MAKING SCIENCE AFFORDABLE

PACE®/PACE 2.0 GENOTYPING MASTER MIX TRIAL KIT USER GUIDE

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CONTENT

1. PRODUCT DETAILS - PACE GENOTYPING MASTER MIX

PRODUCT	PRODUCT VOLUME	PART NUMBER	NUMBER OF REACTIONS AT 10 µL	CONCENTRATION
PACE® Genotyping Master Mix (**Standard ROX** – 150 nM)	2.5 mL	001-0001	500	2x
	25 mL	001-0002	5,000	2x
	250 mL	001-0003	50,000	2x
	1,000 mL	001-0004	200,000	2x
PACE® Genotyping Master Mix (**Low ROX** – 25 nM)	2.5 mL	001-0005	500	2x
	25 mL	001-0006	5,000	2x
	250 mL	001-0007	50,000	2x
	1,000 mL	001-0008	200,000	2x
PACE® Genotyping Master Mix (**High ROX** – 500 nM)	2.5 mL	001-0009	500	2x
	25 mL	001-0010	5,000	2x
	250 mL	001-0011	50,000	2x
	1,000 mL	001-0012	200,000	2x
PACE® Genotyping Master Mix (**no ROX**)	2.5 mL	001-0013	500	2x
	25 mL	001-0014	5,000	2x
	250 mL	001-0015	50,000	2x
	1,000 mL	001-0016	200,000	2x
PACE® Genotyping Master Mix (1x concentration, **Standard ROX** – 150 nM)	250 mL	001-0017	25,000	**1x**
	1,000 mL	001-0018	100,000	**1x**
	25 mL	001-0019	2,500	**1x**

PRODUCT DETAILS - PACE 2.0 GENOTYPING MASTER MIX

PRODUCT	PRODUCT VOLUME	PART NUMBER	NUMBER OF REACTIONS AT 10 µL	CONCENTRATION
PACE® 2.0 Genotyping Master Mix (**Standard ROX** – 150 nM)	2.5 mL	003-0001	500	2x
	25 mL	003-0002	5,000	2x
	250 mL	003-0003	50,000	2x
	1,000 mL	003-0004	200,000	2x
PACE® 2.0 Genotyping Master Mix (**Low ROX** – 25 nM)	2.5 mL	003-0005	500	2x
	25 mL	003-0006	5,000	2x
	250 mL	003-0007	50,000	2x
	1,000 mL	003-0008	200,000	2x
PACE® 2.0 Genotyping Master Mix (**High ROX** – 500 nM)	2.5 mL	003-0009	500	2x
	25 mL	003-0010	5,000	2x
	250 mL	003-0011	50,000	2x
	1,000 mL	003-0012	200,000	2x
PACE® 2.0 Genotyping Master Mix (**no ROX**)	2.5 mL	003-0013	500	2x
	25 mL	003-0014	5,000	2x
	250 mL	003-0015	50,000	2x
	1,000 mL	003-0016	200,000	2x
PACE® 2.0 Genotyping Master Mix (1x concentration, **Standard ROX** – 150 nM)	250 mL	003-0017	25,000	**1x**
	1,000 mL	003-0018	100,000	**1x**
	25 mL	003-0019	2,500	**1x**

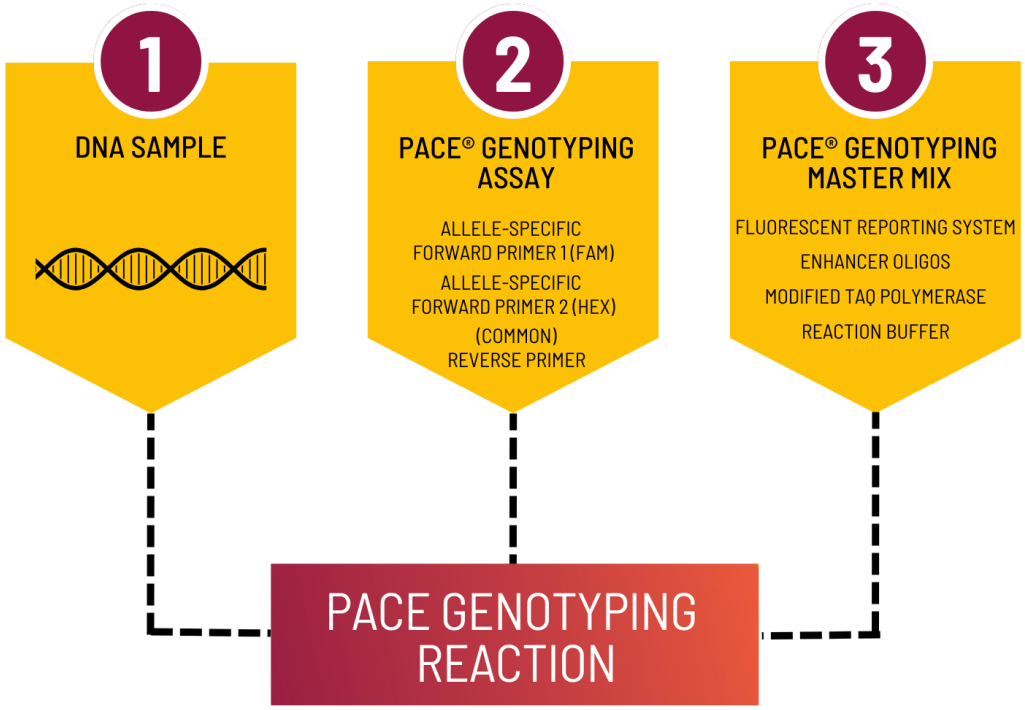
2. DESCRIPTION

PACE (**P**CR **A**llelic **C**ompetitive **E**xtension) genotyping chemistry is a homogeneous, PCR-based allele-specific technology for the analysis of Single Nucleotide Polymorphisms (SNPs) and insertion/deletions (Indels).

The PACE genotyping chemistry is comprised of two parts:

1. PACE Genotyping Assay: comprising two allele-specific forward primers and one common, reverse primer.
2. PACE Genotyping or PACE Genotyping 2.0 Master Mix: containing all components required for PCR and generation of fluorescent signals.

When combined with template DNA, these components create a PACE Genotyping Reaction.



3. ADDITIONAL BENEFITS OF PACE 2.0 GENOTYPING MASTER MIX

- Higher signal-to-noise, giving higher fluorescent values, enabling the user to see improved group separation during analysis.
- Inhibitor resistance enables this mix to be used with crudely extracted DNA samples as well as purified DNA samples.

4. STORAGE AND SHELF LIFE

PACE & PACE 2.0 Genotyping Master Mix is shipped on blue ice. Upon arrival, store at -20˚C/-80˚C (stable for two years); multiple freeze/thaw cycles are not recommended. PACE & PACE 2.0 Genotyping Master Mix can be aliquoted into light-protective tubes to reduce the need for repeated freeze-thaw cycles. The mix can also be stored at 4˚C for two weeks (protected from light).

5. SAFETY WARNINGS AND PRECAUTIONS

This product should only be handled by trained laboratory personnel. It is advisable to wear suitable personal protective equipment (PPE) when using the product. In case of contact with skin or eyes, wash immediately with water.

6. KIT COMPONENTS

1. **PACE or PACE 2.0 Genotyping Master Mix**: A single brown plastic tube containing 500 µL of PACE or PACE 2.0 Genotyping Master Mix (supplied at 2x concentration), with the relevant ROX normalisation dye already added (See section 7).

2. **Trial DNA samples**: Three colourless plastic tubes with coloured lids, each containing 50 µL of a known DNA sample at the correct working concentration (no need to dilute):
    - DNA 1 (Homozygous HEX/HEX, red lid)
    - DNA 2 (Heterozygous HEX/FAM, green lid)
    - DNA 3 (Homozygous FAM/FAM, blue lid)

3. **PACE Genotyping Assay**: A single 2D-barcoded assay tube with a yellow lid containing 20 µL of PACE Genotyping Assay
    - PACE Genotyping Assay (72x concentration) reporting with FAM and HEX

ADDITIONAL COMPONENTS REQUIRED

- Fluorescent plate reader or qPCR machine capable of reading the fluorophores in *Table 1*
- PCR plate or equivalent and appropriate optically clear seal
- PCR-grade water

FLUOROPHORE	EXCITATION (nM)	EMISSION (nM)
FAM	485	520
HEX	520	560
ROX *	580	610

Table 1. Excitation and Emission values for the fluorophores used in the PACE genotyping chemistry.

* Only required where appropriate. See section 7.

7. ROX COMPATIBILITY

In this trial kit, PACE/PACE 2.0 Genotyping Master Mix is supplied with a custom level of ROX normalisation dye (Standard, Low, High or No ROX) according to the requirements of your instrument. The ROX Instrument Compatibility List for PACE genotyping master mixes can be found here.

8. MECHANISM OF ACTION

A video explaining PACE genotyping chemistry mechanism of action can be found on our website www.3crbio.com/#pace

PACE genotyping chemistry uses a novel, universal, fluorescent reporting cassette to produce machine-readable fluorescent signals corresponding to genotypes. A PACE Genotyping Assay is comprised of two competitive allele-specific forward primers (which differ in their terminal 3′ bases and unique 5′ tail sequences) and a common, reverse primer. PACE and PACE 2.0 Genotyping Master Mix contains a quenched fluorescent reporting cassette for the fluorophores FAM and HEX.

When PCR is initiated, the allele-specific primers bind with their 3′ ends at the SNP of interest. Both allele-specific primers will bind if the SNP is heterozygous, whereas only one or other of the primers will bind if the SNP is homozygous. At the same time, the common reverse primer will bind on the opposite strand. As PCR proceeds, the tail sequences of allele-specific forward primers become incorporated into the amplicon and the corresponding tail sequence complement is generated. At this point the quenched, fluorescent reporting cassettes bind to their appropriate tail sequence complements, becoming unquenched and producing a light signal. If the genotype of the SNP is homozygous, only one of the possible fluorescent signals will be generated, whereas if the SNP is heterozygous, the result will be a mixed fluorescent signal. In this trial kit, samples representing the three possible genotypes are included for you to run and view example data (see Figure 1, Diagram of a typical genotyping cluster plot).

9. REACTION PROCEDURE

A. SUMMARY

- Dispense each of the DNA samples in triplicate into a PCR plate using suggested volumes in *Table 2*.
- In a separate tube, combine the recommended volume of PACE or PACE 2.0 Genotyping Master Mix and PACE Genotyping Assay as shown in *Table 3*.
- Mix well, then dispense the combined reaction mix into all 12 sample wells containing DNA samples plus no template controls. Spin, seal, then run the plate using the recommended thermal cycling conditions given in *Table 4*.
-Read the plate and compare data produced with the expected results.

	VOLUME PER WELL 96-WELL PLATE	VOLUME PER WELL 384-WELL PLATE	VOLUME PER WELL 1536-WELL PLATE
DNA 1 (red lid)	5 µL	2.5 µL	1.5 µL
DNA 2 (green lid)	5 µL	2.5 µL	1.5 µL
DNA 3 (blue lid)	5 µL	2.5 µL	1.5 µL
PCR-grade water (No template control)	5 µL	2.5 µL	1.5 µL

Table 2. Volume of DNA sample required per well. Each DNA sample should be pipetted in triplicate to give 12 reaction wells in total.

		96-WELL PLATE	384-WELL PLATE	1536-WELL PLATE
1	**PACE/PACE 2.0 Genotyping Master Mix**	65.0 µL	32.5 µL	13 µL
2	**PACE Genotyping Assay**	1.8 µL	0.9 µL	0.36 µL
	Total	66.8 µL	33.4 µL	13.36 µL
3	**Volume of this Reaction Mix added to each of the 12 test wells**	5.0 µL	2.5 µL	1 µL

Table 3. Volume of PACE/PACE 2.0 Genotyping Master Mix and PACE Genotyping Assay to be combined in a separate tube and added to each of the 12 test wells containing the DNA samples.

B. STEP-BY-STEP GUIDE

Step 1. Dispense each of the three trial DNA samples (DNA 1, 2 and 3) in triplicate onto a PCR plate using volumes given in *Table 2*.

Step 2. Combine appropriate volumes of PACE/PACE 2.0 Genotyping Master Mix with PACE Genotyping Assay in a tube, as detailed in *Table 3*, then mix.

Step 3. Dispense the combined mixtures into each of the wells containing DNA using volumes indicated in table. Each test now contains a complete PACE genotyping reaction.

Step 4. Seal the PCR plate with an optically clear seal and centrifuge to ensure all components are at the bottom of the wells.

Step 5. Thermally cycle the reaction plate using the thermal cycling conditions in *Table 4*.

STEP	DESCRIPTION	TEMP.	TIME	NO. CYCLES
1	Enzyme activation	94˚C	15 min	1
2	Template denaturation	94˚C	20 secs	10
	Annealing and extension	65-57˚C	60 secs (drop 0.8˚C per cycle)	
3	Denaturation	94˚C	20 secs	30
	Annealing and extension	57˚C	60 secs	

Table 4. Thermal cycling conditions for PACE/PACE 2.0 Genotyping Reactions.

C. FLUORESCENT SIGNAL DETECTION

After thermal cycling is complete, the fluorescent signal data should be collected using an appropriate fluorescent plate reader or qPCR machine in endpoint mode. *Table 5* details the optimal suggested wavelength and bandwidth settings for each filter to minimise crosstalk between the different filters.

FLUOROPHORE	EXCITATION (nM)	EMISSION (nM)
FAM	485 (10 nM)	520 (10 nM)
HEX	520 (10 nM)	560 (10 nM)
ROX	580 (10 nM)	610 (10 nM)

Table 5. Suggested excitation and emission filter settings (with bandwidth in brackets) for the fluorophores used in the PACE genotyping chemistry.

It is important that the fluorescent signal is read at or below 40˚C. If using a qPCR instrument, an additional temperature-controlled reading step should be included after the final PCR step or used separately to it. The temperature-controlled reading step should be used with the main PCR (*Table 5*). If using a fluorescent plate reader, the addition of this temperature-controlled reading step to the thermal cycling protocol should not be necessary as the PCR plate will have cooled sufficiently by the plate-reading stage.

D. INTERPRETATION OF DATA

The HEX and FAM fluorescence signal data produced by PACE Genotyping Reactions should be analysed and interpreted as cluster plots using cluster analysis software or with Microsoft Excel (see *Figure 1*). The ROX passive reference dye is included to eliminate the effect of well-to-well liquid volume differences from the resulting cluster plot data. This inclusion leads to tighter clustering and, as a result, more accurate scoring of data. When viewing the genotyping data, NTCs should show no amplification and remain around the origin of the cluster plot, giving confidence that any amplification observed is real.

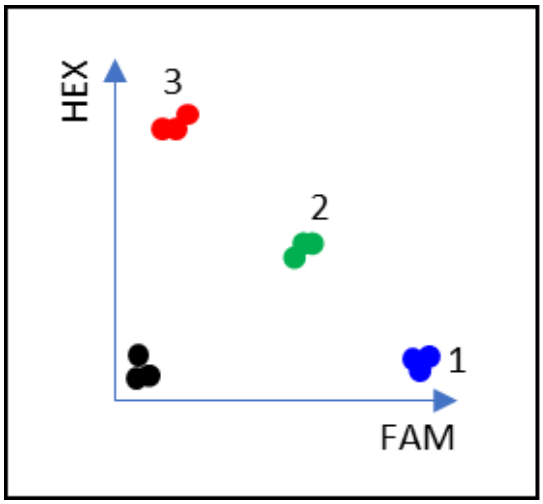


Figure 1. Diagram of typical genotyping cluster plot data generated from a PACE/PACE 2.0 Genotyping Reaction. Black samples at the origin are the no-template controls (NTCs).

10. ORDERING INFORMATION

For ordering details, please visit www.3crbio.com

11. SUPPORT

If you require any support with the use of PACE or PACE 2.0 Genotyping Master Mix or other 3CR Bioscience products, please contact our Technical Support team on support@3crbio.com.

12. LEGAL INFORMATION

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

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