

Production of Human Insulin by Genetic Engineering

Recombinant DNA Technology for Therapeutic Insulin

Insulin is a 51-residue peptide hormone secreted by the beta cells of the pancreatic islets of Langerhans; it lowers blood glucose by promoting glucose uptake into cells, and its deficiency or ineffective action causes diabetes mellitus. Before genetic engineering, insulin for therapy was extracted from the pancreases of slaughtered cattle and pigs. Animal insulin works in humans, but bovine insulin differs from human insulin at three amino-acid positions and porcine insulin at one, and these small differences made some patients develop immune reactions (antibody formation, injection-site allergy, and gradually reduced effectiveness) after years of use. Supply also depended entirely on the meat industry - roughly two kilograms of pancreas glands were needed to obtain a few hundred milligrams of purified insulin. **Recombinant human insulin (marketed as Humulin) was approved in 1982, becoming the first genetically-engineered drug approved for human use;** it is manufactured in bacteria or yeast programmed with the human insulin gene, so the product is chemically identical to natural human insulin.

1. Advantages of Producing Insulin by Genetic Engineering

(a) Medical advantages

- **Identical to natural human insulin:** the recombinant product has exactly the human amino-acid sequence, unlike bovine or porcine insulin, which differ from human insulin by one to three residues.
- **Fewer immune reactions:** because the sequence is not foreign, patients are far less likely to develop anti-insulin antibodies, injection-site allergy, or the gradual loss of effectiveness seen with long-term use of animal insulin.
- **Consistent purity and potency:** a fermentation-based process gives batch-to-batch uniformity that is difficult to guarantee when extracting from variable animal tissue.

(b) Supply and economic advantages

- **Unlimited, scalable supply:** production depends on bacterial or yeast fermentation, not on the number of animals slaughtered, so supply can expand with demand.
- **Lower long-term cost at scale:** a single fermenter can replace the pancreas glands of a very large number of animals, improving the economics of large-volume production.
- **Independent of the meat industry:** production is not tied to animal agriculture, which also removes any religious or dietary objection to an animal-derived medicine.

(c) Safety advantages

- **No risk of animal-pathogen transmission:** because no animal tissue is used, there is no risk of transmitting animal viruses or prions through the drug.
- **Defined, controllable composition:** the chemical identity of the product is known precisely and can be verified analytically at every batch, simplifying quality control and regulatory approval.

(d) Technical/platform advantages

- **A reusable expression platform:** once a host organism is engineered to secrete insulin, the same recombinant-DNA approach can be adapted to make modified insulin analogues (for example, rapid-acting or long-acting insulins) by altering specific residues in the synthetic gene.

Key point

- The clinical case for recombinant insulin rests on identity to the natural human hormone, not merely on cheaper manufacturing - it is the amino-acid match that removes the immunogenicity problem seen with animal-derived insulin.

2. Structure of Human Insulin

Mature insulin is made of two short polypeptide chains: the **A chain** (21 amino acids) and the **B chain** (30 amino acids), held together by two inter-chain disulfide bonds (A7-B7 and A20-B19); a third, intra-chain disulfide bond (A6-A11) stabilises a loop within the A chain. Inside the pancreatic beta cell, insulin is first made as a single-chain precursor, **proinsulin** (signal peptide - B chain - connecting or C-peptide - A chain), which folds so that the disulfide bonds form correctly while the chain is still continuous; proteolytic enzymes then excise the C-peptide, releasing mature, two-chain insulin. This natural folding advantage - correct disulfide pairing is easier when the A and B chain sequences are held close together on one chain - is exactly why a single-chain (proinsulin) strategy is also used in industrial recombinant production (Section 3.2).

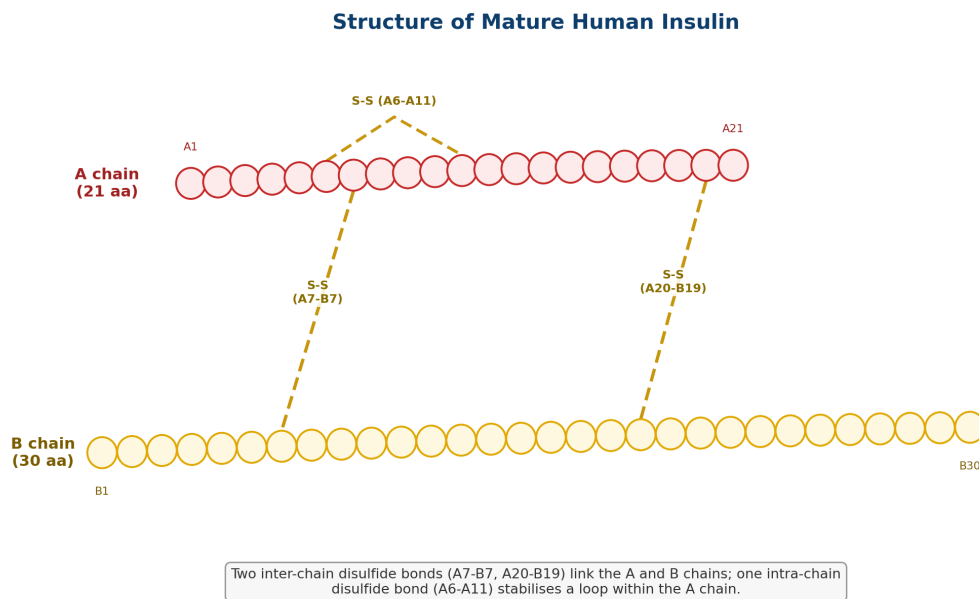


Fig. 1 - Mature human insulin: A chain and B chain joined by two inter-chain disulfide bonds, with one intra-chain disulfide bond in the A chain.

3. Strategies for Recombinant Production

3.1 The two-chain (separate A- and B-chain) method

This was the original method used by Genentech and licensed to Eli Lilly to create Humulin. The A-chain and B-chain coding sequences are synthesised as two separate genes and expressed in two separate *Escherichia coli* cultures as fusion proteins; the two chains are purified independently and then combined chemically outside the cell. Because folding and disulfide-bond formation happen *in vitro* after purification, this route gives the chemist direct control over the final product, at the cost of an extra combination/purification step (see Section 4 for the full sequence).

3.2 The single-chain (proinsulin) method

A later, now widely used approach expresses the complete proinsulin sequence (or a close analogue of it) as a single polypeptide from one synthetic gene - in *E. coli* or, in some industrial processes, in the yeast *Saccharomyces cerevisiae*, which can secrete the folded protein directly into the culture medium. Because the A and B chain portions are already linked, the correct disulfide bonds form more reliably during folding, and a single downstream enzymatic step - cleavage by trypsin and carboxypeptidase B - removes the connecting C-peptide (and any extra linker sequence) to leave mature, correctly-folded insulin. This method needs fewer separate fermentations than the two-chain route, which is one reason it is preferred in current large-scale manufacturing.

Key points

- Two-chain method: two separate fermentations and genes, chains combined chemically after purification - historically important as the original Humulin process.
- Proinsulin (single-chain) method: one gene, one fermentation, folding occurs naturally within the chain, followed by enzymatic removal of the C-peptide - closer to how the human body itself makes insulin, and the basis of most modern industrial processes.

4. Step-by-Step Process — the Two-Chain Method

- **Gene synthesis:** the amino-acid sequences of the A chain and B chain are already known, so their coding sequences are chemically synthesised as two separate DNA fragments, using codons preferred by *E. coli* (there is no need to isolate the natural human gene, which in any case contains introns that bacteria cannot process).
- **Vector construction:** each synthetic gene is ligated into a plasmid vector (originally pBR322), immediately downstream of the *lacZ* gene (beta-galactosidase) and its lac promoter/operator, with a codon for the amino acid methionine placed exactly at the fusion junction. The plasmid's antibiotic-resistance genes (for example, ampicillin and tetracycline resistance) serve as selectable markers, and insertion at one resistance gene (insertional inactivation) helps identify successfully recombined plasmids.
- **Transformation and selection:** the recombinant plasmids (one batch carrying the A-chain construct, a separate batch carrying the B-chain construct) are transformed into separate *E. coli* host cultures; cells that have taken up the plasmid are selected using the antibiotic-resistance markers.
- **Induction and fermentation:** the cultures are grown in a fermenter and induced with IPTG, which switches on the lac promoter; each culture then expresses a beta-galactosidase-methionine-insulin-chain fusion protein, which typically accumulates inside the cell as insoluble inclusion bodies.
- **Extraction:** cells are harvested and lysed, and the inclusion bodies containing the fusion protein are recovered and solubilised.
- **Cyanogen bromide (CNBr) cleavage:** CNBr cleaves polypeptides specifically at methionine residues; because the fusion protein was designed with a methionine exactly at the beta-galactosidase/insulin-chain junction, CNBr treatment releases the free A chain (from one culture) and the free B chain (from the other) from beta-galactosidase.
- **Chain purification:** the free A chain and free B chain are separately purified by chromatography.
- **Chain combination (renaturation):** the purified chains are mixed under controlled oxidative sulfitolysis conditions (commonly via S-sulfonated intermediates and a controlled reoxidation step), which allows the correct inter-chain disulfide bonds (A7-B7, A20-B19) and the intra-chain bond (A6-A11) to form, reconstituting biologically active, two-chain insulin.
- **Final purification and formulation:** the combined product is purified further, commonly by HPLC, to remove mis-paired or incompletely combined material, then formulated, tested for potency and sterility, and packaged as the finished drug.

Production of Human Insulin by Genetic Engineering — the Two-Chain Method

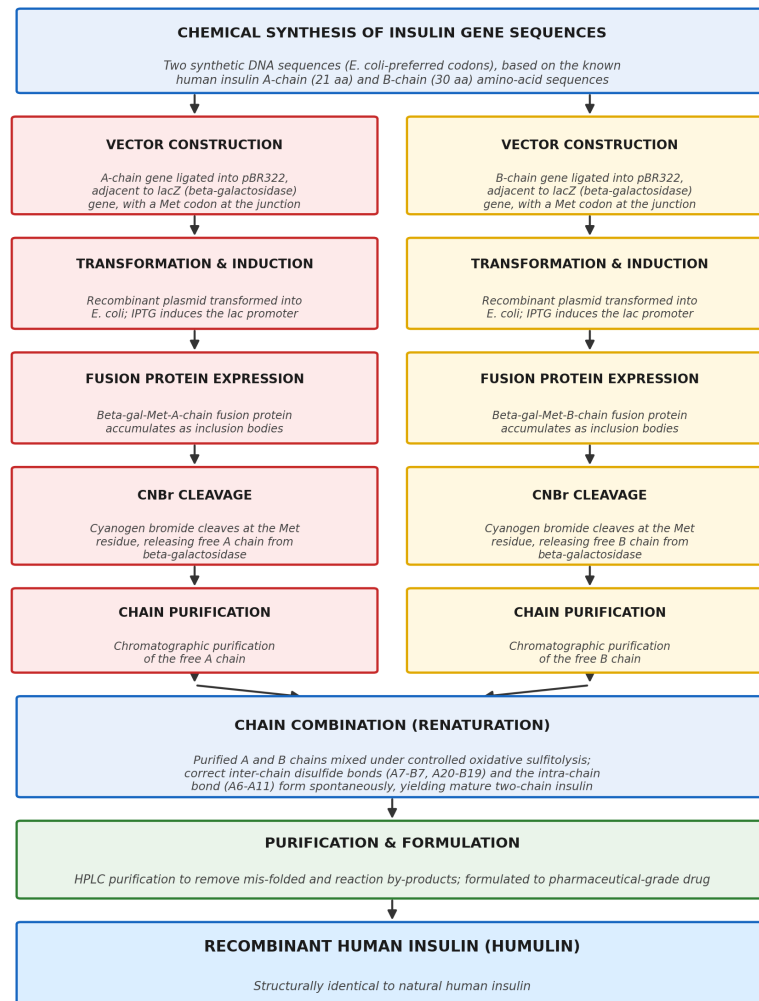


Fig. 2 - Process flow for recombinant human insulin production by the two-chain method.

5. Animal-Derived vs Recombinant Human Insulin

Aspect	Animal-derived insulin	Recombinant human insulin
Source	Pancreas glands of slaughtered cattle/pigs	Genetically engineered <i>E. coli</i> or yeast fermentation
Amino-acid sequence	Differs from human insulin (bovine: 3 residues; porcine: 1 residue)	Identical to natural human insulin
Immunogenicity	Higher risk of antibody formation, allergy and reduced effectiveness over time	Substantially lower immune reaction risk
Supply	Limited by number of animals processed	Scalable by fermentation volume
Batch consistency	Variable, tissue-dependent	High, process-controlled
Pathogen transmission risk	Present (animal-derived tissue)	Not applicable

Notes prepared for study purposes. The two-chain method is described as the original, historically significant process; most current industrial production uses proinsulin/single-chain or related improved methods. Consult current primary literature for details required in formal citation.