ACTIVITY REPORT 2016 - 2021

FACULTY:	**DEPARTMENT/ COMMITTEE**	**IQAC ACTIVITY No:**

NAME OF THE ACTIVITY: "NATIONAL WORKSHOP ON TECHNIQUES IN PLANT SCIENCES"			
DATE	**FACULTY**	**DEPARTMENT/COMMITTEE**	**COORDINATORS NAME**
4-5 dec. 2019	Dr. Sunila Khurana (Convener) Dr. Amit Vashishtha (Co-Convener) Dr Neeti Mehla (Co-Convener) Dr Aditi Kothari-Chhajer (Co-Convener)	Botany	Dr. Sunila Khurana
TIME	**VENUE**	**NUMBER OF PARTICIPANTS**	**NATURE: Outdoor/Indoor**
9.30 am to 5.30 pm	Sri Venkateswara College, Delhi University	25	Indoor
SUPPORT/ASSISTANCE:	UGC		

BRIEF INFORMATION ABOUT THE ACTIVITY (**CRITERION NO. -**):

TOPIC/SUBJECT OF THE ACTIVITY	TECHNIQUES IN PLANT SCIENCES
OBJECTIVES	The workshop aimed at providing hands-on-training in the field of in-vitro morphogenesis of medicinal plants and study of genetic fidelity through molecular markers. A session on practical exposure to HP-TLC technique was conducted along with the brains storming session on question paper setting for the faculty.
METHODOLOGY	Interactive sessions were facilitated by subject experts. The methodology included discussion, demonstration and hands-on-training.
OUTCOMES	The workshop aimed at providing hands-on training in the field on *in-vitro* morphogenesis of medicinal plants and study of genetic fidelity through molecular markers. The workshop aimed to create awareness about the conservation of biodiversity and medicinal plants. Moreover, hands-on training enabled the participants to be able to perform *in-vitro* studies in various plant systems. Genetic integrity of the plants derived through micro

	propagation becomes crucial if genetic transformation studies have to be carried out and hence, hands-on training in this field hugely benefited the faculty by providing an overall understanding of this area of plant biotechnology. Phytochemical analysis of extracts derived from Medicinal plants can be qualitatively studied using HP-TLC. A session on practical exposure to this technique was highly beneficial in expanding the horizons of the teachers attending the workshop. A brain storming session on question paper setting helped teachers create challenging and concept-based exercises.

PROOFS & DOCUMENTS ATTACHED (Tick mark the proofs attached):

Notice & Letters ✓	**Student list of participation** ✓	**Activity report ✓ (Workshop Mannual)**	**Photos** ✓	Feedback form
Feedback analysis	News clip with details	Certificate	Any other	

IQAC Document No:	Criterion No:	Metric No:
Departmental file no	IQAC file No;	

NAME OF TEACHER & SIGNATURE	NAME OF HEAD/ COMMITTEE INCHARGE & SIGNATURE	IQAC COORDINATOR (SEAL & SIGNATURE)
Dr. Sunila Khurana Dr Amit Vashishtha Dr Neeti Mehla Dr. Aditi Kothari	Dr. Sunila Khurana	

For Reference

Criterion I	Curricular Aspects (planning & Implementation)		Criterion V	Student Support & Progression
Criterion II	Teaching Learning & Evaluation		Criterion VI	Governance
Criterion III	Research, Innovations & Extension		Criterion VII	Institutional Values & Best Practices
Criterion IV	Learning Resources and Infrastructure			

ACTIVITY 1 : NATIONAL WORKSHOP ON TECHNIQUES IN PLANT SCIENCES

Date: 4-5 Dec. 2019 **Time**: 9.30am-5.30 pm **Venue: Sri Venkateswara College** **Criterion No: I/ II/ III/ V/ VII**

LIST OF PARTICIPANTS

1. Dr. Mayuresh Joshi, Ramnarain Ruia College, Mumbai
2. Ms. Yugandhara Patil, Ramnarain Ruia College, Mumbai
3. Ms. Sushma Bhosale, Ramnarain Ruia College, Mumbai
4. Ms. Swati Singh, Ramnarain Ruia College, Mumbai
5. Ms. Vimal Temkar, Ramnarain Ruia College, Mumbai
6. Dr. Prabhavati, Shivaji College, Delhi

7. Dr. Deepali, Miranda House College, Delhi
8. Dr. Pratima Rani Sardar, Shivaji College, Delhi
9. Dr. Ranjana Singh, Bulandshahar, UP
10. Dr. Pooja Gupta, Ramjas College, Delhi
11. Ms. Kajol Nayak, Helix Biogenesis, Delhi
12. Dr. Anita Singh, University of Delhi, Delhi
13. Ms. Shivaji Tyagi, Mewar University, Rajasthan
14. Mr. Vivek Chopra, Hindu College, Delhi
15. Dr. Nidhi Gautam, DDU College, Delhi
16. Dr. Tapan Dutta, Jawaharlal Nehru College, Boko, Assam
17. Dr. Dhiraj Kumar Das, Jawaharlal Nehru College, Boko, Assam
18. Dr. Alok Das, Jawaharlal Nehru College, Boko, Assam
19. Mr. Pinaki Kr. Rabha, Jawaharlal Nehru College, Boko, Assam
20. Dr. Habibur Rahman, Jawaharlal Nehru College, Boko, Assam
21. Dr. Nuruddin Ahmed, Jawaharlal Nehru College, Boko, Assam
22. Dr. Apurba Kumar Goswami, Jawaharlal Nehru College, Boko, Assam
23. Dr. Tapan Kr. Deka,Jawaharlal Nehru College, Boko, Assam
24. Dr. SUsanta Kr. Bhuyan, Jawaharlal Nehru College, Boko, Assam
25. Dr. Ranjit Baishya, Jawaharlal Nehru College, Boko, Assam

Name of the Coordinators and signature

Head of the Department

NATIONAL WORKSHOP
ON
TECHNIQUES IN PLANT SCIENCES

4-5 DECEMBER,2019

Chief Guest

Prof. S. K. Gakhar

Vice Chancellor,Indira Gandhi University
Meerpur-Rewari
Former Vice Chancellor Sri Sri University
Cuttack-Orissa
Founder Vice Chancellor, Ch. Bansi Lal
University,Bhiwani

Guest of Honour

Dr. Suman Govil

Advisor, Department of
Biotechnology
Govt. of India

Inaugural address

Prof. K.S Rao

Head, Department of Botany
University Of Delhi

RESOURCE PERSONS

Prof. Veena Aggarwal
Department of Botany
University of Delhi

Prof. Sunita Shailajan
Dean-Research Consultancy
and Innovation
Ruia College
University of Bombay

Dr. Sasikumar Menon
Member, National Steering
Committee
Science Olympiads,
HBCSE-TIFR,
DAE, GOI

PATRON

Dr. P. Hemalatha Reddy
PRINCIPAL
Sri Venkateswara College

CONVENOR

Dr. Sunila Khurana

CO-CONVENORS

Dr. Aditi Kothari-Chhajer
Dr. Amit Vashishtha
Dr. Neeti Mehla

ORGANIZED BY

SRI VENKATESWARA COLLEGE

(Accredited by NAAC with 'A' Grade)

UNIVERSITY OF DELHI

ADVISORY COMMITTEE

Dr. Kalyani Krishna
(Department of Botany, SV College)
Dr. GPC Rao
(Department of Botany, SV College)
Dr. Shukla Saluja
(Department of Botany, SV College)
Dr. N. Latha
(Department of Biochemistry, SV College)
Dr. Anju Kaicker
(Department of Biochemisty, SV College)

ORGANISING TEAM

Dr. Pooja Gokhale Sinha
Dr. Tabassum Afshan
Dr. Yogendra Kr. Gautam
Dr. Pamil Tayal
Dr. Sunita Yadav
Dr. Neer Komal Singh

THEME OF WORKSHOP

The workshop aims at providing hands-on training in the field of *in-vitro* morphogenesis of medicinal plants and study of genetic fidelity through Molecular Markers. Plant tissue culture is a part of Plant Biotech which is the collection of many techniques that are used to maintain and grow plants under controlled aseptic conditions. Genetic integrity of the plants derived through micropropogation becomes crucial if genetic transformation studies have to be carried out and hence hands-on training in this field shall provide an overall understanding of this area of plant biotechnology. With the changing phase of education it is imperative for teachers to stay abreast with the latest trends in higher education to meet the emerging challenges & opportunities. In order to enhance this facet of teaching -learning process, this workshop provides an opportunity to interact with leading faculty for some brainstorming sessions for the development of questions with higher order thinking skills.

WORKSHOP AT A GLANCE

- Hands on training
- *In-vitro* morphogenesis in Medicinal Plants.
- Genetic fidelity using Molecular markers.
- HP-TLC.
- Brainstorming session on question paper setting involving higher order thinking skills.

Who can attend the workshop–

Faculty from Colleges/Universities.

Registration Fees - Rs. 750/-

Last date For Registeration-

1st December,2019

Registration Link –(Double tap to open the link)

https://docs.google.com/forms/d/e/1FAIpQLScmxs5LtvK9n79AehnC72m8RS4X2AKLc3QuxWWf_mPxSlV9fA/viewform?vc=0&c=0&w=1

For any query,Contact:
Amit Vashishtha- 8860302550
8708043830
E-mail :vashishtha24@gmail.conm

Inaugural Address

Prof. K.S. Rao

Head, Department of Botany

University of Delhi

RESOURCE PERSONS

Prof. Veena Agrawal

Professor,
Department of Botany,
University of Delhi

Prof. Sunita Shailajan

Ramnarain Ruia College,
Mumbai

Dr. Sasikumar Menon

Member, National Steering Committee,
Science Olympiads,HBCSE-TIFR
Ramnarain Ruia College,
Mumbai

CHIEF GUEST

Prof. Surendra Gakhar

Vice Chancellor, Indira Gandhi University, Meerpur- Rewari
Former Vice Chancellor Sri Sri University, Cuttack -Orissa
Founder Vice Chancellor, Ch. Bansi Lal University, Bhiwani

GUEST OF HONOUR

Dr. Suman Govil

Former Sr. Advisor, Department of Biotechnology
Govt. of India

PATRON

Dr. P. Hemalatha Reddy

(Principal, Sri Venkateswara College)

CONVENOR

Dr. Sunila Khurana

CO-CONVENORS

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(Co-ordinator, DBT-STAR)
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Dr. Neer Komal Singh

CONTACT – Dr. Amit Vashishtha
(8860302550 , 8708043830)
(vashishtha24@gmail.com)

NATIONAL WORKSHOP ON TECHNIQUES IN PLANT SCIENCES

4 – 5 DECEMBER 2019

Organized By
Department of Botany
Sri Venkateswara College, Delhi University

"THE SCIENCE OF TODAY IS THE TECHNOLOGY OF TOMORROW"

- EDWARD TELLER

WORKSHOP AT A GLANCE

- **Hands-on training**
- ***In-Vitro* morphogenesis in Medicinal Plants.**
- **Genetic Fidelity Using Molecular Markers.**
- **HP-TLC.**
- **Brainstroming session on question paper setting involving higher order thinking skills.**

WHO CAN ATTEND THE WORKSHOP –

Faculty from Colleges/Universities.

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1st December 2019

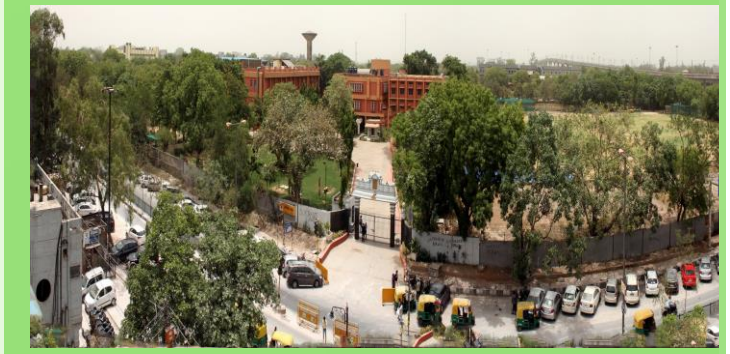
Registration Link –

https://docs.google.com/forms/d/e/1FAIpQLScmxs5LtvK9n79AehnC72m8RS4X2AKLc3QuxWWf_mPxSlV9fA/viewform?vc=0&c=0&w=1

Registration Fees - Rs. 750/-

ABOUT THE COLLEGE

Sri Venkateswara College is one of the premier colleges under the University of Delhi. Filled with the zeal of promoting the cause of education, the college was set up at New Delhi under the benign auspices of the Tirumala Tirupati Devasthanam (TTD). The college is **NAAC-A accreditated** and has been conferred upon the coveted **STAR STATUS COLLEGE** by the Department of Biotechnology, Government of India in 2016. Sri Venkateswara College couples sound academic achievement with an extensive, vibrant co-curricular programme that includes the fine arts, sports, and leadership training programmes. The institution believes in imparting education that is an amalgamation of strong academic foundation and moral values. It imparts value-based education to students through innovative pedagogy and state-of-art research facilities.



THEME OF WORKSHOP

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With the changing face of education it is impertive for teachers to stay abreast with the latest trends in higher education to meet the emerging challenges & opportunities. In order to enhance this facet of teaching - learning process, this workshop provides an opportunity to interact with leading faculty for some brainstorming sessions for the development of questions with higher order thinking skills.

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NATIONAL WORKSHOP ON TECHNIQUES IN PLANT SCIENCES

4 – 5 DECEMBER 2019

Organised by

SRI VENKATESWARA COLLEGE

(Accredited by NAAC with 'A' grade)

University of Delhi

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Dr. Neer Komal Singh

Programe Schedule	
4th December 2019	
9.30 am	Invocation and Lighting of Lamp
9.40 am	Address by DBT-STAR College Status Co-ordinator, **Dr. Nandita Narayansamy**
9.45 am	Principal's address, **Dr. P. Hemalatha Reddy**
9.55 am	Address by the **Chief Guest** **Prof. S.K. Ghakhar** **Vice Chancellor** **Indira Gandhi University** **Meerpur - Rewari, Haryana**
10.10 am	Inaugural address by **Prof. K.S. Rao** **Head** **Department of Botany** **University of Delhi, Delhi**
10: 30 – 11:00 am	High tea and Interaction with faculty
Session –I	
11:00 -11:45 am	Session Chair: **Dr. Suman Govil, Former Senior Advisor, Department of Biotechnology, Government of India** Talk on "Applications of HP-TLC in Medicinal plants" by **Prof. Sunita Shailajan, Ramnarain Ruia Autonomous College, Mumbai**
11:45-1:30 pm	Hands on Training in HP-TLC by **Prof. Sunita Shailajan**
1:30-2:00 pm	Lunch
2:00-3:00 pm	HP-TLC Contd….
3:00-5:00 pm	Session on Question Paper setting by **Dr. Sasikumar Menon, , Ramnarain Ruia Autonomous College, Mumbai**
5th December 2019	
Session – II	
9:30 am	Introductory Talk on Plant Tissue culture by **Prof. Veena Agrawal** **Department of Botany** **University of Delhi**
10:30 am	Tea break

10:45-2:00 pm	Hands-on training in Plant Tissue Culture Techniques by **Prof. V. Agrawal**
2:00-2:30 pm	Lunch
Session -III	
2:30- 4:30 pm	Hands-on training on Genetic Fidelity using Molecular Markers by **Prof. Veena Agrawal**
4:30 pm	Distribution of Certificates
5:00 pm	Tea

SESSION – I

Hands on training on HP-TLC

PROTOCOL FOR QUALITY EVALUATION OF *Pushyanuga churna*

Resource Person: Prof. Sunita Shailajan

Ramnarain Ruia Autonomous College, Mumbai

Introduction

Pushynuga churna is ayurvedic polyherbal formulation composed of twenty-five plant ingredients and one mineral described in Ayurvedic Formulary of India. Ayurvedic texts prescribe it for various female reproductive disorders like Asrgandha (menorrhagia), Svetapradara (leucorrhoea), Rajodosa (Menstrual disorders), Arsa (Piles) and Yonidosa (disorders of female genital tract).Owing to its clinical efficiency, *Pushyanuga Churna* is being prepared and marketed by different manufacturers like Dabur, Baidyanath, Arkashala, Dhootpapeshwar, Patanjali and Kottakkal etc. **(Ref.:** *The Ayurvedic Formulary of India (AFI). Part-I, Second ed., Vol I Controller of Publications Civil Lines, Delhi-110054, India. 2007 p55.* **Berberine** is an isoquinoline-derivate alkaloid reported to have pharmacological uses including lowering of blood glucose, increasing insulin sensitivity. It has positive role in the management of PCOS and female reproductive disorders.

The Problem

Pushynuga churna samples are provided to you. Using TLC technique, evaluate the quality of these samples provided to you.

You are provided with the following:

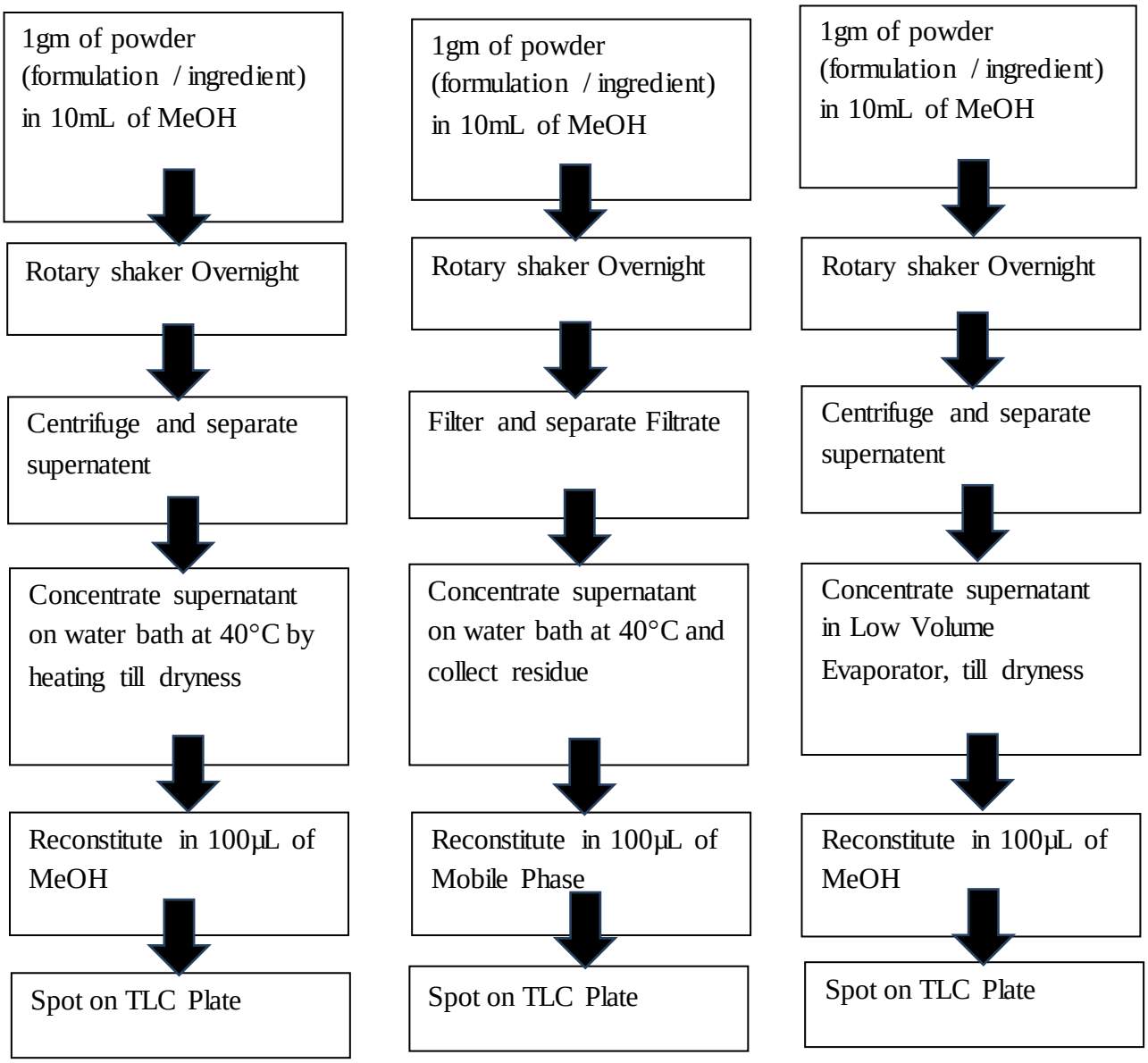
Extracts of *Pushynuga churna,* Berberine (100ppm) each in MeOH, and Lab-ware for conducting TLC analysis

TASK ONE:

- Make a schematic representation of the TLC plate with tracks / lanes marked for the specific samples to be spotted. Arrange the spots on TLC plate such that comparison of developed fingerprint can be made unequivocally. Show the Schematic to the Lab. Experts.

TASK TWO

- An analyst has to make a choice of a procedure from the following three extraction flowcharts (i, ii, and iii). Help him to make the choice;



Show your answer to the Lab- Expert.

Now ask for Task Three.

TASK THREE: (to be started only after completing Tasks One and Two)

- Carry out the TLC as stated in protocol and provide your findings in the table below;

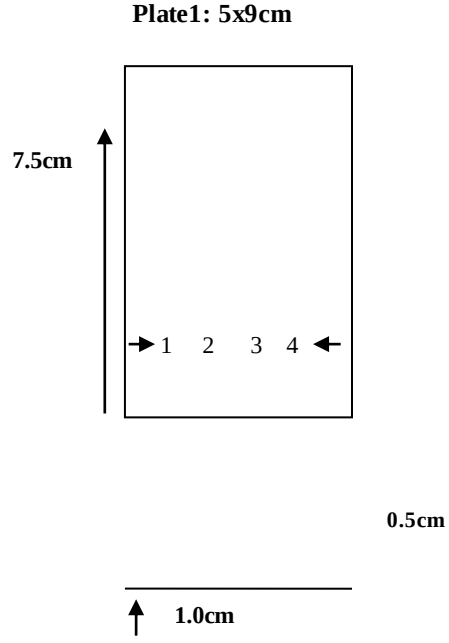
1) For *Pushynuga churna*

Parameters		**PC-01**	**PC-02**	**PC-03**	**Berberine**
Lane No.					
No. of Spots seen at 366nm					
R_f of spots seen (numbered from Sample spot)	**1.**				
	2.				
	3.				
	4.				
	5.				
	6.				
	7.				
	8.				
	9.				
Number of spots common in samples					
Number of spots NOT common in samples					

In my opinion, sample _____ is better in quality.

Protocol for TLC

- Measure the dimension of the plate and cut the plate of the required size (i.e 5x9cm for *Pushyanuga churna*).
- Leave 1cm space from bottom and 0.5cm from both sides, mark 7.5cm as solvent front and label the spots accordingly (Note: Use only pencil for markings on plate)
- Load the sample with the help of fused capillary on to the TLC plate in form of Spot/band (Note: Use separate fused capillary for every sample to avoid contamination. load the sample at least five times, dry the spot before next loading).
- Prepare the mobile Phase, toluene: ethyl acetate: methanol: formic acid (6:6:2:1v/v/v/v) for quantitation of Berberin from *Pushyanuga churna* and vortex it.
- Pour the mobile phase in the beaker and cover it with glass lid, allow it to saturate it for 20 minutes.
- Gently place the TLC plate into the mobile phase of the saturated beaker, with the spotted end of the plate towards the bottom close to the solvent. Submerge the bottom edge of the plate into the solvent, allowing the solvent to run upto the marked solvent front (7.5cm).
- Remove the plate and allow it to air dry (Note: Allow the solvent to evaporate from the plate.)
- Visualize the plate at 366nm in UV-Visible visualizing chamber and calculate the Retardation Factor (R_f) and note them is the results sheet.



PROTOCOL FOR QUALITY EVALUATION OF *Triphala churna* and *Eclipta alba*

Resource Person: Prof. Sunita Shailajan

Ramnarain Ruia Autonomous College, Mumbai

Introduction

Triphala churna is an age old commonly used Ayurvedic powdered preparation (*churna*) in Indian systems of medicine. It is one of the most famous and widely used Ayurvedic products and is available in herbal powder form. *Tri* means three, *Phala* means fruit. *Triphala churna* is made of three fruits. Ayurvedic formulary of India gives the specification for the composition of *Triphala churna*. The dried powder of fruit rind of three fruits namely *Terminalia chebula*, *Terminalia belerica* and *Embellica officinalis* are mixed in equal proportions.

Eclipta alba (L.) Hassk (synonym *Eclipta prostrata*) is an annual herbaceous plant; erect or prostrate, belonging to the Asteraceae family in Ayurveda has been generally utilized for a very long time as a part of conventional prescription for ailments especially related to the liver and hair. *Eclipta alba*, also known as *maka* locally, is the major ingredient in several hair tonics. A well known hair tonic *Bhringaraj hair oil* is used for keeping hair dark and for regaining lost hair. It is also termed as 'king of the hair' **(Ref.:** *Bhavaprakasha Nighantu, Haritakyadi Varga, 42,43* and Yadav *et al.*, 2017). Gallic acid (also known as 3, 4, 5-trihydroxybenzoic acid or trihydroxybenzoic acid) is a type of phenolic acid found in many medicinal plants as bioactive phytoconstituent.

The Problem

Triphala churna and *Eclipta alba* samples are provided to you. Using TLC technique, evaluate the quality of these samples provided to you.

You are provided with the following:

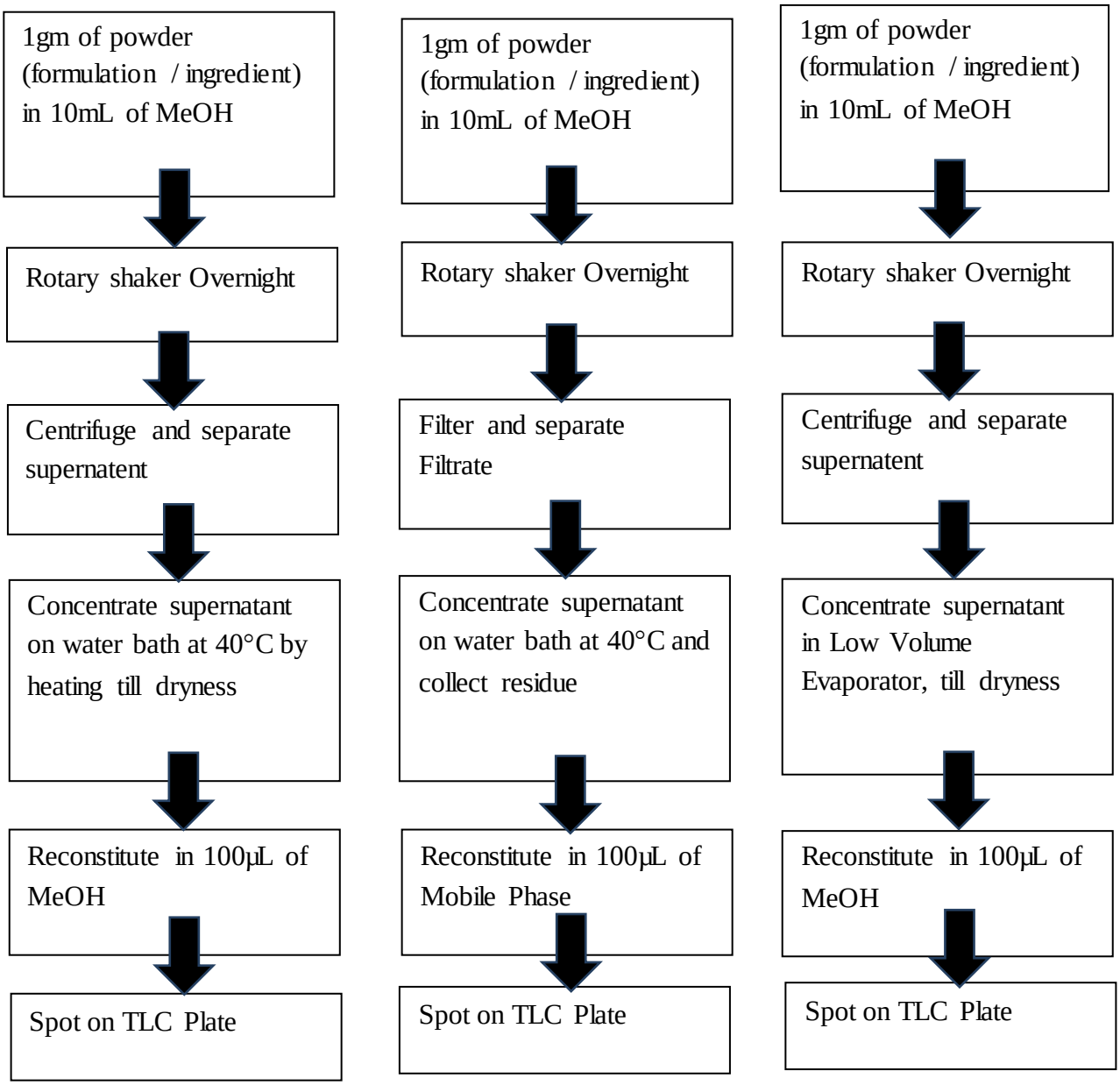
Extracts of marketed *Triphala churna,* Gallic Acid (100ppm) each in MeOH, MeOH extract of *Terminalia chebula*, MeOH extract of *Terminalia belerica,* MeOH extract of *Embellica officinalis,* MeOH extract of *Eclipta alba* and Lab-ware for conducting TLC analysis

TASK ONE:

- Make a schematic representation of the TLC plate with tracks / lanes marked for the specific samples to be spotted. Arrange the spots on TLC plate such that comparison of developed fingerprint can be made unequivocally. Show the Schematic to the Lab. Experts.

TASK TWO

- An analyst has to make a choice of a procedure from the following three extraction flowcharts (i, ii, and iii). Help him to make the choice;



Show your answer to the Lab- Expert.

Now ask for Task Three.

TASK THREE: (to be started only after completing Tasks One and Two)

- Carry out the TLC as stated in protocol and provide your findings in the table below;

1) For *Triphala churna*

Parameters		*Terminalia chebula*	*Terminalia belerica*	*Embellica officinalis*	*Triphala Churna* (A)	*Triphala Churna* (B)	Gallic acid
Lane No.							
No. of Spots seen in visible							
No. of Spots seen at 366nm							
No. of Spots seen at 254`nm							
R_f of spots seen (numbered from Sample spot)	**1.**						
	2.						
	3.						
	4.						
	5.						
	6.						
	7.						
	8.						
	9.						
Number of spots common in samples and formulations							
Number of spots NOT common in samples and formulation							
Number of spots from ingredients of the formulation							

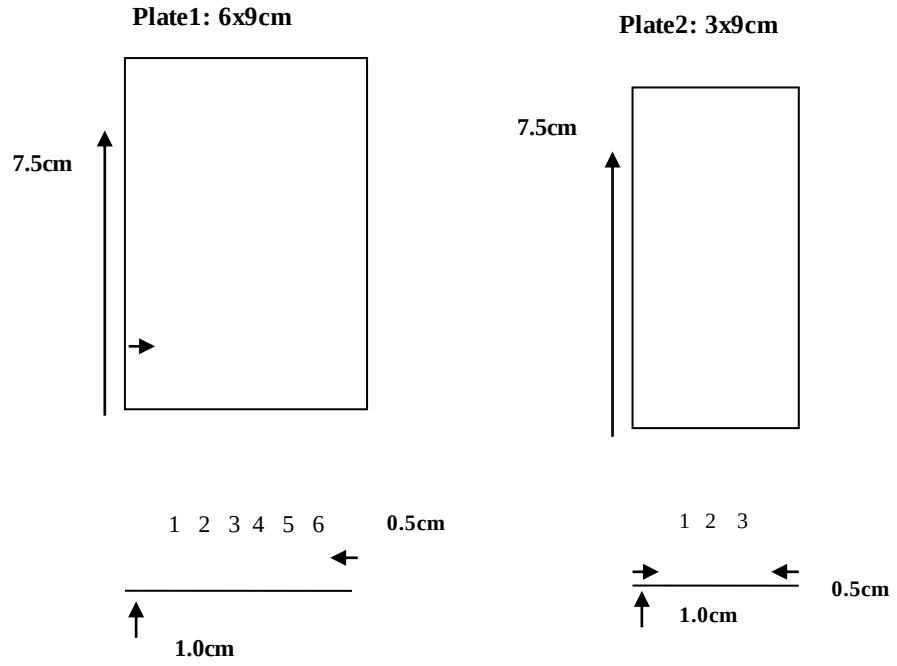
In my opinion sample _____ is better in quality than sample _____

2) For *Eclipta alba*

Parameters		*Eclipta alba* (1)	*Eclipta alba* (2)	*Eclipta alba* (3)
Lane No.				
No. of Spots seen in visible				
No. of Spots seen at 366nm				
No. of Spots seen at 366nm (After derivatization)				
No. of Spots seen at 254`nm				
R_f of spots seen (numbered from Sample spot)	**1.**			
	2.			
	3.			
Number of spots common in samples				
Number of spots NOT common in samples				
Number of spots common in samples after derivatization				

Protocol for TLC

- Measure the dimension of the plate and cut the plate of the required size (i.e 6x9cm for *Triphala churna* and 3x9 cm for *Eclipta alba*).
- Leave 1cm space from bottom and 0.5cm from both sides, mark 7.5cm as solvent front and label the spots accordingly (Note: Use only pencil for markings on plate)
- Load the sample with the help of fused capillary on to the TLC plate in form of Spot/band (Note: Use separate fused capillary for every sample to avoid contamination. load the sample at least five times, dry the spot before next loading).
- Prepare the mobile Phase, toluene: ethyl acetate: formic acid (2:7:1v/v/v) for quantitation of gallic acid from *Triphala churna* and toluene: n hexane (9:1.2 v/v) for fingerprint of *Eclipta alba* and vortex it.
- Pour the mobile phase in the beaker and cover it with glass lid, allow it to saturate it for 20 minutes.
- Gently place the TLC plate into the mobile phase of the saturated beaker, with the spotted end of the plate towards the bottom close to the solvent. Submerge the bottom edge of the plate into the solvent, allowing the solvent to run upto the marked solvent front (7.5cm).
- Remove the plate and allow it to air dry (Note: Allow the solvent to evaporate from the plate.)
- Visualize the plate at 254nm and 366nm in UV-Visible visualizing chamber and calculate the Retardation Factor (R_f) and note them is the results sheet.
- Derivatize the (*E. alba*) plate by Dipping the plate in 10% Methanolic sulphuric acid reagent for 50 seconds, heat the plate at 110°C using hot air oven for 5minutes).
- Visualize the plate at 366nm in UV-Visible visualizing chamber and note them in the results sheet.



SESSION – II

Hands on training on *In-vitro* morphogenesis in medicinal plants

PROTOCOL FOR *IN VITRO* CLONAL PROPAGATION OF ECONOMICALLY IMPORTANT PLANT TAXA

Resource Person: Prof. Veena Agrawal

Plant biotech. Lab Department of Botany, University of Delhi, Delhi - 110 007

❖ **Requirements:**

- **Plant Material:** Any plant system (herb, shrub or tree) of economic value such as medicinal, neutraceutical, crop or commercial importance.

- **Chemicals:** Teepol, bavistin, cefotaxime, citric acid, mercuric chloride, salts of macro- and micro elements of Murashige and Skoog's (1962) and Knop's (1865) basal media, sucrose, agar,1N NaOH, 1N HCL, plant growth regulators [2,4-dichlorophenoxyacetic acid (2,4-D), α-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 6-benzyladenine (BA), thidiazuron (TDZ), kinetin (kn), 2-iP [6-(gamma, gamma-Dimethylallylamino) purine] and absolute alcohol. All the chemicals should be of Sigma-Aldrich, Merck./Hi- Media, SRL. and of standard companies.

- **Glass wares:** Beakers (500, 250, 1000 ml), measuring cylinders (10, 50, 100, 500, 1000 ml capacity), conical flasks.

- **Equipments:** Laminar air flow cabinet, pH meter, oven/ heater, stirrer, autoclave, pipettes, tips, Petri plates, forceps, scalpel, blades.

- **Miscellaneous:** Aluminum foil, tissue paper, butter paper, cotton plugs, spirit lamp, discard, dip, match box, marker, rectified spirit, autoclaved distilled water and glass plates/ Petri-plates for inoculation.

❖ **Methods:**

- **Preparation of Stock Solutions:**

Stock solutions were prepared according to the salt composition given below:

Table 1. Composition of Murashige and Skoog (1962) and Knop's (1865) medium

S. No.	Nutrients	MS (1962)	Knops (1865)
Major (g/1000 mL)			
1	KNO_3	38	3
2	NH_4NO_3	33	-
3	KH_2PO_4	3.4	3
4	$MgSO_4.7H_2O$	7.4	3
5	$CaCl_2.2H_2O$	8.8	-
Minor (mg/1000 mL)			
6	$MnSO_4.H_2O$	446	500
7	H_3BO_3	124	200
8	$ZNSO_4.7H_2O$	172	10
9	KI	16.6	-
10	$Na_2MoO_4.2H_2O$	5	-
11	$CuSO_4$	0.5	-
12	$CoCL_2.6H_2O$	0.5	-
Organic (mg/100 mL)			
13	Glycine	20	-
14	Meso-inositol	100	100
15	Nicotinic acid	5	-
16	Pyridoxine HCl	5	-
17	Thiamine HCl	1	10
Iron (mg/100 mL)			

18	$FeSO_4.7H_2O$	278	55.6
19	Na_2EDTA	373	74.4

- **Preparation of stock solution of growth regulators:**

  Weigh 10 mg of the plant growth regulator in an Eppendorf tube or other glass container. Add 1-2 drops of solvent (ethanol/NaOH) to dissolve the powder. Once completely dissolved, make volume up to 10 ml with autoclaved distilled water. Store the stock solution in the fridge for further use.

Table 2. List of some Important plant growth regulators and their storage

S.N.	Plant growth regulators	Molecular weight (g/mol)	Solvent	Stock Storage
1	2,4-D (2, 4- Dichlorophenoxyacetic acid)	221	Ethanol (Pure)	2-8 ºC
2	NAA (α-Naphthaleneacetic acid)	186.2	Ethanol / 1N NaOH	2-8 ºC
3	IAA (Indole-3-acetic acid)	175.2	Ethanol / 1N NaOH	0 ºC
4	IBA (Indole-3-butyric acid)	203.2	Ethanol / 1N NaOH	0 ºC
5	BAP (6-Benzylaminopurine)	225.3	1N NaOH	2-8 ºC
6	Kinetin	215.2	1N NaOH	0 ºC
7	2-iP [6-(gamma,gamma-Dimethylallylamino] purine	203.2	1N NaOH	0 ºC
8	TDZ (Thidiazuron)	220.25	1N NaOH	0 ºC

- **Preparation of Culture media:**

Semi-solid MS basal medium was prepared using above mentioned stock solutions containing 0.8 % (w/v) agar-agar (bacteriological grade, Thermo Fisher Scientific®) and 3 % (w/v) sucrose (DCM, Daurala, India) as given hereunder:

1. Weigh 8 g of agar and 30 g of sucrose and put these in the conical flask of 1L containing 300 ml of distilled water. Melt the agar and sucrose by keeping the flask on the hot plate/ heater.
2. In a separate measuring cylinder of 1/ 2L add 50 ml stock solutions each of micro and macro salts and 10 ml each of iron and organic prepared above (Table 1).
3. Add the mixture of agar and sucrose (prepared at step 1) to this measuring cylinder containing MS salts and make the volume 1L by adding hot distilled water. Shake gently till homogenous solution is prepared. Transfer the medium to conical flask to set the pH.
4. Add growth regulator if required prior setting pH.
5. The pH of the medium was adjusted to 5.8 with 1 N NaOH and 1 N HCl (Thermo Fisher Scientific®) prior to autoclaving at 121°C and 15 lbs psi for 15 min.
6. Take out the test-tubes from the autoclave and wait till the medium temperature come down to around 60°C.
7. Make slants of the medium by tilting the test tubes and place these in the culture room for inoculation.

- **Surface sterilization of explants:**

1. Nodal explants (0.5-1.0 cm in length) were cut from the fresh twigs.
2. These explants were washed thoroughly under running tap water for 15-20 min, followed by rinsing with 5% v/v Teepol (Reckitt and Colman, Mumbai, India) for 10-15 min.
3. Subsequently, the explants were treated with 2% (w/v) citric acid to minimize browning effect from the cut ends of nodal explants.
4. To remove any fungal and bacterial contaminants adhered to the surface, explants were treated with 0.5% (w/v) Bavistin® fungicide (Bavistin®, Carbendazim 50% WP, BASF India Ltd., Mumbai, India) + 150 mg/L cefotaxime (Alkem, Sikkim, India) for 10 min and then rinsed three times with distilled water.
5. Final surface sterilization is to be done with 0.1% (w/v) $HgCl_2$ for 2 min in laminar air flow cabinet followed by rinsing 3 or 4 times with autoclaved double distilled water.

❖ **Induction of morphogenesis and multiple shoots:**

- **In vitro regeneration(micropropagation) can be achieved through either organogenesis or somatic embryogenesis:**

1. **Direct regeneration: Cotyledonary nodes, stem n**odal segments and shoot tip explants are the ideal material for direct induction of multiple shoots.For indirect organogenesis root hypocotyle, cotyledon and leaf, explants are used.
2. Initially, there is a bud break and elongation of axillary shoot takes place on MS basal medium.
3. For multiple shoot induction MS medium supplemented with different plant growth regulators (BAP, KN, TDZ, or 2-iP) at concentrations ranging from 0.1, 1, 5, 10 & 20 µM is beneficial. **(See Figs 1&2).**
4. The cultures are daily observed for 28 days and data pertaining to shoot number and shoot length recorded weekly on observation book. Average number of shoot and shoot length are calculated and statistically analyzed.

❖ **Induction of roots in the excised shoots (Rhizogenesis):**

1. For root induction, *in vitro* raised shoots were excised and placed on MS medium supplemented with different concentrations (0.1, 1, 5, 10 µM) of IAA, IBA, or NAA. Emergence of direct roots is initiated with in one weeks from the basal cut ends. However, if there is an intervening callus then some additives such as PVP or activated charcoal may be added to the rooting medium.
2. Average number of roots per shoot and average root length were recorded after 4 weeks of culture.

❖ **Indirect organogenesis:** Explants such as root, hypocotyle, cotyledonary leaf, stem leaf are cultured on MS /B5 basal medium augmented with auxin (IAA,NAA,IBA or 2,4-D) in different concentration ranging from 0.1-20 µM to induce callus. Such callus pieces are then sub cultured on cytokinin (BA,Kin.,2iP,Zeatin) supplemented basal medium for induction and proliferation of shoots. These micro shoots are subsequently excised and transferred to rooting medium given above for induction of roots. Finally these are hardened and acclimatized to soil and fields**.(See Fig.1)**

❖ **Somatic embryogenesis:** Somatic embryos are embryo like structures derived from somatic tissues and mimic all the stages of zygotic embryos. These are also considered as best

material for clonal propagation as the embryo directly develops in to shoot and root in one step. Explants are first cultured on auxin supplemented MS/B5 medium to induce callus. The callus either organize embryos or shoot buds. The embryogenic calluses are microscopically identified with the embryo-like structures and are further sub cultured on maturation medium (abscisic acid/proline/sucrose/PEG) for proper developments. Such mature embryos are then transferred to basal medium for germination in to complete plantlets.(See Figs 1&3).

❖ ***In vitro*** **hardening and field acclimatization:**

1. After rhizogenesis, healthy plantlets with well developed roots were removed from medium and washed under running tap water to remove the adhering medium.
2. They were treated with 1 % bavistin (BASF, Mumbai, India) solution to prevent any fungal infection, before being transferred to plastic pots (5 cm diameter) containing autoclaved soil.
3. The plantlets were irrigated with one fourth MS salts for one week and one-tenth for next one month.
4. The plantlets were covered with polythene bags having, 3–5 holes to maintain the humidity.
5. The potted plants were maintained inside the glass house at controlled conditions till they are established well in the soil.
6. After 45 days, the plantlets were transplanted to earthen pots (25 cm diameter) containing garden soil and were maintained there for 2-3 months.
7. The plantlets were finally transferred to soil-bed for field acclimatization till maturity.

❖ **Recording of data and statistical analysis:**

1. For *in vitro* regeneration, the average number of shoots per explant, the average shoot length, the average number of roots per shoot and the average root length has been represented as mean values along with standard error (Mean ± SE).
2. The mean values were calculated on the basis of a minimum of 24 replicates in each experiment and repeated at least for once or twice.
3. The data expressed as mean ± SE have been statistically analyzed using ANOVA (Analysis of Variance) through SPSS (Statistical Package for Social Sciences) version 16.0. The differences between means were tested for significance by Duncan's multiple range test (DMRT) at $P \leq 0.05$.

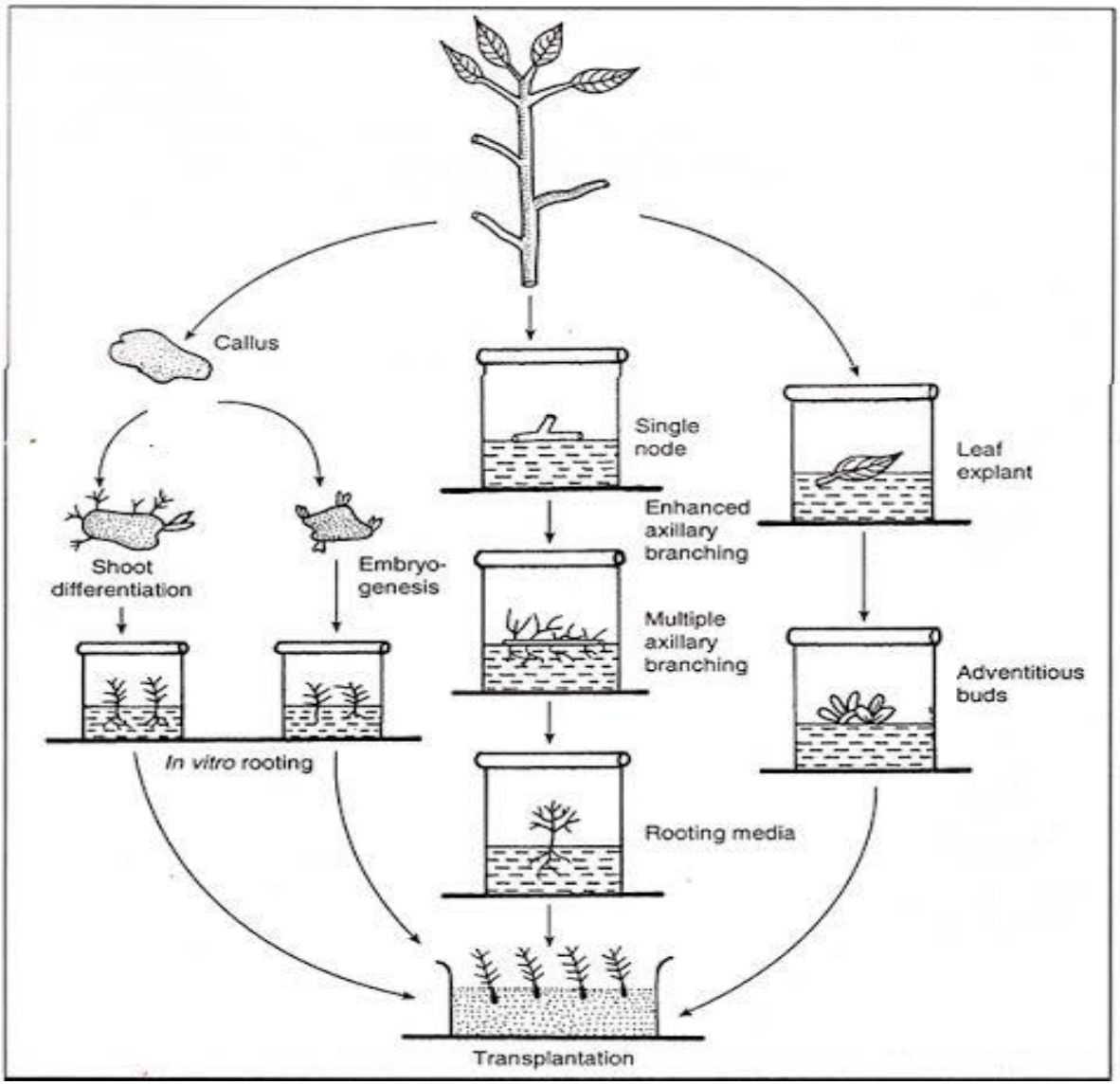


Fig.1. An overview of Micropropagation revealing direct and indirect *in vitro* regeneration

Fig. 2. Direct organogenesis through nodal explants of *Spilanthes calva* DC. after 6 weeks of culture. (a) Field raised mature plant (4-month-old) of *Spilanthes calva* (inset shows nodal explant at the time of inoculation); **(b)** Single axillary shoot after 6 weeks of culture on MS basal medium; **(c)** Differentiation of multiple shoots from nodal explants within 6 weeks of culture on MS+10 µM BA; **(d)** Culture showing stunted shoots and formation of callus at the basal end of the nodal explant on MS+20 µM BA; **(e)** Excised *in vitro* shoot inducing root on MS (1/2)+ 0.1 µM IBA; **(f)** Tissue culture-derived plant acclimatized to soil in glass house; **(g)** Three month old tissue culture raised plants in garden.

Fig. 3. (A-T) *In vitro* differentiation of secondary somatic embryos and regeneration of emblings in *Callinadra tweedii* (Benth.) (A) A flowering twig (insert : intermodal segment). (B) Primary embryogenic callus induced on intermodal segment on MS + 1 uM 2iP after 16 weeks of culture (bar – 5mm). (C) Aggregartyes of primaery somatic embryos on MS + 1 µM 2iP (bar – 2mm). (D and E) Diffrentiation of heart shaped (D; bar- 4 mm) and torpedo shaped (E; bar – 2 mm) somatic embryo. (F) Two cotyledonary stage somatic embryos (bar – 2mm), (G) Primary cotyledonary embryos differentiating secondary embryos on MS + 1 µM 2iP after 6 weeks of culture (bar – 1mm). (H) Secondary embryos developing from both the radicular (r) and plumular (p) ends (bar – 2mm). (I) Secondary globular embryos (ge) differentiated from all over the explant surface (bar – 2mm). (J) Heart shaped embryos (hs; bar – 2mm). (K-N) Asynchronous development of secondary somatic embryos of different stages: ge – globular embryo; hs – heart shaped stages; lhs – late heart shaped; ts – torpedo stage; cs – cotyledonary stage embryo (bar – 2mm). (O) Fully developed secondary cotyledonary stage embryo after maturation on 5 µM ABA (bar – 2mm). (P) A 15 day old secondary somatic embryo germinating with distinct cotyledonary (cot) leaves and radicle end on half-strength MS medium (bar – 2mm). (Q) Plantlet growing on half strength MS with induced intervening callus between shoot and root (bar – 1.5cm). (R) Plantlet reared on half- strength MS + 0.25% activated charcoal.

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3. Heikrujam, M.; Kumar, D.; Kumar, S.; Gupta, S.C. & **Agrawal, Veena. 2014**. High efficiency cyclic production of secondary somatic embryos and ISSR based assessment of genetic fidelity among the emblings in *Calliandra tweedii*- an ornamental woody legume**. Sci. Hortic. 177: 63–70. Elsevier, Netherlands.**
4. **Knop, N., 1865.** Quantitative untersuchungan uber die Ernahrungsprozesse der pflangen Land Wortsch. Vers. Skn. **7:** 93-109.
5. **Murashige, T., and Skoog, F., 1962.** A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. **15:** 473-497.
6. Pandey, Vibha & **Agrawal, Veena. 2009.** Efficient micropropagation protocol of *Spilanthes acmella* L. possessing strong antimalarial activity**. In Vitro Cell Dev. Biol**.Pl. **45: 491-499. Springer, USA. (I.F. 1.057).**
7. **Razaq, M.; Heikrujam, M.; Chetri, S. K. & Agrawal, Veena. 2012.** In vitro clonal propagation and genetic fidelity of the regenerants of *Spilanthes calva* DC. using RAPD and ISSR marker. Physiol. Mol. Biol. Plant **19:** 251-260. (IF: 1.539), Springer, India.

SESSION – III

Hands on training on Genetic fidelity using molecular markers

EVALUATION OF GENETIC FIDELITY OF *In-vitro* REGENERATED PLANTS

Resource Person: Prof. Veena Agrawal

Plant biotech. Lab Department of Botany, University of Delhi, Delhi - 110 007

Requirements:

Plant material: Fresh leaves of tissue cultured plants and mother plant.

Chemicals: Liquid nitrogen, CTAB, EDTA, TRIS, PVP, β-mercaptoethanol, isopropanol (chilled), chloroform: isoamylalcohol (24:1 v/v),TE buffer, ethanol (70%), HCl (conc.) NaOH pellets and double distilled water, DNA universal markers SSR, SRAP, SCoT, RAPD, etc), agarose powder, TBE buffer, DNA ladders, gel loading dye (Bromophenol blue), sterile water.

CTAB buffer components (Stock Solutions)	Final conc. in CTAB buffer	CTAB buffer(100 ml)
5% CTAB	2% CTAB	40 ml
5 M NaCl *	1.4 M NaCl	28 ml
1 M Tris-HCl (pH 8.0) *	100 mM Tris-HCl	10 ml
0.5 M EDTA (pH 8.0) *	20 mM EDTA	4 ml
2-mercaptoethanol	0.2% 2-mercaptoethanol	200 µl

***To be autoclaved**

TE Buffer: 10 ml of 1 M Tris HCl pH 8.0 and 2 ml of 0.5 M EDTA. Make the final volume to 1 L with ddH_2O.

1 M Tris HCl pH 8.0: Dissolve 121.1 g of Tris in about 700 ml of H_2O. Adjust the pH to 8.0. Make the final volume to 1 L with ddH_2O.

0.5 M EDTA: Dissolve186.12 g of EDTA in about 700 ml of H_2O. Add 16-18 g of NaOH pellets. Adjust pH to 8.0. Make the final volume to 1 L with ddH_2O.

5 M NaCl: Dissolve 292.2 g of NaCl in about 700 ml of H_2O. Make the final volume to 1 L.

Instruments: Water bath, refrigerated centrifuge, ph meter, spectrophotometer, gel electrophoresis unit, PCR machine, GEL-DOC.

Miscellaneous: Micro-pestle, water bath maintained at 60°C, centrifuge, micropipettes, vortex mixer, 2 ml microcentrifuge tubes, weigh boats or equivalents, spatulas, weighing balance, loops, rack for microcentrifuge tubes, optional: vacuum desiccator to dry DNA pellets, mortar and pestle (autoclaved), spatula, forceps, pipettes with tip box, ice-box, eppendorfs with stands, butter paper, tissue papers, aluminium foil, gloves, markers.

I. Genomic DNA Isolation

Principle: Lysis, extraction and precipitation

Plant cells can be lysed with the ionic detergent cetyltrimethylammonium bromide (CTAB), which forms an insoluble complex with nucleic acids in a low-salt environment. Under these conditions, polysaccharides, phenolic compounds and other contaminants remain in the supernatant and can be washed away. The DNA complex is solubilized by raising the salt concentration and precipitated with isopropanol.

a. **Lysis of the cell membrane:** The first step of DNA extraction is the rupture of the cell wall and nuclear membrane. For this purpose, the homogenised sample is first treated with the extraction buffer containing EDTA, Tris/HCl and CTAB. All biological membranes have a common structure comprising lipid and protein molecules held together by non-covalent interactions (**Fig. 1**).

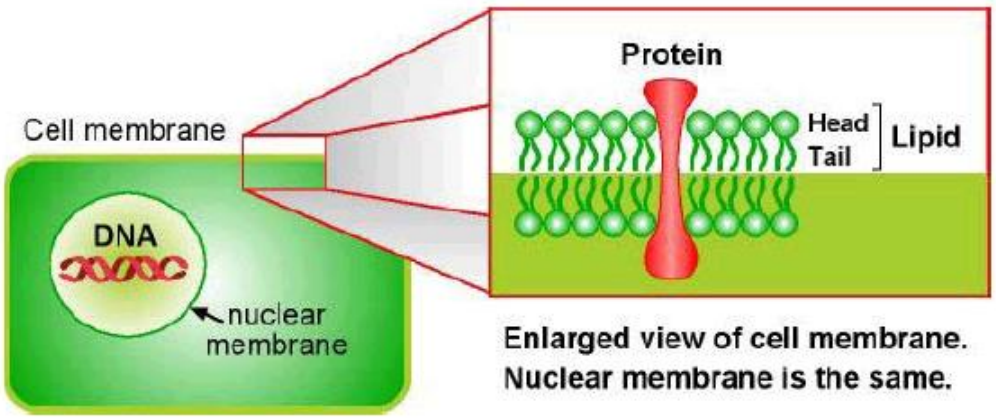

Figure 1: Simplified representation of the cell membranes

The lipid molecules are arranged as a continuous double layer in which the protein molecules are embedded or remain superficial. The lipid molecules are constituted by hydrophilic ends called "heads" and hydrophobic ends called "tails". Because of the similar composition of both the lipids and the detergent, the CTAB component of the extraction buffer has the function of capturing the lipids constituting the cell and nuclear membrane (Fig. 2).

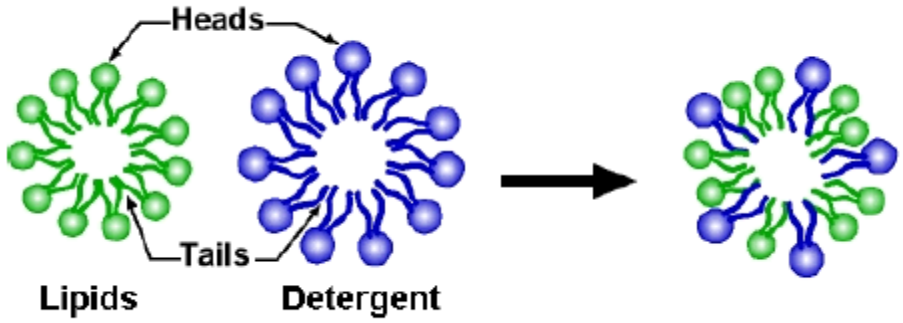

Figure 2: Mechanism of lipid solubilisation using CTAB

During the lysis phase, the detergent captures the lipids and the proteins allowing the release of the genomic DNA (Fig. 3). In a specific salt (NaCl) concentration, the detergent forms an insoluble complex with the nucleic acids. EDTA, a chelating component, has high affinity towards divalent ions like Ca2+, Mn2+, Mg2+. Magnesium is a cofactor for DNase. By binding Mg with EDTA, the activity of present DNase is reduced. The broken cell is thus, treated with EDTA to chelate the Mg2+ ions so that DNase and other nucleases lose their function and we are able to get good yield of DNA. NaCl provides Na+ ions that will block negative charge from phosphates on DNA. DNA molecules repel one another because of the preponderance of negatively charged phosphate groups. The Na+ ions will form an ionic bond with the negatively charged phosphates on the DNA, neutralizing the negative charges and allowing the DNA molecules to come together. Tris/HCl gives the solution a pH buffering capacity (a low or high pH damages DNA). It is important to notice that since nucleic acids can easily undergo degradation at this step of extraction, the time between homogenization of the sample and addition of CTAB buffer should be minimal.

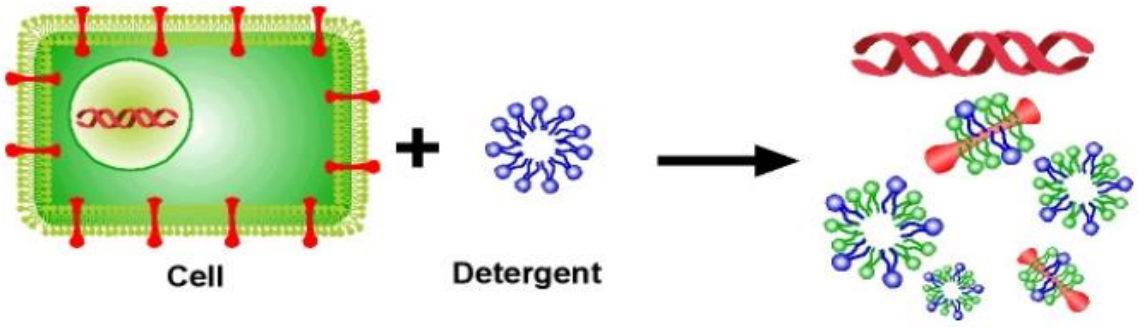

Figure 3: Disruption of the cellular membrane and extraction of genomic DNA

b. **Extraction:** In this step, polysaccharides, phenolic compounds, proteins and other cell lysates dissolved in the aqueous solution are separated from the CTAB nucleic acid

complex. The elimination of the polysaccharides as well as phenolic compounds is particularly important because of their capacity to inhibit a great number of enzymatic reactions. Under low salt concentration (< 0.5 M NaCl), the contaminants of the nucleic acid complex do not precipitate and can be removed by extraction of the aqueous solution with chloroform. The chloroform denatures the proteins and facilitates the separation of the aqueous and organic phases. Normally, the aqueous phase forms the upper phase. However, if the aqueous phase is dense because of salt concentration (> 0.5 M), it will form the lower phase. In addition, the nucleic acid will tend to partition into the organic phase if the pH of the aqueous solution has not been adequately equilibrated to a value of pH 7.8 - 8.0. If needed, the extraction with chloroform is performed two or three times in order to completely remove the impurities from the aqueous layer. To achieve the best recovery of nucleic acid, the organic phase may be back-extracted with an aqueous solution that is then added to the prior extract.

c. **Precipitation:** Once the nucleic acid complex has been purified, the last step of the procedure, precipitation, can be performed. In this final stage, the nucleic acid is liberated from the detergent. For this, the aqueous solution is first treated with a precipitation solution comprising a mixture of CTAB and NaCl at elevated concentration (> 0.8 M NaCl). The salt is needed for the formation of a nucleic acid precipitate. Sodium acetate maybe preferred over NaCl for its buffering capacity. Under these conditions, the detergent, which is more soluble in alcohol than in water, can be washed out, while the nucleic acid precipitates. The successive treatment with 70% ethanol allows an additional purification, or wash, of the nucleic acid from the remaining salt.

Procedure:

The procedure requires sterile conditions. Contamination may be avoided during sample preparation by using single-use equipment, decontamination solutions and avoiding dust. The genomic DNA extraction steps are carried out as follows:

1. Place a small amount of Cauliflower curd (*Brassica oleracea*) material (~ 200 mg) in a sterile 2 ml micro centrifuge tube and add 1000 µl of warmed 2 X CTAB extraction buffer. Grind to a fine powder with a sterile plastic micro-pestle.

2. Incubate the micro centrifuge tube at 65^{o}C for ~ 30 min (in a water bath, or on a controlled heating block), inverting tubes occasionally to mix contents.

3. Cool briefly and then add 750 µl of chloroform-isoamyl alcohol (24:1). Mix the contents for about 2 min by inverting the tubes.

4. Centrifuge at ~ 10,000 rpm for 5 min. Carefully (without disturbing the bottom layer) pipette out the aqueous (top) layer into a new sterile 2 ml micro-centrifuge tube that already contains ~ 2/3rds of a volume of ice-cold isopropanol. Gently mix the contents by a few inversions. White threads of DNA will probably become evident. Place samples in a –20^{o}C freezer for 30 min or longer.

5. Spool ot the DNA, wash with 70% ethanol and dry under vacuum. The DNA is finally suspended in 2 ml of TE (10 mM Tris-HCl and 1 mM EDTA) buffer.

Precautions:

a. Sterilize bottles, tools and solutions with an autoclave at 15 psi for 15 minutes at 121°C. Do not sterilize DNA or plant material by autoclaving or heating.
b. Do not autoclave CTAB and 2-mercaptoethanol.
c. Do not inhale fumes of chloroform, isoamyl alcohal and phenol.
d. Avoid physical contact with above mentioned reagents.
e. Tip of Cauliflower (Cauliflower curd) has to be taken as tissue.
f. While incubation, water bath has to be maintained at 60°C.
g. At the time of incubation, 2ml tubes are to be inverted at regular interval so as to mix the content.

Reference:

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II. DNA Quantification and Quality Analysis

After isolation of DNA, quantification and analysis of quality are necessary to ascertain the approximate quantity of DNA obtained and the suitability of DNA sample for further analysis. This is important for many applications including digestion of DNA by restriction enzymes or PCR amplification of target DNA. The most commonly used methodologies for quantifying the amount of nucleic acid in a preparation are: (i) gel electrophoresis; and (ii) spectrophotometric analysis. If the sample amount is less, the former method is usually preferred.

A. Agarose gel electrophoresis

Agarose gel electrophoresis is a powerful separation method frequently used to analyze DNA fragments generated by restriction enzymes, and it is a convenient analytical method for separating DNA fragments of varying sizes ranging from 100 bp to 25 kb. Agarose gel electrophoresis can also be used to separate other charged biomolecules such as RNA and proteins. This method of quantification is based on the ethidium bromide fluorescent staining of DNA. Ethidium bromide is a fluorescent dye, which intercalates between the stacked bases. The fluorescent yield of the dye: DNA complex is much greater than the unbound dye. UV irradiation at 254nm is absorbed by the DNA and transmitted to the dye and the bound dye itself absorbs radiation at 302nm and 366nm. This energy is retransmitted at 590nm, the reddish-orange region

of the visible spectrum. In case of genomic DNA, the nucleic acids are electrophoretically separated on a 1% agarose gel containing ethidium bromide at a final concentration of 0.5 μg/ml. The quantity of DNA can be estimated by comparing the fluorescent yield of the samples with a series of standards, for instance, lambda (λ) DNA at varying known concentrations. This provides a very rapid and sensitive means of estimating the nucleic acid concentration. A large number of samples with as little as 1-5ng of DNA can be quantified. Besides quantification, it also allows provides the advantage of analyzing the quality of the DNA preparation. Native DNA, which migrates as a tight band of high molecular weight (≥40 kb), presence of RNA, and degraded/sheared DNA, if any, can be visually identified on the gel.

Principle:

DNA has a distinct chemical structure, in which the nucleobases, the letters of the DNA code, are joined by a backbone of a sugar, deoxyribose, and a phosphate group. DNA contains a negative charge for every nucleobase present, making the mass-to-charge ratio of DNA the same across different fragment sizes. Because of this negative charge, when we apply an electrical current (anodal, negative) to a solution containing DNA, the DNA molecules will migrate towards the positively charged electrode (cathodal).

The Gel Matrix

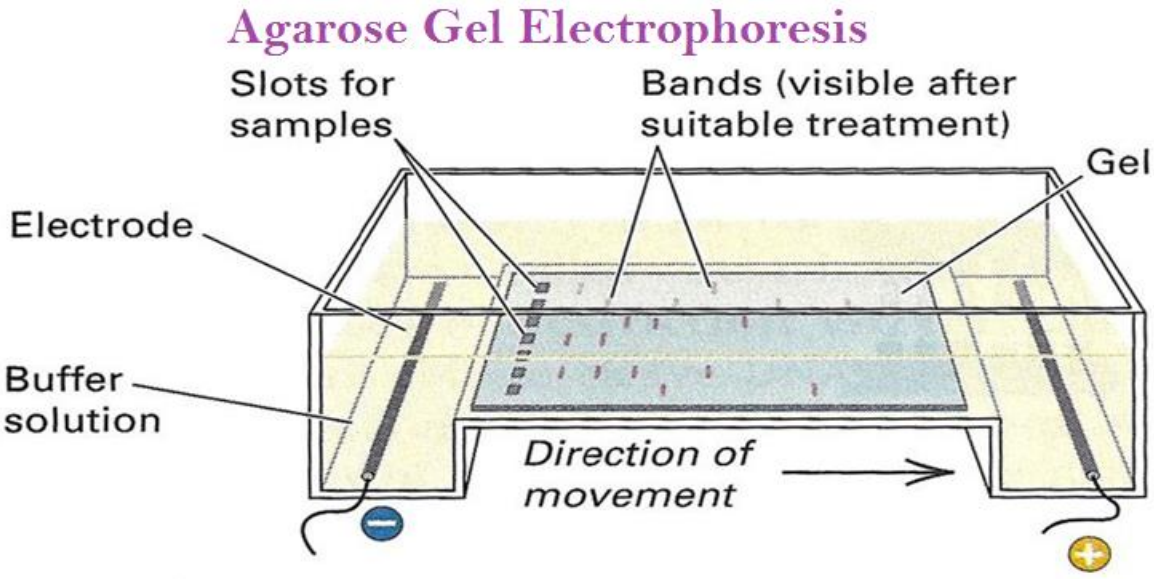
In agarose gel electrophoresis we introduce a gel matrix, imagine several layers of sieves or netting, which the DNA migrates through along the voltage gradient towards the positive electrode. This matrix creates resistance and means that smaller molecules migrate more quickly while larger molecules migrate more slowly. The difference in migration rate is how we separate the different sizes of DNA molecule to determine their length. The gel matrix is created by dissolving a natural polysaccharide called agarose, derived from a type of seaweed, in a conductive buffer typically at around 1% agarose, and allowing it to set into a gel. The pore size in this gel matrix is well suited to the separation of DNA and the speed of migration can be influenced by increasing or decreasing the percentage of agarose in the mixture.

Agarose is a polysaccharide extracted from the seaweed. The long chain of (polymer) agarobiose creates the agarose sugar. Agaropectin is removed from the agar to form agarose.A linear agarose polysaccharide is made up of the monomeric unit of D-galactose and 3, 6-anhydro-L-galactopyranose disaccharide.The agarose powder is only soluble in the water on boiling. After cooling, it undergoes hydrogen bonding (cross-linking) which consequence in polymerization. By forming the hydrogen between an adjacent molecule, it creates a three-dimensional matrix of pores.The size of the pores varies as the concentration of agarose in the gel varies. The pores create a channel in the gel from where the DNA can migrates.

The melting temperature of the agarose is nearby the boiling temperature (~ 95ºC) whereas the gelling temperature is ~ 37ºC – 43ºC.

Running the gel

For a typical agarose gel electrophoresis procedure, the gel matrix is cast as a horizontal slab. Plastic combs are used to create indentations, or wells, into which the DNA is loaded. Before loading, the DNA is mixed with a loading dye that weighs down the sample in the solution, so it does not leave the well, and also includes a visible marker to track the progression of the run. Unknown samples are often run alongside a DNA Ladder, containing known lengths of DNA for comparison. The gel is then placed in a container, called a gel tank or box, filled with conductive, pH controlled buffer solution, usually Tris-acetate-EDTA or Tris-Borate-EDTA and an electrical field is applied along the length of the gel. Electrophoresis buffer facilitates the liquid medium for the migration of DNA into the gel. The electrophoresis buffer also maintains a constant pH during the run.



DNA gel loading dye

DNA gel loading dye contains Bromophenol blue and glycerol. The BPB runs ahead of the DNA. We can monitor the migration by gel loading dye. Also, the glycerol present into the DNA gel loading dye gives density to the DNA which settled DNA at the bottom of the well.

EtBr

Ethidium bromide is a fluorescent dye used for tagging DNA. DNA does not have their own colour, EtBr intercalates with the DNA bases and gives fluorescent colour under UV light.

Precautions:

- Agarose powder is hazardous hence always wear gloves, face mask and goggles while preparing of an agarose gel.
- During the boiling of agarose wear oven-gloves (heat resistant). Do not use plastic gloves, it will burn your hand.
- EtBr is a carcinogenic and mutagenic therefore take necessary precautions.

B. Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is a method widely used in molecular biology to make several copies of a specific DNA segment. Using PCR, copies of DNA sequences are exponentially amplified to generate thousands to millions of more copies of that particular DNA segment. The vast majority of PCR methods rely on thermal cycling. Thermal cycling exposes reactants to repeated cycles of heating and cooling to permit different temperature-dependent reactions – specifically, DNA melting and enzyme-driven DNA replication.

Principle:

PCR uses the enzyme DNA polymerase that directs the synthesis of DNA from deoxynucleotide substrates on a single-stranded DNA template. DNA polymerase adds nucleotides to the 3` end of a custom-designed oligonucleotide when it is annealed to a longer template DNA. Thus, if a synthetic oligonucleotide is annealed to a single-stranded template that contains a region complementary to the oligonucleotide, DNA polymerase can use the oligonucleotide as a primer and elongate its 3` end to generate an extended region of double stranded DNA.

Components:

- **DNA template is** the sample DNA that contains the target sequence. At the beginning of the reaction, high temperature is applied to the original double-stranded DNA molecule to separate the strands from each other.
- **DNA polymerase is** a type of enzyme that synthesizes new strands of DNA complementary to the target sequence. The first and most commonly used of these enzymes is*Taq*DNA polymerase (from*Thermisaquaticus*), whereas*Pfu*DNA polymerase (from*Pyrococcusfuriosus*) is used widely because of its higher fidelity when copying DNA. Although these enzymes are subtly different, they both have two capabilities that make them suitable for PCR: 1) they can generate new strands of DNA using a DNA template and primers, and 2) they are heat resistant.
- **Primers are** short pieces of single-stranded DNA that are complementary to the target sequence. The polymerase begins synthesizing new DNA from the end of the primer.

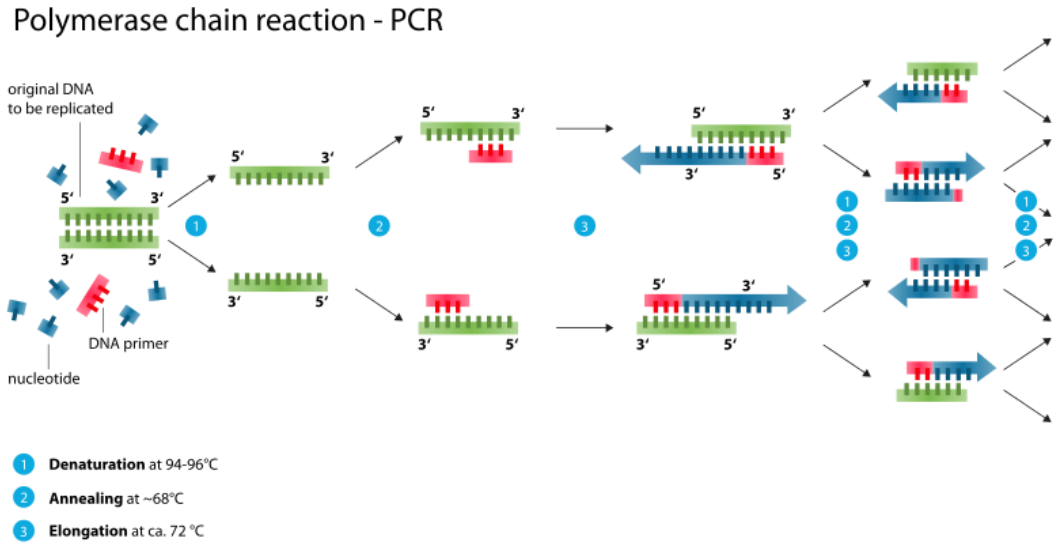
- **Nucleotides (dNTPs or deoxynucleotide triphosphates) are** single units of the bases A, T, G, and C, which are essentially "building blocks" for new DNA strands.

Procedure:

Typically, PCR consists of a series of 20–40 repeated temperature changes, called thermal cycles, with each cycle commonly consisting of two or three discrete temperature steps.

The individual steps common to most PCR methods are as follows:

1. **Initial Denaturation for 2 minutes at 94°C:** This initiation step heats the double stranded DNA template strand to the point where the strands start denaturing and the hydrogen bonds are broken between the nucleotide base pairs.
2. **Denature 30 seconds at 94°C:** Continued denaturation of double stranded DNA.
3. **Anneal primers for 30 seconds at 55°C:** The forward and reverse primers are stable within this temperature range to anneal to each of the single stranded DNA template strands. The DNA polymerase is also stable enough to now bind to the primer DNA sequence.
4. **Extend DNA for 1 minute at 74°C:** The *Taq* polymerase has an optimal temperature around 70-75°C so this step enables the DNA polymerase to synthesize and elongate the new target DNA strand accurately and rapidly.
5. **Repeat steps 2-5 25-30 times.**
6. **Final Extension for 5 minutes at 74°C:** A final extension to fill-in any protruding ends of the newly synthesized strands.
7. **Final hold:** The final step cools the reaction chamber to 4–15 °C (39–59 °F) for an indefinite time, and may be employed for short-term storage of the PCR products.



Problems and Precautions:

- Since PCR can amplify as little as a single molecule of DNA, problems of contamination become paramount. To minimize the risk of contamination, one must always wear gloves when setting up reactions. Use solutions that are dedicated solely to PCR (it is best to make up solutions and dispense them in small aliquots, for single use only), pipetters and tips dedicated solely to PCR and new glassware and plasticware.

- Both positive and negative controls should always be run in conjunction with any target reaction. A positive control should include a small amount of the appropriate target sequence and a negative control should include all reaction components except the DNA template.

- In addition to potential problems of PCR contamination, variations in sequences obtained from a single PCR reaction occur. This is because Taq DNA polymerase lacks any proofreading activity and therefore occasionally makes an error during template extension.

III. Spectrophotometric Determination

Analysis of UV absorption by the nucleotides provides a simple and accurate estimation of the concentration of nucleic acids in a sample. Purines and pyrmidines in nucleic acid show absorption maxima around 260nm (eg.,dATP: 259nm; dCTP: 272nm; dTTP: 247nm) if the DNA sample is pure without significant contamination from proteins or organic solvents. The ratio of OD_{260}/OD_{280} should be determined to assess the purity of the sample. This method is however limited by the quantity of DNA and the purity of the preparation. Accurate analysis of the DNA preparation may be impeded by the presence of impurities in the sample or if the amount of DNA is too little. In the estimation of total genomic DNA, for example, the presence of RNA,

sheared DNA etc. could interfere with the accurate estimation of total high molecular weight genomic DNA.

Principle:

The Beer-Lambert Law relates the absorption of light to the properties of the material through which the light travels. This law states that there is a logarithmic dependence between the transmission of light through a substance and the product of the absorption coefficient of the substance and the path length. For DNA and RNA, the heterocyclic rings of nucleotides (adenine, guanine, cytosine and thymine/uracil) result in nucleic acid molecules absorbing ultraviolet (UV) light maximally at 260nm ($\lambda max = 260nm$).

$$A = \varepsilon \cdot b \cdot c$$

A = absorbance at a particular wavelength
ε = extinction coefficient
b = path length of the spectrophotometer
c = concentration of sample

The Beer-Lambert law can be used with the appropriate extinction coefficients to determine nucleic acid concentration.

For most commercial instruments, reliable spectrophotometric quantification (A260) readings lie between 0.1 and 1.5. While nucleic acids absorb maximally at 260nm, other common contaminants absorb strongly at wavelengths near 260nm. Solvents such as guanidine and phenol, as well as salts, have maximal absorbance around 230nm, while proteins contribute at 230nm and 280nm. These neighbouring peaks, if present in a sample, will inflate the reading at 260nm.

Procedure:

1. Take 1 ml TE buffer/ dd H_2O in a cuvette and calibrate the spectrophotometer at 260nm as well as 280nm.
2. Add 10 µl of each DNA sample to 990µl TE (Tris-EDTA buffer)/ / dd H_2O and mix well.
3. Use TE buffer// dd H_2O as a blank in the other cuvette of the spectrophotometer.
4. Note the OD_{260} and OD_{280} values on spectrophotometer.
5. Calculate the OD_{260}/OD_{280} ratio.

- A ratio between 1.8- 2.0 denotes that the absorption in the UV range is due to nucleic acids.
- A ratio lower than 1.8 indicates the presence of proteins and/or other UV absorbers.
- A ratio higher than 2.0 indicates that the samples may be contaminated with chloroform or phenol. In either case (<1.8 or >2.0) it is advisable to re-precipitate the DNA.

6. The amount of DNA can be quantified using the formula:

DNA concentration (mg/ml) = OD260 x dilution factor x 50 μg/ml

Example of Calculation

A sample of dsDNA was diluted 50X. The diluted sample gave a reading of 0.65 on a spectrophotometer at OD_{260}. To determine the concentration of DNA in the original sample, perform the following calculation:

- dsDNA concentration = 50 μg/mL × OD_{260} × dilution factor
- dsDNA concentration = 50 μg/mL × 0.65 × 50
- dsDNA concentration = 1.63 mg/mL

Spectrophotomteric Conversions for Nucleic Acids:

1 A 260 of ds DNA = 50 μg/ml

1 A 260 of ss oligonucleotides = 33 μg/ml

1 A 260 of ss RNA = 40 μg/ml

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PHOTOS


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