Forensic Science International: Genetics Supplement Series 6 (2017) e357–e358

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/fsigss

Development of a *SNaPshot® Multiplex* system for the typing of single nucleotide polymorphisms (SNPs) involved in the adaptive response to high altitude hypoxia

G. Burgos[a,*], M. Ruano[b], M. Badin[a], M. Camargo[c], E. Tejera[a], C. Paz-Y-Miño[d]

[a] Escuela de Medicina, Facultad de Ciencias de la Salud, Universidad de Las Américas (UDLA), Quito, Ecuador
[b] Facultad de Ingeniería y Ciencias Agropecuarias, Universidad de Las Américas (UDLA), Quito, Ecuador
[c] Laboratorio de Genética, Instituto Nacional de Medicina Legal y Ciencias Forenses, Cali, Colombia
[d] Centro de Investigación Genética y Genómica, Facultad de Ciencias de la Salud, Universidad Tecnológica Equinoccial, Quito, Ecuador

ARTICLE INFO

Keywords:
Hypoxia
SNP
Genotyping
SNaPshot
rs1868092
rs1867728
rs10187368
rs13419896
rs4953354
rs895436
rs1867785

ABSTRACT

HIF2-α is the leading gene involved in adaptive response to hypoxia. The codification of this gene regulates the expression of more than 100 genes. SNPs in this gene, affecting the protein HIF2-α, have been linked to the regulation of several tissues proliferation; in multiple diseases and even oncogenic processes. For this reason, the major aim of the study was to develop the first genotyping protocol using *SNaPshot®* system in Ecuador. To fulfil the objectives of the present work, a *SNaPshot®*-based system was designed to typify seven SNPs. In this manner, commercial control DNAs were used, based on which the conditions of PCR and *SNaPshot® Multiplex* were adjusted, finally, the results were validated using *Gold Standard* method. It was concluded that *SNaPshot® Multiplex* system has the same sensitivity and specificity as the *Gold Standard*, but it is extremely economical, fast, and feasible to implement in developing countries.

1. Introduction

The main gene responsible in adapting the organism to hypoxia conditions is Hypoxia Inducible Factor 2-α (HIF2-α). This gene is located at position 2p21, with a size of 89302 bp [1]; it codes for the second isoform of the alpha subunit of the transcriptional factor HIF (HIF2-α), regulating the expression of about 100 metabolic pathways.

SNPs in HIF2-α gene affecting the protein (HIF2-α) have been linked to regulation of angiogenesis and proliferation of liver, pancreas and placenta cells [2]; production of erythropoietin and oncogenic processes (e.g., VEGFR-2 in melanoma).

The Gold Standard method for determination of SNPs is the enzymatic sequencing fluorinated by the Sanger method sized by capillary electrophoresis. However, it involves considerable investment of money, time and resources, since it requires sequencing the two strands of the DNA fragment for each SNP located when a distance greater than 1000 bp from another.

To overcome these drawbacks SNaPshot® technology was consolidated, allowing the genotyping of up to 20 SNPs even in different chromosomes, in a single reaction of Multiplex PCR and run of capillary electrophoresis [3].

SNaPshot® approach is well received since it can be used in several fields of research such as forensic genetics, biomedicine, plant breeding, animal diagnostics, and molecular epidemiology among others. It requires less quantity of DNA than Gold Standard method and provides selected information, and it can be used even in degraded forensic samples.

In the present study, a SNaPshot® System (Primers and Probes) was designed to genotyping the seven intronic SNPs of the HIF2-α gene: rs1868092, rs1867728, rs10187368, rs13419896, rs4953354, rs895436 and rs1867785; Reactions Multiplex PCR and SNaPshot® conditions were standardized in commercial DNA samples: 9948 M, 9947A, K562, C007.

2. Methods

2.1. Primers and probes design

Primers and probes were designed according to Assay Design Suite V 2.0, based on the sequence of HIF-2α gene, available in NCBI and

* Corresponding author.
E-mail addresses: hgermanburgosf@yahoo.com, german.burgos@udla.edu.ec (G. Burgos).

http://dx.doi.org/10.1016/j.fsigss.2017.09.111
Received 3 September 2017; Accepted 19 September 2017
Available online 21 September 2017

Table 1
Sequences of primers and probes used for genotyping with *SNaPshot*® system in *HIF2-α* gene.

Polymorphism	Forward Primer	Reverse Primer	Probes – SBE
rs1868092	AGCCGCCTCCAAATTGAAGT	GTTTGAGAGCCACACTGTTC	ctctcTTCGGGACCTCCATC
rs1867782	TCGACGTGTGAACATTCTGG	CCAAATGCAATATGGGCAG	ctctctctTCTGCCCACTTCTTGA
rs10187368	CAGCGTGGCAGCTAATATCA	TTGGAGAGGAAACCTCAACG	ctctctctctctctAGTTTGCTTCCTGTGATA
rs13419896	CCTGAACCAGAGTCAGTAAC	TGGTTGAGTAGGCCAGTGTC	ctctctctctctctctctctctctGAGTCAGTAACCAATCCTAG
rs4953354	GCAGCATCTAGGATCATGTA	AAGAGGCGAAATGTGCAGAC	ctctctctctctctctctctctctctctAAAGTATTTATGGAGCATATCT
rs895436	GGCACTAAGTTTGGTGATGG	AGACCTCATCAGGTGTGGAC	tctctctctctctctctctctctctctTGTTAAGAAAGGAAAAATAAAGTAG
rs1867785	TTTCGAATGGCAGCGTTCAC	GCTCTGCAGAGGACTTCTC	tctAGCTGGCTGAGGTC

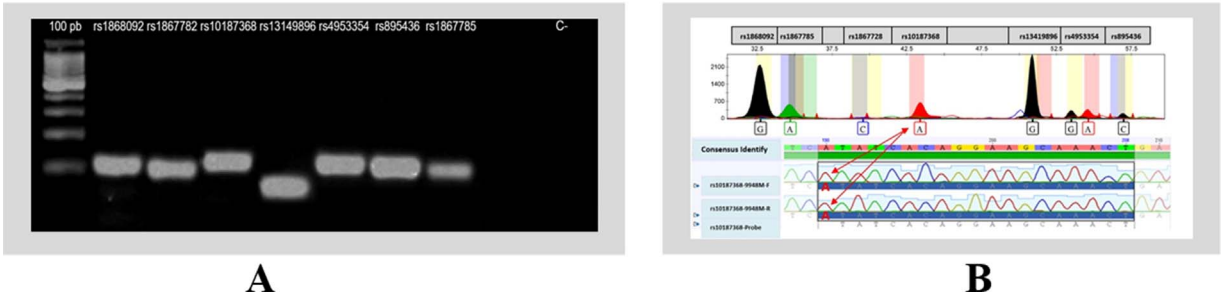

Fig. 1. A. Amplicons from Singleplex PCR standardization of the seven pairs of primers used in the *HIF-2α* gene; **B.** *SNaPshot® Multiplex* and Sanger sequencing reactions of 9948 M control DNA, in the box is observed the homozygous genotype A/A of rs10187368 in both readings.

dbSNP databases. They were evaluated for dimers and hairpins with Autodimer y OligoAnalyzer V 3.1, and later in silico PCR was performed, using Primer-BLAST, UCSC in silico PCR and Multiple Primer Analyzer (Table 1).

2.2. Multiplex PCR reaction

Before Multiplex reaction, it was performed seven reactions of Singleplex PCR, for each pair of primers. Later, for the joint amplification of all amplicons in one Multiplex PCR reaction, the conditions used were: QIAGEN® Multiplex PCR Master Mix 1 X, Primers 1 μM each, DNA 10 ηg/μL. Thermal profile: 95 °C × 15 min, 40 cycles at 94 °C × 1 min, 62,7 °C × 1 min and 72 °C × 1:30 min; and finally, 72 °C × 10 min.

2.3. SNaPshot® reaction

PCR products were digested with 1 U SAP 1 X + 1 U EXO-I 1 X both Illustra™ ExoProStar™, for 37 °C × 1 h and 80 °C × 15 min. Conditions for reaction were: SNaPshot® Multiplex kit 1 X, probes 0.1 to 1 μM and 1,5 μL of purified PCR product. It was subjected to 34 cycles of Single Base Extension of 96 °C × 10 s, 45 °C × 5 s and 60 °C × 30 s; and then SAP purification. Finally, end products were run on ABI 3130 genetic analyzer with LIZ® 120 ladder and analysed with GeneMapper® V. 3.2.

3. Results and discussion

Amplicons of each Singleplex PCR were visualized on 2% agarose gels (Fig. 1A). In that manner, primer pairs were approved for Multiplex PCR, since it was determinated that the primers and probes were working correctly.

The SNaPshot® system designed enabled the joint identification of the seven selected genotypes. Then, those genotypes were validated with Gold Standard method (Fig. 1B). It could be appreciated that the reading of homozygotes and heterozygotes in SNaPshot® reaction is simpler than Gold Standard method. It was determined that Sanger sequencing and SNaPshot® technology have the same sensitivity, but the later was almost 98.4% cheaper.

4. Conclusions

It is the first SNaPshot® Multiplex system designed and developed in Ecuador, and it would allow access to a greater amount of genetic information, with the same sensitivity as the Gold Standard sequencing method, but with significantly lower costs; Also, it would contribute to the forensic sciences in the country since it works in any organism, with lower DNA concentration.

Role of funding

The financial support was provided by MED.GB.17.06 Grant from Dirección General de Investigación, Universidad de Las Américas, Quito, Ecuador. The writing of the report and the decision to submit the paper for publication was taken from Germán Burgos and Mateo Ruano.

Conflict of interest

None.

Acknowledgements

We thank to Dr. Jeppe Dyrberg for primer multiplex and probe design assistance.

References

[1] D. Maglott, J. Ostell, K.D. Pruitt, et al., Entrez gene: gene-centered information at NCBI, Nucleic Acids Res. 33 (2005) D54–D58 (Database issue).
[2] S.A. Patel, M.C. Simon, Biology of hypoxia-inducible factor-2alpha in development and disease, Cell Death Differ. (2008) 2008.
[3] J.J. Sanchez, C. Phillips, C. Borsting, et al., A multiplex assay with 52 single nucleotide polymorphisms for human identification, Electrophoresis 27 (9) (2006) 1713–1724.