PTC - C11 (Genetic Transformation) Cheat Sheet

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Introduction

Genetic transformation is a process that involves the introduction and expression of foreign genes in a host organism

This expression can result from the extrachromosomal, or episomal, presence of genes in nuclei that may persist if the introduced DNA has a mechanism for replication

Genetic Transformation Methods

1. Using Calcium Phosphate
2. Microinjection
3. Lipofection
4. Electroporation
5. Bombardment
6. Polyethylene glycol (PEG)-mediated transformation
7. Agrobacterium mediated transformation

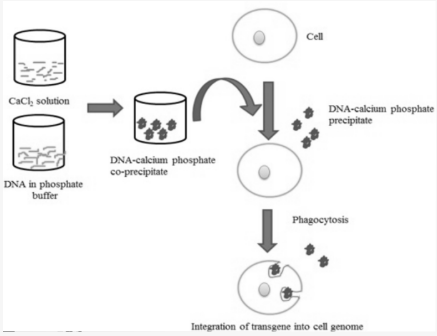
1. Calcium Phosphate

-HEPES-buffered saline solution is mixed with a calcium chloride solution containing DNA for transfection to form a fine precipitate of calcium phosphate with DNA.

-The suspension of the precipitate is then added to the monolayer of cells.

-The cells take up the calcium-phosphate-DNA complexes by endocytosis and express genes.

Calcium Phosphate Transformation

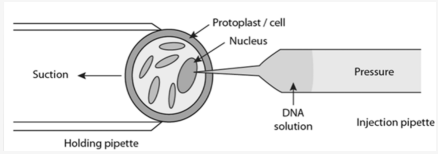


2. Microinjection

-DNA is directly injected into the nucleus using a fine glass capillary under a microscope.

-However this method acquire a great effort as each and every cell has to be injected individually.

Microinjection Process



3. Lipofection

-use of cationic lipids for DNA transfection into mammalian cells

-safer than viral vectors

-can be produced in large quantities

-can deliver large DNA fragments of up to several megabase pairs long into cells

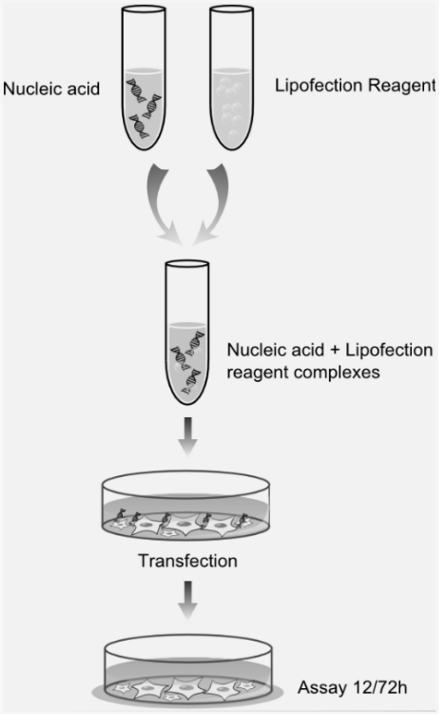
-There are many formulations of lipid reagents for transfection, but they normally contain a positively charged moiety attached to a neutral lipid component.

a) On mixing of these reagents with DNA, the charged head groups are drawn towards the phosphate backbone of DNA and form lipid-DNA complexes.

b) When the suspension of these complexes is added to the cells, the positively charged head groups of the lipid are attracted to the negatively charged cell membrane.

c) The end-result is that the lipid-DNA complex is either fused to the cell membrane or enters the cell by endocytosis, transferring its DNA load into the cell.

Lipofection Process



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4. Electroporation

-Host cells and selected molecules are suspended in a conductive solution, and an electrical circuit is closed around the mixture.

-An electrical pulse at an optimized voltage and only lasting a few microseconds to a millisecond is discharged through the cell suspension.

-This disturbs the phospholipid bilayer of the membrane and results in the formation of temporary pores.

-The electric potential across the cell membrane simultaneously rises to allow charged molecules like DNA to be driven across the membrane through the pores

-This technique can used for protoplast, intact cell & tissue (callus culture, immature embryos, influorescence material)

-Efficiency is depend on condition of plant and tissue treatment conditions chosen.

-Linear DNA may can improve efficiency of electroporation

Process

1. Material incubated in a buffer solution containing DNA and subjected to controlled, millisecond high-voltage electrical pulses 100-200 V for 1-2 ms

2. High-voltage- induce transient pore in the cell membrane and allow DNA migrate through plasma membrane and integrate with genome.

3. After pulsing, cell membrane reseals and left unharmed.

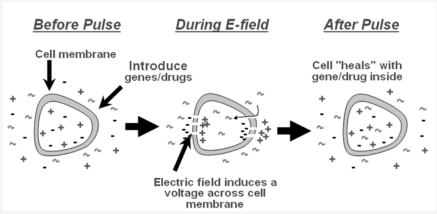
4. Plant materials may require pre- and post-electroporation incubation in buffer of high osmotic pressure.

Advantages	Disadvantages
1. Produced transformants with low transgene copy numbers	1. Low efficiency; requires careful optimization
2. High deliver rate	-
3. Transformed cells will not damage due to transformation	-

*Protoplasts are cells stripped of their cell walls and maintained in culture

*Transgene copy numbers is defined as the number of exogenous DNA insert(s) in the genome.

Electroporation Process



5. Bombardment

Principle: Using a gene gun directly shoot a piece of DNA into recipient plant tissue.

Also known as: Biolistics, Particle bombardment, Microprojectile bombardment, Particle inflow gun

-Particles should be high enough mass in order to possess adequate momentum to penetrate into plant cell and achieve particle delivery to plant cells

-Metals should be chemically inert to prevent adverse reaction with DNA and cell component. Eg. gold, tungsten, palladium, rhodium, platinum and iridium

-Plant cell are competent cell for transformation

-After bombardment, cells require a “healing” period under special condition of light, temperature, and humidity.

Process

1. Separation of the protoplast from leaf

2. DNA-coated microcarriers are loaded on the macrocarrier

3. Microcarriers are shot towards target tissue during helium gas decompression.

4. A stopping screen placed allowing the DNA-coated microcarriers to pass through and reach the target.

5. Transfer to the solid media

6. Transfer of the transgenic plant in a greenhouse

Advantage

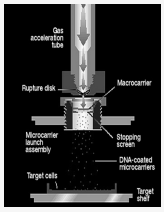
1. Unlimited host range

2. Not limited by ability to regenerate from single cells

3. Immature embryos from seeds will continue to develop

4. Transgenic plants selected

Bombardment Process



6. PEG-Mediated Transformation

PEG: Polyethylene glycol

-Transformation of naked DNA done by treatment with PEG in presence of divalent cations

-PEG and divalent cations destabilize the plasma membrane of plant protoplast and render it permeable to naked DNA.

Advantage	Disadvantage
1. simple and efficient, allowing a simult-aneous processing of many samples	1. Plant protoplasts are not easy to work with, and the regeneration of fertile plants from protoplasts is problematic for some species.
2. yields a transformed cell population with high survival and division rates	2. The DNA used is also susceptible to degradation and rearrangement.
3. helps to overcome a hurdle of host range limitations of Agrobacte-rium-mediated transformation.	

7. Agrobacterium-mediated Transformation

-ability of an organism to transfer its T-DNA into the host cells effici-ently

-components: T-DNA present on the plasmid called Ti (tumor-in-ducing) plasmid along with other functional components like virulence (vir), conjugation (con), and origin of replication (ori); T-DNA consists of 25 bp repeats that end at the T-region & virulence (vir) region composed of seven major loci

-transfer of a piece of plasmid by the bacteria into the plant cells during infection

-plasmid then integrates into the nuclear genome in order to express its own genes and affect the hormonal balance in the host cell

-bacteria also produce a number of enzymes that are involved in the synthesis of opines that is then used by the bacteria as nutrients

7. Agrobacterium-mediated Transformation (cont)

Bacterial Infection Process:

1. entry of the bacteria through wounded sites

2. The binding of bacteria to the plant cells is enhanced by the release of phenolic acetosyringone (AS) by the injured plant cells

3. The AS activates the VirA proteins on the bacteria, which activates VirG via phosphorylation of its aspartate residue.

4. The activated form of VirG then binds to other vir genes, inducing their expression. VirD activated by this process stimulates the T-strand generation (a single-stranded copy of the T-DNA).

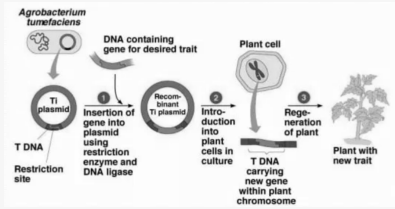
5. The VirD2 covalently binds to the 5’ end of the T-strand as the 5’ end is the leading end during the transfer. Other factors like VirE2 and VirB proteins also bind to the T-strand, forming a T-complex.

6. The complex is then passed into the nucleus by the nuclear target signals released by the Vir proteins.

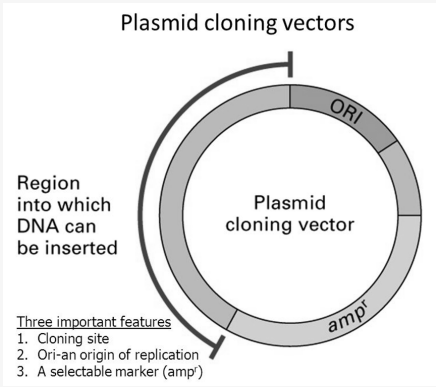
7. T-DNA strand is integrated into the plant genome randomly as either a single copy or multiple copies

8. The integration usually occurs in the transcription active or repetitive regions of the genome by the process of recombination.

Agrobacterium-mediated transformation



Plasmid Cloning Vectors



Agrobacterium-mediated Transformation of Tobacco

5 basic protocols used for any Agrobacterium-mediated transformation in tobacco

1. Suitable tobacco plant tissue	-in this case leaves must be removed from a donor plant and sterilized to be used as explants source.
2. Co-cultivation	-Cutting the leaf tissue into smaller pieces, placing it into culture of Agrobacterium for approximately 30 minutes.
	-During this incubation period, the bacteria will attach to the plant cells.
	-Remove the explants and blot the excess bacterial culture off and then place into solid Murashige and Skoog (MS) medium with no selective agent.
3. Incubate MS medium with the explants for 2 days - T-DNA can be transferred to plant cells.	-
4. Remove explants from the medium and wash in antibiotic solution to kill the Agrobacterium cells.	-
5. Transfer explants to fresh solid medium with a few selective agents	-(kanamycin) So that growth of non-transformed plant cells can be inhibited
	-(cefotaxime) So that growth of any extra surviving Agrobacterium can be killed.
	-Auxins and cytokinins added.

Genetic Transformation Screening

1. Blue white screening

-DNA of interest is ligated into a vector. The vector is then transformed into competent bacterial cells. The competent cells are grown in the presence of X-gal. If the ligation was successful, the bacterial colony will be white; if not, the colony will be blue. This technique allows for the quick and easy detection of successful ligation

2. Restriction enzyme screening

-First, restriction mapping should be performed to identify which restriction enzymes can be used to easily identify the presence of your insert within the plasmid. After isolating a plasmid DNA from an overnight bacterial culture, digest the purified plasmid DNA from recombinant clones using restriction enzymes. Once digested, run the plasmid on an agarose gel to verify that the vector backbone and insert are of the expected sizes

3. Antibiotic resistance screening

-After transformation, cells are grown in a medium containing the said antibiotics to screen out transformants carrying antibiotic resistance gene and gene of interest.

Reasons for screening after gene transformation

1. To identify transformants with the gene insert of interest from those without gene insert of interest in the vector transformed into the host

2. To identify for sense and antisense gene insert in the vector transformed into the host

3. To identify host that expresses the gene of interest from those that does not expresses the gene of interest