CURRENT

Category: General SOP for preparation and performing the horizontal gel electrophoresis in Genetics laboratory.
Title: Performing the horizontal gel electrophoresis for digested PCR products in the Department of Clinical Pharmacology, K.E.M Hospital, Mumbai.
SOP No.: Post-PCR method -04
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.

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Version: 05

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Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the method for performing the gel electrophoresis for Digested Polymerase Chain Reaction Products in Department of Clinical Pharmacology (DCP), Seth GS Medical College and KEM Hospital, Mumbai.

Scope: This SOP is limited for performing the horizontal gel electrophoresis for digested Polymerase Chain Reaction Products.

Responsibilities:

Primary Responsibility: Divya Bhere
Lab Technician
Dbhere 30 DEC 2024

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

References: Molecular Cloning: A laboratory manual 3rd Edition (JF Sambrook & DW Russel)

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Detailed Instructions:

Important: This procedure should be carried out wearing sterile gloves

1. For 3% gel preparation, dissolve 3g of agarose in100mL of 1X TAE buffer in a bottle by heating the solution in microwave oven.
2. Heat till agarose powder is fully dissolved. (I.e. till solution becomes clear).
3. Now add 15µL of Ethidium bromide in gel and mix the solution.
4. Cool agarose for 3-4 minutes till all the vapour is gone, keeping its cap open.
5. Pour the gel in casting tray and allow it to solidify.
6. Arrange the horizontal gel electrophoresis tray by placing the comb at least 1cm above the surface of the tray. Now place the electrode plates according to the charge i.e. red plate (positive) opposite to the well and black plate (negative) near the wells.
7. Connect the electrodes to the power pack (voltage suppliers) with the help of connecting wires to the respective plates and close the tank with the lid.
8. After the gel has solidified, pour 1X TAE buffer on it (till the gel is completely covered by buffer) and wait for 10-15 mins.
9. Then remove the comb carefully such that wells should remain intact.
10. Place the solidified gel in the buffer tank.

11. Pour 1XTAE buffer in the tank such that all wells are immersed in it.
12. Load 5 µL DNA ladder.
13. Load 15 µL of PCR product in the well.
14. Now turn ON the voltage supplier.
15. Press Manual option key of power pack.
16. Now put 100V using the number keys.
17. Now press the RUN button.
18. Let the sample run till the bands and the DNA ladder is separated as per the product size i.e. approximately 3/4th of the gel.
19. Press STOP button to stop running gel.
20. Now remove the gel from the tray.
21. Place the gel in and observe the bands (Follow the SOP no. Post-PCR INS -02)

NOTE: The gel percentage can vary with gene being studies. For example, if digested PCR products are less than 100bp, 4% gel should be used and if they are more than 1kbp, 2% gel should be used.

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Gel Picture after Restriction Digestion of PCR product