Standardization of SSP-PCR protocol for genotyping of HPA 1,2,3,4,5 &15 in North Indian blood donor population

Dr Mridula Pathak 1 , Mr. Charan Sai Ramireddi 2, Prof Dheeraj Khetan 1, Alok Kumar 2 ,Prof Priti Elhence 1
1 Department of Transfusion Medicine, 2 Department of Molecular Medicine
Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India

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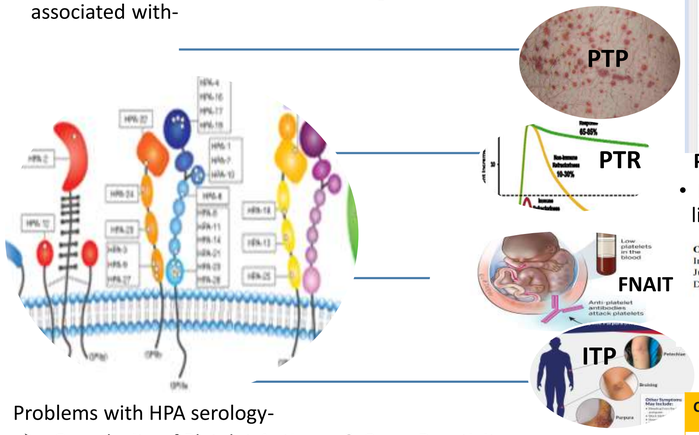
ISBTI (GUJARAT CHAPTER)

INTRODUCTION

- Encoded by the HPA gene cluster located on chromosome 17.
- Till date, 35 antigens categorized among which HPA-1, HPA-2, HPA-3, HPA4, HPA-5, and HPA-15 are biallelic, and expressed on GPIIIa, GPIba, GPIIb, GPIIIa, GPIa, and CD109, respectively
- HPA-1-5 & HPA-15 are of particular interest in the context of transfusion medicine, as mismatches in these antigens have been most often associated with-

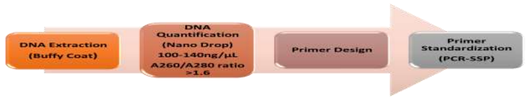


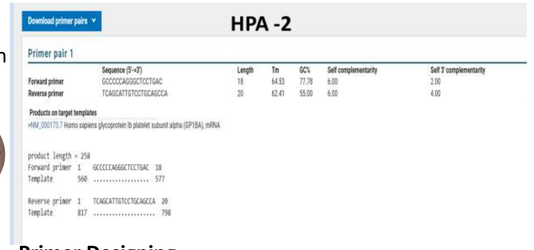
- Problems with HPA serology-
  - Complexity of Platelet Antigens & Cross-Reactivity
  - Low Sensitivity and Specificity of Antibody Detection
  - Limited Availability of Specific Reagents & Lack of Standardized Methods
- Situation in India- due to diverse & genetically complex population of India, there exists a need for a robust and standardized genotyping method to accurately identify the presence or absence of these platelet antigens.
- Advantages of SSP-PCR

OBJECTIVES

Develop a Standardized SSP-PCR Protocol for HPA Genotyping (HPA 1-5 & 15)

METHODS





Primer Designing-

- Primer designing was done based on published literatures(https://doi.org/10.18502/ijaai.v20i3.6333)

ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
June 2021; 20(3):350-363.
Doi: 10.18502/ijaai.v20i3.6333

Genotyping of Human Platelet Antigen-1 to -5 and -15 by Polymerase Chain Reaction with Sequence-specific Primers (PCR-SSP) and Real-time PCR in Azeri Blood Donors

PCR Reaction-

Component	Vol/Reaction
DNA(100-1400 ng/µL)	0.2µL
GoTaq Master Mix (Promega)	5µL
HPA Forward Primer	0.4µL
HPA Reverse Primer	0.4µL
Nuclease free water	4µL
Total	10µL

Gradient PCR

HPA	Mean tempr -1	Mean tempr	Mean tempr +1
HPA 1	59	60	61
HPA 2	61	62	63
HPA 3	61.8	62.8	63.8
HPA 4	56.5	57.5	58.5
HPA 5	53.2	54.2	55.2
HPA 15	48.7	49.7	50.7

Agarose Gel Electrophoresis

Preparation of loading sample (For primer standardization)
•2ul of 2X loading dye + 5ul of sample + 3ul NFW
•For ladder : First add 2ul of ladder then add 2ul of 2X dye and 6ul of NFW
Preparation of Running buffer
Prepared 1.5L of 1X TBE for running Buffer
Parameters:
To run the gel set power accordingly : **90V for 45 minutes.**

World Health Organization NIBSC

PCR-SSP Protocol for HPA-1 to -5 + -15

Primer	Sequence	Product size
HPA -1a HPA -1b Common	5' TCACAGCGAGGTGAGGCCA 3' 5' TCACAGCGAGGTGAGGCCG 3' 5' GGAGGTAGAGAGTCGCCATAG 3'	90bp
HPA-2a HPA-2b Common	5' GCCCCCAGGGCTCCTGAC 3' 5' GCCCCCAGGGCTCCTGAT 3' 5' TCAGCATTGTCCTGCAGCCA 3'	258bp
HPA-3a HPA-3b Common	5' TGGACTGGGGGCTGCCCAT 3' 5' TGGACTGGGGGCTGCCCAG 3' 5' TCCATGTTCACTTGAAGTGCT 3'	293bp
HPA-4a HPA-4b Common	5' GCTGGCCACCCAGATGCG 3' 5' GCTGGCCACCCAGATGCA 3' 5' CAGGGGTTTTCGAGGGCCT 3'	253bp
HPA-5a HPA-5b Common	5' AGAGTCTACCTGTTTACTATCAAAG 3' 5' AGAGTCTACCTGTTTACTATCAAAA 3' 5' CTCTCATGGAAAATGGCAGTACA 3'	252bp
HPA-15a HPA-15b Common	5' TTCAAATTCTTGGTAAATCCTGG 3' 5' TTCAAATTCTTGGTAAATCCTGT 3' 5' ATGACCTTATGATGACCTATTC 3'	226bp
HGH Controls	5' GCCTTCCCAACCATTCCCTTA 3' 5' TCACGGATTTCTGTTGTGTTTC 3'	429bp

The below protocols (1 & 2) works well in some laboratories but not all, and this is presumably due to local differences in PCR machines or reagents , here insufficient PCR product was obtained . So different PCR parameters with the annealing temperatures was used to improve specificity (protocol-3)

Protocol -1

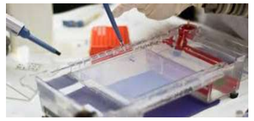
1 cycle 96°/60 sec

5 cycles 96°/25 sec, 70°/45 sec, 72°/30 sec

20 cycles 96°/25 sec, 65°/45 sec, 72°/30 sec

8 cycles 96°/25 sec, 55°/45 sec, 72°/30 sec

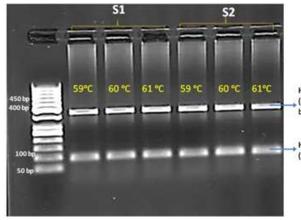
1 cycle 72°/3min

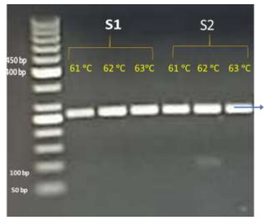


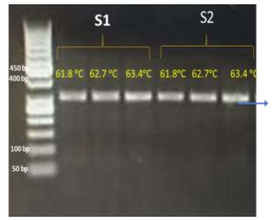
XY Loper, Unitech, Cambridge

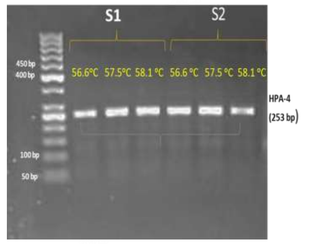
Biometra -PCR

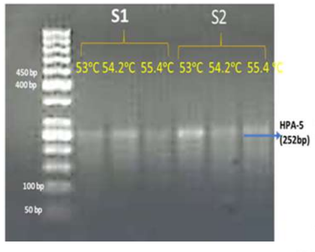
RESULTS (Gradient PCR experiments for HPA primers)

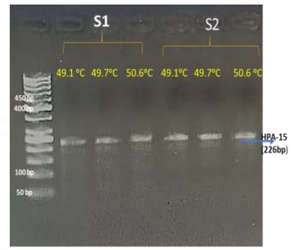












Protocol -2

1 cycle; 96°/60 sec

5 cycles; 96°/25 sec, 68°/45 sec, 72° /30 sec

28 cycles; 96°/25 sec, 61°/45 sec, 72° /30 sec

1 cycle; 72°/3min

Protocol -3

	Temperature	Time	No of Cycles
Activation	96 °C	1 min	1X
Initial Amplification (HPA1-5&15)	96 °C 68 °C 72 °C	25 sec 45 sec 30 sec	5X
Amplification			
Denaturation	96 °C	25 sec	20X
Annealing	HPA 1=60 .0°C HPA 2=62.0 °C HPA 3=62.8 °C HPA 4=57.5 °C HPA 5=54.2 °C HPA 15= 49.7 °C	45 sec	
Extension	72°C	30 sec	
Final Amplification (HPA1-5&15)	96 °C 51 °C 72 °C	25 sec 1 min 2 min	8X
Final Extension (HPA1-5&15)	4 °C	∞	1X

CONCLUSION

SSP-PCR protocol for HPA 1, 2, 3, 4, 5, and 15 was successfully standardized. This will enable us determine frequency of different HPA alleles in our population and further develop HPA typed panels for development of platelet serology.

References

1.(https://www.versiti.org/products-services/human-platelet-antigen-hpa-database)
2.https://www.nibsc.org/science_and_research/biotherapeutics/molecular_immunology/blo od_immunology.aspx
3. https://ijaai.tums.ac.ir/index.php/ijaai/article/view/2885
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Acknowledgment

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