**Transient Plant Cell Transformation by Particle Bombardment**
**(from Wong Hann Ling Sept 2006)**

**<Reagents>**

2.5 M CaCl2 (Filtered, store at -20°C)
0.1 M Spermidine (Filtered, store at -20°C)
0.8% Agarose plates (Can be substituted with wet filterpaper in Petri dishes)

**<Gold particle preparation>**

**1) Weigh gold particles**
0.6 mg particle (diam. 1.6 μm for onion and rice, 1.0 μm for Arabidopsis)/1 shot
**2) Sterilize gold particles**
Add 300μl 70% EtOH into weighed gold particles and vortex.
Briefly spin down and discard EtOH.
Add 300μl of 100% EtOH, spin down and discard EtOH.
**3) Coating DNA onto gold particles**
(NOTE: Following is the most important step in this experiment. Prepare all the solution suck in a tip respectively, and add them one by one seamlessly)
Add sterile dH2O and briefly sonicate.
(NOTE: total volume of water and DNA together is 10 μl)
Add 5 μg/shot of DNA, vortex briefly.
Add 10 μl/shot of 2.5 M CaCl2 solution and vortex briefly.
Add 4 μl/shot of 0.1 M Spermidine and vortex briefly.
Incubate at room temp. for 30 min.
Spin down and discard supernatant.
Add 100 μl 70%EtOH and sonicate briefly, spin down and remove supernatant.
Add 15 μl 100%EtOH/ shot and briefly sonicate.
Spot resuspended samples on microcarrier and allow to them dry.

**<Operation particle gun (BioRad PDS-1000/He)>**

1) Turn on Vacuum pump.
2) Turn on He meter gauge.
3) Turn on He meter valve.
4) Turn the gauge to the left of He meter and set it at 1300 psi (depending on plant tissue and rapture disk types)
5) Place rapture disk (e.g. 1100 psi type) on Gas Acceleration Tube Stand and tighten with Torque wrench.
6) Place Stopping Screen and Microcarrier on Microcarrier Launch Assembly in main chamber (at 2$^{nd}$ level).
7) Place plant tissue on agarose plate and set them on the 4$^{th}$ level in main chamber.
8) Shut the door of chamber apply vacuum by pressing <VAC> down until vacuum reached 28.5~29.0, then quickly press down <HOLD>.
9) Press down <FIRE> to launch bombardment. (A 'ponk' sound with be emitted when bombardment occurred.)
10) Release vacuum by placing switching knob at <VENT> position.
11) Repeat steps 4~8 if required.
12) To finish, turn off he valve, shut the cahmber, and set <VAC> and <FIRE> simultaneously until vacuum He pressure returned zero.
13) Turn off He meter valve.
14) Add a little water to bombarded plates and store in dark at 30$^{o}$C.

*2 DNA amount is depending on the plant. If you are using Arabidopsis or onion, you may use 0.5~1 μg, but bombarding into rice requires more DNA