SJ-500 PORTABLE

GENE GUN

DRAWELL

Artist of Science

User Manual

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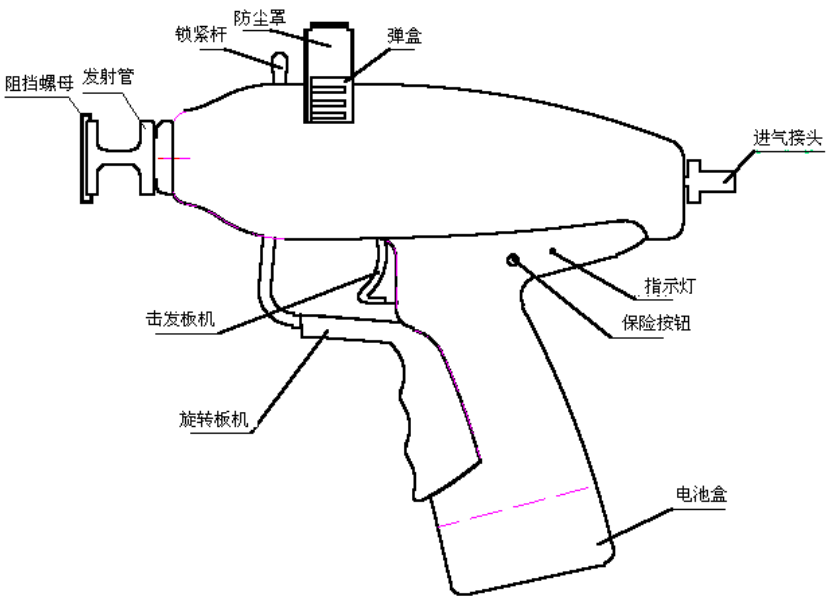
A. Principle and Structures

1. Principle: SJ-500 Portable Gene Gun is a gene transfer apparatus for directly shooting DNA bullet. It is used helium as a power to carry DNA bullet to the body. It is fitting to use in plant in vivo, plant tissue, and cultured cells.

2. Structures



B. Before experiment (Dry environment)

阻挡螺母：Stop nut　　发射管：aunch tube:

锁紧杆：Lock lever　　防尘罩：dust cover:

弹盒： Bomb case　　进气接头：Inlet connector:

指示灯：Indicator　　保险按钮：Insurance button:

电池盒： Battery box:　　旋转板机：rotating button

击发板机：shooting botton

1. Prepare all the apparatus, materials and tools needed.

2. Unscrew the shoot tube and soaking in 75% ethanol for 15 minutes, and try. Clean the gun with 75% ethanol and try. After cleaning the shoot tube, make sure to screw it tightly. Clean the magazines base, bar nut, and bar

net with 75% ethanol and try.

3. Clean the high-pressure tube. When use a new gene gun, first connect one end of the tube with the helium steel bottle, let the air clean the tube for 1-2 seconds. Then connect the other end of the tube with gene gun.

4. Prepare the sterilized solutions, tools and apparatus (such as ethanol, cleanse desk, tips etc.)。

5. Prepare cover DNA bullets.

6. Prepare cells.

▲Prohibit open the gene gun.

C. Install and operation

1. Connect tube: Use two special wrenches connect the tubes connecting the gene gun and the helium steel bottle. Make sure it is airproof and no leak. Open the valve of air steel bottle. Record the indicator value of high-pressure meter, and adjust the pressure at 0.4-3.5Mpa to 5.0Mpa.

2. Install magazine Pull down the dustproof cover (Use thumb and forefinger to catch the cover using). Use right hand to pull back rolling trigger to the end, and use left hand to pull fore the lock pole and orientation to the right. Loose rolling trigger, put the empty magazine to the base (notice the direction, concave is fore and protruding is rear). The magazine tightly clings to the fore end of the base. Put in the base and move back to 2mm. Press close to the back end, Lock pole reposition, and Lock magazine. Use left hand to put down magazine and move right and left slightly till a “click” sound. Cover the dustproof cover.

3. Try shoot: Press the safety button till the indicator light bright (finish charge), then press shoot trigger. If first use, you can shoot empty to make sure the air and the electricity are at normal situation. Pull out the empty magazine.

4. Set bar net: Screw the bar nut in the shoot tube end, and put in bar net slice. And then screw the bar nut tightly.

5. Set DNA bullet: Use tip to transfer bullet to the cavity. Put in a culture dish containing dryer. When it little dry, can be used.

6. Put the bullet cavity into magazine as step 2, and set dustproof.

7. Shoot: Hold gun, use thumb to press safety button. The indicator light will light and then turn to red, put the shoot tube in the 0~2cm site of the receptor. For living animals, can touch the skin. Mouse (belly or ear)、rat (belly, ear or thigh), dog (nose or other sites), pig (nose or other sites). The hairs should be got rid of using shaver or depilitant. Use 75% ethanol clears the skin. The press of helium is set at 2.5~3.5Mpa. If use tissues or cells, the press is set at 0.8~1.2Mpa. Press the trigger and shoot.

8. Second shoot: Press rotation trigger, let magazine turn, and a "toot" sound will be heard. Press trigger.

9. Repeat step 8 till the twelve shoots (Note: when the indicator light is off, need press the safety button to recharge).

10. Pull the dustproof cover, and drop out the magazine. In case contamination, suggest use bullet once.

11. For the second cycle, change new magazine.

D. After experiment management

1. Fasten up the high-pressure valve of steel bottle. Shoot with no bullet to let remain air out. Pull gun, high-pressure tube, pressure meter, and valve etc. (according to the use manual of steel bottle to maintain the steel bottle) . If long time no use, pull out the batteries.

2. Use 75% ethanol to clear the parts and put into box.

E. Encasement list

1. Portable Gene Gun	1	2. magazine (siliconlizated)	50
3. magazine turn shelf	1	4. special wrench(14*17)	2
5. movement wrench 300*36	1	6. 9V batteries	2
7. high-pressure tube	1	8. decompress valve	1
9. bar steel net	50	10. gland	10
11. special tool for gland	1	12. guarantee card	1
13. user manual	1	14. quality certification	1

Note: golden power ϕ1.6μm, ϕ1.0μm can be selected

For reference (confidential materials, only for the user)

(B) the preparing of DNA bullet

Step 1 : Clear the golden powder

The golden powder used in animals are usually ϕ1.0~ϕ1.6μm, and for plants are ϕ1.0μm.

(1) put 60mg golden powder into siliconlizated Eppendorf tube, add absolute ethanol.. Use ultrasonic crusher them till hot temperature (about 3~5 minutes) . Transfer the golden powder into another siliconlizated Eppendorf tube.

(2) centrifugation at 4000~6000rpm, discard the suspend.

(3) add 1ml absolute ethanol, vortex 3~5 minutes. Transfer the golden powder into clear siliconlizated Eppendorf tube.

(4) quiescence 1minute.

(5) centrifugation discard the suspend.

(6) add 1ml sterilized water, vortex, Transfer the golden powder into clear siliconlizated Eppendorf tube. centrifugation discard the suspend. (repeat 3 times)

(7) put 1ml 50% glycerol into the precipitation.

Second Step: the preparing of DNA bullet.

(1) Vortex the golden powder in 50% to become suspended solution.

(2) pipette 100μl suspend solution into centrifugation tubes (each tube enough for 12 guns; According to the quantity of sample to determine the tube numbers needed).

(3) add 10-20μl plasmid DNA (1μg/μl) into the tube, vortex 30 sec., and then add 40μl Spermidine (0.1M) , vortex 30 sec., add 100μl $CaCl_2$ (2.5M) while vortex, 30 sec. ~ 1 min., quiescence for 1min, then transfer the golden powder into a clear tube. Centrifugation, and discharge the suspended solution.

(4) add 300μl 70% ethanol, mix with fingers (no need vortex). If the deposition disperses uniformity, centrifugation, and discharge the suspended solution, add absolute ethanol and then go to next step. If the deposition cannot be dispersed, the ultrasonic need be used (add 70% ethanol till 700μl, 100~200W, 1 sec, intermission 1 sec, repeat 4~6 times,. Transfer the golden powder into a clear tube siliconlizated Eppendorf tube. Centrifugation, and discharge the suspended solution.

(5) add 300μl absolute ethanol, not disrupt the deposition, quiescence 1 min, and then discharge the suspends.

(6) add 120μl ethanol and vortex to suspend solution. If the solution disperse uniformity, pipette 10μl into bullet (enough for 12 bullets). First put the bullets into the mixer, run slowly (make the golden powder to the tube wall). Make sure the suspend solution go to the bullet and not let the golden powder at the below 3 mm of gas hole. When the solution in the hole is dry, bombardment can begin. The bombardment starts from the control.

For each bullet, 0.5mg gold, 0.8μg DNA (10μl plasmid DNA). More plasmid DNA may be better. Too high concentration of DNA may make the particle congregate.

(C) The transformation mechanism of gene gun

The gene gun technology is a novel technology of delivering genetic materials into tissues, cells and organelles with high speed.

SJ-500 Portable Gene Gun has a special apparatus to give rise to high-pressure gas wave from 0.4Mpa to 5Mpa. This mechanism makes the DNA into the receptors with “cold” gas and avoids damage cells by “hot” gas wave.

SJ-500 Portable Gene Gun can be used in plant in vivo and provide stable and high-efficiency transformation.

(D) The Physical and Chemical factors of affecting the gene gun transformation

The physical and chemical factors mean some parameters of gene gun such as pressure, distance, bombardment times, the concentration and purification of DNA, the concentration of DNA precipitator ($CaCl_2$, Spermidine) etc. According to the characteristics of receptors, these parameters may be adjusted.

(1) The pressure selected will affect the penetrability. If he receptors are different, the pressure will different. As a result, according to the materials used to adjust the pressure.

(2) The distance is another important factor. At the same power, longer distance means lower penetrability. And shorter distance means bigger damage to the cells. As a result, suitable distance should be considered.

(3) Experiments have proved that the bombardment times will affect the transformation. Multiple bombardments will increase the instantaneous expression. However, too many bombardments may damage the cells. For these reasons, 2 to 3 guns is suitable for one position.

(4) The higher purification of DNA, the higher efficiency of transformation. However, the transformation efficiency is not consistence with the concentration of DNA. Too high of DNA concentrations will make

them to congestion and low the efficiency. The most common used concentrations of DNA are 1μg/μl.

(5) The effect of DNA precipitator is very big. The suitable concentrations of CaCl2 and spermidine are 1.9-2.5M (suggestion 2.5M) and 100mM, respectively.

★ Cautions: When operate gene gun, defend glasses and glove are needed. No person is allowed to stand on the back of the target.

(E) Notes

(1) When the battery valve cannot be open and the indicator light cannot be turned red, this means the power insufficiency and need change battery (9V).

(2) When gas pressure is low and gas leaks, screw the beam tube and check the airproof ring. When change the rear ring, please use special tool.

(3) The prepare and store of DNA bullets should not be affected with damp. Put dryer into the bullet box.

(4) The beam trigger does not work; the rotation trigger cannot be moved.

(F) Experiment conditions

Name of the receptor	mouse	rat
Shoot position	abdomen	Liver or kidney
Pressure	2.5Mpa	1-1.5 Mpa
Gas	Helium	Helium

Shoot Distance	cling	1-3mm
Metal Particle	ϕ1.6μ golden or tungsten	ϕ1.6μ golden or tungsten
remark	Fuda University, Academy of Military Sciences	Institute of Biophysics

Notes: check references for details.

Apparatus, solutions and gas need be prepared before experiments

1. The purity of helium or nitrogen is 99.999%. And their pressure should be above 9Mpa, commonly 15 Mpa.

B. Golden or tungsten powder. (for plant cells, 1.0μm; most animal cells, 1.6~1.7μm; microorganism, 0.6μm).

C. Spermidine (0.1M, filtrated), $CaCl_2$ (2.5M, filtrated), sealed 100% ethanol, 70% ethanol, 50% glycerol, and sterile distilled water.

D. JY92-II ultrasonic crusher, XW-80A vortex, table high-speed micro-centrifuge (12000 rpm) (Scientz provided).

E. Receptor (transformation materials), donator (plasmid DNA, 1μg/μl, about 10kb, had better no more than 15kb) .

The conditions above should be prepared before open the gene gun box. What kind of transformation materials or how to detect the

instantaneous expression, please consult the representatives

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