A simple and cheap device for gradient formation

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We describe a simple and low cost nonlinear gradient former. The apparatus is derived from a 20 ml syringe and was used to build up sucrose gradients (1) for the separation of DNA fragments needed for the construction of a cosmid library. The setup is given in Figure 1. The lower chamber underneath the plunger is the mixing chamber containing a given volume of the lower concentrated solution, the upper part above the plunger is the reservoir for the higher concentrated component. The given volume of the mixing chamber determines the shape of the nonlinear concentration curve (2). Figure 2 shows a partial Sau3A digest of human genomic DNA separated on a sucrose gradient. The mixing chamber was loaded with 7 ml of 10% sucrose, 40 mM Tris-HCl pH 7.4, 1 M NaCl, the reservoir contained 40% sucrose, 40 mM Tris-HCl pH 7.4, 1 M NaCl. Gradients were centrifuged in 17 ml polyallomer tubes in a Kontron TST 28.17 rotor for 20 hrs at 21,000 rpm.

REFERENCES

Figure 1. Gradient former. No. 1 = plunger; 2 = syringe; 3 = small triangle cut in plunger (about 1 mm²); 4 = magnet; 5 = silicone tubing; 6 = tube clamp; 7 = centrifuge tube; 8 = glass capillary; 9 = magnetic stirrer.

Figure 2. DNA fractions from a gradient formed with the described set up. 0.4 ml fractions (#4–#14) were collected and aliquots run on a 0.35% agarose gel. Numbers, numbers of fractions; M, molecular weight marker.