

Tagging of Laboratory Mice Using Electronic p-Chips

PharmaSeq, Inc. has developed and implemented the world's first light-activated microtransponder (p-Chip) that can be used in a wide variety of applications to label, track, and authenticate items. The p-Chip is part of an integrated system that can identify laboratory mice and other small animals during research and pre-clinical trials. The system allows researchers to track experimental subjects in a more cost-effective and reliable manner than previously possible. The p-Chip also can be used to tag animals shortly after birth creating the opportunity for chain-of-custody tracking. This paper explains how the PharmaSeq p-Chip system works and how it can be used to track mice and other small laboratory animals.

The p-Chip has also been used to study the social habits of ants [1], to diagnose a genetic disease [2] and to study cell growth [3].

The p-Chip System for Tagging

The PharmaSeq method is based on tiny, silicon-based integrated circuits called p-Chips that, when activated by laser light, transmit their serial 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**), providing safe and confident confirmation of the animal's identity and its experimental role throughout its lifetime. The p-Chip is differentiated from other RFID tags used for laboratory animals by being much smaller and far less expensive. The PharmaSeq system offers a number of advantages over conventional tagging methods including *no need for anesthetics* upon tagging and subsequent handling, less stress for the animal, and greater system reliability. These products allow institutions to secure the value of a major experimental asset, laboratory animals, and to guarantee the integrity of experimental results.

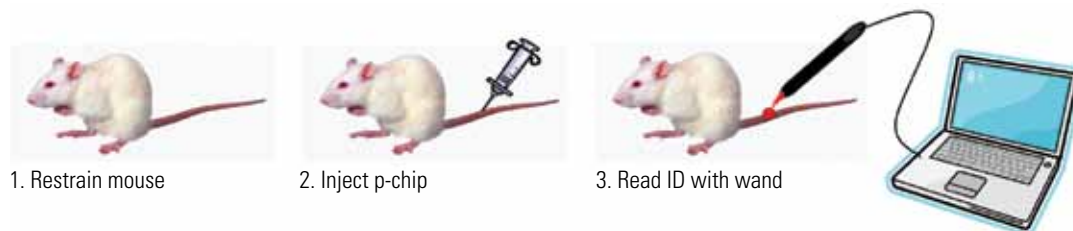
The PharmaSeq system for small animal tagging consists of the following components:

- p-Chips
- injectors
- an ID reader (wand) for p-Chips with a personal computer and application-specific software, "MouseTrack"



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Figure 1.
PharmaSeq system for tagging laboratory mice.



p-Chips

The heart of a p-Chip-based system is an ultra-small electronic device having a unique ID. It is a monolithic integrated circuit built on a tiny silicon chip, the nominal dimensions of which are 500 x 500 x 100 microns (see **Figure 2**). An essential part of the p-Chip are photocells that, when illuminated by light, provide power for its electronic circuits. Each p-Chip contains an on-chip antenna that transmits its unique ID number through a variable magnetic field created close to the tag as a result of modulated current. A key aspect of p-Chip's cost effectiveness and reliability is due to the fact that there is no need to attach an external antenna. The current in the antenna is controlled by logic circuitry and the contents of the p-Chip's electronic memory (ROM). The memory capacity allows for over 10^{15} unique codes, i.e. more than one trillion different IDs. The potential ID range is sufficient, therefore, to distinguish any two p-Chips produced.

Stability of p-Chips is a key feature for their use in laboratory animals and other biochemical applications. The reliability of the RF transmitting functions 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, and after a 15 day exposure, 80-100% of the chips maintained their RF performance in all of the organic solvents tested. The stability is due, in part, to the p-Chip's silicon dioxide surface, which is deposited during the electronic 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, which is important 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.

p-Chips have excellent stability 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). 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 (15 cycles). The stability of p-Chips to various environmental factors far exceeds the requirements for tagging laboratory animals. Further details on the PharmaSeq p-Chip system and their use can be found in references 1 and 2.

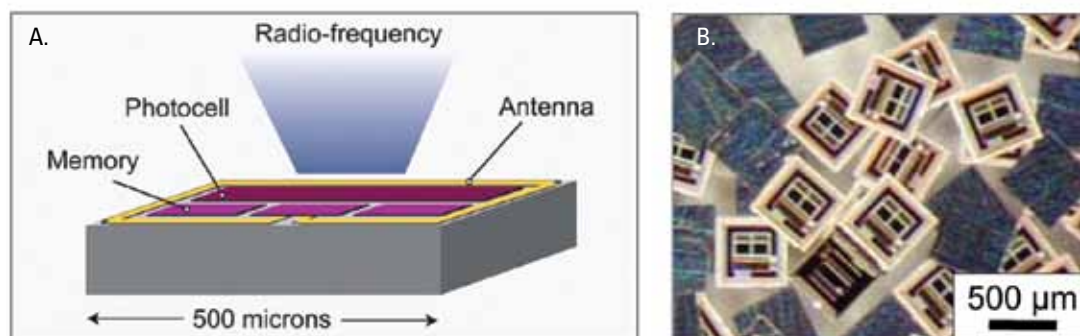


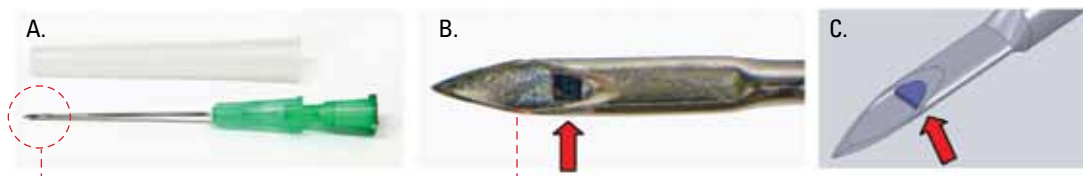
Figure 2.
p-Chips. *Panel A:* Simplified view of a p-Chip. *Panel B:* The integrated circuit side and the back of the p-Chip (dark gray squares) are shown.

Injectors

p-Chips are injected subcutaneously in the tail of small laboratory animals using a specially designed injector (**Figure 3**). Each p-Chip is pre-loaded in a disposable, sterile needle. No assembly or programming is required. All one does is remove the cap, implant the p-Chip, and dispose of the injector. p-Chips can be read immediately after insertion and the subject animal ID registered into the MouseTrack software.

Figure 3.

The injector for p-Chips. *Panel A:* The flat-tip needle with a plunger and a plastic cover. *Panel B:* A photograph of the flat-tip needle with the p-chip inside. *Panel C:* A drawing of the flat-tip needle. The p-Chips are marked with red arrows.



ID Reader (Wand)

The PharmaSeq ID reader (**Figure 4**) is a small, light-weight device capable of reading the ID of individual p-Chips. It is connected to a conventional PC using a USB cord that provides power and communication to the device. The ID reader can be hand-held, although 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. The ID is read when the implantable chip is placed in line with a beam of light emitted by the reader which contains a laser emitting 5 to 90 mW average of optical power in the range of 658 nm. The device is integrated with the following components: a USB 2.0 microcontroller, an FPGA, power regulators, a laser diode and programmable current laser driver, an optical focusing module and an air coil RF receiver. Properties of the ID reader including the coil dimension, enclosure tolerances, and specifications of the optical path, have been carefully optimized (see Specifications). The reader – when placed over a p-Chip – activates it, determines the ID from the RF signals, and reports it via software developed by PharmaSeq. The read time is practically instantaneous resulting in minimum handling time for an animal.

There are two requirements for successful operation of the reader: first, the reader must be placed within range of the p-Chip, typically within 4 mm, and secondly, within “line-of-sight” of the p-Chip. The p-Chip, itself, should not be covered with material that significantly absorbs light, although partially transparent material, such as layers of skin, will not interfere with normal operation.

Figure 4.

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



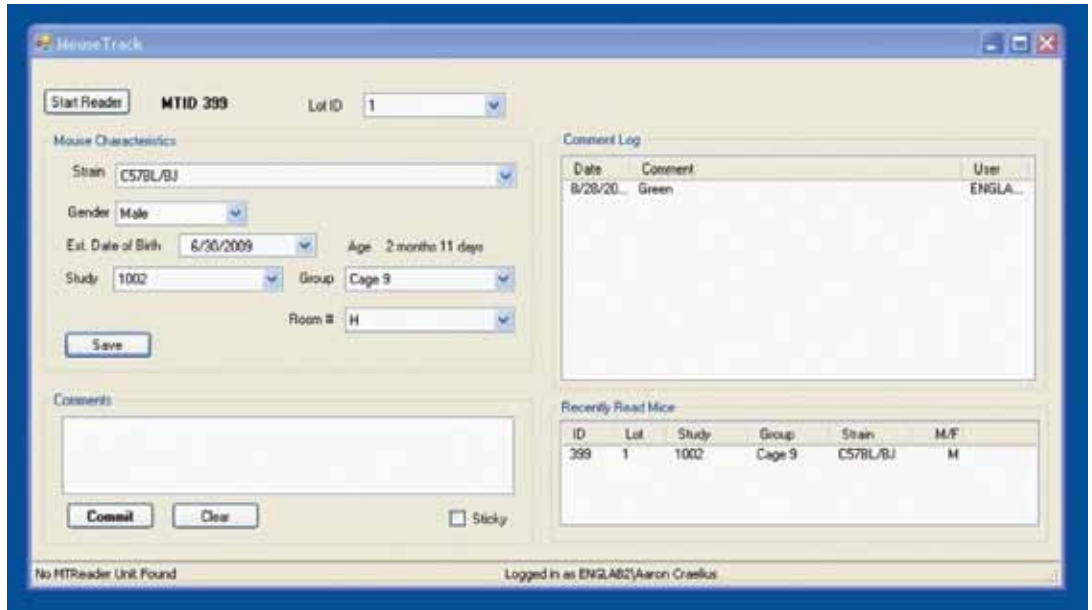
Personal Computer and Software

PharmaSeq’s MouseTrack software is a Windows-based program that is loaded onto the PC to which the ID reader is attached. It receives the serial number of each p-Chip as it is read from an animal. MouseTrack provides a database for tracking mice as well as a log of important activities associated with experiments (**Figure 5**). In order to accommodate a wide variety of possible procedures, a number of customization options are built into the software. In addition, the MouseTrack software can be extended to interface with existing databases as well as popular desktop programs such as MS Excel and Access. In practice, the ID reader and software are integrated into an animal workstation to provide for efficient operations. PharmaSeq provides an option to include an optimally configured laptop PC with the system.



Figure 5.

Screen shot of MouseTrack software. By editing the mouse characteristics fields, information can be associated with new mice or updated for existing mice. The fields to the left provide stored information associated with the p-Chip, such as Strain, Gender, Study, and Group for each mouse. A history of previous log messages will be shown on the right. Each time a mouse is read, a time-stamped log message can be entered in the field labeled Comment.



Typical Tagging Protocol



Figure 6.

The PharmaSeq set-up to tag mice. A stand with the wand is shown during the process of reading the p-Chip ID.

A typical set-up for reading p-Chips is shown in **Figure 6**. The workstation includes a stand with a wand connected to a laptop computer. p-Chips are inserted subcutaneously (**Figure 7**) using standard injection techniques. The p-Chip should be inserted into 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 on the right side of the tail. The pigmentation is lighter in this location making it easier to see and read the p-Chip afterwards. This also prevents nicking the tail vein. The mouse is placed in a plexiglass restrainer (**Figure 7A**) and the tail wiped with 70% ethanol. While holding the tail taut, the line of cartilage along the right side of the tail is then localized by a slight counterclockwise rotation of the tail. The needle is inserted at an angle almost parallel to the tail, 1-2 cm from the base until the entire bevel is under the skin. The plunger is then pushed to gently insert the p-Chip and the needle removed and disposed. The p-Chip is then read using the ID reader, and the animal “registered” in the tracking software. This entire process takes less than one minute and does not require anesthetics or tranquilizers.

To read the ID of the microtransponder in the tail, the mouse is restrained by a two-handed scruff method. This method allows the user to hold the tail straight for easier reading. While holding the mouse with the left hand, the tail is gripped with the right hand and held out straight. The spot in the tail where the p-Chip was inserted is scanned under the laser, almost touching the wand, to read the ID (**Figure 7B**). The laser intensity flickers as the p-Chip comes close to the read point, and emits an audible beep when the ID is successfully read. This entire process typically takes less than fifteen seconds.

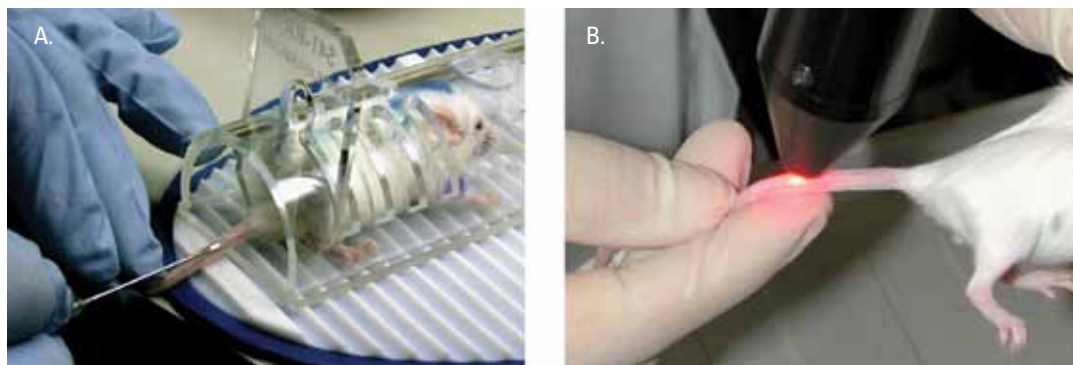


Figure 7.
Panel A: Inserting the p-Chip into the mouse's tail. *Panel B:* Reading the p-Chip ID.

Key Advantages

PharmaSeq system overcomes inadequacies of other tagging methods. Existing methods are either too expensive (other types of microchips) or too error prone (all other methods). The number of codes that can be generated with existing methods (excluding microchips) is too small for unambiguous tracking of animal colonies.

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

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

Accuracy. For all clinical trials, it is necessary to precisely identify clinical subjects in order to perform drug accountability, reconciliation, returns, and destruction (ARRD) activities. 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 IDs are read, leading to costly rework and troubleshooting. The PharmaSeq p-Chip system will increase the reliability of animal tracking and reduce the cost of 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 with inferior properties. The low cost is a result of mass-scale, fully automated production in state-of-the-art silicon foundries that are used to manufacture computer chips.

Intellectual Property

PharmaSeq's p-Chip is patented under US patent 7,098,394 and other related patents both in the USA and world-wide. Appropriate licenses from PharmaSeq, Inc. are required for the fabrication and use of p-Chips.



Frequently Asked Questions (FAQs)

Q. Will the p-Chip harm the animal?

A. No. p-Chips are made from silicon, an inert material. Studies by PharmaSeq with one of its partners 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 under the skin. The entire process, including restraining, injecting, and "registering" the animal by reading the serial number into the database takes less than a minute.

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 that illustrates the entire process.

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. The number of total unique serial numbers is large enough that no two animals will ever need to have the same serial number.

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 a few millimeters of the wand. However, they can be activated through a layer of skin and can be read in mice with black or agouti pigmentation as easily as they are read in albino mice.

Q. Can the ID be erased?

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.

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. As the p-Chip is brought within range of the ID reader, the laser light will flicker, aiding the proper placement of the p-Chip. The actual read time (signal processing by the wand and software) is less than 5 milliseconds.

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

A. Traditional RFID devices are larger, more expensive and are activated by radio frequency. In contrast, the PharmaSeq p-Chip is activated by laser light and is extremely low-power (microwatts). Furthermore, it can be inserted without anesthesia or other discomfort to the animal, is biocompatible, and conforms to normal animal husbandry procedures.

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

A. No. The strength of the radio signal transmitted by p-Chips is so faint that it does not require approval from the US Federal Communications Commission or similar agencies.



Q. Is the laser on the ID harmful?

A. No. The wand is a Class 3R laser device 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) only when it is actually reading a p-Chip. Moreover, the wand is usually mounted on a stand pointing downward and away from the technician. However, as with all laser devices, it is recommended that the laser not be pointed directly into the eyes.

References

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Specifications

p-Chips

Description:	Integrated circuit carrying its own 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: in silicon foundry <i>Power:</i> on-board photodiodes activated by laser light <i>Wavelength range:</i> 500 to 700 nm
Output:	Analog RF signal at 1 mHz <i>Cycle time:</i> 300 microseconds <i>Serialization:</i> current: 10 bits (1,024 numbers) future: 40 bits (~10 ¹² numbers) <i>Memory:</i> write once, read many times (WORM) <i>Read distance:</i> 1-3 mm (typical), 4 mm (maximum) <i>Nominal read volume:</i> ~4 mm ³
Physical characteristics:	Inert to autoclaving (high temperature and pressure)
Safety information:	FCC certification not required, emissions <47CFR15.209

ID Reader

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 Wand contains laser, decode electronics and RF pickup coil
Laser characteristics:	Laser diode, wavelength of 658 nm 90 mW average power in read mode, 5 mW in standby
Laser device class:	3R (protective eyewear not required)
Power:	USB (universal serial bus) 5 V, <500 mA
Computer:	Personal Computer with Microsoft XP or Vista operating system (note: not compatible with Apple operating systems)
Read time:	Less than 5 milisecond (nominal) Less than 15 seconds (including mouse handling)
Data presentation:	p-Chip serial number transferred to Microsoft Excel or database
Dimensions:	175 mm x 28 mm x 28 mm
Weight:	130 g

MouseTrack Software

System requirements:	Microsoft Windows 2000, XP, or Vista with the .NET framework 2.0.
Database:	MS SQL Compact Edition-based database.
Built-in fields:	p-Chip ID, Study, Group, Mouse Strain, Gender, Date of Birth, and Room Number.
General features:	Time-stamped log messages can be stored for each p-Chip reading. Input from PharmaSeq ID reader. Data exportable to various formats.



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