Tagging of Laboratory Mice Using Electronic p-Chips

Introduction

PharmaSeq, Inc. has developed and exclusively offers a light-activated p-Chip (microtransponder) for a wide variety of applications to label, track, and authenticate items. This integrated system is used to identify laboratory mice and other small animals during research and clinical trials [1], and enables chain-of-custody tracking. This White Paper explains how the PharmaSeq p-Chip System works and discusses its reliability and cost effectiveness.

The p-Chip has also been used to study the social habits of ants [2, 6] and honey bees, in bioassays [3, 5] and to study cell growth [4].

The p-Chip System for Tagging

The key element of the PharmaSeq method is a tiny, silicon-based integrated circuit called a p-Chip. When activated by laser light, the p-Chip transmits a unique identification number via a reader to tracking software on a standard PC. The p-Chip is inert, durable, and easy to insert under the skin in the mouse's tail. Once implanted, the p-Chip can be repeatedly and rapidly read at a workstation where other experimental parameters are being recorded (**Figure 1**). This provides safe and confident confirmation of the animal's identity and experimental role throughout its lifetime.

The p-Chip is differentiated from other, traditional RFID (radio frequency identification) tags used for laboratory animals by being much smaller and far less expensive. This is why the PharmaSeq system offers a number of advantages over conventional tagging methods including ***no need for anesthetics*** during tagging and subsequent handling, less stress for the animal and greater system reliability. The system allows institutions to secure the value of a major experimental asset, laboratory animals, and to guarantee the integrity of experimental results.

The PharmaSeq system for small animal tagging consists of the following two key components:

- p-Chips preloaded in injectors, sterile, with optional injector handles
- a PharmaSeq Wand (ID reader)

The Wand is connected to a PC or a laptop running PharmaSeq's p-Chip Reader software.

PHARMASEQ

PharmaSeq, Inc.
11 Deer Park Drive, Suite 104
Monmouth Junction, NJ 08852
info@pharmaseq.com
www.pharmaseq.com

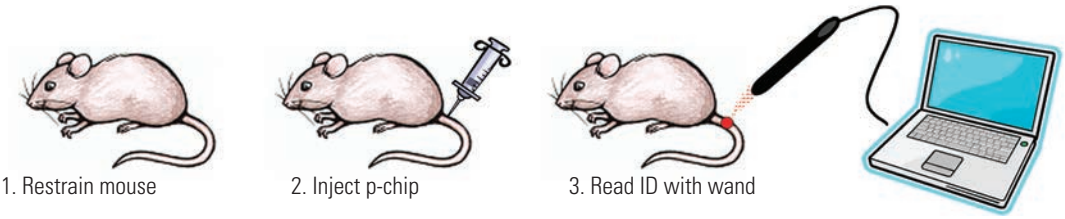

Figure 1.
Three key steps in PharmaSeq procedure to tag laboratory mice.

p-Chips

The p-Chip is an ultra-small electronic device (500 x 500 x 100 microns) that carries a unique identification number (ID) (**Figure 2**). An essential part of the p-Chip are photocells that, when illuminated by light from the Wand, provide power for the chip's electronic circuits. Each p-Chip contains an on-chip antenna that transmits the chip's ID through a variable magnetic field created close to the tag as a result of modulated current in the antenna loop. The current in the antenna is driven by onboard logic circuitry controlled by the contents of the p-Chip's electronic memory (ROM). The memory capacity allows for over one billion different IDs. The ID range is sufficient, therefore, to distinguish between any two p-Chips produced in the majority of applications.

Stability and chemical inertness are key features that enable the use of p-Chips in laboratory animals and other biochemical applications. The reliability of the RF transmitting function was tested by exposing p-Chips to various aqueous solutions and solvents. The results show that the p-Chips are very stable in most neutral aqueous solutions, and moderately stable in acidic or basic solutions (half-life of about 1 day). They are also very stable in organic solvents: after a 15 day exposure, 80-100% of the chips maintained their RF performance in all of the organic solvents tested. The stability and inertness are due, in part, to the p-Chip's silicon dioxide surface, which is deposited during the semiconductor manufacturing process as a final passivation layer. The surface of the device is glass-like, similar to other implantable microchips that come in glass capsules, an important property for biocompatibility. PharmaSeq, along with one of its partners, has performed long-term studies with implanted p-Chips in mice and observed no adverse histological effects [1].

p-Chips have excellent resilience to physical extremes: they can be heated up to 520 °C for 8 hours and still have full RF activity (sample size: 100 devices, all of which were fully active at the end of incubation). They have a lifetime of many years at room temperature or lower (-20 °C and -80 °C were tested). They withstand repeated freezing and thawing in liquid nitrogen. In addition, they are not affected by centrifugation (15 min at about 15,000 g), exposure to microwave radiation (1 hour exposure, standard 700 W microwave oven), or autoclaving (2,000 cycles). The stability of p-Chips in the presence of various environmental factors far exceeds the requirements for tagging laboratory animals. Further details on the PharmaSeq p-Chip system and its use can be found in references [1] through [4].

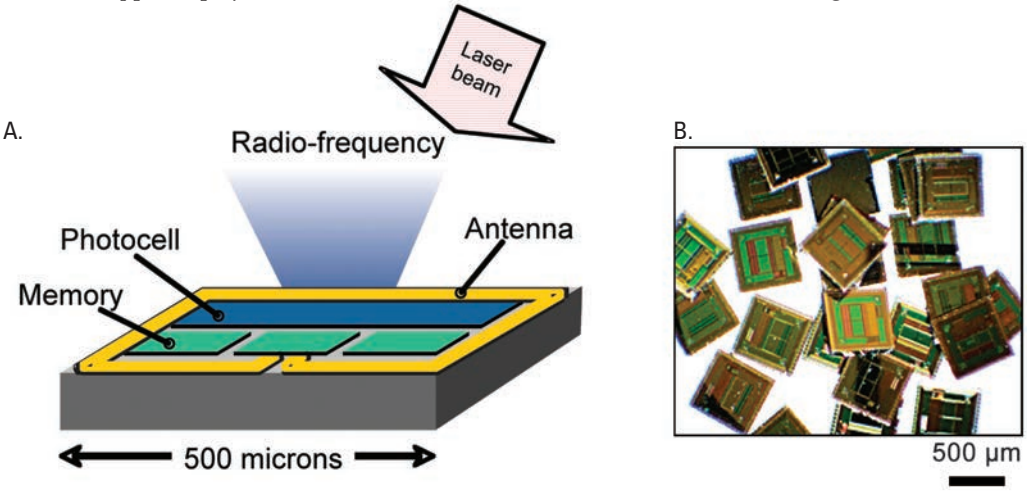

Figure 2.
p-Chips. *Panel A:* Simplified representation of a p-Chip. *Panel B:* The integrated circuit side of the p-Chip.

Injectors

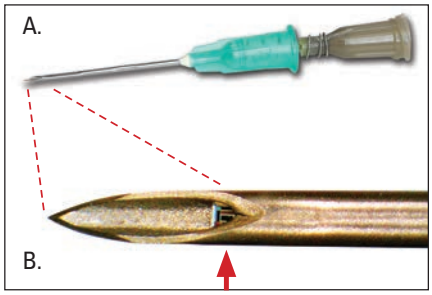

Figure 3.
The injector for p-Chips. *Panel A:* The flat-tip needle with a plunger. *Panel B:* A photograph of the flat-tip needle with the p-Chip inside. The p-Chip is marked with a red arrow.

The p-Chip is injected subcutaneously in the tail of a mouse using a specially designed injector that consists of a disposable hypodermic needle and plunger assembly, all in a sterilized package (**Figure 3**). The injector is preloaded with a single p-Chip. The reusable handle provided snaps onto the injector for the implantation. No other assembly or preparation is required. p-Chips are read immediately after insertion, and the ID of the subject animal is registered into tracking software. Mice can generally be tagged approximately 14 days after birth, or later.

PharmaSeq Wand (ID Reader)

The PharmaSeq Wand (**Figure 4**) is a small, light-weight device capable of reading the IDs of individual p-Chips. It is connected to a conventional PC or laptop using an included USB cable that provides power and communication to the device. For mouse tagging it is usually mounted on a fixed stand to allow a technician to have both hands free for working with the animal. It can also be handheld. The ID is read when the implanted p-Chip is placed in the beam of light emitted by the Wand. Partially transparent material, such as a layer of skin, will not normally prevent reading.

The Wand consists of the following components: a laser diode with programmable laser driver, an optical focusing module, an air coil pickup connected to an RF receiver, an FPGA, a USB 2.0 microcontroller and power regulators. The laser emits 5 to 60 mW of optical power at 660 nm. Key characteristics of the Wand are listed in the Specifications section below.

The Wand – when placed over a p-Chip – energizes the p-Chip, determines the ID from the RF signals using internal onboard firmware, and reports the decoded ID via p-Chip Reader software. The ID readout from a valid RF signal is practically instantaneous (milliseconds), thus minimum handling time is required for an animal. To read an ID, the Wand must be placed within the p-Chip range, typically less than 7 mm, and a sufficient amount of light has to reach the p-Chip.

Figure 4.
The PharmaSeq Wand (ID reader) compared to the size of a U.S. quarter.

Personal Computer and Software

PharmaSeq's p-Chip Reader software is a Windows-based program that is installed on the PC to which the Wand is attached. The software receives and processes the ID of each p-Chip as it is read from the animal. p-Chip Reader software is compatible with most Windows desktop applications such as Excel and Access, as well as enterprise software for animal colony and experimental management. An example of the software window is shown in **Figure 5.**

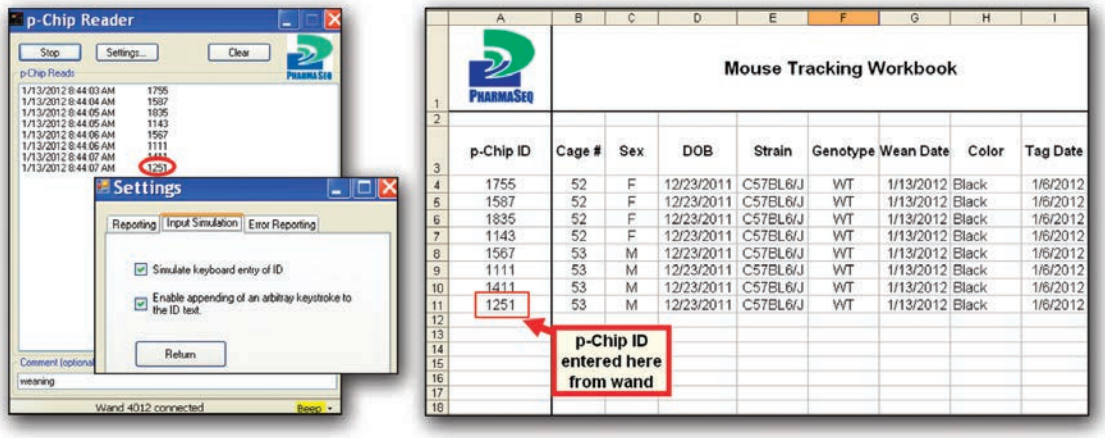

Figure 5.
Software. *Panel A*: Screen shot of p-Chip Reader software. *Panel B:* Demonstration of direct input into Microsoft Excel. p-Chip Reader places the ID into the cursor location of any Windows application that accepts keyboard input.

Typical Tagging Protocol

A typical workstation for reading p-Chips includes a stand with a Wand connected to a laptop computer. p-Chips are inserted subcutaneously using standard injection techniques. The p-Chip should be inserted into approximately the same location in each mouse to facilitate subsequent readings. We recommend inserting the p-Chip on top of the section of cartilage that is in the right side of the tail. The pigmentation is lighter in this location making it easier to see and read the p-Chip. This also prevents nicking the tail vein.

The mouse is placed in a tail accessible restrainer (**Figure 6**) and the tail wiped with 70% ethanol. A polycarbonate restrainer is recommended for its ability to withstand cleaning. While holding the tail taut, the line of cartilage along the right side of the tail is located by a slight counterclockwise rotation of the tail. The needle is inserted in a direction almost parallel to the tail, 1-2 cm from the base of the tail, until the entire bevel is under the skin. The plunger is then pushed to gently insert the p-Chip and the needle is removed and disposed. The p-Chip is then read using the Wand, and the animal "registered" in the tracking software. This entire process takes less than one minute and does not require anesthetics or tranquilizers. A video clip demonstrating the procedure is available at www.pharmaseq.com/RFID.

To read the ID of the p-Chip in the tail, the mouse is restrained by a two-handed scruff method or, in some cases, by merely holding the animal in a cupped hand. The spot in the tail where the p-Chip was inserted is scanned under the laser to read the ID. The laser intensity flickers as the p-Chip comes close to the read point. The computer sounds an audible beep when the ID is successfully read. This entire process typically takes less than fifteen seconds.

1. Restrain mouse **2.** Inject p-Chip **3.** Read ID with Wand **4.** Automatically register ID

Figure 6.
Procedure to implant the p-Chip in the mouse's tail.

Key Advantages

PharmaSeq system overcomes inadequacies of other tagging methods. Existing methods are either too expensive (other types of RFID microchips) or too error prone (all other methods, such as ear piercing or toe clipping). In addition, the number of codes that can be generated with existing methods (excluding microchips) is too small for unambiguous tracking of animals in colonies.

Small size. The p-Chip is extremely small, just one half millimeter on a side and very thin. This permits implantation subcutaneously within a mouse's tail where it can be easily located for identification, and is essentially imperceptible to the mouse itself. In addition, the injector used is of a much smaller gauge (21 gauge) than those of other microchips (18 to 16 gauge) causing much less pain and stress to the animal and eliminating the need for anesthetics.

Ease of use. Implanting is performed by a single person using a simple subcutaneous injection technique. The procedure can be rapidly taught to technicians with no prior experience.

Accuracy. For all animal studies, it is necessary to precisely identify individual animals in order to accurately assign genotypes, dose properly, and record clinical manifestations. This includes the growing need to more accurately report the chain of custody of laboratory animals, from their birth until the completion of all analyses performed on the animal.

Cost savings. Currently inadequate animal tagging methods lead to cumbersome, time consuming, and costly experimental designs. In addition, many errors are generated when mice

Figure 7.
PharmaSeq Basic Laboratory System with Computer for small animal tagging.

are read, leading to costly rework and troubleshooting. The PharmaSeq p-Chip System increases the reliability of tracking and reduces the labor cost associated with working with the animals.

The cost of the p-Chip itself is very low, an order of magnitude less than competing RFID tags, even when compared to some having inferior properties. This low cost is a direct result of the p-Chip having a monolithic design realized in a mass-scale, fully automated production process using state-of-the-art silicon foundries normally applied to the manufacture of computer chips.

Integrated System. The p-Chip is part of a fully integrated, proven system that has been developed by PharmaSeq for colony tracking and animal maintenance (**Figure 7**).

Intellectual Property

PharmaSeq's p-Chip is patented under US patent 7,098,394 and other related patents and patent applications both in the USA and world-wide. Appropriate licenses from PharmaSeq, Inc., are required for the use of p-Chips. A license to use the PharmaSeq System for research purposes is provided with the purchase of a Wand and injectors.

Frequently Asked Questions (FAQs)

Q. Will the p-Chip harm the animal?

A. No. p-Chips are made from silicon, a biologically inert material. Studies by PharmaSeq with one of its partners [1] show that there are no adverse histological effects from inserting p-Chips under the skin of a mouse's tail. Further, the injector design allows insertion of the device without anesthetics and with minimal handling.

Q. How is a p-Chip inserted into a mouse and how long does it take?

A. The device is implanted with a fine needle (21 gauge) under the skin of the tail. The entire process, including restraining, injecting, and "registering" the animal by reading the ID into a database takes less than a minute.

Q. Do p-Chips ever fall out after being injected?

A. Rarely. PharmaSeq's experience is that greater than 99.5% of properly injected p-Chips can be read reliably over the lifetime of the animal.

Q. Is it difficult to learn how to inject p-Chips into small animals?

A. No. A technician can become proficient at injecting and reading p-Chips in less than one hour. A video clip is available from PharmaSeq (www.pharmaseq.com/RFID) that illustrates the entire process.

Q. What is the youngest age a mouse can be chipped?

A. In our experience, p-Chips can be inserted into pups once their tails are at least 40 mm long. The minimum age at which a successful implantation of the p-Chip was observed by PharmaSeq is 11 days, with the average age being between 14 and 18 days.

Q. Can the injector or p-Chip be reused?

A. Both the injector and p-Chip are designed for low-cost, one-time tagging and should not be reused. Proper placement of a p-Chip in an injector requires special instrumentation, procedures, and sterility, and reuse should not be attempted.

Q. Is the number of available IDs large enough?

A. Yes. The number of total possible unique serial numbers is in billions in the current (Gen 2) PharmaSeq p-Chip. The number is large enough that no two animals will ever have the same ID.

Q. Can I read the p-Chip from far away?

A. No. p-Chips are activated by a low-power laser and have to be placed within seven millimeters of the wand. However, they can be activated and read through translucent surfaces such as a layer of skin.

Q. Can the ID be erased? Can I set my "own" IDs?

A. No. IDs are "written" into p-Chips during the fabrication process. The p-Chips are known as WORM ("write once, read many times") devices. The ID of a p-Chip is associated with the experimental number of an animal at the time of injection when the animal is indexed into a database. Providing users with the ability to program numbers could lead to duplicates and possible confusion between various groups in a facility. All colony management software programs allow the users to maintain a local "experimental code" and a unique, non duplicated serial number. One can think of PharmaSeq IDs as being analogous to the vehicle identification number (VIN) found on automobiles.

Q. How long does it take to read a p-Chip?

A. An adept animal handler can typically "scruff" an animal and read the p-Chip with the wand in less than fifteen seconds. Many users find that reading can also be accomplished after "cupping" the animal in their hand. As the p-Chip is brought within the range of the Wand, the laser light will flicker, aiding the proper placement of the p-Chip. The actual read time (signal processing by the Wand) is under 1 millisecond.

Q. What is the difference between traditional RFID chips and p-Chips?

A. Traditional RFID devices are larger (**Figure 8**), more expensive and communicate by radio frequency backscatter using multi-watt excitation transmitters. In contrast, the PharmaSeq p-Chip actively transmits its ID using energy from laser light and is extremely low-power (microwatts). Furthermore, it can be inserted into the tail without anesthesia or other discomfort to the animal, is biocompatible, and conforms to normal animal husbandry procedures. A key enabler of the p-Chip's cost effectiveness and reliability is the fact that there is no need to attach an external antenna.

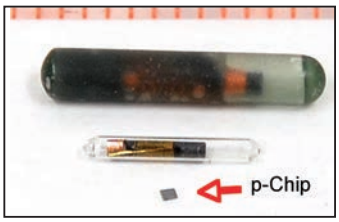

Figure 8.
Size and shape differences between the PharmaSeq p-Chip and competing microchips. A millimeter scale is shown on the top of the picture.

Q. Is the radio frequency signal from the p-Chip harmful?

A. No. The radio signal transmitted by p-Chips is so faint that approval from the US Federal Communications Commission or similar agencies is not required. Emissions fall well below FCC radiated emission limits defined in CFR 47, 15.209.

Q. Is the laser light emitted by the Wand harmful?

A. No. The Wand is registered with the Food and Drug Administration as a Class 3R laser device (similar to a standard laser pointer) and the light beam intensity is relatively low. Furthermore, the laser is in a low-power state when the Wand is idle, and the light intensity increases briefly (for a few milliseconds at a time) only when the Wand is actually reading a p-Chip. Finally, the Wand is usually mounted on a stand pointing downward and away from the technician further reducing exposure. However, as with all laser devices, it is recommended that the laser not be pointed directly into the eyes.

Q. I am already using a database program, do I have to change?

A. No. The p-Chip Reader software is compatible with, and easy to interface with, most Windows based programs. Keyboard simulation functionality is built-in. For custom applications, a Software Developer's Kit (SDK) can be obtained from PharmaSeq. In addition, PharmaSeq offers custom system installation and training. Call us for details.

References

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Contact Information

For further information regarding sales and service please contact us at:

PharmaSeq, Inc.
11 Deer Park Drive, Suite 104
Monmouth Junction, NJ 08852
info@pharmaseq.com
www.pharmaseq.com
Tel. (732) 355-0100 ext 10
Fax (732) 355-0102

Specifications

p-Chips (Gen 2)

Description:	Integrated circuit carrying a serial number (ID)
Dimensions:	500 x 500 x 100 microns (nominal, size of integrated circuit) 600 x 600 x 100 microns (actual, size of p-Chip) Weight: 85 micrograms
Construction:	Monolithic CMOS IC Manufactured: Silicon foundry Power: On-board photodiodes activated by laser light Wavelength range: 500 to 700 nm
Output:	Analog RF signal at 1 MHz Cycle time: 512 microseconds Serialization: 30 bits (> 10 IDs) Memory: write once, read many times (WORM) Read distance: 2-5 mm (typical), 7 mm (maximum) Read volume: ~7 mm^3
Physical characteristics:	Inert to autoclaving (high temperature and pressure), multiple cycles Range of operation: -186 °C to 600 °C
Safety information:	FCC certification not required, emissions lower than those described in 47CFR15.209

PharmaSeq Wand (Series 4300 or later)

Description:	Electronic device specifically designed to read PharmaSeq p-Chips
Configuration:	Designed to be incorporated into portable tagging workstation
Construction:	Hand-held device connected via USB to computer (2 meters maximum) Wand contains laser and RF pickup coil
Laser characteristics:	Laser diode, wavelength of 660 nm 60 mW average nominal read mode power
Laser device class:	3R (protective eyewear not required) (FDA registered)
Operating temperature range:	10 °C to 35 °C
Power:	USB 2.0 (universal serial bus) 5 V, <500 mA
Computer:	Personal computer with Windows XP or later 32 or 64 bit operating system and .NET 4.0. (note: not compatible with Apple operating systems)
Read time:	Less than 1 millisecond (nominal); less than 15 seconds (including mouse handling)
Data presentation:	p-Chip serial number transferred to Microsoft Excel/Access, or as keyboard simulation to other database programs
Dimensions:	175 mm x 28 mm x 28 mm
Weight:	130 g

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PHARMASEQ

PharmaSeq, Inc.
11 Deer Park Drive, Suite 104
Monmouth Junction, NJ 08852
info@pharmaseq.com
www.pharmaseq.com