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FCGR3A V158F HRM kit

MBK0039

50 Reactions (42 tests)

Store at -20°C

Intended use

For identification of FCGR3A V158F polymorphism, due to a T599G transversion (rs 396991), by High Resolution Melt (HRM).

Introduction

FCGR3A gene encodes a receptor for the Fc portion of immunoglobulin G, and it is involved in the removal of antigen-antibody complexes from the circulation, as well as other antibody-dependent responses. The receptor encoded by this gene is expressed on natural killer (NK) cells as an integral membrane glycoprotein anchored through a transmembrane peptide, mutations in this gene have been linked to susceptibility to recurrent viral infections, susceptibility to systemic lupus erythematosus, and alloimmune neonatal neutropenia.

Particularly the FCGR3A V158F variant is associated with susceptibility to rheumatoid arthritis¹, with the response rate to rituximab and freedom from progression in patients with follicular lymphoma², and finally with response to cetuximab in colorectal cancer patients³ (the F allele carriers was associated with longer progression-free survival).

Product description

The FCGR3A V158F HRM kit is an easy-to-use master mix dedicated for the use with the Rotor-gene 6000 instrument (Corbett Research) and Rotor-Gene Q (Qiagen). The kit contains reagent, enzyme and genotype controls for the detection of FCGR3A V158F SNP located in the of FCGR3A gene by HRM.

Kit contents

1 x FCGR3A V158F 2X master mix (650 µl)
1 x DNA polymerase (10 µl)
2 X FCGR3A V158F T/T CONTROL (10 µl)
2 X FCGR3A V158F T/G CONTROL (10 µl)
2 X FCGR3A V158F G/G CONTROL (10 µl)

Storage

The product should be stored at -20°C immediately upon arrival. When stored under the recommended conditions and handled correctly, the kit is stable up to the expiry date indicated in the attached label.

Note: The product is guaranteed for two thawing steps. For an intermittently use we suggest to subdivide Ile105Val 2xMaster mix in small aliquots.

Precautions

The user should always pay attention to:

- use pipette tips with aerosol-preventive filters, deionized DNA-free water and gloves;
- store positive material (plasmidic DNA controls) separately from all other reagents and, if possible, add it to the reaction mix in a separated space;
- do not use the same precision pipettes for reaction mix and DNA;
- thaw all components samples at room temperature before starting an assay;
- when thawed, mix the components and centrifuge briefly.
- Protect Ile105Val 2xMaster mix from light.

Procedure

PREPARING HRM GENOTYPIC CONTROLS

Genotype controls must be prepared by 1:10 dilution of stock solution in PCR -grade water (not provided).

Genotype controls are provided to characterize the HRM curves of unknown amplicons.

PCR SETUP

Total volume per reaction is 25 µl.

Before each use, thaw all reagents completely, mix and centrifuge.

Pipet mastermix and the Taq polymerase in the quantity needed for the planned reactions into a 1.5 ml reaction tube and mix as indicated in table 1.

Moreover include provided positive controls for each assay as references for genotype assignment.

Table 1

	1 reaction	50 reaction
PCR Mastermix 2X	12.5 µl	625 µl
Taq Diatheva (5U/µl)	0.125 µl	6,25 µl
H ₂ O	10,375	518,75
Total volume	23 µl	1150 µl

Aliquot 23 µl of master mix into each PCR reaction tube before adding 2 µl sample DNA*, or 2 µl of each provided positive controls (FCGR3A V158F T/T-T/G-G/G genotypes).

*Employ 10 ng DNA/reaction (2 µl of a 5ng/µl concentrated DNA).

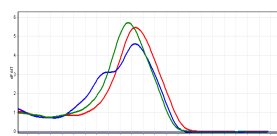
DNA extracted employing **QIAamp DNA Blood Mini Kit** and **DNeasy Blood & Tissue Kit** –QIAGEN can be used without quantitation step, simply diluting the sample 1:2 in water.

(DNA quality is a fundamental factor for HRM genotyping: kit has been optimized with DNA extracted by **DNeasy Blood & Tissue Kit** - **QIAamp DNA Blood Mini Kit** -QIAGEN and **Blood genomic DNA isolation mini Kit** – NORGEN).

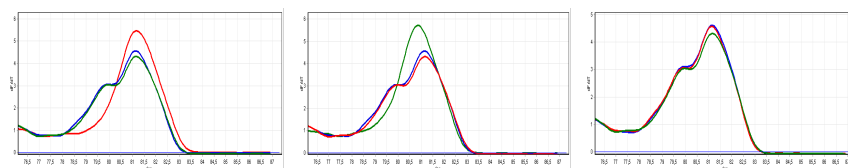
!! Attention !! Final elution step must be carried out using distilled water instead the elution buffer supplied with the respective kits.

Alternative isolation methods have to be validated from the user. In any case we suggest to elute purified DNA in water.

Negative controls (NTCs) are not required for this analysis since contaminations can be easily detected through positive controls curves behaviour: if the reaction is not contaminated positive controls generate 3 distinct curves, while if contaminated, controls generate two or three heterozygous curves (see the graphs below)



Example of not contaminated mix: the 3 positive controls generate 3 different curves.



Example of contaminated mix: the 3 positive controls generate 2 or 1 curves only.

THERMAL PROFILE

HRM analysis setting is a prerequisite for accurate results. For details, please refer to the manual provided with your HRM real-time PCR instrument.

Program the HRM instrument according to the operator's manual and table 2

Table 2: optimized cycling protocol for rotor-gene 6000

STEP	TEMPERATURE	TIME	CICLES
Initial denaturation	95°C	10 min	1X
Denaturation	95°C	10 sec	40 X
Annealing / Extension	59°C	40 sec	
Acquire on the GREEN Channel in the Annealing / Extension step			
High Resolution melting	Ramp 81°-91°C	Rise temp 0.2°C/step; wait for 90 sec pre-melt conditions; wait for 2 sec for each step afterwards; For optimal acquisition of fluorescence data, set the gain to	

		70% of saturation in the highest fluorescent signal.
Acquire on the HRM channel		

ANALYSIS SETUP

For HRM analysis set confidence level at 90%.

Due to the comparison between controls and unknown samples HRM profiles, the software instrument performs an automatic genotyping showing a confidence level for each sample. Results with a confidence level lower than 90% are defined "variation" and have to be reject.




Troubleshooting

No signal, poor Rn value (PCR or HRM) or signal detected late in PCR	Pipetting error or missing reagent	Check the storage conditions of the reagents, Repeat the assay.
	Problems with starting template DNA	Check the concentration, storage conditions, and quality of the template and control DNA ($\lambda_{260}/\lambda_{280}$ ratio of DNA samples must be over 1,7.) Efficient removal of PCR inhibitors is essential for optimal results; purify nucleic acids from your sample using an appropriate purification method. Insufficient or degraded template DNA, increase the amount of template DNA if possible.

References

- Lee YH, Ji JD, Song GG.. **Associations between FCGR3A polymorphisms and susceptibility to rheumatoid arthritis: a metaanalysis.** *J Rheumatol.* 2008 Nov;35(11):2129-35
- Weng WK, Levy R **Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma.** *J Clin Oncol.* 2003 Nov 1;21(21):3940-7.
- Zhang W, et al **FCGR2A and FCGR3A polymorphisms associated with clinical outcome of epidermal growth factor receptor expressing metastatic colorectal cancer patients treated with single-agent cetuximab.** *J Clin Oncol.* 2007 Aug 20;25(24):3712-8.

Example images for HRM analysis Images

 A-A
 A-C
 C-C

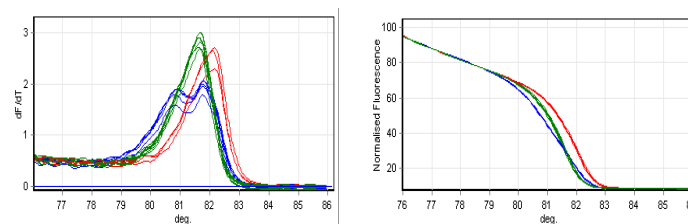


Fig. 1: Graphical visualizations for the identification of the MTHFR A1298C polymorphisms.
A) Melting curves show heteroduplexes formation in the A-C population (in blue) and temperature shift between A-A and C-C genotypes (green and red curves, respectively).
B) Normalized melting curves of the three genotypes.

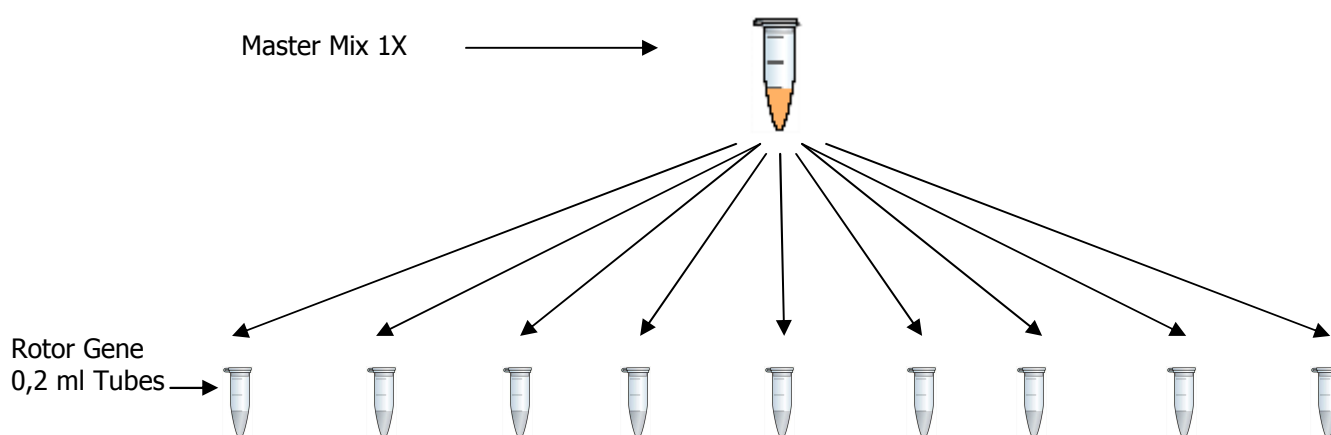
Example of experiment set-up for 6 unknown samples:

1) Prepare each positive controls and samples as described in procedure paragraph and keep them on ice.

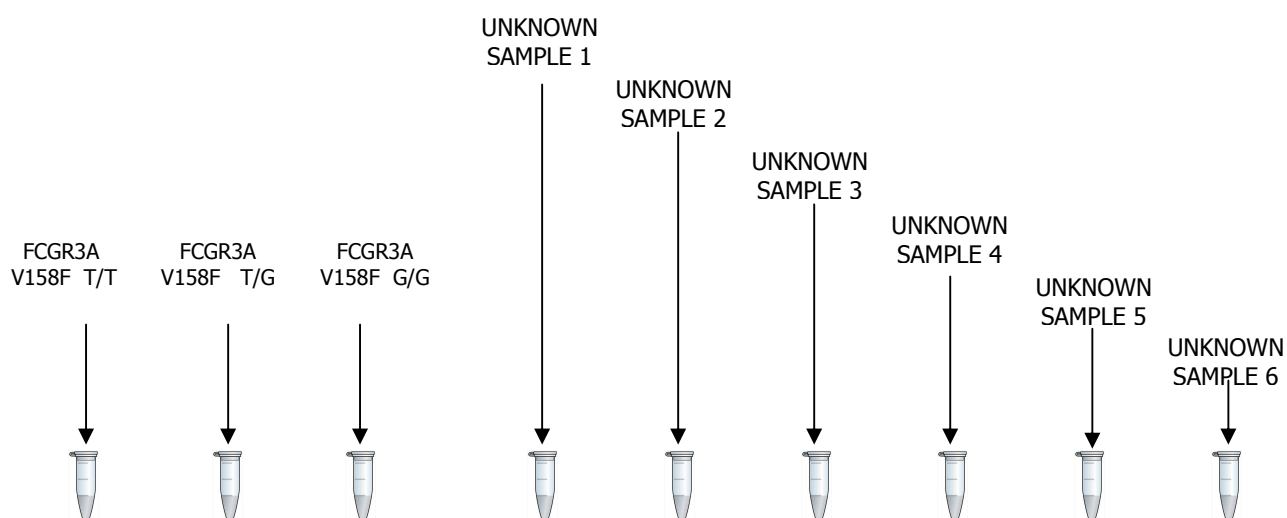
2) prepare Master Mix 1X for 10* reaction (the 3 positive controls + 6 unknown samples + 1):

	1 reaction	10 reaction
GSTP1 Ile105Val 2X PCR Master Mix	12.5 µl	1255 µl
DNA Polymerase (5U/µl)	0.125 µl	1,25 µl
H ₂ O	10.375	103,75 µl
Total volume	23 µl	230 µl

3) aliquot 23 µl in each rotor-gene 0,2 ml tubes.



4) Dispense in each PCR tubes 2 µl of positive controls or samples prepared in step 1.



5) Start thermal cycling as described in procedure paragraph.

*In order to eliminate pipetting errors we suggest to consider a reaction excess for every Master Mix constitution.