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Extraction of genomic DNA from different grass species using a modified Qiagen method

Rita kumari, Anjali kumari
Dr, Student
Rameshwar Mahavidyalaya , BRABU Muzaffarpur

Background

Effective DNA extraction is a critical step in genetic research. The Caigen method is a well-known technique for isolating high-quality DNA. However, modifications to this method can enhance DNA yield and purity, particularly when working with challenging sample types such as dry plant materials. Grass species are fundamental components of many ecosystems and play a crucial role in agriculture. Analyzing DNA from diverse grass species helps in understanding genetic diversity, adaptation mechanisms, and evolutionary patterns, which are essential for conservation and agricultural improvement. In this study, we have employed a modified Caigen method to extract DNA from dry grass samples collected from various regions across India. The modification aimed to improve DNA concentration and quality, addressing the specific challenges posed by dry, fibrous plant tissues. Our research focuses on 37 different grass species, providing valuable genetic insights and contributing to the broader understanding of grass genetics and biodiversity.DNA molecular techniques are mainly based on polymerase chain reaction (PCR) assay that requires isolation of genomic DNA of suitable purity. Various extraction protocols have been established in order to isolate pure and intact whole genomic DNA from plant tissues.While handling grass samples initially we faced problems to extract a good quantity of DNA . We tried different methods of extraction like Edward's method, Ctab method but this method gave best results. We modified it accordingly so that impurities are minimized and we get a good graph. For analyzing the concentration of DNA we used nanodrop Spectrophotometer. This study's findings are expected to support conservation efforts and enhance knowledge about the genetic diversity of grass species, which are vital for both ecological balance and agricultural productivity.

Materials and Methods

Plant materials

Thirty seven Plant samples were collected from all over India. For this we went to different places like Gir, Hingolgadh, Shokhrukh, Kudrevatti, Jessore, Sengeltheri , Bangalore, NSTR, Laldhang ,Sonpur,Lakkidi,Masinagudi,Osmanabad and Eravikulam. We are trying to sample all the grass species for building phylogeny.

QIAshredder spin column

DNeasy Mini spin column

Reagents

Buffer AP1

Buffer P3

Buffer AW1

Buffer AW2

Buffer AE (Elution Buffer)

Modified DNA extraction protocol

1. Disrupt samples using Mortar and pestle.
2. Add 400 µl Buffer AP1. Vortex and incubate for 1 hour. (Invert the tubes 2-3 times during incubation)
3. Add 130 µl of P3 Buffer. Mix and incubate for 30 mins on ice.
4. Centrifuge the lysate for 5 mins at 14000 rpm.
5. Pipet the lysate into a QIAshredder spin column placed in a 2ml collection tube.

   Centrifuge for 10 mins at 13000 rpm.
6. Transfer the flowthrough into a new tube without disturbing the pellet if present. Add 1.5 volumes of Buffer AW1 (ethanol mixed) and mix by pipetting.
7. Transfer 650 µl of the mixture into a DNeasy mini spin column placed in a 2ml collection tube. Centrifuge for 1 min at 8000 rpm. Discard the flowthrough and repeat this step with the remaining sample.
8. Place the spin column into a new 2 ml collection tube. Add 500 µl of Buffer AW2(ethanol mixed). Now centrifuge it for 1 min at 6000 rpm . Discard the flowthrough.
9. Add another 500 µl of buffer AW2(ethanol mixed). Centrifuge for 5 mins at 13000 rpm. NOTE:Remove the spin column carefully so that the column does not come in contact with the flowthrough.

10. Transfer the spin column to a new 1.5 or 2 ml microcentrifuge tube.
11. Add 100 µl of AE buffer for elution.Incubate for 5 mins at room temperature. Centrifuge for 1 min at 6000 rpm.
12. Repeat step 11.

Quantitative and qualitative analysis of DNA extracted by established Caigen method and modified protocol

DNA concentration, purity, and quality

DNA concentration was determined spectrophotometrically at 260 nm (A260) absorption using NanoDrop1000 (Thermo Scientific). Purity of DNA from protein and polysaccharide contamination (Wilson and Walker 2005) was assessed by estimating the absorbance ratio at A260/A280 and A260/A230 respectively. The quality of the extracted DNA using both protocols was also evaluated by electrophoresis separation for all DNA samples on 1% agarose gel stained with ethidium bromide (1 µg/ml).

Now here is the list and concentration of DNA , I have got after extracting

Extract ID	Specie	Place	Concentration ng/microlitre	S no
DIRE-JE-1	D.retroflexa	Jessore	368.3	1
CYCA-SE-3	C caesius	Sengeltheri	131	2
SENE-HI-1	S.nervosum	Hingolgadh	73.8	3
CHZI-LA-1	C.zinzinoides	Laldaang	91.2	4
CHHA-KU-1	C.haeckelii	Kudrevetti	117.4	5
CHOR-KU-2	C orientalis	Kudrevetti	115.6	6
THSP-KU-1	Themeda sp	Kudrevetti	152	7
THECA-SH-1	Taenaxia cachemyriana	Shokhrakh	128.8	8
THUDI-UN-2	D.annaelatum	NSTR	96.3	9
THUCY-GI-1	C gidarba	NSTR	112.3	10
CYFL-SE-1	C flexous	Sengeltheri	89.6	11
UG1SH1	Rhododendron understory	Shokhrakh	90.9	12
LAKTH-SA-1	T sabarimalayan	Lakkidi	212.2	13
EUTH-LA-1	Eulalia twatsi	Lakkidi	885.6	14
ISKU-LA-2	I kunthiana	Lakkidi	549.8	15
THECA-SH-1	Danthonia sp	Shokhrakh	86.3	16
HISE-NE-1	S nervosa	Hingolgadh	98.1	17

KUDSO-UN-1	Sorghum sp	Kudrevetti	234.3	18
JAAUN-UN-3	Understory grass	Gir	92.1	19
JAAAP-MU-3	Apluda sp	Gir	160.3	20
MASCY-UN-1	Cynodon sp	Masinagudi	167.2	21
MAS-BR-UN-1	Brochiaria sp	Masinagudi	108.3	22
ISSP-SO-2	Ishaemum sp	Sonpur	186.2	23
CAHU-GI-1	C hugleii	Gir	81.9	24
MINBO-UN-1	Brothiochloa	Minew	224	25
MHAUN-UN-1	Wetland grass	Osmanabad	254.3	26
PESPB-A1	Pennisetum	Bangalore	701	27
ERV-DI-OL-1	Dicanthimum oliganthum	Ervikulum	89.4	28

Gel electrophoresis

To separate DNA using agarose gel electrophoresis, the DNA is loaded into pre-cast wells in the gel and a current is applied. The phosphate backbone of the DNA (and RNA) molecule is negatively charged, therefore when placed in an electric field, DNA fragments will migrate to the positively charged anode.

Here we have made 1% agarose gel. So for that

1) Measure 1g of Agarose
2) Mix agarose powder in 100ml 1x TAE microwave flask.
3) Microwave it for 5 minutes until the agarose is completely dissolved.
4) Let Agarose solution cool down so that we can hold it with our hands.
5) Pour the agarose in gel tray with well comb in place.
6) Now let it solidify at room temperature for 30 mins.

Loading and Preparing samples on butter paper

1) Take 2 microlitre of ladder and add 2 microlitre of dye to it.
2) Now add 2 microlitre of sample and add 2 microlitre of dye to it.

   Like this with all the samples.

3) Now carefully load the ladder into the 1st lane of gel.

   We use ladder because we know the size of the ladder . Manufacturer tells us the size of each band. So taking that as reference we can know the size of remaining bands.

4) Likewise load other samples in remaining lanes.
5) Now run the gel at 80-150 V until the dye is 50% way down the gel . For this approx time taken will be 40-45 minutes.

Analyzing gel using gel doc

A Gel Documentation System (GDS) is a laboratory instrument used to visualize and document DNA, RNA, and protein samples that have been separated by gel electrophoresis. We just need to place our gel inside and it automatically processes the image. And here is the result of what I got.

Possible reasons behind Smear formation and How did we find out?

1) The extraction kit is too old or expired long back. So unknowingly I extracted DNA from that kit and I got good DNA concentration too. So I did labeling and stored it but after a month when I checked it's concentration then I found it's concentration to have decreased drastically.
2) Maybe the modification of protocol wasn't good enough for bands to occur and using RNAse is MUST.
3) Poor sample quality could be a possible reason because fresh samples showed bands and samples more than an year old didn't show bands. Maybe the DNeasy Plant mini kit is not made for dried and very old grass samples.
4) Maybe the normal refrigerator temperature(4 degree) isn't suitable for storing it .And it should be stored at -20 degree celsius. So, storage can also create problems.

   Degradation of DNA has many reasons and we can't exactly predict what is the correct reason. I assumed all these to be reasons based on my extraction. But maybe the reason could be something else .

NOTE : For herbarium sheet type, DNA modification is needed if we are doing this method then problems may come .

https://sci-hub.st/https://link.springer.com/protocol/10.1007/978-1-0716-0997-2_4

We should look at this. They have extracted herbarium DNA and protocol is different . So this is the reason I think .

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