Obtaining DNA for transgenesis

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Obtaining DNA for transgenesis

There are two ways of isolating a gene:

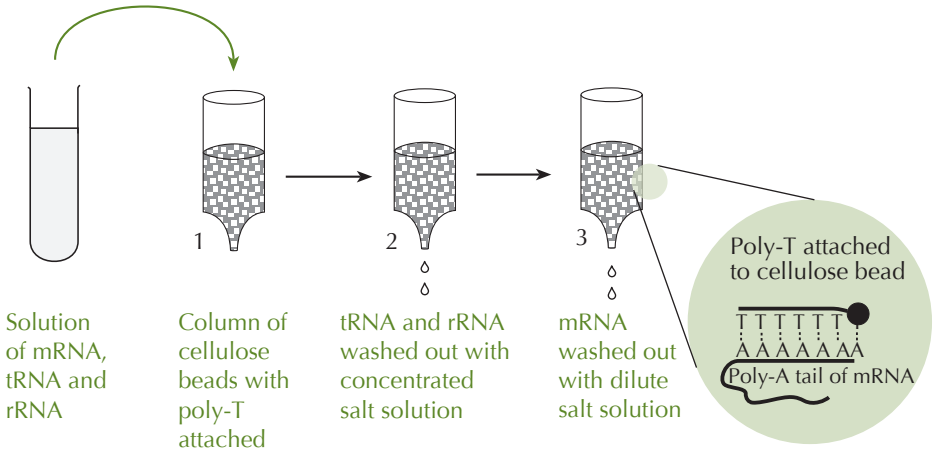
- indirectly by making the DNA for the gene from its mRNA
- directly from the DNA.

Genes from mRNA for transgenesis

The most frequently used method is to extract mRNA and produce a DNA copy using reverse transcriptase. This method has two important advantages:

- it avoids the problem of introns, since these are not present in the final mRNA product
- the various kinds of mRNA represent copies of only part of the genome, so the desired gene represents a higher proportion of the cDNA than it would of the total genome.

Though mRNA is only a small proportion (about 1%) of the total RNA in a cell, it can be extracted using the fact that most eukaryotic mRNA has a poly-A tail on the 3′ end. A poly-T nucleotide is added to the surface of a column of cellulose or other inert material. A solution containing the total cellular RNA is then poured through the column. The poly-A tails of the mRNA bind to the poly-T nucleotide, the rest of the RNA passing straight through. The mRNA is then removed by chemical treatment.



Extracting mRNA using poly-T

The mRNA is used to make cDNA. The DNA fragments are spliced into a suitable cloning vector and amplified by introducing them into a host bacterium. Many different clones will be produced, only a tiny proportion of which contain the gene of interest. Between them, these clones contain copies of all the genes that were transcription products active in the tissue from which the mRNA had been extracted. Collectively, these clones constitute transcription products of the active genes of the donor tissue and hence represent a cDNA library.

A cDNA library represents only a small part of the total (genomic) DNA. Some genes are transcribed by all the cells of the body. These 'housekeeping' genes are those coding for the enzymes concerned with basic functions such as respiration and protein synthesis. Others are only translated in particular kinds of cell (e.g. the genes for haemoglobin are only active in developing red blood cells from the bone marrow).

Obtaining a gene directly from the DNA for transgenesis

This involves extracting the entire genome and is thus a more indiscriminate method than the mRNA method. If promoter and other controlling sequences are required, this method must be used since these are not transcribed into mRNA.

The DNA is extracted and digested by restriction enzymes to yield many different fragments. These are then cloned as described for cDNA. The resulting **genomic DNA library** is considerably larger than a cDNA library.

Finding the right gene for transgenesis

Having obtained either a cDNA or genomic DNA library, the next step in transgenesis is to locate the desired gene.

The result of cloning a gene is an agar plate containing a large number of clones. If a plasmid is used as a cloning vector, the clones take the form of bacterial colonies. If a bacteriophage is used, the clones will be plaques on a 'lawn' of host bacteria.

Only a tiny proportion of these clones will contain the desired gene. The most common way of finding it is to use a **gene probe**. If bacteriophage is used as the vector, the essentials of the technique are similar.

A gene probe is a length of nucleic acid with a base sequence complementary to at least part of the gene sought. Each probe can thus base-pair with only DNA containing a complementary base sequence. The probe has to be long enough to contain a unique base sequence that will only pair with the DNA of interest.

A 20-base sequence is sufficient.

There are several ways of preparing a DNA probe.

- Extracting an mRNA copy of the gene and using reverse transcriptase to make a DNA copy. mRNA is most easily extracted from cells specialised for making a very small number of proteins, in which there are correspondingly few types of mRNA.
- Determining the sequence of seven or eight of the amino acids of the protein, and using the genetic code to deduce the DNA base sequence. A short length of DNA is then made artificially (degeneracy of the code means several alternative sequences may have to be tried).

A probe that has found its target (its complementary sequence) must be identifiable. This is made possible by first making the probe radioactive by labelling the 5′ end with ^{32}P.

A piece of nitrocellulose filter or nylon is gently pressed on the agar containing the colonies. The membrane is then pierced through to the agar to provide orientation marks for subsequent alignment of positive hybridisation signals. The membrane is then carefully lifted off. Some of the bacteria adhere to the filter, producing an exact replica of the pattern of colonies.

1. The bacteria are then lysed (broken open) chemically to free the DNA.

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2. The DNA is then denatured by alkali to make it single-stranded.

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3. The probe is added and allowed to hybridise with the DNA on the filter.

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4. The filter is then washed to remove any unbound probe.

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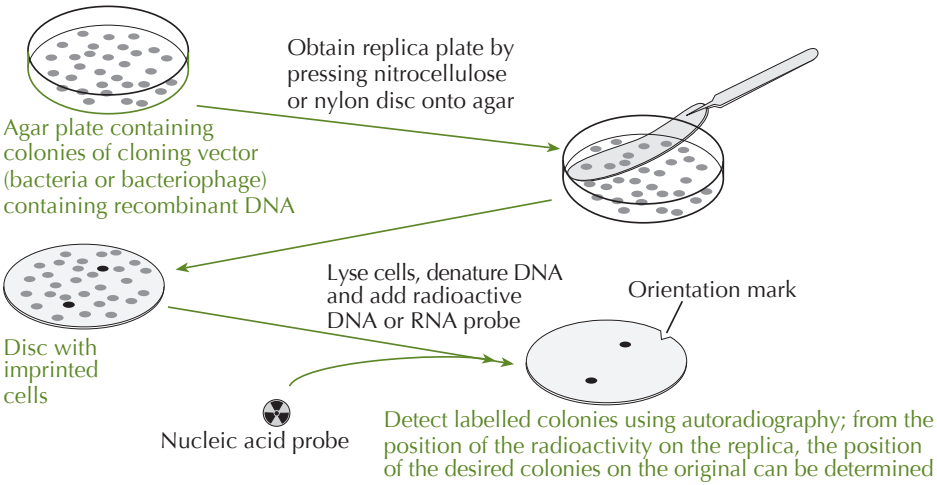
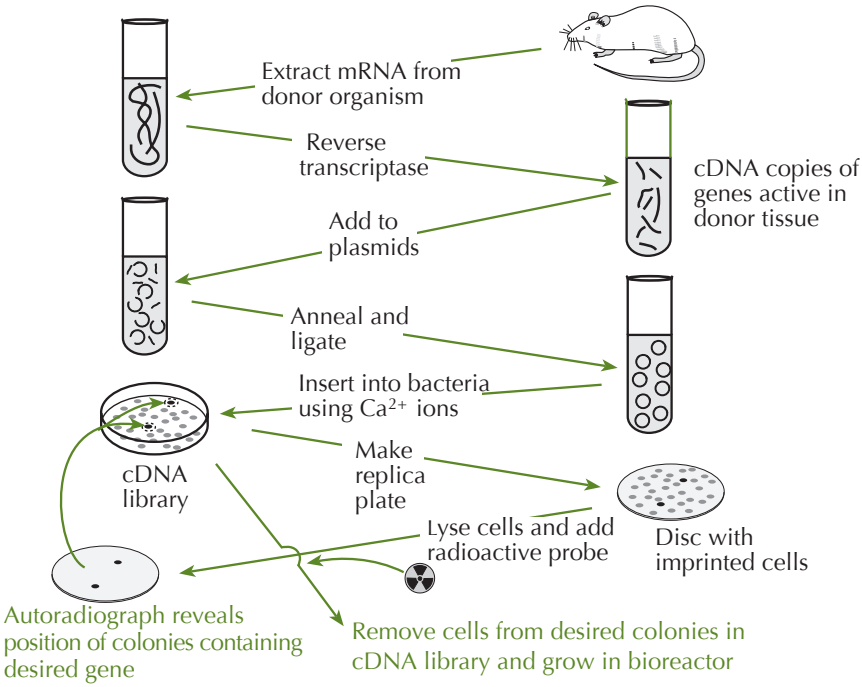
5. The position of the bound probe is then revealed by leaving it in contact with X-ray film. After development, the position of the hybridised probe is revealed, and the colonies on the agar carrying the DNA fragment of interest therefore identified.



Using a gene probe to find the desired gene

An alternative to using a gene probe is to use an antibody to the protein product of the gene. The method is similar to that shown for a gene probe except that the nitrocellulose replica plate is treated with radioactively labelled antibody. An autoradiograph enables the colonies producing the protein of interest on the original plate to be identified.



Summary of the stages in transgenesis, starting from mRNA