CURRENT

Category: General SOP for Extraction of genomic DNA from whole blood in Genetics laboratory.

Title: Extraction of genomic DNA from whole blood by phenol-chloroform method in the Department of Clinical Pharmacology, K.E.M Hospital, Mumbai.

SOP No.: Pre PCR-Method- 03

Date first effective: 1st January 2025 **Review date:** 31st December 2025

Department of Clinical Pharmacology, 1st floor, New MS building Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012.

Category: General SOP for Extraction of genomic DNA from whole blood.

Title: Extraction of genomic DNA from whole blood by phenol-chloroform method in the Department of Clinical Pharmacology, K.E.M Hospital, Parel, Mumbai.

SOP No.: Pre PCR-Method- 03 **Total pages: 06**

Date first effective: 1st January 2025 **Review date:** 31st December 2025

Version: 05

Author: Dr.Sheetal Kudtarkar
Project Scientist

Signature with date: 30/12/24

Reviewer 1: Dr.Bhaskar Krishnamurthy
Assistant Professor

Signature with date: 30/DEC/2024

Dr. Bhaskar Krishnamurthy
Assistant Professor,
Department of Clinical Pharmacology,
Seth GSMC and KEMH,Mumbai -400 012.

Reviewer 2 Dr. Mahesh Belhekar
Associate Professor

Signature with date: 30/Dec/2024

Dr. Mahesh N. Belhekar
Associate Professor
Department of Clinical Pharmacology
New MS Building, First Floor,
Seth GS Medical College and KEM Hospital
Acharya Donde Marg, Parel,
Mumbai - 400 012. India

Approved by: Dr. Nithya Gogtay
Professor and Head

Signature with date: 30.12.24

Dr. Nithya Gogtay
Professor & Head
Department of Clinical Pharmacology
1st Floor, MS Building,
Seth GS Medical College & KEM Hospital
Parel, Mumbai - 400 012.

Category: General SOP for Extraction of genomic DNA from whole blood in Genetics laboratory.

Title: Extraction of genomic DNA from whole blood by phenol-chloroform method in the Department of Clinical Pharmacology, K.E.M Hospital, Mumbai.

SOP No.: Pre PCR-Method- 03

Date first effective: 1st January 2025 **Review date:** 31st December 2025

Department of Clinical Pharmacology, 1st floor, New MS building Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012.

Table of contents

Category: General SOP for Extraction of genomic DNA from whole blood in Genetics laboratory.

Title: Extraction of genomic DNA from whole blood by phenol-chloroform method in the Department of Clinical Pharmacology, K.E.M Hospital, Mumbai.

SOP No.: Pre PCR-Method- 03

Date first effective: 1st January 2025 **Review date:** 31st December 2025

Department of Clinical Pharmacology, 1st floor, New MS building Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012.

Purpose: This standard operating procedure (SOP) describes the method for extraction of genomic DNA from whole blood.

Scope: This SOP is limited to extraction of genomic DNA from whole blood.

Responsibilities:

Primary Responsibility: Divya Bhere
Lab Technician
30/Dec/2024

Secondary Responsibility: Dr.Sheetal Kudtarkar
Project Scientist
30/12/24

References: JIPMER (Pondicherry) Workshop 2012 manual

Category: General SOP for Extraction of genomic DNA from whole blood in Genetics laboratory.
Title: Extraction of genomic DNA from whole blood by phenol-chloroform method in the Department of Clinical Pharmacology, K.E.M Hospital, Mumbai.
SOP No.: Pre PCR-Method- 03
Date first effective: 1st January 2025 **Review date:** 31st December 2025
Department of Clinical Pharmacology, 1st floor, New MS building Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012.

Detailed Instructions:

Important: This procedure should be carried out wearing sterile gloves

1. Collect 5mL blood in a 15mL fresh polypropylene tube containing 100µL of 10% EDTA disodium salt.
2. Collected blood sample is stored at -20°C and should be analyzed within two weeks.
3. Add 10mL of RBC lysis solution, mix vigorously for 5mins and keep the tube in deep freezer for 10mins (-20°C)
4. Centrifuge the tube at 2500 rpm in cooling centrifuge for 10 mins at 4°C
5. Discard the upper layer in bucket containing 10% hypochlorite solution by tilting the tube slowly in such a way that the pellet should remain in the tube
6. Add 6 to 8mL of RBC lysis solution, mix and follow the Steps 5,6 & 7 till the RBCs are lysed completely **(Till solution becomes clear; NOT more than 4 washes)**
7. Add 2.25 mL of WBC lysis solution in the tube. Mix gently with the help of rotospin machine for 20 mins at 21 rpm.
8. Add 125 µL of 10% Sodium dodecyl sulphate, 50 µL of Protienase K, and 75 µL of MilliQ water to it and mix it with the help of Rotospin for 20 mins at 21rpm.
9. Mix the above solution and incubate at 37°C for overnight in Incubator
10. Next day at 10:00am check whether the lysis is complete (If **lysis is complete the solution will be homogenous and will freely move when tilted to and fro)**
11. If lysis is incomplete invert the tube for 10 times gently and incubate at 37°C for 3- 4 hrs in Incubator
12. Add 1mL of saturated NaCl and mix.
13. Add 3.5mL of Chloroform and mix by to and fro movement (gently) with the help of Rotospin for 20 mins at 21 rpm till it becomes homogenous mixture.
14. Centrifuge at 4000 RPM at 4°C for 20 mins

Category: General SOP for Extraction of genomic DNA from whole blood in Genetics laboratory.
Title: Extraction of genomic DNA from whole blood by phenol-chloroform method in the Department of Clinical Pharmacology, K.E.M Hospital, Mumbai.
SOP No.: Pre PCR-Method- 03
Date first effective: 1st January 2025 **Review date:** 31st December 2025
Department of Clinical Pharmacology, 1st floor, New MS building Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012.

15. Take the upper layer by micropipette **(by slightly rotating the pipette by the sides of the wall of the test tube)** without disturbing the lower layer and transfer the upper layer to a fresh tube (**use new tips for each sample)**
16. Add 2.5mL of equilibrated phenol. Mix gently with the help of rotospin for 20 mins at 21 rpm and centrifuge at 4000 RPM for 20 min at 4°C
17. Take upper layer using micro pipette without disturbing lower layer and transfer to a fresh tube.
18. Add 3 mL of Chloroform: Octanol **(ratio 24:1)** and mix gently with the help of Rotospin for 20 mins at 21 rpm and centrifuge at 4000 RPM for 20 min at 4°C
19. Transfer the upper layer to a fresh tube **(If the interphase is not clear at this stage keep the tube standing for 5-10 mins) Interphase: A thin layer between upper and lower layers**
20. Add 6mL of ice-cold absolute ethanol which is kept in ice box in -20°C deep freezer and mix by inverting the tubes for 6 to 8 times. High molecular weight DNA will precipitate at this step
21. Remove DNA using a clean pipette tip and wash it with 70% ethanol in 1.5 mL v-shaped polypropylene tube by tapping the tube five to six times
22. Remove the DNA from the ethanol tube with a pipette and air dry
23. Transfer the DNA in a 1.5mL v-shaped micro centrifuge tube containing 200 µL of Tris EDTA buffer (pH 7.4).
24. Incubate at 37°C for 2-3 days in Incubator
25. Prepare two aliquots
26. Store the one aliquot of DNA sample at -20°C **(for 4 to 5 years)** and the second aliquot at 4°C **(one year)**

Category: General SOP for Extraction of genomic DNA from whole blood in Genetics laboratory.

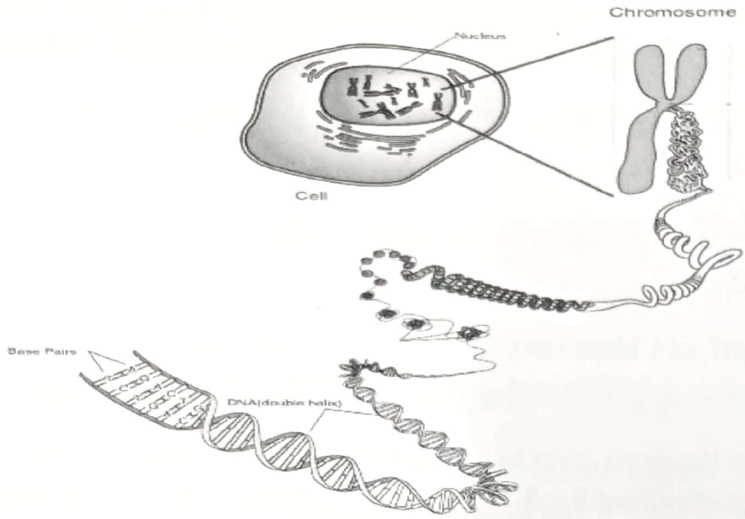
Title: Extraction of genomic DNA from whole blood by phenol-chloroform method in the Department of Clinical Pharmacology, K.E.M Hospital, Mumbai.

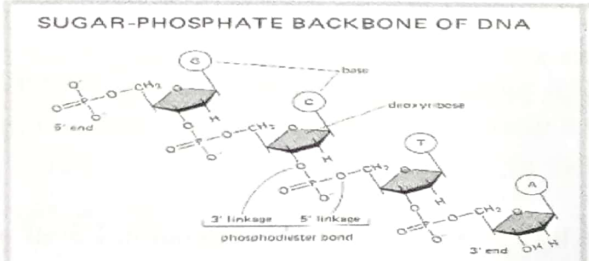
SOP No.: Pre PCR-Method- 03

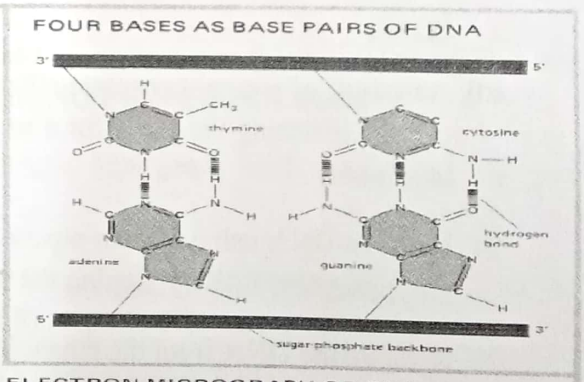
Date first effective: 1st January 2025 **Review date:** 31st December 2025

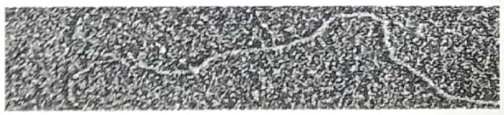
Department of Clinical Pharmacology, 1st floor, New MS building Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012.

DNA Structure:









ELECTRON MICROGRAPH OF DNA