Convenient, Sensitive and High-Throughput Method for Screening Botanic Origin

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Supporting Information

Materials and instruments: Poly[(9,9-bis(6′-*N*,*N*,*N*-triethylammonium)hexyl)fluorenylene phenylene] (PFP) was synthesized according to Feng et al. [18]. Large-fragment *Bst* DNA polymerase and inorganic (yeast) pyrophosphatase were purchased from New England Biolabs Inc.(Connecticut, USA). Shrimp alkaline phosphatase (SAP), Exonuclease I, and Taq DNA polymerase were purchased from TaKaRa Biotechnology Co. Ltd (Dalian, China). *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES) and dimethyl sulfoxide (DMSO) were purchased from Merck Co. Ltd.(Darmstadt, Germany). Fluorescein-12-ddUTP (ddUTP-Fl) and cyanine 3-dGTP (dGTP-Cy3) were purchased from Perkin Elmer. (Hong Kong, China). Primers were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China), with a purified method of ULTRA polyacrylamide gel electrophoresis. Double-distilled water (ddH_2O) was autoclaved at 120 ºC for 15 min. Fresh *Lonicera* spp. plants were sampled from Beijing, Anhui, Guangxi, Shandong, Tianjin, Henan, and Guizhou provinces, China. Dried *Lonicera japonica* buds and twelve patented Chinese drugs were collected from pharmacies in Beijing and Nanjing, China. Fluorescence spectra were measured using a F-4500 spectrofluorimeter from Hitachi Inc. (Tokyo, Japan) equipped with a xenon lamp excitation source. Photographs of polymer solutions were taken using a Canon EOS 550D Digital camera under a hand-held UV lamp (ZF-7A, Shanghai Gucun Electron Optic Instrument Factory) with λ_{max} = 365 nm. For high-throughput product testing, the fluorescence signals were read on a microplate reader (Bio-Tek Synergy HT, USA) equipped with an excitation filter of 380/20 nm. The emission filters were 440/30 nm for PFP, 528/20 nm for Fluorescein and 574/20 nm for cyanine 3.

Samples: Fresh *Lonicera* spp. plants, dried buds (medicinal materials), and twelve patented Chinese drugs were collected **(Table S1, S2)**.

Supplementary Table 1. Plant and medicinal materials samples in this paper

Code	Species	Producing area	Number	surveyor
Plant				
LJ2012001	*Lonicera japonica*	Hefei, Anhui prov.	1	Peng HS
LJ2012002-LJ2012003	*Lonicera japonica*	Wuming, Guangxi prov.	2	Wu QH
LJ2012004-LJ2012048	*Lonicera japonica*	Linyi, Shandong prov.	45	Li SB
LJ2012049-LJ2012058	*Lonicera japonica*	Xiaozai, Henan prov.	10	Liu HY
LJ2012059- LJ2012070	*Lonicera japonica*	Xinmi, Shandong prov.	12	Zhou FQ
LJ2012071- LJ2012094	*Lonicera japonica*	Fengqiu, Henan prov.	24	Liu HY

LJ2012095- LJ2012106	*Lonicera japonica*	Huangdezheng, Henan prov.	12	Zhou FQ
LJ2012107- LJ2012108	*Lonicera japonica var. chinesis*	Beijing	2	Yuan QJ
LH2012001- LJ2012009	*Lonicera hypoglauca*	Henxiang, Guangxi prov.	9	Wu QH
LH2012010- LJ2012017	*Lonicera hypoglauca*	Mashan, Guangxi prov.	8	Wu QH
LH2012018- LJ2012026	*Lonicera hypoglauca*	Ziyuan, Guangxi prov.	9	Wu QH
LH2012027- LJ2012036	*Lonicera hypoglauca*	Ningming, Guangxi prov.	10	Wu QH
LM2012001-LM2012033	*Lonicera macranthoides*	Linyi, Shandong prov.	33	Li SB
LM2012034-LM2012039	*Lonicera macranthoides*	Wuming	6	Wu QH
LM2012040-LM2012048	*Lonicera macranthoides*	Ziyuan, Guangxi prov.	9	Wu QH
LM2012049-LM2012056	*Lonicera macranthoides*	Guiyang, Guizhou prov.	8	Zhou T
LF2012001-LF2012010	*Lonicera fulvotomentosa*	Guilin, Guangxi prov.	10	Wu QH
LF2012011-LF2012018	*Lonicera fulvotomentosa*	Guiyang, Guizhou prov.	8	Zhou T
LC2012001-LF2012005	*Lonicera confusa*	Guiling, Guangxi prov.	5	Wu QH
LC2012006-LF2012008	*Lonicera confusa*	Linyi, Shandong prov.	3	Li SB
LO2012001-LO2012002	*Lonicera similis*	Linyi, Shandong prov.	2	Li SB
LO2012003	*Lonicera microphylla*	Beijing	1	Hao JD
LO2012004	*Lonicera tragophylla*	Linyi, Shandong prov.	1	Li SB
LO2012005	*Lonicera tragophylla*	Beijing	1	Peng HS
LO2012006- LO2012013	*Lonicera tatarica*	Beijing	8	Hao JD
LO2012014-LO2012018	*Lonicera dasystyla*	Lingui, Guangxi prov.	5	Wu QH
LO2012019-LO2012021	*Lonicera dasystyla*	Nanning, Guangxi prov.	3	Wu QH
LO2012022-LO2012031	*Lonicera dasystyla*	Mashan, Guangxi prov.	10	Wu QH
LO2012032	*Lonicer akawakamii*	Beijing	1	Hao JD
LO2012033-LO2012037	*Lonicera maackii*	Beijing	5	Hao JD
LO2012038	*Lonicera maackii*	Hefei, Anhui prov.	1	Peng HS
LO2012039-LO2012064	*Lonicera maackii*	Tianjin	26	Peng HS
LO2012065-LO2012069	*Lonicera fragrantissima*	Beijing	5	Hao JD
LO2012070-LO2012074	*Lonicera pampaninii*	Guiyang, Guizhou prov.	5	Zhou T
Medicinal materials (dried buds)				
LJ2012109-LO2012113	*Lonicera japonica*	Beijing TongRenTang pharmacy	5	Hao JD
LJ2012114-LO2012118	*Lonicera japonica*	Beijing YongAnTang pharmacy	5	Hao JD

Supplementary Table 2. Patented Chinese drugs samples in this paper

Chinese patent drug	Identification method in Chinese Pharmacopoeia	Raw materials number
Qing Re An Chuang Wan	No method	9
Li Yan Jie Du Ke Li	TLC	16
Jian Nao Bu Shen Wan	microscopy	25
Jin Sang San Jie Wan	microscopy	16
Niu Huang Qing Gong Wan	microscopy,TLC	18
Xiao Er Yan Bian Ke Li	TLC	8
Gan Mao Zhi Ke Ke Li	No method	9
Lian Qiao Bai Du Wan	TLC	19
Zhi Zi Jin Hua Wan	microscopy	8
Qing Guo Wan	microscopy	8
Fu Fang Zhen Zhu An Chuang Pian	No method	15
Xiao Er Gan Mao Ning Tang Jiang	No method	15

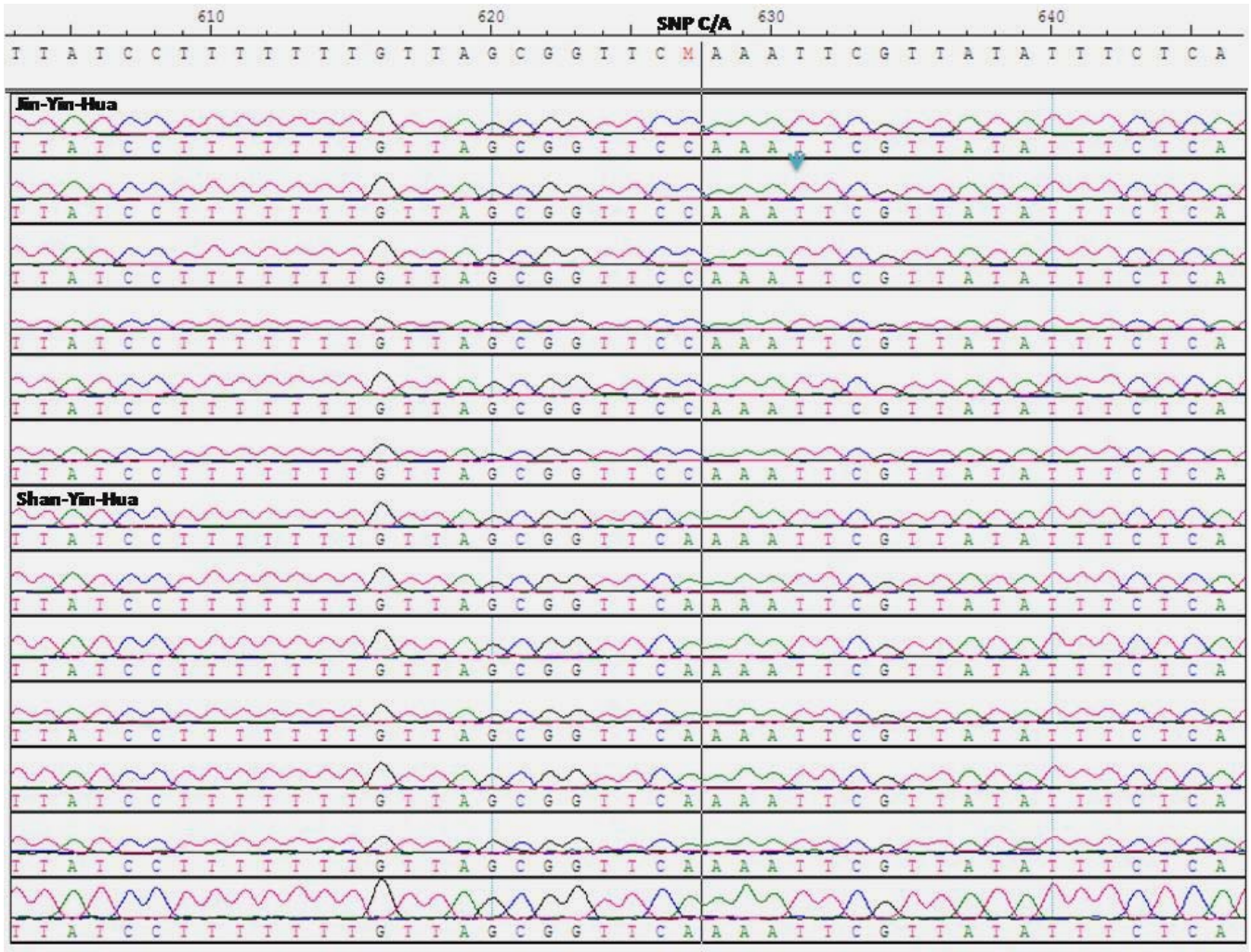

Figure S1. The sequencing results of Jin-Yin-Hua and Shan-Yin-Hua samples at SNP site C/A

Supplementary Table 3. Primers for allele-specific PCR detection

Primer Name	**Primer sequences**	**Amplicons Size(bp)**	Reaction conditions
trnL *trnF*	CGAAATCGGTAGACGCTACG ATTTGAACTGGTGACACGAG	1050	94℃ 5 min, 94℃ 30 s, 54℃ 30 s, 72℃ 45 s, 35 cycles, 72℃ 7 min
trnL.F *trnL*.R	GGTTCAAGTCCCTCTATCC ATTTGAACTGGTGACACGAG	424	94℃ 5 min, 94℃ 30 s, 54℃ 30 s, 72℃ 45 s, 30 cycles, 72℃ 7 min

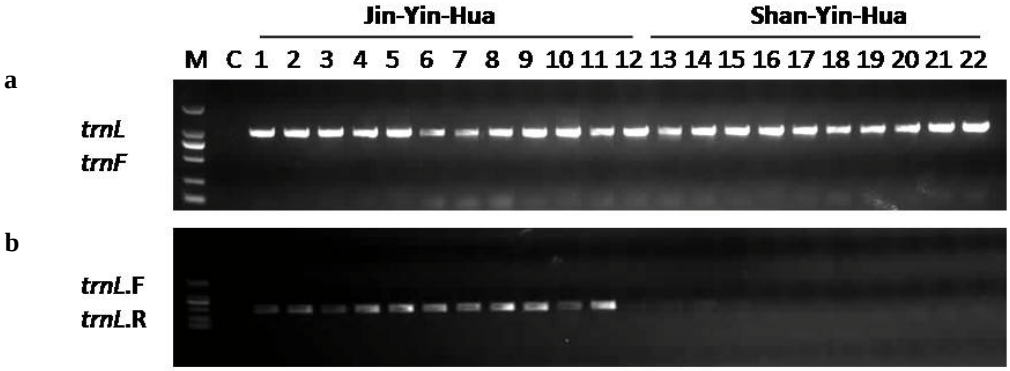

Figure S2. Allele-specific PCR detection using *trnL-trnF* (a) and trnL.F-trnL.R (b) primers. M, 2000bp DNA Ladder. 1-11, Jin-Yin-Hua samples.12-22, Shan-Yin-Hua samples. A no-template control (NTC) was used as the blank.

Supplementary Table 3. Primers for LAMP

Primer Name	**Primer sequences (5'→3')**	**Amplicons Size (bp)**
trnL-FIP	GGAGGATAAATAATTGGGGAGTCAATTTAGAAATCGTGAGGGTTCA	92
trnL-BIP	AGCGGTTCCAAATTCGTTATATTTCAAAACATTTCCGCTCAGATC	
trnL-F3	GTAAGAGGAAAATCCGTCGA	
trnL-B3	TCACAAGACTTGTGATAAGAGA	

Supplementary Table 4. Primers for single base extension

Primer Name	Primer sequences
trnL.SBE	AGTAAGGTGGATGAGAAATATAACGAATTT

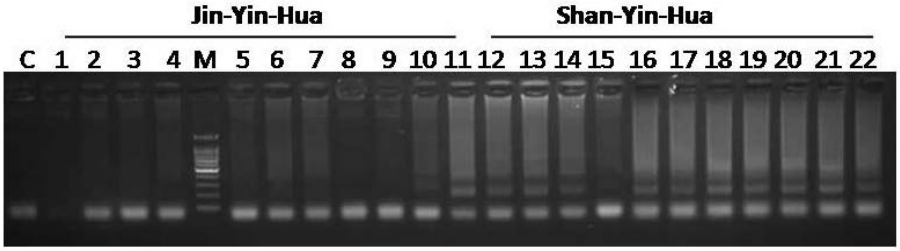

Figure S3. LAMP detection of Jin-Yin-Hua(1-11) and Shan-Yin-Hua(12-22) samples at SNP site C/A. M, 100 bp DNA Ladder.

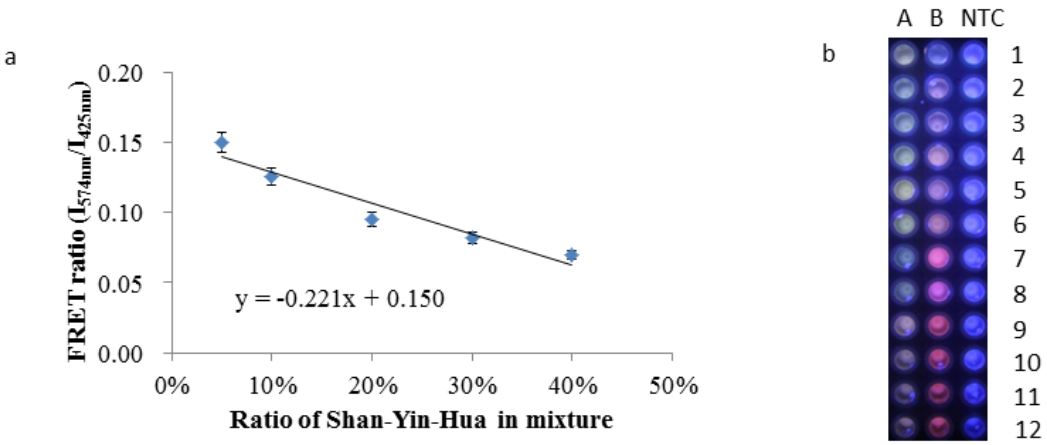

Figure S4. (a) FRET ratios from solutions containing PFP and extension products containing various percentages of Shan-Yin-Hua in mixture. (b) A photograph of fluorescence pattern on a microplate corresponding to mixture containing various percentages of Jin-Yin-Hua and Shan-Yin-Hua, including A1-3, 0:100 (only Shan-Yin-Hua), A4-6, 50:50 (50% of Shan-Yin-Hua), A7-9, 60:40 (40% of Shan-Yin-Hua), A10-12, 70:30 (30% of Shan-Yin-Hua), B1-3, 80:20 (20% of Shan-Yin-Hua), B4-6,90:10 (10% of Shan-Yin-Hua), B7-9, 95:5(5% of Shan-Yin-Hua), and B10-12, 100:0 (only Jin-Yin-Hua).