 1)
- Alignment Marker: QX 15 bp-3 kb (circled with 2)

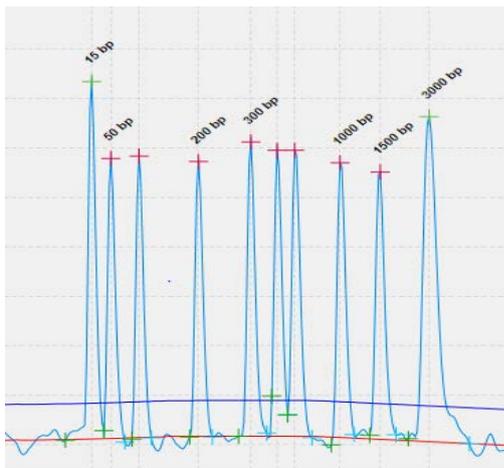
The 'Total conc.' is set to 5 ng/μl (circled with 3). A table of marker sizes and concentrations is shown on the right:

Size [bp]	Conc. [ng/μl]
15	--
50	0.625
100	0.625
200	0.625
300	0.625
400	0.625
500	0.625

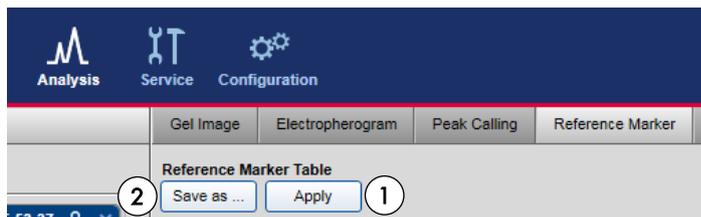
- Perform the run. Upon completion, enter the **Analysis** environment of the ScreenGel software. Select the size marker lane (1) and click **Reference Marker** (2).



- Open the size marker **Electropherogram** to ensure that all peaks were identified correctly.



- Return to the **Reference Marker** tab and click **Apply** (1) and then **Save as** (2).



7. Save the Reference Marker Table.

Reference Marker Table Name

DM80 v20_50bp-1500bp

OK Cancel

This saved Reference Marker Table can be used as long as cartridge and the method used for analysis of the samples is the same as those used to generate the Reference Marker Table.

Note: We recommend refreshing the Reference Marker Table every 2 months. Simply delete the Reference Marker Table file, run and analyze the size marker anew and save the results with the same name.

To find the Reference Marker Table file, click **File** in the Main Menu of the ScreenGel software and select **Open Data Directory** and then **Application data**. The file will be in the folder **Reference Marker Table**.

8. To use the saved Reference Marker Table in subsequent runs, select the corresponding option (1) in the **Marker Selection** dialog of the ScreenGel software and select the Reference Marker Table from the drop-down menu (2).

Profile Definition

Process Profile

Run Parameters

Analysis

Marker

Peak Calling

Report/Export

Start a Process

Sample Selection

Sample Information

Run Check

Save process profile as ... Run

Marker Selection

No Marker

Reference Marker Table (1)

Run size marker side by side with sample

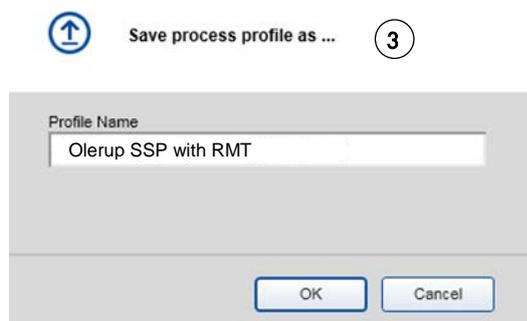
Reference Marker

DM80 v20_50bp-1500bp (2)

Reference Marker

Rel.	NA	Size [bp]	Conc. [ng/μl]
0.0000	--	15	--
0.0768	0.012876	50	0.40
0.1622	0.013473	100	0.40
0.2475	0.013830	150	0.40
0.3368	0.014193	200	0.40
0.4251	0.014566	250	0.40
0.5085	0.032069	300	1.00
0.6477	0.013873	400	0.40
0.7415	0.013896	500	0.40
0.7839	0.014090	600	0.40
0.8179	0.014180	700	0.40
0.8343	0.014399	800	0.40
1.0000	--	3000	--

9. To couple this Reference Marker Table with the process profile **Olerup SSP HLA typing**, change the Marker settings in the process profile. Choose **Reference Marker Table** instead of **Run size marker side by side with sample**, and save the process profile (3) with a new name, e.g., **Olerup SSP with RMT**.



As a result, 2 process profiles will be available:

- **Olerup SSP HLA typing** to be used when running the size marker.
- **Olerup SSP with RMT** to be used when running samples without a size marker and using the data saved as the Reference Marker Table.

10. After defining the folder **QIAxcel-SCORE** as the export directory (see "Export Settings", page 10), save the process profile as **Olerup SSP complete**.

References

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Ordering Information

Product	Contents	Cat. No.
QIAxcel DNA Fast Analysis Kit (3000)	QIAxcel DNA Fast Analysis Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, Alignment marker, DNA Size Marker, 12-Tube Strips	929008
QX Nitrogen Cylinder (6)	6 QIAxcel Nitrogen Cylinders	929705
Accessories		
QX 0.2 ml 12-Tube Strip (80)	80 x QX 0.2 ml 12-Tube Strips	929703
QX 0.2 ml Color 12-Tube Strip (80)	80 x QX Color 0.2 ml 12_Tube Strips	929704

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