ECOGPT TRANSFORMATION PROTOCOL

Seed approximately 10^6 cells on 100 mm plates (in Dulbecco's modified Eagle medium (DME) containing 5% fetal calf serum and penicillin and Streptomycin) and twenty-four hours later, transfect the culture with 10-20 µg of the plasmid-Ecogpt DNA according to Parker and Stark's modification of Graham and Vander Eb's procedure omitting the addition of salmon sperm DNA as carrier.

After 3 days at 37°C in DME containing 5% fetal calf serum, trypsinize the cell monolayers and disperse 5 x 10^5 cells on 100 mm plates in DME containing 10% dialyzed fetal calf serum, 250 µg/ml xanthine, 15 µg/ml thymidine, 2 µg/ml aminopterin, and 25 µg/ml mycophenolic acid (sodium salt). Twenty-four hours later, replace the medium with fresh medium containing the same supplements, and fluid change, thereafter, every 3 days. Colonies are visible in 7-10 days.

Note:

1. Mycophenolic Acid (MPA) can be obtained without cost (so far) from Eli Lilly Co., Indianapolis, Indiana. Since MPA is relatively insoluble, the sodium salt is prepared by making a 25 mg/ml stock in 0.1 N NaOH and titrating it to neutrality with HCl. An alternative to MPA is ribavirin (Virazole), ICN Pharmaceuticals, Irvine, California, another inhibitor of IMP dehydrogenase; a formal written proposal is necessary to obtain this drug. The effective kiling concentration (10-25 µg/ml) appears to be similar to that of MPA with the cell lines tested.
2. Xanthine is also relatively insoluble in water and therefore stock solutions (5 mg/ml) are prepared in 0.1 N NaOH. After preparing a bottle of selective medium, the contents are flushed with CO_2 to bring the pH back to the proper range.
3. Dialyzed fetal calf serum (as opposed to undialyzed) may not be obligatory. The amount of free guanine (which can be salvaged by the animal cells' HGPRT to produce GMP and potentially overcome the selective block) in different sera varies and in some cases, may be negligible. In addition, some sera have been reported to contain large amounts of guanine deaminase which might convert any guanine in the sera to xanthine.
4. With some cell lines, the inhibition of cell growth in the presence of MPA is somewhat delayed. The addition of aminopterin speeds up the selective inhibition, perhaps because it reduces the amount of de novo synthesized IMP which could be potentially converted to XMP before MPA exerts its full effect.
5. An alternative to the selective media described above is Hams F12 media (GIBCO) supplemented with the above concetrations of MPA, aminopterin, and xanthine. This medium has recently been used successfully for CHO cells and we assume it should work for all lines.
6. In applying the selection to other lines, the optimal inhibitor and supplement concentrations should be determined. In HGPRT cells, the specificity of MPA toxicity can be evaluated by titration of the drug in the absence and presence of increasing concentrations of guanine (which should overcome the growth inhibition). Similarly, optimal concentrations of adenine or hypoxanthine for generation of AMP should be determined.

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