GJ-1000 High-Pressure Gene Gun

User Manual

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Please read the manual before installation and operation.

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Instructions for GJ-1000 High-Pressure Gene Gun

- **Read this instruction manual carefully before operation!**
- **Operators must abide by the regulations of using high-pressure gas in laboratory!**

The gene gun provides a completely new technique for transferring genetic material directly into cells, tissues and organ cells. The GJ-1000 high-pressure gene gun (Chongqing Drawell Instrument Co., Ltd. is a device that can indirectly bombard DNA "micro-bullet" into a range of targets. Compressed nitrogen or helium can be used as pulse stream for plant cells. It costs less when nitrogen is applied, and the microparticles can be accelerated to required velocities. It is necessary to use helium in the studies of nerve cells or cells with tight cell walls.

1 Structures and Principles (See Figure 1 in Appendix 6)

Compressed gas (nitrogen or helium) of grade 99.999% is used to generate a cool high-pressure pulse into sample chamber of gene gun. Rupture disks with different thickness are used to adjust the gas pressure by varying different thickness (3.5 MPa, 4.5MPa, 6.5 MPa and 7MPa). For example, when a 9 Mpa pressure is needed, the assembly of two pieces of 4.5 MPa rupture disks could meet the requirement. Upon the pressure of the gas reaching the critical pressure, the disk will be broken. Thus the gas pulse generated via a special structure will force the macrocarrier and accelerate the microparticles (carried DNA) with high speed and directly bombard the target cells on the bottom of the sample chamber.

The whole system is sealed in a cylindric vacuum chamber operating by double vacuum suction. The vacuum can be reached upto -0.095 Mpa. The maximal input pressure is 10 Mpa.

2. Installations

2.1 The Gene Gun must be set up on the clean bench.

2.2 Attach the Gene Gun to the high-pressure gas tank (Pressure hose and gauge assembly are provided by Drawell). The whole pressure system must be airproof. Before assembly, a little stream of gas is released to flush the hose for 1-2 seconds. If the system makes noise during flushing, it's probably because of air leaking. Daubing suds around the connection can also test the system. If air bubbles appear, the system is not airproof. Tighten the nut of connection again to eliminate air leak.

2.3 Attach the Gene Gun to the hose of the vacuum pump (one plastic vacuum hose is

provided). The whole vacuum system must be airproof. The power supply plug of vacuum pump must be plugged into the outlet position located on the back of gene gun.

2.4 Plug the power supply line into the three-phase socket (220AC/50Hz).

2.5 Switch on the valve of the gas tank; turn on the power supply of gene gun then indicator light is on. Press the high-pressure trigger button as soon as possible to make sure that the gas and power are working properly

3 Operation Procedures

3.1 Preparations before experiment

3.1.1 Sterilize the gun chamber, sample cylinder chamber, gas accelerating hose and the connection nut with 75% ethanol.

3.1.2 Sterilize the rupture disks by soaking them in 100% ethanol for 15 min then dry them in asepsis condition. Sterilize the macro carrier with 78% ethanol. Sterilize steel disk in 121^{o}C for 20 min respectively then fully dried (sterilization can also be realized by treatment of the discs by 75% ethanol).

3.1.3 Clean the pressure hose. When new Gene Gun is set up or the Gene Gun is replaced, connect the gas tank with the valve regulator, one inlet of the pressure hose , do not connect with the Gene Gun. Hold the end of hose toward floor, discharge gas from the gas tank with small flux for 1-2 seconds to clean the hose. And then attach it to the Gene Gun.

3.1.4 Sterilize the operating tools.

3.1.5 Prepare for DNA coating. .

3.1.6 Please read the instruction manual for the operation of the vacuum pump

3.2 Operation Procedures

3.2.1 Pull the push bar outward and lock it in a proper position and take off the sample cylinder chamber and macrocarrier holder.

3.2.2 Choose appropriate rupture disks and place them on the inner bore slot of the nut, connect the nut with snail of gas chamber. Use wrench to tighten to ensure airproof.

3.2.3 Place netty disk holder into macrocarrier holder, followed by the netty disk and O-ring (as shown in Figure 2 in Appendix 6).

a. Choose steel disk assembly when nerve cells are targets. Place steel disk holder into steel disk ring. Coat DNA particles onto the steel disk (this step can be operated on steel disk frame), then wait it dry for a short time. Place the steel disk with tweezers (particles side down) to its holder, followed by the the steel disk ring, and tighten the seal nut.

b. For general experiments, choose macrocarrier assembly. Place the macrocarrier into its holder, coat the macrocarrier with DNA microparticles. After drying, place the macrocarrier and its holder onto the netty disk ring with DNA side down and tighten the seal nut. (The microparticles can be daubed on six macrocarrier at one time in general.)

3.2.4 Put the sample vessel by tweezers on the sample frame of Gene Gun. Choose proper position for the frame (the distance between the underside of stopper screen and the sample plane is varied from 45 to 125).

3.2.5 Place the macrocarrier holder (assembled with macrocarrier in step **3.2.3**) above the

sample frame, fix the two objects on the lifter holder. After the lifter bar was pulled out, the sample chamber ascends and combines with the macrocarrier holder and sample frame. All the objects are sealed by O-rings.

3.2.6 Switch on the vacuum pump (Caution: Before operating pump, check the oil level. Operating without oil is strictly forbidden. See the instruction of vacuum pump for details. When the pump starts working, the vacuum gauge indicates the decrease of the pressure. A -0.085 ~ -0.095Mpa pressure is required. The sample chamber must be airproof during taking in vacuum. (The lax connection of mentioned three objects may cause the decrease of the vacuum. Gently move the three objects to seal the system.)

3.2.7 Switch on the trigger button to allow the pressurized gas to enter the cartridge and observe the pressure value through the pressure gauge. When the pressure reaches the critical pressure of rupture disks, an air pulse is generated instantly to strike the macrocarrier, and simultaneously the bombardment (DNA coated microparticles) on the macrocarrier maintain a high velocity through the netty disk and enter the target cells. (A vacuum of -0.5 Mpa is needed when animal cell experiments are performed)

3.2.8 Turn off the vacuum pump and discharge the vacuum, take out the sample. The gene transfer experiment is finished.

3.2.9 Take out the sample chamber and macrocarrier holder following step **3.2.1**. Take out the sample from the sample shelf. Mark the sample and record the experiments in detail.

3.2.10 Take the macrocarrier out from the macrocarrier holder, loosen the nut and take the rupture disk out from the inner bore slot.

3.2.11 Repeat steps **3.2.3** to step **3.2.10** to complete all samples.

3.2.12 Shut down the Gene Gun system: After shutting down the Gene Gun, close the valve on the gas tank. Discharge the residual gas from the pressure hose by pressing the trigger button. Switch off the power and disconnect the power supply.

3.2.13 Clean up with 75% ethanol in general. Please store the seal rings of sample chamber with vacuum grease.

* Before experiment, the sample chamber must be wiped up with 75% ethanol to avoid pollution.
* follow the instruction manual to maintain and operate the gas tank
* Do not use grease in nitrogen system.

4 Packing List

(1) Gene Gun (including sample frame) 1
(2) Power Supply Line 1
(3) Vacuum Pump Power Supply Line 1
(4) Vacuum Grease 1
(5) Special Wrench (17×19 and14×17) 2
(6) Special Wrench (round) 1
(7) 5/64” Coach Wrench 250×20 1
(8) Fuse 5A 2
(9) Vacuum Hose (plastic) 1

(10) Pressure regulator/Gauge (with pressure hose) 1
(11) Rupture Disks
a. Steel Disk (100); b. flying disk (100)
(12) Netty Disk (50) 1
(13) Flying Diskholder 6
(14) O-Ring (with vacuum grease) Φ5×120 4
(15) Instruction Manual 1
(16) Conformity certificate 1
(17) Warranty Card 1
(18) Steel Disk Frame 1
(19) Tungsten Powder 1

Appendix 1

DNA Bullet Preparations

(Only for Reference)

Attention: The ambient humidity should be below 20%. Put $CaCl_2$ as desiccantand with a piece of filter paper in the incubation plate, on which the flying disk holder is placed. This procedure is used to avoid agglomeration when coating the microparticles.

Step 1: Cleaning of Gold or Tungsten Power

(1) Put 60mg of gold or tungsten particles with 100% of ethanol in the tube, sonicated for 10 sec in an ultrasonic bath to break up gold clumps until it fells warm.
(2) Centrifugate the mixture and discharge the supernatant.
(3) Add 1 ml anhydrous ethanol and vortex for 3-5 min.
(4) Wait for 1 min.
(5) Centrifugate the mixture and discharge the supernatant.
(6) Add 1 ml sterilized distilled water and vortex, centrifugate the mixture and discharge the supernatant. Repeat the above for 3 times.
(7) Add 50% of glycerol into the sediment (gold or tungsten particles) to obtain gold or tungsten particles with a concentration of 60mg/ml.

Step 2: Preparations for DNA Bullets

(1) Shake the gold or tungsten particles in 50% glycerol to gain a suspension.
(2) Take 50ul of the suspension into the tube (this quantity can be used to bombard for 6 times, according to experiment to decide the number of tubes).
(3) Add 5-10 ul of plasmid DNA (1ug/ul) to the carrier suspension, with continuous vortexing for 30 seconds, add 20 µl of spermidine (0.1 M) and vortex for 30 seconds. Simultaneously add 50 µl of $CaCl_2$ (2.5 M) with continuous vortexing for 30-60 sec. Wait for 1 min. Centrifugation and discharge the supernatant.
(4) Add 150 ul of ethanol (70%) into the sediment. If the sediment disperses uniformly, centrifugate the mixture (3000 rpm for10 seconds) and remove the supernatant. Anhydrous ethanol to the sediment and go to next step. If the deposition cannot disperse uniformly, it should be dissolved into 70% ethanol to 600 ul and sonicated for 1 sec (200 W, 4-6 times). Then centrifugate the mixture and remove the supernatant.
(5) Add 150ul of anhydrous ethanol into the deposition and avoid destroying the sediment. Then wait for 1 min and remove the supernatant.
(6) Add 60ul of anhydrous ethanol into the deposition to obtain suspension. Take 10ul of suspension to the macrocarrier (6 macrocarriers could be used). Each piece contains 0.5 mg gold or tungsten particles with 0.8ug DNA (calculated with 5ul plasmid DNA. The more plasmid, the better).

Appendix 2

Factors affecting the delivery of genes of the gene gun

The delivery efficiency of gene gun can be affected by many factors. It has been studied systematically by many researchers./ The key factor to improve the efficiency of gene gun is to allow the intacted DNA as much as possible to enter the target cells, and on the other hand, to reduce the damaged target cells. The factors shown below can be the reference for researchers.

1. System parameters

System parameters refer to some bombardment parameters for the gene gun, including the pressure of rupture disk, range, available vacuum in the sample chamber, times of bombardment, the purity and concentration of DNA, and the concentration of DNA precipitator (calcium chloride and spermidine) etc. It is necessary for each laboratory to determine the optimal parameters according to their particular target cells.

(1) Optimize rupture disk pressure: Different type of cells may need different bombardment pressure that can affect the delivery of bullet to acceptor. Thus according to particular acceptor type, rupture disk pressure should be optimized.

(2) Optimize range: The range is an important factor. For example, only certain cells in plant organism are able to reproduce themselves. So it is possible to adjust the range to improve the transformation efficiency of these cells. If the drive power is fixed, the longer the range is, the smaller the penetrability is, *vice versa*.

(3) Optimize the vacuum in sample chamber: Holding certain vacuum in sample chamber can reduce the air resistance to bullets. There is a positive correlation between vacuum and transformation. But the target cells have limited tolerance for vacuum. It is suitable to use 0.095 Mpa vacuum to bombard the plant cells with GJ-1000 Gene Gun.

(4) Optimize the times of bombardment: Experiments results indicate that too many times of bombardments can harm the target cells, and the optimum numbers are 2-3 times.

(5) Optimize the DNA Loading Ratio: The higher purity of DNA will bring better transformation. However increasing the concentration of DNA does not result in a proportional increase in gene transformation. On the contrary, excessive final concentration of DNA will form coagulation with bullet thus decrease the efficiency of transformation.

(6) Optimize the precipitator: For example, eliminating $CaCl_2$ may greatly decrease the transformation ratio. The optimum concentration of Calcium chloride and spermidine is about 2.5 M and 0.1 M respectively.

(7) Please use fresh 100% ethanol only! (100% ethanol is prone to absorb water, which can cause bullet agglomerating and prevent from gene expression). The bullet must be moisture proof in or after preparation and stored in dry environment.

2. Biological parameters

Before or after bombardment, the culture conditions may greatly affect the transformation ratio. And material in different physiological phase can be observed different efficiency of DNA infection. It is generally considered that tissues and cells with high physiological activity will be benefit to DNA transformation. For example, high osmotic and dehydration treatment can improve the transformation efficiency.

Because the gene gun technology is a complex integrated and experienced technology. It is necessary for the operator to accumulate experience and optimize parameters in operating process.

Appendix 3

Troubleshooting

Problem	Possible reasons and solutions
Bullets agglomerateion after coating with DNA	Concentration too high or low purity of DNA, molecular weight of DNA too big, ethanol or spermidine not fresh, speed of centrifuge too high. Solution: The last centrifugation could be adjusted to 3000rpm,10-20second; sonicate for 1 sec (200W, 2-3 times)
Vacuum not attainable	The accelerate hose, sample cylinder chamber and base are not connected properly. There is air leak in vacuum connection.
Air leak in high-pressure system	The gas tank and hoses are not correctly connected. Check the joint by using suds and tighten the nut of joint. Strictly prohibit using oil in nitrogen system.
Attached cells are lifting off the center of the plate after delivery of gold particles	The pressure of gas is too high. Replace the suitable rupture disk or adjust the range.
Rupture disk is not cracked.	The pressure of gas tank is too low or the rupture disk is not properly fixed. Check the thickness and numbers of rupture disk. Pressing trigger button for 1 second.

Appendix 4

Bombardment Parameters

(Only for Reference)

Cell Type	Vacuum (MPa)	Pressure (MPa)	Gas	Range (cm)	Particle Size (μm)	macrocarrier	Notes
Corn embryos	-0.095	10	Nitrogen	30-60	1.0W	Steel disk	China Agriculture Univ.

Corn callus	-0.095	6.5-7	Nitrogen	50-60	1.0W	Steel disk	China Agriculture Univ.
Tea-leaf callus	-0.095	6.5-7	Nitrogen	60	1.0W	Steel disk	Zhejiang Univ.
Nerve cell	-0.5	6.5-7	Helium	30-40	1.6Gold	Flying disk	Chinese Academy of Sciences
Lettuce-leaf	-0.095	4.5-6	Nitrogen	60-80/40	1.0Gold	Flying disk / Steel disk	Institute of Genetics, Chinese Academy of Sciences
Elm-leaf	-0.095	6	Nitrogen	50-60/30-40	1.0Gold	Flying disk / Steel disk	

※ To check whether the gene gun works properly: Add tungsten power into 100% of ethanol as bullet and lettuce or tender leaf as acceptor, observe the result using microscope after bombardment.

※ Advantages of tungsten power: tungsten power is less expensive and can be used to bombard plant cells. Disadvantages: tungsten power is prone to be oxidized, damp, agglomeration and be toxic.

※ Advantages of steel disks: stabilization, less harmful to cells. But the penetrability is a little low.

Appendix 5

Materials and Components for Experiment

- GJ-1000 high-pressure gene gun
- Helium or Nitrogen Tank (Grade 99.999%, 15Mpa)
- Purified Plasmid DNA (1 ug/ul, 10kb-15kb length)
- JY92-II Ultrasonic Cell Crusher
- Vortexer
- Tabletop Centrifuge
- Sterilized distilled water
- 2.5mol/1 $CaCl_2$ (Sterilized with filtration)
- 0.1mol/l Spermidine (Sterilized with filtration)
- Fresh 100% ethanol
- Tungsten or Gold powder, rupture disk, macrocarrier etc.

Appendix 6

Diagrammatic View

Figure 1

Pressure gauge	Switch of vacuum pump	Back of instrument panel
Macrocarrier holder	Switch of power	Power of vacuum
Sample chamber	Vacuum of gauge	Fuse station
Plate	Outlet valve of vacuum	
Plate Station		Inlet of high-pressure gas
Lifter station	High-pressure trigger button	Inlet of vacuum
Bar of lifter		

Figure 2 **Sketch map of assembling macrocarrier**

Choose the appropriate macrocarrier or steel disk according to acceptor type.

1. Place the macrocarrier or steel disk into the frame, insert the frame into the holder, daub the macrocarrier or steel disk with bullet until use.
2. Place the Nett disk holder, steel Nett disk, the sealed ring of Nett disk into the macrocarrier holder (with bullet underside). Tighten the nut to seal.
3. If the steel disk is needed, displace the macrocarrier holder with the steel disk holder, place the steel disk daubed with bullet into its holder (with bullet underside), tighten the nut.

Macrocarrier ⟶ Macrocarrier holder (6) ⟶ Seal Ring of Netty disk (1) ⟶ Netty disk ⟶ Netty disk holder (1)

Sealed ring of Steel disk ⟶ Steel disk ⟶ Netty disk holder (1)

Notes:

Please use desiccant on the sample plate to avoid agglomeration of the bullets. Put the lid on the steel disk as soon as the steel disk is coated with bullets or the flying disk is put on the holder. As shown in the Figure.

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