BIOLISTIC®

PDS-1000 / He

Particle Delivery System

The Biolistic PDS-1000/He system is a unique transformation instrument that uses helium pressure to accelerate DNA-coated microparticles toward target cells. The physical nature of the technique makes it extremely versatile and easy to use; high-velocity microprojectiles have been used to transfect cells, tissues and organelles without dependence on DNA interaction with the cell surface. Targets as diverse as insect and fish embryos, cultured animal cells, animal tissues *in situ*, cultured plant cells, intact plant tissues, pollen, algae, fungi, bacteria, mitochondria, and chloroplasts have been successfully transformed. Both transient and stable transformation efficiencies have been achieved with a wide variety of cell types.

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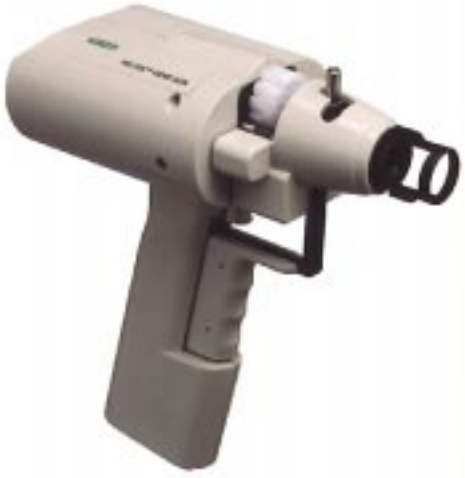
Why Microprojectile Bombardment?

Of the several transformation methods available today, the Biolistic process has the advantage of being applicable to a wider range of cell and tissue types than any other method. For example, cultured mammalian cells do not need to be trypsinized to remove them from their support, thus their morphology is maintained. Biolistic bombardment is much easier and less time-consuming to perform than the tedious task of microinjecting embryos such as C.elegans. Some species, most notably the agriculturally important cereal crops, had been without a transformation method altogether before the advent of particle delivery. Even intracellular organelles have been transformed with microprojectile bombardment. Neuronal cells and brain tissue sections have been successfully transformed (request bulletin 2087). Bio-Rad's Helios Gene Gun uses Biolistic technology for *in vivo* applications as well.

Fig. 1. The assembled PDS-1000/He system. *For bombardment, the door is closed, and a vacuum is drawn in the chamber. Activation of the "fire" switch then allows helium to flow into the gas acceleration tube at a rate regulated by the helium metering valve and monitored by the helium pressure gauge.*



Helios Gene Gun

Instrument Design

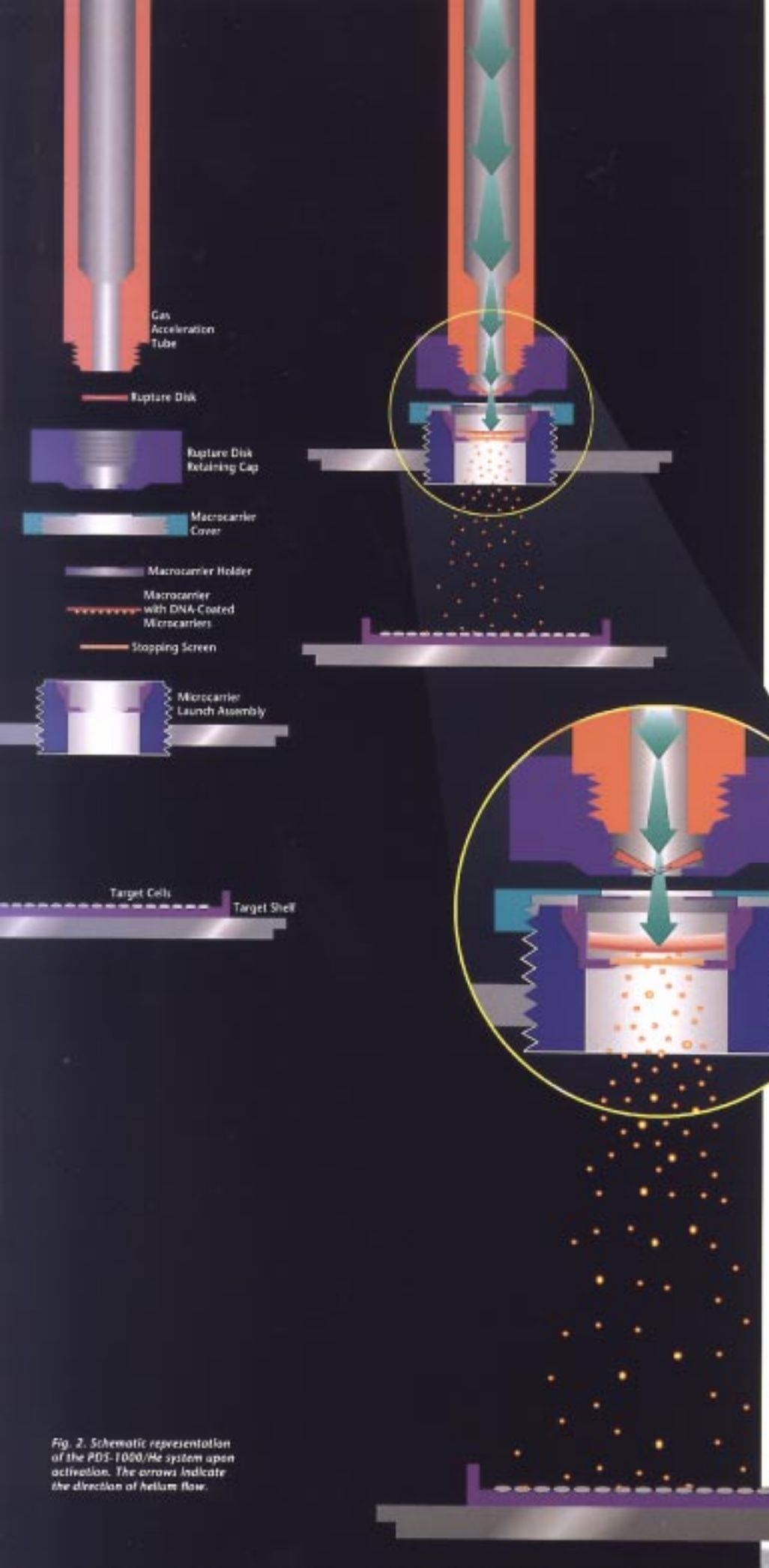


Fig. 2. Schematic representation of the PDS-1000/He system upon activation. The arrows indicate the direction of helium flow.

The PDS-1000/He instrument is capable of accelerating subcellular-sized microprojectiles coated with DNA over a range of velocities necessary to optimally transform many different cell types. By this process, thousands of cells can be transformed simultaneously. The efficiency of the bombardment is determined by the nature and size of the microparticles, and by several instrument parameters. One important parameter is the helium pressure, which determines the velocity to which the microprojectiles are accelerated. The helium pressure that enters the chamber from the gas tube varies with the choice of plastic rupture disks. Each of the nine different rupture disks is designed to rupture at a specific pressure, ranging in rating from 450 pounds per square inch (psi) to 2200 psi. Helium flows into the top of the instrument and is held in the gas acceleration tube until the pressure of the rupture disk is reached (Figures 1 and 2). When the disk bursts, the ensuing helium shock wave drives a second plastic disk (the macrocarrier) a short distance toward the stopping screen. The macrocarrier carries the DNA-coated microcarriers (subcellular-sized particles of tungsten or gold) on its front surface. The stopping screen retains the macrocarrier while allowing the coated particles to pass through to penetrate the target cells at the bottom of the bombardment chamber.

In addition to the choice of rupture disk, this system offers several other ways to optimize the particle penetration. Bio-Rad provides technical bulletins to help you determine the appropriate starting conditions for your biological system.

- Seven sizes of microparticles are available.
- The chamber vacuum can be regulated.
- There are also several adjustments that can be made within the chamber that affect the velocity of the microparticles (Figures 1 and 2):
  - the distance that the helium shock wave travels before reaching the macrocarrier (between the rupture disk and the microcarrier launch assembly);
  - the distance that the macrocarrier travels before reaching the stopping screen;
  - the distance that the microcarriers travel (between the stopping screen and the target cells).

Applications

Plant Systems

The particle bombardment technique is especially useful for cells and tissues that are refractory to other transformation methods, such as the agriculturally important monocotyledonous plants (Tables 1 and 2). Seedlings, embryos, cultured cells, leaves, epidermal tissues, apical meristems, and floral tissues are among the many targets that have been transformed. Figure 3 shows maize suspension cells bombarded with tungsten carrying plasmid containing the ß-glucuronidase (GUS) gene. Figure 4 shows a tobacco leaf bombarded with particles coated with plasmid containing the luciferase gene. GUS activity may also be easily assayed in bombarded cauliflower epidermal sections (see bulletin 1688 for details).

Animal Systems

Besides being useful for plant applications, the Biolistic process has also been used successfully for the transformation of intact animal tissues, animal cells in culture, and animal embryos (Tables 1 and 2). Particle delivery is a convenient method for transforming these sensitive cells since very little pre- or post-bombardment manipulation is neccesary. See Figure 5.

Other Biological Systems

The physical nature of the Biolistic process is its strongest virtue, making the PDS-1000/He instrument extremely versatile. In addition to plant and animal work, the technology has been applied to targets as diverse as chloroplasts, mitochondria, bacteria, fungi, algae, and pollen (Tables 1 and 2). Figure 6 shows *Saccharomyces cerevisiae* that has been successfully transformed using the Bio-Rad Yeast Optimization Kit.

A wealth of additional information is available from a number of primary literature references that have been published over the past few years. A comprehensive bibliography is also available from Bio-Rad.

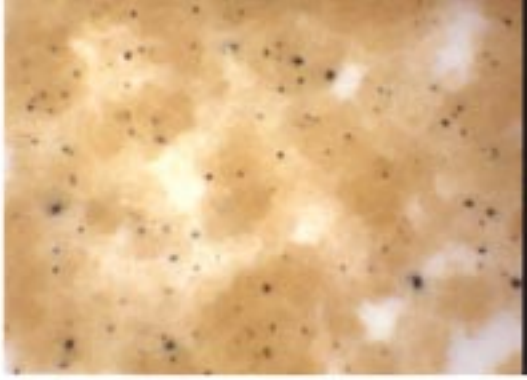


Fig. 3. Maize Cultured Cells.
GUS activity displayed in maize suspension culture cells. PDS-1000/He bombardment conditions were 1550 psi burst pressure at a distance of 12 cm from the stopping screen. Photo courtesy of E. Dunder, CIBA-GEIGY Agricultural Biotechnology, Research Triangle Park, NC.

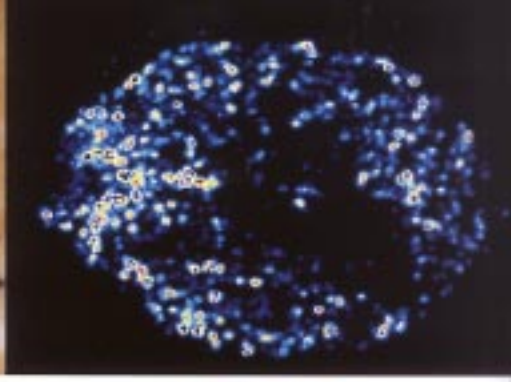


Fig. 4. Tobacco Leaf.
Expression of luciferase reporter gene as monitored by photon counting imaging. Millar, A., Hiratsuka, K., Chua, N.-K., and Kay, S.A., Plant Molec. Biol. Rep., 10, 324-337 (1992).

Table 1. Stable Transformation by High Velocity Microprojectiles.

	Stable Transformation	
Transgenic Plants	Maize	embryogenic cultures
	Rice, indica and japonica	immature embryos
	Wheat	embryogenic cultures
	Sugarcane	embryogenic cultures
	Soybean	meristems, embryogenic cultures
	Cotton	meristems, embryogenic cultures
	Poplar	callus cultures
	Cranberry	stems
	Papaya	immature embryos
	Tobacco	callus cultures
	Arabidopsis thaliana	root cultures
Transgenic Animals and Insects	Loach	*Misgurnis fossils*, embryos
	Rainbow Trout	*Salmo gairdneri*, embryos
	Zebrafish	*Brachydanio rerio*, embryos
	Fruitfly	*Drosophila melanogaster*, embryos
Algae and Fungi	*Chlamydomonas reinhardtii*	
	Cryptococcus neoformans	
	Neurospora crassa	
	Podospora anserina	
	Saccharomyces cerevisiae	
	Saccharomyces pombe	
	Uncinula necator	
Bacteria	*Agrobacterium tumefaciens*	
	Bacillus megaterium	
	Erwinia amylovora	
	Erwinia stewartii	
	Escherichia coli	
	Pseudomonas syringae pv. syringae	
Organelles	Chloroplasts	*C. reinhardtii*, tobacco
	Mitochondria	*S. cerevisiae*

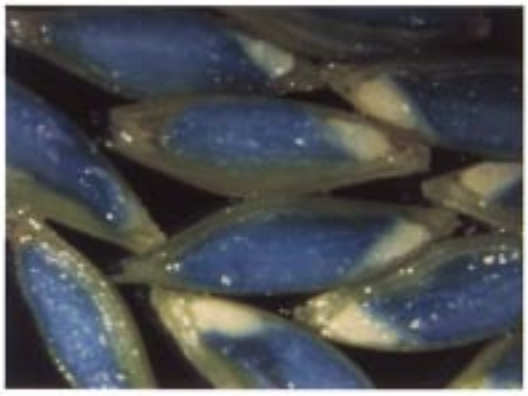


Fig. 7 Barley Seeds.
Histochemical enzyme assay for ß-glucuronidase on T_1 seeds from a stably transformed line containing gusA driven by a barley endosperm-specific promoter (M.-J. Cho, unpublished).

Fig. 5. Chicken Skeletal Muscle Cells.
Primary chick muscle cells bombarded at 800 psi with a plasmid containing the ß-galactosidase gene coated onto 1.0 µ gold particles. The target distance was 12 cm. Photo courtesy of S. Johnston, University of Texas Southwestern Medical Center, Dallas, TX.

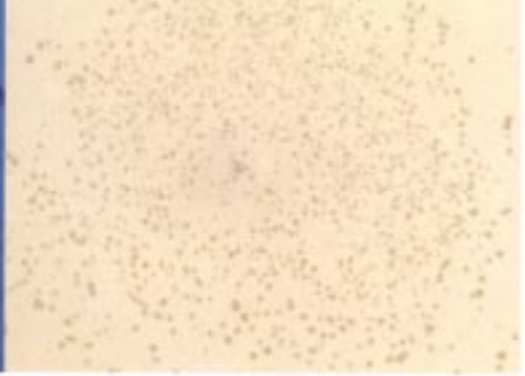


Fig. 6. Fungi.
S. cerevisiae strain 948 transformed with the YEp352 plasmid is capable of growth on uracil-deficient selection media. See bulletin 1688.

Table 2. Transient Expression by High Velocity Microprojectiles.

	Transient Expression	
Plants	Gene Regulation	anthocyanin
	Heterologous Gene & Promotor Function	pollen
	Mechanical Viral Infection	TMV, BGMV, BDMV
	Tissue Specificity of Promotor Function	meristems, leaves, stems, roots, coleoptiles, microspores/pollen, anthers, petals, embryos, scutella, endosperm, aleurones, tissue culture
Animals	Kidney	mouse, rat
	Liver	mouse, rat
	Mammary Ductal Segments	mouse, rat, human
	Muscle	mouse
	Skin	mouse
	Tissue Cultured Cells	
	Chicken	Fibroblasts
	Hamster	CHO
	Human	HT1080, MCF-7, 143B
	Mouse	NIH3T3, EL4

For more detailed information on the bombardment of these biological systems, please refer to the Biolistic Bibliography (bulletin 1687), or contact your local Bio-Rad office.

Recent Review Articles

Birch, R. G., and Franks, T., Development and optimization of microprojectile systems for plant genetic transformation, *Aust. J. Plant. Physiol.*, **18**, 453 - 469 (1991).

Christou, P., Genetic transformation of crop plants using microprojectile bombardment, *Plant J.*, 2, 275 - 281 (1992).

Klein, T. M., Arentzen, R., Lewis, P. A., and Fitzpatrick-McElligott, S., Transformation of microbes, plants, and animals by particle bombardment, *Bio/Technol.*, **10**, 286 - 291 (1992).

Sanford, J. C., Smith, F. D., and Russell, J. A., Optimizing the Biolistic process for different biological applications, *Meth. Enzymol.*, **217**, 483-509 (1993).

Literature

Suggested Reading

The following publications describe work done with the Biolistic PDS-1000/He. Supplementary references (including additional review articles) are listed in the continually-updated Biolistic Bibliography, bulletin 1687.

Fitzpatrick-McElligott, S., Gene transfer to tumor-infiltrating lymphocytes and other mammalian somatic cells by microprojectile bombardment, *Bio/Technol.*, **10**, 1036-1040 (1992).

Russell, J. A., Roy, M. K., and Sanford, J. C., Major improvements in Biolistic transformation of suspension cultured tobacco cells, *In Vitro Cell. Develop. Biol.*, **28P**, 97 - 105 (1992a).

Russell, J. A., Roy, M. K., and Sanford, J. C., Physical trauma and tungsten toxicity reduce the efficiency of biolistic transformation, *Plant Physiol.*, **98**, 1050 - 1056 (1992b).

Sanford, J. C., DeVit, M. J., Russell, J. A., Smith, F. D., Harpending, P. R., Roy, M. K., and Johnston, S. A., An improved, helium-driven biolistic device, *Technique*, **3**, 3 - 16 (1991).

Shark, K. B., Smith, F. D., Harpending, P. R., Rasmussen, J. L., and Sanford, J. C., Biolistic transformation of a procaryote, *Bacillus megaterium*, *Appl. Environ. Microbiol.*, **57**, 480-485 (1991).

Smith, F. D., Harpending, P. R., and Sanford, J. C., Biolistic transformation of prokaryotes: factors that affect biolistic transformation of very small cells, *J. Gen. Microbiol.*, **138**, 239 - 248 (1992).

Ye, G.-N., Daniell, H., and Sanford, J. C., Optimization of delivery of foreign DNA into higher-plant chloroplasts, *Plant Molec. Biol.*, **15**, 809-819 (1990).

System Components

Instrument

The Biolistic PDS-1000/He instrument is rather simple in design since its primary function is solely to accelerate the coated microparticles to velocities effective to penetrate the target cells, located in the lower portion of the chamber. The important features of the instrument are the bombardment chamber, the gas acceleration tube, and the microcarrier launch assembly. See Figure 1.

The bombardment chamber can be evacuated to subatmospheric pressures; the sample to be transformed is placed on the target shelf. The vacuum gauge monitors the pressure in the chamber, while the vacuum flow rate and vent flow rate knobs control the rates at which the vacuum is drawn and released. The gas acceleration tube fills with helium from the top. It is fitted at the bottom with a rupture disk retaining cap which holds a rupture disk in place, temporarily closing off the gas tube from the bombardment chamber. The helium flows from the helium regulator through the helium tubing and helium metering valve to the gas tube. The pressure in the tube is monitored by the helium pressure gauge. The microcarrier launch assembly houses the microcarrier-coated macrocarrier and its holder, and the stopping screen and its support.

The pressure (helium) and vacuum circuits work together to effectively accelerate the microcarriers. A safety feature requires that a small vacuum be drawn in the chamber in order for the unit to be activated. The vacuum serves the additional purpose of reducing the frictional drag of the particles as they are accelerated toward the target cells.

Fig. 7. Inserting rupture disk.
An appropriate rupture disk is chosen and placed into the rupture disk retaining cap. Next, the cap is tightened onto the end of the gas acceleration tube.

Fig. 8. Loading DNA samples.
The DNA-coated microcarriers are pipetted onto the macrocarrier seated in the macrocarrier holder.

Table 3. Summary of Numerous Experiments Comparing Transformation Efficiency, in Gunpowder-driven versus Helium-driven Systems

	Number of Transformants		
Target	Gunpowder	Helium	Fold Increase
Yeast (colonies)	81	1792	22x
Bacillus megaterium (colonies)	0.8	265	331x
Escherichia coli (colonies)	3.8	380	100x
NT1 (tobacco) (blue spots)	540	3115	6x
NT1 (tobacco) (Km' calli)	3.6	23	6x
Chicken Myotubes (luc activity)	112k	1920k	11x
Mouse Skin (luc activity)	300	1543	5x
Mouse Ear (luc activity)	1312	5563	4x
Mouse Liver (luc activity)	0	309	-----

From Sanford, J. C., DeVit, M. J., Russell, J. A., Smith, F. D., Harpending, P. R., Roy, M. K., and Johnston, S. A., *Technique*, 3, 3 - 16 (1991). Reprinted with permission from the author and the publisher.

Fig. 11. Consumables for the PDS-1000/He Instrument.
Macrocarriers (large brown disks), stopping screens (silver, foreground), nine different rupture disks (small disks), and seven different microcarriers (vials in background) are available.

Fig. 9. Microcarrier launch assembly. *Once the microcarrier slurry is completely dry, the holder is inverted (such that the microcarriers face the target cells) and placed into the microcarrier launch assembly. Note the stopping screen near the bottom of the assembly.*

Fig. 10. After bombardment. *Disassembly of the chamber components reveals that the helium pressure has forced the macrocarrier from its holder (now empty, bottom left) onto the stopping screen. Note the imprint of the screen on the macrocarrier.*

Additional Kits & Information

Disposable Items

There are four other small consumable parts needed in addition to the instrument to complete a functional system: rupture disks, macrocarriers, microcarriers, and stopping screens (see Figure 11). The macrocarriers and stopping screens are each available in one standard size and are appropriate for all Biolistic applications.

The choice of rupture disk depends on the biological system. In general, the higher the rupture disk rating, the greater the helium shock wave, and therefore the higher the velocity the microparticles can achieve. Higher particle velocities are most desirable for recalcitrant cells such as intact plant cells with thick cell walls. Best results may be achieved with a rupture disk of a lower pressure rating for more fragile cells and tissues.

Microcarriers are also chosen for the particular system beign studied. Three sizes of gold and five sizes of tungsten are available. Both metals have high densities and have been used effectiviely to penetrate a wide range of cell types. It is recommended that the smallest microcarrier that will penetrate the cell be chosen. Tungsten has been commonly used in the past, alhhough it has been observed to be toxic in some cells (Russell et al., 1992b). Recently gold has become the more popular microcarrier due to its inert nature, size uniformity, consistent spherical shape, and low incedence of agglomeration. 6 µ gold microcarriers are now also available.

Additional Requirements

In addition to the Biolistic parts available through Bio-Rad, the user must also have access to high-quality helium, a vacuum source, and various laboratory supplies and reagents. Please request our Biolistic Checklist for more detailed information on additional requirements.

Optimization

Bio-Rad offers two optimization kits to help you fine-tune the bombardment conditions for your cells of interest. The 500 Optimization Kit provides material sufficient for 500 bombardments. It includes 100 of each of the nine different rupture disks, samples of both the 1.0 µ and 1.6 µ diameter gold microcarriers, 500 macrocarriers, and 500 stopping screens. These consumables allow you to try several experimental conditions to determine exactly the conditions that are optimal for your cells. Once the best bombardment conditions have been defined, Standard Pressure Kits containing only the desired rupture disks and microcarriers are available. Replacement kit components are also offered.

The Yeast Optimization Kit is available as well. This kit contains the biological components needed to grow, transform, and assay *Saccharomyces cerevisiae*, a system that is easily bombarded and assayed in a short period of time, as shown in Figure 6. The Yeast Optimization Kit is designed to demonstrate the effect of varying certain biological and bombardment conditions. The kit allows first-time users to become familiar with the Biolistic instrument, and is also helpful for experienced users wishing to periodically standardize their bombardment conditions. Material sufficient for 60 assays is provided. See Figure 12.

Improved Technology

The original Biolistic device was gunpowder driven, and it is clear that cell types which have been successfully transformed with the older technology are transformed to the same or higher efficiencies with the new helium design (Table 3). The helium technology provides the additional advantages of being more reproducible, cleaner, and safer to use, as well as inflicting less tissue damage. The improved control of microprojectile velocity with helium stems from the use of rupture disks that burst at defined pressure.

Bio-Rad offers detailed application notes outlining biological and bombardment conditions for several cell types. Contact you local Bio-Rad office for your complimentary copies of our Biolistic technical bulletins. Including information on the Helios Gene Gun for in vivo applications.

Fig. 12. Yeast Optimization Kit. *This kit allows the user to become familiar with the entire Biolistic process in less than a week.*

Acquisition of the PDS-1000/He

The Biolistic PDS-1000/He system is available worldwide for lease or sale depending on research circumstances at your institution. Contact your local Bio-Rad office or representative for details on acquiring the instrument for your laboratory. Bio-Rad representatives are readily available for consultation throughout the acquisition, installation, and optimization processes.

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