Model Answers
Int UG/PG V Sem Examination 2013-14
Biotechnology Paper I (Recombinat DNA Tech)

Answer to Question 1 (Objective Type):

(i) (d)
(ii) (b)
(iii) (a)
(iv) (a)
(v) (d)
(vi) (d)
(vii) (a)
(viii) (b)
(ix) (d)
(x) (b)

Subjective/Long Answer Question

Qn2. : List of vectors used in RDT with suitable Examples:

Definition: Vectors are used in RDT to clone, amplify and express the genes (fragments of genomic DNA or cDNA) into a suitable host cells.

A number of different types of vectors have been constructed based on the desirable properties like, low molecular weight DNA, an origin of replication (***ori***), a multiple cloning site, preferably in the reading frame of the selectable markers so that the recombinants may be selected on the basis of insertional inactivation of the markers. The expression vector additionally contains a strong promoter, upstream from the selectable marker, to drive the expression of a gene cloned downstream.

Sopme of the common clong/expression vectors used in different organisms are described as follows:

(A) Bacterial plasmids as vectors

(a) pBR322: The first artificial cloning vector constructed for *E.coli* (size: 4363 bp) by Bolivar & Rodriguez (1975), having two selectable markers (amp^R and Tc^R) running in opposite orientation. Recombinants may be selected by insertional inactivation of either amp^R or Tc^R genes. The vector may also be used for detecting promoter like sequences using HindIII site which lies in the promoter of Tc^R.
(b) pUC 8-19: A series of vectors under the name pUC, having lacZ gene as the selectable marker and a multiple cloning site in the reading frame of lacZ so that the recombinant may be screened using blue-white screening.
(c) Ti Plasmid: The tumor inducing Agrobacterium tumefaciens plasmids are natural genetic engineer for plants. The Ti plasmids have been modified suitably to clone DNA to transformed in plants to improve

their traits.

(B) Viral/Bacteriophage vectors:

A number of improved vectors have been designed using the manipulated lambda phage DNA, based on insertion or replacement of fragment. The vectors thus developed may accommodate fragments averaging 12-15 Kb.

Cosmids : These are developed by combining the cos site along with adjacent genes for head, tail, packaging and lysis and bacterial plasmid so that the manipulated DNA may be packaged in phage head *in vitro* and transfected to suitable E.coli strains. Cosmids are particularly useful for cloning fragments averaging 35-45 Kb for the construction of genomic DNA library.

(C)Yeast Cloning Vectors: These vectors have been designed to clone eukaryotic genes in an eukaryotic hosts. This is particularly useful in cloning very long DNA and in the light of post-transcriptional and post-translational modifications of expressed products. The common vectors for yeasts are YIP, YCP, YEP etc.

(D) Animal Cloning Vectors: A number of viral vectors have been designed to transfer genes in animal model systems, mostly use the helper virus for transfection.

Qn 3: Western Blot and its application:

Western blot is molecular technique based on the antigen (protein) and antbody interaction on a solid support (nitrocellulose/nylon filters). The method is based on the fact that every protein is antigenic and may be detected if antibody specific to the protein is available.

The protocol of Western Blot may be described as follows:

Cells grown in culture→Centrifuge at 10k→Pellets/cells disruption or homogenisation→DNAase/RNAase treatment→Protein extraction and purification→Loading on SDS-PAGE→Electrophoretic run→Protein bands visualised on gel by staining with coommassie blue. A parallel gel is used for transblotting on nitrocellulose/nylon filter, as the stained gel cannot be used for hybridization.

The filter containing protein bands are dipped in the solution of antibody prepared in suitable buffer. The signals produced by immuno-reactions may be visualized by gel doc.

Application:
(a) Detection of proteins used as marker in disease diagnosis
(b) Location of specific protein expressed in tissues under different conditions
(c) Testing the auto-immunological disorders etc

Q 4. What is ultrasonication technique and for what purpose it is used?

Answer:
Ultrasound has been used for diagnostic imaging in clinical fields without producing any significant adverse effects. This method also have considerable potential for the introduction of macromolecules like DNA into cells of both plant and animals. Ultrasonication can alter the transient permeability of plasma membrane to facilitate uptake. Compared to other direct DNA delivery methods, such as particle gun bombardment, electroporation and microinjection..

Procedure:

- Immersion of cells/tissue in sonication buffer containing the plasmid
- Sonicated with an ultrasonic pulse generator at 0.5w/cm2 acoustic intensity for 30 mins
- Samples were rinsed in a buffer solution
- Cultured for growth and differentiation

Factors affecting the method:

- Cell density
- Cell status
- pH of the buffer
- Ultrasonic pulse
- Time intensity

Application:

- Mild ultrasound irradiation has been proved an efficient method for transfection in animal cells and tissues in vitro and in vivo.
- The use of ultrasound has been described for stimulating uptake of foreign DNA by plant protoplast and also leaf segments
- Ex- Development of herbicide resistance Potato using bar gene.
- This method is also used for extraction of secondary metabolites
- This is also used for nanoparticle dispersion
- Used for gene therapy

Merits and demerits

- This technique has the advantages of being simple, inexpensive and multifunctional equipment.
- One can use standardized conditions there is no requirement of tissue culture expertise
- The ultrasound treatment may be simpler to carry out, however, could cause cell damage or ever rupture, so it is important to optimize the conditions for uptake without causing damage to the cells.

Q 5. Explain how plant viruses are used as episomal expression vector with suitable examples.

Answer:

- Viral vectors are episomal vectors as its genome doesn't integrate into plant genome, therefore they have high copy number per cell and they are not subjected to the "position effect". The gene product is very rapidly accumulated. Viral genome sequences are excellent source of promoters, enhancers and other components useful for designing gene vectors. Virus is systemically spread in the plant body. Generally have Broad host range, virulence, easy mechanical transmission and seed transmission. Carry additional genetic information within the packaging limit. Viruses that are rod shaped or have multipartite genome or

contain a helper or satellite component offer potential for carrying extra genetic material

Types of Viruses

Basically three types of viruses were used as the episomal vector for transfer of gene in plant, these are

1. Caulimoviruses 2. Gemini viruses 3.Tobacco mosaic virus

1. Caulimoviruses

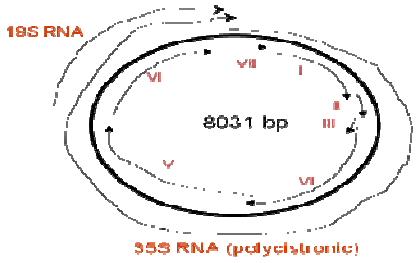
- CaMV (Cauliflower mosaic virus) is the most commonly used viral vector used for transfer of gene to the targeted plant.Several properties of the cauliflower mosaic virus (CaMV) indicate that it could provide a useful vector for gene transfer in higher plants:

(1) It has a relatively small double-stranded genome that can be easily manipulated *in vitro*;

(2) cloned viral DNA is infectious when rubbed onto healthy leaves

(3) virus spreads throughout the plant and can be found in most cells at high copy number.

(4) Two regions of the CaMV genome—open reading frames (ORFs) II and VII—do not seem to be essential for infection, as both can be either deleted or expanded by small inserts of foreign DNA



Gene I: plasmadesmata movement, Gene IV: translation transactivation, Gene V: reverse transcriptase, Gene III/IV: assembly, Gene II/VI: inclusion bodies

Examples: Expression of DHFR in plant

Likely explain the other types of viruses in short on the following head

-its genomic composition

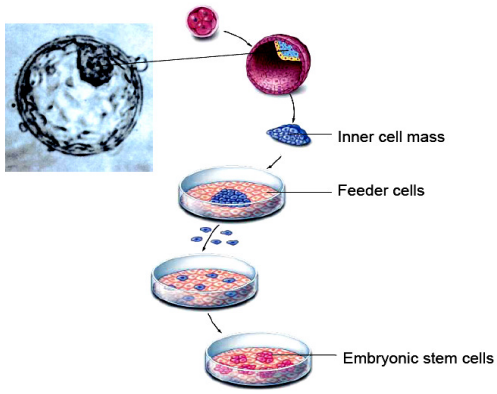
-mode of infection

Qn 6: What are embryonic stem cells and how are they derived? Describe the embryonic stem cell transfer technology for the production of transgenic mice and discuss its various applications and limitations.

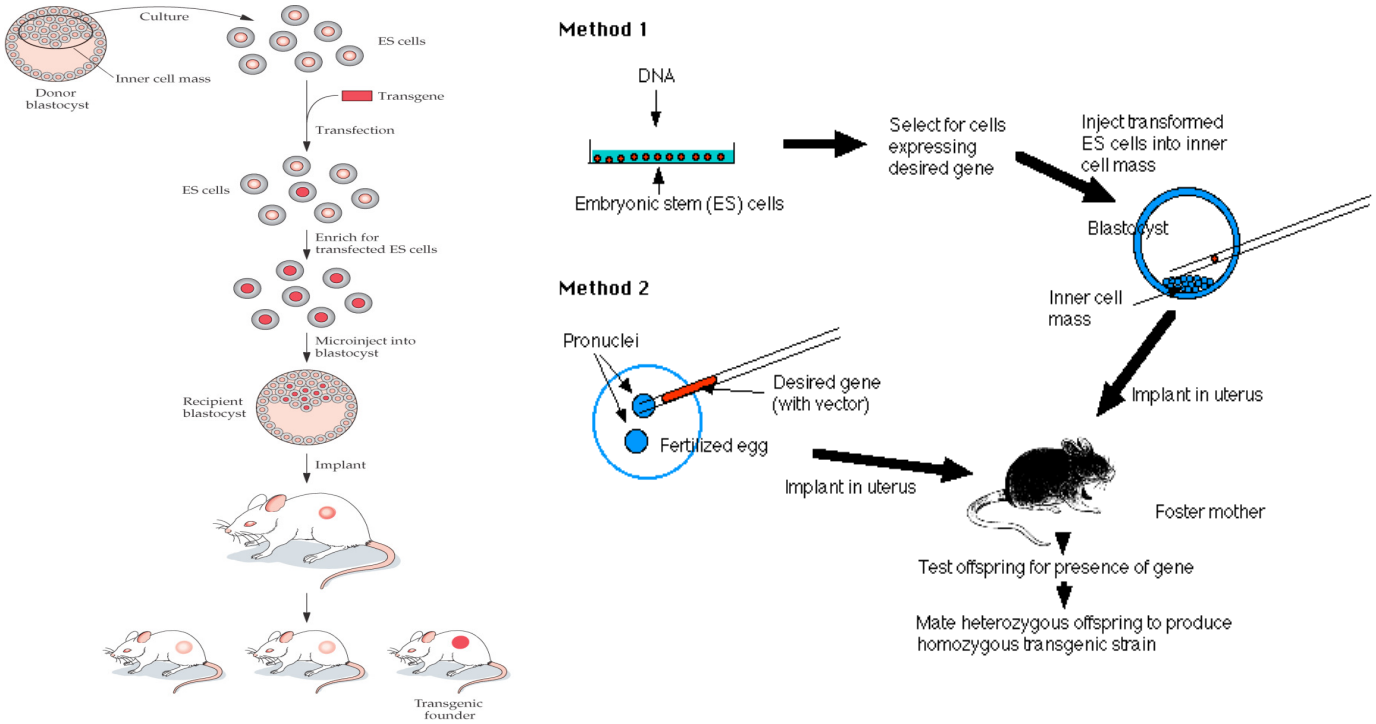
Answer:

Embryonic stem cells and their derivation:

Embryonic stem cells are derived from embryos at a developmental stage before the time that implantation would normally occur in the uterus. Zygote becomes blastocyst (embryonic stage) at day 5-6 after several divisions. Blastocyst has clear cut trophoectoderm and inner cell mass. Cells of Inner cell mass isolated and cultured in controlled condition known as as embryonic stem cells. These cells are pluripotent in nature which can be differentiated into any cell type of three germ layers. These cells are derived by microinjection method from inner cell mass and cultured on gamma-rays irradiated fibroblast feeder layer.

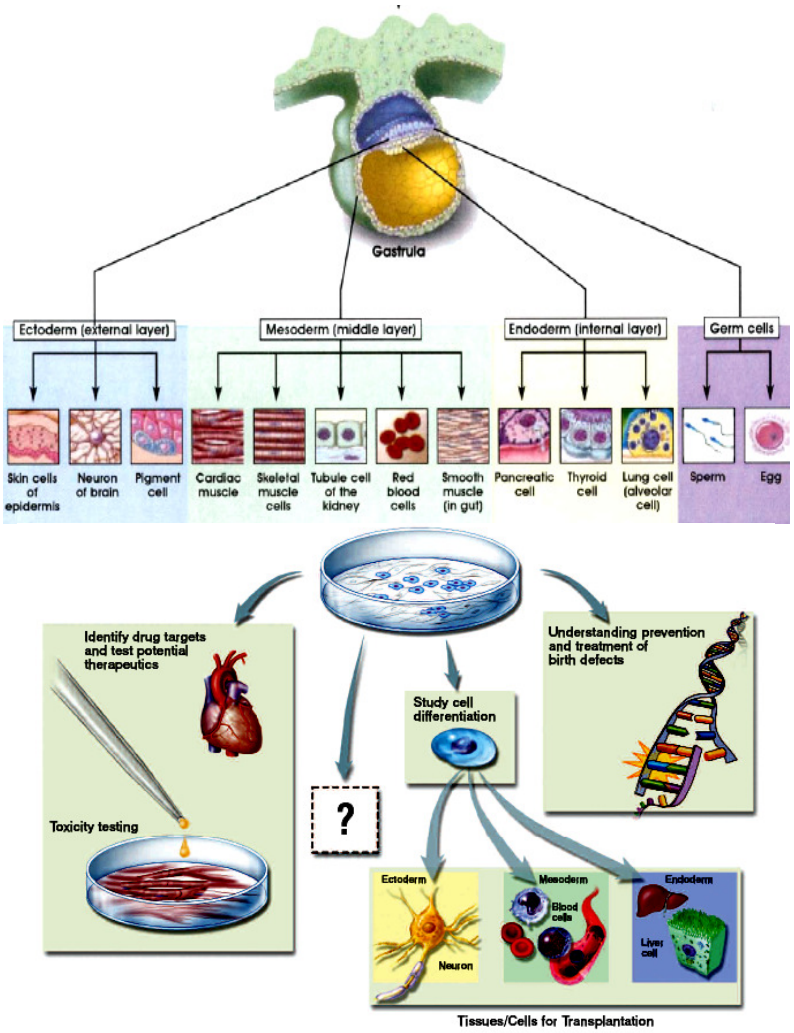


Embryonic stem cell transfer technology for the production of transgenic mice:



Application and limitation of embryonic stem cells:

1. Embryonic stem cells are pluripotent in nature and these cells can be differentiated into any cells type and thus can be used for regenerative medicine.
2. Embryonic stem cells are used for regenerative cloning and therapeutic cloning.
3. Embryonic stem cells are used for transgene transfer and gene targeting by Cre-loxP system.
4. Embryonic stem cells are also used to study mechanism of organism development, cell differentiation, ageing, maintenance of cell integrity etc. There are various other applications depicted in following figure.



Limitations:

1. There are social and ethical concerns in using embryonic stem cells.
2. There are strong chances of graft rejection after transplantation of embryonic stem cells
3. There are chances of teratoma formation with embryonic stem cells.
4. Use of embryonic stem cells needs longer time and expertise.

Qn. 7 : Short notes:

(i) <u>Structural Features of Linkers and Adapters</u>

<u>Linkers:</u> These are chemically synthesized small decameric DNA molecules containing internally located site for a particular enzyme, as exemplified below:

GG**GGATTC**CC
CC**CCTAAG**GG BamH! Linker

Linkers are added to the two ends of blunt ended DNA using T4 ligase followed by restriction digestion by BamHI to create cohesive termini compatible with BamHI digested fragments.

Adapters : These are chemically synthesized small DNA molecules having one blunt end and one pre-formed cohesive end for a particular Restriction enzyme. The 5' cohesive end is dephosphorylated to prevent dimerization.
Adapters are used are particularly useful when the blunt ended DNA to cloned contanms internally located site for the same enzyme by which vector has been linearlized.
The adpters are ligated at the two ends using T4 ligase followed by treatment with enzyme polynucleotide kinase in presence of dATP.

(b) Artificial Insulin :

Modern biotechnology began when recombinant human insulin was first marketed in the United States in 1982. The effort leading up to this landmark event began in the early 1970's when research scientists developed protocols to construct vectors, by cutting out and pasting pieces of DNA together to create a new piece of DNA (recombinant DNA), that could be inserted into the bacterium, Escherichia coli (transformation).
If one of the pieces of the new DNA included a gene which produced a protein enzyme that broke down a particular antibiotic, the bacterium would be resistant to that antibiotic and could grow in a medium containing it. To the piece of DNA that conferred resistance of Escherichia coli to a particular antibiotic was added the human gene for the making of insulin. If this recombinant DNA containing the human insulin gene was used to transform Escherichia coli,and the bacteria were plated on an agar plate containing the antibiotic, the bacteria that grew contained not only the antibiotic resistant gene but also the insulin gene.
Additional new pieces of DNA were then added to promote the expression of the human insulin gene so that this new recombinant DNA (expression vector) could be used to transform Escherichia coli. Thus, large quantities of human insulin messenger RNA were formed, which in turn were translated into large quantities of the human insulin protein which could then be harvested from the medium in which the transformed Escherichia coli was grown. This insulin could then be marketed as the human derived insulin that is on the market today. Various types of insulin (Regular, NPH, Ultralente, Lente, Glargine etc. could be made by changing the DNA vector which was inserted into the E. coli bacteria or modifying the insulin once it was produced. This would change the structure of the insulin protein molecule which would result in longer or shorter acting insulins.

(iii) Northern Blotting and its significance:

Same as Western Blotting but this technique was developed by Alwine et al for detection of RNA using radioactive cDNA as the probe by X ray auto radiography.

The hybridization experiment is performed using nitrocellulose/nylon filter containing blotted RNA followed by detection with radiolabeed cDNA.

This is particularly useful for detecting the level of expression of transcripts of any gene under different environmental (physical/chemical) conditions.