A CTAB procedure of total genomic DNA extraction for medicinal mushrooms

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Introduction

Molecular activities are getting important and advanced technology in fingerprinting required a pure total genomic DNA. Further, appearance of new high-throughput sequencing technologies also involves development of high-throughput methods of DNA extraction from mushroom. As mushroom is getting popular for nutraceutical, nutriceutical, pharmaceutical and cosmeticeutical, the DNA fingerprinting is required in assessing species and certifying strains for industrial used. Isolation of intact, high-molecular-mass genomic DNA is essential for many molecular biology applications including long Polymerase Chain Reaction (PCR), endonuclease restriction digestion, Southern blot analysis, and genomic library construction. The most important and prerequisite towards reliable molecular biology work is a good quality of total genomic DNA. The objective of this works is to optimize total genomic DNA extraction of medicinal mushrooms towards high quality intact genomic DNA for molecular activities.

Methodology

Ganoderma lucidum

Schizophyllum commune

Pleurotus sajor caju

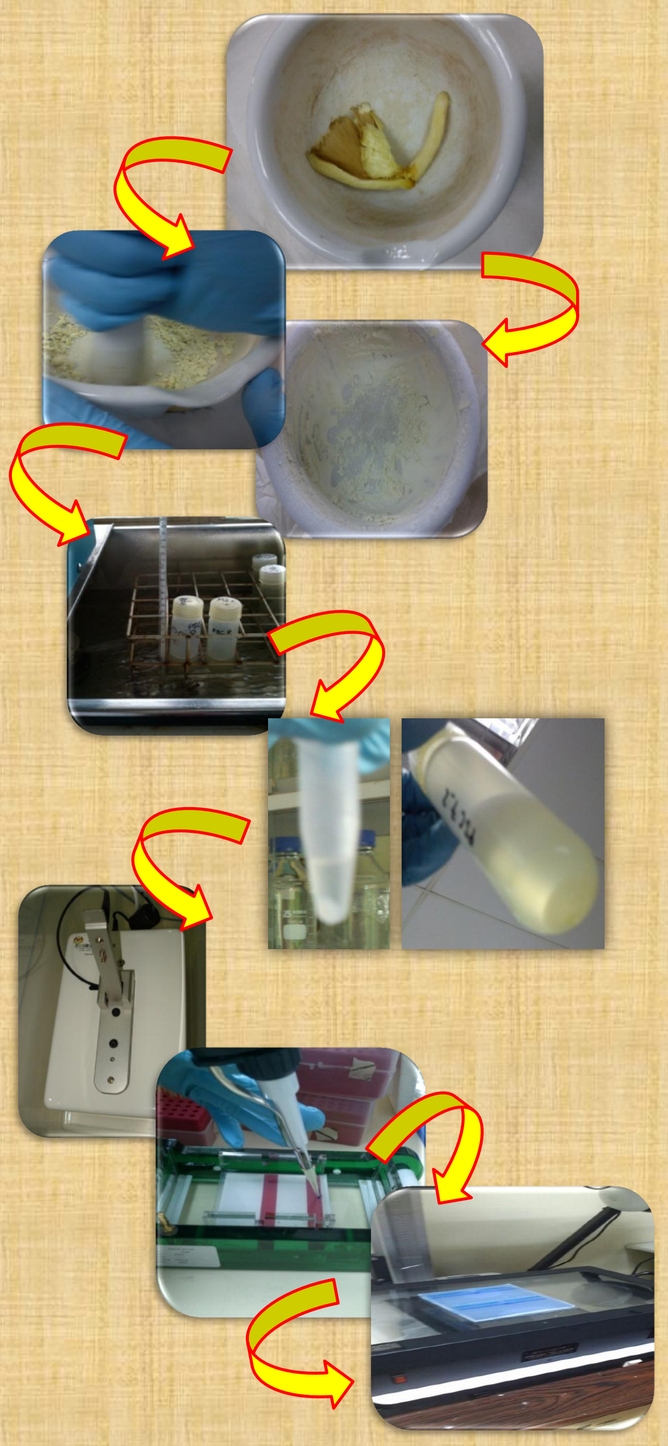
Lentinus edodes

Pleurotus cystidiosus

Hericium erinaceus

Auricularia polytricha

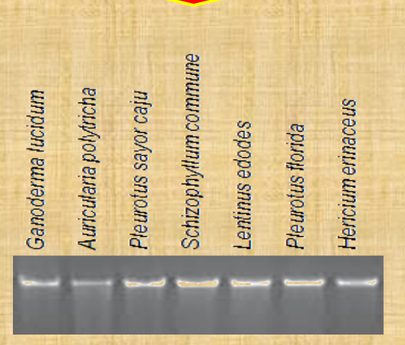
A standard Doyle and Doyle protocol of CTAB was revised in optimizing the total genomic DNA from the medicinal mushroom. The modification parameters were percentage of CTAB, incubation period and temperature. 5 mg of each sample were used for DNA extraction and prepared in 5 replications.



Results

The results revealed that each sample required a certain combinations of time and period of incubation period. Percentage of CTAB in buffer was found significant in giving high yielding of extracted total genomic DNA. The extracted total genomic DNA from the medicinal mushroom yielded from 39.7 ng/ul to 919.1 ng/ul. Different yield among mushroom species found to be corresponded to polysaccharide content in the medicinal mushrooms.

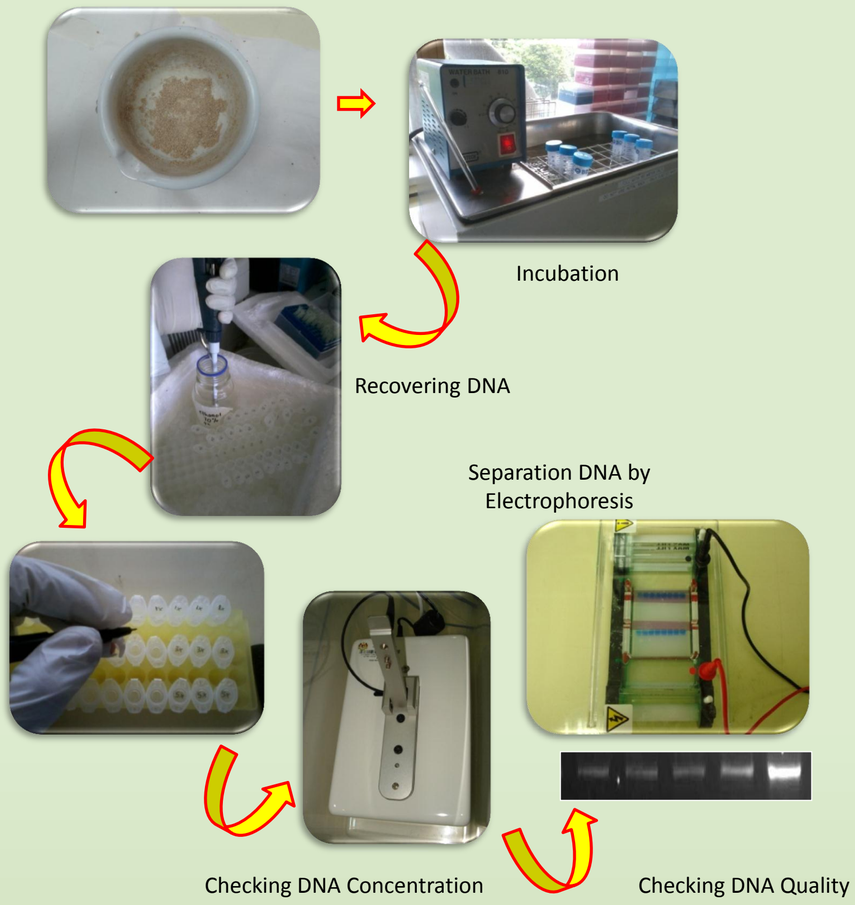
Samples	Treatment	OD $_{260/280}$	Concentration (ng/µl)	Total volume (µl)
Ganoderma lucidum	3% CTAB; 60min; 60C	2.2 ± 0.01	340.4 ± 22.5	1500
Auricularia polytricha	1% CTAB; 30min; 65C	1.6 ± 0.14	68.4 ± 24.9	1500
Pleurotus sayor caju	3% CTAB; 60 min; 60C	1.9 ± 0.01	800 ± 12.1	1500
Schizophyllum commune	1% CTAB; 60min; 65C	2.0 ± 0.04	4040± 148.7	1500
Lentinus edodes	5% CTAB; 30min; 60C	2.0 ± 0.07	648.7 ± 83.6	1500
Pleurotus florida	3% CTAB; 30min; 60C	2.2 ± 0.01	1160 ± 13.7	1500
Hericium erinaceus	3% CTAB; 30min; 60C	2.0 ± 0.06	161.3 ± 24.9	1500



Total genomic DNA derived from seven medicinal mushrooms separated by 1% Agarose gel in TBE buffer at 70V for 30 minutes

Conclusion

The CTAB procedures have described a simple, safe, reliable, and cost-efficient CTAB DNA extraction method that provides high-quality DNA from medicinal mushrooms containing elevated concentrations of polysaccharide and polyphenolics compounds
This method is recommended even in low-technology laboratories for high-throughput sample preparation suitable for various molecular analytical techniques.


Incubation
Recovering DNA
Separation DNA by
Electrophoresis
Checking DNA Concentration
Checking DNA Quality