DNA extraction methods for quality assurance of ginseng and herbal materials

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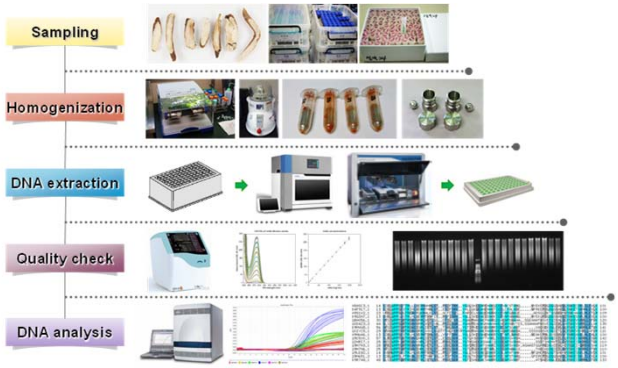
Introduction

- The genetic authenticity of ginseng and herbal resources is one of the most the important issues in the quality assurance of herbal medicines and food supplements.
- The Korea Ginseng Corporation (KGC) has hired the DNA barcoding techniques for confirmation of herbal genetic origin and an automated liquid handling systems for construction of high-throughput analysis systems.



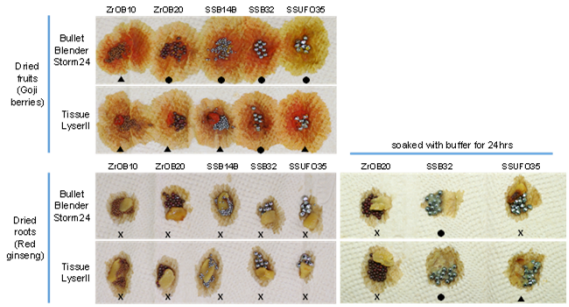
- As plat tissue is very robust, the lysis procedure is most effective with well homogenized, powdered samples. Developing suitable methods include grinding with steel beads is critical for the high-throughput plant genomic DNA extraction.
- There are two kinds of commercially available nucleic acid purification system such as spin column and magnetic beads. These purification systems should be evaluated for using automated liquid handling systems.

Materials and Methods

- Herbal materials : 0.1 g of herbal tissues were used for DNA extraction.
  - Red ginseng was derived from steamed root of 6-year-old *Panax ginseng*
  - Goji was derived from dried fruit of *Lycium chinense*
  - Angelica was derived from dried root of *Angelica gigas*
  - Licorice was derived from dried root of *Glycyrrhiza glabra*
- For grinding samples, various kinds of steel beads were used; ZrOB10 (zirconium oxide beads with 1.0 mm diameter), ZrOB20 (zirconium oxide beads with 2.0 mm diameter), SSB14B (stainless steel beads with 1.4 mm diameter), SSB32 (stainless steel beads with 3.2 mm diameter), and SSUFO35 (3.2 mm stainless steel beads in cone ball shape).
- For lysis of tissue, freshly prepared cetyltrimethylammonium bromide (CTAB) extraction buffer or CTAB-based buffer provided by commercial extraction kit were used.
- For evaluating the spin column-based nucleic acid purification system, NucleoSpin 96 Plant II (Macherey-Nagel, Germany) and DNeasy 96 Plant (Qiagen, Germany) were used. NucleoMag Plant (Macherey-Nagel, Germany) and Ex-DNA Plant (Tianlong, China) were used for testing the magnetic beads-based system.
- To isolate genomic DNA automatically, Microlab Starlet (Hamilton, USA) and Libex (Tianlong, China) were used.

Results and Discussion

Comparison of grinding beads



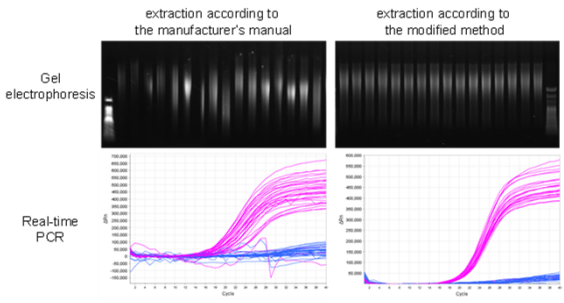
- Sample tissues were effectively disrupted as the beads with over 3.2 mm diameter were used (●). In the case of robust tissue such as red ginseng root, additional soaking step with extraction buffer for 24 hours was required.

Comparison of DNA purification system and extraction buffer

Purification System		Filter Column System						Magnetic Beads System					
Extraction Buffer		provided by extraction kit			CTAB, freshly prepared			provided by extraction kit			CTAB, freshly prepared		
DNA		Average	MAX	MIN	Average	MAX	MIN	Average	MAX	MIN	Average	MAX	MIN
Red Ginseng	Concentration (ug/mL)	24.42 ± 24.86	106.44	7.02	47.20 ± 33.33	175.58	6.83	27.32 ± 11.94	67.12	11.82	69.61 ± 15.76	104.42	47.21
	260/280	1.59 ± 0.19	1.86	1.26	1.91 ± 0.09	1.97	1.67	1.15 ± 0.17	1.53	0.95	1.71 ± 0.15	2.19	1.37
	260/230	0.46 ± 0.10	0.60	0.28	1.46 ± 0.41	2.15	0.85	0.49 ± 0.09	0.61	0.34	1.24 ± 0.19	1.52	0.87
Goji Berries	Concentration (ug/mL)	31.85 ± 30.32	127.50	2.21	42.31 ± 19.37	161.06	6.34	46.80 ± 16.73	161.44	20.00	68.07 ± 24.53	295.87	33.76
	260/280	1.52 ± 0.28	1.94	0.93	1.47 ± 0.32	2.89	1.14	1.22 ± 0.17	1.60	1.00	1.30 ± 0.19	1.72	1.04
	260/230	1.05 ± 0.52	2.26	0.36	1.09 ± 0.45	2.18	0.40	0.84 ± 0.24	1.32	0.49	1.09 ± 0.27	1.81	0.70
Angelica	Concentration (ug/mL)	42.26 ± 18.16	56.63	11.35	101.55 ± 42.42	176.44	65.77	110.08 ± 25.16	141.15	70.77	318.62 ± 97.74	464.52	191.54
	260/280	1.81 ± 0.07	1.88	1.71	1.66 ± 0.18	1.91	1.42	1.36 ± 0.13	1.49	1.14	2.00 ± 0.14	2.12	1.76
	260/230	1.51 ± 0.32	1.66	0.87	1.49 ± 0.30	1.87	1.14	1.03 ± 0.20	1.27	0.68	2.01 ± 0.13	2.12	1.79
Licorice	Concentration (ug/mL)	33.75 ± 19.51	96.44	5.96	76.46 ± 41.91	199.23	33.17	43.08 ± 28.22	168.37	13.85	236.09 ± 98.34	464.71	104.04
	260/280	1.50 ± 0.64	1.55	0.40	1.18 ± 0.11	1.46	1.01	1.37 ± 0.14	1.71	1.16	1.47 ± 0.21	1.84	1.13
	260/230	1.02 ± 0.59	1.90	0.14	0.49 ± 0.12	0.77	0.31	0.84 ± 0.19	1.58	0.56	1.05 ± 0.22	1.49	0.77

- Although the indicators of DNA purity (260/280 and 260/230) were slightly lower than the spin column-based system, the magnetic beads-based purification system with freshly prepared CTAB buffer provided the largest amount of genomic DNA from herbal tissues.

High-throughput analysis using the modified DNA extraction methods



- The modified DNA extraction methods showed improved quantity and quality of isolated DNA which are suitable for automated high-throughput analysis.

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