Start with the desired DNA

Recombinant DNA joins DNA from different sources. Genetic engineering deliberately changes genetic material to alter characteristics; transferring a gene is one way to do this.

1. THREE WAYS TO OBTAIN A GENE

A. Donor DNA	Isolate a DNA region from an organism. A eukaryotic genomic gene may include introns.
B. Donor mRNA	Use reverse transcriptase to make complementary DNA (cDNA) from an expressed, processed mRNA.
C. Chemical synthesis	Assemble a chosen DNA sequence from nucleotides when the required sequence is known.

2. WHY USE PROCESSED mRNA?

Processed mRNA
introns removed

→

First cDNA strand
reverse transcriptase

→

Double-stranded DNA
DNA polymerase

After the RNA template is removed, DNA polymerase can make the complementary DNA strand. DNA copied from fully processed mRNA lacks introns, which bacteria generally cannot splice out of a eukaryotic transcript.

3. A CODING SEQUENCE IS NOT THE WHOLE SYSTEM

A cDNA copy does not include the original DNA promoter upstream of the gene. The transferred sequence must be placed under suitable expression control in the new host. Carrying the DNA and producing its protein are different outcomes.

Recombinant DNA: National Human Genome Research Institute (NHGRI).
cDNA and reverse transcription: NHGRI glossary.

Cambridge Biology 9700 syllabus: 19.1.1-7, p. 47.

Cut, pair and seal

Restriction endonucleases recognise specific DNA base sequences and cut the sugar-phosphate backbones. Some produce sticky ends; others produce blunt ends without overhangs.

1. STAGGERED CUTS CAN MAKE STICKY ENDS

Example: EcoRI recognition site

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5' ...   G|A A T T C   ... 3'
3' ...   C T T A A|G   ... 5'
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Vertical bars mark cuts in the two strands. Separating the products leaves complementary single-stranded overhangs. EcoRI produces a four-base 5' overhang: AATT.

The strands are antiparallel. Read a recognition sequence with its strand direction, not by treating both lines as 5' to 3'.

2. BASE PAIRING ALIGNS THE FRAGMENTS

Compatible sticky ends on the vector and insert can pair by complementary bases, forming hydrogen bonds. Cutting both with the same suitable restriction enzyme is one way to produce compatible ends. Pairing alone does not seal the DNA backbone.

3. LIGASE MAKES THE JOIN PERMANENT

DNA ligase forms phosphodiester bonds between adjacent DNA ends in the sugar-phosphate backbone. It seals the aligned vector and insert to form recombinant DNA; it does not join the fragments by adding hydrogen bonds.

Keep enzyme roles separate: reverse transcriptase copies RNA into DNA; DNA polymerase synthesises DNA; restriction enzymes cut; ligase seals.

EcoRI recognition site and cleavage: New England Biolabs (NEB).
DNA ligase and phosphodiester bonds: NEB.
Restriction-based cloning: Addgene, Molecular Cloning Techniques.

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A vector carries the gene

A plasmid is a small DNA molecule, usually circular, that can replicate independently of the bacterial chromosome. An engineered plasmid can act as a vector carrying an inserted gene into a host cell.

1. FEATURES HAVE DIFFERENT JOBS

Origin of replication	Allows the plasmid to be copied in a compatible host.
Insertion site	A chosen restriction site or cloning region where the DNA fragment is added.
Promoter	A DNA sequence recognised by the host transcription machinery; controls transcription of the gene.
Marker gene	Produces an identifiable trait used to select or screen cells carrying the construct.

The donor promoter may not work in the new host, so a suitable promoter may need to be transferred with the desired gene.

2. KEEP THE EXPRESSION SEQUENCE IN ORDER



Transcription proceeds in the appropriate direction.

3. TRANSFER, MULTIPLY AND EXPRESS

Join the insert to the vector, then introduce the recombinant plasmid into host cells: uptake by bacteria is called transformation. Selected cells can divide and copy the plasmid. With suitable control sequences, transcription and translation can produce the gene product.

Plasmid features and promoters: Addgene, Molecular Biology Reference.
Vector construction and gene cloning: Addgene, Molecular Cloning Techniques.

Cambridge Biology 9700 syllabus: 19.1.1-7, p. 47.

Check what actually happened

Not every host takes up a plasmid, and not every plasmid contains the intended insert. DNA uptake, the correct construct and gene expression require different evidence.

1. SELECTION IS NOT COMPLETE CONFIRMATION

A selectable marker such as antibiotic resistance allows cells carrying a functional marker to grow under selection. An empty vector can also carry that marker. Growth alone does not prove that the desired insert is present, correctly oriented or expressed.

2. FLUORESCENT MARKERS REPORT EXPRESSION

A marker gene can code for a fluorescent protein such as GFP. When it is transcribed and translated, the product fluoresces under suitable illumination. A reporter under the promoter being tested provides evidence that this promoter is active.

Interpret the design: an independent GFP marker confirms its own expression, not automatically the expression or function of another gene. Linking the reporter to the target expression system makes it informative about that system.

3. GENE EDITING: CHANGE A SPECIFIC SITE

Gene editing is genetic engineering that inserts, deletes or replaces DNA at selected sites in a genome. Unlike simply adding a gene on a plasmid, editing targets an existing genomic location; it need not introduce a gene from another species.

Use evidence matched to the claim: DNA analysis checks the sequence; a suitable reporter tests expression; testing the resulting protein or trait checks function.

Next: PCR, electrophoresis and DNA profiling.

Fluorescent reporters: Addgene, Green Fluorescent Protein (GFP).
Genome editing: National Human Genome Research Institute.

Cambridge Biology 9700 syllabus: 19.1.1-7, p. 47.