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Optimization of Biolistic® Transformation Using the Helium-Driven PDS-1000/He System

William Heiser, Ph.D., Research and Development Department, Genetic Systems Division, Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547

Summary

We have found that the most important factors which must be optimized in Biolistic transformation are target distance and microparticle size. Changing most other parameters, within a broad range, usually has minimal effect on transformation efficiencies. In this report we describe a strategy for optimizing Biolistic transformation. We use two model systems to show how this strategy can be employed by developing optimal conditions for stable transformation of yeast and transient expression in cauliflower stem sections.

Introduction

The Biolistic process, first reported by Sanford, *et al.* (1987), is a method by which foreign substances are introduced into intact cells and tissues via high-velocity microprojectiles. Transformation of biological material using the Biolistic method has been shown to be a valuable technique for delivering DNA into the cells of plant (Klein, *et al.*, 1988 a, b), animal (Williams, *et al.*, 1991), and microbial species (Shark, *et al.*, 1991), as well as into subcellular organelles (Johnston, *et al.*, 1988; Daniell, *et al.*, 1991). Achieving high rates of DNA expression in each of these cases often requires that time be spent in optimizing some of the parameters involved in transformation. However, this task can be difficult, considering the large number of parameters which can be varied in the system. In addition, there has been a recent modification of the commercially available instrument which now uses a helium-powered acceleration system (Sanford, *et al.*, 1991). This report outlines an approach to optimizing microparticle and particle accelerator parameters with Bio-Rad's PDS-1000/He system using two biological systems, transient expression of the ß-glucuronidase (GUS) gene in cauliflower epidermal cortex, and stable expression of a wild-type *URA* 3 gene in an auxotrophic mutant of *Saccharomyces cerevisiae*. The microparticle variables include the size, amount, and type of microparticles; the accelerator parameters include the helium pressure, the distance between the rupture disk and macrocarrier (the gap distance), the distance between the macroprojectile and the stopping screen (the macrocarrier travel distance), and the distance between the stopping screen and the biological target (the target distance). See Figure 1 for a graphic representation of these variable distances. In order to obtain the maximum number of transformants, it is also necessary to optimize the biological parameters associated with the target tissue. These factors include the state of growth of the target cells, the cell density, the osmolarity of the bombardment medium, the treatment of the cells post-bombardment, and the expression vector being used for transformation (Sanford, *et al.*, 1992).

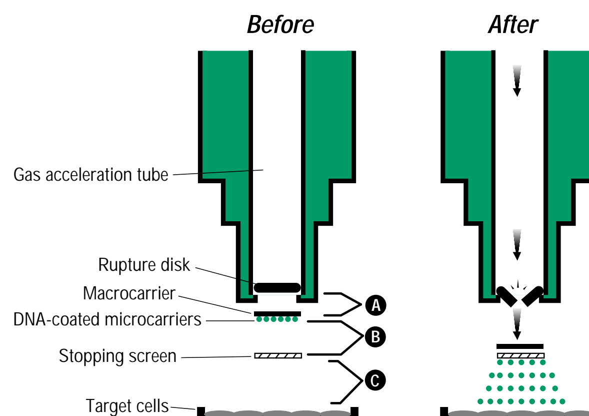

Fig. 1. Schematic representation of the PDS-1000/He system before and after activation (not to scale). A, B, and C are the adjustable distances that influence the velocity with which the microcarriers hit the target cells. **A.** rupture disk - macrocarrier gap distance. **B.** macrocarrier travel distance. **C.** target distance. The arrows indicate the direction of helium flow.

Methods

Preparation of Yeast and Cauliflower

The yeast auxotroph, *S. cerevisiae* st 948 (Armaleo, *et al.*, 1990), contains non-reverting mutations in the *ura* 3 and *leu* 2 genes, and was used for all experiments with yeast. Yeast were prepared for bombardment as described by Klein, *et al.* (1991). Briefly, cells were grown to early stationary growth phase in YEPD media. Cells were concentrated 100-fold by centrifugation, and 10^8 cells were spread on uracil-deficient agar plates containing 0.75 M sorbitol and 0.75 M mannitol. Seeded plates were kept for 2-4 hours at room temperature before transformation. After bombardment, the cells were incubated for 60 hours at 30 °C, at which time the colonies were counted.

Cauliflower was obtained from a local produce market. The main stems were sectioned into approximately 1 cm squares (2-4 mm thick), and nine longitudinal sections were placed in a 3 x 3 array in the center of 100 mm Petri plates containing 1% agar (Klein, *et al.*, 1991). The agar acted as a support to

help the tissue sections absorb the shock from the bombardment and also kept the tissues moist during overnight incubation. After bombardment of the epidermal cells, the tissue was incubated for 24 hours at 24 °C. The location of GUS-expressing cells was visualized using a histochemical reaction (Jefferson, *et al.*, 1987). Cauliflower sections were transferred to 16 mm wells containing 0.8 ml of GUS assay buffer (0.5 mg/ml 5-bromo-4-chloro-3-indoyl-ß-D-glucuronic acid, 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide, 0.06% Triton X-100, 0.1 M sodium phosphate buffer, pH 7.0) and incubated at 37 °C. After 18 hours, the tissue sections were evaluated for their level of GUS expression (see Table 1, footnote c).

Plasmids

Plasmid YEp352 (Hill, *et al.*, 1986), which contains a wild-type *ura* 3 gene, was used for transformation of yeast. Cauliflower tissue was bombarded with the plasmid pBI221, which contains the GUS gene expressed from the cauliflower mosaic virus 35S promoter (Jefferson, *et al.*, 1987). Both plasmids were prepared by alkaline lysis (Maniatis, *et al.*, 1982) followed by cesium chloride purification and ethanol precipitation.

Biolistic Transformation

We recommend using conditions previously reported to transform a similar biological system as a starting point for the optimization of Biolistic transformation. For example, Armaleo, *et al.* (1990) report the use of 1.1 µm tungsten particles (equivalent to M17 tungsten) and a vacuum of 0.03 atmospheres (equivalent to 29 inches Hg) to transform yeast cells with the gunpowder-powered PDS-1000 unit (the precursor to the PDS-1000/He system). These parameters were used as starting conditions for optimizing bombardment of yeast with the helium-driven PDS-1000/He system. Preliminary experiments had indicated that a helium pressure of 1,300 pounds per square inch (psi) would produce large numbers of transformants (R. Arentzen, personal communication). Starting conditions for transformation of cauliflower sections were chosen as 1 µm diameter gold or M17 tungsten microparticles and a helium pressure of 1,300 psi, based on experiments using other plant systems (T. Klein, pers. comm.).

Unless otherwise noted, the following parameters were kept constant in all of the experiments described here:

Chamber vacuum	28″ Hg (0.06 atm)
Gap distance	1/4″
Macrocarrier travel distance	8 mm (stopping screen at middle level)
Microcarriers/bombardment	500 µg
DNA/bombardment	833 ng

These conditions have proven to be “standard” for Biolistic experiments involving most cell types (J. Sanford, pers. comm.).

Both gold and tungsten microparticles were prepared, and coated with supercoiled plasmid essentially as described by Sanford, *et al.* (1992; see Appendices 1 and 2). Microparticles were dried onto macrocarriers in individual “desiccators” (Petri dishes containing $CaSO_4$) immediately upon application, and were used within 2 hours of drying.

Results

Cauliflower

An example of cauliflower sections transformed with the plasmid pBI221 and stained for GUS-expressing cells is shown in Figure 2. Each section in the array was scored separately and given a semi-quantitative score based on the relative number of GUS-positive cells (see Figure 2 and Table 1, footnote c). Results of an experiment to determine the effects of microparticle size, helium pressure, and target distance are shown in Table 1. Several observations were made from the initial experiment (Table 1, Experiment 1): (1) while both M10 (median particle diameter of 0.7 µm) and M17 tungsten microparticles may be used for cauliflower transformation, the latter are consistently more effective; (2) tissue sections bombarded at level 2 (6 cm target distance) are transformed more efficiently than sections bombarded at level 4 (12 cm target distance); and, (3) changing the helium pressure between 1,100 and 1,550 psi has little effect on transformation efficiency. Subsequent results confirmed these conclusions and also indicated that similar transformation efficiencies were achieved with all pressures from 900 to 1,550 psi (Table 1, Experiments 2 and 3). Transformation results using 1.0 µm and 1.6 µm gold microparticles to bombard tissue sections at level 2 or 4 also indicated that the highest transformation efficiency was obtained using the larger particles with the shorter target distance. As with the tungsten microparticles, varying the helium pressure between 900 and 1,550 psi had little effect on transformation efficiency.

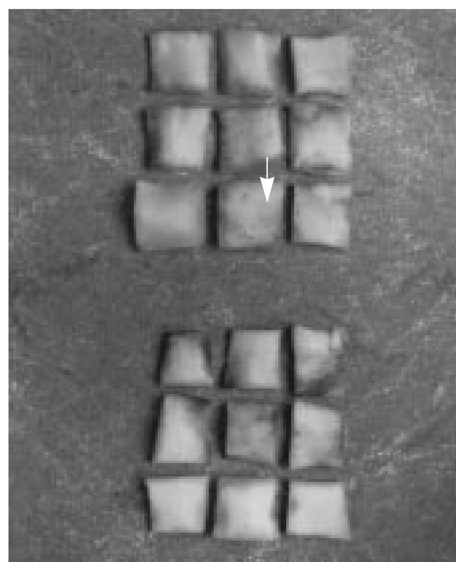

Fig. 2. Example of GUS expression in cauliflower epidermal sections. Cauliflower sections were bombarded with pBI221 coated onto 1.6 µm gold microparticles and stained with GUS assay buffer as described in the Methods section. Each cauliflower section is placed in its location, but not necessarily its actual orientation, at the time of bombardment. The spot marked by the arrow represents a typical positive response.

Table 1. Effects of microparticle size, helium pressure, and target distance on transformation of cauliflower tissue

Microparticles	Helium Pressure[a]	Target Distance[b]	GUS Score[c] Expt. 1	Expt.2	Expt. 3
M10 tungsten	1,100	6	+		
	1,300	6	+++		
	1,550	6	+		
	1,100	12	0		
	1,300	12	+		
	1,550	12	0		
M17 tungsten	900	6	++++		
	1,100	6	++++		+++++
	1,300	6	++++		++++
	1,550	6			+++++
	1,100	12	+++		
	1,300	12	0		
	1,550	12	+		
1.0 µm gold	1,100	6		++	
	1,300	6		+	
	1,550	6		++	
	1,100	12		+	
	1,300	12		+	
	1,550	12		+	
1.6 µm gold	900	6		++++	
	1,100	6		++++	++++
	1,300	6		++++	++++
	1,550	6			+++
	1,100	12		++	
	1,300	12		+++	++
	1,550	12		++++	+++
	1,800	12			++

a Burst pressure of rupture disk in psi.

b Approximate distance (in cm) from the stopping screen to the biological sample. The biological target may be placed at one of four positions, approximately 3 cm (level 1), 6 cm (level 2), 9 cm (level 3), or 12 cm (level 4), below the stopping screen.

c The GUS Score is a semi-quantitative estimation of the level of GUS expression in all nine cauliflower sections in a bombarded plate; each score is the average obtained from three bombardments. Each cauliflower section was given a low (0, +), intermediate (++, +++), or high (++++, +++++) value based on the number and density of the blue spots which were visible on the surface of the tissue (see Figure 2). The scores in each column are from separate experiments.

The effect of changing the gap width and macrocarrier travel distance on GUS expression was assayed using optimal conditions determined in the initial experiments (Table 2). At a target distance of 6 cm and bombardment pressures of either 900 or 1,300 psi, changing the gap width or macrocarrier travel distance had very little effect on transient expression. These experiments indicate that for Biolistic transformation of cauliflower sections using the PDS-1000/He system, optimum transient expression is obtained when the sample is placed at level 2 (6 cm target distance) and bombarded at a helium pressure of 900 psi with either M17 tungsten or 1.6 µm gold microparticles.

Stable transformants can not be isolated from cauliflower tissue sections. However, these transient assays may be used as starting conditions for optimizing stable transformation. A rigorous experiment for resolving the optimal conditions for isolating stable transformants could be determined in a fractional factorial experiment as outlined in Table 4. Such an experiment should be undertaken only if experimental quantitation is relatively simple. A more practical approach would be to vary those parameters which have the greatest effect on transient expression, in this case helium pressure and target level. In optimizing for stable transformation, the most severe Biolistic conditions which are used should be the mildest conditions which produce a high level of transient expression (e.g., helium pressure of 900 psi and target at level 2). Optimization of stable transformation should use lower helium pressures and/or longer target distances than conditions for transient expression. (Also see comments in the Discussion section.) Since similar levels of GUS expression were obtained using M17 tungsten and 1.6 µm gold microparticles, it may be preferable to use gold microparticles for producing stable transformants due to the demonstrated toxicity of tungsten in some plant cells (Russell, *et al.*, 1992b).

Table 2. Effect of gap distance or macrocarrier travel (stopping screen) distance on GUS expression in cauliflower tissue bombarded with pBI221[e]

Microparticles	Pressure[a]	Gap Width[b]	Stopping Screen Position[c]	GUS Score[d]
1.6 µm gold	900	1/8″	Middle	+++++
		1/4″	Middle	+++++
		3/8″	Middle	++++
	1,300	1/8″	Middle	++++
		1/4″	Middle	+++++
		3/8″	Middle	+++++
M17 tungsten	900	1/4″	Top	+++
		1/4″	Middle	++++
		1/4″	Bottom	++++
	1,300	1/4″	Top	++++
		1/4″	Middle	+++++
		1/4″	Bottom	++++

a Burst pressure of rupture disk in psi.

b Distance between the rupture disk retaining cap and the macrocarrier cover lid as measured with the PDS-1000/He gap tools.

c Location of the stopping screen support within the fixed nest. Top, Middle, and Bottom refer to the stopping screen support above both spacer rings, between the two spacer rings, and below both spacer rings, respectively. Moving the stopping screen support through the three positions changes the macrocarrier flight distance from approximately 3 mm (Top) to 8 mm (Middle) to 13 mm (Bottom).

d See Table 1, footnote c.

e All bombardments were performed with the tissue samples 6 cm from the stopping screen.

Yeast

The effects of microparticle size, helium pressure, and target distance on transformation of the uracil auxotroph *S. cerevisiae* st 948 with the uracil-containing plasmid YEp352 are shown in Table 3. The initial experiment (Table 3, Experiment 1) shows that both M10 and M17 tungsten microparticles are effective in transforming *S. cerevisiae*, that cells on level 2 are transformed >500-fold more efficiently than cells on level 4, and that changing the helium pressure between 1,100 and 1,550 psi has very

little effect on transformation efficiency. A second experiment (Table 3, Experiment 2) confirmed these conclusions, and also suggested that bombardment with M17 tungsten microparticles consistently produced more transformants than did bombardment with M10 tungsten microparticles. Results with 1.0 µm gold and 1.6 µm gold microparticles indicated that the former were approximately equivalent to M10 tungsten microparticles in transformation efficiency, but that larger gold particles had a 100-fold lower transformation rate than the other three types of microparticles. This suggests that the 1.6 µm gold particles, the largest particles used in this experiment with an average diameter of 1.6 µm, probably destroy the cells upon bombardment.

Table 3. Effects of microparticle size, helium pressure, and target distance on transformation of *S. cerevisiae* with YEp352 DNA

Micro-Particles	Helium Pressure[a]	Target Distance[b]	Colonies Average[c]	±	St. Dev.
Experiment 1					
M10	1,100	6	639	±	269
M10	1,300	6	876	±	400
M10	1,550	6	952	±	346
M17	1,100	6	1,800	±	150
M17	1,300	6	1,300	±	361
M17	1,550	6	1,307	±	190
M10	1,100	12	2	±	2
M10	1,300	12	18	±	31
M10	1,550	12	1	±	1
M17	1,100	12	2	±	2
M17	1,300	12	1	±	2
M17	1,550	12	2	±	3
Experiment 2					
M10	1,100	6	985	±	169
M10	1,300	6	720	±	284
M10	1,550	6	1,280	±	218
M17	1,100	6	1,967	±	231
M17	1,300	6	2,193	±	170
M17	1,550	6	2,047	±	185
1.0 µm Au	1,100	6	1,096	±	107
1.0 µm Au	1,300	6	792	±	131
1.0 µm Au	1,550	6	878	±	72
1.6 µm Au	900	6	5	±	6
1.6 µm Au	1,100	6	7	±	2
1.6 µm Au	1,300	6	9	±	8

a Burst pressure of the rupture disk in psi.

b Approximate distance (in cm) from the stopping screen to the Petri plate containing the yeast.

c The number of transformed colonies are the averages obtained on three or four replicate dishes bombarded at each set of conditions.

Subsequent experiments with *S. cerevisiae* used a fractional factorial design to optimize both gap distance and macrocarrier travel distance as a function of both helium pressure and target distance. In a fractional factorial experiment (Kempthorne, 1983) interactions between several factors are measured at selected points. A statistical model is then used to predict the optimum conditions. An example of this type of experiment, in which the macrocarrier travel distance was varied along with the helium pressure and target distance, is shown in Table 4. Analysis of the three variables indicates that the maximum number of transformants is obtained with the sample at level 2 and the helium pressure between 900 and 1,500 psi. The macrocarrier travel distance had little effect on either increasing or decreasing the transformation efficiency. Similar results were found when the gap width was varied as a function of the helium pressure and target distance; neither increasing nor decreasing the gap width affected the number of transformants produced (results not shown). These results were further verified by changing either the gap width or the macrocarrier travel distance under conditions which were optimal for transformation (M17 tungsten microparticles, target at level 2, and helium pressure of 1,100 psi). Again, there was no significant effect on the number of yeast transformants (results not shown).

Table 4. A fractional factorial experiment designed to optimize helium pressure, target distance, and macrocarrier flight distance for yeast transformation[e]

Stopping Screen Position[a]	Helium Pressure[b]	Target Distance[c]	Colonies Average[d]	±	St. Dev.
Middle	900	6	459	±	54
Middle	900	12	7	±	9
Top	900	9	112	±	31
Bottom	900	9	111	±	21
Bottom	1,300	12	31	±	9
Bottom	1,300	6	354	±	37
Top	1,300	12	46	±	12
Top	1,300	6	306	±	62
Middle	1,300	9	407	±	72
Bottom	1,800	9	8	±	6
Top	1,800	9	1	±	2
Middle	1,800	6	228	±	93
Middle	1,800	12	2	±	1

a Location of the stopping screen support within the fixed nest (see Table 2, footnote c).

b Burst pressure of the rupture disk in psi.

c Approximate distance (in cm) from the stopping screen to the Petri plate containing the yeast.

d The number of transformed colonies are the averages obtained on three or four replicate dishes bombarded at each set of conditions.

e All bombardments were performed using M17 tungsten microparticles.

Discussion

Transient expression of the GUS gene in cauliflower stem sections and stable transformation of a yeast *ura* auxotroph with the wild type *URA* gene were used as model systems to identify those bombardment parameters which are important for optimization of Biolistic transformation. The parameters and their optimum conditions are summarized in Table 5 for both systems. In both cases, the most important parameter to optimize is target distance; there is a significant decrease in expres-

sion when the target level is changed from the optimum of 6 cm. This is apparent when cauliflower sections are transformed with M17 tungsten microparticles (Table 1) and when yeast are transformed with M10 and M17 tungsten or 1.0 µm gold microparticles (Table 3). This reduced transformation efficiency is most likely due to the lower velocity of the microparticles at longer travel distances.

Table 5. Summary of optimum conditions for stable transformation of *S. cerevisiae* and transient expression in cauliflower stem sections using the PDS-1000/He system

Parameter	Yeast	Cauliflower
Microcarrier	M17 tungsten	M17 or 1.6 µm gold
Rupture disk-macrocarrier gap	1/4″	1/4″
Macrocarrier travel distance	8 mm	8 mm
Chamber vacuum	28 in Hg	28 in Hg
Helium pressure	900 psi	900 psi
Target distance	6 cm	6 cm

Microparticle size also has a significant effect on transformation efficiency, probably for different reasons, however. In the case of cauliflower stem sections, when the optimum particle accelerator parameters were used, the 1.6 µm gold and M17 tungsten (median diameter 1.1 µm) microparticles produced more GUS-expressing cells than did the smaller 1.0 µm gold and M10 tungsten (median diameter 0.7 µm) microparticles. Furthermore, using the 1.6 µm gold microparticles, significant GUS expression was obtained when the cauliflower sections were bombarded at level 4. This may be attributable to the larger size of the 1.6 µm gold microparticles compared to the other microparticles assayed; the velocity of the larger microparticles is less affected by the travel distance. On the other hand, in the case of yeast transformation, using optimum particle accelerator parameters, the M17 tungsten microparticles were slightly better than the 1.0 µm gold and M10 tungsten microparticles in producing *ura*$^+$ transformants. However, almost no transformants were found when the 1.6 µm gold microparticles were used. This effect is most likely the result of the larger 1.6 µm gold microparticles causing significant damage to the yeast cells (average diameter 6 µm) upon penetration. Because of the heterologous size distribution of the tungsten microparticles, their biological effect is due to a subset of small diameter microparticles.

The final parameter which should be verified in optimizing Biolistic transformation is the helium pressure. While the fungal and plant experiments reported here show that the range over which optimum transformation occurs is quite broad (there is little difference in transformation efficiency between 900 and 1,550 psi), this is not always the case. In other systems, such as the stable transformation of bacteria (Smith, *et al.*, 1992) or plants (Russell, *et al.*, 1992a), the optimum helium pressure range is narrower. Because microparticle bombardment will result in cell damage, the most gentle conditions which give a high transformation efficiency should be used. This is particularly true when transient gene assays are performed as preliminary experiments for determining conditions to use in generating stable transformants (see below).

The experimental results obtained here indicate that two of the parameters which can be varied on the PDS-1000/He instrument, the gap distance and the macrocarrier travel distance, may have little effect on the transformation efficiency. Varying either of these parameters and subsequently assaying their effect on yeast transformation (Table 2) or transient expression in cauliflower (Table 4) failed to show a significant effect. Table 6 summarizes the effect of changing the particle accelerator parameters on microparticle velocity and transformation efficiency.

Biolistic transformation of yeast and cauliflower stem sections has not been reported previously using the PDS-1000/He instrument. However, transformation of yeast and other plant tissues has been reported using the gunpowder-driven PDS-1000 unit. Armaleo, *et al.* (1990) described optimized biological parameters for yeast, and recommended using 1.1 µm tungsten microparticles. Starting with the biological conditions reported by this group, we have optimized microparticle accelerator parameters for the helium-powered unit. In the case of transient expression in plants, earlier work with the gunpowder unit (Klein, *et al.*, 1988c) had shown that high levels of transient expression in maize could be achieved using 1.2 µm tungsten microparticles, a target distance of 6 cm, and a chamber vacuum of 28 inches Hg. These conditions formed the basis for the starting conditions used in these assays, and, in fact, are the optimal conditions which we found for transient expression in cauliflower stem sections. We recommend using a similar strategy of starting with previously reported conditions when optimizing parameters for other cell types of interest.

When extrapolating from transient to stable expression, it is important to remember that the optimum transformation conditions may be different in the two cases. For example, Biolistic transformation may damage cells such that they score positive in transient expression assays, but can not divide, and therefore would not produce a stable transformant. As a result, conditions which are found to be optimum for transient expression may be too harsh to produce the maximum number of stable transformants. Therefore, when optimal conditions for transient expression are known, it may be necessary to use more gentle conditions to achieve maximum stable expression. The transformation process may be made less harsh by reducing the velocity of the microcarriers. This may be achieved by reducing the helium pressure, the macrocarrier travel distance, and the size and number of microparticles, and/or by increasing the target distance and the gap distance (Table 6). In addition, using smaller microparticles and reducing the chamber vacuum may result in less tissue damage. Initial starting conditions which may be used for the PDS-1000/He with various types of biological systems are given in Table 7. These conditions, though probably not optimal, may be used in any initial experiments to assess optimal biological parameters (e.g., expression vector, cell type, etc.) prior to optimizing the conditions described here.

Table 6. Particle accelerator parameters which affect transformation with the PDS-1000/He

Parameter	Theoretical Effect on velocity	Observed Effect on transformation
Microparticle size	Inc vel w/ inc size	Major[a]
Target distance	Dec vel w/ inc dist	Major
Vacuum	Inc vel w/ inc vac	Major[b]
Helium pressure	Inc vel w/ inc press	Variable[c]
Gap distance	Dec vel w/ inc dist	Minor
Macro. flight distance	Inc vel w/ inc dist	Minor

a The effect on transformation is probably more dependent on the size of the target cells than on the velocity of the microparticle.

b The smaller the particle, the more pronounced the effect.

c See Discussion section.

Finally, the helium pressures reported here are all based on the rating of the rupture disk which was used in each bombardment. The actual pressures at which the rupture disks burst were monitored using the gauge on the PDS-1000/He in approximately half of the 500+ bombardments performed in the course of these experiments. In all cases, the disks burst within 100 psi of their rated pressure. In order to correctly monitor the pressure at which the rupture disks burst, it is important to set the helium pressure at the regulator valve 200 psi over the pressure rating of the rupture disk being used, and to adjust the helium metering valve so that the helium flow rate into the gas acceleration tube occurs at a rate such that a 1,550 psi rupture disk will burst in 12 $\pm$ 1 seconds. Additionally, in all of the bombardments performed in this study, there was only one rupture disk failure. In order to obtain this low failure rate, it is very important to tighten the rupture disk retaining cap onto the gas acceleration tube as much as possible (the torque on the cap should be between 40 and 50 in-lb).

Conclusions

Stable expression in yeast and transient expression in cauliflower tissue sections have been demonstrated using the PDS-1000/He instrument. Transformation efficiencies can be maximized by optimizing the microparticle size, target distance, and helium pressure. In both systems studied here, the first two parameters are the most important. Little difference in results was found using helium pressures between 900 and 1,550 psi. This report also shows that these conditions may be optimized in one or two experiments; because of shot-to-shot variability, it is important to perform at least three to five replicates for each set of conditions. The optimum parameters found for the yeast and cauliflower systems assayed here are summarized in Table 6. When optimizing other systems, we suggest to begin by using conditions given in Table 7, then examine in order: target distance, microparticle size and type, and helium pressure.

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Table 7. Suggested starting conditions for optimizing several types of biological systems using the PDS-1000/He system[a]

Biological system	Microcarrier[b]	Vacuum[c]	Helium pressure[d]	Target distance[e]	References
Bacteria	0.8 µm tungsten	29	1,100	6 cm	Shark, *et al.*,1991 Smith, *et al.*, 1992
Yeast	1.1 µm tungsten	28	1,100	6 cm	Armaleo, *et al.*, 1990 This report
Algae	1.2 µm tungsten	27	900	12 cm	K. Kindle, pers. comm.
Plant cells/tissues	1.2 µm tungsten	28	1,100	9 cm	Klein, *et al.*, 1988c Russell, *et al.*, 1992a
Animal cell cultures	1.0 µm gold	15	2,000	9 cm	S. McElligott, pers. comm.
Subcellular organelles[f]					
Macrocarrier	1.0 µm tungsten	28	900	6 cm	Ye, *et al.*, 1990
Gas entrainment	1.0 µm tungsten	28	1,500	8 cm	G.-N. Ye, pers. comm.

a In all cases, the rupture disk-macrocarrier gap distance was set at 1/4 inch and the macrocarrier travel distance at 8 mm (one spacer ring above and one below the stopping screen support). To optimize Biolistic transformation, begin with the parameters given in Table 7 and test conditions both above and below those listed in Table 7 and described in the Discussion section. In all cases, the most important parameters to examine are the target distance, the microparticle size, and the helium pressure.

b Mean particle size of the tungsten and gold microcarriers (there is lot-to-lot variation in the size of microcarriers, particularly with tungsten). The M10 and M17 tungsten particles used in this study have a median size of 1.1 and 1.7 µm, respectively.

c Chamber vacuum in inches Hg. Some cell types are more sensitive to reduced atmospheric pressure. If reduced cell viability from excessive vacuum is suspected, test this in a separate experiment.

d Burst pressure of rupture disk in psi.

e Approximate distance from the stopping screen to the biological sample; the four levels for the sample holder in the PDS-1000/He instrument correspond to distances of approximately 3, 6, 9, and 12 cm.

f Transformation of organelles may be accomplished by launching microparticles from a macrocarrier as described in the PDS-1000/He instrument manual. Ye, *et al.* (1990) have also reported efficient results using gas entrainment.

Appendices

The procedures described in Appendices 1 and 2 were developed by Sanford, *et al.* (1992).

Appendix 1. Microcarrier preparation

For 120 bombardments using 500 µg per bombardment

1. In a 1.5 ml microfuge tube, weigh out 60 mg of microparticles.
2. Add 1 ml of 70% ethanol, freshly prepared.
3. Vortex on a platform vortexer for 3-5 minutes.
4. Incubate for 15 minutes.
5. Pellet the microparticles by spinning for 5 seconds in a microfuge.
6. Remove the liquid and discard.
7. Repeat the following steps three times:
    a. Add 1 ml of sterile water.
    b. Vortex for 1 minute.
    c. Allow the particles to settle for 1 minute.
    d. Pellet the microparticles by spinning for 2 seconds in a microfuge.
    e. Remove the liquid and discard.
8. Add sterile 50% glycerol to bring the microparticle concentration to 60 mg/ml (assume no loss during preparation).
9. Store the microparticles at room temperature for up to 2 weeks.

Appendix 2. Coating DNA onto microcarriers

The following procedure is sufficient for six bombardments; if fewer bombardments are needed, prepare enough microcarriers for three bombardments by reducing all volumes by one-half. **When removing aliquots of microcarriers, it is important to vortex the tube containing the microcarriers continuously in order to maximize uniform sampling.**

1. Vortex the microcarriers prepared in 50% glycerol (60 mg/ml) for 5 minutes on a platform vortexer to resuspend and disrupt agglomerated particles.
2. Remove 50 µl (3 mg) of microcarriers to a 1.5 ml microfuge tube.
3. While vortexing vigorously, add in order:

    5 µl DNA (1 µg/µl)

    50 µl $CaCl_2$ (2.5 M)

    20 µl spermidine (0.1 M)
4. Continue vortexing for 2-3 minutes.
5. Allow the microcarriers to settle for 1 minute.
6. Pellet the microcarriers by spinning for 2 seconds in a microfuge.
7. Remove the liquid and discard.
8. Add 140 µl of 70% ethanol without disturbing the pellet.
9. Remove the liquid and discard.
10. Add 140 µl of 100% ethanol without disturbing the pellet.
11. Remove the liquid and discard.
12. Add 48 µl of 100% ethanol.
13. Gently resuspend the pellet by tapping the side of the tube several times, and then by vortexing at low speed for 2-3 seconds.
14. Remove six 6 µl aliquots of microcarriers and transfer them to the center of a macrocarrier. An effort is made to remove equal amounts (500 µg) of microcarriers each time and to spread them evenly over the central 1 cm of the macrocarrier using the pipette tip. Desiccate immediately.

References

Armaleo, D., Ye, G.-N., Klein, T. M., Shark, K. B., Sanford, J. C. and Johnston, S. A., Biolistic nuclear transformation of *Saccharomyces cerevisiae* and other fungi, *Curr. Genet.*, **17**, 97-103 (1990).

Daniell, H., Krishnan, M. and McFadden, B. F., Transient expression of β-glucuronidase in different cellular compartments following Biolistic delivery of foreign DNA into wheat leaves and calli, *Plant Cell Rep.*, **9**, 615-619 (1991).

Hill, J. E., Myers, A. M., Koerner, T. J., and Tzagoloff, A., Yeast/ *E. coli* shuttle vectors with multiple unique restriction sites, *Yeast*, **2**, 163-167 (1986).

Jefferson, R. A., Kavanagh, T. A. and Bevan, M. W., GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants, *EMBO J.*, **6**, 3901-3907 (1987).

Johnston, S. A., Anziano, P. Q., Shark, K., Sanford, J. C. and Butow, R. A., Mitochondrial transformation in yeast by bombardment with microprojectiles, *Science*, **240**, 1538-1541 (1988).

Kempthorne, O., The Design and Analysis of Experiments, R. E. Krieger Publishing Co., Malabar, Florida (1983).

Klein, T. M., Fromm, M., Weissinger, A., Tomes, D., Schaaf, S., Sletten, M. and Sanford, J. C., Transfer of foreign genes into intact maize cells with high-velocity microprojectiles, *Proc. Natl. Acad. Sci. USA*, **85**, 4305-4309 (1988a).

Klein, T. M., Harper, E. C., Svab, Z., Sanford, J. C., Fromm, M. E. and Maliga, P., Stable genetic transformation of intact *Nicotiana* cells by the particle bombardment process, *Proc. Natl. Acad. Sci. USA*, **85**, 8502-8506 (1988b).

Klein, T. M., Gradziel, T., Fromm, M. E. and Sanford, J. C., Factors influencing gene delivery into *Zea mays* cells by high-velocity microprojectiles, *Bio/Technol.*, **6**, 559-563 (1988c).

Klein, T. M., Knowlton, S. and Arentzen, R., *Gene transfer by particle bombardment*, Plant Tissue Culture Manual, pp D1: 1-12 (Lindsey, K., ed.), Kluwer Academic Publishers, The Netherlands (1991).

Maniatis, T., Fritsch, E. F. and Sambrook, J., Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1982).

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., Klein, T. M., Wolf, E. D. and Allen, N., Delivery of substances into cells and tissues using a particle bombardment process, *Part. Sci. Technol.*, **5**, 27-37 (1987).

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).

Sanford, J. C., Smith, F. D. and Russell, J. A., Optimizing the Biolistic process for different biological applications, *Meth. Enzymol.*, (in press, 1992).

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).

Williams, R. S., Johnston, S. A., Riedy, M., DeVit, M. J., McElligott, S. G. and Sanford, J. C., Introduction of foreign genes into tissues of living mice by DNA-coated microprojectiles, *Proc. Natl. Acad. Sci. USA*, **88**, 2726-2730 (1991).

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).

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