Annals of Forest Science (2018) 75: 43
https://doi.org/10.1007/s13595-018-0705-y

ORIGINAL PAPER

Rapid and economical protocols for genomic and metagenomic DNA extraction from oak (*Quercus brantii Lindl.*)

Elahe Ahmadi[1,2] · Mojegan Kowsari[1] · Davoud Azadfar[2] · Gholamreza Salehi Jouzani[1]

Received: 15 July 2017 / Accepted: 30 January 2018 / Published online: 28 March 2018

Abstract

• ***Key message*** **Two new efficient, fast and low-cost metagenomic DNA extraction methods were developed for different Persian oak tissues (leaf, stem, root, and rhizospheric) and soil samples.**
• ***Context*** The new "omics" studies on the genus *Quercus* are of importance to help finding efficient strategies for overcoming environmental challenges, and to do this, presence of efficient DNA extraction protocols for different *Quercus* species are very critical.
• ***Aims*** The objective of the present study was to develop new efficient methods for extraction of metagenomic DNA (mDNA) from of Persian oak (*Quercus brantii* Lindl.) tissues.
• ***Methods*** The efficiency of two newly developed mDNA extraction methods, including indirect SDS-based (ISB or concentrate method) and one spin column-based method (SCB) were compared to that of two classical direct methods, including CTAB-based and SDS-based methods, and two commercial mDNA extraction kits.
• ***Results*** The maximum average yield of mDNA for all samples (leaf, stem, root, bulk, and rhizospheric soils) was obtained by SCB (258 ng/μl) and ISB (189 ng/μl) methods, respectively. Successful PCR amplification for 16Sr*RNA* and ITS sequence was consistently observed for ISB, SCB, and kit-extracted mDNAs, which confirmed the high purity of mDNA extracted by these methods. The new methods showed more than 96% quantitative PCR efficiency, and partial restriction digestion and metagenomic library construction confirmed the high efficiency of the newly developed methods.
• ***Conclusion*** It could be concluded that two new protocols enhanced efficiency (yield, purity, and cost) of mDNA extraction from different tissues of Persian oak.

Keywords Indirect SDS-based method (ISB) · Metagenomic DNA extraction · Microbiome · Oak decline · *Quercus brantii* · Spin column-based method (SCB)

Handling Editor: Ricardo Alia

Contribution of the co-authors All the co-authors have made contribution to the manuscript. Eng. Elahe Ahmadi collected the oak samples, developed the mDNA extraction protocols and performed the data analysis.
Dr. Mojegan Kowsari supervised the work and contributed in designing the experiments. Dr. Davoud Azadfar supervised the work and contributed in designing the experiments. Prof. Gholamreza Salehi Jouzani contributed in designing the experiments and wrote the manuscript.

✉ Gholamreza Salehi Jouzani
gsalehi@abrii.ac.ir

Elahe Ahmadi
Ahmadi_silva@yahoo.com

Mojegan Kowsari
Kowsari@abrii.ac.ir

Davoud Azadfar
Azadfar.d@gmail.com

[1] Microbial Biotechnology Department, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Fahmideh Blvd, P.O. Box: 31535-1897, Karaj, Iran

[2] Department of Silviculture and Forest Ecology, Faculty of Forest Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

1 Introduction

The genus *Quercus* is one of the most important clades of woody angiosperms in the northern hemisphere in terms of species diversity, ecological dominance, and economic value. This genus provides important economic benefits and high sociocultural value, and its role in water and soil protection in different regions of the world, such as Europe (e.g., UK, Slovakia, Czech Republic, Hungary, Austria, Germany, Italy, France, Ukraine, Belarus, and Moldova), North and Central America (e.g., USA, Mexico, Belize, Canada, Colombia, Guatemala, and El Salvador), and Asia (e.g., Iran and Indonesia), led to increasing attention to this genus (Nixon 2006; Heydari et al. 2016). *Quercus* is the most frequent genus of the Fagaceae family in forests of Iran (Panahi et al. 2012), and several species of oaks grow abundantly in Zagros forests in the west region of Iran. The most important species of the region exhibiting remarkable morphological variation are *Quercus brantii* Lindl., *Quercus libani* Oliv., and *Quercus infectoria* Oliv. (Khalyani and Mayer 2013; Rahmani et al. 2015). Persian oak (*Q. brantii* Lindl.) is the most dominant tree species in the natural forest ecosystems over large areas of the western region of Iran, covering an approximate area of 5 million hectares and have at least 5500-year-old antiquities (Ahmadi et al. 2014). The *Q. brantii* is a complex species which contain 12 taxa, including 7 species and 5 varieties (Djavanchir Khoie 1967). During the last two decades, new molecular biology and high-throughput "omics" methods, such as next-generation sequencing (NGS: the Illumina HiSeq and TruSeq platforms), genomics, transcriptomics, proteomics, metabolomics, metagenomics, genetic mapping, and population genomics have opened new windows for forestry scientists to explore more deeper phylogeny and evolutionary relationships among different forest species and ecotypes, characterize physiological responses to biotic and abiotic stresses in trees at molecular level, study plant-microbe interactions, and to detect metabolic pathways in trees for production of different metabolites. They also help forest pathologists to detect, identify, and monitor forest pathogens and sequence their entire genome, examine global distributions of forest pathogens and their hosts, assess the diversity and structure of host and pathogen populations, and evaluate the structure and function of genes as well as their levels of expression within species and within communities (Kaul et al. 2016; Plomion et al. 2016; Ross-Davis et al. 2013). Recently, these new technologies, such as NGS, have been applied for oak species to evaluate their phylogeny and evolutionary relationships (e.g., Alexander and Woeste 2014; Fitz-Gibbon et al. 2017; Schroeder et al. 2016; Sork et al. 2016a; San Jose-Maldia et al. 2017), explore structure of different populations (Degner 2014), study their molecular and physiological mechanisms for biotic and abiotic stress tolerance (e.g., Magalhães 2015; Guerrero-Sanchez et al. 2017; Plomion et al. 2016; Rellstab et al. 2016; Sork et al. 2016b; Usié et al. 2017), characterize chloroplast genomes of oaks (Yang et al. 2016), and to examine plant-microbe interactions in these trees (e.g., Caravaca et al. 2015; Fernandes et al. 2014; Moore et al. 2015; He et al. 2016; Koide et al. 2017).

To achieve reliable molecular data in oak studies, efficient DNA extraction protocols are required for different species and ecotypes. Therefore, isolating high quantities of contaminant-free genomic DNA for downstream applications from different tissues of species, especially those species rich in different contaminants, such as sugars, polyphenolics, and terpenoids, prompt the urgent need to revisit, adapt, and improve DNA extraction protocols (Barta et al. 2017). Previously, many molecular studies have been performed on different species of oak trees using classical DNA extraction procedures, such as SDS or CTAB (e.g., Pandey and Tamta 2015; Toader et al. 2009; Makela et al. 2016) and different commercial kits (e.g., Hipp et al. 2014; Barta et al. 2017; Vranckx et al. 2014). However, oaks show large phenotypic and genotypic variations (e.g., physical size, leaf and stem type, metabolites, and genome size and structure), and DNA extraction from some species mainly from warmest climates is not as efficient as for temperate oaks or it is even deficient (Finkeldey et al. 2010; Finch-Savage 1992; Sunderlíková et al. 2009). For example, it has been confirmed that some oak species such as *Q. robur* and *Q. petraea* are suitable for efficient extraction and purification of genomic DNA, plastid DNA, and RNA which facilitated the development of a large number of genomic resources. However, some other species, such as *Q. pyrenaica*, *Q. pubescens* x *Q. faginea* hybrids, certain *Q. ilex* ecotypes, etc., present more difficulties for DNA extraction/purification (Bodénès et al. 2012; Goicoechea et al. 2015), due to several factors, such as pubescence, glue-like components in the cuticle, and some inhibitor metabolites. In addition, presence of polysaccharides and phenolic compounds prevent the use of DNA for molecular biology purposes, such as PCR, restriction digests, or sequencing by inhibiting the action of polymerases or endonuclease (Sahu et al. 2012; Healey et al. 2014; Rawat et al. 2016).

The classical DNA extraction methods are extremely time-consuming, either relying on long incubation steps, use hazardous chemicals, nuclei pre-extraction that increases handling time, or requiring multiple DNA washes and precipitations that decrease overall yield (Tibbits et al. 2006; Chabi Sika et al. 2015). Kit-based extraction methods, which are intended to easily remove contaminants, are often expensive, which prevents their use especially when large numbers of samples are required. Furthermore, it has been previously proved that the classical protocols enabled access to more diverse endophytic bacterial communities than commercial kits (Niu et al. 2008; Sahu et al. 2012). Robust lytic processes (e.g., SDS-treatment) are able to rupture a broader range of bacterial communities (Yuan et al. 2012; Maropola et al. 2015). Metagenomics is known as one of the most important NGS methodologies recently used for plant-microbe interactions and associations studies. However,

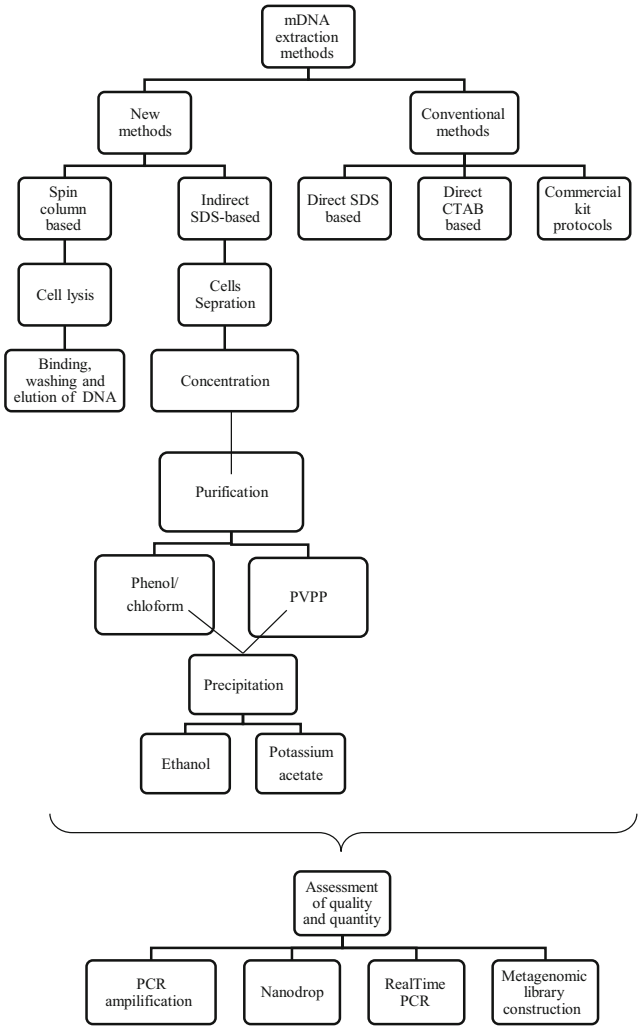

Fig. 1 Flow chart showing experimental design of different mDNA extraction methods from different plant tissues and soil samples (leaf, root, shoot, rhizospheric, and bulk soil)

this new technology has not been widely used for those studies in oak species. Up to now, a few such studies have been focused on determination of microbial communities associated with oak species. For instance, it has been used to characterize microbiome associated with acute oak decline (Denman et al. 2017) and English oak trees (*Q. robur*) with different ages and locations (Meaden et al. 2016). It has been also used to determine the diversity of a *Q. pyrenaica* Willd. rhizospheric microbiome in the Mediterranean mountains (Cobo-Díaz et al. 2017) and to evaluate dynamics of fungal communities in a temperate oak forest soil (Voříšková et al. 2014).

These events show that achieving more efficient genomic and metagenomic DNA extraction protocols for oak species, especially for those recalcitrant species (in view of DNA extraction problems), is of importance. Therefore, the objective of the present study was to increase the efficiency of genomic DNA and metagenomic DNA extraction procedures for a "recalcitrant" species from the region (*Q. brantii*). To do this, two strategies, including indirect SDS-based methods (ISB) and spin column-based (SCB), were used. The first strategy (ISB) included the early extraction of bacterial and fungal cells from the matrix, and then cell lysing and DNA purification. The second strategy (SCB) was based on the in situ lysis (the direct disruption of the microbial cell walls without primary separation of microbial cells from tissues or samples) of bacteria and fungi prior to DNA recovery and purification using spin column to limit mechanical shearing of the DNA, contact between DNA and sample (soil or tissues) components, and DNA degradation.

2 Material and methods

2.1 Sampling site and procedures

Zagros forests, as primary oak forests, stretch along the Zagros Mountains in western Iran from north to south. Oak tree samples were collected from Eastern Hillside of the Tange Dalab situated in Ilam province (S26°44,302′ E027°05584′, Ilam Province, western Iran) during the summer season (June, 2016). Three oak trees with decline symptoms were selected following a random sampling technique. Stem, root, and leaf samples were excised from each tree and placed in the plastic bags. After labeling of the samples, they were frozen in the liquid nitrogenous and stored at − 80 °C. The same samples (root, shoot, or leaf) from different trees were pooled and aseptically ground to a fine powder in liquid nitrogen using autoclaved pestle and mortar. The sampling strategy from soil (bulk and rhizosphere) consisted of taking randomized soil samples from the depth of 10–15 cm below the soil surface of four areas of the plot. The soil samples were manually homogenized by thorough physical mixing, immediately placed on ice, and transported to the lab where they were stored at − 80 °C prior to processing. The mDNA extraction procedure was repeated three times for each sample.

2.2 Metagenomic DNA extraction procedures

Two new mDNA extraction methods, including indirect SDS-based (ISB or concentrate method) and one spin column-based method (SCB), were developed in the present study. Two previously reported classical direct methods, including direct CTAB-based (DCB) (Doyle and Doyle 1987) and SDS-based (DSB) for tissue (Zhou et al. 1996) and soil (Qu et al. 2009) samples, and two commercial kits (MoBio PowerSoil® DNA Isolation Kit for soil samples and QIAGEN DNeasy Plant Mini Kit for tissue samples) were used as control.

The direct mDNA extraction methods were consisted of direct cell lysis within samples, whereas in the indirect method (ISB) at the first step, microbial cells were separated via a concentration method by two consequent centrifugations in PBS buffer, and then mDNA was extracted (Fig. 1). At the

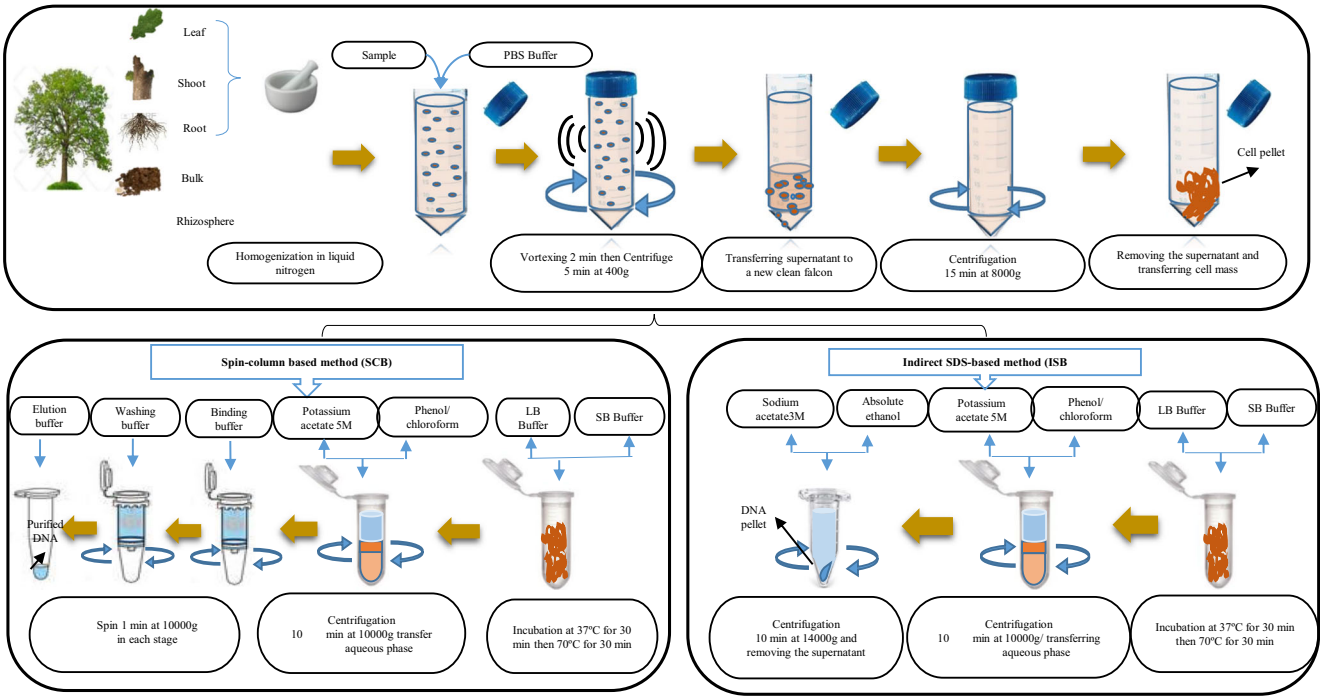

Fig. 2 The schematic of two newly developed mDNA extraction methods, including indirect SDS-based (ISB or concentrate method) and one spin column-based method (SCB)

final stage, the extracted mDNA was air dried at room temperature for 30 min and dissolved in 50 μl of TE buffer (Tris 10 mM, EDTA 1 mM) for normalization purposes. All mDNA extraction methods were carried out in triplicate.

2.3 New ISB mDNA extraction method

Three grams of rhizospheric or bulk soil, powdered leaf, shoot, or root samples were suspended in 50 ml of the PBS as cell extraction buffer (Uquillas et al. 2011). The soil and tissue suspensions were continuously mixed for 3 min at 25 °C on tube rotator (SLM-TR-100, Bangalore GeNei) with the speed of 160 rpm. Then, the homogenous mixture was centrifuged at lower speed of 400×*g* for 5 min at room temperature. The supernatant was collected in a clean falcon, and the cell mass was harvested at 8000×*g* for 15 min at room temperature. After centrifugation, the PBS buffer was poured off, and the bacterial and fungal cells remained a pellet at the bottom of the tube. The mDNA was extracted by a two-step cell lysis using a combination of chemical (enzymatic lysis and hot detergent lysis) and physical (vortex and grinding) methods. Initially, the obtained cell mass was transferred to a 2-ml tube, resuspended in 500 μl of the suspension buffer (SB: 10 mM Tris–HCl; 1 mM EDTA; lysozyme 20 mg/ml^{-1}; proteinase K 30 μl, 20 mg/ml^{-1}) by vortexing and inverting the tubes, and incubated at 37 °C for 30 min. The resultant cell lysate was further lysed with 500 μl of lysis buffer (LB: 100 mM Tris–HCl; 50 mM EDTA; 0.5 M NaCl; 4% SDS; 2% polyvinylpolypyrrolidone (PVPP)). The lysis buffer was added over the SB buffer and kept at 70 °C for 30 min with intermittent mixing at every 5-min interval, followed by addition of 250 μl potassium acetate (5 M, PH = 5.5). Equal volume phenol/chloroform was added to each tube and mixed by inversion. Top aqueous phase containing DNA was collected after centrifugation. The extracted mDNA was precipitated from the aqueous phase by adding 60 μl of sodium acetate (3 M, pH 5.2) and two volumes of absolute ethanol and was incubated for 5 min under ambient conditions. The final DNA precipitate was pelleted at 14,000×*g* (Sigma 1-14 k Refrigerated Centrifuge, Osterode am Harz, Germany) for 10 min.

Table 1 The primer sets used in the study

Type	Primer sequences	Product size (bp)	Reference
Fungal ITS	ITS1 F: 5′ TCCGTAGGTGAACCTGCGG 3′ ITS4 R: 5′ TCCTCCGCTTATTGATATGC 3′	600 to 800	Verma and Satyanarayana (2011)
Bacterial 16S rDNA	27F: 5′-AGAGTTTGATCCTGGCTCAG-3’ 1541R: 5′-AAGGAGGTGATCCAGCC-3′	1500	Embarcadero-Jiménez et al. (2014)

Table 2 Key differences in various DNA extraction protocols used for mDNA extraction from soil and tissue sample

DNA extraction method	Method code	Processing time (h)	Cost	Extraction buffer use	Separation phase	DNA precipitation
Direct CTAB-based	DCB	5	Low	1 ml CTAB buffer	β-mercaptoethanol0.2% + 10 mM ammonium acetate 5 M + 1 vol C/IA	1 vol cold isopropanol
Direct SDS-based	DSB	5–6	Low	1 ml SDS buffer	1 vol C/IA	2 vol cold absolute ethanol
Indirect SDS-based	ISB	1:30	Low	PBS (or TEN) + SDS + LB Buffer	PVPP +250 μl Potassium acetate 5 M+ 1 vol P/C/IA	2 vol cold absolute ethanol and 60 μl sodium acetate 3 M
Spin column-based	SCB	1:30	Medium	PBS + SDS + LB Buffer	PVPP + 250 μl Potassium acetate 5 M+ 1 vol P/C/IA	None
Commercial kit	CK	45 min	High	Kit supplied	Kit supplied	None

2.4 SCB method

This method relies on the fact that nucleic acids bind to the solid phase of silica under certain conditions. The column-based DNA extraction was adapted using the first three steps of the concentrate method, followed by the use of a silica-based DNA-binding spin-bind columns (EconoSpin (TM), Epoch Life Science, Texas, USA). Briefly, the samples were processed similar to the concentrate method till the third step when lysed cells were treated with chloroform/phenol/isoamylalcohol solution. Then, the solutions were spun at 13,000 rpm for 10 min prior to the transfer of supernatant to equilibrated columns. Top aqueous phase containing DNA was transferred to spin columns. Equal volume of the binding solution (5 M guanidinium hydrochloride, 20 Mm Tris, 0.2 M NaCl, absolute ethanol) was added to the spin column, and after centrifugation, the flow through was removed. Then, 750 μl washing buffer (80% ethanol and 10 mM Tris–HCl) was added to the column. The washing buffer was removed, and 50 μl elution buffer (TE buffer) was added to the column. Total DNA was separated from the membrane using the elution buffer and collected from the bottom of the column. The schematic process of two newly developed mDNA extraction methods, including ISB and SCB, is shown in Fig. 2.

2.5 Assessment of yield and purity of the extracted mDNAs

Equal volume (5 μl) of all mDNAs extracted by different methods was loaded in 1% agarose gel along with 1 μl of 1 Kb DNA ladder, and the bands were visualized using UVP Multidoc IT digital imaging system (UVP LCC, California, USA). The purity and concentration of mDNAs was determined by spectrophotometry analysis (*UV Spectrophotometer*, *Eppendorf* North America).

The extracted mDNAs were quantified on a Nanodrop spectrophotometer (Implen GmbH, 140 München, Germany), and their purity was expressed as ratios of absorption at A260/A280 and A260/A230. Expected 260/230 values are commonly in the range of 2.0–2.2. If the ratio is appreciably lower than expected, it may indicate the presence of polyphenolics, salts, and humic acid contaminants which absorb at 230 nm whereas 260/280 nm ratio of ~ 1.8 is generally accepted as pure for DNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol, or other contaminants that absorb strongly at or near 280 nm (Sharma et al. 2014).

Table 3 Comparison of the yield of mDNA (in ng/μl) obtained by different methods for different sample

Sample/method	DSB	DCB	ISB	SCB	CK
Shoot	261.6 + 44.9a[a]	193.6 + 3.2b	211.4 + 34.7ab	195.2 + 0.4b	269.1 + 2.9a
Root	120.9 + 0.9d	260.6 + 52.9b	305 + 44.1a	197 + 1.5c	61.2 + 0.2e
Leaf	61.2 + 0.2d	37.9 + 6.2e	205 + 5.62b	401 + 2.4a	157.3 + 8.7c
Bulk	106.7 + 1.9b	4.2 + 0.6e	47.9 + 0.5c	297.2 + 15.5a	16.1 + 1.4d
Rhizosphere	165.4 + 0.4c	16.1 + 1.4e	175.3 + 7.4b	201.3 + 0.4a	37.9 + 8.2d

The means followed by different lowercase letters within each row are significantly different ($p < 0.01$)

[a] The statistical analysis was separately performed between different methods for each sample

Table 4 Purity of metagenomic DNA obtained by different methods for different samples ($A_{260/280}$ and $A_{260/230}$)

	$A_{260/280}$					$A_{260/230}$				
Sample/method	Leaf	Shoot	Root	Rhizosphere	Bulk	Leaf	Shoot	Root	Rhizosphere	Bulk
DSB	1.29 ± 0.08b*	1.86 ± 0.04a	1.71 ± 0.03ab	1.41 ± 0.03c	1.44 ± 0.04c	2.45 ± 0.4a	2.23 ± 0.2c	2.55 ± 0.2a	2.033 ± 0.1bc	2.36 ± 0.2a
DCB	1.27 ± 0.04b	1.52 ± 0.04b	1.63 ± 0.04b	1.3 ± 0.03d	1.48 ± 0.026c	0.64 ± 0.0c	1.7 ± 0.2d	1.39 ± 0.1c	0.58 ± 0.1d	0.37 ± 0.0c
ISB	1.82 ± 0.02a	1.86 ± 0.04a	1.6 + 0.12b	1.75 ± 0.03b	1.78 ± 0.03b	2 ± 0.1b	2.86 + 0.2a	2.25 + 0.1b	2.39 ± 0.1a	2.12 ± 0.1a
SCB	1.76 ± 0.04a	1.82 ± 0.36a	1.85 ± 0.04a	1.74 ± 0.04b	1.85 ± 0.035a	2.1 ± 0.11b	2.76 ± 0.2a	2.25 ± 0.1b	1.92 ± 0.1c	1.92 ± 0.1b
CK	1.86 ± 0.036a	1.89 ± 0.02a	1.77 ± 0.065a	1.82 ± 0.037a	1.81 ± 0.03a	2.55 ± 0.1a	2.51 ± 0. 1b	2.64 ± 0.1a	2.16 ± 0.1b	2.36 ± 0.2a

The means followed by different lowercase letters within each column are significantly different ($p < 0.01$)

[a] $A_{260/280}$ represents protein contamination and the expected values are commonly around 1.8. $A_{260/230}$ is an indication for salts and humic acid presence, and the expected values are commonly in the range of 2.0–2.2

2.6 PCR amplification and quantitative PCR analysis of microbial populations

All the extracted DNAs were used for further molecular analysis through qualitative and quantitative polymerase chain reaction (PCR and Q-PCR). Amplification of the bacterial *16SrDNA* and fungal ITS sequences was conducted on mDNAs with starting materials of 1 μl per reaction for all the methods. PCR amplification was performed for all the methods (one sample per triplicate) using the respective sets of primers (Table 1). The reaction mix consisted of 50 ng of the mDNA as the template, 10 pmol of each primer, 0.2 U Taq polymerase (Thermo Fisher Scientific, USA), 5 μl of 10× Taq buffer [10× buffer composition: Tris–HCl pH 9.0; PCR enhancers; KCl; 20 mM MgCl2], and 10 mM dNTP mix (Thermo Fisher Scientific, USA). The final mixture was adjusted to 30 μl by addition of sterile, purified water. The amplification steps includes initial denaturation at 95 °C for 7 min, 35 cycles of denaturation at 95 °C for 50 min, annealing at 57 °C for 1 min, and extension at 72 °C for 50 s min with the final extension of 72 °C for 5 min. Amplification products were confirmed by loading 3 μl of the samples along with 1 Kb DNA ladder on 1% agarose gel.

PCR efficiency of the extracted mDNAs using the new indirect method was analyzed for soil sample by qPCR (Lightcycler 480 system, Roche Life Sciences, US) using a 350-bp fragment of *16S rDNA*. The qPCR reactions were performed in a total volume of 20 μl, consisting of 1 μl of target DNA and 15 μl of amplification mixture containing PCR reaction buffer (BioFACT™ 2× Real-Time PCR Master Mix (For SYBR Green I), primers, and molecular biology grade water. The reactions were performed in triplicate for each sample. The reaction without the template served as a non-template control (NTC). The amplification conditions were 95 °C for 15 min as initial denaturation followed by 40 cycles of 95 °C for 10s, 60 °C for 20 s, and 72 °C for 20s.The amounts of DNA per reaction tube ranged from 10 ng to100 pg.

2.7 Partial restriction digestion and metagenomic library construction

Partial restriction digestion was performed using the enzyme *Hind*III (Thermo Fisher Scientific, USA) on mDNA extracted from leaf samples by the different methods to examine the suitability of the methods for downstream DNA manipulation. Four microliters of each mDNA template was digested with 1.5 U of the enzyme in a 30-μl reaction containing 3 μl of 10× assay buffer R (Thermo Fisher Scientific, USA) [1× buffer composition: 10 mM Tris–HCl pH 8.5; 10 mM MgCl2; 100 Mm KCl; 0.1 mg/ml BSA] for 5 h at 37 °C. Then, 5 μl

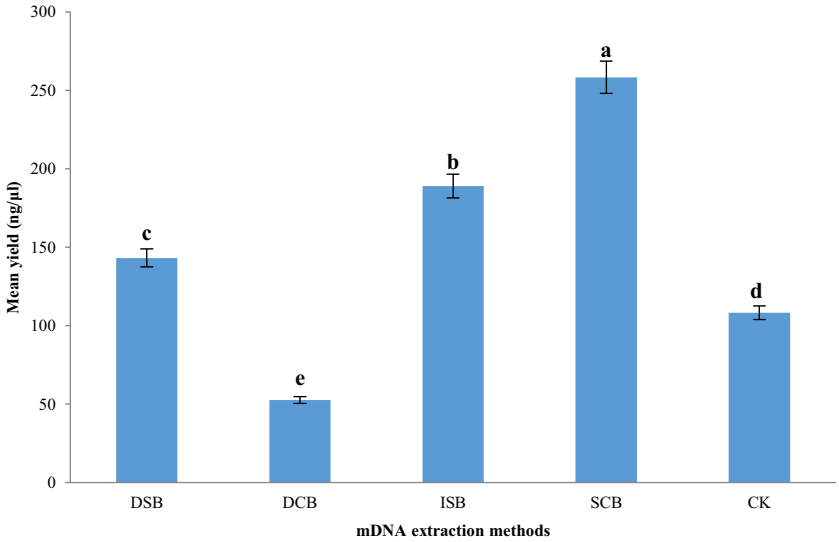

Fig. 3 The mean yield efficiency of the different studied mDNA extraction methods for all samples. The means followed by different lowercase letters within each column are significantly different ($p < 0.01$)

of the digested products was analyzed on 1% agarose gel along with 1Kb DNA ladder. The metagenomic library was constructed with the mDNA isolated by concentrate method (ISB) using the pUC19 vector. The library was constructed in the following manner: 2 to 10 kb fragments from the partially digested mDNA were gel purified using GeneJET Gel Extraction Kit (Thermo Fisher Scientific, USA). The pUC19 plasmid was linearized with enzyme *Hind*III. The linearized vector was ligated with the insert [vector: insert molar ratio (1:3)] using 2 µl of T4 DNA ligase (Thermo Fisher Scientific, USA) in a 30-µl reaction containing 3 µl of ligase buffer [1× buffer composition 400 mM Tris–HCl, 100 mM MgCl2, 100 mM DTT, 5 mM ATP (pH 7.8 at 25 °C)] for 1 h at room temperature and at 4 °C overnight. Five microliters of the ligation mixture was then added to 100 µl of chemically competent *Escherichia coli* DH5α cells and incubated on ice for 20 min. Heat shock was applied at 42 °C for 40 s, then transferred to ice for 5 min followed by addition of 1 ml of

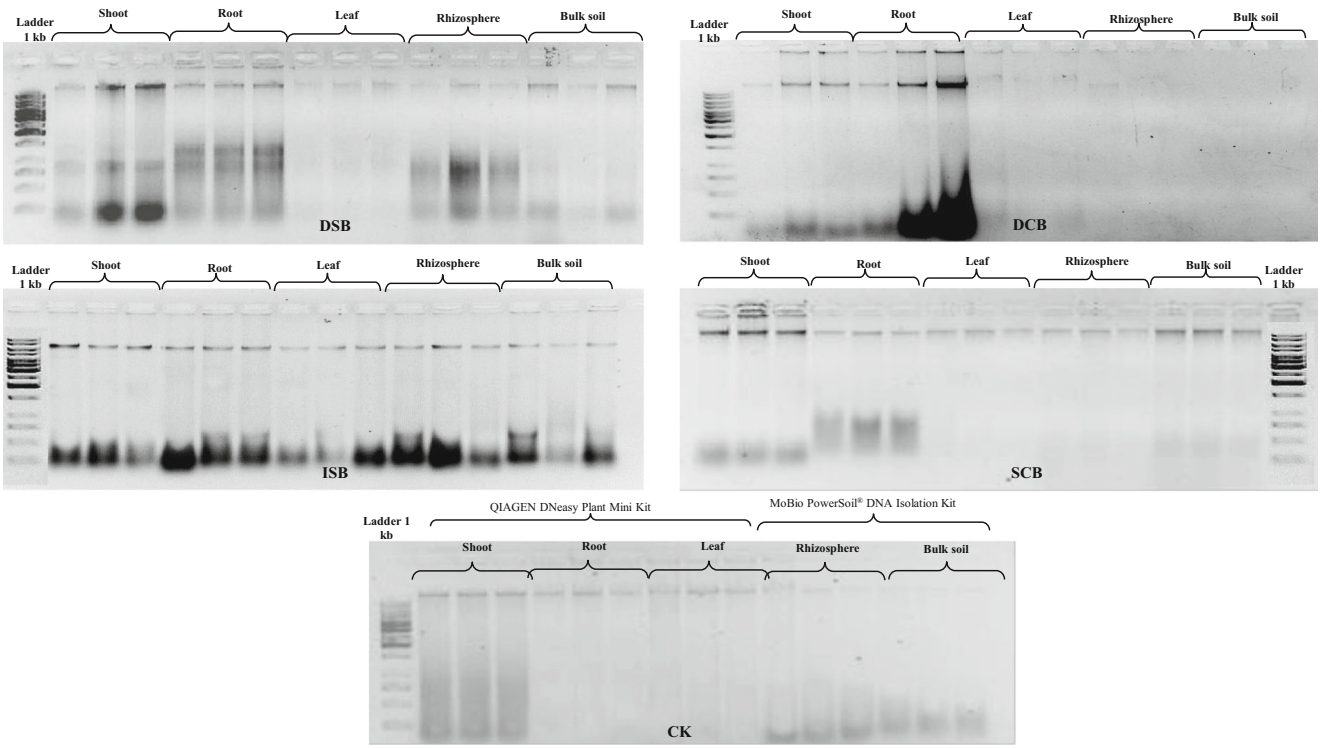

Fig. 4 The Oak mDNAs extracted by different methods from different tissues and samples. DNA was extracted from stem, root, and leaf tissues and bulk soil and rhizosphere using classical protocols (DSB and DCB), concentrate method (ISB), spin column-based (SCB), and commercial kit (CK). Five microliters from each sample was visualized by electrophoresis (90 V, 1 h) on 1% agarose gels. DNA molecular size was determined by comparison to molecular markers, 1Kb DNA ladder

LB broth, and then, cells were allowed to grow at 37 °C for 2 h. One hundred microliters of cells was plated on LB media containing ampicillin (50 μg/ml), IPTG (0.1 mM), and X-gal (40 μg/ml). The recombinant colonies were re-plated on LB plate with ampicillin (50 μg/ml).

2.8 Statistical analysis

The experiments were performed in triplicate (the replicates of each sample were taken from pooled tissues of three different trees), and the mean and standard deviations were estimated for each experiment. Analysis of variance (GLM) was performed with the software SPSS21 to determine the significant effects of extraction methods and sample types on DNA yield. Duncan test was used to compare means within and among methods. The comparisons were considered significant if $p < 0.05$.

Data availability The authors declare that the data supporting the findings of this study are available within the article; however, more detailed raw data of the study will be available on request from the corresponding author.

3 Results

3.1 Comparison of DNA yield and purity using various methods

Table 2 shows key differences of five different mDNA extraction methods used in the present study. The price paid for the reactive compounds in our institute (ABRII) was used to calculate the cost of each method. Duration of each method was calculated by measuring the minutes necessary to perform each method in the laboratory.

Analysis of variance revealed that the different mDNA extraction methods as well as various sample types showed significant effects on the mDNA yield ($p < 0.05$) (Tables 3 and 4). Both the method of DNA extraction and sample type significantly affected the yield of the extracted mDNAs ($F = 270.79$, $p < 0.05$ and $F = 124.43$, $p < 0.05$) and purity of DNA based on $A_{260/280}$ ($F = 147.29$, $p < 0.05$ and $F = 72.20$ $p < 0.05$) and $A_{260/230}$ ($F = 124.69$, $p < 0.05$ and $F = 51.59$, $p < 0.05$) (Table 4). The efficiency of different mDNA extraction methods for different samples is shown in Table 3. Totally, the ISB with PBS buffer (ranging from

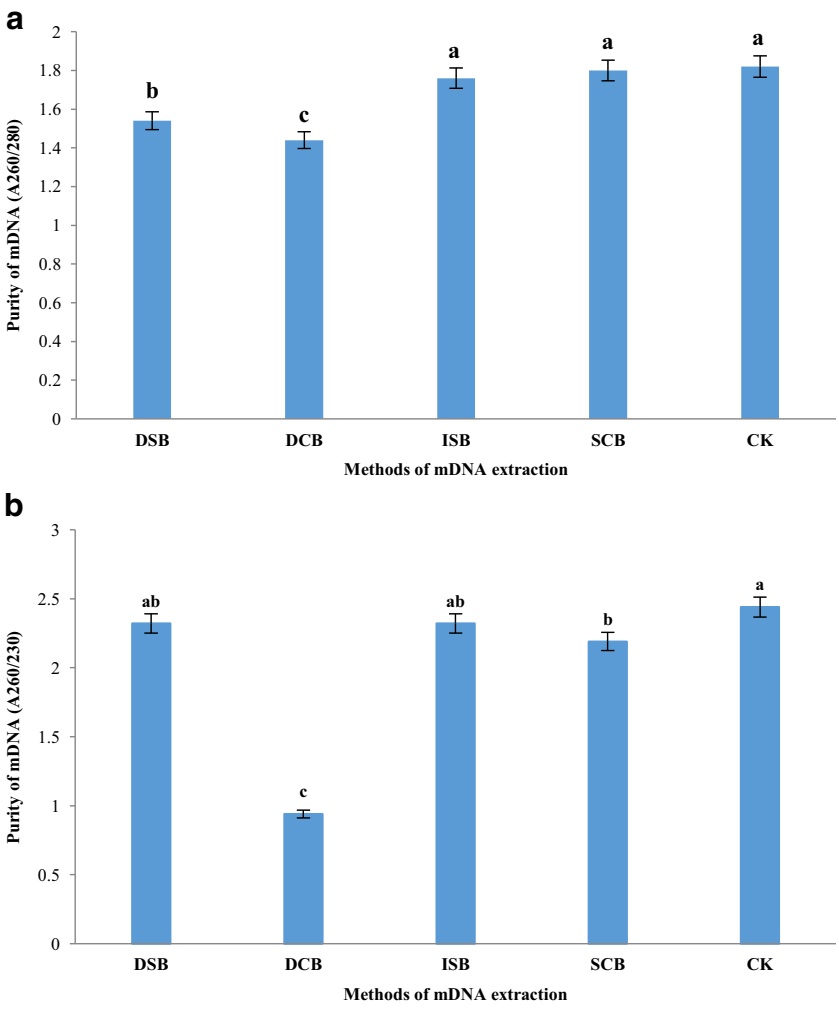

Fig. 5 Purity of mDNAs obtained by different methods. **a** Based on protein contamination. **b** Based on humic acid contamination

47.9 + 0.55 to 304.96 + 44.1 ng/μl) and SCB (ranging from 195.2 + 0.36 to 401 + 2.4 ng/μl) methods showed the maximum efficiency for mDNA extraction for all samples, respectively, which were significantly higher than those of commercial kits (ranging of 37.86 + 8.2 to 269.1 + 2.93) and other control methods (Fig. 3). In addition, they showed more molecular weight (ranging between 10 and 20 kb) compared to other methods. As it is shown in Fig. 4, the ISB with PBS buffer, SCB, and DSB methods showed more intact and bright bands on 1% agarose gels for all samples especially for shoot and bulk samples. The method DCB showed bands for the extracted DNAs from shoot and root samples (Fig. 4). Further illustrating the results confirmed that among samples, shoot sample showed the highest yield of DNA in all methods (Table 3). Method DCB showed the brightest bands for root samples rather than other methods; however, it did not produce a good band for remain samples and showed lowest yield for soil samples (Fig. 4). The SCB (258 ng/μl) and ISB (with PBS buffer; 189 ng/μl) produced the maximum yield of mDNA for all samples, followed by the DSB (143.17 ng/μl) and CK (108.32 ng/μl) methods (Fig. 3 and Table 3). The maximum quantity of mDNA was extracted from leaf sample by SCB method (401 ng/μl) and root (304.96 ng/μl) by ISB (with PBS buffer) method (Table 3). The assessment of purity and quality of the extracted mDNAs demonstrated that the SCB, ISB (with PBS buffer), and DSB retrieved higher-quality mDNA, respectively (Fig. 5).

3.2 PCR amplification and quantitative PCR analysis for 16SrDNA and ITS sequences

Successful PCR amplification for *16S rDNA* and ITS sequence was consistently observed for ISB (PBS buffer), SCB, and kit-extracted mDNAs and indicated that minimal PCR-inhibiting compounds were co-extracted, therefore confirming the high purity of mDNA for all the samples extracted with (Figs. 6 and 7). Contrastingly, PCR inhibition was more frequently observed when the DCB-extracted mDNA was used as template. The DCB method did not provide amplification for any of the soil and leaf samples indicating that those methods would require further purification of DNA to remove humic substances. This method provided amplification for a few samples (Figs. 6 and 7); however, these methods would require additional purification to be suitable for soil and leaf samples.

The coefficient of correlation (R2), angular coefficient (slope), and efficiency of real-time PCR amplification are shown in Fig. 8. The slope of the calibration curves indicates the amplification efficiency and the optimal value of − 3.414, which corresponds to 100% efficiency. The average PCR efficiencies of 96.3% for the *16S rDNA* gene target using mDNA extracted by ISB (with PBS buffer) method and of 98.1% using mDNA extracted by SCB method are not statistically different from each other ($p > 0.05$).

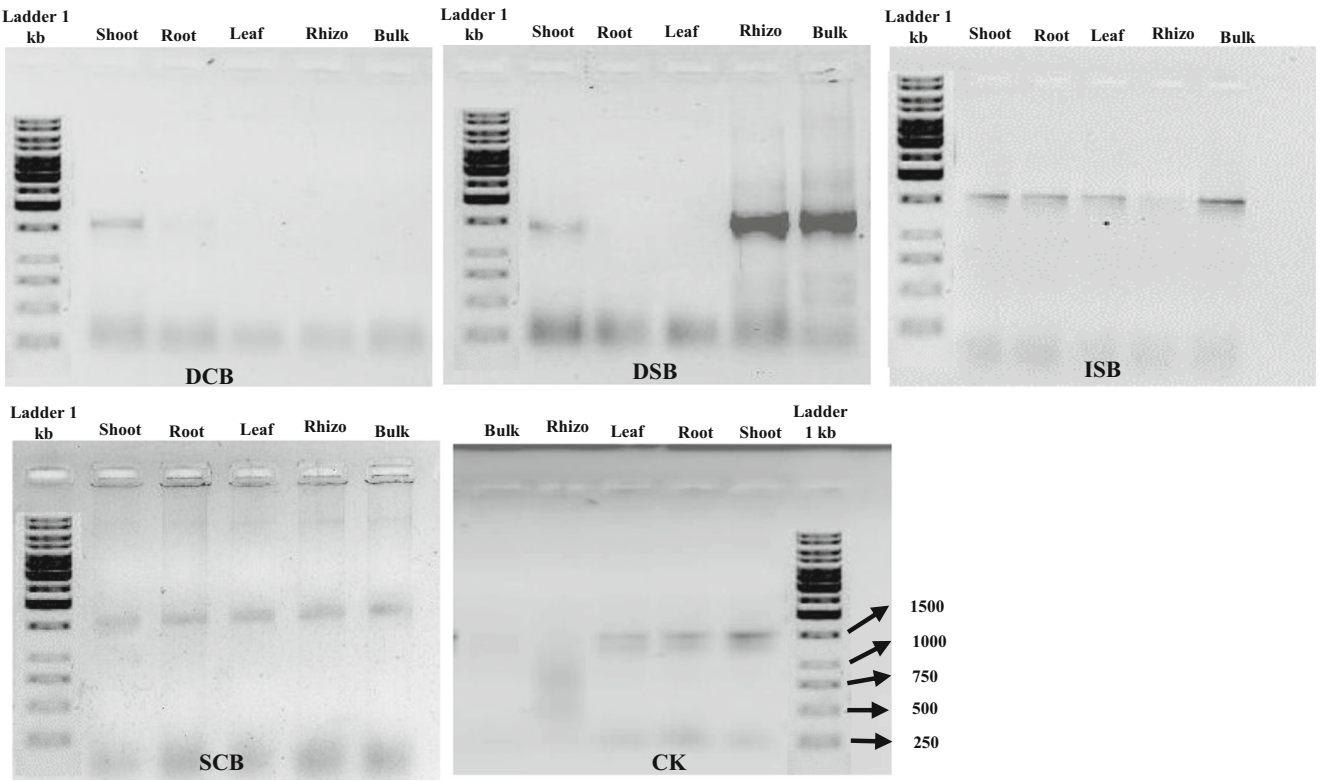

Fig. 6 Amplification of bacterial *16SrRNA* gene using the oak mDNAs extracted from shoot, root, leaf, and rhizosphere and bulk samples by different methods. The PCR products (5 μl) were visualized via electrophoresis (90 V, 1 h) on 1% agarose gels, and size determined by comparison to DNA ladder 1 kb

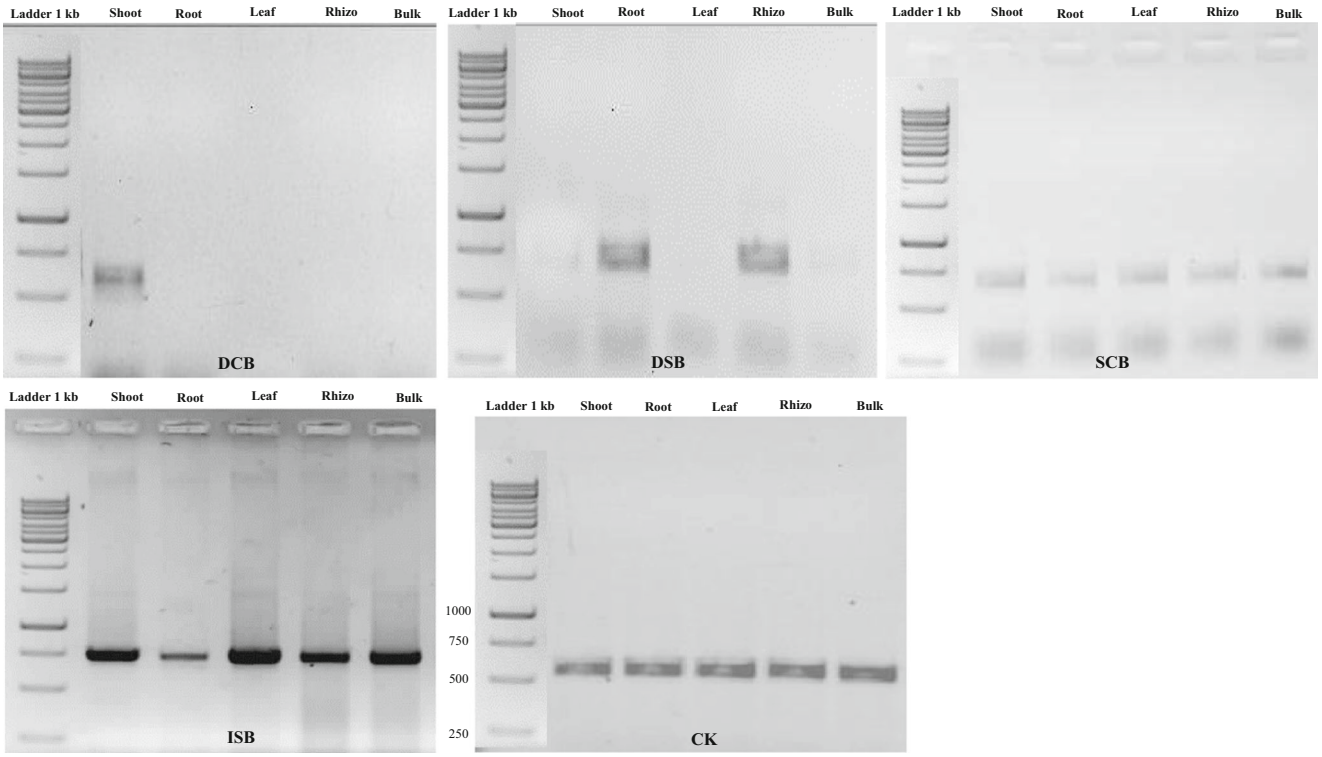

Fig. 7 Amplification of fungal ITS sequence using oak mDNAs extracted from shoot, root, leaf, rhizosphere, and bulk samples by different methods. The PCR products (5 μl) were visualized via electrophoresis (90 V, 1 h) on 1% agarose gels, and size determined by comparison to DNA ladder 1 kb plus

3.3 Partial restriction digestion and metagenomic library construction

The mDNA samples extracted from leaf samples using ISB method SCB, DCB, DSB, and CK were partially digested with the enzyme *Hind*III for metagenomic library construction (Fig. 9). The mDNAs extracted by ISB method were also suitable for digestion process, whereas those of the DCB method were not subjected to efficient restriction digestion and resulted in shear-degraded DNA. The partially digested ISB mDNA was cloned successfully to construct a metagenomic library containing 4×10^6 CFU ml^{-1}, which confirmed the purity of the extracted mDNA. The mDNA extracted from all the samples could be preserved at − 20 °C without any loss in yield or change in purity for 6 months.

4 Discussion

The present study was firstly performed to develop efficient protocols for genomic and metagenomic DNA extraction from different tissues of Persian oak trees and soil samples. The results showed that the newly improved procedures (ISB and SCB) were efficient for different oak samples, including rhizospheric and bulk soil, leaf, stem, and root samples, whereas the traditional methods (DCB, DSB, and commercial kits) were efficient only for one or two specific tissue samples.

The purity of mDNA extracted by ISB was significantly higher than that of other methods (Table 4). Probably, application of the PBS buffer in the ISB method helps in dissociation of cells which enhance the efficiency of cell extraction compared to the conventional microbial cell extraction methods which extract only up to 50% of the cells (Narayan et al. 2016; Robe et al. 2002). It also proves that addition of PBS buffer and PVPP in this procedure may play an important role in proper chemical lysis of the cells as compared to other chemicals like CTAB, SDS, EDTA, etc. (Fatima et al. 2014).

Moreover, grinding and vortexing of the tissues and soil samples in the ISB procedure may help in creating non-oxidative environment and also homogenizing of thick cell walls in the buffer. Proper grinding of the sample ruptures the cell wall thereby releasing the cellular DNA from the inner compartment. During homogenization, polyphenols are released from vacuoles which rapidly react with cytoplasmic enzymes, then PVPP purges and forms complex hydrogen bonds with polyphenols and gets precipitated which can easily be separated from DNA by centrifugation (Frostegard et al. 1999; Rawat et al. 2016). Because of these advantages, PVPP has been successfully used in the SDS-based extraction methods to absorb phenolics and prevent their oxidation for other recalcitrant plant species (Tibbits et al. 2006; Healey et al. 2014). Furthermore, to increase the rate of precipitation in both the ISB and SCB protocols, a high concentrated salt solution (potassium acetate) was added before final precipitation of mDNA by absolute cold ethanol.

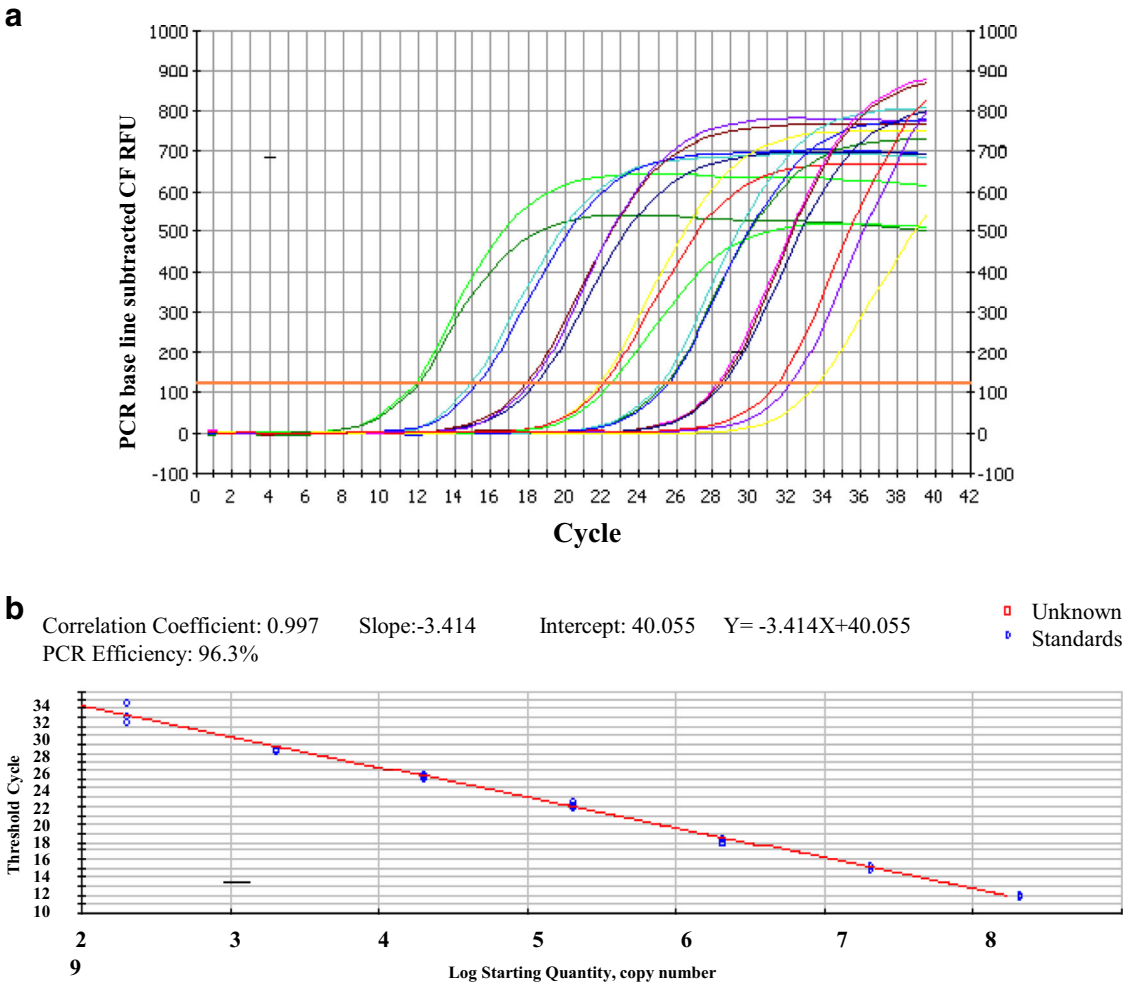

Fig. 8 The qPCR analysis for method ISB. **a** The qPCR reaction graph with fluorescence against cycles. **b** PCR efficiency standard graph for the template prepared using method ISB. The PCR efficiency is 1.986 against 2 which is equal to 99.3%

Polysaccharides have a similar solubility to DNA and co-precipitate in either isopropanol or ethanol, inhibiting downstream molecular application (Tibbits et al. 2006). The addition of a high concentrated salt buffer increases their solubility in ethanol, allowing their removal once the DNA has been precipitated and pelleted (Healey et al. 2014). Use of SDS, lysozyme, or mechanical force enhances extraction of archaeal and bacterial DNA but fails for fungal cells; however, it has been previously shown that combination of SDS, lysozyme, and vigorous shaking can successfully release fungal DNA (Melo et al. 2006).

The PCR amplifications also confirmed high efficiency of the newly developed methods compared to the control

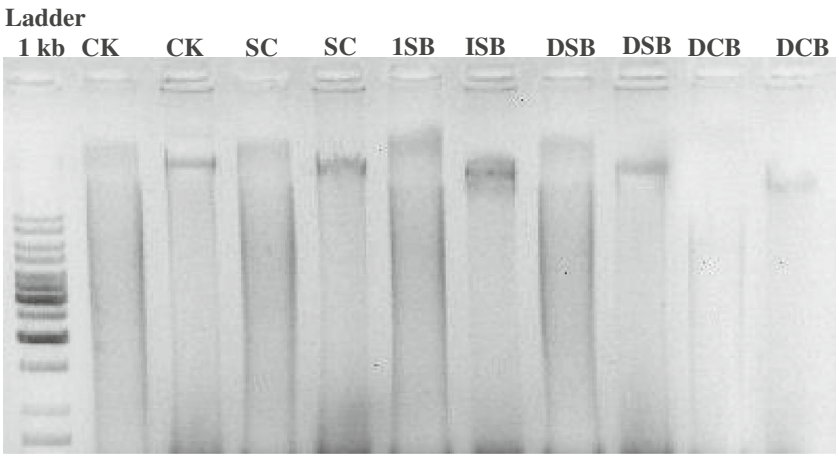

Fig. 9 The partial restriction digestion of the mDNAs extracted by all the methods using *Hind*III. The samples were analyzed on 1% agarose gel in 1× TAE buffer. For each method, the first lane is restricted mDNA, and the second lane is the unrestricted mDNA

methods. The low efficiency of the DCB method may be because of high concentration of co-extracted plant polyphenolics and polysaccharides which are known to bind to DNA, making it inaccessible to the polymerase enzyme (Demeke and Jenkins 2010; Mornkham et al. 2012). The direct SDS-based method (DSB) retrieved high yields compared to the commercial kit protocols; however, the isolated mDNA using this method did not indicate sufficient purity; therefore, it was not considered as suitable method for PCR amplification when tested on different soil samples (Figs. 6 and 7). A potential reason for low performance of cell disruption in soil samples by bead beating of power soil kit may be due to the increase of viscosity and the presence of a high concentration of insoluble materials during the beating process (Devi et al. 2015). High losses of input DNA during the application of commercial kits have been reported (Roossinck et al. 2010; Vo and Jedlicka 2014; Sadeghi et al. 2013).

The mDNAs isolated using the ISB method (using PBS buffer) displayed a high qPCR efficiency, greater than 99%, where the accepted PCR efficiency for qPCR analysis ranges from 90to 110% (Roche Life Sciences, USA). In addition, the results of restriction digestion assay and metagenomic library construction confirmed the sufficient purity of mDNA extracted by the ISB method. As a final added advantage, the overall processing time for the ISB method was shorter (1:30 h) than 5 to 7 h of the earlier reported methods (Sagar et al. 2014). The commercial kit (CK) took the least time for DNA extraction (Table 3); however, the processing cost of 1 g of soil or tissue for a single reaction is about US$7 which is quite high if large numbers of samples are needed to be processed (Devi et al. 2015).

The second newly developed method (SCB) also showed high efficiency in quality and quantity of the extracted mDNA and also reduction of process time. Thechaotropic agents (for instance guanidinium thiocyanate) used in this method would have played a role to remove DNA-binding proteins, allowing for better absorption in the spin columns (Boom et al. 1989). The results confirmed that the use of cell lysis buffer and following elution over a column could greatly enhance the amount of the extracted pure mDNAs, which was previously reported also by other researchers (Poh and Gan 2014; Telfer et al. 2013). In addition to the technical efficiency, the SCB protocol is significantly more cost-effective (~ US$ 1 per reaction) compared with the commercial kits (~ US$ 7 per reaction), which makes this method suitable for molecular biology studies, such as PCR amplifications.

5 Conclusion

Two new procedures (ISB and SCB) with high productivity and efficiency for genomic and mDNA extraction from different Persian oak tissues and soil samples were developed. The minimal process time and costs and high quality of the obtained DNA make these two methods ideal for extraction of genomic and mDNA from different oak tissues and soil samples to facilitate the molecular biology studies in these important trees, especially when a large number of plant samples are needed to be analyzed in lab settings with limited resources.

Acknowledgements The authors are very grateful to Eng. Ebrahim Karimi, Eng. Morteza Ebrahimi Rastaghi, Eng. Saadi Karami, and Eng. Amin Alidadi for their kind technical assistances in development of the protocols.

Funding information The study was financially and technically supported by the Forest, Rangeland and Soil of Forests, Range and Watershed Management Organization and Agricultural Biotechnology Research Institute of Iran (ABRII).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Ahmadi R, Kiadaliri H, Mataji A, Kafaki S (2014) Oak forest decline zonation using AHP model and GIS technique in Zagros forests of Ilam Province. J Biodivers and Environ Sci 4:141–150

Alexander LW, Woeste KE (2014) Pyrosequencing of the northern red oak (*Quercus rubra* L.) chloroplast genome reveals high quality polymorphisms for population management. Tree Genet Genom 10(4):803–812. https://doi.org/10.1007/s11295-013-0681-1

Barta CE, Bolander B, Bilby SR, Brown JH, Brown RN, Duryee AM, Edelman DR, Gray CE, Gossett C, Haddock AG, Helsel MM (2017) *In situ* dark adaptation enhances the efficiency of DNA extraction from mature pin oak (*Quercus palustris*) leaves, facilitating the identification of partial sequences of the 18S rRNA and isoprene synthase (IspS) genes. Plant 6(4):52. https://doi.org/10.3390/plants6040052

Bodénès C, Chancerel E, Gailing O, Vendramin GG, Bagnoli F, Durand J, Goicoechea PG, Soliani C, Villani F, Mattioni C, Koelewijn HP (2012) Comparative mapping in the Fagaceae and beyond with EST-SSRs. BMC Plant Biol 12(1):153. https://doi.org/10.1186/1471-2229-12-153

Boom R, Sol CJA, Salimans MMM, Jansen CL, Wertheim-Van Dillen P, Noordaa JVD (1989) Rapid and simple method for purification of nucleic acids. J Clin Microbe 28:495–503

Caravaca F, Maboreke H, Kurth F, Herrmann S, Tarkka MT, Ruess L (2015) Synergists and antagonists in the rhizosphere modulate microbial communities and growth of *Quercus robur* L. Soil Biol Biochem 82:65–73. https://doi.org/10.1016/j.soilbio.2014.12.004

Chabi Sika K, Kefela T, Adoukonou-Sagbadja H, Ahoton L, Saidou A, Baba-Moussa L, Baptiste LJ, Kotconi SO, Gachomo EW (2015) A simple and efficient genomic DNA extraction protocol for large scale genetic analyses of plant biological systems. Plant Gene 1:43–45. https://doi.org/10.1016/j.plgene.2015.03.001

Cobo-Díaz JF, Fernández-González AJ, Villadas PJ, Toro N, Tringe SG, Fernández-López M (2017) Taxonomic and functional diversity of a *Quercus pyrenaica* willd. rhizospheric microbiome in the Mediterranean mountains. Forests 8(10):390. https://doi.org/10.3390/f8100390

Degner JC (2014) Using a genotyping-by-sequencing (GBS) approach to elucidate population structure in Garry oak (*Quercus garryana*). Thesis in the University of British Columbia

Demeke T, Jenkins GR (2010) Influence of DNA extraction methods, PCR inhibitors and quantification methods on real-time PCR assay of biotechnology-derived traits. Anal Bioanal Chem 396(6):1977–1990. https://doi.org/10.1007/s00216-009-3150-9

Denman S, Doonan J, Ransom-Jones E, Broberg M, Plummer S, Kirk S, Scarlett K, Griffiths AR, Kaczmarek M, Forster J, Peace A (2017) Microbiome and infectivity studies reveal complex polyspecies tree disease in acute oak decline. ISME J 12(2):386–399. https://doi.org/10.1038/ismej.2017.170

Devi SG, Fathima AA, Radha S, Arunraj R, Curtis WR, Ramya M (2015) A rapid and economical method for efficient DNA extraction from diverse soils suitable for metagenomic applications. PLoS One 10(7):e0132441. https://doi.org/10.1371/journal.pone.0132441

Djavanchir Khoie K (1967) Les chęnes de l'Iran. PhD Thesis, Univ. Montpellier, Faculté des Sciences, Montpellier, 223 pp.

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Embarcadero-Jiménez S, Yang FL, Freye-Hernández R, Trujillo-Cabrera Y, Orduña FNR, Yuan HL, Wang ET (2014) An improved protocol for extraction of metagenomic DNA from high humus, alkaline and saline soil of chinampa for T-RFLP fingerprinting analysis. British Microbiol Res J 4(7):821–830. https://doi.org/10.9734/BMRJ/2014/9670

Fatima F, Pathak N, Rastogi S (2014) An improved method for soil DNA extraction to study the microbial assortment within rhizospheric region. Mol Biol Inter, Article ID 518960: doi:https://doi.org/10.1155/2014/518960

Fernandes I, Alves A, Correia A, Devreese B, Esteves AC (2014) Secretome analysis identifies potential virulence factors of *Diplodia corticola*, a fungal pathogen involved in cork oak (*Quercus suber*) decline. Fungal Biol 118(5):516–523. https://doi.org/10.1016/j.funbio.2014.04.006

Finkeldey R, Leinemann L, Gailing O (2010) Molecular genetic tools to infer the origin of forest plants and wood. Appl Microbiol Biotechnol 85(5):1251–1258. https://doi.org/10.1007/s00253-009-2328-6

Finch-Savage WE (1992) Seed development in the recalcitrant species *Quercus robur* L.: germinability and desiccation tolerance. Seed Sci Res 2(1):17–22

Fitz-Gibbon S, Hipp AL, Pham KK, Manos PS, Sork VL (2017) Phylogenomic inferences from reference-mapped and de novo assembled short-read sequence data using RADseq sequencing of California white oaks (*Quercus* section *Quercus*). Genom 60(9):743–755. https://doi.org/10.1139/gen-2016-0202

Frostegard A, Courtois S, Ramisse V (1999) Quantification of bias related to the extraction of DNA directly from soils. Appl Environ Microbiol 65(12):5409–5420

Goicoechea PG, Herrán A, Durand J, Bodénès C, Plomion C, Kremer A (2015) A linkage disequilibrium perspective on the genetic mosaic of speciation in two hybridizing Mediterranean white oaks. Heredity 114(4):373–386. https://doi.org/10.1038/hdy.2014.113

Guerrero-Sanchez VM, Maldonado-Alconada AM, Amil-Ruiz F, Jorrin-Novo JV (2017) Holm oak (*Quercus ilex*) transcriptome. De novo sequencing and assembly analysis. Front Mol Biosci 4:70. https://doi.org/10.3389/fmolb.2017.00070

Healey A, Furtado A, Cooper T, Henry RJ (2014) Protocol: a simple method for extracting next-generation sequencing quality genomic DNA from recalcitrant plant species. Plant methods 10(1):21

He F, Yang B, Wang H, Yan Q, Cao Y, He X (2016) Changes in composition and diversity of fungal communities along *Quercus mongolica* forests developments in Northeast China. Appl Soil Ecol 100:162–171. https://doi.org/10.1016/j.apsoil.2015.12.014

Hipp AL, Eaton DA, Cavender-Bares J, Fitzek E, Nipper R, Manos PS (2014) A framework phylogeny of the American oak clade based on sequenced RAD data. PLoS One 9(4):e93975. https://doi.org/10.1371/journal.pone.0093975

Heydari M, Rostamy M, Najafi A (2016) Effect of fire severity on physical and biochemical soil properties in Zagros oak (*Quercus brantii* Lindl.) forests in Iran. J. Forest Res:1–10

Kaul S, Sharma T, Dhar MK (2016) "Omics" tools for better understanding the plant–endophyte interactions. Front Plant Sci 7. https://doi.org/10.3389/fpls.2016.00955

Khalyani AH, Mayer AL (2013) Spatial and temporal deforestation dynamics of Zagros forests (Iran) from 1972 to 2009. Landsc Urban Plan 117:1–12. https://doi.org/10.1016/j.landurbplan.2013.04.014

Koide RT, Ricks KD, Davis ER (2017) Climate and dispersal influence the structure of leaf fungal endophyte communities of *Quercus gambelii* in the eastern Great Basin, USA. Fungal Ecol 30:19–28. https://doi.org/10.1016/j.funeco.2017.08.002

Magalhães AMP (2015) RNA-seq analysis of the *Quercus suber* root response to drought (doctoral dissertation)

Makela M, Michael P, Theriault G, Nkongolo KK (2016) High genetic variation among closely related red oak (*Quercus rubra*) populations in an ecosystem under metal stress: analysis of gene regulation. Gene Genom 38(10):967–976. https://doi.org/10.1007/s13258-016-0441-3

Maropola MKA, Ramond JB, Trindade M (2015) Impact of metagenomic DNA extraction procedures on the identifiable endophytic bacterial diversity in *Sorghum bicolor* (L. Moench). J Microbiol Meth 112:104–117. https://doi.org/10.1016/j.mimet.2015.03.012

Meaden S, Metcalf CJE, Koskella B (2016) The effects of host age and spatial location on bacterial community composition in the English oak tree (*Quercus robur*). Environ Microbiol Rep 8(5):649–658. https://doi.org/10.1111/1758-2229.12418

Melo SCO, Pungartnik C, Cascardo JCM, Brendel M (2006) Rapid and efficient protocol for DNA extraction and molecular identification of the basidiomycete *Crinipellisperniciosa*. Genet Mol Res 4:851–855

Moore D, Van Stan J, Rosier CL, Gay TE, Wu T (2015) Canopy structural alterations to nitrogen functions of the soil microbial community in a *Quercus virginiana* forest. Poster presentation in Georgia Southern University

Mornkham T, Wangsomnuk PP, Wangsomnuk P, Jogloy S, Pattanothai A, Fu YB (2012) Comparison of five DNA extraction methods for molecular analysis of Jerusalem artichoke (*Helianthus tuberosus*). Genet Mol Res 11(1):572–581. https://doi.org/10.4238/2012.March.8.5

Condition in Europe. UN / ECE-EC Technical Background Report. Brussels, Geneva: ECUN / ECE

Narayan A, Jain K, Shah AR, Madamwar D (2016) An efficient and cost-effective method for DNA extraction from athalassohaline soil using a newly formulated cell extraction buffer. 3 Biotech 6(1):62. https://doi.org/10.1007/s13205-016-0383-0

Nixon KC (2006) Global and neotropical distribution and diversity of oak (genus *Quercus*) and oak forests. In Ecology and conservation of neotropical montane oak forests (pp 3–13). Springer, Berlin, Heidelberg

Niu C, Kebede H, Auld DL, Woodward JE, Burow G, Wright RJ (2008) A safe inexpensive method to isolate high quality plant and fungal DNA in an open laboratory environment. Afr J Biotech 7:2818–2822

Pandey A, Tamta S (2015) High-molecular-weight DNA extraction from six Quercus species of Himalaya, India. Int J Advance Res 3:30–34

Panahi P, Jamzad Z, Pourmajidian M, Fallah A, Pourhashemi M (2012) Foliar epidermis morphology in *Quercus* (subgenus *Quercus*, section *Quercus*) in Iran. Acta Bot Croat 71(1):95–113

Plomion C, Bastien C, Bogeat-Triboulot MB, Bouffier L, Déjardin A, Duplessis S, Fady B, Heuertz M, Le Gac AL, Le Provost G,

Legué V, Lelu-Walter MA, Leplé JC, Maury S, Morel A, Oddou-Muratorio S, Pilate G, Sanchez L, Scotti I, Scotti-Saintagne C, Segura V, Trontin GF, Vacher C (2016) Forest tree genomics: 10 achievements from the past 10 years and future prospects. Ann Forest Sci 73(1):77–103. https://doi.org/10.1007/s13595-015-0488-3

Poh JJ, Gan SK (2014) Comparison of customized spin-column and salt-precipitation finger-prick blood DNA extraction. Biosci Rep art. e00145 doi:https://doi.org/10.1042/BSR20140105

Qu YY, Zhang Q, Wei L, Ma F, Zhou JT, Pi WQ, Gou M (2009) Optimization of metagenomic DNA extraction from activated sludge samples. Asia Pac J Chem Eng 4(5):780–786. https://doi.org/10.1002/apj.338

Rahmani MS, Alikhani L, Shabanian N, Khadivi-Khub A (2015) Genetic differentiation in Quercus infectoria from northwest of Iran revealed by different nuclear markers. Tree Genet Genomes 11(1):800. https://doi.org/10.1007/s11295-014-0800-7

Rawat S, Joshi G, Annapurna D, Arunkumar AN, Karaba NN (2016) Standardization of DNA extraction method from mature dried leaves and ISSR-PCR conditions for Meliadubia Cav. A fast growing multipurpose tree species. Am Plant Sci 7:437–445

Rellstab C, Zoller S, Walthert L, Lesur I, Pluess AR, Graf R, Bodénès C, Sperisen C, Kremer A, Gugerli F (2016) Signatures of local adaptation in candidate genes of oaks (*Quercus spp.*) with respect to present and future climatic conditions. Mol Ecol 25(23):5907–5924. https://doi.org/10.1111/mec.13889

Ross-Davis AL, Stewart JE, Hanna JW, Kim MS, Knaus BJ, Cronn R, Rai H, Richardson BJ, McDonald GI, Klopfenstein NB (2013) Transcriptome of an *Armillaria* root disease pathogen reveals candidate genes involved in host substrate utilization at the host–pathogen interface. Forest Pathol 43(6):468–477. https://doi.org/10.1111/efp.12056

Roossinck MJ, Saha P, Wiley GB (2010) Ecogenomics: using massively parallel pyrosequencing to understand virus ecology. Mol Ecol 19: 81–88. https://doi.org/10.1111/j.1365-294X.2009.04470.x

Robe P, Nalin R, Capellano C, Vogel TM, Simonet P (2002) Extraction of DNA from soil. Eur J Soil Biol 39:183–190

Sadeghi MMH, Abedi D, Mohmoudpour HR, Akbari V (2013) Comparison of five methods for extraction of genomic DNA from a marine archaea, *Pyrococcus furiosus*. Pakistan J Medic Sci 29:90–394

Sagar K, Singh SP, Goutam KK, Konwar BK (2014) Assessment of five soil DNA extraction methods and a rapid laboratory-developed method for quality soil DNA extraction for *16S rDNA*-based amplification and library construction. J Microbiol Meth 97:68–73. https://doi.org/10.1016/j.mimet.2013.11.008

Sahu SK, Thangaraj M, Kathiresan K (2012) DNA extraction protocol for plants with high levels of secondary metabolites and polysaccharides without using liquid nitrogen and phenol. ISRN Molecul Biol 2012:1–6. https://doi.org/10.5402/2012/205049

San Jose-Maldia L, Matsumoto A, Ueno S, Kanazashi A, Kanno M, Namikawa K, Yoshimaru H, Tsumura Y (2017) Geographic patterns of genetic variation in nuclear and chloroplast genomes of two related oaks (*Quercus aliena* and *Q. serrata*) in Japan: implications for seed and seedling transfer. Tree Genet Genom 13(6):121

Schroeder H, Cronn R, Yanbaev Y, Jennings T, Mader M, Degen B, Kersten B (2016) Development of molecular markers for determining continental origin of wood from white oaks (*Quercus L. sect. Quercus*). PloS One 11(6):e0158221

Sharma S, Sharma KK, Kuhad RC (2014) An efficient and economical method for extraction of DNA amenable to biotechnological manipulations, from diverse soils and sediments. J Appl Microb 116(4):923–933. https://doi.org/10.1111/jam.12420

Sork VL, Fitz-Gibbon ST, Puiu D, Crepeau M, Gugger PF, Sherman R, Stevens K, Langley CH, Pellegrini M, Salzberg SL (2016a) First draft assembly and annotation of the genome of a california endemic oak *Quercus lobata* Née (Fagaceae). *G3:* Gen Genom Genet 6(11): 3485–3495

Sork VL, Squire K, Gugger PF, Steele SE, Levy ED, Eckert AJ (2016b) Landscape genomic analysis of candidate genes for climate adaptation in a California endemic oak, *Quercus lobata*. American J Botany 103(1):33–46. https://doi.org/10.3732/ajb.1500162

Sunderlíková V, Salaj J, Kopecky D, Salaj T, Wilhem E, Matusíková I (2009) Dehydrin genes and their expression in recalcitrant oak (*Quercus robur*) embryos. Plant Cell Rep 28(7):1011–1021

Tibbits JFG, Mcmanus LJ, Spokevicius AV, Bossinger G (2006) A rapid method for tissue collection and high-throughput isolation of genomic DNA from mature trees. Plant Mol Biol Rep 24(1):81–91. https://doi.org/10.1007/BF02914048

Telfer E, Graham N, Stanbra L, Manley T, Wilcox P (2013) Extraction of high purity genomic DNA from pine for use in a high-throughput genotyping platform. New Zeal J Forest Sci 43(1):3. https://doi.org/10.1186/1179-5395-43-3

Toader V, Moldovan IC, Şofletea N, Abrudan IV, Curtu AL (2009) DNA isolation and amplification in oak species (Quercus spp.) Bull Transilvania Uni Braşov 2:5167

Uquillas JA, Kishore V, Akkus O (2011) Effects of phosphate buffered saline concentration and incubation time on the mechanical and structural properties of electrochemically aligned collagen threads. Biomed Mat 6(3):035008. https://doi.org/10.1088/1748-6041/6/3/035008

Usié A, Simões F, Barbosa P, Meireles B, Chaves I, Gonçalves S, Folgado A, Almeida MH, Matos J, Ramos AM (2017) Comprehensive analysis of the cork oak (*Quercus suber*) transcriptome involved in the regulation of bud sprouting. Forests 8(12):486. https://doi.org/10.3390/f8120486

Verma D, Satyanarayana T (2011) An improved protocol for DNA extraction from alkaline soil and sediment samples for constructing metagenomic libraries. Appl Biochem Biotechnol 165(2):454–464. https://doi.org/10.1007/s12010-011-9264-5

Vo ATE, Jedlicka JA (2014) Protocols for metagenomic DNA extraction and Illumina amplicon library preparation for fecal and swab samples. Mol Ecol Res 14(6):1183–1197.69. https://doi.org/10.1111/1755-0998.12269

Voříšková J, Brabcová V, Cajthaml T, Baldrian P (2014) Seasonal dynamics of fungal communities in a temperate oak forest soil. New Phytol 201(1):269–278. https://doi.org/10.1111/nph.12481

Vranckx G, Mergeay J, Cox K, Muys B, Jacquemyn H, Honnay O (2014) Tree density and population size affect pollen flow and mating patterns in small fragmented forest stands of pedunculate oak (*Quercus robur* L.) For Ecol Manag 328:254–261. https://doi.org/10.1016/j.foreco.2014.05.044

Yang Y, Zhou T, Duan D, Yang J, Feng L, Zhao G (2016) Comparative analysis of the complete chloroplast genomes of five *Quercus* species. Front Plant Sci 7. https://doi.org/10.3389/fpls.2016.00959

Yuan S, Cohen DB, Ravel J, Abdo Z, Forney LJ (2012) Evaluation of methods for the extraction and purification of DNA from the human microbiome. PLoS One 7(3):e33865. https://doi.org/10.1371/journal.pone.0033865

Zhou J, Bruns MA, Tiedje JM (1996) DNA recovery from soils of diverse composition. Appl Environ Microb 62:316–322