COLLEGE OF SCIENCE | THE UNIVERSITY OF UTAH

SCHOOL OF BIOLOGICAL SCIENCES

THE MARIO CAPECCHI APPARATUS

Aline W. Skaggs Biology Building

Click here to view video of Dr. Capecchi in action with the apparatus

Simple equipment used in early experiments by Mario Capecchi to inject foreign DNA fragments directly into nuclei of cultured mammalian cells.

Such direct injection greatly increased the efficiency with which information from that DNA was incorporated into the cells' genomes. Success of the technique led to further discoveries and developments in Capecchi's lab, eventually resulting in a general approach to 'gene targeting' in mice–gene-editing procedures providing the ability to create mice carrying virtually any desired modification of any gene. All of that work was carried out here, in the University's Dept. of Biology, now the School of Biological Sciences, and, based on it, Capecchi shared the 2007 Nobel Prize in Physiology or Medicine.

A Brief Outline of Mario Capecchi's Nobel Prize-winning Work

There were at least four key ideas underlying the development of a practical approach to 'gene targeting' in mammals for which Mario Capecchi shared the 1977 Nobel Prize for Physiology or Medicine. The following summary is intended to provide some background in what Mario set out to, and did, accomplish.

1. DNA-mediated homologous transformation, long known as a useful tool in bacterial genetics, could also work for mammalian cells. This is a process in which pieces of externally provided DNA get into a cell, and information from them (often parts of their physical material) find their way to, and replace, essentially matching (“homologous”) regions of a cell's own DNA (“genome”). The original genetic code of the cell is thus changed by the new code carried by the introduced DNA, 'transforming' the cell's genetic information. It was widely doubted that mammalian cells, with their much larger, more complex genomes, had the machinery required to convey the outside DNA to the matching (homologous) regions and to incorporate the new information there, at the “right place.” Capecchi thought otherwise, believing that mammalian cells probably do possess that ability.

2. Even if successful, the frequency of successful transformation in mammalian cells would likely be very low, but could be usefully increased by a direct, technical approach. A known bottleneck in DNA-mediated transformation in bacteria was getting the outside DNA through the cell membrane and into the cells. That bottleneck greatly reduces the frequency of successful transformation events in a cell populations exposed to outside DNA. Capecchi supposed that the bottleneck would be even worse for mammalian cells, which are “eukaryotes,” having genomes further protected within a membrane-bound nucleus, thus presenting two membrane barriers to be passed by the outside DNA. It was Capecchi's idea that injection of DNA directly into the nuclei of mammalian cells, via a glass micropipette used as an injection needle, could overcome both barriers, increasing the frequency of successful transformations.

3. “Mis-located” transformation events, with new information inserted at the 'wrong' (“non-homologous”) places in the genome could be screened out by a genetic approach. Capecchi employed a clever “positive-negative” selection idea to deal with the problem of non-homologous transformation events. He had found that unwanted, non-homologous “insertions’ of new information occurred at a much higher frequency than the desired, homologous ones, but that the non-homologous insertions included information from the ends of the introduced DNA pieces, while desired, homologous, 'replacement' events eliminated those ends, replacing the cell's original information at the homologous site with information from central parts of the matching (homologous) region of the introduced DNA. Capitalizing on that observation, he included extra information with the central part of the added DNA that allowed drug-based survival (“positive” selection) of cells that had been successfully transformed, whether by homologous or non-homologous events, eliminating the larger un-transformed population without any code from the added DNA. Other information, included at the ends of the introduced DNA pieces, could be used for drug-based killing (“negative” selection) of cells transformed by unwanted, non-homologous insertions, leaving essentially only those cells expressing the wanted, homologous replacements.

4. Homologously-transformed mammalian cells of the right type could be used to produce entire organisms–transformed adults, i.e., animals with chosen genetic modifications 'targeted' to the correct place in their genomes. In dramatic experiments in the 1960s Beatrice Mintz had demonstrated that mouse embryos fused at the early, 8-cell blastula stage could develop into “chimeras,” in which essentially every part of the new mouse contained contributions from both the original blastulae. Capecchi supposed that, if DNA-mediated homologous transformation could be carried out on cells of a “blastula type,” those modified cells might be introduced into a recipient blastula, eventually contributing to all parts of a new mouse, including the germ line, permitting new generations of 'transformed mice' to be generated by ordinary mating. Martin Evans developed techniques for recognizing and culturing the correct cell type–“embryonic stem cells”–capable of developing into any part of a final embryo. He taught the techniques to Mario and shared the 2007 Nobel Prize.

Successful demonstration of the correctness of the technically critical second idea was reported by Capecchi in a ground-breaking 1980 paper, “High Efficiency Transformation by Direct Microinjection of DNA into Cultured Mammalian Cells” (*Cell 22*: 479-488).

Experiments for that paper employed a very simple apparatus, borrowed from my lab, where it had been designed for injections of various substances into cultured chick-embryo neurons. That equipment is displayed here.

Once the necessary technical solution to the transformation-frequency problem was in hand, subsequent papers, in 1986-1988, co-authored by other members of Capecchi's lab–Kim Folger, Susan Mansour, Kirk Thomas, Geoff Wahl, and Eric Wong–reported dramatic, successful verifications of the other three ideas.

Since the homologous recombination can involve foreign DNA synthesized to include any desired change of the code present at the 'targeted' homologous site originally present in the cells, the combination of the four key ideas verified in Mario's lab provided geneticists with usable tools for creating experimental mice with essentially any desired alteration “targeted” to the correct location in their genomes, i.e., a usable approach to targeted gene editing of the mouse genome.

Early and very wide application of Capecchi's approach involved creation of mice carrying a chosen gene deliberately disabled (“knocked out”) by recombination with carefully garbled code in the homologous foreign DNA. These were the so-called “knockout mice,” created in many labs to investigate the functions of a broad variety of genes. However, the general power of the approach extends to creation of mice carrying arbitrary changes in chosen genes. These include correction of defective mouse genes suspected of involvement in disease and more subtle modifications of genes permitting higher resolution of their roles in development or disease. Many mice with such alterations have come to be called “knock-in” mice. Even further, in combination with other genetic tricks, the approach permits creation of mice with specific genes able to be turned on or off at chosen times during embryonic or post-natal development or in the adult, or only in chosen cell types.

Dr. Mario Capecchi’s approach thus opened an extremely broad spectrum of possibilities for exploration and understanding of mammalian genetics, promising new insights from mice ultimately likely to be of great value to developments in human medicine.

Larry Okun
October, 2018

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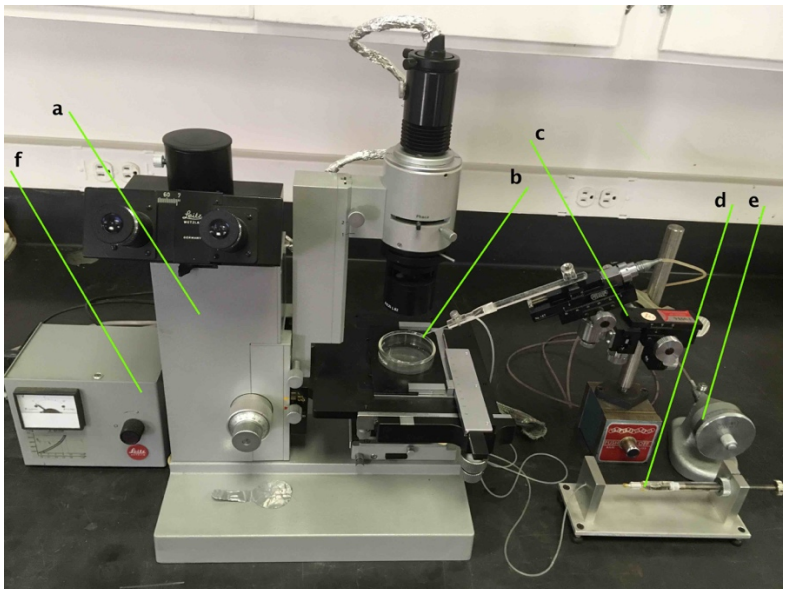
COMPONENTS OF THE EQUIPMENT



Equipment displayed here as used for Mario's early (1980) experiments testing direct injections of DNA into nuclei of cultured mouse cells:

The key components labeled in the accompanying photograph are the following:

1. A generally available 'inverted' microscope (**a**) permitting cells in a culture dish to be observed through the dish's bottom, leaving the area above the dish accessible to a micropipette (**b**) for injections. The micropipettes are prepared with a small lab device that heats thin glass capillary tubing about 1mm in diameter and pulls it to yield tips on the order of 0.5 - 1 micron in diameter, around 1/100th the thickness of a human hair.

2. A micromanipulator (**c**) holding the micropipette and custom modified from a commercial model to allow both movement of the micropipette from cell to cell in the horizontal (X-Y) plane of the culture dish, but above the cells, and a hydraulically controlled "axial" micro-drive positioned at a convenient angle, allowing the pipette to be carefully driven into the nucleus of a selected cell, and connected to the manual driving control (**e**) by a very flexible oil-filled "hydraulic" line.

3. A screw-driven injection syringe (**d**), mounted on a simple holder and connected to the micropipette by very flexible fluid-filled tubing.

4. A control for microscope-lamp brightness (**f**), connected to the lamp by a flexible cable.

5. A "stable table," on which the microscope and micromanipulator are mounted, isolating them, via the flexible control lines, from the manually operated axial-drive control and injection syringe. This prevents inadvertent injury to the cells by vibrations introduced during the pipette penetration and injection operations. Such stable tables typically involve a heavy steel plate (enabling use of manipulator mounts on magnetic bases), supported by air cushions (e.g., inner tubes!) or foam, on another heavy table. The heavy lower table resists vibrations introduced via the floor of the room, while, as noted, the steel plate isolates the critical equipment from operationally-introduced vibrations. (The heavy plate may be represented here by a thinner one resting directly on the display bottom without support by an air or foam cushion.)

Historical Note: Later, careful measures of transformation frequencies demonstrated that direct injections of DNA into cell nuclei indeed provided dramatic increases in the frequency of success - up to 10,000 - 100,000 fold increases in the frequency of total transformations per injected cell, and increases of 100-1000 fold in the fraction of homologous vs. total events, compared to another common approach ('DNA-calcium phosphate precipitation') then in use for introducing DNA into mammalian cells. However, even with later use of more easily operated and more precise (albeit more expensive) manipulators than the one I'd provided, the injection technique required hours of highly skilled, painstaking work to yield adequate numbers of transformed cells

for subsequent growth and selection. Despite the widespread attention it received, Mario was not wedded to the technique. He later employed an 'electroporation' approach, developed elsewhere, that got DNA into cells by use of brief, strong electrical shocks. While far less efficient on a per-cell basis, electroporation allowed treatment of around 10 million cells per experiment, greatly in excess of what could be achieved by even many hours of hand-controlled injection. Even if 1000-10,000 fold less efficient per cell, exposure of 10 million cells at a time yielded greatly increased numbers of usable transformed cells per practical experiment, with subsequent genetic selection during growth compensating for the lower efficiency.

The display is intended to show, once again, that major advances in science can be achieved by clever application of quite simple equipment.

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