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Development of LAMP-colorimetric assay for detecting hemoglobin Constant Spring and hemoglobin Pakse'

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Wittaya Jomoui[1]

[1]Srinakharinwirot university

Wittaya Jomoui
Srinakharinwirot university

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We use this protocol and it's working

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Keywords: developed lamp colorimetric phenol red assay, colorimetric assay, detecting hemoglobin constant spring, hemoglobin constant spring, novel molecular detection method, colorimetric phenol, red assay, colorimetric lamp with phenol, colorimetric lamp, using primer explorer v5 software, lamp primer set, primer explorer v5 software, specific with hb c, probe in this study, hb ps for the first time

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Abstract

A novel molecular detection method based on a colorimetric LAMP with phenol red assay was developed to detect Hb CS and Hb PS for the first time. All LAMP primer sets and probe in this study were designed using Primer Explorer V5 software (http://primerexplorer.jp /lampv5e/index.html). The common primer set including the outer primers (F3 and B3) and the inner primers (FIP and BIP) was shown in the **Table**. Furthermore, two loop probe (LP) used in this study were designed for specific with Hb CS (LP-CS) and Hb PS (LP-PS) as also shown in **Table** . These primers set and probes were protected according to petty patent submission number 2203001286 (Thailand). The developed LAMP colorimetric phenol red assay was performed with reaction mixture and temperature as detailed. The LAMP mixture was incubated at an isothermal temperature of 65 °C for 35 minutes. The positive result was observed as yellow color by the naked eye compared with negative with pink color.

LAMP-colorimetric assay for detecting hemoglobin Constant Spring

1 All LAMP primer sets and probe in this study were designed using Primer Explorer V5 software (http://primerexplorer.jp /lampv5e/index.html).

2 Primers for Hb Constant spring

	A	B
	Primers & Probes	**Primer's sequence (5'> 3')**
	F3	TCCCTGGACAAGTTC
	B3	GTTGGCACATTCCGGGATA
	FIP	CGGGCAGGAGGAACGGCTACGAGCACCGTGCTGACCT
	BIP	CCTTGCACCGGCCCTTCCTGAGAACCCAGGCACACAC
	LP-CS	CAGCTTGACGGT

Primers for Hb Pakse'

	A	B
	Primers & Probes	Primers's sequence (5'>3')
	F3	TCCCTGGACAAGTTC
	B3	GTTGGCACATTCCGGGATA

	A	B
	FIP	CGGGCAGGAGGAACGGCTACGAGCACCGTGCTGACCT
	BIP	CCTTGCACCGGCCCTTCCTGAGAACCCAGGCACACAC
	LP-PS	CCAGCATAACGGTA

3 **Reaction mixture**: Hb Constant Spring or Hb Pakse'
A total of 12.5 µL contained 1.5 µL of (20-50) ng/µL genomic DNA, 6.25 µL of WarmStart® Colorimetric LAMP, 1.0 µL of 5M Betaine, 0.2 µL of 10 µM each primer of F3 and B3, 0.4 µL of 40 µM each primer of FIP and BIP, 1.5 µL of 10 µM of LP-CS, and the remainder distilled water.

4 **Isothermal temperature**: 65 ^{0}C, 35 minutes

5 The positive result was observed as yellow color by the naked eye compared with negative with
pink color.