The American Journal of Applied sciences
(ISSN – 2689-0992)
VOLUME 04 ISSUE 04 Pages: 1-6
SJIF IMPACT FACTOR (2020: 5. 276) (2021: 5. 634) (2022: 6. 176)
OCLC – 1121105553 METADATA IF – 7.987
Publisher: The USA Journals

Journal Website: https://theamericanjournals.com/index.php/tajas

Research Article

STAB SEPARATION FROM PLANT ISSUE BY STAB

Submission Date: March 26, 2022, **Accepted Date:** April 02, 2022,
Published Date: April 16, 2022 |
Crossref doi: https://doi.org/10.37547/tajas/Volume04Issue04-01

Dilshod Akhmadovich Mullayev
Doctor of Philosophy (PhD), Acting Associate Professor, Department of "Biology and its teaching methods", Tashkent State Pedagogical University named after Nizami, Uzbekistan

Khusnorahon To'lqin qizi Tojiboyeva
Student, Tashkent State Pedagogical University named after Nizami, Uzbekistan

ABSTRACT

This article provides information on DNA extraction from plant tissues, their methods, and the effective STAB method. It also provides detailed information on the tools required to implement the STAB method, the reagents, and the sequence of operations.

KEYWORDS

DNK, plant, genotype, method, tissue, stab, supernatant, sediment, leaf, homogenization.

INTRODUCTION

Methods of DNK extraction from plant cells

The first step in analyzing plant genotypes is to perform DNK extraction from plants. In doing so, researchers used a number of methods. Examples include:

1. Phenol-chloroform method. This is an old but useful method. The downside is that the reagents used are dangerous to human health and do not always contain quality DNK for PCR and sequins.
2. STAB method. It is a very useful method for the plant. In practice, total plant DNA can be isolated.

The American Journal of Applied sciences
(ISSN – 2689-0992)
VOLUME 04 ISSUE 04 Pages: 1-6
SJIF IMPACT FACTOR (2020: 5. 276) (2021: 5. 634) (2022: 6. 176)
OCLC – 1121105553 METADATA IF – 7.987
Publisher: The USA Journals

On the plus side, reagents used are less dangerous to human health and cheaper than other methods. The downside is that it takes a lot of time.

3. KIT method. In this method, several steps are shortened by the addition of reagents in accordance with each other, compared to other methods. On the plus side, DNK is of good quality and breaks down very quickly. The downside is that it is very expensive. [1]

There are a number of difficulties in extracting pure and high-quality DNA from plants. For example, due to the high molecular weight compounds present in plants, a lot of time and reagents are spent on the separation of nucleic acids [2].

Most plant species contain high molecular weight polysaccharides, polyphenols, several pigments, and other secondary metabolites.

It is very important to select the leaf tissue of plants to isolate DNK. DNK extraction is effective due to the low content of polysaccharides and polyphenols in young eagles [3].

There are some ways to reduce the stages of DNK extraction, but they involve the use of large amounts of plant tissue and liquid nitrogen [4].

The method used by Doyle and Doyle (1987) to extract DNK from plants using liquid nitrogen is widely used. In recent years, various scientists have been working more effectively to modify this method. The STAB method, modified by Doyle and Doyle (1990), has been successfully applied to many plant species [5].

A number of scientists have also made some changes to the KIT method to increase its effectiveness.

DNK separation STAB method.

In our study, we used the STAB method and its modification, which are more useful and popular, to separate genome DNK from a young leaf of cotton.

DNK buffers, their composition, and preparation:

2 x STAB	**300ml**	
100mM Tris pH-8.0	1MTris pH-8.0	30ml
20mM EDTA pH-8.0	0.5M EDTA pH-8.0	12ml
1.4M NaCl	NaCl	24.5448 g
2% STAB	STAB	6 g

The American Journal of Applied sciences
(ISSN – 2689-0992)
VOLUME 04 ISSUE 04 Pages: 1-6
SJIF IMPACT FACTOR (2020: 5. 276) (2021: 5. 634) (2022: 6. 176)
OCLC – 1121105553 METADATA IF – 7.987
Publisher: The USA Journals

Hold in the autoclave for 30 minutes.

10 x STAB (STAB/NaCl)	**100ml**	
0.7M NaCl	NaCl	4.1 g
10%STAB	STAB	10 g

Hold on to the autoclave for 30 minutes.

STAB presipitation	**100мл**	
50mM Tris pH-8.0	1M Tris pH-8.0	5ml
10mM EDTA pH-8.0	0.5M EDTA pH-8.0	2ml
1% STAB	STAB	1 g

Hold on to the autoclave for 30 minutes.

Highly salty TE	**200ml**	
10mM Tris pH-8.0	1M Tris pH-8.0	2ml
0.1mM EDTA pH-8.0	0.5 M EDTA pH-8.0	40mkl
1M	NaCl	11.68 g

Hold on to the autoclave for 30 minutes.

RNKaza

10 ml of RNA is added to 1 ml of dH 2O and soaked in 100° C water for 10 minutes

- Stored at 20° C.

TE	**50 ml**	
10mM Tris	1M Tris pH 8.0	0.5 ml
1mM EDTA	0.5M EDTA pH 8.0	0.1 ml

The American Journal of Applied sciences
(ISSN – 2689-0992)
VOLUME 04 ISSUE 04 Pages: 1-6
SJIF IMPACT FACTOR (2020: 5. 276) (2021: 5. 634) (2022: 6. 176)
OCLC – 1121105553 METADATA IF – 7.987
Publisher: The USA Journals

0.5 M EDTA pH 8 (Hold on to the autoclave for 30 minutes.)

dH2O	700 ml
$Na_2EDTA2H_2O$	186.1 g
10N Na OH	pH=8.0
dH_2O	1litr

3M NaOAc pH 5.2 (Hold on to the autoclave for 30 minutes.)

dH_2O	300 ml
$NaC_2H_3O_23H_2O$(NaOAc)	408 g
Acetic acid concentration	pH=5.2 + dH_2O 1 liter
Xloroform/Izoamil	24ml xloroform 1ml izoamil

REQUIRED EQUIPMENT.

The following laboratory instruments and equipment were used in the dissertation:

1. Dallas (Pipetman, USA)
2. Centrafuga (Eppendorf, Germany)
3. Thermostatic water bath (LKB, Sweden)
4. Vortex (Genie Scientific Industries, Inc., USA)
5. Autoblod (Bellco Glass, Inc., USA)
6. Horizontal electrophoresis equipment (Stratagene, USA)
7. Thermostatic mixer (Eppendorf, Germany)
8. Concentrator (Eppendorf, Germany)
9. Thermostat (Heraeus, Germany)
10. Microwave oven (Ferette, Italy)
11. Scale (Sartorius, USA)
12. Image Documentation Equipment Alpha Imager 3400 (Alpha Innotech Inc., USA)

SEPARATION OF GENOM DNA FROM PLANT ISSUE BY STAB.

1. The leaf is frozen in liquid nitrogen and homogenized.
2. Fill 650 µl 2xCTAB and place on AUTOBOT for 20 minutes (stirring every 5 minutes).
3. Add 650 µl of chloroform: isoamyl (24: 1) and mix vigorously for 4 minutes.
4. Centrifuge at 5 min / 10,000 rpm and transfer 550 µl from the top of the supernatant to another solution.
5. Add 550 µl of chloroform: isoamyl (24: 1) and mix vortex.
6. Centrifuge at 5 min / 10,000 rpm and transfer 500 µl from the top of the supernatant to another solution.
7. Add 300 µl of High Salt to the precipitate and vortex for 5 minutes, then keep it at room temperature for 15 minutes.
8. Add 250 µl of isopropanol and mix slowly for 2 minutes and refrigerate at -200 ° C.

The American Journal of Applied sciences
(ISSN – 2689-0992)
VOLUME 04 ISSUE 04 Pages: 1-6
SJIF IMPACT FACTOR (2020: 5. 276) (2021: 5. 634) (2022: 6. 176)
OCLC – 1121105553 METADATA IF – 7.987
Publisher: The USA Journals

9. Centrifuge for 15 min / 10,000 rpm and pour supernatant.
10. Add 500 µl of 70% alcohol to the precipitate and mix for 5 minutes.
11. Centrifuge at 3 rpm / 14,000 rpm and pour in the alcohol.
12. 500 µl of 70% alcohol is added to the sediment.
13. Centrifuge for 3 min / 14,000 rpm and pour in the alcohol.
14. Sediment is dried in a DNK concentrator for 10-15 minutes.
15. Vortex 100 µl of TE buffer onto the sink and place in a short centrifuge and -200 C refrigerator.

EXAMINATION OF DNA SAMPLES BY ELECTROPHORESIS.

DNK electrophoresis in agarose gel is a standard method used for the purification and identification of DNK fragments.

The method of electrophoresis of DNK on horizontal agarose gel plates is widely used in molecular genetics and biochemistry. Because the reagents of this method are easy to find, the simplicity of the method and the low cost of the equipment make it possible to obtain sufficient information from very small amounts of untreated material.

Therefore, the experiments use the method of electrophoresis on horizontal agarose gel plates.

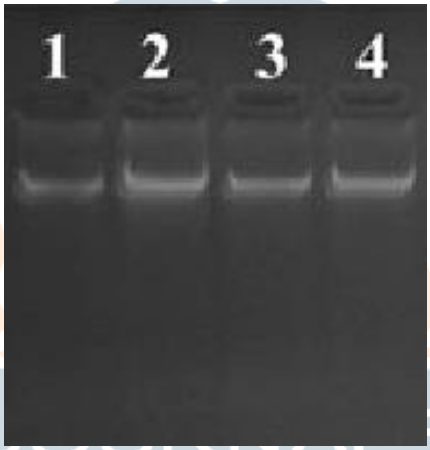

Figure 1 DNK isolated using the Stab method.

Plant DNK samples isolated using the standard STAB method of DNK separation were electrophoresed in 09% agarose gel. The electrophoresis gel was stained using Ethidium Bromide and photographed on a UV Transilluminator (Innotech Inc., USA) (Figure 1).

REFERENCES

1. Shermatov Sh. E. “biologik ashyolardan DNK ajratish usullari” // Genom va bioinfarmatika markaz o’quv matreali // Toshkent. 2009 yil 138-141 bet.

The American Journal of Applied sciences
(ISSN – 2689-0992)
VOLUME 04 ISSUE 04 Pages: 1-6
SJIF IMPACT FACTOR (2020: 5. 276) (2021: 5. 634) (2022: 6. 176)
OCLC – 1121105553 METADATA IF – 7.987
Publisher: The USA Journals

2. Ali Dehestani, S.K. Kazemi Tabar A Rapid Efficient Method for DNA Isolation from Plants with High Levels of Secondary Metabolites // Asian Journal of Plant Sciences // Asian Journal of Plant Sciences, June 2007, DOI:10.3923/ajps.2007.977.981.
3. Jinfa Zhang, James Mcd, Stewart Economical and rapid method for extracting cotton genomic DNA // Journal of Cotton Science// 4(3):193-201 January 2000.
4. Raul Tapia-Tussell, Andres Quijano-Ramayo, Rojas Herrera Rafael, Alfonso Larqué-Saavedra A Fast, Simple, and Reliable High-Yielding Method for DNA Extraction From Different Plant Species // Molecular Biotechnology // November 2005 31(2): 137-9, DOI:10.1385/MB:31:2:137.
5. Doyle, J.J.; Doyle J.L. Isolation of plant DNA from fresh tissue. Focus, v.12, p.13-15, 1990.