INSTRUCTIONS FOR USE

Imegen® Turkey MC1R genotyping Kit

Ref. IMG-189

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Guide overview

The information in this guide is subject to change without notice.

Health in Code, S.L. guarantees that its products are free from defects, both in used materials as in its manufacturing process. This warranty is extended to the expiration date, as long as the storage conditions specified in this manual are met. Our products are designed for research use only. The user of the product is responsible for validating the usefulness of the protocol proposed by Health in Code, S.L. These protocols are considered a guide only. Health in Code, S.L. does not offer any other warranty, express or implied, which extends beyond the proper functioning of the components of this set. Health in Code S.L., sole obligation in respect of the preceding guarantees, will be to replace the product or return the purchase price thereof, as desired by the customer, as long as the existence of a defect in the materials test, or in the manufacture of its products. Health in Code, S.L. will not be responsible for any damage, direct or indirect, resulting in economic losses or damages resulting from the use of this product by the purchaser or user.

All products sold by Health in Code, S.L. are subjected to rigorous quality control (App. A). The **Imegen® Turkey MC1R genotyping Kit** has passed all internal validation tests, ensuring the reliability and reproducibility of each assay.

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

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Instructions for Use (IFU) modifications

V.06	NOV 2024	Transcription error: modification of no of cycles in table 5. Content revision in 2.1.
V.05	JAN 2024	Contents review. Modification of the storage temperature of the General Master Mix. Change of the manufacturer's identification, going from Imegen to Health in Code, S.L.
V.04	AUG 2022	Contents review
V.03	JUL 2019	Contents review

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01

Product information

01.1 General description

Association between turkey plumage color and MC1R genotype let to obtain valuable information to improve turkey breeding and production.

By sequencing the coding region of the turkey melanocortin 1 receptor (MC1R) gene, it has been detected three relevant mutations for bronze, black or black-winged bronze turkey plumage color genetics determination (c.96 G>A, c.364 A>T and c.887 C>T). These mutations are organized in 4 different haplotypes (MC1R*1 to MC1R*4). These haplotypes correlate perfectly with the 3 alleles of the bronze locus (B, b+, and b1). Following a pattern of dominance B>b+>b1 (Vidal et al. 2010).

Table 1. Correlation between haplotypes and bronze locus alleles.

MC1R haplotypes	Bronze locus allele
MC1R*1	b+
MC1R*2	B
MC1R*3	b1
MC1R*4	b1

References

Vidal O, Viñas J and Pla C. Variability of the melanocortin 1 receptor (MC1R) gene explains the segregation of bronze locus in turkey (Meleagris gallopavo). Poultry Science Association, Vol. 89, Nº 8, 1599-1066. August 1, 2010.

01.2 Intended use

Imegen® Turkey MC1R genotyping Kit allows knowing plumage colour phenotype (bronze, black or black-winged bronze) by genotyping three positions of the turkey melanocortin 1 receptor (MC1R) gene.

This kit uses Real-Time PCR technology and contains all the reagents required to amplify and genotype these three positions (c.96 G>A, c.364 A>T and c.887 C>T) of the *MC1R* gene in turkey DNA.

Each of these reactions amplifies a specific region by way of a single PCR. Regions comprising 96, 364 and 887 MC1R positions are amplified. Each reaction also contains two probes whose fluorescence signal is detected using two channels on the thermal cycler (FAM™ and VIC™). Each probe will be bound specifically to DNA chains which contain a determined base in a determined position.

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01.3 Content and storage conditions of the kit

Imegen® Turkey MC1R genotyping Kit contents the necessary reagents to perform 48 reactions:

MC1R-96A/ MC1R-96G Master Mix	Master Mix with specific oligonucleotides, fluorophore-labelled hydrolysis probes (FAM™ probe for which detects amplicons that has an adenine in position 96 of the MC1R, and the other labelled with VIC™ to detect amplicons that has a guanidine in that position) and nuclease-free water.
MC1R-364T/ MC1R-364A Master Mix	Master Mix with specific oligonucleotides, fluorophore-labelled hydrolysis probes (FAM™ probe for which detects amplicons that has a thymine in position 364 of the MC1R, and the other labelled with VIC™ to detect amplicons that has an adenine in that position) and nuclease-free water.
MC1R-887T/ MC1R-887C Master Mix	Master Mix with specific oligonucleotides, fluorophore-labelled hydrolysis probes (FAM™ probe for which detects amplicons that has a thymine in position 887 of the MC1R, and the other labelled with VIC™ to detect amplicons that has a cytosine in that position) and nuclease-free water.

General Master Mix	Master Mix of PCR with nucleotides, $MgCl_2$, DNA polymerase and buffer needed to carry out RT- PCR.

Table 2. IMG-189 Imegen® Turkey MC1R genotyping Kit components and description.

Reagents	Color indicator	Quantity	Conservation
MC1R-96 Master Mix*	Yellow pad	400 µl*	-20 °C
MC1R-364 Master Mix*	Black pad	400 µl*	-20 °C
MC1R-887T Master Mix*	Red pad	400 µl*	-20 °C
General Master Mix*	White pad	3 x 660 µl*	-20 °C upon receipt. 2 - 8 °C after initial use. Store protected from light.

(*) See the expiration date on the box and tubes.

01.4 Equipment, reagents and materials required but not supplied

Equipment

- Real-Time PCR Thermal Cycler with channels for detection of FAM™ (520 nm) and VIC™ (550 nm)
- Micropipettes (10 µl, 20 µl and 200 µl)
- Tabletop centrifuge with adaptors for 96 well PCR plates and/or 0.2 ml tubes
- Vortex

Materials

- Optical 96-well reaction plates or 0.2 ml optical tubes
- Optical adhesive film for 96 well plates or optical caps for 0.2 ml tubes
- Disposable micropipette filter tips (10 µL, 20 µL and 200 µL)
- 1.5 ml sterile tubes
- Powder-free latex gloves

Reagents

- Nuclease-free water

02 Methods

02.1 Preparation of the amplification reactions

Imegen® Turkey MC1R genotyping Kit is designed to perform 48 reactions.

The PCR Master Mix contains:

- **Specific Master Mix**
- **General Master Mix (2X)**

The recommended protocol for preparation of amplification reactions is shown below:

01. Thaw the Master Mixes, and DNA samples.
02. Vortex each reagent and keep cold.
03. Add into a 1.5 mL tube (one for each PCR master mix preparation), the following reagents (Table 2). To estimate the amount of necessary reagents, we recommend to make calculations taking into account the number of samples to be simultaneously analyzed, and then considering one more reaction.

Table 3. Reagents and volumes needed to perform PCR reactions.

Reagents	Amount per reaction
Specific Master Mix	7.5 µL
General Master Mix	12.5 µL

04. Vortex and spin the 1.5 mL tube and dispense 20 µl per well or tube of 0.2 ml.
05. Add 5 µl of each DNA sample at 10 ng/µl, 5 µl of Beef Positive Control and 5 µl of the Negative Controls* into the appropriate wells.
06. Cover the well plate with optical film or the tubes with optical cover and spin in the centrifuge.

(*) We strongly recommend using an **extraction negative control** for each run of extractions carried out. This control consists in one tube to which no sample is added, and which is summited to the same extraction process as the other samples. Likewise, we recommended using a **PCR negative control** for each PCR run; this tube contains no DNA but all PCR reagents.

02.2 Settings for the Real-Time PCR program

This kit is compatible with the Real-Time PCR platforms 7500 FAST, StepOne Real-Time PCR System (Thermo Fisher Scientific) and QuantStudio™ 5 Real-Time PCR system.

Table 4. IMG-189 Imegen® Turkey MC1R genotyping Kit probes and specifications.

Target	Receptor	Quencher
MC1R-96A	FAM™	MGB
MC1R-96G	VIC™	MGB
MC1R-364T	FAM™	MGB
MC1R-364A	VIC™	MGB
MC1R-887T	FAM™	MGB
MC1R-887C	VIC™	MGB

The following instructions should be taken into account in order to setup the amplification program:

- **Reaction volume:** 25 µL.
- **Targets:** FAM™ and VIC™.
- In case the quencher has to be defined, select MGB for all probes. If the real time PCR system does not take into account the quenchers, select only the receptors (FAM™ and VIC™).
- If the Real-Time PCR system is a 7500 Fast, a StepOne Real-Time PCR system (Thermo Fisher Scientific) or a QuantStudio™ 5 Real-Time PCR system, select Quantitation - Standard curve as a type of experiment and include ROX™ as a reference.
- **Ramp rate:** standard
- **Optimal program:**

Table 5. Optimal PCR program.

Fields	Step 1 Enzyme activation	Step 2 PCR	
No. of cycles	1 initial cycle	40 cycles	
		Denaturation	Primers binding/extension
Temperature	95°C	95°C	60°C
Time	10 minutes	15 seconds	1 minute *

(*) Fluorescence detection.

02.3 Analysis of results

To analyze the results, it is recommended to establish the Threshold at 0.1 and to keep the default Baseline value to minimize the residual signal in the detection channels.

Ct settings	Threshold	0.1
	Baseline	AUTO

Before analyzing the sample results, the following considerations should be taken into account:

- **Negative controls:** amplification should only be detected in the VIC™ channel. In this channel an internal positive control (IPC) is detected, which determines the absence of inhibition in the sample.
- *MC1R* is not a specific gene of Turkeys. Therefore, if it is not certain that samples to be analyzed are turkey samples, it is recommended using the **Imegen® Turkey ID Kit** (IMG-267) to make a pre-test to be sure of that.

FAM Homozygous	It is observed a FAM™ channel amplification and a residual VIC™ cannel amplification.
Heterozygous	It is observed amplification in a FAM™ channel, superior to the VIC™ channel amplification.
VIC Homozygous	It is observed a VIC™ cannel amplification and a residual FAM™ channel amplification.

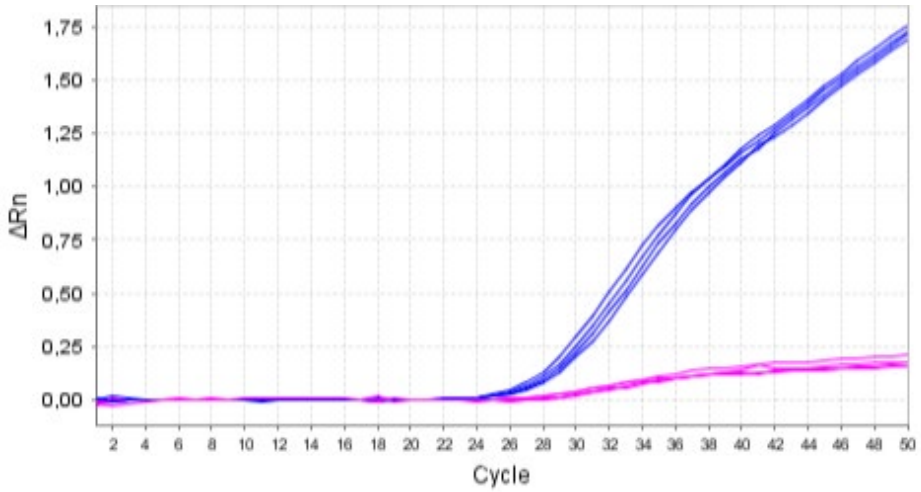

Figure 1. Type of amplification in FAM Homozygous.

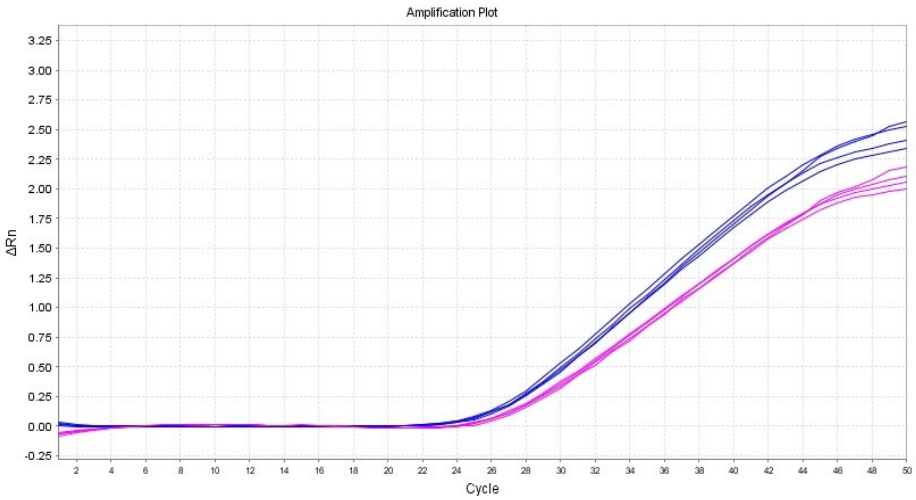

Figure 2. Type of amplification in Heterozygous.

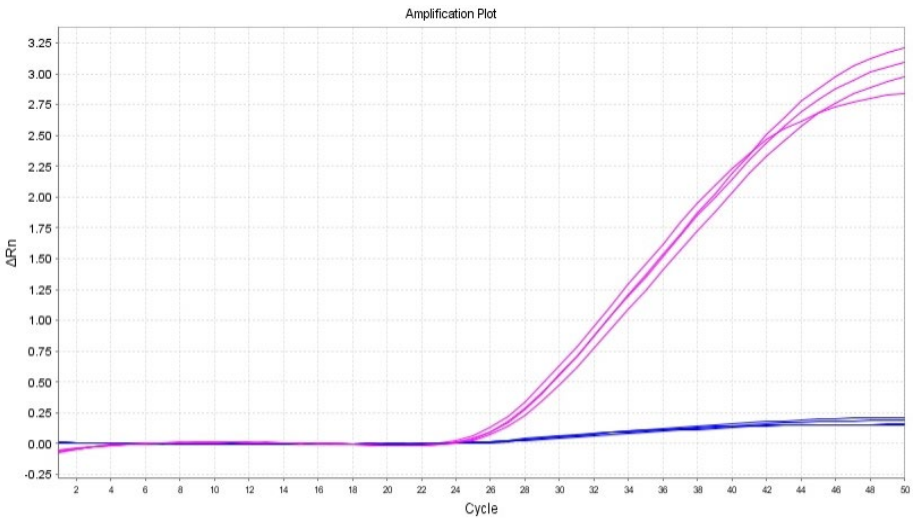

Figure 3. Type of amplification in VIC Homozygous.

The following table shows graphically the results that may be obtained from one sample analysis, as well as the interpretation that should be done from the obtained result:

Table 6. Results interpretation.

Position	Results	Allele 1 Base	Allele 2 Base	Alleles
MC1R-96	FAM Homozygous	A	A	A/A
	Heterozygous	A	G	A/G
	VIC Homozygous	G	G	G/G
MC1R-364	FAM Homozygous	T	T	T/T
	Heterozygous	T	A	T/A
	VIC Homozygous	A	A	A/A
MC1R-887	FAM Homozygous	T	T	T/T
	Heterozygous	T	C	T/C
	VIC Homozygous	C	C	C/C

The following table lists the interpretations of the results based on the described alleles and haplotypes combinations:

Table 7. Results interpretation.

Genotype. Position			Haplotypes	Alleles	Plumage colour pattern
MC1R-96	MC1R-364	MC1R-887			
G/G	A/A	C/C	MC1R*1/MC1R*1	b+/ b+	Bronze
G/G	A/T	C/C	MC1R*1/MC1R*2	b+/ B	Black
G/A	A/T	C/C	MC1R*1/MC1R*3	b+/ b1	Bronze
G/G	A/A	C/T	MC1R*1/MC1R*4	b+/ b1	Bronze
G/G	T/T	C/C	MC1R*2/MC1R*2	B/ B	Black
G/A	T/T	C/C	MC1R*2/MC1R*3	B/ b1	Black
G/G	T/A	C/T	MC1R*2/MC1R*4	B/ b1	Black
A/A	T/T	C/C	MC1R*3/MC1R*3	b1/ b1	Black-winged bronze
A/G	T/A	C/T	MC1R*3/MC1R*4	b1/ b1	Black-winged bronze
G/G	A/A	T/T	MC1R*4/MC1R*4	b1/ b1	Black-winged bronze

NOTE: New haplotypes, different from the haplotypes described above, cannot be assigned to a plumage colour pattern. Although, potentially, haplotypes which has an adenine in 96 position of MC1R of turkey has the same effect than MC1R*3 due to the mutation originates a stop codon in the protein. The putative protein coded by these alleles is then truncated at 32 amino acids independently of the bases in the other positions (364 and 887).

03 Troubleshooting

The following table shows graphically the results that may be obtained from the analysis of different assay controls, as well as the interpretation that should be done from the obtained result:

Table 8. Possible results and their interpretation.

Controls	Specific Master Mix		Interpretation
	FAM™	VIC™	
Sample	+	+	Expected result
	+	-	Expected result
	-	+	Expected result
	-	-	[1] PCR Amplification Failure
Extraction Negative Control	-	-	Expected result
	-	+	[2] Turkey DNA contamination in the meat DNA extraction procedure
	+	-	[2] Turkey DNA contamination in the meat DNA extraction procedure
	+	+	[2] Turkey DNA contamination in the meat DNA extraction procedure
PCR Negative Control	-	-	Expected result
	+	-	[3] PCR contamination with Turkey DNA
	-	+	[3] PCR contamination with Turkey DNA
	+	+	[3] PCR contamination with Turkey DNA

(1) PCR Amplification Failure: check amplification program and configuration of fluorescence capture. Amplification failure may be due to a setup technical problem.

(2) Contamination in the Turkey DNA extraction procedure: contamination may be due to some error made in the process of sample handling, reagents contamination, or environmental contamination. Check DNA extraction protocol, wipe the laboratory where DNA extraction process was performed and take care to avoid any contamination during sample homogenization. If necessary, use new aliquots of the reagents used in DNA extraction.

(3) PCR contaminations with Turkey DNA: contamination of PCR reactions may be due to an error made in the process of sample handling, contamination of the reagents or environmental contamination. Thoroughly clean the laboratory where the PCR process was performed, as well as equipment. If necessary, use new aliquots of the reagents used in the PCR. Prepare the PCR reaction containing the Positive Control last to avoid cross contamination.

04 Limitations

04.1 Equipment

Imegen® Turkey MC1R genotyping Kit has been validated using the following Real-Time PCR systems:

- 7500 FAST Real-Time PCR System (Thermo Fisher Scientific)
- StepOne™ Plus Real-Time PCR System (Thermo Fisher Scientific)
- QuantStudio5™ Real-Time PCR System (Thermo Fisher Scientific)

Technically, this kit is compatible with any Real-Time PCR systems that enable the detection of the fluorescence emitted by FAM™ and VIC™ fluorophores.

If a PCR system different from the systems described in this section is going to be used, it is possible that the PCR program might need to be readjusted. In this case, please contact our Technical Support Team for more details.

04.2 Reagents

Imegen® Turkey MC1R genotyping Kit has been validated using the reagents included in the kit and the DNA polymerase recommended by the supplier of the Real-Time PCR systems used in the validation as follows:

- TaqMan Environmental Master Mix 2.0 (Thermo Fisher Scientific)

If a PCR master mix (DNA polymerase) different from the DNA polymerase used in the validation is going to be used to perform the analysis, a validation with the new reagents is recommended beforehand. Please, contact our Technical Support Team if you request any further information.

04.3 Product Stability

The optimal analytical functioning of this product is confirmed as long as the recommended storage conditions are applied as specified on Section 01.3 (Contents and Storage Conditions) from the reception of the kit until the expiry date assigned to each production batch.

Appendix
A

Supplemental information

AA.1 Quality certifications

- Health in Code, S.L. is certified against the standard UNE-EN ISO 9001:2015 "Quality management systems" for the design, development, manufacture, and commercialization of kits for genetic analysis.
- Health in Code, S.L. is certified against the standard UNE-EN ISO 14001:2015 “Environmental Management Systems” for the design, development, manufacture, and commercialization of kits for genetic analysis.

Appendix B

Safety warnings and precautions

	Strictly follow the instructions of this manual, especially regarding the handling and storage conditions.
	Do not pipette by mouth.
	Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
	You must properly protect any skin condition, as well as cuts, abrasions and other skin lesions.
	Send down the drain only those materials found on the safe list. Compounds not listed are not suitable for drain disposal. Use waste containers according to the local legislation and manage their treatment through an authorised waste manager.
	In case of an accidental release of any of the reagents, avoid contact with skin, eyes and mucous membranes and clean with abundant water.
	The materials safety data sheets of all hazardous components contained in this kit are available on request to Health in Code, S.L.
	This product could require the handling of samples and materials of human and animal origin. You should consider all human and animal source materials as potentially infectious and handled in accordance with OSHA Biosafety Level 2 of bloodborne pathogens or must use other relevant biosafety practices for materials containing or suspect that they may contain infectious agents.
	Reagents included in this kit are non-toxic, neither explosive, infectious, radioactive, magnetic, corrosive nor environmental polluters.

	This kit has been validated with specific equipment under certain conditions, which could be different in other laboratories. It is recommended that each laboratory performs an internal validation when the kit is used for the first time.
	The manufacturer is not responsible for the malfunction of the assay when one or more 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 employs reagents not validated by Health in Code, S.L., considering them equivalent to those provided in the kit.

Appendix C

Documentation and support

AC.1 Food safety support

Please, visit our website for the latest services, orders and support information:

imegenagro.es

Health in Code, S.L. certificates of analysis and other product documentation:

portal.imegen.es/en/certificate-of-analysis/

AC.2 Customer and technical support

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

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NOTE: For SDSs for reagents and chemicals from other manufacturers, please contact the appropriate manufacturer.