Introduction to Animal Biotechnology

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Reproductive Biotechnology
Factors affecting reproductive efficiency

* Early development of the conceptus
* Genetic and environmental factors
* Nutrition
* Breeds
* Fertility and temperature
* Culling strategies
* Stress
* Effect of antibiotics and hormones
Reproductive-biological procedure

- AI (artificial insemination)
- Estrous synchronization
- Regulation of parturition
- ET (embryo transfer)
- Cryopreservation of gametes and embryos
- Sexing of sperm and embryos
- IVP (in vitro production of embryos)
- Embryo bisection
- NT (nuclear transfer)
- Microinjection technology (Sperm, DNA, RNA...
Estrus Detection
Stages of the estrous cycle
Cow

Swine
Artificial Insemination, AI
Advantages of AI

- **Genetic improvement** e.g., widespread use of outstanding sires; improving accuracy of selection through progeny test; permitting crossbreeding; introduction of new genetics

- **Availability of accurate breeding records**

- **Control of veneral diseases**

- **Economic service**

- **Safety through elimination of dangerous male**

- **Use of deep-frozen semen after a donor is dead**

- **Gender control**
Semen Collection & Artificial Insemination

- Bull semen collection by artificial vagina
- Bull semen collection by electrostimuation
- Boar semen collection by massage
- Cow insemination
- Swine insemination
Cryopreservation of gametes and embryos
Cryopreservation

Freezing injury

- destroy cell function irreversibly
- intracellular ice crystal formation and salt deposits
- changes in the composition of surrounding milieu at thawing phase
- cytotoxicity of cryoprotectants
Cryoprotective agents

* high water solubility; high permeability; low toxicity
* alcohols, amines, sugars and proteins
* dimethyl sulfoxide (DMSO); 1,2-propanediol (PROH); ethylene glycol (EG); glycerol

Antifreeze proteins

* many animals and plants in nature use cryoprotective agents
* production of antifreeze proteins, mainly glyopeptides
Boar semen cooling

Bull semen cooling and freezing
Embryo Transfer
Molecular breeders

Nuclear transfer
Mammalian embryogenesis
by asexual reproduction

Cell transplantation
Male gametes have potential of
developing in a surrogate environment
Transgenic Animals
normal mouse  transgenic mouse

Promoter: metallothionine-1

Transgene: rat GH

Science 1982, 385:810-813
The Aims of producing transgenic animals

- Biological, biomedical, veterinary and genetic research
- Agriculture: enhance growth and development
- Increase disease resistance
- Produce foreign proteins
- Xenotransplantation
- Gene therapy
The Methods to produce transgenic animals

- Microinjection
- Retroviral vector infection
- Sperm vector
- Embryonic stem cell (ES cells): particle gun / transfection / electroporation
- Nuclear transfer
DNA microinjection
Nuclear Transfer

Enucleation  Nuclear transfer
Improvement of agriculture traits

- Dietary modifications of animal products
- Environmentally friendly farm animals
Total phosphorus content (on a dry matter basis) of fecal matter from non-transgenic pigs (■) and transgenic pigs (□) of line WA fed different levels of soybean meal as the sole source of dietary phosphorus.

DM, Dry matter content of feces.
Transgenic animals in biomedicine

As models for human diseases

Alzheimer’s disease

Amyloid precursor protein (APP)
Presenilins (PS1 or PS2)

Huntington’s disease (HD)

HD is caused by the expansion of cytosineadenine-guanine (CAG, translated into glutamine) trinucleotide repeats in the first exon of the human huntingtin (HTT) gene.
For pharmaceutical production

Proteins produced in the mammary gland of transgenic farm animals

<table>
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<tr>
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<th>Developmental phase</th>
<th>Production species</th>
<th>Therapeutic application</th>
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<tr>
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<td>Phase III</td>
<td>Goat</td>
<td>Genetic heparin resistance</td>
<td>2005</td>
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<tr>
<td>TPA</td>
<td>Phase II / III</td>
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<td>Dissolving coronary clots</td>
<td>➢2006</td>
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<tr>
<td>α-AT</td>
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<td>Goat / sheep</td>
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<td>Experimental</td>
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<tr>
<td>HAS</td>
<td>Phase I</td>
<td>Cattle</td>
<td>Hemophilia A</td>
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<tr>
<td>Various Abs</td>
<td>Phase I / II</td>
<td>Goat</td>
<td>Blood substitute</td>
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</table>

AT III: antithrombin; TPA: tissue plasminogen; α-AT: antitrypsin; hFVIII: human clotting factor; Human serum albumin;
Procedure for generation of transgenic birds

Genomics
Age of -omics

Genomics: the quantitative study of genes, regulatory and noncoding sequences.

Transcriptomics: RNA and gene expression.

Proteomics: protein expression.

Metabolomics: metabolites and metabolic networks.

etc......
DNA, the molecule of life

Trillions of cells
Each cell:
- 46 human chromosomes
- 2 meters of DNA
- 3 billion DNA subunits (the bases: A, T, C, G)
- Approximately 35,000 genes code for proteins that perform most life functions

(Brooks et al., 2004)
DNA 2003: 50 years of DNA discovery

2003 marks the 50th anniversary of the discovery of the double helix structure of the DNA molecule, by James Watson and Francis Crick, in Cambridge in 1953. These pages gather together elements of the history of the discovery, the science and the scientists, look at some of the new research and developments and show how Cambridge has marked the anniversary. They also provide links to the resources available in Cambridge and elsewhere for learning more about DNA and its impact.

J. Watson and F. Crick
Overview of gene expression

(Brooks et al., 2004)
Defining Genes

A complete chromosomal segment responsible for making a functional product

- **Open Reading Frame (ORF):** a string of codons bounded by start and stop signals.

- **Evidence of transcription:** RNA or protein expression is a hallmark of a gene product. Methods: microarray, cDNA mapping, sequencing of EST clones, etc.

- **Sequence conservation:** conserved DNA sequences suggest importance of the gene product.
Genome

The entire collection of genes encoded by a particular organism.

Determination of an entire genome sequence is a prerequisite to understanding the complete biology of an organism.
Mitochondrial genome

- Animal has 15-17kb, no intron
  - Nearly always circular with 37 genes: 13 for proteins, 22 for tRNA and 2 for rRNA.

- Plant has 200kb-2Mb, with intron
  - 51 genes, not too many more than animal.
  - Besides introns, duplication of genes.
  - Gain genes from chloroplast and lose gene to nucleus.

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Genomics

Genomics is the scientific study of structure, function and interrelationships of both individual genes and the genome in its entirety (Bazer and Spencer, 2005)

The field has evolved from identifying short nucleotide strings of DNA to the sequencing of an organism’s complete genome.
- **Structural genomics**: construction of sequence data and gene map.

- **Functional genomics**: functions of genes, and their regulation and products.

- **Comparative genomics**: compare genes from different genomes to elucidate functional and evolulational relationship.
功能性基因體的研究方法

• 基因表現全貌: EST 基因庫、差異展現法、雜合扣除法、DNA microarray 和 proteome 等

• 突變篩選: generation of random mutation in the genome, ENU, RNAi, T-DNA, EP-transposon, and so on...

• 轉基因或基因剔除: generation of target mutation, knockout
Current status of farm animal genomics
U.S. Livestock Species Genome Projects

Supported by

NATIONAL ANIMAL GENOME RESEARCH PROGRAM

HORSE
SHEEP
CATTLE

CHICKEN
SWINE
AQUACULTURE

BIOINFORMATICS

http://www.animalgenome.org/
• CHICKGBASE site at the Roslin Institute

Supported, in part, by the USDA-CSREES National Animal Genome Research Program in order to serve the poultry genome mapping community.

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# NAGRP Cattle Genome Coordination Program

## Research Activities
- Cattle Genome Sequencing Project (Baylor)
- Cattle Genome Sequencing White Paper
- ANGENMAP (Discussion Group | Members)
- Upcoming Meetings, Workshops, etc.

## News and Updates
- New genome assembly available (Dec. 1, 2006)
- GOA Release for Bovine (Mar. 8, 2007)
- Bovine Genome: Next Phase (Oct. 1, 2005)
- NRSP-8 Species Priorities (Jul. 23, 2005)
- Cattle Genome Assembled (Oct. 6, 2004)

## Databases
- Bovine Genome Sequence Archive (ECM)
- Golden Track Cattle Genome (UCSC)
- Ensembl Cattle Genome (Sanger/EMBL)
- Cow GOA (EMBL) | Cow GO (MS State U.)
- Bovmap Database (INRA, France)
- Bovine SNPs from 6x Genome seq. (Baylor)
- Interactive Bovine In Silico SNP DB (CSIRO)

## Gene Maps
- Bovine Genome Browser (TXAM Univ.)
- Bovine Genome Assembly (Univ. Maryland)
- Bovine Genome Assembly Browser (CSIRO)
- Cattle Genome Maps (NCBI | MARC)
- The ArkDB - Cattle Map (Roslin Inst.)
- RH Maps (SUNb map | UIUC | COMRAD)
- Bovine BAC Finger Print Map (U. Alberta)
- QTL Maps (Texas A&M | U. Sydney | Iowa State U.)

## Resources
- NCBI Cew Genome Resources
- Blast Servers (NCBI | NAGRP)
- Cattle Gene Index (BtGI)
- Bovine Oligo Array
- Animal Genome Projects / Databases

## Community Links
- NCBI Cattle Genome Project
- Belur Bovine Genome Project
- TAMU Cattle Genome Database
- EAAP Cattle Network
- NAGRP Bioinformatics
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<td>UK Sheep Genome Mapping Project</td>
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WELCOME

The Horse Genome Project is a cooperative international effort by over 100 scientists in 20 countries to define the genome, the DNA sequence, of the domestic horse. With this knowledge, scientists can begin to understand the genetic aspects of equine physiology and disease. Genetic tools have the potential to help researchers find new therapies and treatments for diseases such as tumors, respiratory diseases, etc.

Some horsemen may worry that once the horse genome is sequenced, all the mystery and magic will be gone from horse breeding and ownership—colorful characters, around a racetrack or replaced by computer printouts and test tubes. Fear not. The study of the horse genome is more the studying the weather than investing a sports car. Consider the following. All our research on the weather has shown we can experience a sunny day in Florida knowing that a hurricane will...
The potential of farm animal genomics

- The meat industry can now use cow and chicken genomic data to **confirm the quality** of meat products. For example, meat producers can now confirm the parentage of an Angus cattle breed by performing a genetic blood test or attempt to identify the SNPs associated with high-quality beef.

- Using genomic information to **determine disease-resistant genes** in shrimp and then are selectively mating the shrimp that carry them in order to create disease-resistant strains.

  (Dove, 2005).
In the healthcare arena, farm animal genomic work will aid in enterprises such as xenotransplantation (the transfer of animal tissues or organs into humans).

Though animal organs may be used someday to satiate organ donor shortage, genomic work in this area is still in its early stage (Cascalho and Platt, 2001).

Many of the immediate practical applications of farm animal genomics show potential for growth in this field.
ArkDB is one of the major sources of information on farm animal genomics.

The database system is hosted at Roslin Institute aims to provide a comprehensive public repository for genome mapping data from farmed and other animal species.
Applications of genomics in Animal Production

➢ For decades, breeders have altered the genomes of farm animals by first searching for desired phenotypic traits and then selecting for superior animals to continue their lineage into the next generation (Harlizius et al., 2004).

➢ This genomic work has already facilitated a reduction in genetic disorders in farm animals, as many disease carriers are removed from breeding populations by purifying selection (Andersson, 2001).
By studying diverse phenotypes over time, researchers can now monitor mutations that occur as wild species become domesticated (Andersson and Georges, 2004).

Farm animal food safety will remain a concern for some time; however, advancements such as the discovery of Escherichia coli resistant genes in the pig (Edfors-Lilja, 1995) can mediate most of the problems.
Moreover, resources devoted to investigating the genomes of farm animals can bring eventual economic benefits.

For example, isolation of DNA from animal tissue can be used as an inexpensive method for tracking the origin of a meat sample, providing the recipient with the quality assurance of that food (Brem, 2004).
DNA分子資訊的應用

- 協助育種者選拔特定的主要突變（如大腸桿菌粘附抵抗力）或對抗負面的突變（像負面的鹵乙烷對偶基因或RN-對偶基因）。
- 用於數量性狀的選拔（包含那些可以用傳統方法選拔的性狀），例如利用ESR之B對偶基因增加窩仔數及MC4R來降低飼料採食。
- 增加選拔的準確性、容許對受性別限制性狀或銘印基因做選拔及容許選拔像肉質這類的性狀。
基因晶片的發展與應用

- 數萬個基因或ESTs序列固定在一小塊面積的基材(即微陣列或基因晶片)上，例如載玻片，對某種生物材料進行研究，快速比對出mRNA具有差異表現的基因(Craig et al., 1990)。

- 利用DNA晶片或微陣列來檢測基因表現將提供動物性狀改良的新契機。
Proteomics
mRNA level ≠ expressed protein level nor does it indicate the nature of the functional protein product.
A: Correlation of protein and mRNA across all 165 protein spots

(Chen et al., 2002)
Definition of Proteome

The \textbf{PROTE}in complement expressed by the \textbf{genOME} of an organism

(Wilkins, 1994; Wasinger et al., 1995)
Definition of Proteomics

The study of protein properties on a global, integrated view of biological processes.

**Expression Proteomics:**
Study of global changes in protein expression

**Cell-map Proteomics:**
Study of protein-protein interactions through the isolation of protein complex

(Blackstock and Weir, 1999)
Fields for proteomics related market

* Drug discovery
* Diagnostics
* Equipment supply
* Protein/antibody chip
Essential technology needed for proteomics

1. Separate large numbers of proteins
2. Identification of proteins
3. Study protein modifications
4. Progress from protein sequence to 3D structure and functions
5. Handle massive datasets - bioinformatics
Key Technologies Driving Proteomics

- Reproducible 2D Gel Technology
- Staining and Scanning Technology
- Mass Spectrometry for Identification
- Databases (protein and genome)
- Database Searching Algorithms

The key challenge in proteomic research is the automation and integration of these technologies

(Winter, 2000)
Fig. 2 The current status of proteomic technologies. The different data typically collected in proteomic research and the available technologies are listed. The relative maturity of the proteomic technologies and other key discovery science tools is apparent from the position of the respective technology on the graph.

(Patterson and Aebersold, 2003)
Fig. 4 Time line indicating the convergence of different technologies and resources into a proteomic process. Advances in mass spectrometry and the generation of large quantities of nucleotide sequence information, combined with computational algorithms that could correlate the two, led to the emergence of proteomics as a field.
Experimental approach for proteomic studies

(Winter, 2000)
Key equipments necessary for proteomic studies.

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Figure 1. Proteomics analysis by (a) gel-based and (b) gel-free approaches. By the gel approach, the protein mixture is separated by two-dimensional electrophoresis, first by isoelectric focusing followed by SDS-PAGE. After spot visualization, proteins are extracted from the gel, digested, and analyzed by mass spectrometry for further identification by database searching. By the gel-free approach, the protein mixture is directly digested into a peptide mixture separated by multidimensional separation methods. Peptides are next analyzed by mass spectrometry. Proteins are identified from the generated mass spectra using database searching.

(Fournier et al., 2007)
Proteome databases on internet
1. ExPASy (Expert Protein Analysis System) proteomics server

   a. SWISS-PROT and TrEMBL - Protein sequences
   b. PROSITE - Protein families and domains
   c. SWISS-2DPAGE - Two-dimensional polyacrylamide gel electrophoresis
   d. SWISS-3DIMAGE - 3D images of proteins and other biological macromolecules
   e. SWISS-MODEL Repository - Automatically generated protein models
   f. CD40Lbase - CD40 ligand defects
   g. ENZYME - Enzyme nomenclature
   h. SeqAnalRef - Sequence analysis bibliographic references
2. **Protein Data Bank**: the Research Collaboratory for Structural Bioinformatics

3. **The Protein Information Resource**: International Protein Sequence Database

4. **WORLD-2DPAGE**: Index to 2-D PAGE databases and services

5. And many others
Applications of proteomics
Biomedicine (Clinical and Biomedical)
Drug discovery
Protein-protein interaction
Biological Application
Clinical and Biomedical Application

*Clinical diagnostics
analysis of body fluid and tissue
monitor disease process
discover new disease markers
Drug discovery

* identify disease specific protein
* target validation and signal transduction
* drug mode-of-action
* formal drug toxicology
Biological Application of Proteomics (I)

* Tracking complexity
  # host-pathogen interaction

* Immunogenetic proteins
  # identify immuno-reactivity proteins
  # identify allergens

* Improve agricultural products
  # identify proteins important for disease protection
  # identify proteins related to economically important traits
* **Value added agriculture products**
  
  # dairy industry: seeking useful proteins
  # abattoir wastage: discovery of useful proteins
  # food industry: cereal protein replace casein

* **Quality control**
  
  # bring precision and definition to a new level of protein-based products
  # identify genetic features of a cell line
  # detect the presence of a disease in biotechnology products