

Goodgal

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DEPARTMENT OF BIOCHEMISTRY

July 11, 1957

Dr. Joshua Lederberg
Professor of Medical Genetics
Genetics Building
University of Wisconsin
Madison 6, Wisconsin

Dear Josh:

Thank you for your letter. I'll try to comply with your request. I am in the process of writing up the technical procedures which we use. They are somewhat different from the procedures which we borrowed from Alexander and Leidy (J. Exptl. Med. 1953, 97, 17). Shaeffer (Ann. Inst. Pasteur, Oct. or Nov., 1956) uses procedures which are very similar to ours, but we find the use of a blood medium to be superior to his medium in our hands. I am sending you a copy of some laboratory exercises which we have used successfully for two years with some additional comments which may be helpful.

(1) Use defibrinated blood. We use sheep's blood which we collect ourselves at the slaughter house. The blood is defibrinated by means of a rough wooden paddle which is turned by an ordinary household mixer motor, for about 10 minutes. The blood is then strained and stored in the deep freeze. We have also used commercial rabbit blood and horse blood which are far too expensive for large scale use.

(2) We have developed a procedure for preparing highly competent cells. The frequencies which we obtain are close to the frequencies reported for Pn. Unfortunately, the problem has not been exploited to any great extent, and we have not yet published. The procedure may be useful in testing for competence in other organisms. Consequently, we should not like to spread the word around, at least until we check a few organisms and publish the results. Would you, therefore, not disclose further the method for the time being. The technique is quite simple: Grow the receptor cells from an inoculum of 10^7 /ml to 1×10^9 /ml with good aeration (we use an A. H. Thomas rotary shaker with a flask about 1/6 to 1/10 full). Then transfer the cells to a smaller flask and grow without aeration or shaking an additional 90 min. Then 15% glycerol is added to the cells which are transferred to small vials (usually 2 ml.) and frozen with dry ice and alcohol. The vials are stored in a dry ice chest and remain perfectly viable and competent to within 50% of their original competence for at least 5-6 months. Incidentally, a number of coli strains, including K12, have been kept approximately 100% viable for over 12 months.)

(3) To perform a transformation the cells are thawed in tap water and diluted into a mixed volume of media containing transforming factor (we use 3 ml.). The mixture is allowed to shake for a fixed period of time at 36°C. and DNase added. The addition of DNase is unnecessary if one chooses to permit the mixture to incubate for 30 min. by which time very little further transformation occurs. The cells are then diluted in Eugonbroth and pour plated with agar media, the plates allowed to set for 5 min., incubated at 37° for 2 hrs. which is ample time for expression of the characteristics which we have, and then the selective agent is added in the form of an additional 10 ml. agar layer.

(4) The H.I. receptor cells (Rd) which we are forwarding were dried some time ago, but should be viable--at least the sample we tested was still viable. In addition, I am sending you a liquid culture of Rd, a sample of sterile Levinthal stock, Eugonbroth, sterile DPN, and T.P. which should be sufficient to get you started.

(5) The T.P. is a multiply marked stock with the following markers:
Streptomycin - resistant to 2000 μ /ml., Rd sensitive to 3 μ /ml.
Erythromycin - " " 10 μ /ml., the Rd strain is sensitive to 3 μ /ml.
Cathomycin - resistant to 8 μ /ml, Rd is sensitive at less than 1 μ /ml.
Viomycin - resistant to 500 μ /ml, Rd is sensitive at about 150 μ /ml.

We have some erythromycin and cathomycin markers which are far more useful but they are not incorporated as yet into purified stocks. I'll be pleased to send you the crude stocks if you so desire.

If you wish to use the erythromycin and cathomycin markers, measure out the bottom layer of the pour plate and also the top layer using flat surfaces to harden the plates. For erythromycin use 12.5 μ /ml in the top layer or pour plate the transformation mixture after 2.5 hrs. with 6.25 μ /ml erythromycin. For best results be sure to use a sufficient concentration of eryth. or catho. DNA (0.1 or 0.01 μ /ml) so that the cells can be diluted 100-fold before plating.

Cathomycin and streptomycin are linked and represent the best markers for general use. The other markers are not linked.

If there are any problems, don't hesitate to write.

Please give my kindest regards to Esther. With best wishes for a pleasant trip to you both,

Sincerely,



Sol H. Goodgal