# Tech-Trends

Volume 1, Series 11

# *In Vivo* Electroporation of Spermatogonia

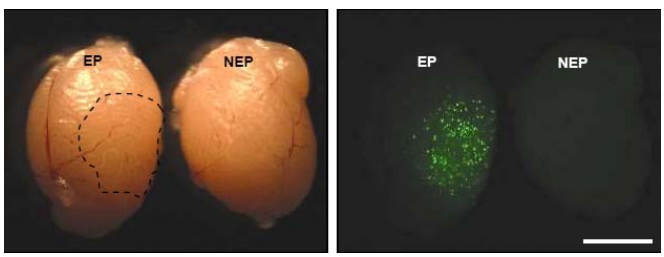
## Transgenesis via permanent integration of genes in repopulating spermatogonial cells

**Abstract**:
Presently production of transgenic mice is a labor intensive process. Requiring skilled personnel, costly infrastructure and the expense of numerous animals. The collection of many zygotes from mice results in the necessary termination of the animals. This technique describes a simple reproducible nonterminal method for transfecting genes in undifferentiated spermatogonia through *in vivo* electroporation of the testis: about 94% of male mice electroporated with different transgenes successfully sired transgenic pups. Such electroporated males provide a valuable resource for continuous production of transgenic founders for more than a year. The technique solves many of the issues regarding cost, simplicity and avoids the termination of animals.

**Methods:**
Using FVB mice, various amount of linearized DNA were injected into the interstitium of one testis in varying areas. Using tweezertrode electrodes the testis was electroporated. The fore founder electroporated males were naturally mated with females and the offspring were screened for the presence of the transgene by PCR. The PCR results for all transgenic lines confirmed genomic integration of transgene.

**Results:**
Seventeen mice were electroporated using four different constructs and all except one produced transgenic pups, indicating a success rate of >94% for our method. The first transgenic progeny can be generated within 60 d of electroporation as compared to 82-177 d after in vivo viral transfection. Notably one of the fore founders produced transgenic offspring as long as 384 d after electroporation, indicating that the transgene was integrated in stem and/or transit cells amplifying undifferentiated spermatogonia at the time of electroporation.

**Conclusions:**
This electroporation-mediated technique for transfection of undifferentiated repopulating spermatogonial cells *in vivo* that resulted in the integration and long term maintenance of the transgene in the germ cell and its transmission during mating. This method contributes to the prolonged ability, in some cases more than a year, of electroporated founders to sire transgenic progeny by natural mating. This methods requires neither assisted reproductive techniques nor sophisticated laboratory set-up and highly trained personnel, results in no termination of animals and is time saving.




Figures: Testis was observed 50 days after interstitial injection of a linearized construct carrying EGFP transgene followed by electroporation. Left panel shows phase contrast image and the right panel shows the image under UV. Dotted line in phase contrast depicts the area that expressed GFP under UV (FITC filter). EP=electroporated testis, NEP=non-electroporated contra-lateral testis. Non-specific fluorescence (red) was not detected under TRITC filter. Scale bar, 2.5 mm.

**ECM® 2001* In Vivo Electroporation Protocol**

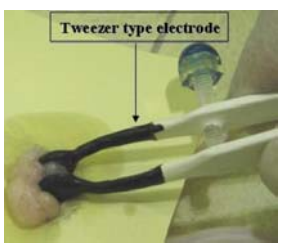
**Tissue preparation:**

FVB male mice @30 d anesthetized and their testes prepared for injection. Solution of DNA containing Trypan blue (0.04%) , (for monitoring the accuracy of the introduction of the DNA), injected into the inter tubular spaces using glass micropipette.

**Electroporation Settings:**

| | |
|---|---|
| Voltage: | DC Only 40V |
| Pulse length: | 50 msec |
| Number of pulses: | 8 applied in alternating directions |
| Interval: | 1s |
| Electrode | 5mm Tweezertrode |

Reference;
Dhup,S.,Majumdar, S., Division of Embryo Biotechnology, National Institute of Immunology, New Dehli, India. 2008 Nature Methods, Transgenesis via permanent integration of genes in repopulating spermatogonial cells *in vivo*


BTX® HARVARD APPARATUS
Molecular Delivery Systems

84 October Hill Rd. Holliston, MA 01746 Toll Free Ph: 800-272-2775 or 508-893-8999
Email: btxinfo@harvardapparatus.com Web: www.BTXonline.com