Drosophila Genomics Resource Center

ADDITIONS TO TISSUE CULTURE MEDIUM

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INTRODUCTION

Most of our cells are grown in M3+BPYE; its recipe can be found in a separate protocol. M3+BPYE is very similar to Schneider's medium, and we believe (but do not guarantee) that all of the lines will grow equally well in Schneider's medium. A few lines are grown in M3 or in CCM-3. The medium must be supplemented with fetal calf serum, and for many lines additional supplements are required. This protocol provides instructions on making and using these supplements. To view the requirements for any individual cell line, go to the web-page describing that line.

FETAL CALF SERUM

We buy the best available grade of fetal calf serum from any of a variety of vendors. Drosophila cells appear to be less finicky about minor variations in serum than mammalian cells are. We always test the lot of serum before buying it, but we have never encountered a lot of serum in which the cells did not do well. Bottles of serum are stored at –20 to –30°C prior to heat-treatment. Before using a bottle of serum, thaw it, bring it to room temperature, and then heat it in a 56°C water-bath for 30-60 min. After heat-treatment, store the serum at 4°C.

Note that the temperature of serum treatment is different from that described in an earlier protocol. Many of the more robust lines (e.g. Kc, S2, S3) grow equally well in serum that is treated at 65º, but many of the more delicate lines (e.g. disc and CNS lines) do not. We surmise that the higher temperature denatures a growth factor that is required by some of the lines.

INSULIN

All of our imaginal disc and CNS lines require addition of insulin to the medium. We use a commercial sterile insulin stock solution (Sigma I9278, supplied at 10 ug/ml); we have no evidence that this product has any particular advantages other than convenience. For the Miyake

laboratory disc and CNS lines, add insulin to a final concentration of 10 μg/ml (a 1:1000 dilution of the commercial stock solution); for the Milner lab lines, add insulin to a final concentration of 5 μg/ml (a 1:2000 dilution of the commercial stock solution).

FLY EXTRACT

Imaginal disc lines made in the Milner laboratory require the addition of a fly extract to the medium. The fly extract is not commercially available; detailed instructions on making the extract follow. This procedure is taken from a Milner lab protocol; for a more colorful description of the same procedure, see the Milner laboratory web-page (hyperlink http://biology.st-andrews.ac.uk/sites/flycell/flyextract.html).The fly extract can be stored indefinitely at -20°. Add fly extract to medium to a final concentratin of 2.5%; medium containing both insulin and fly extract is stable at 4° for at least a month (D. Cottam, personal communication).

1. Flies. Collect adult flies into a capped empty vessel (we use 50 ml plastic disposable centrifuge tubes. Put them in the freezer for at least 45 minutes. They flies can be used once they are quiescent, or stored in the freezer for future use. The genotype of the flies is unimportant; discards from genetic screens are a good source. You will need approximately 300 flies (about 0.35 g) for each 100 ml of final medium.
2. To make the extract, you will need the following items:
    - A glass homogenizer, chilled on ice
    - A refrigerated low-speed centrifuge
    - A 60º water bath.
3. Weigh the flies by transferring them to a tared tube. Transfer the flies plus 6.8 ml medium per gram of flies into a glass homogenizer, and homogenize (1 pass of the plunger is sufficient). Be careful to keep the homogenate cold, since tyrosinase is activated during homogenization and melanization can ruin the extract.
4. Spin the homogenate at 1500 × g at 4º C for 15 min. Decant the supernatant into fresh tubes. Discard the pellet.
5. Incubate the supernatant at 60° C for 5 min; this step will inactivate tyrosinase.
6. Spin at 1500 × g at 4º C for 90 min. Collect the supernatant; this is the fly extract
7. Filter-sterilize the extract through a 0.22 μm filter. Store 2.5 ml aliquots at -20º; each aliquot is appropriate for 100 ml of tissue culture medium.

SELECTION AGENTS USED FOR ESTABLISHMENT OF STABLY TRANSFORMED LINES

Transformed cell lines carrying a transgene are in general maintained in the presence of the same selection agent that was used to establish the transformed line; this ensures that the transgene is

not lost. As of this writing, our collection includes lines selected for resistance to methotrexate and for resistance to hygromycin

Methotrexate

We purchase methotrexate from Sigma (cat. no. A6770). Dissolve the solid reagent in 0.25 M Na_2CO_3 at a concentration of 4×10^{-4} M, sterilize by filtration through a 0.22 μ filter, and store aliquots at -20º. Add the freshly thawed stock solution to medium to give a final concentration of 2×10^{-7} M (1:2000 dilution of the stock). Medium containing methotrexate should be stored in the dark and used within 2 weeks; with time, the methotrexate loses potency as a selective agent, and it breaks down into something which is toxic even to methotrexate-resistant cells.

> **Warning:** Methotrexate is light-sensitive. The dry reagent, stock solutions and medium containing methotrexate must be protected from light.

Hygromycin

The following protocol is based on one from the Nusse laboratory. We purchase hygromycin from Sigma (cat. no. H3274). Determine the actual amount of hygromycin in the bottle from the % purity listed on the label, and dissolve the contents in water to give a hygromycin concentration of 125 mg/ml. Sterilize by filtration through a 0.22 μ filter, and store in aliquots at -20º. A thawed aliquot may be stored up to a month at 4º. Add the stock solution to medium to give a final concentration of 125 μg/ml, and use the hygromycin-containing medium within a few weeks.

> **Warning:** Hygromycin is light-sensitive. The dry reagent, stock solutions and medium containing methotrexate must be protected from light.