Improved method for genomic DNA extraction for *Opuntia* Mill. (Cactaceae)

César Ramiro Martínez-González¹, Rosario Ramírez-Mendoza², Jaime Jiménez-Ramírez², Clemente Gallegos-Vázquez³ and Isolda Luna-Vega¹*

**Abstract**

**Background:** Genomic DNA extracted from species of Cactaceae is often contaminated with significant amounts of mucilage and pectin. Pectin is one of the main components of cellular walls, whereas mucilage is a complex polysaccharide with a ramified structure. Thus, pectin- and mucilage-free extraction of DNA is a key step for further downstream PCR-based analyses.

**Results:** We tested our DNA extraction method on cladode tissue (juvenile, adult, and herbaria exemplars) of 17 species of *Opuntia* Mill., which are characterized by a large quantity of pectin and mucilage.

**Conclusion:** We developed a method for the extraction of gDNA free of inhibitory compounds common in species of *Opuntia* Mill., such as pectin and mucilage. Compared to previously extraction protocols, our method produced higher yields of high-quality genomic DNA.

**Keywords:** DNA quality, DNA quantity, Genomic DNA, Mucilage, *Opuntia*, Pectin

**Background**

Present-day DNA-based molecular studies are useful tools with a wide-range of applications in different biological disciplines. Molecular studies, especially in species with similar morphologies, can be used to characterize and differentiate species [1, 2]. Such studies have used molecular techniques involving PCR amplification of DNA [3, 4] to successfully solve taxonomic and phylogenetic controversies [5]. More specifically, DNA analyses have been used at different taxonomic levels, from communities of bacteria, fungi, yeast, plants and animals, to the cloning of specific genes [6]. High-quality DNA extraction is a necessary first step to conduct molecular studies. This can be performed using conventional methods or commercial kits specifically designed for particular types of samples. Most commercial kits efficiently capture DNA using extraction columns and resins, but the cost of these kits limits their application to large numbers of samples [7].

Conventional methods of DNA extraction involve three basic steps: (1) lysis of cellular walls and membranes; (2) removal of cell debris and other molecular compounds (e.g., polysaccharides, secondary metabolites, proteins, tannins, alkaloids, and polyphenols); (3) DNA precipitation and purification [8]. Currently, fast and cost-efficient DNA extraction protocols yielding large quantities of high-quality DNA are key to the study of species' molecular genetics [9]. For example, DNA extracted from species of cacti (Cactaceae) are often contaminated with high quantities of mucilage and pectin [10–15].

In these species, pectin is the major component of the cellular wall and its composition often varies among species (e.g., *Opuntia*), location and environments. The main molecular components of pectin are α-(1 → 4) chains linked to ³-galacturonic acid interspersed by the insertion of (1 → 2) residues linked to adjacent or alternate residues of L-rhamnopyranosyl. The lineal segments are predominantly composed of homogalacturone [16].

Mucilage is an organic component present in large cells (idioblasts) in the chlorenchyma and adjacent
water-retaining parenchymal cells [17, 18]. Mucilage is composed of complex polysaccharides with ramified structures [16] containing varying proportions of different sugars (e.g., l-arabinose, pyranose, furanose, d-galactose, l-rhamnose and d-xylose) and galacturonic acid. The primary structure of the molecule consists of lineal repetitive chains of 1,4-β-d-galacturonic acid and α-1,2-l-rhamnose with a trisaccharide of β-1,6-d-glucose with a lateral chain joined to O-4-l-residues of rhamnose [19, 20]. Mucilage is found throughout all body parts, including flowers [11]. In most species of cacti, mucilage is secreted in response to wounds and during the DNA extraction process. More specifically, during the DNA extraction process mucilage appears as soon as the tissue is pulverized, which significantly hinders the efficiency of the extraction and purification [21].

Generally, extraction and purification of high-quality genomic DNA (gDNA) is hindered by the presence of pectin that precipitates alongside DNA [22], thus reducing the quality and yield of the extraction process [23]. Although efficient DNA extraction is crucial for downstream PCR-based analyses, there are relatively few studies focusing on gDNA extraction efficiency in species of cacti [11, 13, 22, 24–27]. In this context, the aim of the present study was to develop a simple and cost-effective method to obtain large yields of high-quality gDNA from cladode tissue of Opuntia species.

Methods

We obtained tissues samples from the national Opuntia collection of the Botanical Garden at Instituto de Biología, Universidad Nacional Autónoma de México.

Protocol

CTAB 2X buffer

1. Prepare CTAB 2X buffer solution (Tris 10 mM pH 8.0; EDTA 20 mM, pH 8.0; CTAB 2; NaCl 1.4 M) and preheat to 80 °C for 5 min.
2. Pulverize 2–3 mg of tissue using liquid nitrogen.
3. Mix the pulverized tissue with 700 µl of CTAB 2X in a 2 mL eppendorf tube. Mix vigorously for 20 s.
4. Heat to 85 °C for 2 h and mix vigorously for 20 s.
5. Add 750 µl of chloroform: isoamyl alcohol (24: 1) and mix vigorously for 20 s.
6. Centrifuge for 60 min at 12,000 g (4 °C).
7. Transfer the aqueous phase to a 1.5 mL eppendorf tube.
8. Add 400 µl of isopropyl alcohol previously cooled to −20 °C. Mix gently for 1 min.
9. Centrifuge for 25 min at 10,000 g. Discard the supernatant.
10. Add 500 µl HPLC-grade water to the DNA pellet to dissolve the pectin (evident as a gelatinous substance). Do not mix and discard the dissolved pectin with a micropipette.
11. Resuspend the pellet in 1 mL of ethanol (70) previously cooled to −20 °C.
12. Centrifuge for 5 min at 10,000 g. Discard the supernatant.
13. Air-dry pellet at room temperature for 40 min.
14. Resuspend the pellet in 50 µl of HPLC-grade water.
15. Heat to 60 °C for 15 min.

Integrity of the extracted DNA

We analyzed the integrity of extracted gDNA from 17 species of Opuntia by electrophoresis (1 h with a 87 V cm−3 current) using 1.5 agarose gels prepared with TAE buffer (Tris Acetate-EDTA) and stained with Gel red (Biotium, USA). DNA bands were visualized under UV light with an Infinity 3000 transilluminator (Vilber Lourmat, Germany), which confirmed the presence of intact high quality gDNA without conspicuous contamination by proteins or other compounds (Fig. 1).

Evaluation of gDNA concentration

We determined gDNA concentration with a spectrophotometry analysis using a NanoDrop 8000 (Thermo, USA) and with a fluorometry analysis using Quant-iT ™ PicoGreen® dsDNA Assay Kit (Invitrogen ™) according to the manufacturer’s instructions.

Evaluation of the quality of gDNA

We assessed the purity of all the gDNA samples by spectrophotometry with a Nanodrop 8000 (Thermo, USA) (Table 1).

PCR amplifications

The purity of gDNA was confirmed through PCR of three different molecular markers: (1) nDNA internal transcribed spacer (ITS, 600 bp) [28–32]; (2) cpDNA RuBisCO gene (rbcL, 500 pb) [33, 34]; (3) mtDNA cytochrome oxidase subunit 3 (cox3, 1000pb) [35]. We used a negative control (without target gDNA) to confirm no contamination with extraneous DNA before the PCR. PCRs were performed on a final volume 25 µL containing 1 × buffer, 0.8 mM dNTPs mix, 20 pmol of each primer, 2 units of GoTaq DNA (Promega, USA) and 100 ng of template DNA. For each gene, PCRs consisted of an initial denaturation step at 96 °C for 2 min, followed by 35 cycles at 94 °C for 1 min, annealing temperature differing according to the primer for 1 min (Table 2), 72 °C elongation temperature for different time durations, depending on the length of the product. PCRs were performed using a Peltier Thermal Cycler PTC-200 (BIORAD, México). Amplification products were subjected to electrophoresis
(1 h with a 87 V cm$^{-3}$ current) using 1.5 agarose gels prepared with TAE buffer (Tris Acetate-EDTA), stained with Gel red (Biotium, USA) and visualized with an Infinity 3000 transilluminator (Vilber Lourmat, Germany). PCR products were purified with the ExoSAP Purification kit (Affymetrix, USA) and sequenced using the Bigdye terminator v.3.1 Cycle Sequencing kit (Applied Biosystems) and an Applied Biosystems 3730 $\times$ L automated sequencer (Applied BioSystems, USA).

**Sequence assembly**

DNA sequences were visualized, edited and assembled using BioEdit vers. 7.0.5 [36]. For each gene, consensus sequences were compared with those deposited in GenBank using the BLASTN 2.2.19 search algorithm [37].

**Comparison with previous methods**

Our protocol was compared with two previous methods [11, 13] using 17 species of *Opuntia*.

![Image of the agarose gel of genomic DNA (gDNA) ran by electrophoresis extracted from 17 tissue samples of *Opuntia* Mill., using the improved extraction method (Promega™ 1 kb DNA Ladder Molecular Weight Marker)](image)

**Table 1** Genomic DNA (gDNA) concentration and quality extracted from 17 tissue samples of *Opuntia* Mill. using the improved extraction method

| Species                        | PicoGreen ng/µl | NanoDrop ng/µl | C B ratio PicoGreen concentration/ NanoDrop concentration | $A_{260}/A_{280}$ NanoDrop | $A_{260}/A_{230}$ NanoDrop |
|-------------------------------|----------------|---------------|-------------------------------------------------------------|----------------------------|-----------------------------|
| 1. Opuntia auberi Pfeiff.     | 1250           | 1500          | 0.83                                                        | 1.9                        | 2.1                         |
| 2. Opuntia decumbens Salm-Dyck| 3199           | 3642          | 0.87                                                        | 1.9                        | 2.2                         |
| 3. Opuntia delafuentiana Martínez-González et al. | 8021 | 8126          | 0.98                                                        | 1.9                        | 2.2                         |
| 4. Opuntia depressa Britton and Rose | 2191 | 2588          | 0.84                                                        | 1.9                        | 2.0                         |
| 5. Opuntia durangensis Britton and Rose | 8220 | 8853          | 0.92                                                        | 1.8                        | 2.1                         |
| 6. Opuntia ficus-indica Mill. | 5898           | 6196          | 0.95                                                        | 1.9                        | 2.1                         |
| 7. Opuntia heliabraoana Scheinvar | 8341 | 9147          | 0.91                                                        | 2.0                        | 1.9                         |
| 8. Opuntia huapajpenss Bravo  | 3624           | 4497          | 0.80                                                        | 1.9                        | 2.1                         |
| 9. Opuntia joconostle F. A. C. Weber | 1091 | 1304          | 0.83                                                        | 1.8                        | 2.2                         |
| 10. Opuntia lasiaccantha Pfeiff. | 1892 | 2088          | 0.90                                                        | 1.9                        | 2.1                         |
| 11. Opuntia leischeinianana Martínez-González | 4799 | 5407          | 0.88                                                        | 1.9                        | 2.2                         |
| 12. Opuntia leucotricha DC.    | 6258           | 7000          | 0.89                                                        | 1.9                        | 2.2                         |
| 13. Opuntia matudaef Scheinvar | 2354           | 2802          | 0.84                                                        | 1.9                        | 2.2                         |
| 14. Opuntia megacantha Salm-Dyck | 6895 | 7861          | 0.87                                                        | 1.8                        | 2.1                         |
| 15. Opuntia microdasys Pfeiff. | 7526           | 8592          | 0.87                                                        | 1.9                        | 2.1                         |
| 16. Opuntia oligacantha Förster | 1548           | 1897          | 0.81                                                        | 1.8                        | 2.2                         |
| 17. Opuntia olmeca Joel Pérez et al. | 2112 | 2568          | 0.82                                                        | 1.9                        | 2.1                         |
Only one species (Opuntia ficus-indica) was shared with the protocol of Mondragón et al. [11].

**Results**

The list of the 17 species of Opuntia studied is shown in Table 1.

Our new extraction method allowed us to obtain high quality gDNA from young and mature cladodes using standard protocols using CTAB (Cetyl Trimethyl Ammonium Bromide), which efficiently extracts polysaccharides from leaf tissue. The Agarose gel electrophoresis showed the presence of large quantities of gDNA free of contaminants (Fig. 1). Accordingly, the large amount of gDNA was confirmed with two different methods (i.e., spectrophotometry and fluorimetry). These analyses yielded a mean gDNA ratio (PicoGreen concentration/Nanodrop concentration) of 0.80–0.98 ng/µl for all of the samples tested (Table 1). We obtained reliable absorbance readings from the spectrophotometric analysis.

The estimation of the $A_{260}/A_{280}$ absorbance ratio is a common way to measure DNA purity. Nucleic acids have a maximum absorbance at a wavelength of 260 nm, thus absorbance at this wavelength is directly proportional to DNA concentration. On the other hand, proteins show a maximum absorbance at 280 nm wavelength (mainly resulting from tryptophan residues), thus absorbance readings at 280 nm measure the concentration of proteins in the sample. Depending on the base composition of DNA, reading for the $A_{260}/A_{280}$ Ratio between 1.6 and 1.9 are indicative of high-quality DNA. In addition, absorbance readings at 230 nm wavelength measure the concentration of salts, carbohydrates and other contaminants, so the $A_{260}/A_{230}$ absorbance ratio should also be considered. Both $A_{260}/A_{280}$ and $A_{260}/A_{230}$ absorbance ratios are typically used to determine the purity of DNA samples that were extracted using biological, organic and inorganic compounds. Sambrook et al. [8] suggested that when measuring double-stranded DNA, the $A_{260}/A_{280}$ and $A_{260}/A_{230}$ absorbance ratios should ideally be in the range of 1.6–1.9 and 2.0–2.2, respectively. Accordingly, our absorbance analysis for all samples yielded values for $A_{260}/A_{280}$ and $A_{260}/A_{230}$ within the ideal range (Table 1), which is indicative of high quality of the extracted gDNA.

PCRs of rbcL, cox3 and ITS regions were successful for all samples (Fig. 2). DNA sequencing for all three regions was successful (Fig. 3), which allowed us to construct high-quality consensus sequences for all three regions.

In order to complement sequence quality assessment, we decided to assess the identity of sequences, at least preliminary, with a basic BLAST search. It has been documented that BLAST is not the proper mean for taxonomical identification, but it provides an easy way to broadly verify if the sequence belongs to the sample (e.g., verifying a potential contamination).

We conducted a BLAST search for each of the 17 sequences and the first hit on each search was recorded (Table 3). All the searches hit in sequences of Opuntia, but only five ITS sequences matched with the corresponding species. The other loci (rbcL and cox3) matched on Opuntia as well, but with non-corresponding species. BLAST results on rbcL and cox3 are due to the fact that those loci have very low variability at species level. Sequence variability was not enough for proper species identity, but sufficient for genera identity.

On the other hand, ITS is a loci with larger variability at species level. We found five searches that matched with the corresponding species. At four searches, the corresponding species were not available in GenBank, and no correct match was possible, but the search hit in Opuntia. The remaining searches on the ITS sequences did not match on the correct species, but did match in Opuntia. This result is due to two main reasons: 1) the BLAST search is not designed for species match, even if the species are available in the database, and in consequence it is not a suitable tool for specimens identification; and 2) because in most cases our sequences are longer (including ITS1 and 2 as well as 5.8S region) than those available in GenBank; this extra length may induce some errors.

**Comparison with previous methods**

We replicated the protocols of Mondragón-Jacobo et al. [11] and Griffith and Porter [13] using the same 17 species of Opuntia (Table 4). We confirmed that our method got better performance (quality and quantity of gDNA), and that it has some advantages over other protocols (Table 5). In addition, our protocol is the cheapest one and considered as a micro-method due to the amounts of reagents and tissue involved.

**Discussion**

Several gDNA extraction protocols were developed recently, but few of these have been focused on the elimination of pectin and polysaccharides. These two

| Table 2 Primers used in the amplification and sequencing of the DNA fragments |
| Locus/segment | Name | Sequence 5′–3′ | Tm (°C) |
|---------------|------|----------------|---------|
| ITS           | ITS5 | GGAAGTAAAGTGCTAACAAGG | 57      |
|               | ITS4 | TCCTCCGGCTTATGATGAC | 57      |
| rbcL          | 1f   | ATGTCAACCAAACAGAAAC | 56      |
|               | 724r | TCGCATGATCCGAGTACAG | 56      |
| cox3          | Cox3f | CCGTAGGAGGTGTTAGTGT | 51      |
|               | Cox3r | CTCCCCACCATTAGTACAG | 51      |

| Locus/segment | Name | Sequence 5′–3′ | Tm (°C) |
|---------------|------|----------------|---------|
| ITS           | ITS5 | GGAAGTAAAGTGCTAACAAGG | 57      |
|               | ITS4 | TCCTCCGGCTTATGATGAC | 57      |
| rbcL          | 1f   | ATGTCAACCAAACAGAAAC | 56      |
|               | 724r | TCGCATGATCCGAGTACAG | 56      |
| cox3          | Cox3f | CCGTAGGAGGTGTTAGTGT | 51      |
|               | Cox3r | CTCCCCACCATTAGTACAG | 51      |
compounds are among the most difficult contaminants to separate from the DNA [38] and significantly interfere with the activity of DNA polymerases. Therefore, the elimination of these compounds during the extraction of gDNA favors the efficiency of PCR amplification [39]. Pectin and mucilage (polysaccharides) are two of the main tissue components tissue in *Opuntia*. More specifically, pectin is the main component of the middle layer of cell walls and mucilage is one of the principal components of the parenchyma.

Mondragón-Jacobo et al. [11] developed a DNA extraction method for several cacti species (*e.g.*, *Cleistocactus* spp., *Echinocereus* spp., *Nopalea* spp., *Opuntia* spp., *Stenocereus* spp.). The amount of tissue used in this extraction protocol is species-dependent due to varying mucilage content among species. Griffith and Porter [13] extracted DNA from epidermal cells from several species of *Austrocylindropuntia, Brasiliopuntia, Consolea, Cumulopuntia, Cylindropuntia, Grusonia, Mathueniopsis, Miqueliopuntia, Nopalea, Opuntia, Pereskiopsis, Pterocactus, Tephorocactus* and *Tunilla*. In recent years, Mihalte et al. [25] showed that the protocol of Pop et al. [30] yielded sufficient amounts of DNA from small amounts of tissue for species of *Rebutia, Mediolobivia, Sulcorebutia* and *Aylostera*. Accordingly, Yu et al. [26] introduced a protocol, similar to that of Pop et al. [30], for reliable DNA extraction from *Hylocereus* spp. Montiel et al. [27] used root tissue from *Opuntia* to extract DNA due to the difficulties encountered during extraction from cladode tissue. Wong et al. [22] developed a method to extract DNA from *Hylocereus* spp. Out of these studies, only those of De la Cruz et al. [10], Mondragón-Jacobo et al. [11], Griffith and Porter [13], Montiel et al. [27] and Fehlberg et al. [40] tested extraction efficiency on species of *Opuntia*.

Our improved gDNA extraction method is based on the protocols of Mondragón-Jacobo et al. [11] and Griffith and Porter [13]. We developed this method for the
extraction of DNA from Opuntia cladodes, which contain large quantities of mucilage and pectin [20]. More specifically, improvements in the method involved changes to centrifugation and incubation steps (e.g., increased times and temperatures), the addition of water to remove pectin and the elimination of various reactive agents, such as polyvinylpyrrolidone (PVP), β-mercaptoethanol and protein and RNA degrading enzymes.

The increased centrifugation times allowed for a better separation of gDNA from fiber cells and non-soluble cellular components, such as proteins. As pectin is water-soluble, the addition of water permitted the extraction of this compound, forming a gelatinous substance over the precipitated gDNA [41, 42].

Generally, polyvinylpyrrolidone (PVP) is used to suppress polyphenolic oxidation during the extraction process [43]. However, PVP was not used because the main issue associated with DNA extraction from Opuntia samples is the presence of pectin and mucilage, and not of phenolic compounds.

The longer time of incubation at higher temperatures results in a more efficient denaturation of the proteins and enzymes found in tissue samples of Opuntia. Therefore, the extra step of incubation with proteinases is not needed.

The β-mercaptoethanol inhibits the activity of DNAs and RNAs and thus protects gDNA from degradation. However, we do not use this compound in our extraction protocol because EDTA (contained in CTAB) forms a molecular complex with Mg2+ ions that prevents the functioning of DNAs [8]. In turn, we do not use RNAse because we included a final drying step for 40 min, followed by 15 min at 60 °C, that allows for the efficient degradation of RNA.

Ribonucleases (RNAses) are abundant in all biological and most of these are fairly stable and difficult to inactivate even when extraction reagents and materials have been autoclaved. Thus, when extracting RNA from biological samples RNAses should be eliminated rapidly with denaturing compounds [8]. The presence of RNA in the samples is controlled with the fluorimetry analysis using the Quant-iT™ PicoGreen® Kit (Invitrogen™), which is an ultra-sensitive method for quantifying double-stranded DNA. The determination of absorbance at 260 nm wavelength is the commonly used technique for measuring the overall concentration of nucleic acids. However, absorbance measures have the main disadvantage of confounding the absorbance contribution of single-stranded nucleic acids, thus being unable to distinguish between DNA and RNA.
Table 3  Blast search for the three markers

| Species number | Description                  | Max score | Total score | Query cover (%) | E value | Ident (%) | Accession       |
|----------------|------------------------------|-----------|-------------|-----------------|---------|-----------|-----------------|
| **ITS**        |                              |           |             |                 |         |           |                 |
| 1              | Opuntia sp.                  | 865       | 865         | 100             | 0.0     | 100       | JF787077.1      |
| 2              | Opuntia bravoana             | 929       | 929         | 100             | 0.0     | 100       | JF87044.1       |
| 3              | Opuntia delafuentiana        | 968       | 968         | 100             | 0.0     | 100       | KM67822.1       |
| 4              | Opuntia depressa             | 822       | 822         | 100             | 0.0     | 99        | JF87089.1       |
| 5              | Opuntia martiniana          | 963       | 963         | 100             | 0.0     | 100       | JF87066.1       |
| 6              | Opuntia ficus-indica         | 1059      | 1059        | 100             | 0.0     | 100       | JF87101.1       |
| 7              | Opuntia robusta              | 1048      | 1048        | 100             | 0.0     | 99        | JF87122.1       |
| 8              | Opuntia velutina             | 850       | 850         | 100             | 0.0     | 100       | HQ872589.1      |
| 9              | Opuntia martiniana          | 1094      | 1094        | 100             | 0.0     | 100       | JF87066.1       |
| 10             | Opuntia pittieri             | 1109      | 1109        | 100             | 0.0     | 100       | JF87105.1       |
| 11             | Opuntia leiascheinvariana   | 970       | 970         | 100             | 0.0     | 100       | KM507353.1      |
| 12             | Opuntia cubensis             | 1027      | 1027        | 100             | 0.0     | 100       | JF87058.1       |
| 13             | Opuntia martiniana          | 1033      | 1033        | 100             | 0.0     | 100       | JF87066.1       |
| 14             | Opuntia pittieri             | 1120      | 1120        | 100             | 0.0     | 100       | JF87105.1       |
| 15             | Opuntia carstenii            | 992       | 992         | 100             | 0.0     | 100       | JF87112.1       |
| 16             | Opuntia oligacantha         | 953       | 953         | 100             | 0.0     | 100       | KX247005.1      |
| 17             | Opuntia bakeri               | 1059      | 1059        | 100             | 0.0     | 99        | JF87101.1       |
| **rbcL**       |                              |           |             |                 |         |           |                 |
| 1              | Opuntia maxima               | 1245      | 1245        | 100             | 0.0     | 100       | HM850212.1      |
| 2              | Opuntia dilleni              | 1262      | 1262        | 99              | 0.0     | 100       | HM850211.1      |
| 3              | Opuntia maxima               | 1254      | 1254        | 100             | 0.0     | 100       | HM850212.1      |
| 4              | Opuntia maxima               | 1262      | 1262        | 99              | 0.0     | 100       | HM850212.1      |
| 5              | Opuntia maxima               | 1258      | 1258        | 99              | 0.0     | 100       | HM850212.1      |
| 6              | Opuntia dilleni              | 1258      | 1258        | 99              | 0.0     | 100       | HM850211.1      |
| 7              | Opuntia dilleni              | 1262      | 1262        | 99              | 0.0     | 100       | HM850211.1      |
| 8              | Opuntia maxima               | 1260      | 1260        | 99              | 0.0     | 100       | HM850212.1      |
| 9              | Opuntia maxima               | 1262      | 1262        | 99              | 0.0     | 100       | HM850212.1      |
| 10             | Opuntia maxima               | 1260      | 1260        | 99              | 0.0     | 100       | HM850212.1      |
| 11             | Opuntia maxima               | 1260      | 1260        | 99              | 0.0     | 100       | HM850212.1      |
| 12             | Opuntia maxima               | 1260      | 1260        | 99              | 0.0     | 100       | HM850212.1      |
| 13             | Opuntia maxima               | 1253      | 1253        | 99              | 0.0     | 100       | HM850212.1      |
| 14             | Opuntia maxima               | 1090      | 1090        | 100             | 0.0     | 100       | HM850212.1      |
| 15             | Opuntia dilleni              | 1262      | 1262        | 99              | 0.0     | 100       | HM850212.1      |
| 16             | Opuntia maxima               | 1085      | 1085        | 100             | 0.0     | 100       | HM850212.1      |
| 17             | Opuntia maxima               | 1254      | 1254        | 99              | 0.0     | 100       | HM850212.1      |
| **cox3**       |                              |           |             |                 |         |           |                 |
| 1              | Opuntia megacantha           | 1117      | 1117        | 100             | 0.0     | 100       | EU930402.1      |
| 2              | Opuntia megacantha           | 1033      | 1033        | 100             | 0.0     | 100       | EU930402.1      |
| 3              | Opuntia megacantha           | 1125      | 1125        | 100             | 0.0     | 100       | EU930402.1      |
| 4              | Opuntia megacantha           | 900       | 900         | 100             | 0.0     | 100       | EU930402.1      |
| 5              | Opuntia megacantha           | 1212      | 1212        | 100             | 0.0     | 100       | EU930402.1      |
| 6              | Opuntia albicarpa            | 1179      | 1179        | 100             | 0.0     | 100       | EU930396.1      |
| 7              | Opuntia megacantha           | 1249      | 1249        | 100             | 0.0     | 100       | EU930402.1      |
| 8              | Opuntia megacantha           | 1175      | 1175        | 100             | 0.0     | 100       | EU930402.1      |
| 9              | Opuntia megacantha           | 1236      | 1236        | 100             | 0.0     | 100       | EU930402.1      |
| 10             | Opuntia megacantha           | 1234      | 1234        | 100             | 0.0     | 100       | EU930402.1      |
| 11             | Opuntia megacantha           | 1201      | 1201        | 100             | 0.0     | 100       | EU930402.1      |
| 12             | Opuntia megacantha           | 1223      | 1223        | 100             | 0.0     | 100       | EU930402.1      |
### Table 3 continued

| Species number | Description    | Max score | Total score | Query cover (%) | E value | Ident (%) | Accession       |
|----------------|----------------|-----------|-------------|-----------------|---------|-----------|-----------------|
| 13             | *Opuntia matudae* | 1225      | 1225        | 100 had          | 0.0     | 100       | EU930401.1      |
| 14             | *Opuntia megacantha* | 1238      | 1238        | 100 had          | 0.0     | 100       | EU930402.1      |
| 15             | *Opuntia megacantha* | 1171      | 1171        | 100 had          | 0.0     | 100       | EU930388.1      |
| 16             | *Opuntia megacantha* | 1173      | 1173        | 100 had          | 0.0     | 100       | EU930402.1      |
| 17             | *Opuntia megacantha* | 985       | 985         | 100 had          | 0.0     | 100       | EU930402.1      |

In this table is only recorded the first hit on each search.

### Table 4 Comparison among three different protocols to obtain total genomic DNA using NanoDrop

| Species                                      | Mondragón-Jacobo et al. [11] | Griffith and Porter [13] | This protocol |
|----------------------------------------------|-------------------------------|----------------------------|---------------|
| Species                                      | DNA yield (ng/µl) OD ratio 260.280 | DNA yield (ng/µl) OD ratio 260.280 | DNA yield (ng/µl) OD ratio 260.280 |
| 1. *Opuntia auberi* Pfeiff.                  | 256 1.4                       | 423 1.7                    | 1600 1.9      |
| 2. *Opuntia decumbens* Salm-Dyck             | 35 1.7                        | 30 1.9                     | 2930 1.9      |
| 3. *Opuntia delafuentiana* Martinez-González et al. | 75 1.6                       | 56 1.9                     | 4937 1.8      |
| 4. *Opuntia depressa* Britton and Tose       | 95 1.9                        | 73 1.8                     | 8755 1.9      |
| 5. *Opuntia duragensis* Britton and Tose     | 134 1.5                       | 123 1.7                    | 5835 1.9      |
| 6. *Opuntia ficus-indica* Mill.              | 34 1.8                        | 258 1.8                    | 3829 2.0      |
| 7. *Opuntia helabravoana* Scheinvar          | 198 1.6                       | 43 1.8                     | 8743 1.8      |
| 8. *Opuntia huapapensis* Bravo               | 57 1.5                        | 78 1.7                     | 1573 1.9      |
| 9. *Opuntia jococotle* F.A.C. Weber          | 86 1.9                        | 196 1.0                    | 8375 1.8      |
| 10. *Opuntia lasiacantha* Pfeiff.            | 67 1.7                        | 356 1.7                    | 2943 1.9      |
| 11. *Opuntia leiascheinvariana* Martinez-González | 110 1.8                      | 98 1.9                     | 3980 1.9      |
| 12. *Opuntia leucotricha* DC.                | 248 1.5                       | 34 1.8                     | 3789 1.9      |
| 13. *Opuntia matudae* Scheinvar              | 93 1.7                        | 63 1.8                     | 7947 1.9      |
| 14. *Opuntia megacantha* Salm-Dyck           | 117 1.6                       | 78 1.8                     | 7000 1.8      |
| 15. *Opuntia microdasys* Pfeiff.             | 44 1.5                        | 39 1.7                     | 6578 1.9      |
| 16. *Opuntia oligacantha* Färster            | 87 1.8                        | 70 1.9                     | 2395 1.8      |
| 17. *Opuntia olmeca* Joel Pérez et al.       | 94 1.5                        | 57 1.7                     | 9200 1.9      |

### Table 5 Advantages of our protocol

| Mondragón-Jacobo et al. [11] | Griffith and Porter [13] | This protocol |
|-------------------------------|--------------------------|---------------|
| They tried to use young tissues, avoiding older ones because their higher content of fiber and cuticular wax. | They tried to use epidermal tissue free of waxes | We can use tissue from any part of the plant |
| They used β-mercaptoethanol 8000 mg of cactus pear tissue | They used β-mercaptoethanol 30–50 mg of epidermal tissue | We did not use β-mercaptoethanol 2–3 mg of tissue from every part of the plant |
| They used more CTAB (25 ml) | They used more CTAB (15 ml) | We used few CTAB (0.7 ml) |
| They used more chloroform-isooamy alcohol (10 ml) | They used more chloroform-isooamy alcohol (5 ml) | We used few chloroform-isooamy alcohol (0.75 ml) |
| They used ethanol (8.7 ml) | They used more isopropanol (5 ml) | We used few isopropanol (0.4 ml) |
| They used bigger and expensive tubes (15 ml) | They used bigger and expensive tubes (15 ml) | We used smaller tubes (2 ml) |
| They used RNase to eliminate RNA | They did not use RNase | We did not use RNase |
The purity of the extracted gDNA was confirmed by spectrophotometry. Generally, a higher A$_{260}$/A$_{280}$ value is indicative of RNA contamination, whereas lower values are indicative of protein contamination. On the other hand, lower A$_{260}$/A$_{230}$ values indicate the presence of phenolic compounds and carbohydrates, whereas higher values are usually associated with calibration errors [44]. The A$_{260}$/A$_{280}$ and A$_{260}$/A$_{230}$ ratios for dsDNA ideally range from 1.6 to 1.9 and from 2.0 to 2.2, respectively [8]. Our analyses showed A$_{260}$/A$_{280}$ and A$_{260}$/A$_{230}$ within these ideal ranges (Table 1), which confirm the purity of the gDNA samples. Through the improvement of DNA extraction protocols, we were able to improve the overall yield and purity of gDNA (1500–9147 ng/μl, Table 1) extracted from different species of *Opuntia*. In addition, with these changes, the extraction protocol becomes cheaper and the use of toxic reagents is diminished. When we compared our method with other two previous protocols [11, 13], we observed that the necessary amount of tissue in these two protocols is huge. Also, both methods need a great amount of expensive chemical reagents, making them impractical. With our new protocol, we obtained a higher DNA performance with high molecular weight (1500 ng/μl), and an average of the ratio A$_{260}$/A$_{280}$ of 1.8.

Our protocol is a good alternative to these methods, since it requires milligrams of tissue and small volumes of reagents, facilitating the handling of a large number of samples. In short, our method is cheaper, quick and simple, and it does not need to carry out additional purification.

**Conclusion**

In this study, we developed a method of DNA extraction that yields high-quality gDNA free of inhibitory organic compounds common in species of *Opuntia*, such as pectin and mucilage. This improved method allowed us to obtain higher yields of gDNA of excellent quality. Our method works in other species of cacti (e.g., *Nopalea schinia* [45]); it will be interesting to test it in other Cactaceae and succulent plants such as Crassulaceae. Finally, we are demonstrating that the addition of RNAses is not necessary to remove RNA from the genomic DNA samples. The use of RNAs is replaced by a heat treatment to remove the RNA making the protocol cheaper.

**Authors’ contributions**

CRMG performed the protocol methodology, standardization, as well as the coordination and integration of laboratory results, RRM provided technical support in the laboratory for standardization of the protocol and protocol replicate the species of interest and conducting PCR’s, JIR performed field identification and collection of the species distributed in the central region of Mexico, COV performed field identification and collection of the species distributed in the North central region of Mexico and ILV performed protocol integration, results and coordinated the development of the final work. All authors read and approved the final manuscript.

**References**

1. Scheinvar L. 2004. Caracteres macroscopicos, microscopicos y moleculares valiosos para la identificacion de los recursos genéticos del nopal. In: Memorias X Congreso Nacional y VIII Congreso Internacional sobre el conocimiento y aprovechamiento del nopal. Agosto. UACh. Texcoco, Mexico City.
2. Reyes-Aguero JA, Aguirre-Rivera JR, Hernández HM. Nota sistemática y una descripción detallada de *Opuntia ficus-indica* (L.) Mill. (Cactaceae). Agrociencia. 2005;39:395–408.
3. Wang X, Falher P, Burrow MD, Peterson AH. Comparison of RAPD marker patterns to morphological and physiological data in the classification of *Opuntia* accessions. J Prof Assoc Cactus. 1998;3:3–14.
4. Srikanth K, Whang S. Phylogeny of Korean *Opuntia* spp. based on multiple DNA regions. Turk J Bot. 2015;39:635–41.
5. Equiarte L, Souza V, Aguirre X. Ecología Molecular. Secretaría de Medio Ambiente y Recursos Naturales, Instituto Nacional de Ecología. Universidad Nacional Autónoma de México. Mexico City; 2007. 594 p.
6. Plaza GA, Upchurch R, Brigmom RL, Whitman WB, Ulfi K. Rapid DNA extraction for screening soil filamentous fungi using PCR amplification. Polish J Environ Stud. 2003;13:315–8.
7. Park O. Genomic DNA isolation from different biological materials. Methods Mol Biol. 2007;353:3–13.
8. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning A laboratory manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press; 1989.
9. Boiteux LS, Fonseca M, Simon PW. Effects of plant tissue and DNA purification method randomly amplified polymorphic DNA-based genetic fingerprinting analysis in carrot. J Am Soc Hortic Sci. 1999;124:32–8.

**Author details**

1 Laboratorio de Biogeografía y Sistématica, Departamento de Biología, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyocán, 04510 Mexico City, México. 2 Laboratorio de Biotecnología de Semillas, Colegio de Postgraduados, Carretera México-Texcoco, 56230 Estado de México, México. 3 Herbario de la Facultad de Ciencias, Departamento de Biología Comparada, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyocán, 04510 Mexico City, México. 4 Centro Regional Universitario Centro Norte, Universidad Autónoma Chapingo, Cruz del Sur núm. 100, Colonia Constelación, El Orito, 98085 Zacatecas, Zacatecas, México.

**Acknowledgements**

The editor of this journal, Ricardo García-Sandoval and three anonymous referees did great suggestions to our manuscript.

**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

Not applicable.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

Not applicable.

**Funding**

This research was financially supported by the Red del Nopal belonging to the Sistema Nacional de Recursos Fitogenéticos para la alimentación y la agricultura del Servicio Nacional de Inspección y certificación de semillas and by the Laboratorio de Biogeografía del Departamento de Biología Evolutiva, Universidad Nacional Autónoma de México.

**Publisher’s Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 7 October 2016 Accepted: 3 October 2017

Published online: 11 October 2017

**Publisher’s Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
27. Montiel D, Ramírez-F, Hernández-H. DNA isolation and amplification from cacti. Plant Mol Biol Rep. 1997;1:19–21.
28. Mondragón-Jacobo C, Doudaeva N, Bordeleau BP. DNA extraction from several cacti. HortScience. 2000;35:1124–6.
29. Nyffeler R. Phylogenetic relationships in the cactus family (Cactaceae) based on evidence from trnK/matK and trnL-trnF sequences. Am. J. Bot. 2002;89:312–36.
30. Griffith MP, Porter JM. Back to the basics: a simple method of DNA extraction for mucilaginous cacti. Bradleya. 2003;21:126–8.
31. Edwards E, Nyffeler R, Doneghue J. Basal cactus phylogeny: implications of Pereskia (Cactaceae) paraphyly for the transition to the cactus form. Am. Naturalist. 2005;165:1177–88.
32. Matsuhiro B, Lillo L, Sáenz C, Urzúa C, Zárate O. Chemical characterization of the mucilage from fruits of Opuntia ficus-indica. Carbohydr. Polym. 2006;63:263–7.
33. Borsch T, Quandt D, Taylor P, Müller F, Barthlott W. What does it take to resolve relationships and to identify species with molecular markers? An example from the epiphytic Rhipsalidae (Cactaceae). Am. J. Bot. 2011;99:847–64.
34. Goycoolea F, Cárdenas A. Pectins from Opuntia spp.: a short review. J. Prof. Assoc. Cactus. 2003;5:17–23.
35. Nobel P, Cavalieri J. Mucilage in cacti: its apoplastic capacitance, associated solutes, and influence on tissue water relations. J. Exp. Bot. 1992;43:641–8.
36. Sepúlveda E, Sáenz C, Aliaga E, Aceituno C. Extraction and characterization of mucilage of Opuntia ficus-indica. J. Prof. Assoc. Cactus. 2003;5:17–23.
37. Nyffeler R. Phylogenetic relationships in the cactus family (Cactaceae) paraphly for the transition to the cactus form. Am. Naturalist. 2005;165:1177–88.
38. Manshadi B, Lillo L, Sáenz C, Urraza C, Zárate O. Chemical characterization of the mucilage from fruits of Opuntia ficus-indica. Carbohydr. Polym. 2006;63:263–7.
39. Guevara-Arauza J, Ornelas-Paz J, Pimentel-González D, Rosales S, Soria-Guerra R, Piz-Maldonado T. Prebiotic effect of mucilage and pectin-derived oligosaccharides from nopal (Opuntia ficus-indica). Food Sci. Biotechnol. 2012;21:997–1003.
40. Aljaniabi SM, Forget L, Dookun A. An improved and rapid protocol for the isolation of polysaccharide-and polyphenol-free sugarcane DNA. Plant Mol Biol Rep. 1999;17:1–8.
41. Wong LM, Silvaraj S, Phoon LQ. An optimised high-salt CTAB protocol for both DNA and RNA isolation from succulent stems of Hylocereus sp. J. Med. Biol. Eng. 2014;3:236–40.
42. Katterman F, Shattuck-VL. An effective method of DNA isolation from the mature leaves of Gossypium species that contain large amounts of phenolic terpenoids and tannins. Prep. Biochem. 1983;13:347–59.
43. Tel-Zur N, Abbo S, Myslabodski D, Mizrahi Y. Modified CTAB procedure for DNA isolation from epiphytic cacti of the genera Hylocereus and Seleneceus (Cactaceae). Plant Mol Biol Rep. 1999;17:249–54.
44. Mihaljević L, Sestras R, Feist G. Assessing genetic variability at different genotypes of cacti plants by means of RAPD analysis. Bull. UASVM Hort. 2008;65:110–5.
45. Yu ZX, Ou GZ, Chen QX, Yuan YF. Study on comparison of methods for dragon fruit total DNA extraction. Chin Agric Sci Bull. 2010;26:300–3.
46. Montiel D, Valadéz-Moctezuma E, Palomino G, Bermúdez M, Fernández F. DNA extraction from roots of xoconostle. J. Prof. Assoc. Cactus. 2012;14:35–40.
47. Kim SR, Yang J, An G, Jena KK. A simple DNA preparation method for high quality polysaccharide chain reaction in rice. Plant Breed. Biotechnol. 2016;4:99–106.
48. Pop IF, Pamfil D, Raica PA, Petricele IV, Botu J, Vicol AC, Harta M, Sisea CR. Evaluation of the genetic diversity of several Corylus avellana accessions from the Romanian National Hazelnut Collection. Not Bot Horti Agrobot. 2010;38:61–7.
49. Rogers SQ, Bendich AJ. Ribosomal genes in plants: variability in copy number and in the intergenic spacer. Plant Mol Biol. 1987;9:509–20.
50. Hamby RK, Zimmerman EA. Ribosomal RNA as a phylogenetic tool in plant systematics. In: Solis PS, editor. Molecular systematics of plants. New York: Springer, Chapman and Hall; 1992. p. 50–91.
51. Weider L, Elser J, Cease T, Mateos M, Cotner J, Markow T. The functional significance of ribosomal (r)DNA variation: impacts on the evolutionary ecology of organisms. Annu. Rev. Ecol. Evol. Syst. 2005;36:219–42.
52. Berg S, Krause K, Krupinska K. The rbcL genes of two Cuscuta species, C. granovi and C. subinclusa, are transcribed by the nuclear-encoded plastid RNA polymerase (NEP). Planta. 2004;219:541–6.
53. Gómez-J, Flexas J, Keys AJ, Cifre J, Mitchell RAC. Rubisco specificity factor tends to be larger in plant species from drier habitats and in species with persistent leaves. Plant Cell Environ. 2005;28:571–9.
54. Dunmil J, Pemonge H, Petit M. A set of 35 consensus primer pairs amplifying genes and introns of plant mitochondrial DNA. Mol. Ecol. Notes. 2002;2:428–30.
55. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 1999;41:95–8.
56. Zhang Z, Schwartz S, Wagner L, Miller W. A greedy algorithm for aligning DNA sequences. J. Comput. Biol. 2000;7:203–14.
57. Murray MG, Thompson WF. Rapid isolation of high molecular weight DNA. Nucleic Acids Res. 1980;8:4321–5.
58. Lodhi MA, Ye GN, Weeden NF, Risbey B. A simple and efficient method for DNA isolation from grapevine cultivars and Vitis species. Plant Mol Biol Rep. 1994;12:6–13.
59. Fehrlberg SD, Allen JM, Church K. A novel method of genomic DNA extraction from Cactaceae. Appl. Plant Sci. 2013;1:1–4.
60. Cárdenas A, Higuera-Ciapara I, Goicoeboa FM. Rheology and aggregation of cactus (Opuntia ficus-indica) mucilage in solution. J. Prof. Assoc. Cactus. 1997;2:152–9.
61. Maddob H, Roudesli S, Picton L, Le Cerf D, Muller G, Grisel M. Prickly pear nopal pectin from Opuntia ficus-indica physicochemical study in dilute and semi-dilute solutions. Carbohydr. Polym. 2001;46:69–79.
62. Porebski S, Bailey LG, Baum BR. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol Biol Rep. 1997;15:8–15.
63. Meyer R. Detection methods for genetically modified crops. In: Keller JK, editor. Genetically engineered food methods and detection. KGA. Hoboken: Wiley; 2003. p. 188–200.
64. Martínez-González CR, Alcántara-Ayala O, Luna-Vega I, García-Sandoval R. Phylogenetic placement and new data on macro and micro morphology of Nopalea phyllanthoides (Cactaceae), an endangered species from Mexico. Phytotaxa. 2015;222(4):241–50.