

BIO BIZ

Biotechnology Transforms

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This month, Bio Biz is taking a look at Animal Biotechnology

By Mandy Latimer

Goats producing spider silk or lactoferrin in their milk, and pigs whose organs can be transplanted into humans are just some of the unusual headlines that have appeared in the news over the last 10 years. Even though man-made genetic modification of organisms is relatively a recent phenomena, the creation of transgenic organisms has been occurring in nature for millions of years. A perfect example of natural transgenesis is *Agrobacterium tumefaciens* (a soil-dwelling bacterium which causes crown gall disease in many ornamental & fruit plants) which uses a special plasmid to transport its DNA into plant cells where it becomes integrated into the plant's chromosome. Molecular biologists have been successfully mimicking this process using man-made plasmids to add specific DNA to the chromosomes of bacteria & plants.

Inserting genes into animal genomes proved to be a little trickier. Again mimicking nature, molecular biologists turned to the virus for help. The resulting transgenic animals have become very useful in many areas of research. For example:

- * Transgenic animals are important in disease research because, by either introducing or inactivating particular genes, researchers can discover the root causes of diseases associated with gene defects. Scientists can then design therapies which act by influencing the expression of these disease causing genes.

- * Transgenic animals are used to test the safety of new medicines and vaccines. Their use in toxicity trials also prevents the subsequent use of a larger number of animals in the development phase of pharmaceuticals.

- * Transgenic animals can produce biological products. Human alpha-1-antitrypsin, a protein used to treat the rare genetic disorder of alpha-1-antitrypsin deficiency, is just one example. Research is underway to breed transgenic sheep which produce the protein in their milk. To date, several human proteins have been produced in sheep, pigs or goats but no commercial development of these proteins has been reported. Some of them and their intended applications include:

Employment opportunities

Athens Research & Technology Inc are looking for Research scientists.
artbio@athensresearch.com

USDA is looking for student workers. Contact Jodie Plumlee for more info:
Jodie.Plumlee@ARS.USDA.GOV

Notice

What should a school do with outdated Chemicals?

Contact the
EPA Schools
Chemical Cleanout
Campaign

www.epa.gov/sc3/

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Milestones (some ideas are not as new as we might think):

4000 BC Egyptians use yeast in bread-making and moulds to produce cheese. Chinese used moldy Soya bean products for treatment of skin infections.

1100 Distillation of alcohol.

1752 Steam engine invented.

1859 Petroleum was discovered.

1886 Carl Benz received a patent for a gas-fueled car.

1890 First use of alcohol as fuel.

1908 Henry Ford was the first to mass produce cars.

1925 Henry Ford states that ethyl alcohol was the “fuel of the future.”

“The fuel of the future is going to come from fruit like that succum by the road, or from apples, weeds, sawdust — almost anything.” he said . “there is fuel in every bit of vegetable matter that can be fermented. There is enough alcohol in one years yield of an acre of potatoes to drive the machinery necessary to cultivate the fields for 100 years.”

1928 Fleming discovers penicillin.

1955 Antibiotic production in most industrialized countries.

1973 Ethanol production program by fermentation for gasohol in Brazil.

1974 Increase in world oil prices begins.

1980's By the mid 1980's, ethyl alcohol peaked in production to over a billion gallons of ethyl alcohol produced per year.

1990's By the early 1990's an oil glut and rock bottom fuel prices resulted in most of the alcohol plants being shut down.

Bill Kovarik, "Henry Ford, Charles F. Kettering and the Fuel of the Future," Automotive History Review, Spring 1998, No. 32, p. 7 - 27. Reproduced on the Web at <http://www.radford.edu/~wkovarik/papers/fuel.html>.

Milestones: looking back EIBE European Initiative for biotechnology education 1999



Additional information about the Biotechnology & Pharmaceutical Manufacturing Technology programs is available at:

www.gabioscience.org

Biotech lab corner

Quick biotechnology -related protocols for teachers to use in their classrooms

This months quick pick is a plant cloning (easier than animal) exercise.

Good luck!

Rose Cloning Made Easy

(By: Mandy Latimer & Michael Garrett)

The Georgia Biotechnology & Technical Institute



(Transgenic Blue roses)

Materials needed:

Mini **Rose** plant

Foil

Potting soil

Equipment:

Forceps (8", stainless)

Scissors (Stainless)

1 liter beaker

Culture tube w/cap (50 ml)

Stainless stir rod

Styrofoam Cups

File cabinet hanging file frame (for improvised sterile hood)

Plastic wrap (for covering frame)

Spray bottle

2 x 1 liter Flask

Parafilm

Gloves

Solutions:

70 % Ethanol or Isopropanol

10% Bleach solution

10% soapy water

Distilled water

Agar (10 g /liter of media)

Rose Stage I medium (MS standard media Murashige & Skoog

Shoot Multiplication Media C -MSP0012,

1 liter packet = 1000 ml enough for 16 students @ 30 ml/tube 2 tubes each student,

www.caissonlabs.com

Rose Stage II medium (Rose stage I media with 6-Benzylaminopurine 1 mg/liter)

Equipment & Media Preparation:

Equipment prep:

Wrap forceps in tin foil and autoclave.

Wrap scissors in tin foil and autoclave.

Rose Stage I media prep:

Reconstitute Rose Stage I media in a 1 liter beaker with 800 mls of distilled water.

Adjust pH to 5.5-5.8 with 1M HCL or 1M NaOH

Add agar to media 9 g / liter.

Mix & bring volume to 1 liter. (note: you may need to heat solution to a boil to dissolve agar.)

Autoclave at liquids setting.

Pour 30 ml autoclaved media into 50 ml tubes.

Place cap on but don't tighten until media has solidified.

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Rose Stage II medium prep:

- 1) Reconstitute Rose Stage I media and follow directions above except add 1 ml of 6-Benzylaminopurine 1 mg/liter solution before adjusting pH.
- 2) Follow steps 2-7 above

Do two preps for each student.

Methods:

Sterile Hood

Assemble frame (can use a file cabinet hanging file frame) and cover all sides with plastic wrap leaving front side with movable flap so you can work inside.

Spray interior with 70% alcohol and let sit for 10 min before using.

Stem prep (only use forceps to handle stem)

- 1) Cut stem below axil and remove any leaves.
- 2) Rinse axil in cold tap water for 10 min.
- 3) Rinse in 10% soapy water with shaking for 30 sec.
- 4) Rinse in sterile DI water.
- 5) Rinse in 70% ETOH 30 sec with shaking.
- 6) Rinse in sterile DI water.
- 7) Rinse in 10% bleach for 5-10 min with slight agitation.
- 8) Rinse the axil in sterile DI water 3 X @ 5 min each rinse.

Plant material must be surface sterilized to remove bacteria & fungal spores.

Stem culturing (done in sterile hood)

The Axil is where the leaf of the plant meets the stem.

Using forceps, place the stem into the culture tubes (containing Growth Media I) up to the axil. Return cap to the culture tube and place in rack under grow lights.

During the next 4-6 weeks record plant growth and development in your notebook.

When your plant is 3 inches tall, transfer it to the culture tubes containing Stage II media. When your plant begins to grow roots, clip off the dead stem and transfer it to a container with potting soil. Once plants are hardened off, you can take home.

This will also work with Carnation, Cauliflower & African Violet plants.



Note: the 50 ml centrifuge tubes can be rinsed out and used again. They are also autoclavable!

The List of Clones (Wikipedia)

The modern cloning techniques involving [nuclear transfer](#) have been successfully performed on several species. Landmark experiments in chronological order:

- **Tadpole:** (1952) Many scientists questioned whether cloning had actually occurred and unpublished experiments by other labs were not able to reproduce the reported results.
- **Carp:** (1963) In [China](#), [embryologist Tong Dizhou](#) cloned a fish. He published the findings in an obscure Chinese science journal which was never translated into English.^[3]
- **Mice:** (1986) was the first successfully cloned mammal; [Soviet](#) scientists [Chaylakhyan](#), [Veprincev](#), [Sviridova](#), [Nikitin](#) had mice "Masha" cloned. Research was published in the magazine "Biofizika" volume XXXII, issue 5 of 1987.^[4]
- **Sheep:** (1996) From early embryonic cells by [Steen Willadsen](#). [Megan](#) and [Morag](#) cloned from differentiated embryonic cells in June 1995 and [Dolly the sheep](#) from a somatic cell in 1997.
- **Human:** (November 1998) hybrid embryo created from leg cells and a cleaned cow egg - not allowed to implant in a womb, nor develop, nor be born due to ethical issues.
- **Rhesus Monkey:** [Tetra](#) (female, January 2000) from embryo splitting
- **Cattle:** [Alpha and Beta](#) (males, 2001) and (2005) [Brazil](#)^[5]
- **Cat:** [CopyCat "CC"](#) (female, late 2001), [Little Nicky](#), 2004, was the first cat cloned for commercial reasons
- **Mule:** [Idaho Gem](#), a john mule born 4 May 2003, was the first horse-family clone.
- **Horse:** [Prometea](#), a Haflinger female born 28 May 2003, was the first horse clone. Wikipedia



Dolly was bred to show that cloned animals were normal.



Dolly & her offspring.



Tetra the Rhesus Monkey



Idaho Gem first cloned mule.



Rainbow & her clone "CC"

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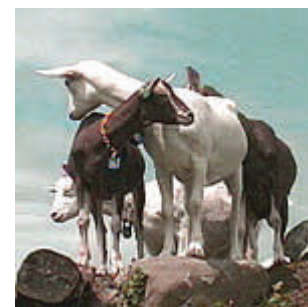
TPA (Tissue Plasminogen Activator), produced in goats for treatment of blood clots.

* Factor VIII and Factor IX, produced in sheep for the treatment of blood clotting disorders such as hemophilia.

* Antithrombin III, produced in goats, also for the prevention of blood clotting.

* CFTR (Cystic Fibrosis Transmembrane Conductance Regulator), for treatment of cystic fibrosis.

* Human protein C produced in pigs for use as an anticoagulant.



*Transgenic pigs with human histo-compatibility genes have been bred in the hope that their "humanized" organs will not be rejected by a patient's immune system, thus providing a new source of organs such as kidneys, livers and hearts, for the many patients awaiting organ transplants.

*Transgenic farm animals are being produced that carry genes that can induce more rapid growth or weight gain, alter milk to reduce lactose or improve shelf life, or increase disease resistance. Most genetic engineering of fish and other aquatic species has focused on enhanced growth, stress resistance, disease resistance and sterility (the latter being an important technique to control the unintentional release of genetically modified organisms into the environment). A few research efforts have concentrated on other applications and uses.

Thus, the tools of biotechnology which have been developed over the last thirty years have clearly opened up dramatic opportunities to create new varieties of plants and animals. At the same time, the novelty of biotechnology has raised questions. Some view biotechnology as a logical and modest extension of conventional plant and animal breeding technologies. Others see it as a novel technology that is dramatically different from traditional breeding. How different is it from traditional plant and animal breeding? How is it being used? What kind of problems is it attempting to solve? What are some of the likely future uses of agricultural biotechnology?

Reference:

Genetic Modification in Nature A Natural Way of Genetically Modifying Organisms, *Agrobacterium tumefaciens* by Jared Keefer
Washington.edu/z490/natural.html

**What does the term
"biotechnology"
mean?**

The term "biotechnology" was first coined in 1917 by Karl Ereky, a Hungarian engineer, to describe the large-scale production of pigs that were fed sugar beets. For much of the last century, it has been the broad term applied to the use of any living organism for a practical purpose—anything from the selective breeding of plants and animals to fermentation of beer or treatment of sewage with organic materials.

The expanding number of genome maps reveals striking genetic commonality among living organisms.

For example, some 10 percent of human genes are clearly related to fruit fly and worm genes; about 99 percent of the overall DNA sequence in humans is similar to that of chimpanzees (Paabo, 2001). To date, scientists and researchers have sequenced forty-eight genomes. These include not only the human genome, but also the flowering mustard plant *Arabidopsis thaliana*, a plant used extensively in agricultural biotechnology research, as well as the fruit fly *Drosophila melanogaster*, pathogenic bacteria and the nematode GOLD 2001.

HARVEST THE HORIZON: FUTURE USES AGRICULTURAL BIOTECHNOLOGY
PREPARED BY THE PEW INITIATIVE ON FOOD AND BIOTECHNOLOGY SEPTEMBER 2001