Transient Gene Expression with the Particle Gun

(protocol modified after BioRad instructions)

Preparation of particles

weigh out 30 mg of tungsten particles in microfuge tube
    wear gloves for this
    recommended sizes: M10 for Arabidopsis, M17 for onion
add 500 µl 70% ethanol
    freshly prepared
vortex for at least 10 min
    speed setting 5, push tube far into holder to particles stay more in bottom
spin particles for ≤ 5 seconds
    don't spin too hard or the particles clump together
remove supernatant
add 500 µl sterile H2O
vortex 1 min
let settle ~ 1 min
spin ≤ 2 seconds, remove SN
repeat H2O wash twice for a total of 3 washes
add 500 µl 50 % glycerol (sterile)
vortex briefly
    particles are stable for at least 1 month

Preparation of tissues

a) onion epidermal peels

    you will need one agar plate (standard MS) per construct
    wear gloves unless you want your hands to smell for three weeks
cut onion in quarters
remove the youngest (innermost) leaves
with a razor blade, slice strips parallel to long axis of leaf
    strips should be about 20 x 5 mm
with a fine forceps, peel epidermis off
lay epidermal strip with upper (outer) side down on agar
    bombardment will occur from the back, viewing from the front
cover the center of the petridish to a size of about a quarter
    use M17 particles

b) Arabidopsis leaves

per construct/shot prepare 1 petridish with a wet filter paper in its center
select young leaves, blades 5 to 15 mm long
leaves should be dark green, healthy and ± flat
place 3 to 5 leaves near center of dish on filter paper upside down
bombardment will occur on abaxial (lower) side
the water on the filter paper will hold the leaves during the shock wave
close the lid to keep from drying out
use M10 particles

Preparation of disks

vortex particles for at least 5 min (speed 5)
this is a good time to prepare the tissues
place plasmids in microfuge tube
total volume should be between 5 and 10 µl
the following steps should be done quickly
add 25 µl particles per tube
add 25 µl 2.5 M MgCl2 (or CaCl2)
add 5 µl 200 mM spermidine *(store in freezer)*
close tube, place back on vortexer
vortex for 3 min (speed 5)
this is a good time to place macrocariers on filter paper in petridish, use 2 or 3 per construct; use millipore forceps
let settle for 1 min
spin 1-2 seconds
be careful to keep this short or you'll get big clumps
remove SN without disturbing the pellet
add 100 µl 70 % ethanol
the pellet should still be intact
remove SN
add 100 µl 100 % ethanol
repeat 100 % ethanol wash once or twice
no need to wait between washes; pellet will stay intact all along
add 25 µl 100 % ethanol
vortex at least 1 min
pipette particles up/down to suspend them evenly
place 8 µl particles an macrocarrier
there should be enough particles for 3 shots
it's usually a good idea to do 2 shots per construct in case one goes wrong
cover macrocarrier dishes and let ethanol evaporate
as soon as the ethanol is evaporated you are ready to shoot
if you need to wait a bit longer, add some dessicant to the dishes to keep moisture low

Bombardment

you will need rupture disks, stopping screens, prepared macrocarriers, prepared tissues, millipore forceps, "red hat"

open helium tank
- open large gray screw by 1/4 turn
- screw in brass regulator until secondary pressure reaches 1300 psi

i.e. ~200 psi above the value of the rupture disks

start vacuum pump
turn on gene gun
the macrocarrier assembly should be on the top shelf
the sample tray should be on the second from the bottom
remove macrocarrier assembly
load rupture disk

make sure holder is firmly tightened or it will blow too soon

open macrocarrier assembly (big screw on top)
remove macrocarrier holding ring
place macrocarrier in ring and snap in place with "red hat"

particles should be facing up

place stopping screen in bottom of assembly
invert and replace ring with macrocarrier

particles should be facing down

slide macrocarrier assembly into top shelf
place sample on lower shelf

don't forget to open the dish

close door
set vacuum switch to top position (VAC)

pull vacuum to about 27.5

flip vacuum switch to lowest position (HOLD)
press (and hold) FIRE switch

pressure in upper chamber will rise to ~1100 psi before disk ruptures

release FIRE switch
vacuum switch to BLEED
open chamber and remove sample, macrocarrier, stopping screen, rupture disk
Repeat for other samples

second and third shots for the same constructs can be done with the same stopping screen; it is helpful to invert it between shots

duplicates can be bombarded into the same tissue sample

Shut down:
clean the gene gun!
pull vacuum to ~25 psi and hold
close big gray screw on helium tank
loosen brass regulator until it turns freely
press FIRE on gene gun to release pressure

keep an eye on the manometers of the helium tank: only the secondary should drop down to zero

release the vacuum by switching to BLEED
set open door, set vacuum switch to VAC
turn off vacuum pump
vacuum switch to bleed
turn off gene gun

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updated on 04.06.30 by Andreas