

Preparing an agarose gel for PCR and ARDRA purposes

1. Subject

ARDRA-fragments can be separated electrophoretically on agarose gels. The size of DNA-fragments (amplicons) can be estimated. According to the expected size of DNA-fragments an agarose gel with the right percentage is casted.

2. Principle

The negative charged DNA will migrate to the positive pole. The velocity of migration depends on the size of the DNA-fragments. An optimal separation can be achieved by combining the optimal agarose percentage and electrical current.

3. Reagents and material

- 3.1 agarose I (Amresco 0710)
- 3.2 Tris(hydroxymethyl)-aminomethane (Merck 8382)
- 3.3 Na-EDTA (Titriplex III, Bio Rad 161-0729)
- 3.4 boric acid (H₃BO₃, Merck 0165)
- 3.5 ethidiumbromide (1,7-diamino-10-ethyl-9-phenylphenanthridiniumbromide, Serva 21238)
- 3.6 sodiumhydroxide pellets, NaOH (Merck 6498)
- 3.7 erlenmeyer-flask 300 ml
- 3.8 a scale
- 3.9 microwave oven 850-1300 W
- 3.10 pipet 10 µl and pipettips
- 3.11 electrophoresis-tray (100 or 250 ml) and combs
- 3.12 tape
- 3.13 latex gloves
- 3.14 TAE 50x (BioRad 161-0743)

4. Solutions

- 4.1 0.5M EDTA:
Add 186.1 g Na-EDTA [3.3] to 800 ml H₂O and stir vigorously on a magnetstirrer. Adjust pH to 8.0 with NaOH (±20 g NaOH pellets); autoclave the solution. EDTA will dissolve at pH 8.0.
- 4.2 TBE-buffer 5x stock:
Add 54 g Tris [3.2] and 27.5 g boric acid [3.4] to 800 ml ultrapuur H₂O. Add 20 ml 0.5M EDTA [4.1]. Adjust volume to 1000 ml with ultrapure H₂O.
- 4.3 TBE-buffer 0.5x:
Before use the TBE-buffer 5x [4.2] has to be diluted 10x: mix 100 ml TBE-buffer 5x [4.2] with 900 ml ultrapure water.
- 4.4 ethidiumbromide:
Dissolve 10 mg ethidiumbromide [3.5] in 10 ml ultrapure H₂O [6.3].
- 4.5 TAE-buffer 1x:
Mix 200 ml TAE 50x [3.14] with 9800 ml ultrapure water.

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5. Protocol

- 5.1 Transfer the appropriate amount of agarose to a 300 ml erlenmeyer-flask (tabel 1).
- 5.2 Add half of the total amount of buffer [6.1], but at least 100 ml to the agarose.
- 5.3 Heat the solution in a microwave oven; at high concentrations agarose will start to foam, stop the cooking process before it boils over.
- 5.4 Rotate the erlenmeyer-flask carefully (Beware! Solution might start cooking). Leave the erlenmeyer-flask for a few min. and repeat step 5.3 with 10-20 sec., until agarose has dissolved completely.
- 5.5 After dissolving, boil agarose 10-15 sec..
- 5.6 Adjust volume with the rest of buffer to the right volume [8.1]. Mix warm and cold solutions. Heat solution in microwave oven if necessary.
- 5.7 Leave agarose to cool to 55-60°C.
- 5.8 Pipet ethidiumbromide (4.4) [8.1] and swirl erlenmeyer-flask [6.2].
- 5.9 Tape geltray on both sides and waterlevel it.
- 5.10 Pour agarose solution into the geltray after cooling (55-60°C).
- 5.11 Swirl erlenmeyer-flask occasionally.
- 5.12 Remove airbubbles with a pipettip and place the comb(s).
- 5.13 Leave agarose to solidify. After solidifying, the agarose can be cooled in a refrigerator.
- 5.14 Remove tapes and place geltray into the electrophoresis-container; the electrophoresis-container is filled with the same buffer as used for the agarose gel [4.3 or 4.5].
- 5.15 Remove comb(s) carefully without damaging the slots.
- 5.16 Add a sufficient amount of buffer to the electrophoresis-container to cover the gel with a thin layer of buffer [6.4].

6. Remarks

- 6.1 At high concentration, TBE (4.2) may precipitate after long-time storage, showing a white layer. To prevent this, 5x solutions of TBE should be kept at roomtemperature in glass bottles. If the buffer shows precipitation, it should be thrown away. In the original protocol a 1x buffer solutions is used for agarose gel electrophoresis, but a 0.5x solution has enough buffer capacity though. For TAE-buffer the concentration should be 1x to have enough buffer capacity.
- 6.2 Ethidiumbromide (4.4) is a potent carcinogen and mutagen. Literature is not always clear about it, but it is advised to wear protective clothes while handling. Use goggles and gloves. By influence of UV-light ethidiumbromide becomes less mutagenic/carcinogenic. Solutions and gels containing ethidiumbromide should be decontaminated before they are disposed of.
- 6.3 In 8.1 the amount of ethidiumbromide given are average values. The usage of a 10 mg/ml stock solution varies within 1-3 µl for a 100 ml tray and 3-5 µl for a 250 ml tray. The migration of DNA is decreasing at higher ethidiumbromide concentrations (> 0.5 µg/ml). When the maximum amount of ethidiumbromide is used (5 µl on 250 ml agarose), the endconcentration will be 200 ng/ml.
- 6.4 The level of buffer above the gel should be as small as possible. With a high level of buffer the currency will not entirely go through the gel but also through the buffer.

7. Literature

- 7.1 Molecular Cloning: A laboratory manual (Sambrook, J., Fritsch, E.F., and Maniatis, T., second edition, 1989)

8. Graphs and Tables

8.1 Concentration and resolution of agarose gels

Percentage	1.0		1.2		1.5		2.0	
TAE [4.5] or TBE [4.3] in ml	100	250	100	250	100	250	100	250
agarose [3.1] in g	1.0	2.5	1.2	3.0	1.5	3.7	2.0	5.0
ethidiumbromide [4.4] in µl	1	2.5	1	2.5	1	2.5	1	2.5
resolution in Kb	0.5-7		0.4-6		0.2-3		0.1-2	

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