

Genetic Manipulation of Chloroplast

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Chloroplast genetic system

- A 50-290 kb double stranded circular molecule
- A pair of 20-30 kb inverted repeat (IR) sequence

Chloroplast transformation

- Expression level of foreign genes is higher than nuclear transformation
- Multiple genes can be introduced as an operon

Stable transplastomic plants

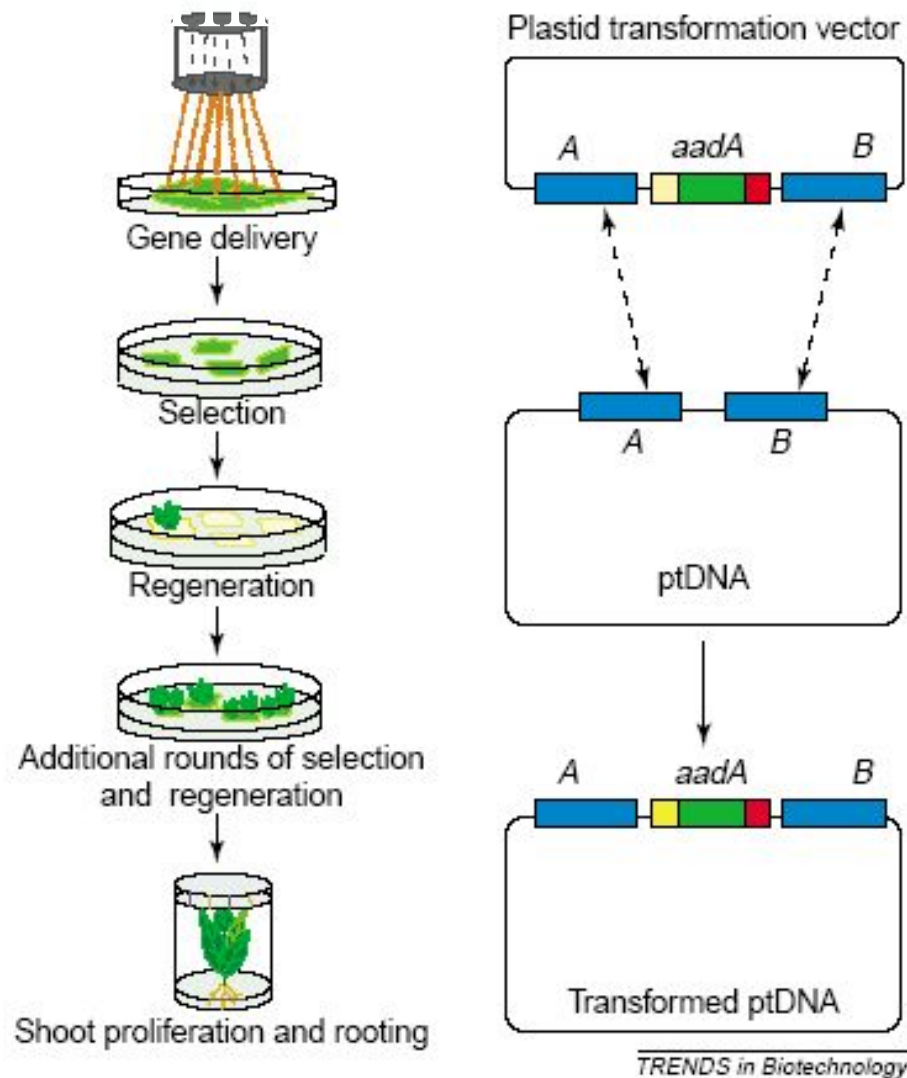
- Transformation of plastids has already been achieved for tobacco , *Arabidopsis*, soybean , cotton, lettuce, cauliflower and potato

Plastid transformed plants

Table I. Plant species for which DNA delivery to plastids

<i>Species</i>	<i>Delivery method</i>
Arabidopsis	Biolistic
Carrot	Biolistic
<i>Nicotiana plumbaginifolia</i>	Protoplast
Tobacco (NT1 suspension cells)	Biolistic
Marigold	Biolistic
Potato	Biolistic
Red pepper	Biolistic
Rice	Biolistic
Tobacco (Petit Havana, Xanthi)	Biolistic, protoplast

Basics of Chloroplast Transformation



Chloroplast Transgenic Production

Chloroplast transformation techniques

- ❑ Biolistic delivery systems
- ❑ Polyethylene glycol (PEG) treatment of protoplast
 - The technique has a lower success rate than biolistic bombardment
 - long selection times required after initial DNA delivery
 - Technically demanding and requires specialized tissue culture skills
- ❑ Femtoinjection technique: injection of DNA material into chloroplasts using syringes with extremely narrow tips
- ❑ *Agrobacterium-mediated plastid* transformation

Particle Delivery System



Advantages and disadvantages of biolistic method

- Relatively high efficiency
- Technical simplicity

Advantages of injection technique

- Cells survive the injection
- Transformed cell can be spotted easily
- Cellular context remains intact

<https://www.youtube.com/watch?v=dX3jmX7qBlw>

Thank You