

## A label-free strategy for visual genotyping based on phosphate induced coloration reaction

Jiaying Zhang,<sup>‡a</sup> Hui Hui,<sup>‡c</sup> Wei Xu,<sup>a</sup> Kai Hua,<sup>a</sup> Yali Cui,<sup>\*a</sup> Xiaonan Liu,<sup>\*a</sup>

<sup>a</sup> College of Life Sciences, Northwest University, Xi'an, 710069, China.

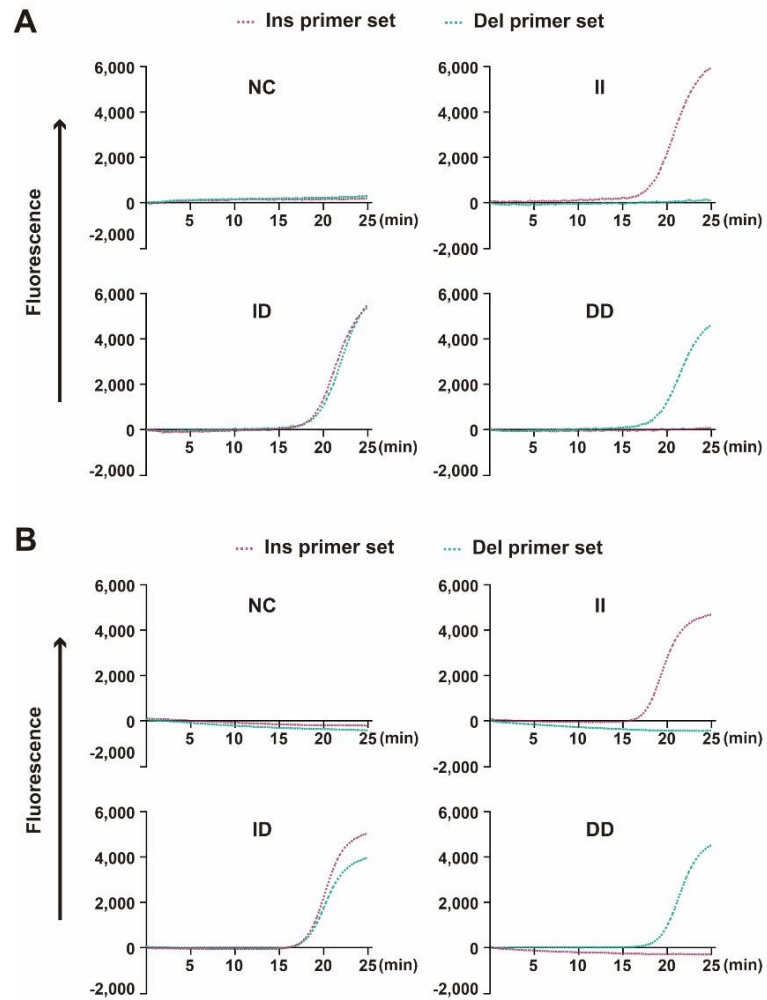
<sup>b</sup> Department of Immunology, The Fourth Military Medical University, Xi'an, 710032, China.

<sup>c</sup> Shanxi Provincial Cancer Hospital, Xi'an, 710061, China.

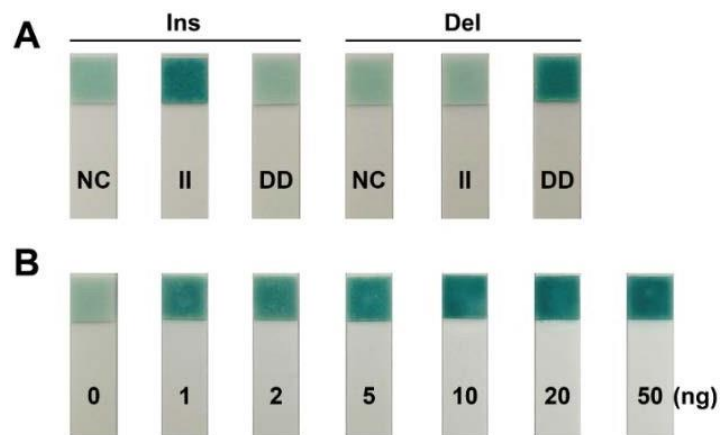
\* Corresponding Authors.

E-mail addresses: yalicui@nwu.edu.cn (Yali Cui), xiaonanliuvip@163.com (Xiaonan Liu).

<sup>‡</sup> Contributed equally.



**Fig. S1.** The amplification kinetics curves of *ACE* indels genotyping with (A) genomic DNA and (B) whole blood respectively.



**Fig. S2.** The response signal with (A) different combinations of primer set and variant and (B)

different concentrations of genome DNA.

**Table. S1.** Primer sequences

Primer	Sequence (5'-3')
F(PCR)	AGGAGAGGAGAGAGACTCAAGCAC
R(PCR)	GGACCCAAGTGCCAGTGATG
FIP-Ins	GTATCACGCCTGTAATCCCAGCACT-CCGGCTAATTTTTTGTATTTTTAGT
FIP-Del	CCACATAAAAGTGACTGTATAGGCAGCAG-AGGCCGGGGACTCTGTAAG
BIP-Ins	TTTCGCCAATTTTATTCCAGCTCTG-CTCCAGCCCTTAGCTCACCT
BIP-Del	TTTCGCCAATTTTATTCCAGCTCTG-CTCCAGCCCTTAGCTCACCT
LF-Ins	AAACGGTGAAACCCCGTCT
LF-Del	AAATGGGAGAAAGGATGGGA
LB-Ins	CTCTGAGCTCCCCTTACAAGC
LB-Del	CTCTGAGCTCCCCTTACAAGC
F3-Ins	GCTCTGTCGCCCAGGCT
F3-Del	AGGAGAGGAGAGAGACTCAAGCAC
B3-Ins	TCAGATCTGGTAGGGGTTTGAAT
B3-Del	TCAGATCTGGTAGGGGTTTGAAT

Note: Ins primer set consists of F3-Ins, B3-Ins, LF-Ins, LB-Ins, FIP-Ins and BIP-Ins, Del primer set consists of F3-Del, B3-Del, LF-Del, LB-Del, FIP-Del and BIP-Del.

**Table S2.** Chromatic difference between the positive and negative results

	Negative 1-Positive 1	Negative 2-Positive 2	Negative 3-Positive 3	Average
$\Delta E^*$	17.7	15.43	15.7	16.28

Note:  $\Delta E^*$  value greater than 6 indicates that chromatic difference can be distinguished by the human eye;  $\Delta E E^* = \sqrt{(\Delta L L^*)^2 + (\Delta a a^*)^2 + (\Delta b b^*)^2}$

**Table S3.** Genotyping results of *ACE* indels

Phosphate induced coloration reaction	PCR-based electrophoresis			Total	Discrepant	Agreement
	II	ID	DD			
II	10	0	0	10	0	100%
ID	0	10	0	10	0	100%
DD	0	0	5	5	0	100%
Total	10	10	5	25	0	100%

**The sequences of *ACE* insertion variant**

AGGAGAGGAGAGAGACTCAAGCACGCCCTCACAGGACTGCTGAGGCCCTGCAGGTGTCTGCAGCA  
TGTGGCCCCAGGCCGGGGACTCTGTAAGCCACTGCTGGAGAGCCACTCCCATCCTTTCTCCCATTCT  
CTAGACCTGCTGCCTATACAGTCACTTTTTTTTTTTTGTAGACGGAGTCTCGCTCTGTGCGCCAGGCTG  
GAGTGCAGTGGCGGGATCTCGGCTCACTGCAAGCTCCGCCTCCCGGGTTACGCCATTCTCTGCCTC  
AGCCTCCAAGTAGCTGGGACCACAGGCGCCGCCACTACGCCCGGCTAATTTTTTGTATTTTTAGTAG  
AGACGGGGTTTACCGTTTTAGCCGGGATGGTCTCGATCTCCTGACCTCGTGATCCGCCCGCCTCGGC  
CTCCCAAAGTGCTGGGATTACAGGCGTGATACAGTCACTTTTATGTGGTTTTCGCCAATTTATTCCAGC  
TCTGAAATTCTCTGAGCTCCCCTTACAAGCAGAGGTGAGCTAAGGGCTGGAGCTCAAGGCATTCAAA  
CCCCTACCAGATCTGACGAATGTGATGGCCACGTCCCGGAAATATGAAGACCTGTTATGGGCATGGG  
AGGGCTGGCGAGACAAGGCGGGGAGAGCCATCCTCCAGTTTTACCCGAAATACGTGGAACATCA  
ACCAGGCTGCCCGGCTCAATGGTGAGTCCCTGCTGCCAACATCACTGGCACTTGGGTCC

**The sequences of *ACE* deletion variant**

AGGAGAGGAGAGAGACTCAAGCACGCCCTCACAGGACTGCTGAGGCCCTGCAGGTGTCTGCAGCA  
TGTGGCCCCAGGCCGGGGACTCTGTAAGCCACTGCTGGAGAGCCACTCCCATCCTTTCTCCCATTCT  
CTAGACCTGCTGCCTATACAGTCACTTTTATGTGGTTTTCGCCAATTTTATTCCAGCTCTGAAATTCTCT  
GAGCTCCCCTTACAAGCAGAGGTGAGCTAAGGGCTGGAGCTCAAGGCATTCAAACCCCTACCAGATC  
TGACGAATGTGATGGCCACGTCCCGGAAATATGAAGACCTGTTATGGGCATGGGAGGGCTGGCGAG  
ACAAGGCGGGGAGAGCCATCCTCCAGTTTTACCCGAAATACGTGGAACATCAACCAGGCTGCCCG  
GCTCAATGGTGAGTCCCTGCTGCCAACATCACTGGCACTTGGGTCC