What is Biotechnology?

- Student answers:

Biotechnology Definition

- the science of altering genetic and reproductive processes in plants and animals

What is Genetic Engineering?

- Student answers

Genetic Engineering

- involves taking a tiny bit of DNA containing the desired gene from one organism and splicing it into the DNA strand of another organism

Why do Genetic Engineering?

- Student answers:

Why Do Genetic Engineering?

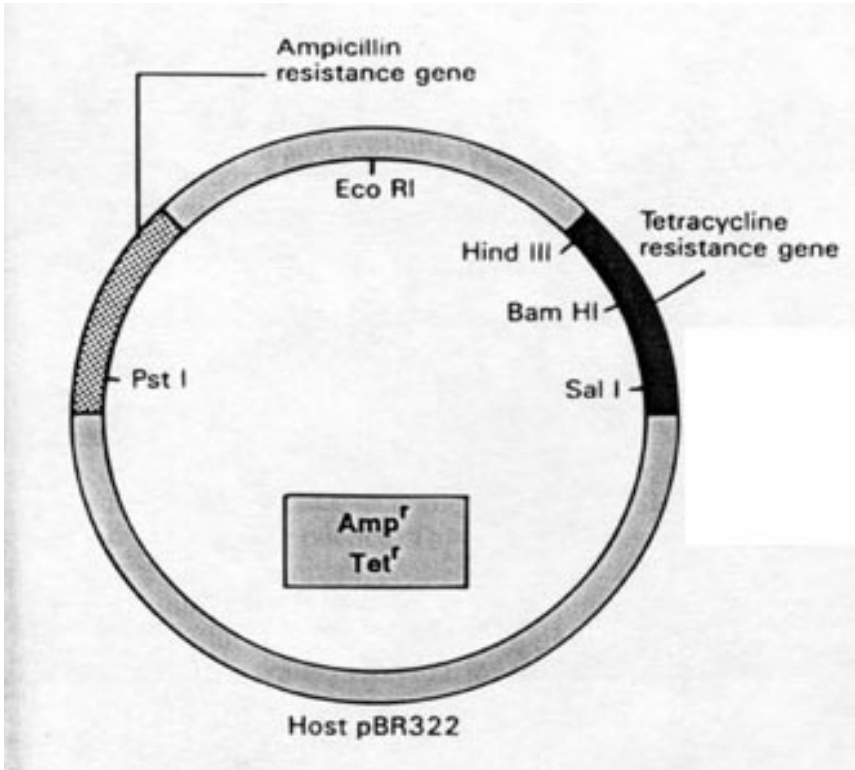
1. Produce desired proteins *in vitro* for therapeutic use.
2. Have rice produce as much starch as a kernel of corn (*in vivo* production).
3. Gene therapy
4. Elucidate the function of proteins of interest

Steps in Genetic Engineering

1. Isolation of gene of interest
2. Isolation of plasmid DNA
3. Manipulation of DNA sequence
   a. Cutting- Restriction enzymes
   b. Splicing- DNA ligase
4. Transformation of bacteria
5. Selection of “correct” bacteria

Plasmid Map

Figure: Harpers Review of Biochemistry



- Ori
- antibiotic resistance gene(s)
- restriction sites

Restriction enzymes

Restriction enzyme- an enzyme which cuts specific DNA sequences, endonuclease

"blunt end" vs. "sticky end"

Cleavage is restricted to specific, 4-6 bp sequences (foreign bacteria); always palindromic sequence

More than 800 are now known

Select Restriction Endonucleases

table 29–2

Recognition Sequences for Some Type II Restriction Endonucleases

*Bam*HI	(5′) G G A T C C (3′) C C T A G G	*Hin*dIII	(5′) A A G C T T (3′) T T C G A A
*Cla*I	(5′) A T C G A T (3′) T A G C T A	*Not*I	(5′) G C G G C C G C (3′) C G C C G G C G
*Eco*RI	(5′) G A A T T C (3′) C T T A A G	*Pst*I	(5′) C T G C A G (3′) G A C G T C
*Eco*RV	(5′) G A T A T C (3′) C T A T A G	*Pvu*II	(5′) C A G C T G (3′) G T C G A C
*Hae*III	(5′) G G C C (3′) C C G G	*Tth*111I	(5′) G A C N N N G T C (3′) C T G N N N C A G

Arrows indicate the phosphodiester bonds cleaved by each restriction endonuclease. Asterisks indicate bases that are methylated by the corresponding methylase (where known). N denotes any base. Note that the name of each enzyme consists of a three-letter abbreviation of the bacterial species from which it is derived (e.g., *Bam* for B*acillus* am*yloli-quefaciens*, *Eco* for E*scherichia* co*li*). The Roman numerals included in the enzyme names (e.g., *Bam*HI) distinguish different restriction endonucleases isolated from the same bacterial species rather than the type of restriction enzyme.

Cloning Vectors

1. Plasmids- 5,000 to 400,000 bp
   useful for putting 0.01-10 kb in
2. Bacteriophages-virus that infects bacteria
   useful for putting 10-20 kb in
3. Cosmids- artificially generated
   useful for putting 20-50 kb in
4. YACs- yeast artificial chromosomes
   useful for putting 500 kb
5. Other, newer exist

GENE MAPPING

- One of the most important processes in gene manipulation is that of finding the location of genes on the chromosomes
- gene mapping involves the finding of the particular location on the strand of DNA that contains the genes that control certain traits
- The arrangement of the nitrogen bases (A,T,C,G) on the molecule of DNA determine the genetic code

Gene splicing

- Once the location of the DNA sequence has been located, scientists can use restrictiion enzymes to separate the DNA at a particular location on the gene
- Once the pieces of DNA are removed other DNA canbe spliced in or recombined with the remaining DNA
  - This results in recombinant DNA

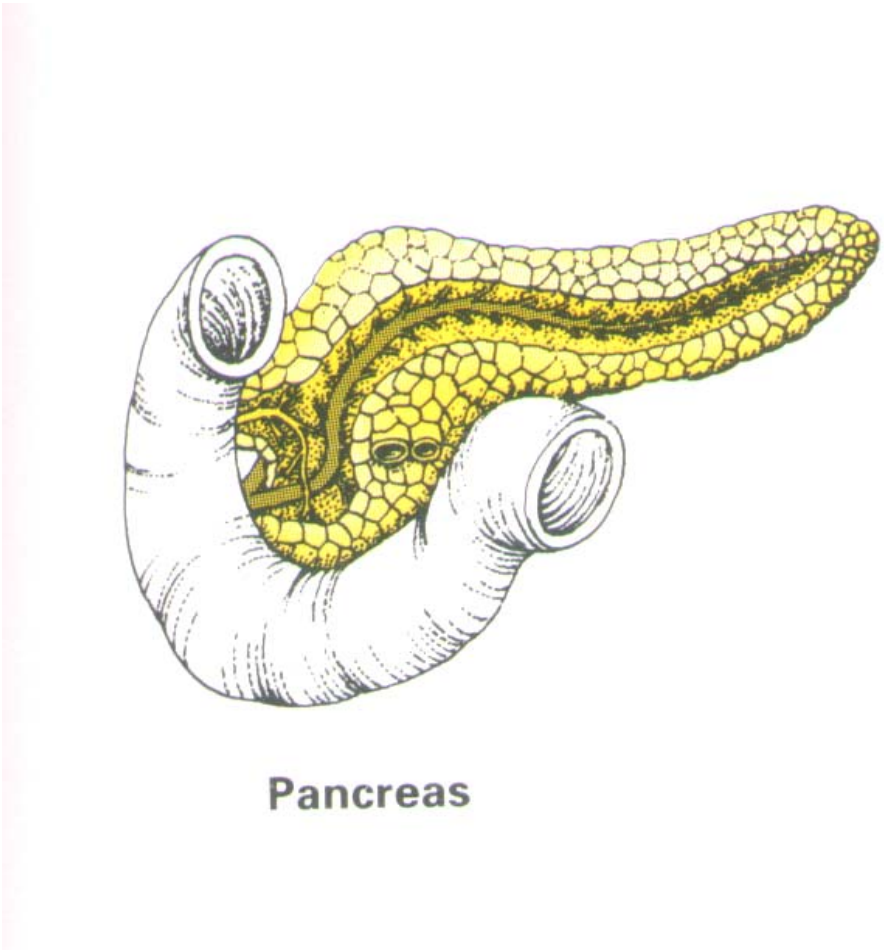
  - The new form of DNA will reproduce with the new characteristics of the introduced DNA
- The first genetic splicing was done using bacteria
  - Bacteria has plasmids (circular shaped pieces of DNA) that float freely in the cell's fluid
  - By selection the proper enzyme, scientists cut out part of the plasmid DNA and insert DNA from another organism
  - The DNA replicates and the new bacteria produced from the spliced DNA holds the desired characteristics

- One of the first uses of gene splicing was the manufacture of human insulin
  - Scientists isolated the DNA sequence that regulates the production of insulin
  - The DNA segment is spliced into the DNA of the E.coli bacteria
  - The bacteria carrying the DNA for insulin production reproduces and passes the capability along to the next generation

- The bacteria are produced in large quantities through a process called fermentation
- When the proper number of bacteria are reproduced, they are removed from the fermentation tanks and are taken apart to retrieve the insulin produced.
- The insulin is then separated, purified, and the remains of the bacteria are destroyed
- This procedure provides a ready relatively inexpensive supply of insulin for those people who need it

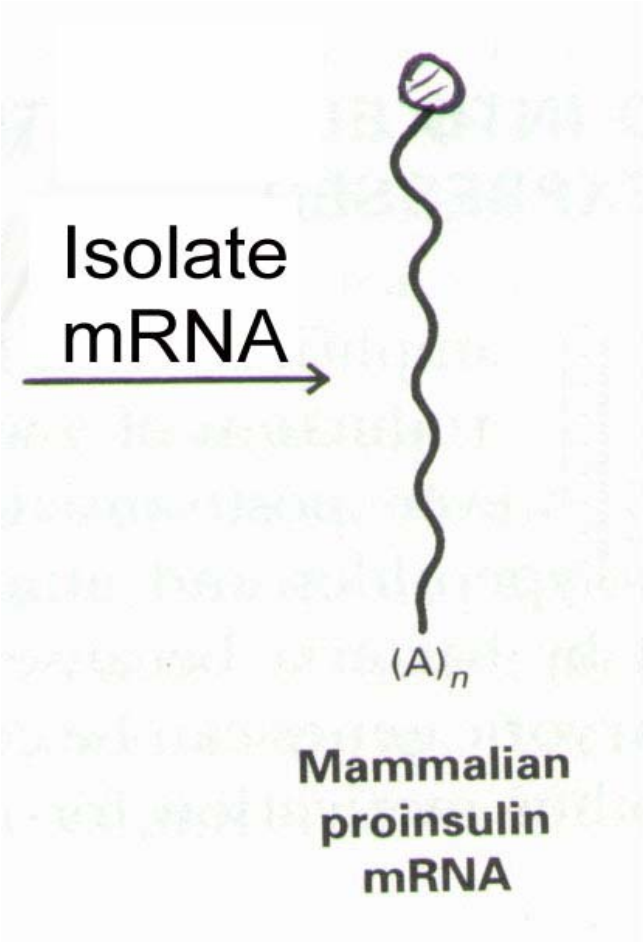
Making Human Insulin

Figure: Stryer, Biochemistry



Making Human Insulin

Figure: Stryer, Biochemistry



Making Human Insulin

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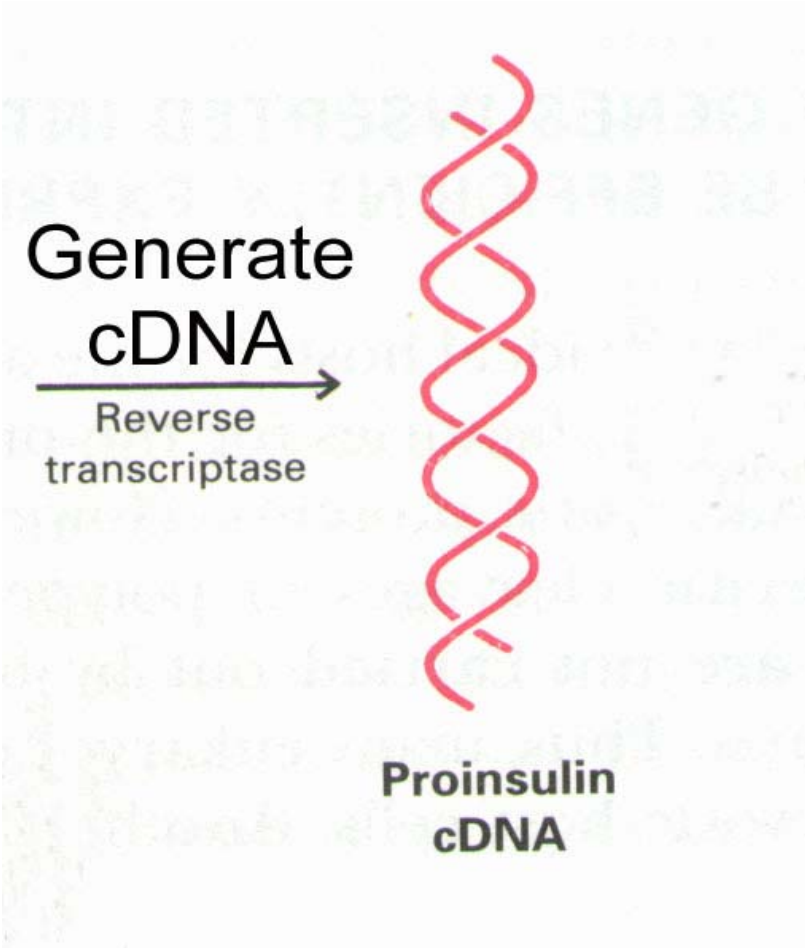

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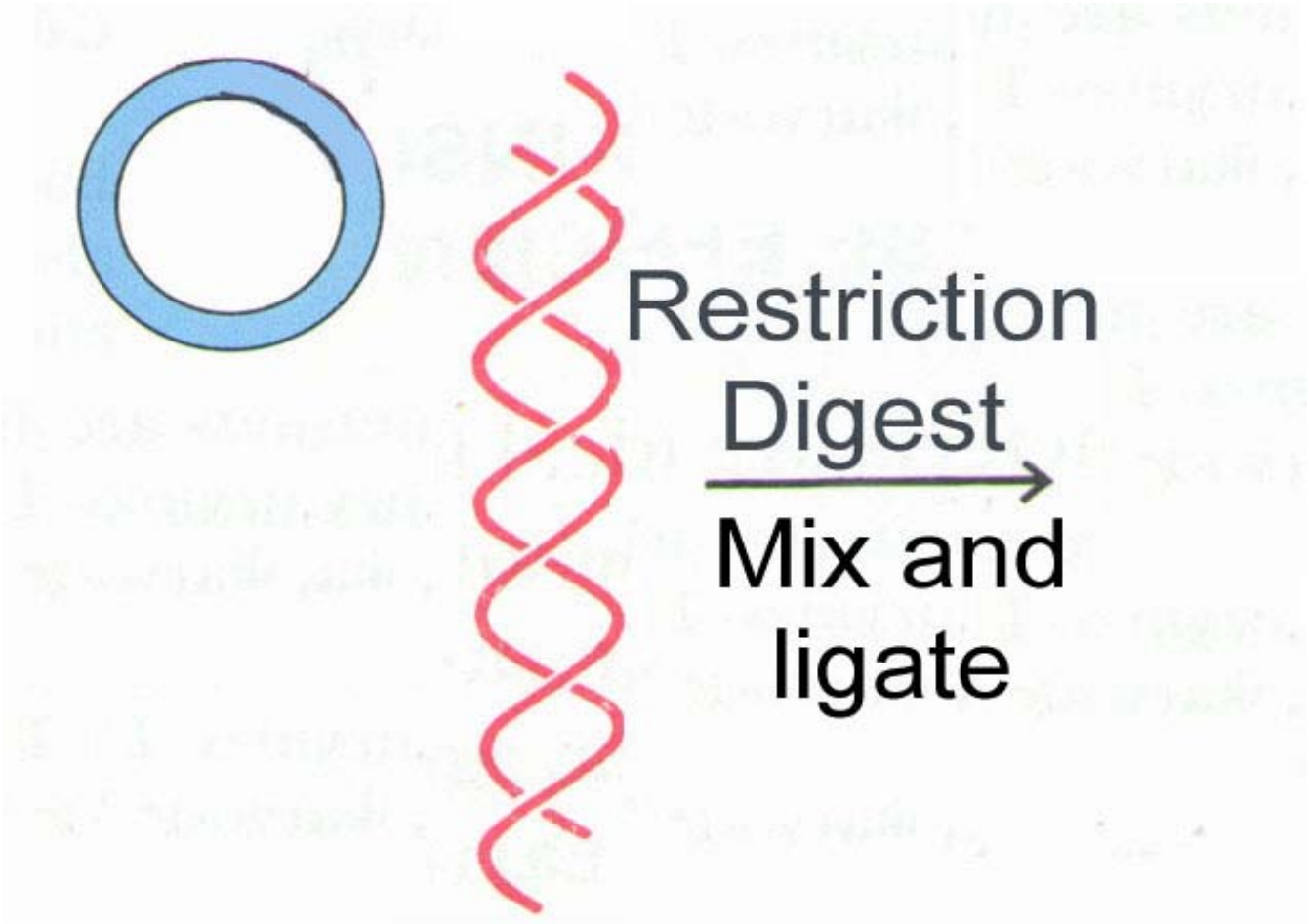

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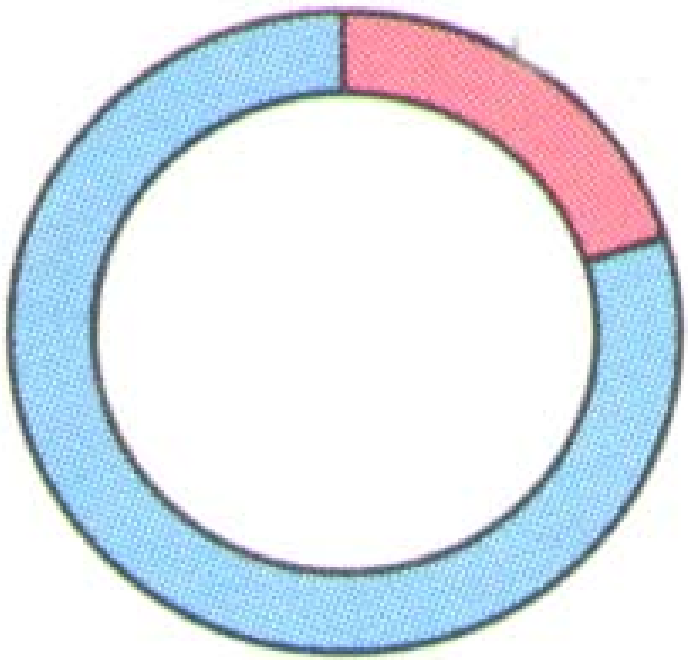

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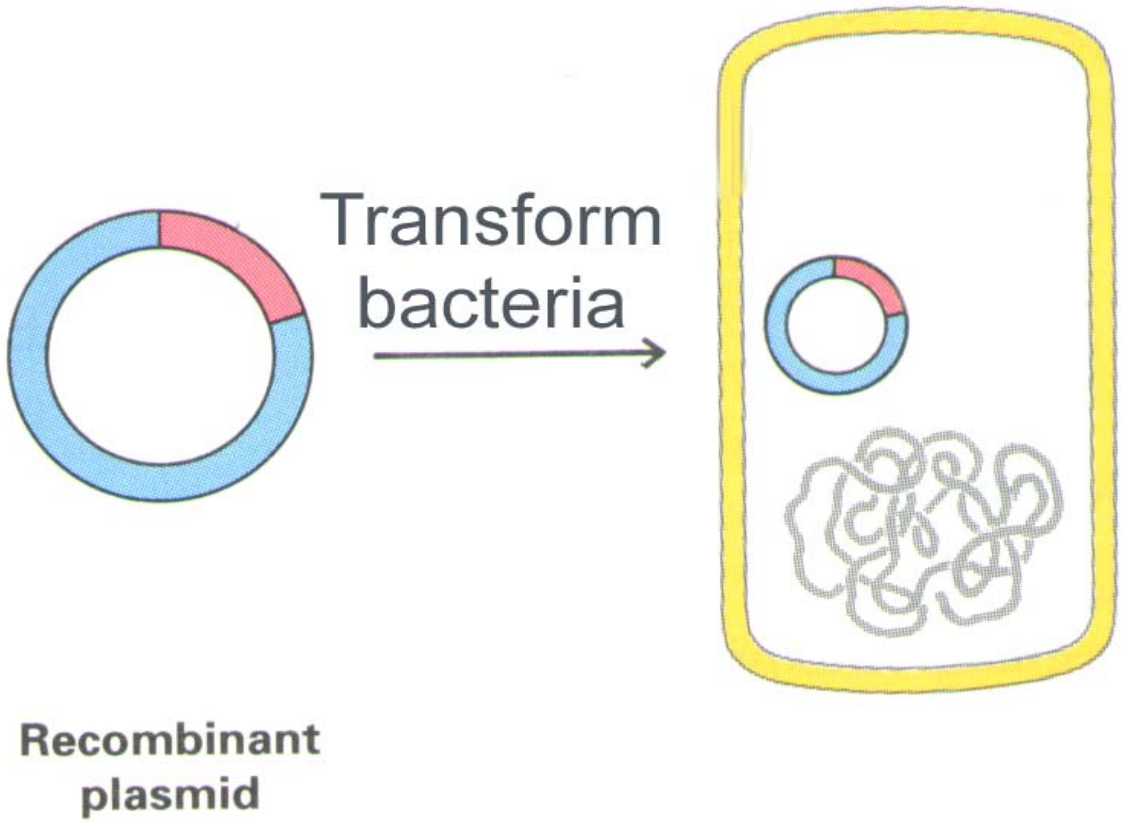

Making Human Insulin

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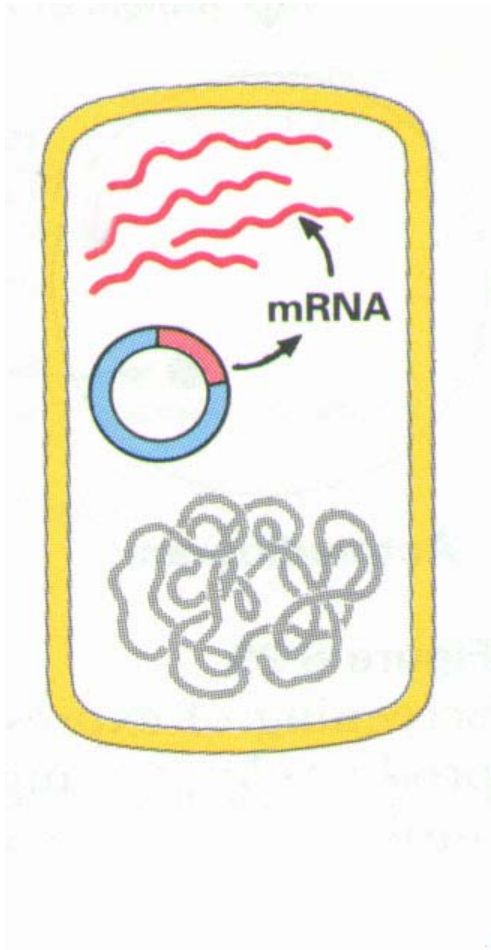

- Bacteria have become the manufacturing centers for many substances that have made the lives of humans better and more productive
  - Vaccines
  - Hormones
- Bovine somatotropin (BST)
  - A relatively recent agricultural innovation using genetic engineering

Biotechnology in Livestock Production

Two areas

- genetic engineering
- embryo transfer

Genetic Engineering

- purpose - to have the recipient organism take on the characteristic controlled by the transferred gene

Examples

- disease resistant animals
- growth regulators
- new drugs and vaccines

Examples

- specify size and sex of animals
- organism that “eats” oil used in the Persian Gulf

BST

- Bovine Somatotropin (Bovine Growth Hormone)
- Somatotropins are proteins that affect the utilization of energy in the body

BST

- causes energy derived from feed to be used for milk production rather than weight gain

BST

- does not reduce energy available for body maintenance
- increases energy available by improving breakdown of fat and increasing appetite

BST

- small amounts of BST are produced naturally in the cow by the pituitary gland

BST

- previously, the only source of BST for research has been from pituitary glands of dead cows

BST

- now, because of genetic engineering, large quantities of BST can be produced

BST

- gene that controls BST production is spliced into the DNA of a bacteria "*Agrobacteria*"
- is injected into a cow
- causing increased BST production in the cow

BST

- research at the University of Wisconsin-Madison and Cornell University in New York has showed an average increase of 40% over lactation (305 days)

Opposition to ‘Biotech’

- Student answers:

Opposition to “Biotech”

- people fear
- production of new uncontrollable disease
- freak animals
- long term adverse effects of environment from products

‘Biotech’ in Crop Production

- Student Answers:

Biotech in Crop Production

- lowered costs and increased yields
- improved feeding values

New Corn Plant

- produces higher levels of tryptophan
- amino acid essential for protein formation in an animals body.
- the first plant patented

Other areas researched

- herbicide resistance
- pest resistance
- frost resistance
- salt tolerant
- drought resistance

Embryo Transfer

- well established in cattle industry, especially dairy

Process

- cow is treated with hormones to cause “superovulation”
  - – can produce as many as 25 eggs
- donor cow then artificially inseminated
- during this process, “Recipient” cows or heifers are treated with hormones to synchronize their heat cycle to be the same as the donor cow.
- after the eggs are fertilized and before they leave the oviduct, the cow is “flushed” with fluid- which washes the fertilized eggs out of the body into a cylinder

Process

- individual embryos are located under a microscope and put into a straw
- embryos may be frozen much the same as cattle semen samples
- first research in the U.S. was done in central WI in 1982
- most transfer work is done non-surgically with success rates of approximately 75%

Splitting

- research has also successfully split embryos resulting in as many as 5 identical calves

Slow Progress

- many characteristics are controlled by multiple genes instead of a single gene

Slow Progress

- lack of money for research
- government regulations
- environmental groups filing lawsuits to stop research and testing

Slow Progress

- many farmers don’t support genetic engineering because they feel we already have surplus production

What are your thoughts?