

STANDARD OPERATING PROCEDURE

Procedure	Use of PDS-1000/HE™ and HEPTA™ systems (Gene gun)
School/Department:	School of Molecular Bioscience
SOP prepared by:	Anna Reid, Hannah Nicholas
Version:	SMB059.1

Section 1 - Personal Protective Equipment (PPE)

1. Enclosed footwear.
2. Wear a lab coat.
3. Hair tied back.
4. Safety glasses must be worn when vacuum in use because there is gas under pressure.

Section 2 – Potential Hazards

1. Release of pressure generated from vacuum may result in flying debris which could cause injury.

Section 3 – Procedure

1. Ensure all users are thoroughly trained and have completed all necessary safety procedures and forms.
2. Follow manufacturer's instructions carefully. Each gene gun and hepta adaptor may be slightly different. Preventative maintenance is performed on some gene guns (contact supplier to see if applicable).
3. Autoclave components (where appropriate – see below) to ensure sterility.
4. When preparing samples for bombardment perform a test run (see below) to ensure everything is working correctly.
5. Check the helium tank to ensure it has enough helium and that the tank valve is in the 'closed' position.
6. Turn on the bombardment apparatus and helium tank regulator. The regulator valve should be set to 'closed'.
7. The following instructions are for the biolistic transformation of *Caenorhabditis elegans* and values will change depending on sample-type and experiment.
8. Open the tank valve. Adjust the regulator valve such that the psi is 200 psi higher than the pressure required to burst the rupture disk (i.e. 1550 psi for a 1350 psi rupture disk).
 - a) *Test run:* Clean the inside of the bombardment apparatus (gene gun) with isopropanol or 70% ethanol. Insert a rupture disk into hepta adaptor. Screw hepta adaptor containing the rupture disk on tightly.
 - b) Close the door (must be tight to draw vacuum). Turn on the gene gun. Switch the Vac/Vent button to 'Vac'. Manually turn the **vac** knob to the open position and watch as the vacuum reaches 27 " Hg. Once it reaches 27 " Hg, switch the Vac/Vent button to 'Hold' and quickly turn the **vac** knob back to a closed position.
 - c) Press and hold the fire button. Watch as the He pressure rises to ~1350 psi. The rupture disk should burst with a loud noise.
 - d) Vent immediately (this is more important when there are worms inside the gene gun!) by turning the Vac/Vent button to 'Vent' and quickly turning the **vent** knob to the open position. The vacuum should drop back to 0 " Hg.
 - e) Open the door and take out the hepta adaptor. Remove the burst rupture disk.
9. *Load the launch assembly:* Using clean (autoclaved) forceps, load autoclaved macrocarriers (after sterilizing briefly in isopropanol) onto the launch assembly, ensuring that they fit into the holds and are fairly flat. Let dry for a few moments. Pipette (using siliconised tips) 11 uL of DNA-coated gold beads onto the middle of each macrocarrier. Let dry (~15 minutes) in a 'chamber' (large plastic petri dish) containing CaCl₂ or near a Bunsen burner flame. Once gold beads are dry (should be a dark brownish colour), insert into launch assembly with stopping screen (autoclaved).
10. Attach the hepta adaptor with fresh rupture disk. Load the complete launch assembly just below the

hepta adaptor, ensuring that the 'holes' of the two components align.

11. Put the sample (large plate of worms) (lid off!) onto the plastic tray at the second slot from the bottom. Close the door and draw vacuum. Fire as above.
12. Immediately after bombardment, vent and remove sample from the gene gun. Ensure that the **vent** knob is in the closed position (or vacuum cannot draw for subsequent bombardments).
13. Wipe the gene gun with 70% ethanol and remove any debris.
14. Close the door.
15. Close regulator adjustment screw (gold valve).
16. Close cylinder valve (brown knob) and then turn a quarter of a turn back.

Section 4 – Disposal / Spills / Incidents

1. All incidents are to be reported to your immediate supervisor.
2. Incidents resulting in injury and any near-miss incidents must be reported using RISKWARE, the online WHS reporting system.
3. Any spills containing *Caenorhabditis elegans* are to be contained and absorbed with paper towel which is then treated as PC2 waste. Contaminated surfaces are to be wiped with 70% ethanol.

Section 5 – Repairs / Certification / Validation

1. Under no circumstances should repairs be attempted on cylinders or regulators. Report all faults to the Service Centre 9351-6006 for assessment.
2. Bio-Rad specialist should be consulted for any repairs.

Section 6 – Relevant safety data sheets (to be available and accessible)

1. Consult the appropriate SDS for the gases you are using. Especially note whether the gases are flammable, corrosive or toxic – these gases pose special risks.

Section 7 - References

1. http://www.bio-rad.com/en-au/product/pds-1000-he-hepta-systems?pcp_loc=lnav

SOP Consultation, Training and Approval

Print names and enter signatures and dates to certify that the persons named in this section have been consulted/trained in relation to the development and implementation of this Standard Operating Procedure. WHS Representative (WHS Committee) certifies that consultation has taken place.

Position	Name	Signature	Date
Supervisor			
employee / student			
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Creation date: 06/07/2015
Last review date: 06/07/15

Next review due: 06/07/2017

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Name Authorising (Printed): DIANNE FISHER.....

Signature:  **Date:** 14/7/15

WHS Committee Representative Name (Printed): MARKUS HOFER.....

Signature:  **Date:** 14/7/15