User Guide

Qexp-MDx ADRB3 PPARG Lyophilized Genotyping Kit

Cat. C9051501-024-O

Research Use Only

Product Information

Contents and Storage

Table 1 ADRB3 PPARG Lyophilized Kit

Label	Component	Amount	Storage
		24 rxns	
Specimen Collection			
	Oral Swab with Collection Tube contains 3mL UTM[a]	24 Pcs	RT
Direct Lysis Assay			
PK	Proteinase K	1 Pc	-20℃
L2	DNA Lysis Buffer 2.0	4800 µL	-20℃
Multiplex PCR Assay			
	Direct PCR Lyo-PreMix-ADRB3 PPARG	3 Pcs (8rxns/Pc)	-20℃
PC	Positive Control-ADRB3 PPARG	300 µL	-20℃
W	Nuclease-Free Water	400 µL	-20℃

[a]Oral Swab with Collection Tube containing 3mL UTM (C9060001-024) should be purchased separately.

- All components will be shipped at ambient temperature. For long-term storage, they should be stored at -20℃.

Guidelines for Sample Collection for Oral Swab

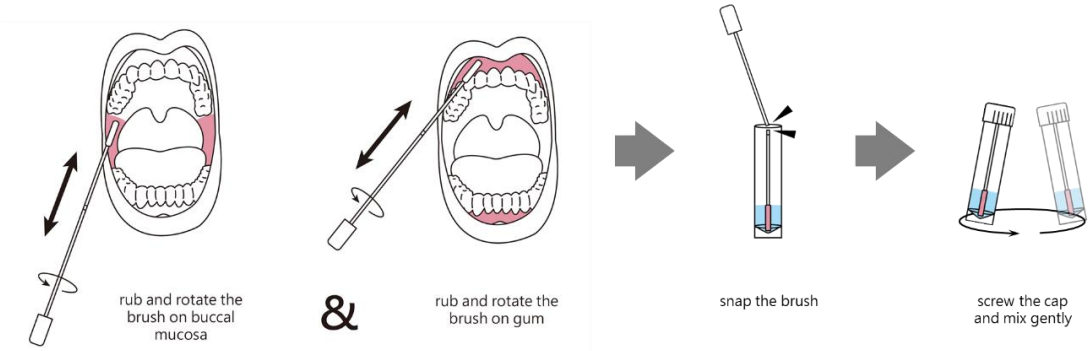
IMPORTANT

- Avoid drinking milk or having food rich in calcium before oral swab collection.
- Before sample collection, rinse the mouth with clean water for 10 seconds to remove microorganisms, food and drink remnants.
- Ensure that there is no eating, drinking, smoking, chewing gum, brushing teeth, or use of mouthwash after rinsing the mouth.

Oral specimen collection from the subject

1. Open the Specimen Collection Brush (Swab).
2. Force the brush a little bit on your buccal mucosa, then rub and rotate the brush on the buccal mucosa and gum 10 times on each side to collect cells.
3. Insert the brush into the supplied Collection Tube with UTM.
4. Snap the brush in the tube, then screw the cap of the Collection Tube.
5. Mix well gently to release the cells on the brush into UTM.

※ Can be stored at -20℃ for a week.



Perform Assay using $Qsep_{Ultra}$™ Otsuka

For the Installation, System Setup, Operation and Cartridge Verification of $Qsep_{Ultra}$, please refer to the operation manual (F0137_QsepUltra Otsuka User Manual) and pay attention to the safety information before use.

Instrument and Software

Table 2 Instrument and software compatibility

Instrument	*Q-Analyzer*™ for $Qsep_{Ultra}$
$Qsep_{Ultra}$™ Otsuka (C500001-O)	Version 1.1.1 or later

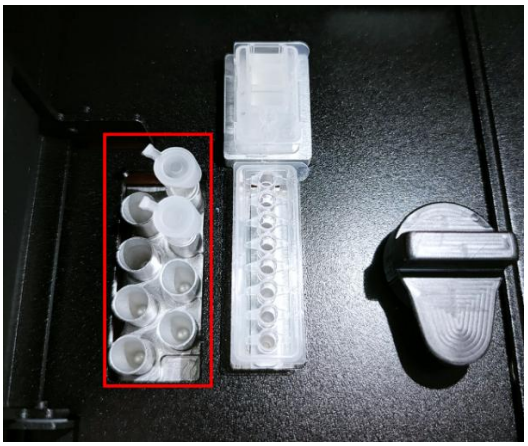
Proteinase K reconstitution

1. Spin down the Proteinase K、Nuclease-Free water.
2. Reconstitute the Proteinase K lyophilized cake with 100 µL Nuclease-free water.
3. Gently pipette and spin down Proteinase K.

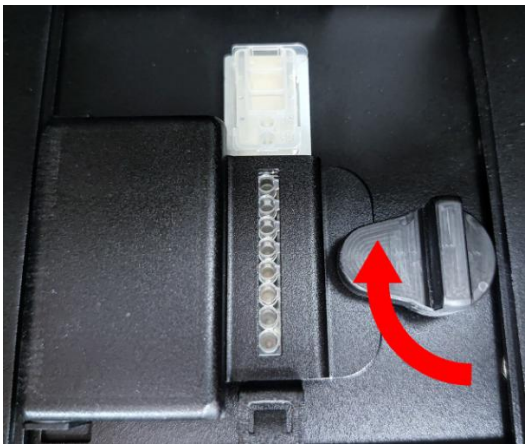
※ Stored at -20℃ after reconstitution.

Prepare oral specimen lysate

1. Gently invert the Collection Tube five times to ensure it mixes well.
2. Take 20 µL mixture from the Collection Tube into a clean PCR tube.
3. Add 2.6 µL Proteinase K, mix well, and incubate at room temperature for 1 min.
4. Set up samples in the well of 0.2 mL PCR tube (marked in red frame as follows).

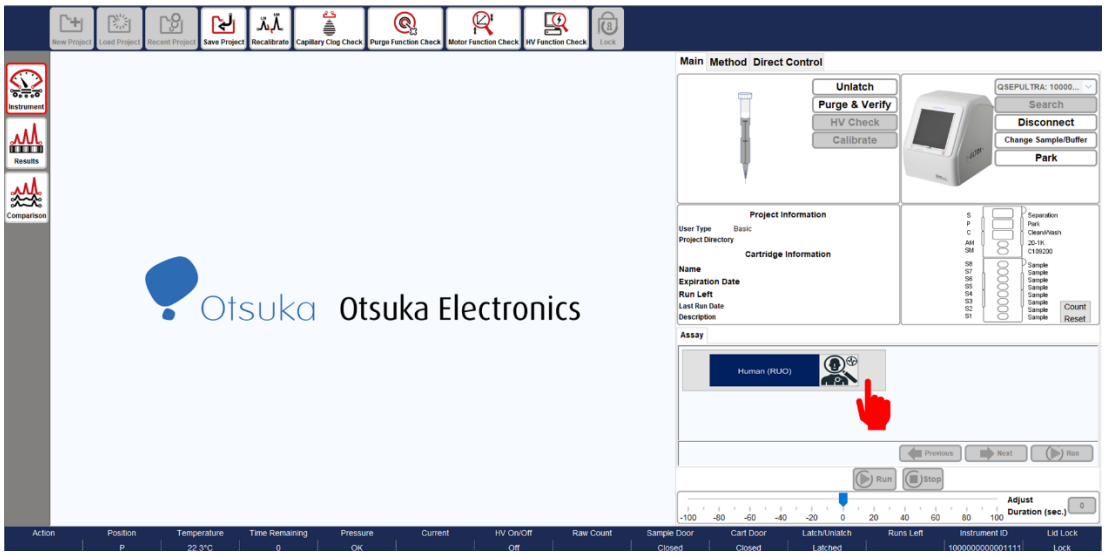


5. Close the lid and twist the lid lock clockwise to ensure the lid is fixed.

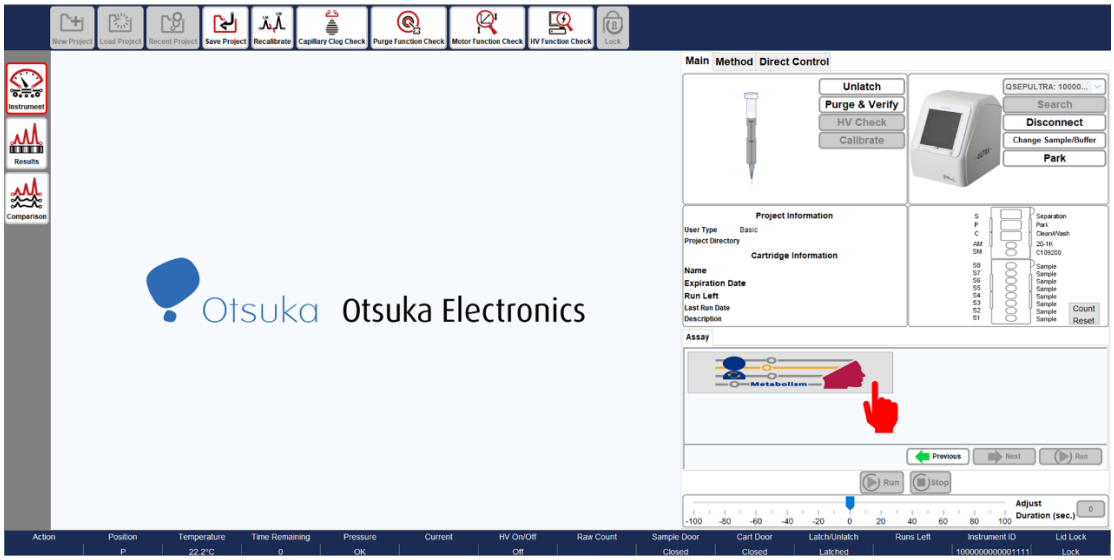


6. Close Sample Door and return to the software.

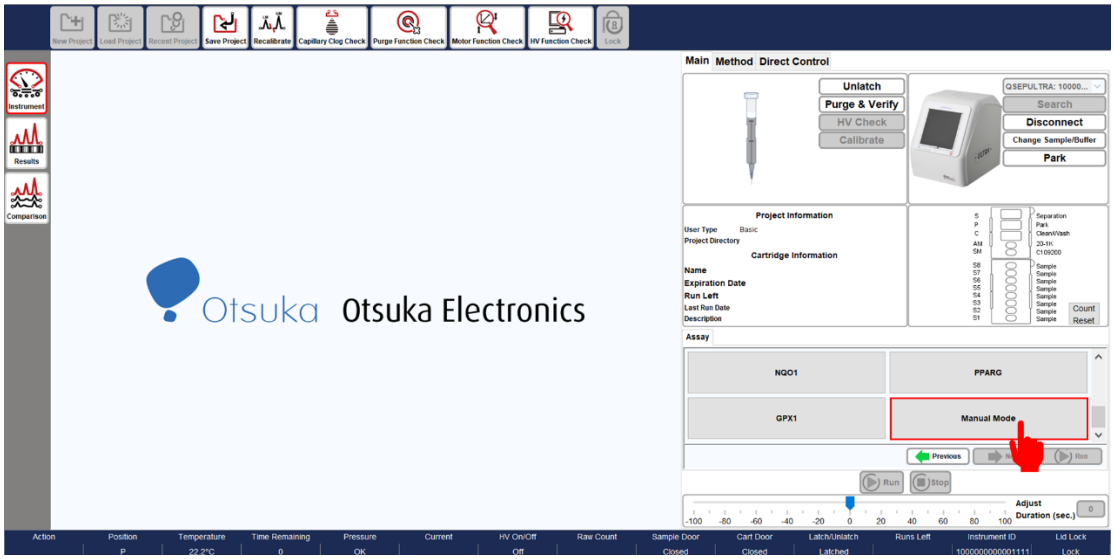
7. Click the icon **"Human (RUO)"**.



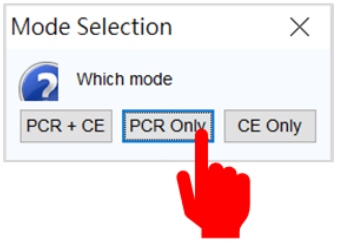
8. Click the icon **"Metabolism"**.



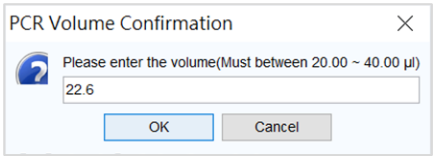
9. Click the icon **"Manual Mode"**.



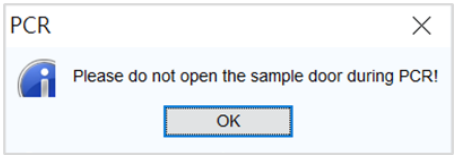
10. Click **"PCR Only"** mode.



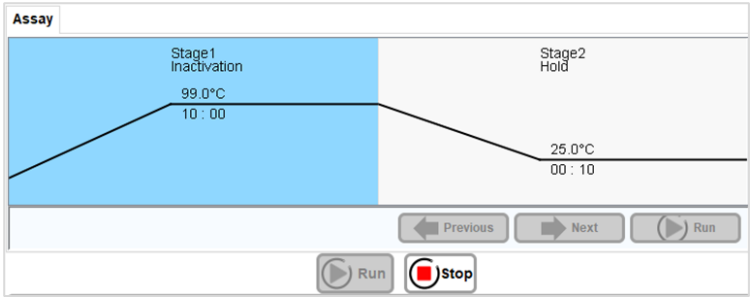
11. Choose the method **"Inactivation"** and open it.
12. Enter the sample volume of **22.6** and then click **"OK"**.



13. The system will mention **"Please do not open the sample door during PCR!"**. Click **"OK"** to start the Proteinase K inactivation.



14. Waiting for the heating process to complete. If there is any emergency while heating, please click **"Stop"** (the button with a red square) to shut down the program.



15. The system will "**beep**" twice when the inactivation step is completed. And the tube holder will move to the door automatically.
16. Click **"Change Sample/Buffer"** to take the inactivated samples out.
17. **Add 180 µL DNA Lysis Buffer** and mix well by gently vortexing or pipetting.
18. Incubate at room temperature for 10 minutes.

Prepare Direct PCR reaction

1. Reconstitute the lyophilized cake as the following table:

Table 3 The preparation list of all PCR reactions

Component	Volume per reaction		
	NTC	Sample reaction	Positive control
Direct PCR Lyo-PreMix-ADRB3 PPARG	1 tube	1 tube	1 tube
specimen lysate	-	25	-
Nuclease-free water	25	-	-
Positive Control-ADRB3 PPARG	-	-	25
TOTAL (µL)	25	25	25

※ Spin down the Lyo-PreMix first before opening.

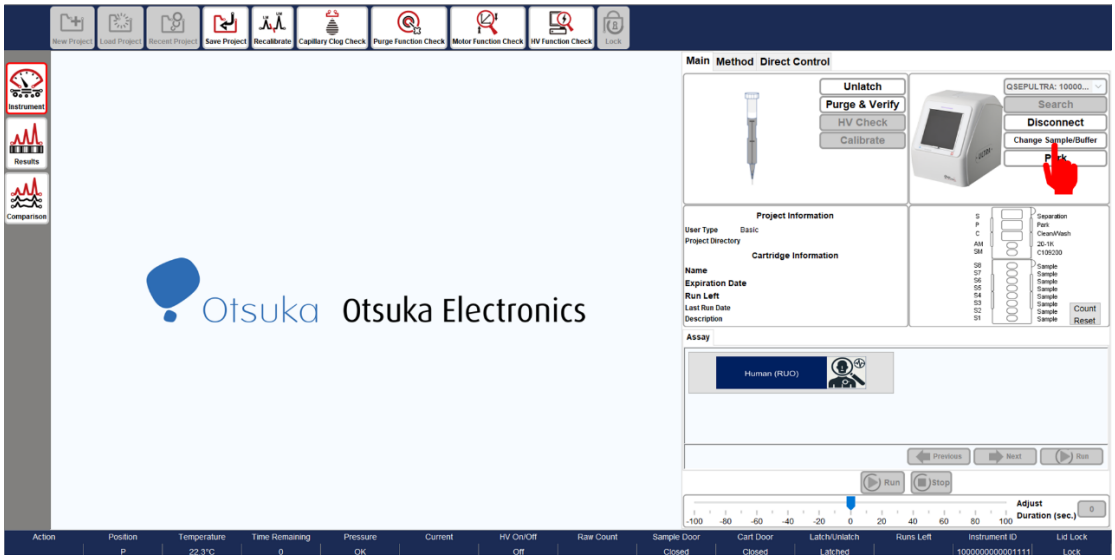
※ Do not use the same pipette to add water, specimen lysates, and Positive control.

※ Always prepare the no template control (NTC) first, and the positive control last to avoid cross-contamination.

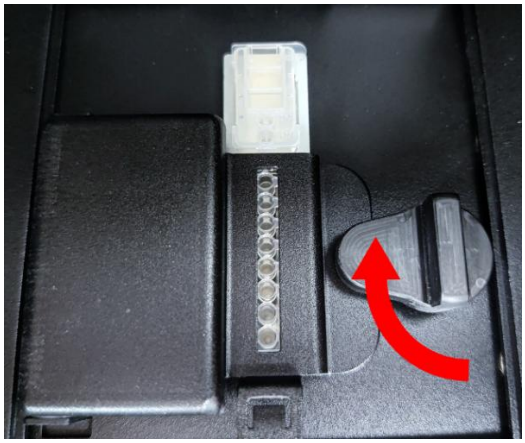
2. Pipetting and spin down (the lyophilisate will dissolve within seconds).
3. Transfer the samples to the $Qsep_{Ultra}$ 8-strip Sample Tube (C104258), then cover every tube with 10 µL mineral oil (provided with BiOptic's cartridge kit).
4. Briefly centrifuge the 8-strip Sample Tube to collect the liquid at the bottom of the tube.

Perform PCR and Analysis

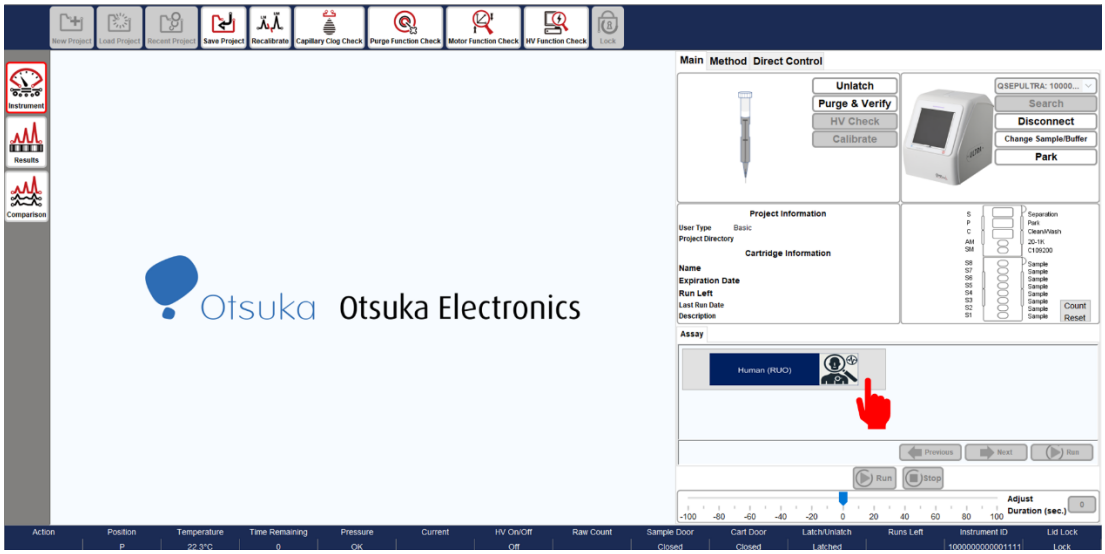
1. Click **"Change Sample/Buffer"** and place the prepared 8-strip Sample Tube (containing prepared 25 µL PCR reaction mix and covered with 10 µL mineral oil) on the tube holder.



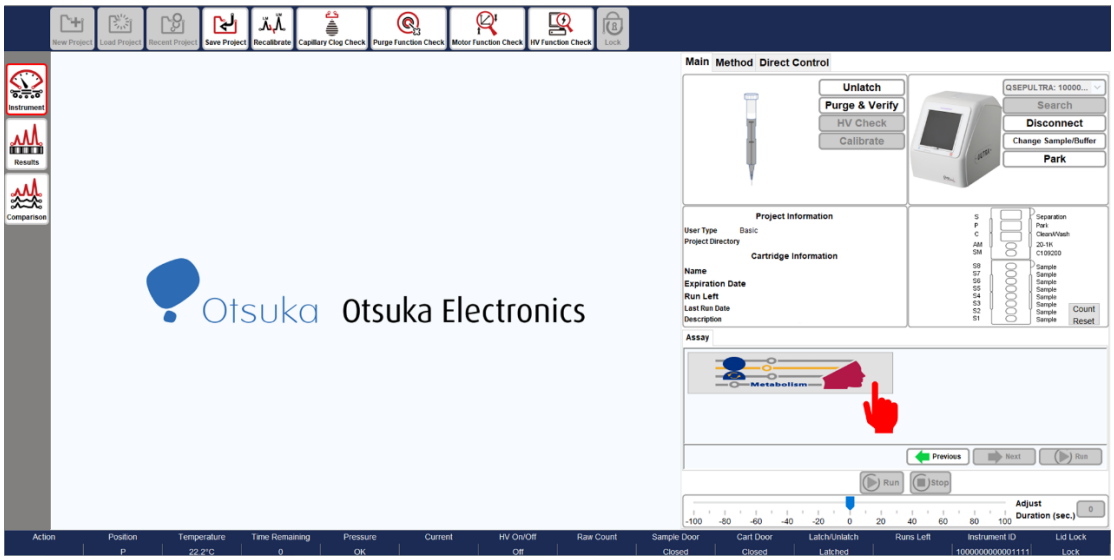
2. Close the lid and twist the lid lock clockwise to ensure the lid is fixed.



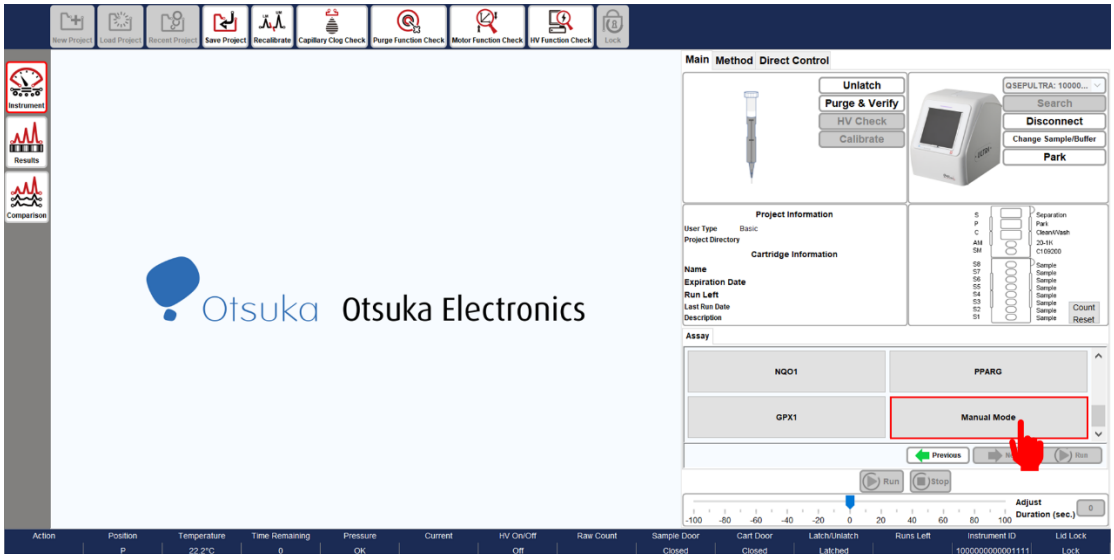
3. Click the icon **"Human (RUO)"**.



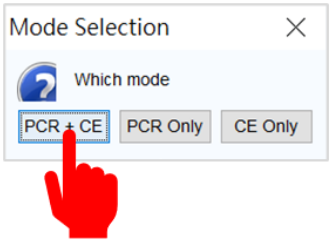
4. Click the icon **"Metabolism"**.



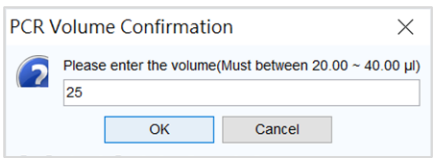
5. Click the icon **"Manual Mode"**.



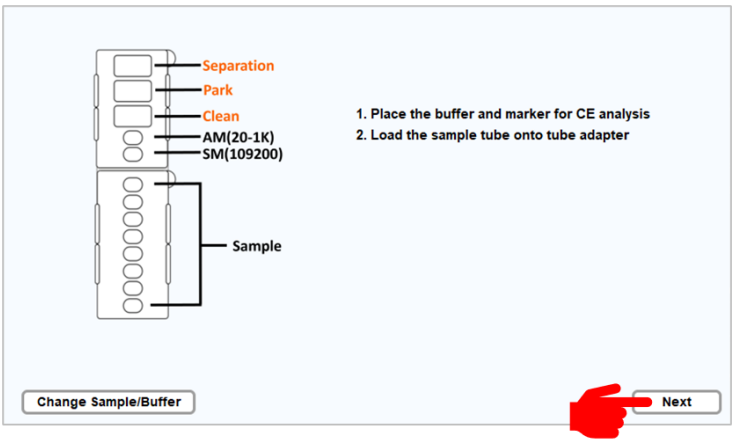
6. Click **"PCR+CE"** mode.



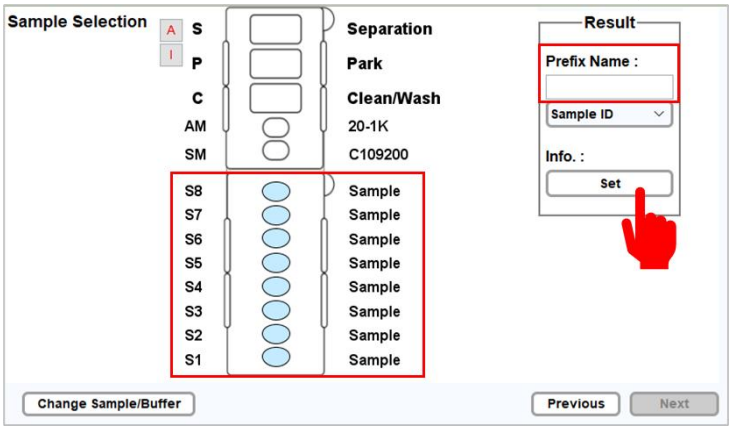
7. Choose the method **"ADRB3 PPARG Lyophilized Genotyping Kit"** and open it.
8. Enter the PCR volume of **25** and then click **"OK"**.



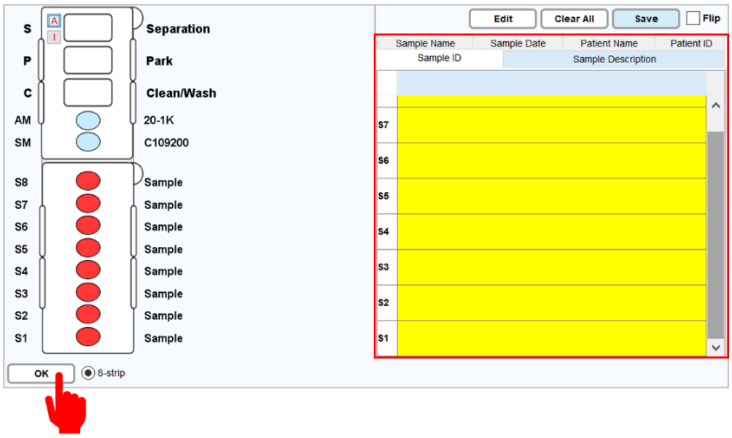
9. Click **"Next"**.



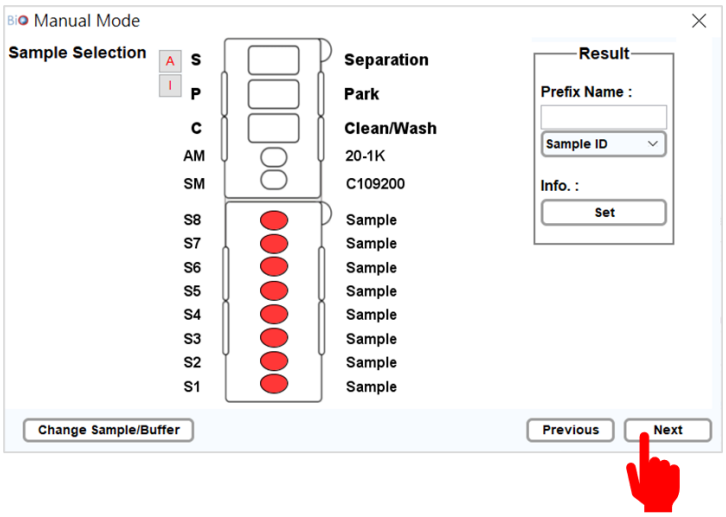
10. Select the sample position and input the result name in **"Prefix Name"** field. Then click **"Set"** to edit the information of the sample if necessary.



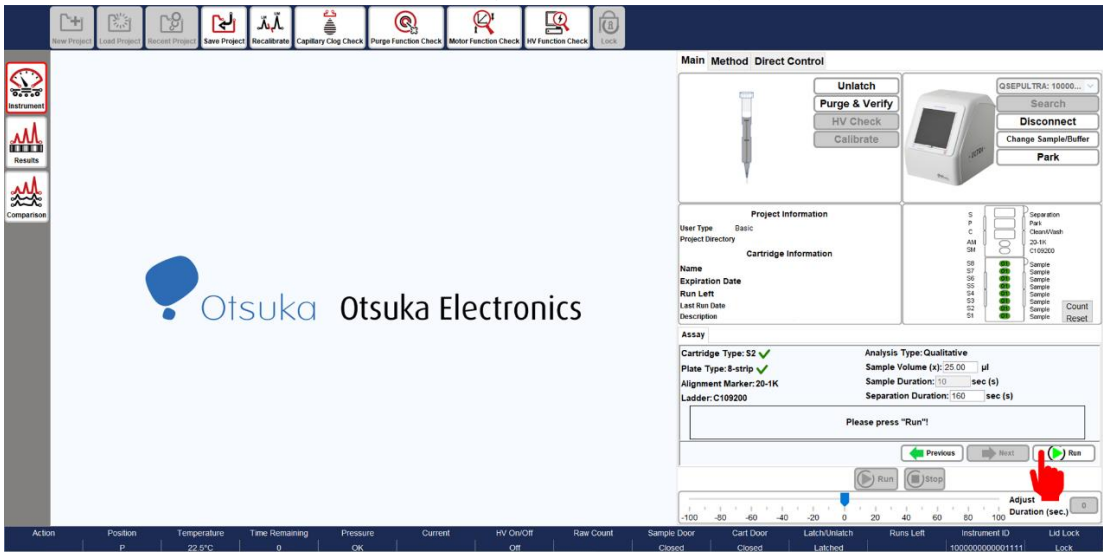
11. Select **"Sample Name"** or any options (Sample Date, Patient Name, etc.) you would like to record, then fill in the field and click **"OK"**.



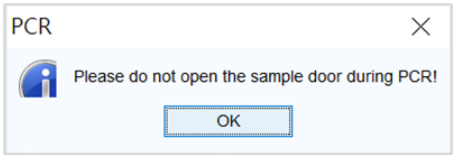
12. Click **"Next"** .



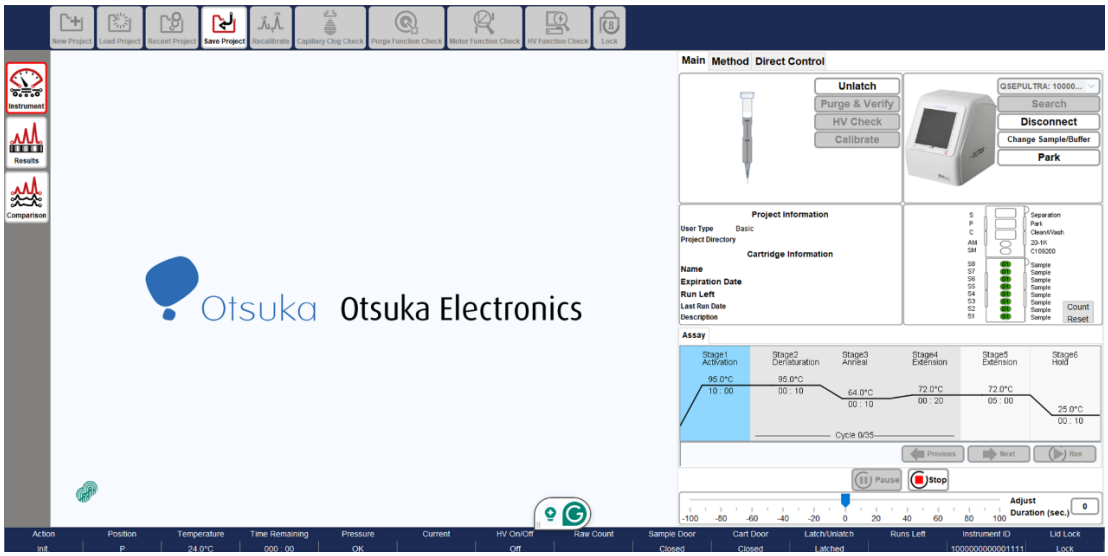
13. Click **"Run"** to start the PCR program and the analysis method.



14. The system will mention **"Please do not open the sample door during PCR!"** .
Click **"OK"** to start the PCR.



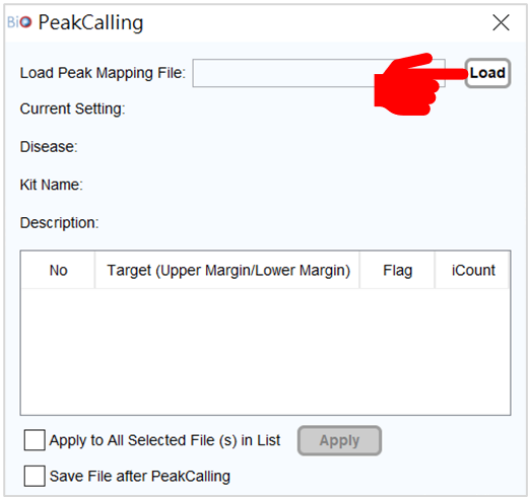
15. Waiting for the heating process to complete. If there is any emergency while heating, please click "**Stop**" (the button with a red square) to shut down the program.



Perform PeakCalling

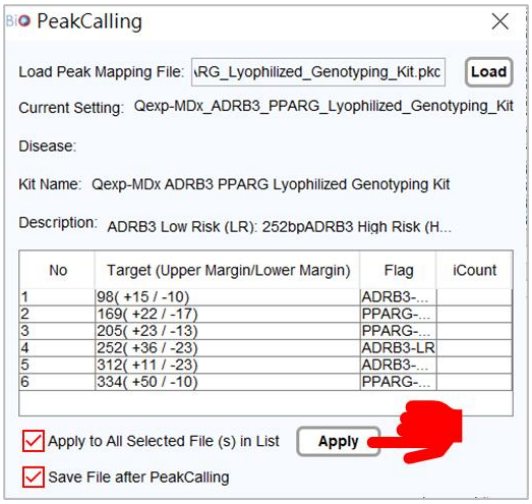
1. Open the results to do PeakCalling.
2. Select and check the results.
3. Click the icon "**PeakCalling**".

4. Click "**Load**".



5. Select "**Qexp-MDx_ADRB3_PPARG_Lyophilized Genotyping Kit**" and then open it.

6. Check the options **"Apply to All Selected File(s) in List"** & **"Save File after PeakCalling"**, and click **"Apply"**.



7. Go back to the results, and click the icon **"Show Size/Legend"**.

8. For sample quality confirmation and genotype determination, please refer to the table below.

Table 4 The principle for confirming sample quality and identifying genotype

Sample Quality		
ADRB3	The S/N value of the ADRB3-LR (252bp) or ADRB3-HR (98bp) should be higher than the criteria of S/N 150. If not, please collect samples again and retest the assay.	
PPARG	The S/N value of the PPARG-LR (169bp) or PPARG-HR (205bp) should be higher than the criteria of S/N 150. If not, please collect samples again and retest the assay.	
Genotype Determination		
ADRB3	Low Risk	There is only a peak of ADRB3-LR, or the S/N of ADRB3-LR is 6 times higher than that of ADRB3-HR.
	Medium Risk	The S/N ratio of ADRB3-LR and ADRB3-HR is below 6.
	High Risk	There is only a peak of ADRB3-HR, or the S/N of ADRB3-HR is 6 times higher than that of ADRB3-LR.
PPARG	Low Risk	There is only a peak of PPARG-LR, or the S/N of PPARG-LR is 6 times higher than that of PPARG-HR.
	Medium Risk	The S/N ratio of PPARG-LR and PPARG-HR is below 6.
	High Risk	There is only a peak of PPARG-HR, or the S/N of PPARG-HR is 6 times higher than that of PPARG-LR.

The results of quality control (NTC and Positive Control)

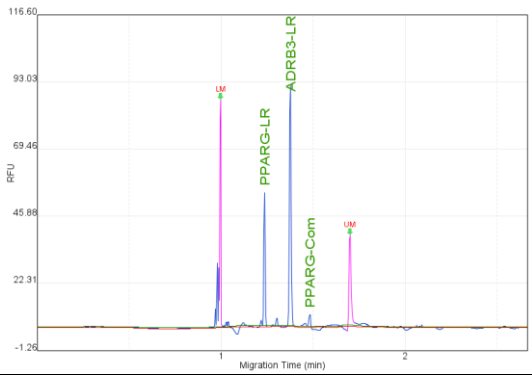
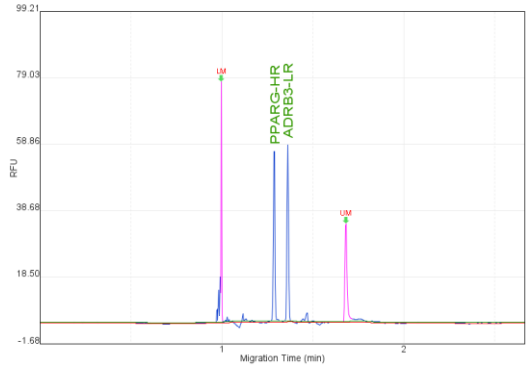
- Ensure that there is no signal of the target sequences revealed in the NTC.
  - If the NTC has any signal, the reagents might be contaminated in the procedure.
- Ensure that all signals of the target sequences are shown in the positive control.
  - If the positive control did not show any signal, it may be caused by the degradation of the reagents. Please contact the distributor in your area.

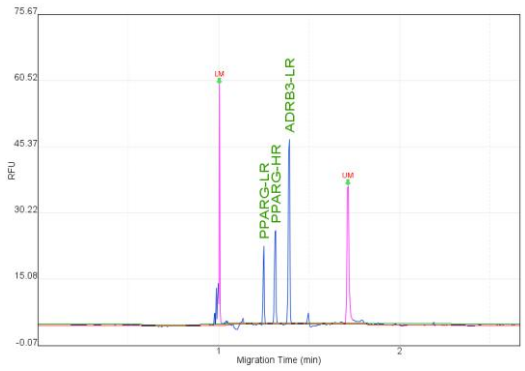
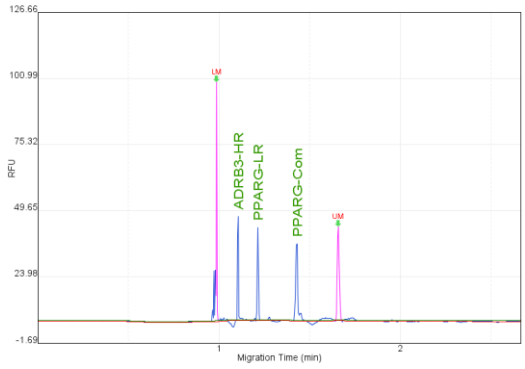
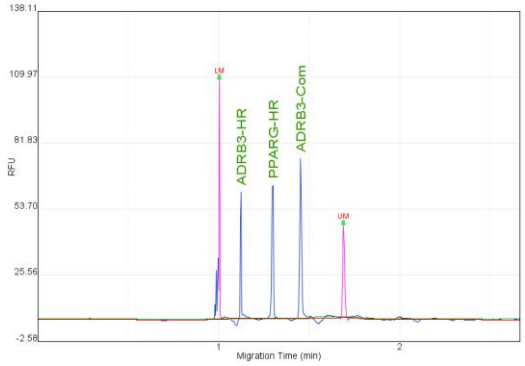
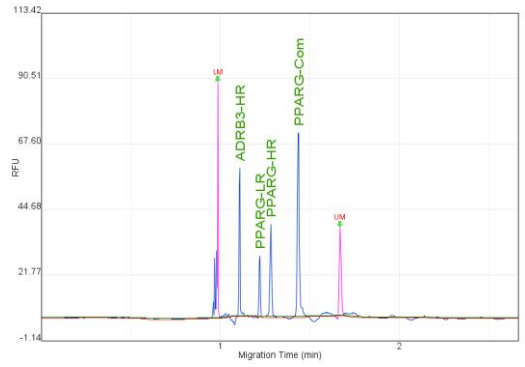
The result of Direct ADRB3 PPARG assay

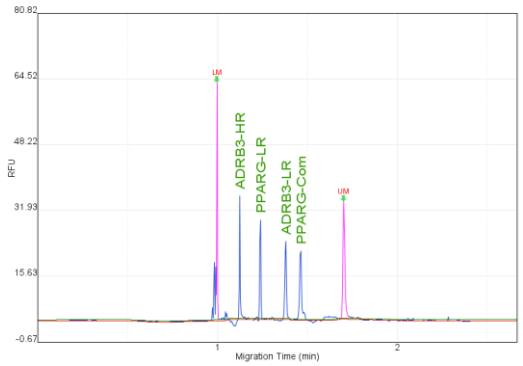
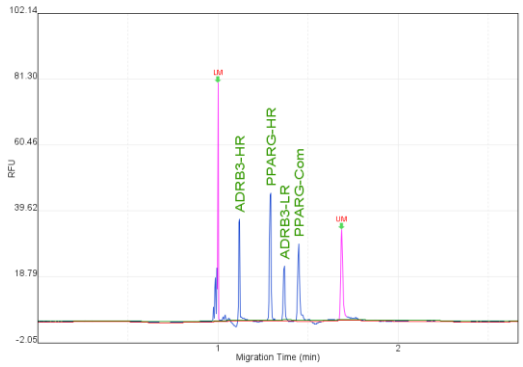
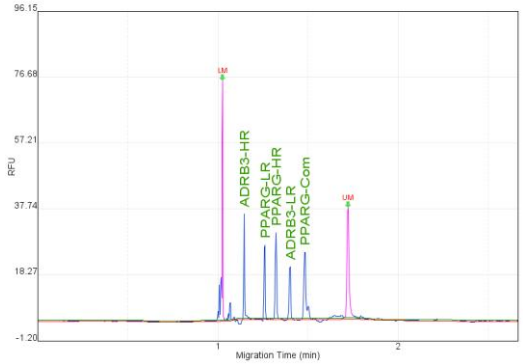
Table 3 The size of the target sequences

		Low Risk (LR)	High Risk (HR)	Common (Com)
Target size	ADRB3	252 bp	98 bp	312 bp
Target size	PPARG	169 bp	205 bp	334 bp

Table 7 The CE result and interpretation of Direct ADRB3 PPARG kit assay

Type	Result data
ADRB3/PPARG Low Risk/Low Risk	
ADRB3/PPARG Low Risk/High Risk	

ADRB3/PPARG Low Risk/Medium Risk	
ADRB3/PPARG High Risk/Low Risk	
ADRB3/PPARG High Risk/High Risk	
ADRB3/PPARG High Risk/Medium Risk	

ADRB3/PPARG Medium Risk/Low Risk	
ADRB3/PPARG Medium Risk/High Risk	
ADRB3/PPARG Medium Risk/Medium Risk