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Generation of transgenic rats using piggyBac transposase mRNA and piezo-assisted microinjection

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We use this protocol and it's working

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Abstract

We present a protocol for generating transgenic rats using the *piggyBac* system. It covers preparation of mammalian codon-optimized piggyBac transposase (mPBase) mRNA and donor plasmid DNA, superovulation and zygote collection, piezo-assisted pronuclear microinjection, and embryo transfer into pseudopregnant females, enabling efficient production of transgenic animals.

Materials

Animals

- Adult females (8–15 weeks old)
- Adult males (over 10 weeks old)

Plasmids and mRNA

- Donor plasmid: transgene between ITRs
- mPBase expression plasmid: pcDNA3.1-EGFP-poly(A)83 containing mPBase cDNA

Reagents

- Xho I
- MEGAscript T7 Transcription Kit (Thermo Fisher Scientific; AM1333)
- Cap Analog (m7G(5′)ppp(5′)G) (Thermo Fisher Scientific; AM8048)
- RNeasy Mini Kit (QIAGEN; 74104)
- Saline
- [des-Gly10, D-Ala6]-LH-RH ethylamide acetate salt hydrate (Sigma-Aldrich)
- Pregnant mare serum gonadotropin (PMSG; Aska Animal Health)
- Anti-inhibin serum (Central Research)
- Human chorionic gonadotropin (hCG; Aska Pharmaceutical)
- HTF medium (ARC Resource)
- EmbryoMax Advanced KSOM Embryo medium (Advanced KSOM) (Merck)
- Rat KSOM (ARK Resource)
- Three types of mixed anesthetic agents ((0.375 mg/kg medetomidine, 2.0 mg/kg midazolam, and 2.5 mg/kg butorphanol)
- Antisedan (Zenoaq)

Equipment

- Inverted microscope (IX73, Olympus)
- PMM4 Piezo impact drive unit (Prime Tech)
- Micromanipulator (Narishige)
- Incubator
- Hot plate

Safety warnings

This protocol involves the use of animals and requires prior approval from the user's Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

Ethics statement

The protocol was approved by the Animal Experimentation Committee and Recombinant DNA Experiment Safety Committee of Kyoto University (Med Kyo 22625, 23016, and 240401).

Preparation of donor plasmid DNA and mPBase mRNA

1 Prepare donor plasmid DNA at 10 ng/µL in nuclease-free water.

2 Linearize pcDNA3.1-EGFP-poly(A)83 containing mPBase cDNA using Xho I by incubating at 37°C overnight.

3 Purify the linearized plasmid DNA by phenol-chloroform-ethanol precipitation.

4 Synthesize mRNA using MEGAscript T7 Transcription Kit and Cap Analog according to manufacturer's instructions.

5 Purify the synthesied mRNA using according to manufacturer's instructions.

6 Prepare mPBase mRNA at 100 ng/µL in nuclease-free water.

7 Mix equal volumes of donor plasmid DNA (final concentration 5 ng/µL) and mPBase mRNA (final concentration 50 ng/µL).

Superovulation and collection of rat zygotes

8 Superovulation is performed following the method described in [1], with slight modifications.

9 Inject adult females intraperitoneally with 0.04 mg [des-Gly10, D-Ala6]-LH-RH in 200 µL saline (day 0, approximately 11:00 AM).

10 48–50 h later (day 2, 11:00 AM–1:00 PM), inject PMSG (150 IU/kg) and anti-inhibin serum (100 µL).

11 48 h after PMSG/anti-inhibin (day 4, 11:00 AM to 1:00 PM), inject hCG (75 IU/kg).

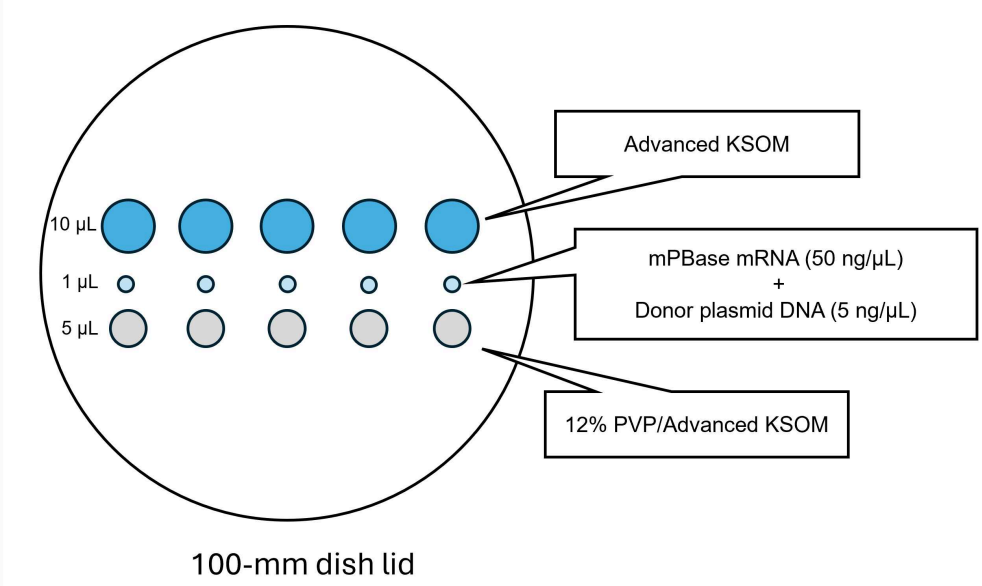
12 Mate hCG-injected females with males the same day.

13 The next day, collect pronuclear-stage embryos from mated females 20–26 h after mating (day 5, 9:00 AM – 1:00 PM).

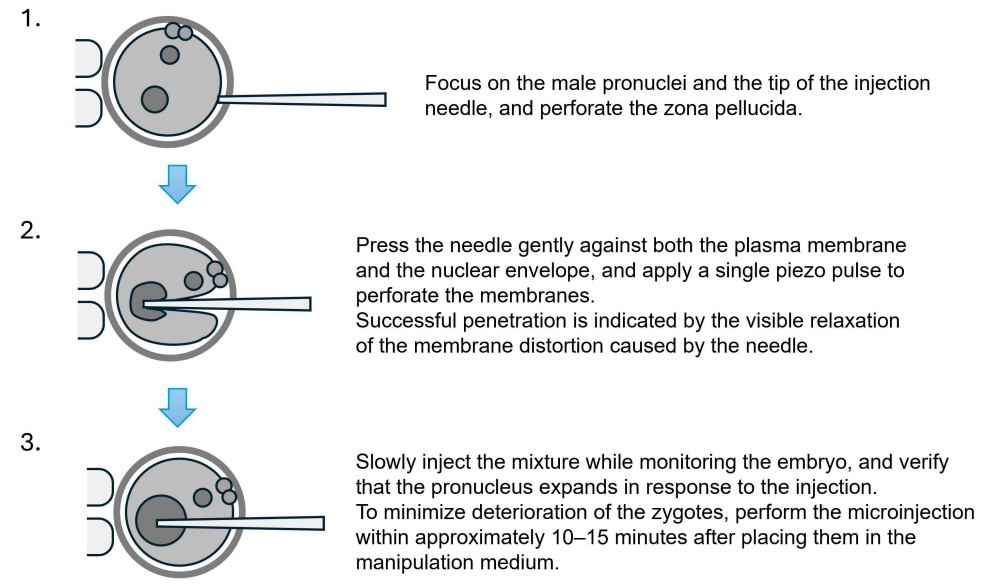
14 Culture zygotes in HTF medium at 37°C under 5% CO_2 prior to microinjection.

Microinjection of mPBase mRNA and donor plasmid into pronuclei of zygotes

15 Prepare drops of Advanced KSOM, the mPBase mRNA/donor plasmid DNA mixture, and 12% PVP in Advanced KSOM on a 100-mm dish lid and cover the drops with paraffin oil.



16 Using an inverted microscope equipped with a PMM4 Piezo impact drive unit and a micromanipulator, microinject the mixture into the male pronuclei of zygotes in Advanced KSOM. Injections can be started approximately between 3:00 PM and 6:00 PM.



17 After injection, place zygotes at room temperature for 5 min.

18 Culture the injected zygotes in Rat KSOM (approximately 50 embryos/50 µL) at 37°C under 5% CO_2 until 2-cell stage.

Embryo transfer

19 Embryo transfer is performed following the method described in [2].

20 Transfer 10–15 embryos per oviduct into pseudopregnant recipient females anesthetized with a mixture of three agents (0.5 mL/100 g body weight).

21 After transfer, administer 3% Antisedan at same volume as anesthetic.

22 Keep recipient female on 37°C hot plate for 3 h.

23 After 22 days of the embryo transfer, pups are delivered naturally or by caesarean section and fostered to surrogate mothers for care.

Protocol references

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