



# Instructions for use

## Imegen<sup>®</sup> Quimera dPCR (Dry)



Ref. IMG-116

Manufactured by:

**HEALTH IN CODE, S.L.**

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All the products marketed by Health in Code, S.L. undergo rigorous quality control. The **Imegen® Quimera dPCR** kit has passed all internal validation tests, which guarantee the reliability and reproducibility of each batch manufactured.

For any questions about the applications of this product or the protocols thereof, please contact our Technical Department:



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Modifications to the instructions for use (IFU)		
Version 09	MAR 2025	Reference added in Section 1.
Version 08	MAY 2024	Update to the contents of Sections 6 and 7.
Version 07	DEC 2023	Review and update of section "3. Technical characteristics".
Version 06	JUN 2023	Change of the kit name throughout the document (from Imegen® Quimera dPCR to Imegen® Quimera dPCR Dry). The reagent has been renamed "Marker Master Mix" instead of "Polymorphism Master Mix". Modification of section 7.1. Preparation of reagents. The rehydration volume has been updated according to the equipment used. Section "11. Performance characteristics" has been added. Change of manufacturer's address: Health in Code S.L., Calle de la Travesía s/n, 15E Base 5, Valencia 46024, Spain.
Version 05	MAY 2022	Change of manufacturer's identification: from Imegen to HEALTH IN CODE, S.L.
Version 04	FEB 2019	Format edition

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# 01 General information

The analysis of molecular chimerism resulting from allogeneic transplantation is now a well-established method for transplant follow-up, as it provides precise, valuable information to orient post-transplant treatment or intervention, with the aim of anticipating possible risks of relapse, rejection or graft-versus-host disease. The approach is extremely useful not only to determine the risk of relapse, rejection or graft-versus-host disease, but also to assess the response to different forms of treatment.

The entire **Imegen® Quimera** kit family has been developed in conjunction with the Málaga Regional University Hospital within the Andalusian Health Service (SAS). As a result of this agreement, Health in Code, S.L. has an **exclusive, worldwide license** for the know-how of the products for their manufacture and exploitation.

## References

- > Jiménez-Velasco A, Barrios M, Román-Gómez J, Navarro G, Buño I, Castillejo J, et al. Reliable quantification of hematopoietic chimerism after allogeneic transplantation for acute leukemia using amplification by real-time PCR of null alleles and insertion/deletion polymorphisms. *Leukemia*. 2005 Mar; 19(3):336-43. Doi: 10.1038/sj.leu.2403622. PMID: 15674363.
- > Stahl T, Böhm M, Kröger N, Fehse B. Digital PCR to assess hematopoietic chimerism after allogeneic stem cell transplantation. *Experimental Hematology*. 2015 Jun; 43(6): 462-8.e1. doi: 10.1016-j.exphem.2015.02.006. Epub 2015 Mar 18. PMID: 25795523.
- > Valero-García J, González-Espinosa MdC, Barrios M, Carmona-Antoñanzas G, García-Planells J, Ruiz-Lafora C, et al. (2019) Earlier relapse detection after allogeneic haematopoietic stem cell transplantation by chimerism assays: Digital PCR versus quantitative real-time PCR of insertion/deletion polymorphisms. *PLoS ONE* 14(2): e0212708. <https://doi.org/10.1371/journal.pone.0212708>

➔ Hematopoietic chimerism analysis procedure:

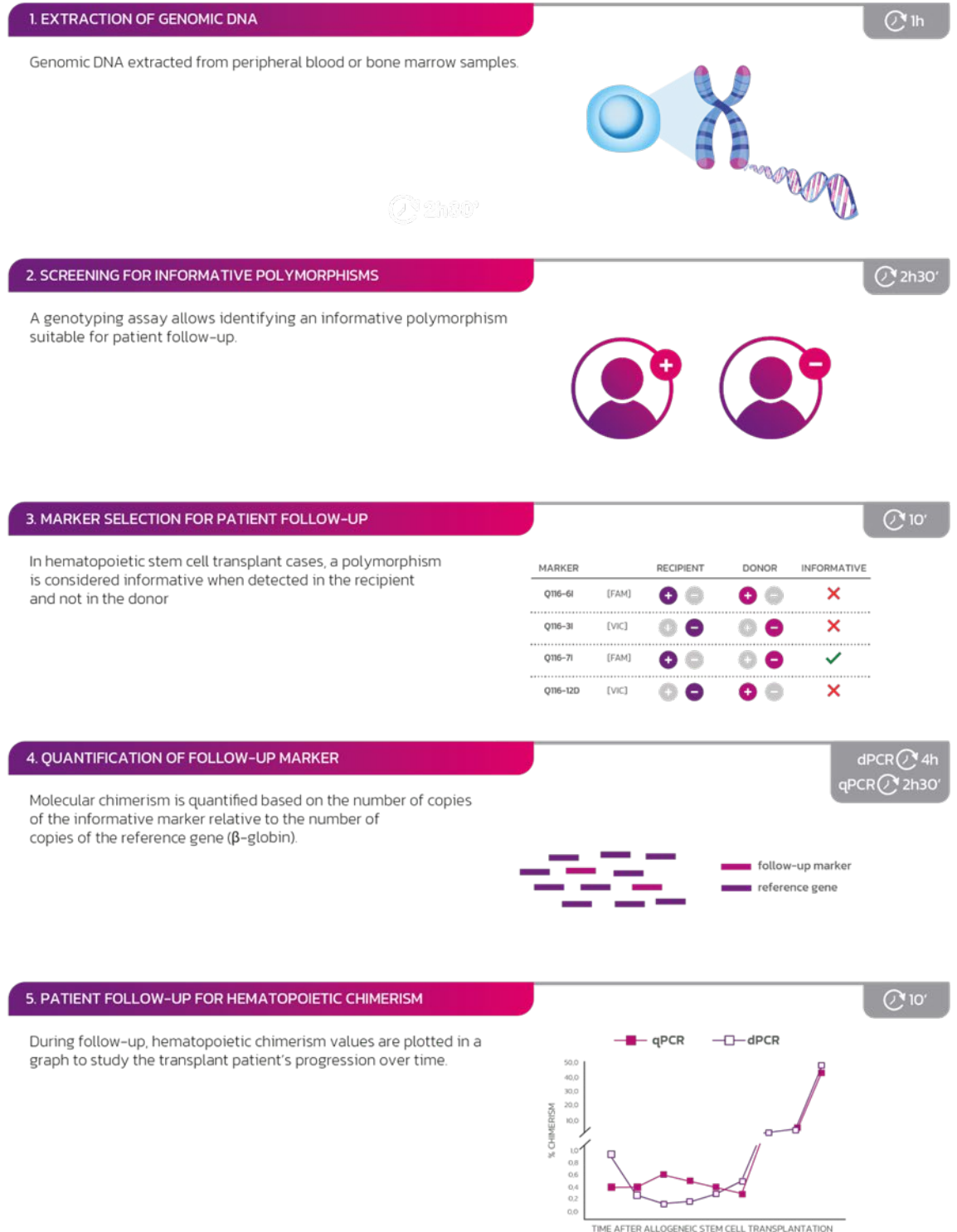


Figure 1. Hematopoietic chimerism analysis procedure

## 02 Intended use

The **Imegen® Quimera dPCR Dry** kit is designed to monitor chimerism in peripheral blood and/or bone marrow after an allogeneic transplant of hematopoietic stem cells.

The assay is based on prior screening of informative polymorphisms/markers, including INDELs (insertions/deletions) and null alleles, which are used to quantify the molecular chimerism as the absolute amount of the informative marker or as the relative amount of the informative marker in relation to the total amount of genomic DNA in the sample (multiplex PCR). This quantification requires analysis of the reference gene ( $\beta$ -globin), which in turn serves as a positive control for amplification.

It is therefore essential to first select the informative polymorphisms in each transplant case. To do this, Health in Code, S.L has validated two references: **Imegen® Quimera Screening Multiplex Plus (IMG-116-26)** and **Imegen® Quimera Screening Multiplex Plus II (IMG-116-25)**. A polymorphism is considered to be informative when it is detected in the transplant recipient and not in the donor.

Once an informative marker has been selected, it can be used for transplant patient follow-up. A total of 33 INDELs have been developed for this purpose, as shown in Table 1.

Markers	Insertion	Deletion	Kit reference
SRY	X		IMG-116-27
RhD	X		IMG-116-28
Q116-6I	X		IMG-116-32
Q116-3I	X		IMG-116-29
Q116-7I	X		IMG-116-33
Q116-12D		X	IMG-116-36
Q116-11I	X		IMG-116-34
Q116-5I	X		IMG-116-31
Q116-4I	X		IMG-116-30
Q116-10I	X		IMG-116-35
Q116-23I	X		IMG-116-37
Q116-28I	X		IMG-116-42
Q116-32I	X		IMG-116-46
Q116-31I	X		IMG-116-45
Q116-30D		X	IMG-116-44
Q116-29D		X	IMG-116-43
Q116-27D		X	IMG-116-41

Markers	Insertion	Deletion	Kit reference
Q116-24I	X		IMG-116-38
Q116-9I	X		IMG-116-48
Q116-20I	X		IMG-116-40
Q116-33I	X		IMG-116-47
Q116-37I	X		IMG-116-49
Q116-38I	X		IMG-116-50
Q116-39I	X		IMG-116-51
Q116-41I	X		IMG-116-52
Q116-42I	X		IMG-116-53
Q116-43I	X		IMG-116-54
Q116-44I	X		IMG-116-55
Q116-45I	X		IMG-116-56
Q116-46I	X		IMG-116-60
Q116-47I	X		IMG-116-57
Q116-49I	X		IMG-116-58
Q116-50I	X		IMG-116-59

Table 1. Imegen® Quimera dPCR Dry kit references.

These instructions for use are suitable for the analysis of any of the 33 markers in Table 1, as they function optimally under the same PCR conditions. Thus, this technique facilitates rapid, effective and simultaneous analysis of multiple polymorphisms.

The Imegen® Quimera dPCR Dry kit is designed for healthcare professionals and researchers, trained in molecular biology techniques in general and in performing digital PCR in particular, who need to differentiate and quantify donor cell populations derived from mixed human DNA samples and post-transplant samples.

## 03 Technical characteristics

**Imegen® Quimera dPCR Dry** kit consists of an assay that facilitates digital PCR quantification of the number of copies of an informative marker for the follow-up of hematopoietic chimerism. This kit is used in combination with specific primers and fluorescent hydrolysis probes to quantify the absolute amount of an informative marker or the relative amount in relation to the amount of the reference gene,  $\beta$ -globin.

The type of sample used in validating the **Imegen® Quimera dPCR Dry** kits is genomic DNA extracted from peripheral blood or bone marrow samples from patients who have undergone allogeneic stem cell transplantation. The technical specifications of the kit are given below:

- ◇ **Type of sample:** Genomic DNA from peripheral blood, bone marrow and cell subtypes.
- ◇ **Quantity of DNA:** 75 ng (Bio-Rad) and 150 ng (ThermoFisher Scientific)
- ◇ **Limit of quantitation:** 0.05%
- ◇ **Number of reactions per sample:** 1
- ◇ **Number of targets:** 2
- ◇ **Manual working time:** 15 min – 1 h 30 min
- ◇ **dPCR program duration:** 1 h 30 min – 3 h
- ◇ **dPCR platforms:**
  - ↳ QuantStudio™ 3D Digital PCR System (ThermoFisher Scientific)
  - ↳ QuantStudio™ Absolute Q™ PCR System (ThermoFisher Scientific)
  - ↳ QX200™ Droplet Digital™ PCR System (Bio-Rad)

## 04 Safety warnings and precautions

- ◇ We recommend strictly following the instructions in this manual, especially regarding the handling and storage conditions for the reagents.
- ◇ Do not pipette by mouth.
- ◇ Do not smoke, eat, drink or apply cosmetics in the areas where kits and samples are handled.
- ◇ Any skin conditions, as well as cuts, abrasions and other skin lesions should be properly protected.
- ◇ Do not pour reagent residues into the drinking water system. It is recommended to use the waste containers set out by the legal regulations and to manage them via an authorized waste manager.
- ◇ In the case of accidental spillage of any of the reagents, avoid contact with skin, eyes and mucous membranes and clean with plenty of water.
- ◇ Material safety data sheets (MSDS) for all hazardous components contained in this kit are available upon request.
- ◇ This product requires the handling of samples and materials of human origin. We recommend all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Biosafety Level 2 standard for bloodborne pathogens or other relevant biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- ◇ The reagents included in this kit are not toxic, explosive, infectious, radioactive, magnetic or corrosive and do not cause biological environmental contamination.
- ◇ This kit has been validated with specific equipment and under specific conditions that may vary significantly in other laboratories. It is therefore recommended that each laboratory perform an internal validation when using the kit for the first time.
- ◇ The manufacturer is not liable for the assay not working properly when the reagents included in the kit are replaced by other reagents not supplied by Health in Code, S.L.
- ◇ The manufacturer does not guarantee the reproducibility of the assay when the user includes reagents not validated by Health in Code S.L., considering them equivalent to those supplied in the kit.

## 05 Content and storage conditions of the kit

This kit contains the lyophilized reagents necessary for 12 digital PCR determinations.

- **Marker\* Master Mix:** oligonucleotides needed to amplify the marker being analyzed (polymorphism) and the reference gene ( $\beta$ -globin), two labeled probes, one labeled with FAM™ to detect the marker and another with VIC™ to specifically detect the  $\beta$ -globin used as an active reference for quantification.

Reagents	Quantity	Storage
<b>Marker Master Mix</b>	2 x 6 rxn	4°C

Table 2. Imegen® Quimera dPCR Dry kit components.

(\*) This reagent is specific for each marker referred to in Table 1.

## 06

# Equipment, reagents and materials not included in the kit

**Equipment:**

- Micropipettes (10 µL, 20 µL and 200 µL)
- Nanodrop Spectrophotometer (Thermo Fisher Scientific)

**QuantStudio™ 3D Digital PCR (Thermo Fisher Scientific)**

- + QuantStudio 3D Digital PCR (chip loader)
- + ProFlex™ 2x Flat PCR System (dPCR thermal cycler)
- + QuantStudio 3D Digital PCR instrument (chip reader)

**QuantStudio™ Absolute Q™ PCR (Thermo Fisher Scientific)**

- + QuantStudio™ Absolute Q Digital PCR System, desktop (dPCR thermal cycler)

**Droplet Digital™ PCR (Bio-Rad)**

- + QX200™ Droplet Digital™ PCR system or QX100™ Droplet Digital™ PCR system
- + PX1™ PCR Plate Sealer
- + C1000 Touch™ Thermal Cycler with 96-Deep Well Reaction Module

**Reagents:**

- Nuclease-free water

**QuantStudio™ 3D Digital PCR (Thermo Fisher Scientific)**

- + QuantStudio 3D Digital PCR Master Mix v.2
- + Absolute ethanol

**QuantStudio™ Absolute Q™ dPCR (Thermo Fisher Scientific)**

- + Absolute Q DNA Digital PCR Master Mix (Ref. A52490)
- + Isolation Buffer (Ref. A52730)

**Droplet Digital™ PCR (Bio-Rad)**

- + ddPCR™ Supermix for probes (No dUTP)
- + Droplet generation oil for probes

**Materials:**

- 0.2 mL and 1.5 mL sterile tubes

- Pipette filter tips (10 µL, 20 µL and 200 µL)
- Powder-free latex gloves

**QuantStudio™ 3D Digital PCR (Thermo Fisher Scientific)**

- + QuantStudio® 3D Digital PCR 20K Chip Kit v2 (12 pack) (Ref.A26316)
- + Precision wipes

**QuantStudio™ Absolute Q™ dPCR (Thermo Fisher Scientific)**

- + MAP16 Plate Kit (Ref. A52865)

**Droplet Digital™ PCR (Bio-Rad)**

- + Droplet Generator Cartridges and Gaskets (Ref.1864007)
- + 96-well plates for ddPCR (Ref.12001925)
- + Perforable thermal film (Ref.1814040)

### Complementary kits

The step before digital PCR informative marker quantification consists of real-time PCR determination of the informativity of possible polymorphisms. To do this, Health in Code, S.L. has designed, developed and manufactured the following kits.

Kit name	Reference
Imegen® Quimera Screening Multiplex Plus	IMG-116-26
Imegen® Quimera Screening Multiplex Plus II	IMG-116-25

Table 3. Marker screening kits.

# 07 Assay protocol

## 07.1 | Preparation of reagents

The first step before starting the assay protocol is to rehydrate the lyophilized reagents with a volume of nuclease-free water as per the chosen dPCR equipment, as specified in Table 4.

Shaking and spinning the tubes containing the reagents and storing them at 4°C for one hour before use is recommended in order to facilitate the resuspension and homogenization of the reagents (\*). If this reagent is not going to be used immediately after rehydration, we recommend storing it at -20°C until used.

Equipment	Volume of nuclease-free water
QuantStudio™ 3D Digital PCR System (ThermoFisher Scientific)	10 µL
QuantStudio™ Absolute Q™ PCR system (ThermoFisher Scientific)	15 µL
Droplet Digital™ PCR system (BIO-RAD)	10 µL

Table 4. Volume of nuclease-free water for the rehydration of reagents in the Imegen® Quimera dPCR Dry kit, depending on the equipment used for the assay.

## 07.2 | Preparation of amplification reactions

The assay should include the following reactions:

- ◇ Reaction of each sample.
- ◇ Recommended: Negative control reaction (reaction containing water instead of DNA to guarantee the absence of contamination in the process).
- ◇ Optional: Analyzing the pre-transplant sample of the recipient is recommended if the cellularity of the selected informative marker is to be determined. This analysis determines whether the recipient is homozygous or heterozygous for the selected marker and permits correction of the allele load to obtain cellularity.

Each PCR mix will consist of:

- + Marker Master Mix (Marker *Master Mix* +  $\beta$ -globin Master Mix)
- + General Master Mix (not included in the kit)

The protocol for preparing the amplification reactions and performing the digital PCR for the platform being used is described below:

01 Thaw the reagents required for the analysis:

- ◇ Genomic DNA samples diluted to the optimum concentration (25 ng/μL; sample quantification by Nanodrop (Thermo Fisher Scientific)).

**NOTE:** Samples of suboptimal quality should be centrifuged at 10,000 x g or at the maximum available speed for 1 minute; the sample should then be drawn from the top of the tube.

- ◇ Marker Master Mix (rehydrated).
- ◇ Nuclease-free water for the negative controls.
- ◇ Master Mix for digital PCR (not supplied).

**NOTE:** It is important to allow the enzyme to reach room temperature before use.

02 Vortex all the reagents for 10 seconds, including the enzyme, and spin.

### ↘ QuantStudio™ 3D Digital PCR System (Thermo Fisher Scientific)

03 Add the indicated amount (Table 5) of each reagent to 1.5 mL tubes to match the number of total reactions. When making the calculations, it is recommendable to add enough reagents to analyze one extra reaction or add 10% more of each of the reagents.

Reagent	Volume per reaction
Marker Master Mix	1.5 μL
QuantStudio 3D Digital PCR Master Mix v.2	7.5 μL

Table 5. Amount of reagents needed per reaction with the QuantStudio™ 3D Digital PCR System

04 Make the PCR mix by pipetting and dispensing 9 μL into the corresponding 0.2 mL tubes, making sure not to create bubbles.

05 Dispense 6 μL of the sample DNA at 25 ng/μL or nuclease-free water (negative control) into the corresponding tubes.

06 Load 14.5 μL of the PCR reaction in the QuantStudio 3D Digital PCR Chip Loader equipment, following the manufacturer’s instructions, to load the chip.

### ↘ QuantStudio™ Absolute Q™ PCR system (Thermo Fisher Scientific)

03 Add the following reagents to a new 1.5 mL tube:

Reagent	Volume per reaction <sup>2</sup>
Marker Master Mix	1.5 μL
Absolute Q DNA Digital PCR Master Mix (5x) <sup>1</sup>	2 μL

Table 6. Amount of reagents needed per reaction with the QuantStudio Absolute Q PCR system

(1) Reagent not supplied by the manufacturer (see section “[06. Equipment, reagents, and materials not supplied](#)”).

(2) Adjust the final volume to the number of reactions, as described at the beginning of the section.

04 Vortex the prepared PCR reaction mix for 10 seconds and then spin for 30 seconds. Dispense 3.5 μL into new 0.2 mL tubes.

- 05 Transfer 6.5  $\mu\text{L}$  of the DNA sample supernatant at 25 ng/ $\mu\text{L}$  or nuclease-free water (for the negative control) to PCR tubes with the reaction mix prepared in point 4.
- 06 Use one of the two options below to add the mix from point 5:
  - ◇ Pipette up and down 10–20 times, taking care not to create bubbles.
  - ◇ Vortex 3–5 times for 1 second at a time.
- 07 Centrifuge for 1 min at 10,000  $\times$  g.
- 08 Hold the pipette at a 45° angle, load 9  $\mu\text{L}$  of the PCR reagent mix into the bottom of each well of the MAPI6 microfluidic array plate and depress until the first pipette stop.
- 09 Next, at a 45° angle, dispense 15  $\mu\text{L}$  of isolation buffer against the side of the well, letting it drip onto the reagents to prevent mixing and bubble formation.
 

**Optional:** wait 15 min before pressing Start in the program and keep the plate inside the device in case bubbles have formed or the reagents have mixed during loading.

### ↘ Droplet Digital™ PCR system (BIO-RAD)

- 03 Add the indicated amount of each reagent (Table 7) to 1.5 mL tubes to match the total number of reactions. When making the calculations, it is recommendable to add enough reagents to analyze one extra reaction or add 10% more of each of the reagents:

Reagent	Volume per reaction
Marker Master Mix	1.5 $\mu\text{L}$
ddPCR™ Supermix for probes (No dUTP)	10 $\mu\text{L}$
Nuclease-free water	5.5 $\mu\text{L}$

Table 7. Amount of reagents needed per reaction for the assay with the Droplet Digital PCR system

- 04 Make the PCR mix by pipetting and dispensing 17  $\mu\text{L}$  into the corresponding 96-well plate, making sure not to create bubbles:
 

**NOTE:** If any of the wells in the plate columns with the samples are left empty, add 20  $\mu\text{L}$  of control buffer or water.
- 05 Dispense 3  $\mu\text{L}$  of sample DNA at 25 ng/ $\mu\text{L}$  or nuclease-free water (negative control) to the corresponding wells.
- 06 Load 20  $\mu\text{L}$  of the PCR reaction with the multichannel pipette in the corresponding loading cartridge wells, following the manufacturer's instructions.

## 07.3 | PCR program setup, loading and reading

### ↘ QuantStudio™ 3D Digital PCR System (Thermo Fisher Scientific)

#### ➤ Load the amplification reactions in the chip.

When loading and preparing all the devices needed to load the chip (consumable not included) with the prepared amplification reactions, we recommend following the manufacturer's instructions for the *QuantStudio 3D Digital PCR Chip Loader* (Thermo

Fisher Scientific). To do this, follow the instructions in chapter 3 of the *MAN0007720 QuantStudio™ 3D Digital PCR System User Guide* available from the web site [www.thermofisher.com](http://www.thermofisher.com).

➤ **PCR program setup.**

For the distribution of the chips in the thermal cycler for dPCR: *ProFlex™ 2x Flat PCR System*, follow the instructions in chapter 4 of the *MAN0007720 QuantStudio™ 3D Digital PCR System User Guide* available from the website [www.thermofisher.com](http://www.thermofisher.com).

◇ **Optimal program:**

Fields	Stage 1 Enzymatic activation	Stage 2 PCR		Stage 3	
<b>No. of cycles</b>	1 initial cycle (denat.)	40 cycles		1 final cycle	Storage
		Annealing/ Extension	Denaturation		
<b>Temperature</b>	96°C	56°C	98°C	60°C	10°C
<b>Time</b>	10 minutes	2 minutes	30 seconds	2 minutes	∞

Table 8. Optimum PCR program for the Imegen® Quimera dPCR Dry kits with the QuantStudio 3D Digital PCR system.

➤ **Chip reading and obtaining results.**

Once the PCR program has terminated, follow the instructions in chapter 5 of the *MAN0007720 QuantStudio™ 3D Digital PCR System User Guide* available from the website [www.thermofisher.com](http://www.thermofisher.com), to obtain the files resulting from the chip reading.

↘ **QuantStudio™ Absolute Q™ PCR system (Thermo Fisher Scientific)**

➤ **Load the microfluidic array plate (MAP).**

Mount the cap strips for the MAP plates in the 4 well columns. Make sure the columns are completely covered and place the MAP plate cap strip in all the columns, including unused ones. Follow the instructions in Chapter 2 of the *MAN0025621 QuantStudio™ Absolute Q™ Digital PCR System User Guide* available from their website [www.thermofisher.com](http://www.thermofisher.com).

➤ **PCR program setup.**

Place the MAP plate in the *QuantStudio™ Absolute Q™ System*, select the protocol from the list and modify the optimum PCR channels and parameters as required. To facilitate the chimerism calculation:

◇ **Experiment setup:**

Analysis	Target	Analysis
FAM	Informative marker	CNV
VIC	Beta-globin	CNV Ref

Table 9. Experiment set up in the QuantStudio Absolute Q PCR system

◇ Optimal program:

Fields	Stage 1 Enzymatic activation	Stage 2 PCR	
<b>No. of cycles</b>	1 initial cycle (denaturation)	40 cycles	
		Denaturation	Annealing/ Extension
<b>Temperature</b>	96°C	96°C	60°C
<b>Time</b>	10 minutes	5 seconds	15 seconds

Table 10. Optimum PCR program for the QuantStudio Absolute Q PCR System.

➤ **Array reading and obtaining results.**

Once execution of the PCR has terminated, follow the instructions in the user guide for the digital PCR *QuantStudio™ Absolute Q* system available from the website [www.thermofisher.com](http://www.thermofisher.com).

↘ **Droplet Digital™ PCR system (BIO-RAD)**

➤ **Load the PCR reactions into the loading cartridge.**

When loading and preparing all the devices needed to load the chip (consumable not included) with the prepared amplification reactions, we recommend following the manufacturer’s instructions for the *QX200™ Droplet Digital™ PCR system* or *QX100™ Droplet Digital™ PCR system* (BIO-RAD). To do this, follow the instructions in chapter 2, section *ddPCR Experimental Workflow>Droplet Generation*, in the *Droplet Digital™ PCR Applications Guide* available from the website [www.bio-rad.com](http://www.bio-rad.com).

➤ **PCR program setup.**

Place the 96-well ddPCR plate in the *C1000 Touch™ Thermal Cycler with 96-Deep Well Reaction Module*:

◇ Optimal program:

Fields	Stage 1 Enzymatic activation	Stage 2 PCR		Stage 3	
<b>No. of cycles</b>	1 initial cycle (denat.)	40 cycles		1 final cycle	Storage
		Denaturation	Annealing/ Extension		
<b>Temperature</b>	96°C	94°C	60°C	98°C	4°C
<b>Time</b>	10 minutes	30 seconds	1 minute	10 minutes	∞

Table 11. Optimum PCR program for the Imegen® Quimera dPCR kits with the BIO-RAD platform.

➤ **Plate fluorescence reading and obtaining results.**

To obtain the files from the plate reading once the PCR program has terminated, follow the instructions in chapter 2, specifically the sections *Setting Up and Experiment in Quantasoft™ Software and Droplet Reading*, in the *Droplet Digital™ PCR Applications Guide* available on the website [www.bio-rad.com](http://www.bio-rad.com). As the experiment type, select the option RED: *rare target sequence detection (rare event detection)*.

## 08 Analysis of results

When analyzing the results, it is important to know how the probes that detect each of the targets analyzed with the kit are labeled:

Probe	Labeling
$\beta$ -globin	VIC™
Informative marker	FAM™

Table 12. Labeling of the probes used

Follow the indications below to ensure the results are analyzed properly:

- **Negative control (NTC).** Confirm there are no amplification signals for any of the targets (FAM and VIC). If amplification is detected, repeat the assay to rule out accidental contamination.
- Hematopoietic chimerism quantification is expressed as a relative measurement of the number of copies of the informative marker in relation to the amount of the reference gene, beta-globin. The molecular chimerism percentage is calculated using the following formula.

$$\% \text{ Chimera} = \frac{\text{Informative marker} \frac{\text{Copies}}{\mu\text{L}}}{\beta\text{-globin} \frac{\text{Copies}}{\mu\text{L}}} \times 100$$

### ↳ QuantStudio™ 3D Digital PCR System (Thermo Fisher Scientific)

The *QuantStudio™ 3D AnalysisSuite™* software by Thermo Fisher Scientific is used to analyze the results. We therefore recommend following the instructions in chapter 5 of the *MAN0007720 QuantStudio™ 3D Digital PCR System User Guide* available from the website [www.thermofisher.com](http://www.thermofisher.com). This manual has a "Troubleshooting" section, which specifies possible solutions for problems arising during the assay.

In addition, follow the indications below to ensure the results are analyzed properly:

- ◇ Check that there is no amplification in the negative controls. If amplification is detected, it is recommendable to repeat the analysis to rule out accidental contamination.
- ◇ If necessary, review and edit the results of the chip reading in the section "Review data" of the software and finally use the data for copies/ $\mu$ L (FAM) and copies/ $\mu$ L (VIC) in the "See results" tab of the analysis software.

### ↳ QuantStudio™ Absolute Q™ PCR system (Thermo Fisher Scientific)

The *QuantStudio Absolute Q Digital PCR* software by Thermo Fisher Scientific is used to analyze the results. To interpret them, follow the instructions in Chapter 3 “Data analysis” in *MAN0025621 QuantStudio™ Absolute Q™ Digital PCR System User Guide* available from [www.thermofisher.com](http://www.thermofisher.com). This guide has a “Troubleshooting” section to solve problems arising during the assay.

- ◇ Manually review and, if necessary, edit the results obtained for each sample in the “Analysis” tab.
- ◇ To analyze and review the results, go to the “Results” tab and export the results to a .csv file.

### ↳ Droplet Digital™ PCR system (BIO–RAD)

To analyze the results, use the *QuantaSoft™ Software* by BIO–RAD. It is advisable to follow the instructions in chapter 2, specifically the *Data analysis* section in the *Droplet Digital™ PCR Applications Guide* available from the website [www.thermofisher.com](http://www.thermofisher.com). This manual has a “Troubleshooting” section to solve problems during the assay.

- ◇ If necessary, review and edit the result. Finally, use the data for “concentration (copies/μL)” for the marker (FAM) and “concentration (copies/μL)” for the reference gene (VIC) in the “Analyze > Concentration” tab of the analysis software.

## ↳ Imegen®-Quimera Software, by Health in Code, S.L Patient follow-up application

Health in Code, S.L. has designed and developed an easy-to-use app (Figure 1) that enables the user to create a patient database and register the results of informative marker screening, informative marker quantifications for different patient follow-up samples and medical actions applied to the patient during follow-up. Users can also view all the medical actions and the patient's course in a graph, and export the results.

For more information, see the video tutorial on the use of the **Imegen®-Quimera** app at the following link: [youtu.be/K38cV3hacm8](https://youtu.be/K38cV3hacm8)

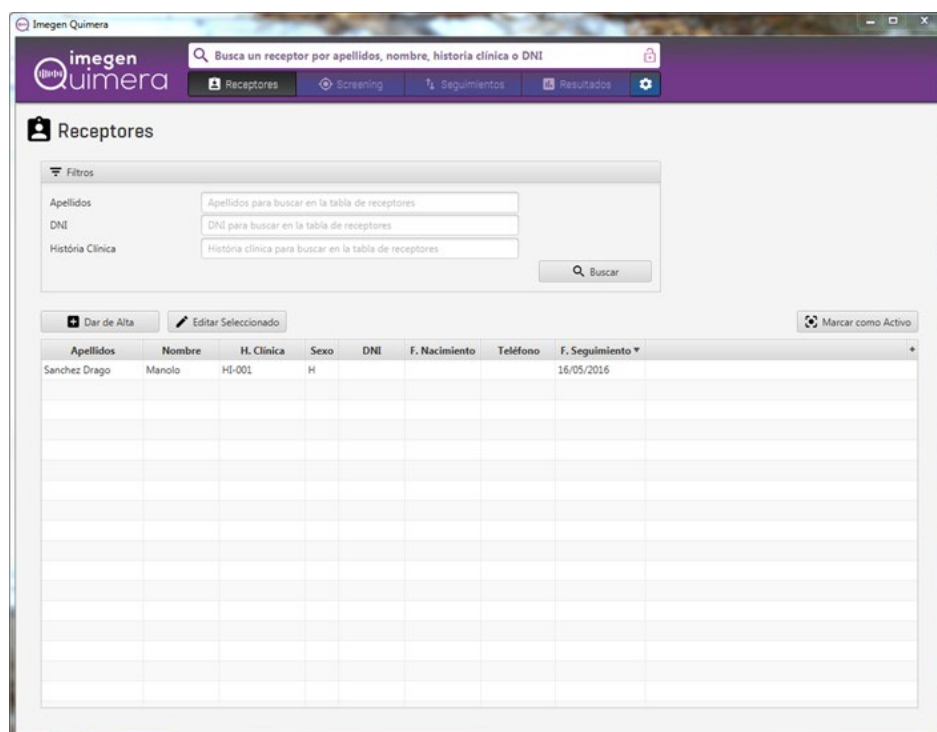


Figure 2. View of the patient follow-up app developed by Health in Code, S.L.

**NOTE:** The Imegen®-Quimera app is not designed for use as a laboratory information management system (LIMS).

## 09 Troubleshooting

Table 13 provides a graphic display of the possible results from analyzing the different controls and a genomic DNA sample in an assay, and the interpretation and causes of the result:

Control	Marker	$\beta$ -globin	Cause
DNA sample	+	+	Expected result
	-	-	Failed sample amplification <sup>1</sup>
PCR negative control	-	-	Expected result
	+	+	Contamination with human DNA <sup>2</sup>

Table 13. Interpretation of possible results obtained from the assay with Imegen® Quimera dPCR Dry

(1) **Sample amplification failure:** check that the DNA concentration or quality in the sample is adequate; if it is, the specified result may be due to the sample being highly degraded. In this situation, a second DNA analysis or extraction would be recommendable before interpreting the results.

(2) **PCR contamination with human DNA:** PCR contamination may be due to mishandling the sample, use of contaminated reagents or environmental contamination. Thoroughly clean the laboratory where the PCR was prepared, as well as the equipment and materials used. If necessary, use new aliquots of PCR reagents.

# 10 Limitations

## 10.1 | Equipment

Imegen® Quimera dPCR Dry has been validated using the following digital PCR platforms:

- + *QuantStudio™ 3D Digital PCR system* (Thermo Fisher Scientific)
- + *QuantStudio™ Absolute Q™ Digital PCR system* (Thermo Fisher Scientific)
- + *QX200™ Droplet Digital™ PCR system and QX100™ Droplet Digital™ PCR system* (BIO-RAD)

If you use another make or model of digital PCR equipment for hematopoietic chimerism quantification, you may need to adjust the amplification program. Please contact the technical department for any questions or clarifications.

## 10.2 | Reagents

The Imegen® Quimera dPCR Dry kits have been validated using the reagents provided in the kit.

It is advisable to use the PCR reagents recommended by the supplier of the thermal cycler you are going to use for the digital PCR assays, specified in section 6 of this manual (equipment, reagents and materials that are not supplied).

Please contact the technical department for any questions or clarifications.

## 10.3 | Product stability

Optimum performance of this product is confirmed, provided that the manufacturer's recommended storage conditions with regard to the optimum product date for each production batch are followed.

# 11 Performance characteristics

## 11.1 | Validation samples

The **Imegen® Quimera dPCR Dry** kit is designed to analyze total genomic DNA (gDNA) extracted from peripheral blood or bone marrow samples.

The analytical validation of the **Imegen® Quimera dPCR Dry** kit was performed with a total of 48 reference samples (Coriell®) or the Health in Code, S.L. internal repository, previously analyzed to identify the presence/absence of the polymorphisms of interest to provide a positive and negative sample of each marker.

## 11.2 | Limit of quantitation (LOQ)

To determine the limit of quantitation (LOQ) of the **Imegen® Quimera dPCR Dry** kit, dilutions of the informative marker positive sample were prepared with a negative one to ensure an allele frequency of the marker at 5%, 0.5% and 0.05%.

Each marker was assayed in triplicate from the prepared dilution. To eliminate possible background noise from the technique, a correction was made including the negative sample for the evaluated marker. The signal produced by the negative sample was extracted from the signal obtained in the mixture. Next, the relative quantification of the marker was calculated in relation to the reference endogenous gene ( $\beta$ -globin).

The results obtained meet the acceptance criteria to establish the limit of quantitation at 0.05% (CV < 25%).

## 11.3 | Specificity

The analytical specificity of the **Imegen® Quimera dPCR Dry** kit was determined by analyzing a negative sample for each marker. In no case was a signal detected for the evaluated marker above the LOQ established in the corresponding negative markers.

## 11.4 | Reproducibility and repeatability

The reproducibility and repeatability parameters for the **Imegen® Quimera dPCR Dry** kit

were evaluated with real samples of gDNA for the three makes of equipment specified in section “10.1 Equipment”.

To do this, 6 replicas of a positive sample previously diluted to the previously established LOQ (0.05%) were analyzed for each marker and make of equipment for a total of 594 reactions. The designed systems for each marker show excellent precision for each make of equipment evaluated (QuantStudio™ 3D and Absolute Q™ Digital PCR system (ThermoFisher Scientific) and QX200™ Droplet Digital™ PCR system (BIO-RAD)) meeting the previously established acceptance criterion (CV < 25%) in all cases. These results are summarized in Table 14.

In addition, the reproducibility tests to assess the performance of 7 of the 33 markers at different frequencies (5% and 0.5%) in the three makes of equipment were extended. As the presence of the analyzed marker increases, the CV decreases. Thus, it may be concluded that at frequencies higher than the LOQ, the systems are also precise and reproducible (CV < 25%) (data not shown).

Marker	QuantStudio™ 3D Digital PCR system		QX200™ Droplet Digital™ (Bio-Rad)		QuantStudio™ Absolute Q™ Digital PCR system	
	Mean (%)	CV (%)	Mean (%)	CV (%)	Mean (%)	CV (%)
SRY	0.05	12.23	0.06	20.70	0.06	21.57
RhD	0.04	24.63	0.05	17.60	0.04	7.20
Q116-6I	0.07	24.03	0.03	22.90	0.05	11.44
Q116-3I	0.05	17.98	0.03	12.90	0.06	3.62
Q116-7I	0.03	10.53	0.03	19.61	0.05	3.26
Q116-12D	0.06	17.8	0.02	10.20	0.06	16.89
Q116-11I	0.08	8.08	0.05	23.10	0.06	7.93
Q116-5I	0.06	6.13	0.05	2.80	0.05	9.29
Q116-4I	0.04	18.16	0.05	18.30	0.04	8.60
Q116-10I	0.05	20.96	0.02	21.00	0.05	7.13
Q116-23I	0.04	6.4	0.05	6.90	0.04	4.87
Q116-28I	0.07	13.4	0.04	5.50	0.05	6.65
Q116-32I	0.05	18.01	0.03	24.00	0.04	7.26
Q116-31I	0.05	13.97	0.03	20.00	0.05	1.65
Q116-30D	0.02	12.79	0.03	19.50	0.05	11.41
Q116-29D	0.06	14.83	0.02	3.20	0.07	8.87
Q116-27D	0.05	2.25	0.04	23.87	0.05	1.77
Q116-24I	0.06	16.88	0.05	23.80	0.05	7.54
Q116-33I	0.05	23.7	0.04	13.26	0.06	4.02
Q116-37I	0.04	21.65	0.05	9.40	0.04	7.44
Q116-38I	0.04	8.57	0.07	10.10	0.06	9.59
Q116-39I	0.07	6.95	0.03	7.50	0.07	13.19
Q116-41I	0.06	9.12	0.03	9.30	0.06	19.85

Marker	QuantStudio™ 3D Digital PCR system		QX200™ Droplet Digital™ (Bio-Rad)		QuantStudio™ Absolute Q™ Digital PCR system	
	Mean (%)	CV (%)	Mean (%)	CV (%)	Mean (%)	CV (%)
Q116-42I	0.05	17.05	0.05	11.40	0.05	16.28
Q116-43I	0.03	14.95	0.05	23.80	0.05	8.70
Q116-44I	0.05	17.64	0.03	15.80	0.05	15.98
Q116-45I	0.04	12.48	0.03	4.00	0.05	1.42
Q116-47I	0.08	17.58	0.12	6.60	0.05	10.46
Q116-49I	0.05	9.39	0.17	15.20	0,047	5.49
Q116-50I	0.06	14.29	0.04	6.30	0.03	21.42
Q116-20I	0.04	13.88	0.03	13.50	0.05	17.04
Q116-9I	0.06	6.09	0.04	11.40	0.05	9.83
Q116-46I	0.05	23.7	0.05	10.70	0.04	7.25

Table 14. Reproducibility of the Imegen® Quimera dPCR Dry kit. The coefficient of variation (CV) is sampled for each marker and piece of equipment evaluated.

Contact our Technical Department for any questions about the applications of this product or its protocols:

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