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# A mammalian cell culture collection for biotechnology

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## INTRODUCTION

Biotechnology culture collections protect patent strains and manufacturing inoculum; standardize biological materials for research, development and manufacturing; and document culture receipt, distribution and transfer. The services provided by a culture collection can be key to the success of a biotechnology company.

## RMS CULTURE COLLECTION

Roche Molecular Systems (RMS) develops products based on the polymerase chain reaction (PCR) [6] in several areas, including infectious diseases, research reagents, environmental products and human genetics. The RMS Culture Collection (RMSCC) has been designed to support all RMS areas of business, providing traditional culture collection services of organism archiving, strain distribution, patent deposits, and species confirmation. In addition, we provide services unique to the needs of our organization.

Due to the highly sensitive nature of PCR, our research laboratories are kept free of large amounts of target DNA and organisms. Our researchers prefer not to grow organisms in their own laboratories, and ask RMSCC to grow microorganisms for delivery as pellets ready for DNA extraction, or as purified genomic DNA.

RMSCC also provides master stocks of control cell lines for RMS diagnostic kits to our manufacturing facility. Master stocks must be certified to be free of bacteria, fungi, mycoplasma and viruses before they are accepted into our manufacturing facility. These tests are done at a contract laboratory under GMP (good manufacturing practices) conditions to satisfy FDA requirements.

## HUMAN GENETICS SUPPORT

Our Human Genetics group studies a wide range of human genetic loci, including human leukocyte antigen (HLA) genes, located on human chromosome 6, which are highly polymorphic. PCR-based detection of genetic variation at the HLA loci is useful in tissue typing to match organs and bone marrow for transplantation [5] and to identify the origin of biological evidence samples for forensic analysis [1].

RMSCC support for our Human Genetics group includes receipt, growth, preservation and distribution of human cell cultures, the majority of which are B-cell derived lymphoblast lines. To create these strains, B cells are purified from blood samples, immortalized by transformation with the Epstein-Barr virus [3], and maintained in culture. These lines are useful in the study of human genetics, for they do not require a tumor to be present to establish a cell line, and allow for a culture to be established from any individual with a genotype of interest. These lines can be expanded and cryopreserved, providing a stable culture for long term studies and for distribution to other scientists.

## LYMPHOBLAST GROWTH

The lymphoblast lines that we receive show variability in growth rate and morphology. We have a standard set of cell culture protocols which must be varied for slow growing lines. Lymphoblast cells grow in suspension, generally in Iscove's modified Dulbecco's medium or RPMI 1640 (Roswell Park Memorial Institute Medium 1640) with 10% fetal bovine serum (FBS) at 37 °C in 5% $CO_2$. Cell lines are examined microscopically every day and conditions are varied if growth is not satisfactory. The FBS concentration may be increased to 15 or 20%, old media may be removed and replaced with fresh, clumps may be broken up with a pipette, and mycoplasma removal agent may be used on a culture if conditions warrant. Vented-cap tissue culture flasks are used to reduce the possibility of contamination, and to increase gas exchange.

## MYCOPLASMA QUARANTINE

A mycoplasma quarantine system is desirable to protect mycoplasma-free lines from contamination. Mycoplasma contamination of a cell line is not visually obvious, as the organisms lack cell walls and do not produce turbidity, but it can be detrimental to the culture, interfering with growth, nucleic acid synthesis, monoclonal antibody production and virus yield [2,4].

Incoming cells that are frozen, and are not certified mycoplasma free are put into a quarantine liquid nitrogen

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tank. When revived, or if received as a growing culture, the new cells are grown in a quarantine tissue culture incubator and manipulated in a quarantine biosafety hood. If the cells are certified mycoplasma free by a repository such as ATCC, or if we determine that the cells are mycoplasma free, we use a mycoplasma-free liquid nitrogen tank, incubator and hood.

A new cell line is grown up, frozen for a master stock, then one vial is revived for viability testing. The viability test flask is subcultured onto mycoplasma test media to detect mycoplasma growth.

Many options are available for mycoplasma testing, including direct tests and growth tests. The combination of both types of testing gives the greatest sensitivity. Growth of mycoplasma allows detection of low level contamination, but is slow, and some strains are not cultivatable. Direct methods can detect non-cultivatable strains and save time, but may require use of a tester cell line.

## PRESERVATION

Mammalian cell lines are stored in the vapor phase of liquid nitrogen to maximize long term viability [7]. As a lymphoblast line is grown for a master stock, the cultures are examined daily, and split or fed with fresh media as needed. Our standard master stock consists of nine vials with one mililiter each of $1 \times 10^7$ cells per ml. Generally enough cells for a master stock can be grown in 100–200 ml of media. An aliquot of the culture is kept growing as a back-up until the viability of the frozen culture is confirmed.

Cells are spun out of the media in a table top centrifuge at $915 \times g$ for 5–10 min, then resuspended in growth medium containing FBS plus 10% dimethyl sulfoxide. Aliquots of the suspension are transferred into sterile cryovials, and cooled in a slow freeze container in a −80 °C mechanical freezer for 4–18 h, then placed in a liquid nitrogen storage vessel.

For DNA extraction, cells from a 1-liter culture are grown, spun down, washed with phosphate-buffered saline, then spun down again into pellets of $1 \times 10^8$ cells per 50-ml conical centrifuge tube. The cells are stored at −80 °C until use.

## VIABILITY TESTING

Newly preserved cells are stored in the vapor phase of liquid nitrogen for one week, then their viability is tested. One vial is thawed by agitation in a 37 °C water bath until the ice melts, then the culture is diluted 1:20 into fresh growth medium.

To determine viable cell count, a 50-$\mu$l aliquot is removed and mixed 1:1 with 0.4% trypan blue. Viable cells exclude the dye, while dead cells stain blue. The stained sample is examined in a hemacytometer, and the number of viable cells counted. An 80% viability is considered normal, and stocks with significantly less viability may be regrown and refrozen if the strain is not easily replaced. The culture is grown for one week and examined daily to detect contamination or post-freeze abnormality. Master stocks for manufacturing are also checked by PCR to confirm the genotype before a stock is approved.

## CONCLUSION

Culture collections are key to the success of biotechnology companies. Protection of patent strains and manufacturing inoculum; standardization of biological materials for research, development and manufacturing; and documentation of organism transfers are essential functions provided by a culture collection in a biotechnology company. Certified, stable mammalian cell cultures will continue to be key in research advances and in manufacturing of biotechnology products in the future.

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