Tips and tricks of the trade

Melting the Way to your Cell Colonies

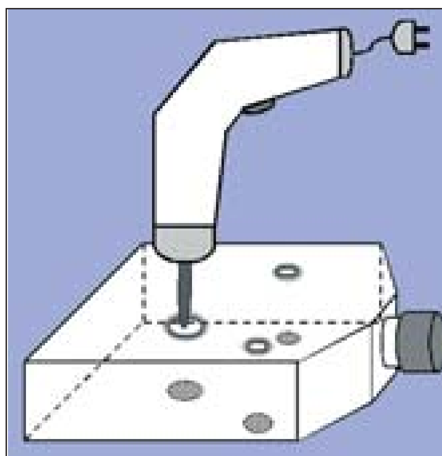
Culturing transformed mammalian cells in large plastic flasks rather then dishes hinders time consuming and frequent media exchanges. But how do you best extract single colonies through a bottle neck? Here's a brilliant but simple solution to that problem.

Lab Hint

Dear Editor,

genetic manipulation of mammalian cells usually requires extensive cell culture. This holds for random induction of mutants after genotoxic stress or for more targeted approaches that aim at a specified outcome like expression of transgenes or generations of knock-out cells. In any case, a small number of suitable clones showing the desired phenotype are selected out of an enormous number of inoculated cells. Those clones eventually need further analysis or another round of genetic manipulation. We raised hundreds of rodent or human clones where we integrated a mutated reporter gene and analysed the reconstitution of the reporter after DNA damage induction. With those or similar types of experiments the investigator faces three problems. (i) The experimental set-up is quite laborious. The entire procedure takes weeks, if not months, and most time is spent with medium exchange. (ii) The long term cell cultures are, hence, prone to infection, which always threatens the whole experiment. (iii) Harvesting the suitable clones may not be easy.

It can be achieved by mechanically scraping the colonies off the support.



A soldering iron melts away the plastic to gain access to individual cell colonies.

Melt the way to their cells with a soldering iron: **Jochen Dahm-Daphie** and **Leonie Schulte-Uentrop**

The simplest way to do this is with a sterile wooden toothpick, analogously to microbial handling. The disadvantage is that cells harvested in lumps may not attach to the next vessel. Today, trypsinization is thus more widely used. With both methods, the individual colonies must be accessible either to the toothpick or the tip of a micropipette. This is easy when using Petri dishes. However, medium exchange in up to hundreds of dishes is time-consuming and repeated lifting of the lid increases the probability of infections. Instead, we are using large cell culture flasks equipped with a 0.2 mm filter top which is of great help in both above respects.

We suggest, here, to employing a soldering iron to harvest the cells instead of trying to manipulate them through the bottle neck. We mark the colonies of interest with a felt tip pen on the bottom and melt 1cm holes in the top of the flasks, exactly above the colonies using a soldering iron (see figure). We use an inexpensive 75 W gun-shaped tool with a finger-trigger (ERSA Multisprint, ERSA, Wertheim, Germany), which needs only seconds to melt the plastic. We have never experienced plastic material dropping down into the culture flask. The 1cm hole is sufficient to harvest the individual colony by micro-trypsinization. The holes can be covered with "parafilm" in case other colonies need to continue growing.

In conclusion, using a soldering iron to gain access to single colonies in plastic flasks is a simple and safe method. It allows the harvest of individual colonies from large culture flasks. The use of large vessels primarily facilitates medium exchange and preserves sterility better during several weeks of cell culture, as opposed to the use of Petri dishes.

JOCHEN DAHM-DAPHI &
LEONIE SCHULTE-UENTROP
(*Lab of Radiobiology and Experimental Radiation Oncology, University Medical Center Hamburg-Eppendorf*)

Do you have any useful tips? Contact us at:

editors@lab-times.org

Cool: Lab Times polo shirt

Available in three colours:
blue, black and bordeaux

Pick your size: S, M, L, XL or XXL

19,95 Euro

(VAT and shipping included)

Please order via email: **subscription@lab-times.org**