LN CryoSystem for Genetic Resources

Ming-Che Wu (吳明哲)

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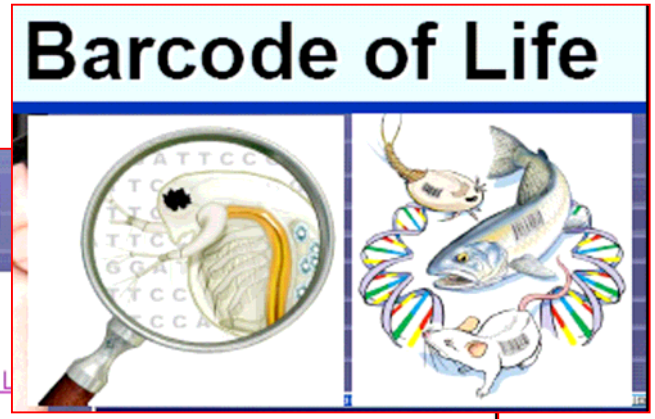
http://www.angrin.tlri.gov.tw
http://www.tlri.gov.tw

Outline

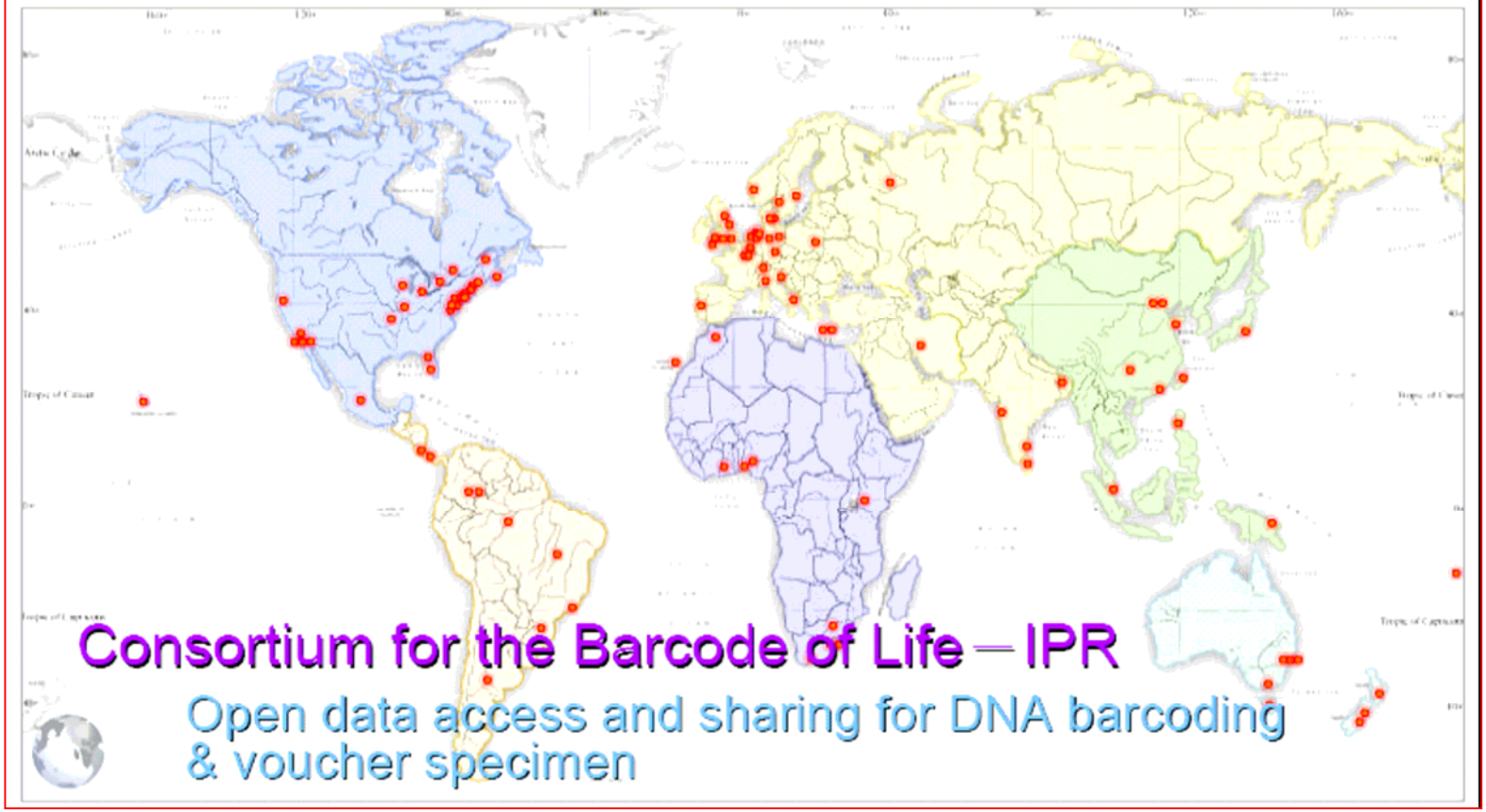
- DNA barcode of life
- From land to DNA banks
- Liquid nitrogen (LN) freeze DNA's life
- Genetic resources in LN
- Backup system of gene Cryobanks



More than 130 institutions have joined

Member Organization Map

Learn more about each CBOL member by clicking on their location, or see a current L



Source: Dr. Kwang-Tsao Shao, Academia Sinica, Taiwan

行政院農業委員會畜產試驗所
Livestock Research Institute
Council of Agriculture, Executive Yuan

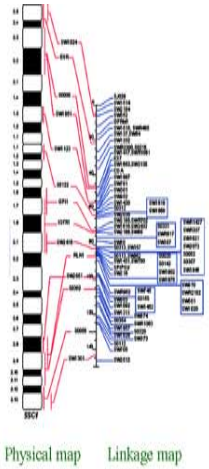
Genomic Breeding
基因選種

Livestock samples

Blood, semen, muscle, milk…etc

DNA extraction

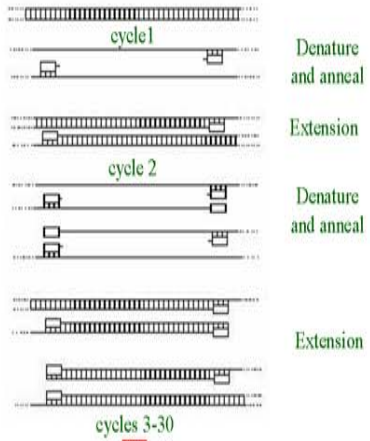
Markers



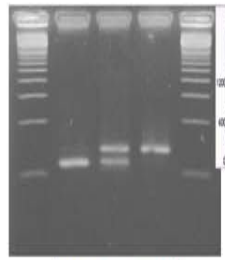
Physical map Linkage map

Amplification

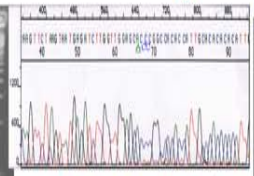
Genetic detection



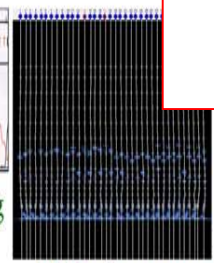
Genotyping Elecrophoresis



Agarose gel



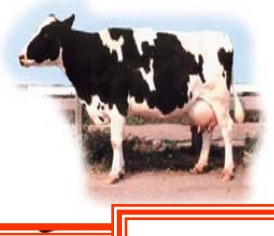
DNA sequencing



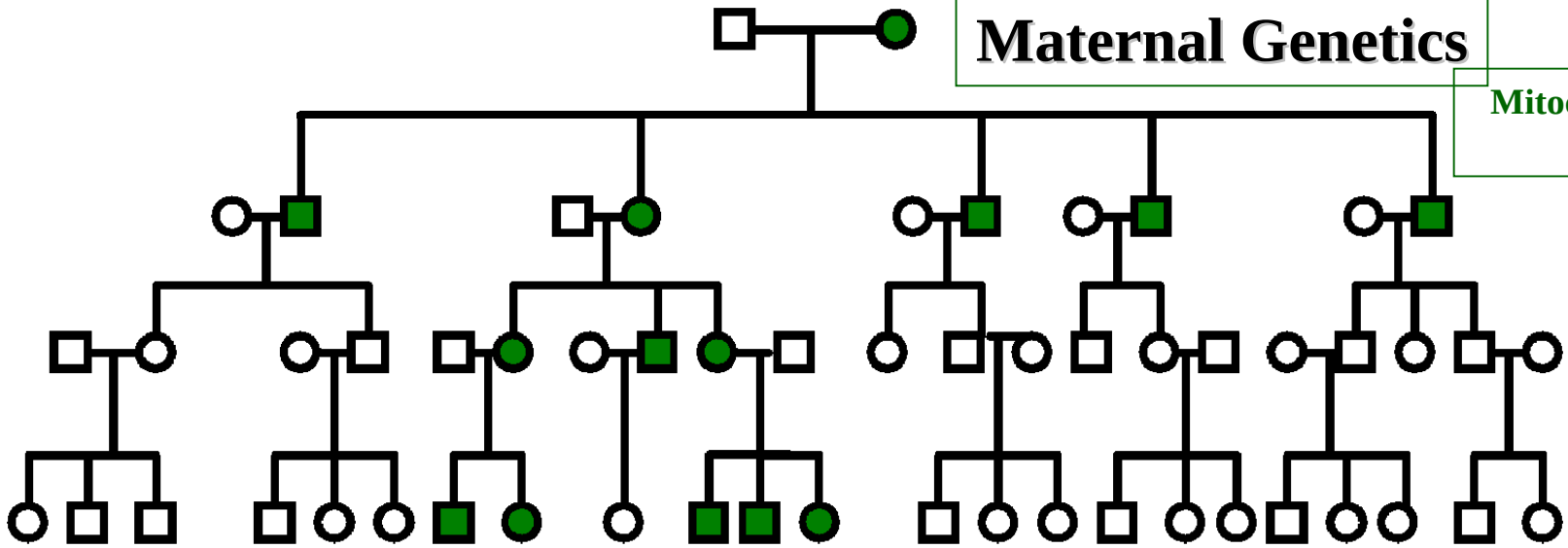
DNA fragment analysis

Statistical analysis

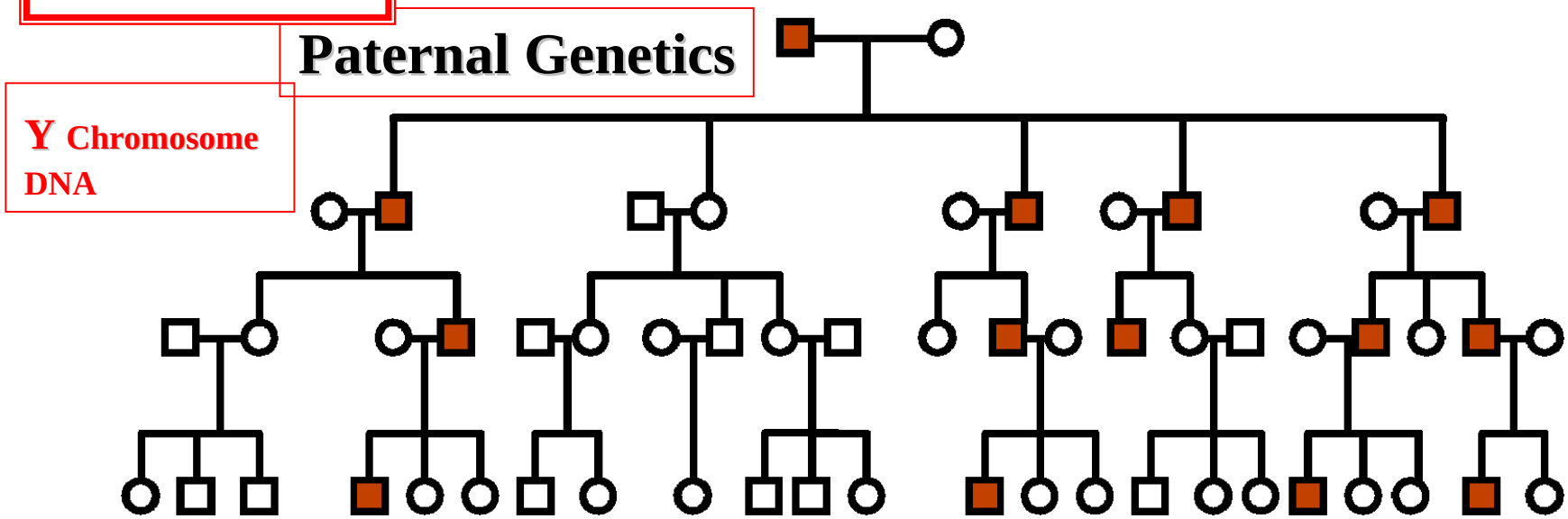
Selection



Elite Animal


Maternal Genetics
Mitochondrial DNA

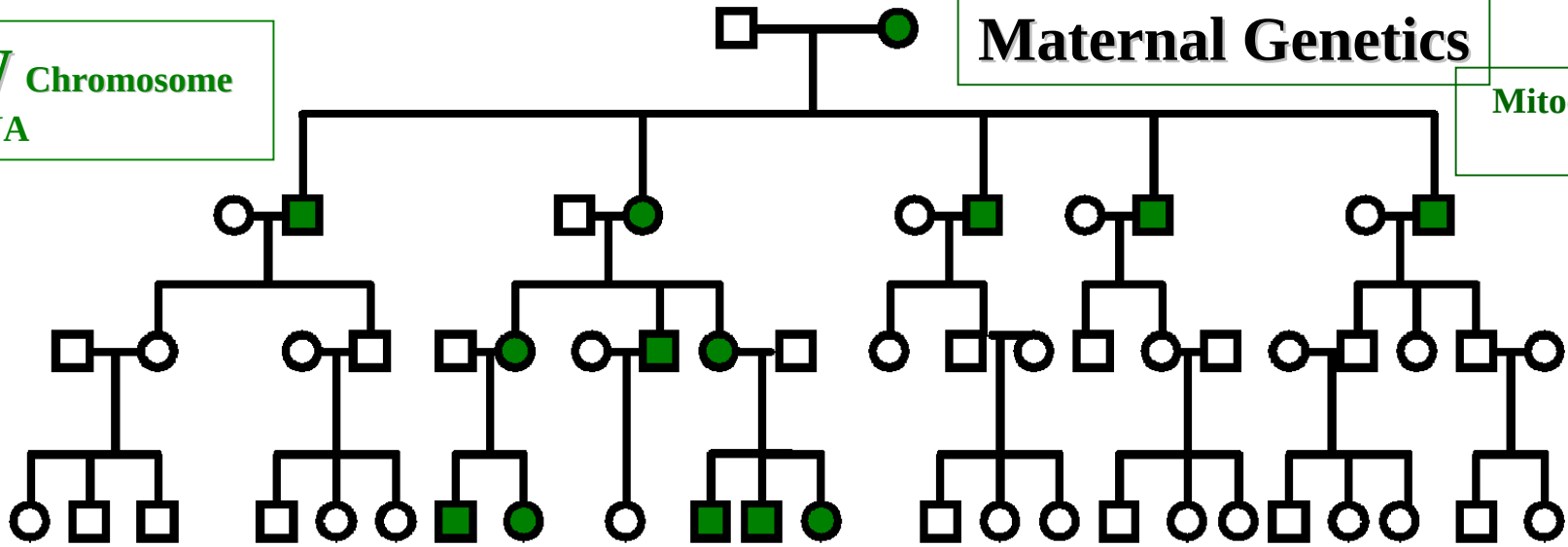
Mammals


Paternal Genetics
Y Chromosome DNA

W Chromosome DNA

Maternal Genetics

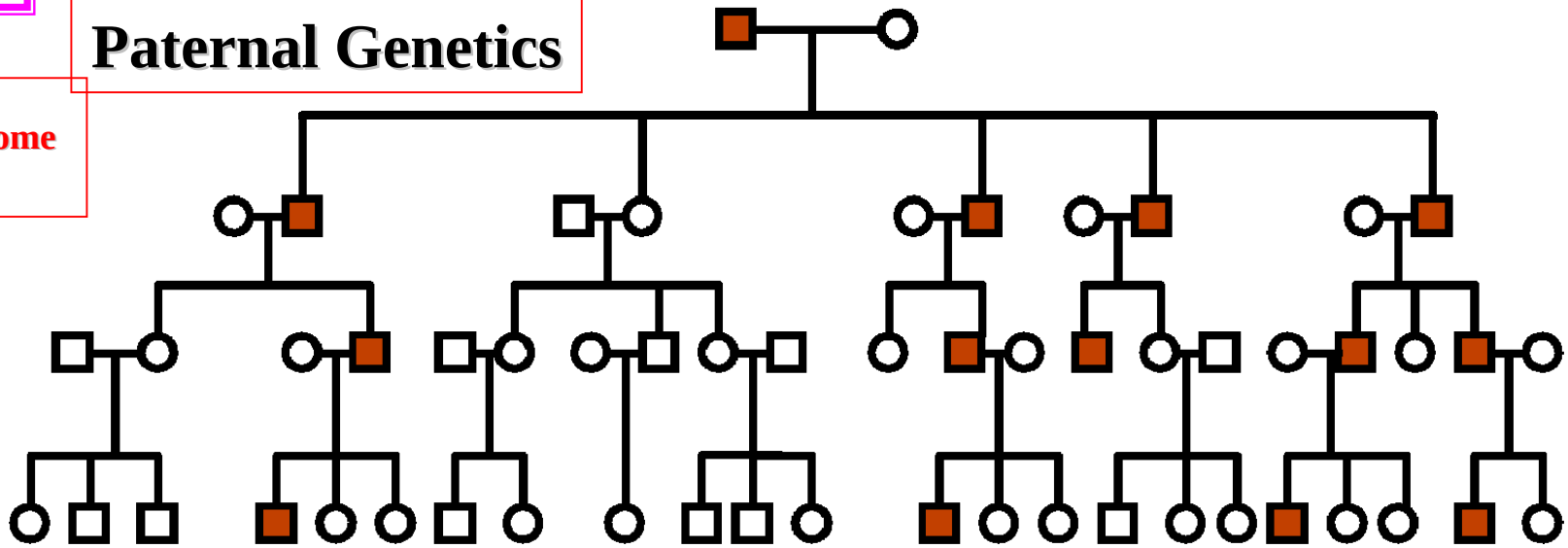
Mitochondrial DNA

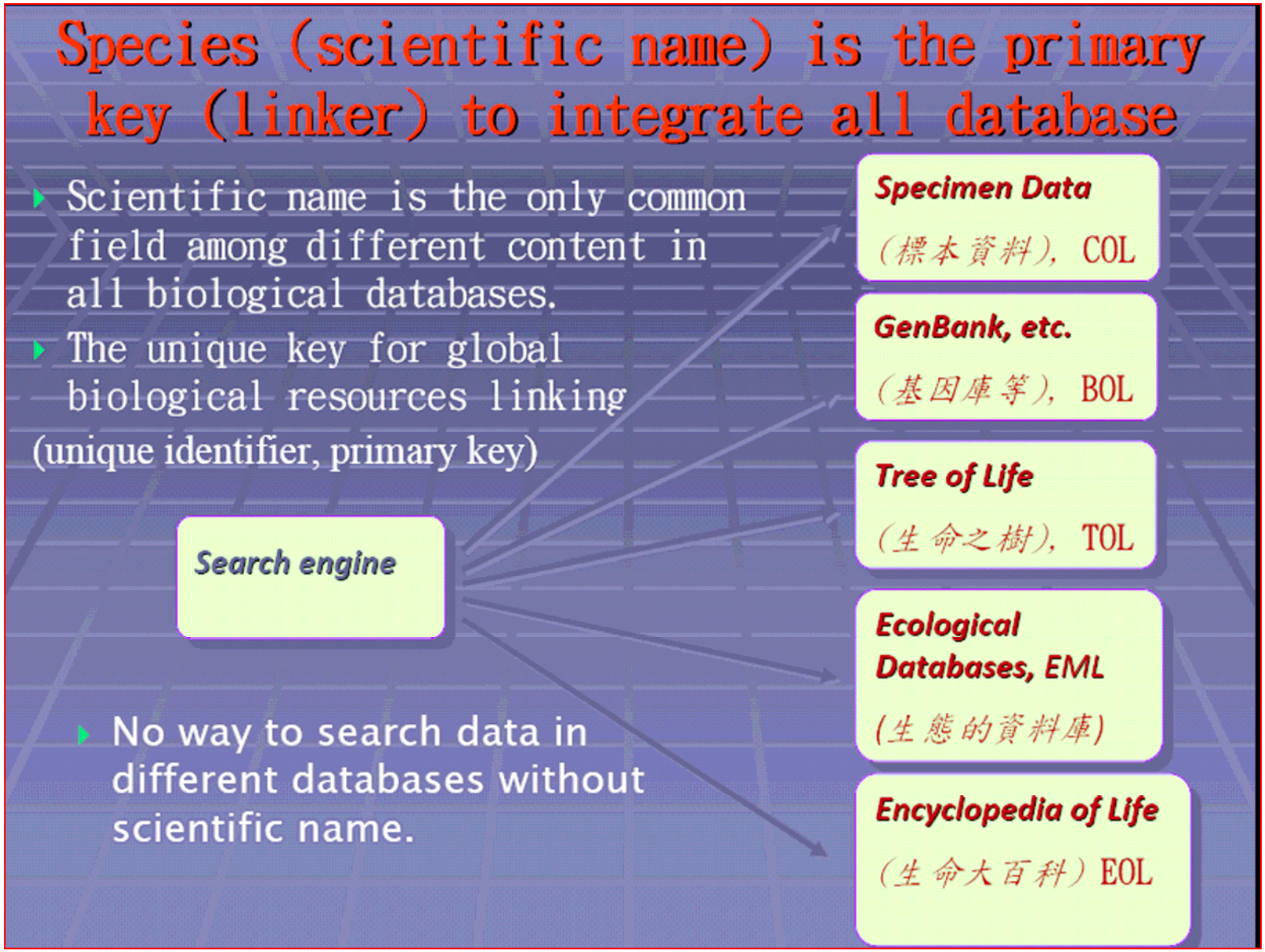


Birds

Paternal Genetics

Z Chromosome DNA





Source: Dr. Kwang-Tsao Shao, Academia Sinica, Taiwan

By the consciousness of the fact that species continuously disappear from the Earth, advanced countries, one by one, start to preserve genetic material for local living forms.

Evolution—Genetic change in a population or species over generations; all the changes that transform life on Earth; the heritable changes that have produced Earth's diversity of organisms.

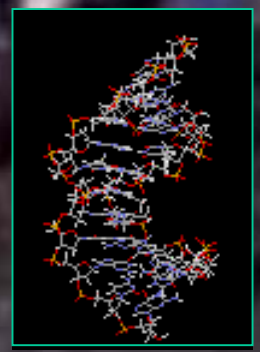
Extinct—The condition when an organism is no longer in existence, when all members of the species have died without leaving a survivor to continue the line.

Feral—A wild animal that originated from domesticated stock.

Food web—A network of interconnecting food chains.

Introduced species—A species that humans move from the species' native location to a new geographic region; sometimes called an exotic species or invasive species.

使全國民眾瞭解農林漁牧業生物多樣性保育之重要性，並身體力行於日常生活中，注重生態保育工作於農村。



From Land to DNA Banks

National Biodiversity Information Network in Taiwan , TaiBNET

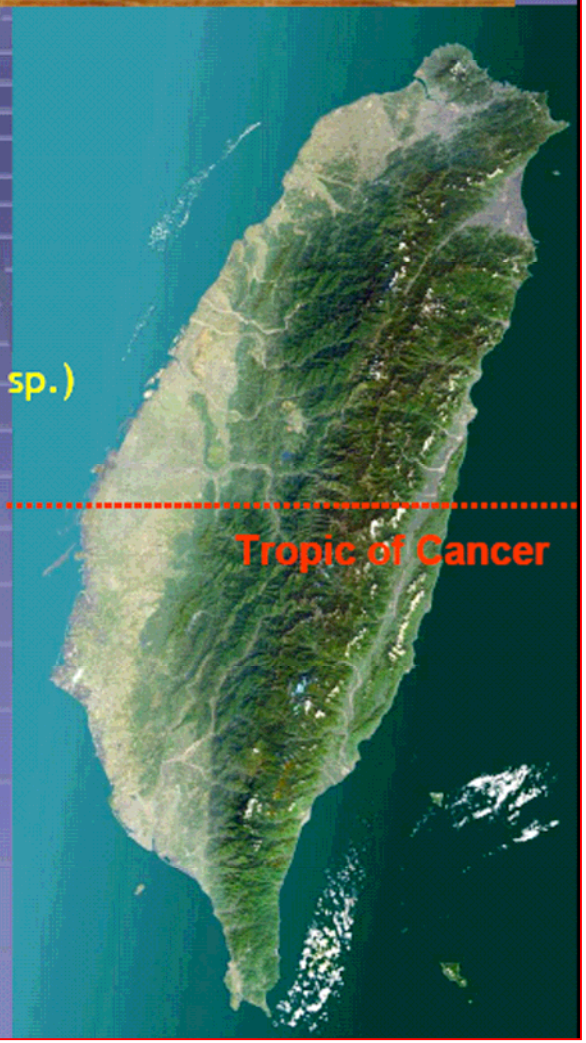
http://TaiBNET.sinica.edu.tw

Source: Dr. Kwang-Tsao Shao, Academia Sinica, Taiwan

Biodiversity & Endemism(%) in Taiwan

- Area of Taiwan : 35,800 km^2
- Area of Taiwan/World : 0.02%
- Total sp. # : 10% (marine); 2% (terrestrial)

- Fishes : 2,900/26,000 = 10% (16% freshwater sp.)
- Birds : 456/9,040 = 4.65% (19%)
- Mammals : 71/4,000 = 1.78% (44%)
- Reptiles : 97/6,300 = 1.54% (32%)
- Amphibians : 34/4.84 = 0.84% (31%)
- Insects : 20,000/751,000 = 2.6% (8-60%)
- Plants : 4,000/200,000 = 2% of global plant diversity (25%)



Source: Dr. Kwang-Tsao Shao, Academia Sinica, Taiwan

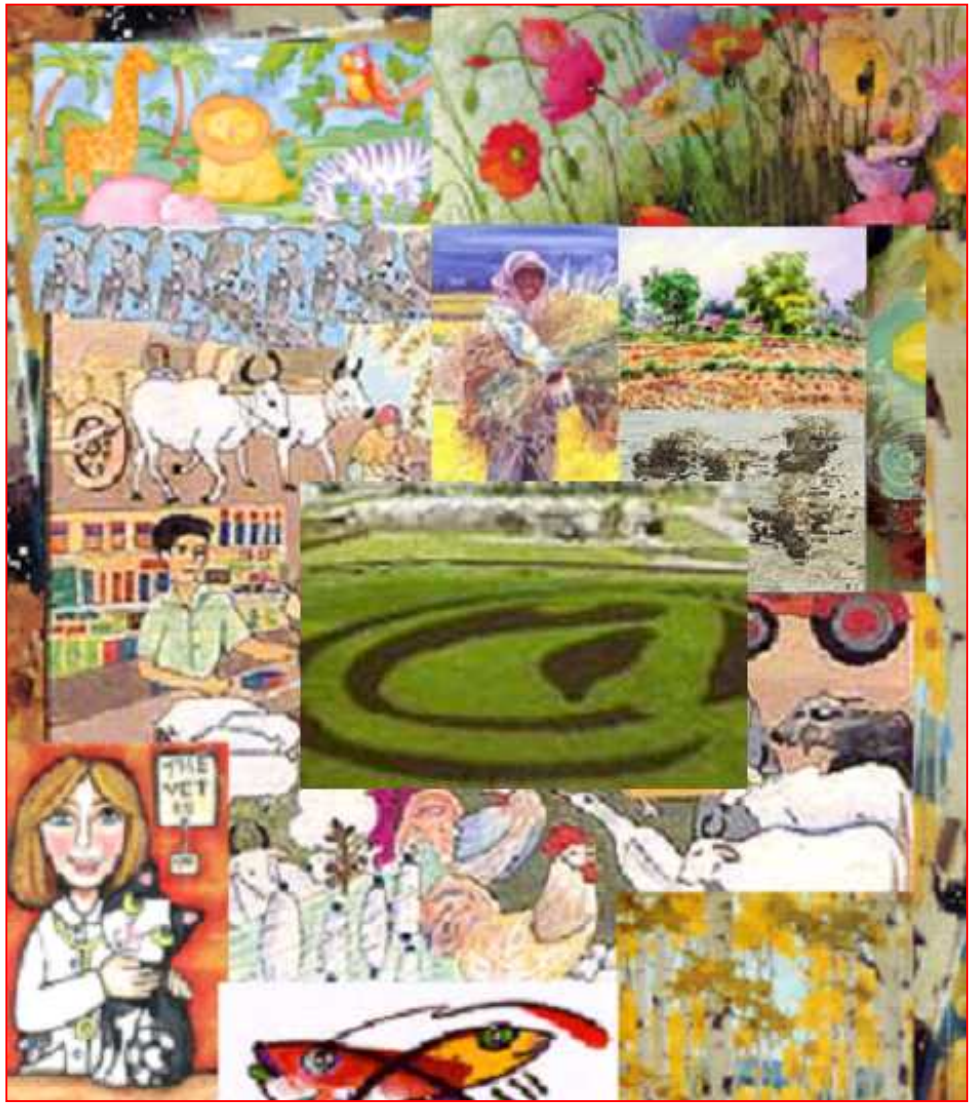
Tissue frozen in high quality can promote permanent preservation of biodiversity as well as provide studying material, including protein, RNA and DNA, for future application in the highly competitive areas of chemistry and biotechnology.

Under the consideration of importance and preservation value of species as well as the difficulties of specimen collection, the principles for genetic material storage and collection are:

(1) Endemic species and subspecies are prior to non-endemic species.
(2) Resident species is prior to non-resident species, such as migrants.
(3) Genus or family with a single subordinate is prior to genus or family with multiple subordinates.
(4) Conservative species is prior to non-conservative species.
(5) Species that are more susceptible to environmental changes is prior to species with a stabilized population.

Genetic Conservation

Living form
Cell/tissue
DNA/RNA/mRNA/cDNA/protein



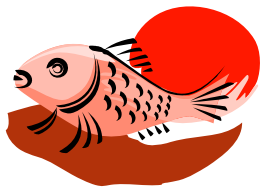
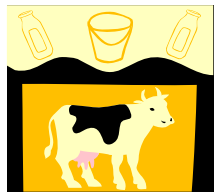
Council of Agriculture

Executive Yuan, Taiwan

- **Agricultural Research Institute**
- **Forestry Research Institute**
- **Fisheries Research Institute**
- **Livestock Research Institute**
- **Endemic Species Research Institute**
- **Animal Health Research Institute**

Research Center for Biodiversity and Computing Center

Academia Sinica, Taiwan

Genetic Materials in Taiwan

2004-2007 sponsored by Forest Bureau, COA

- Research Center for Biodiversity, Academia Sinica: **Fishes and database integration.**
- Council of Agriculture, Executive Yuan, and National Taiwan Normal University: **Aves and Mammals.**
- Taipei Zoo and National Taiwan Normal University: **Reptile and Amphibian.**
- National Nature Science Museum: **Invertebrate**

- Germplasm Center, Livestock Res. Inst, COA: back-up data bank

Source: Dr. Kwang-Tsao Shao, Academia Sinica, Taiwan

-80℃ Refrigerator

Small LN Tank

Facility

- 大型液態氮儲存桶：
  - 兩個，各可以儲存34000件樣本。
- 小型液態氮儲存桶：
  - 兩個，各可以儲存3200件樣本。
- 車攜式液態氮儲存桶：
  - 兩個，各可以儲存300件樣本。
- 野外用液態氮儲存桶：
  - 兩個，各可以儲存30以及50件樣本。

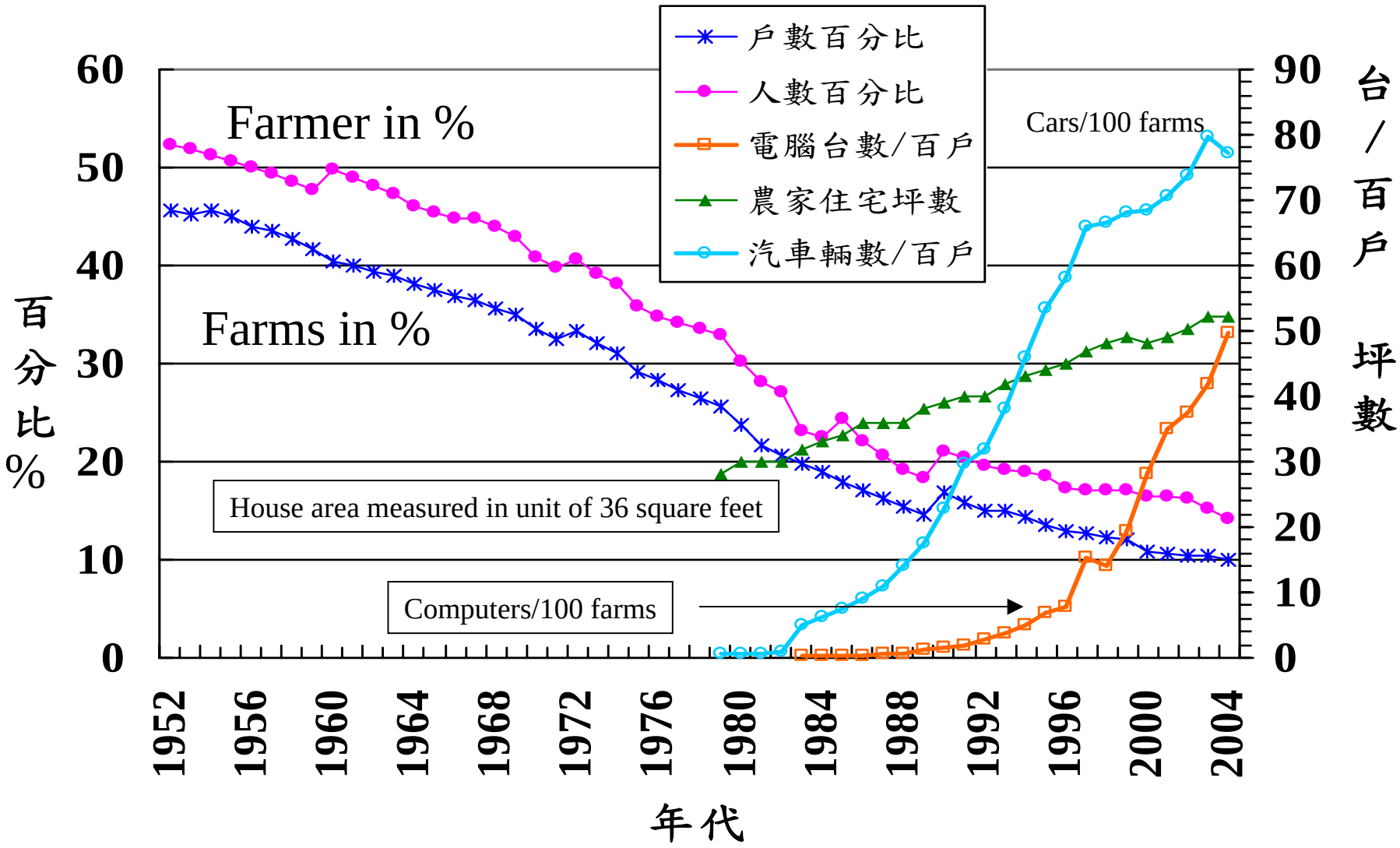
Source: Dr. Kwang-Tsao Shao, Academia Sinica, Taiwan

Genetic Resources of Farm Animals

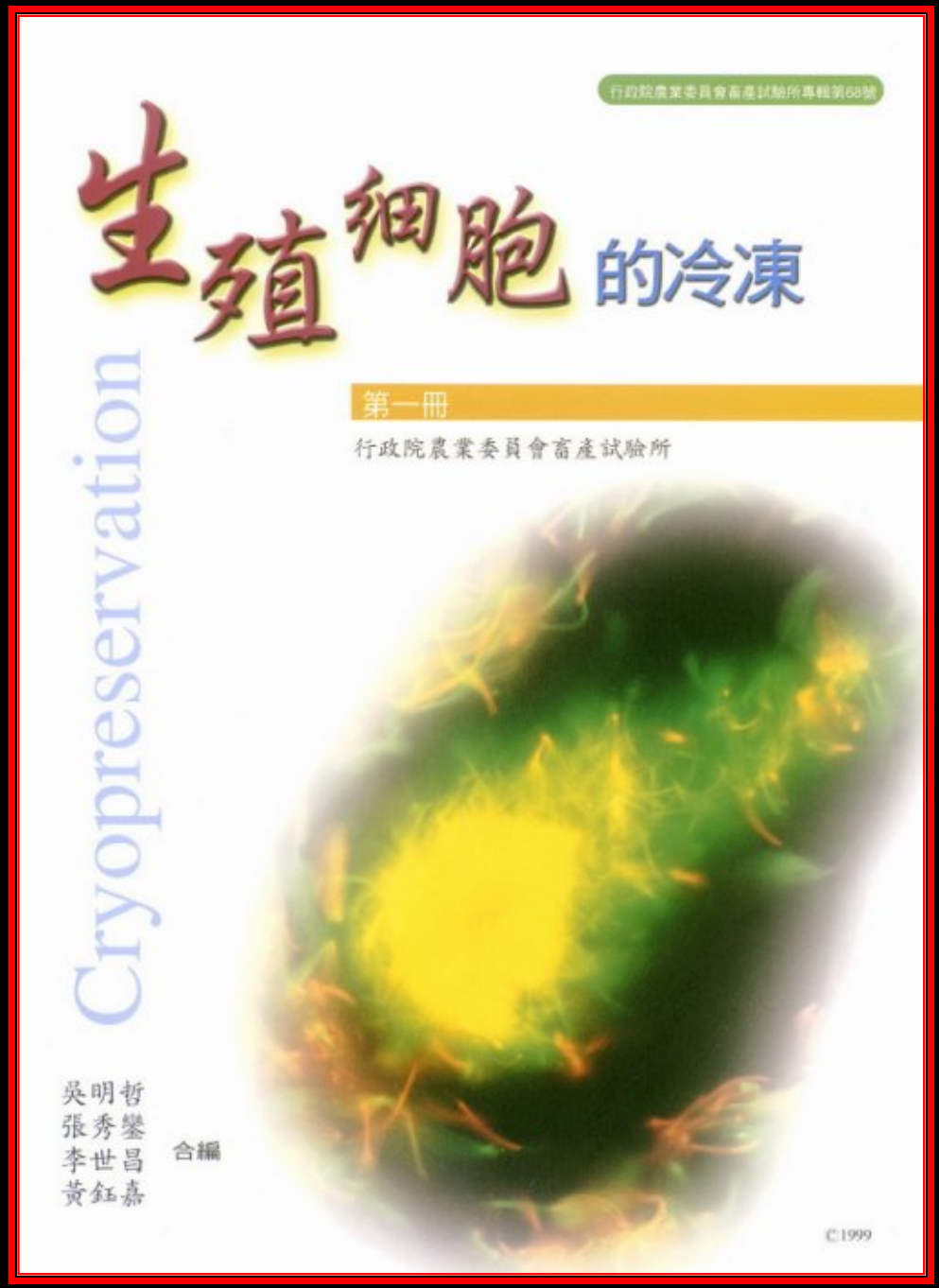
- Conservation farms
- Web site http://ww.angrin.tlri.gov.tw
- *Cryopreservation Bank*
- Molecular techniques
- Genetic resources exchange

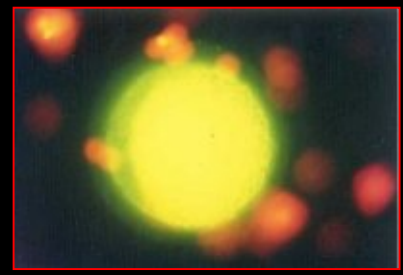
Phase I (1987~1992)：
Conservation farms
Phase II(1993~1998)：
Web site
Phase III(1999~2004)：
Conservation center
Phase IV(2005~2008)：
Research and Utilization
Phase V(2009~2012):
Global Bioidentification System

Living conditions in Taiwan farmer from 1952 to 2004



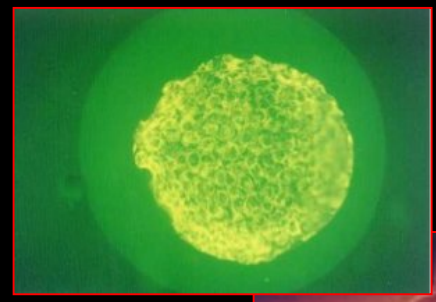
www.angrin.tlri.gov.tw





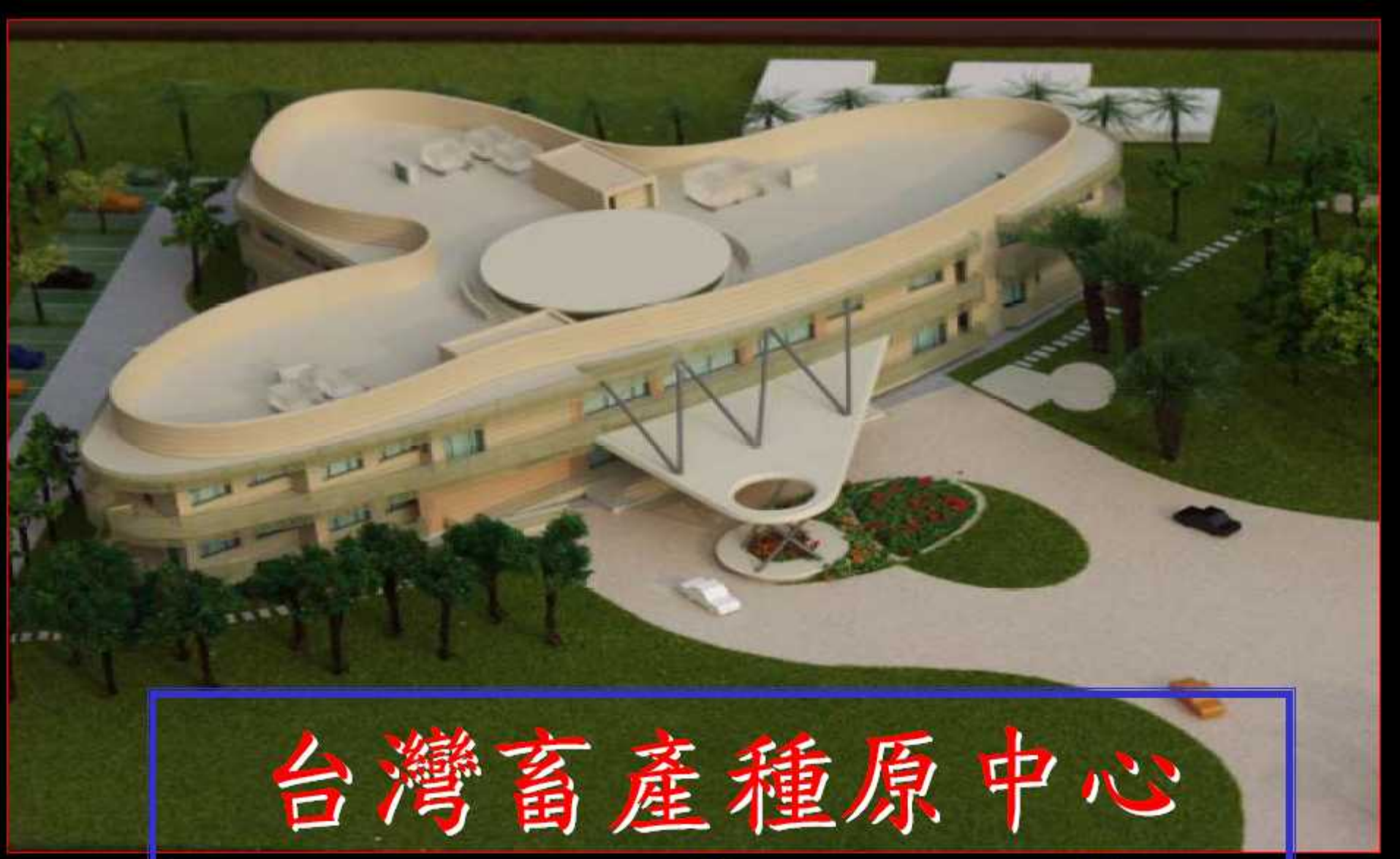
Oocyte

Sperm



Embryo

Cell Bank



台灣畜產種原中心

Taiwan Animal Germplasm Center

http://www.angrin.tlri.gov.tw

earthquake-resistant steel frames

CryoBanking

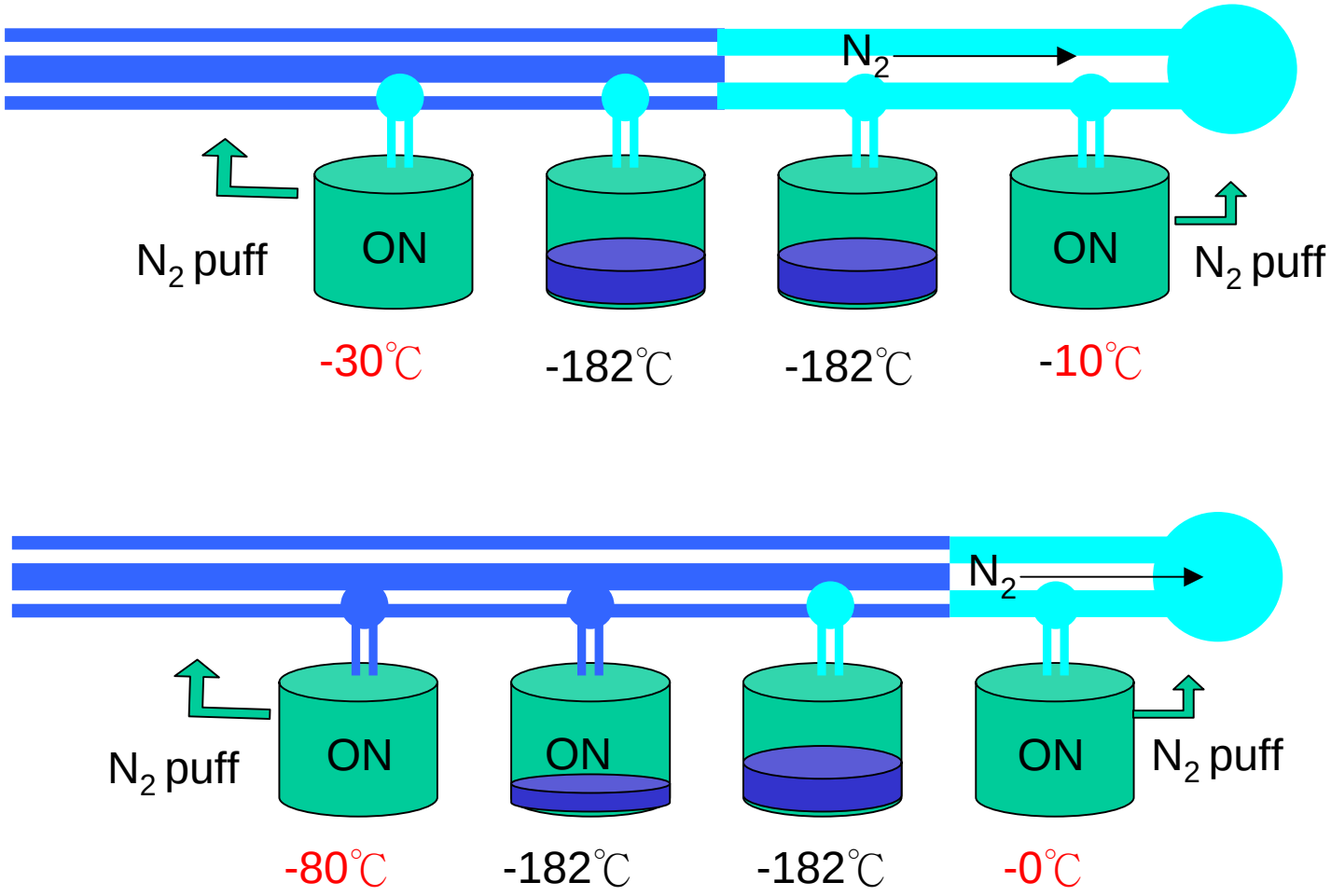
LN2 Tank : SCS-20000
(ADM Grade)

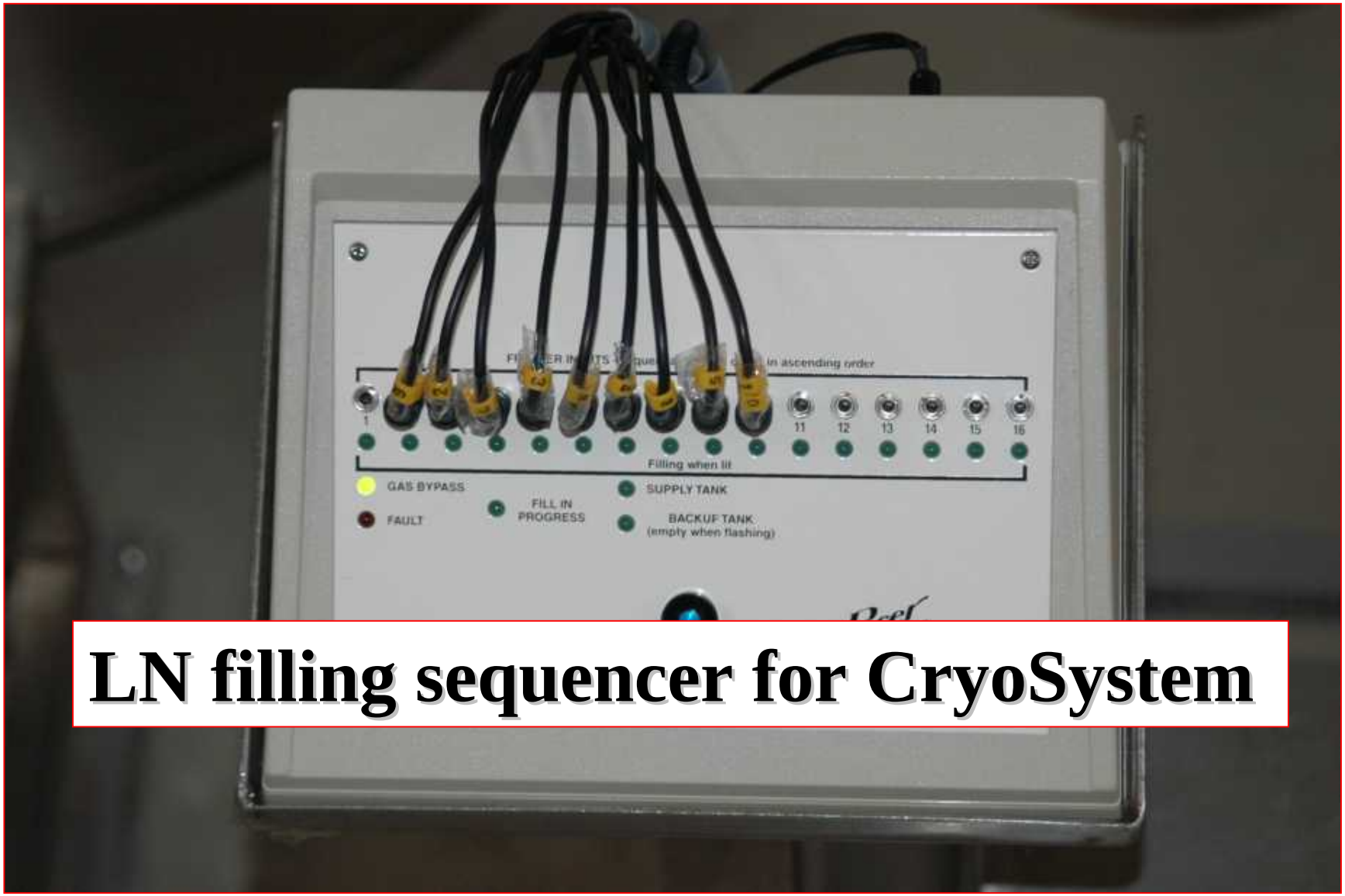
桶	高	1040 cm
直	徑	220 cm
基礎中心直徑		200 cm
全重(含液氮)		37000 kg

Taiwan Animal Germplasm Center
http://www.angrin.tlri.gov.tw/

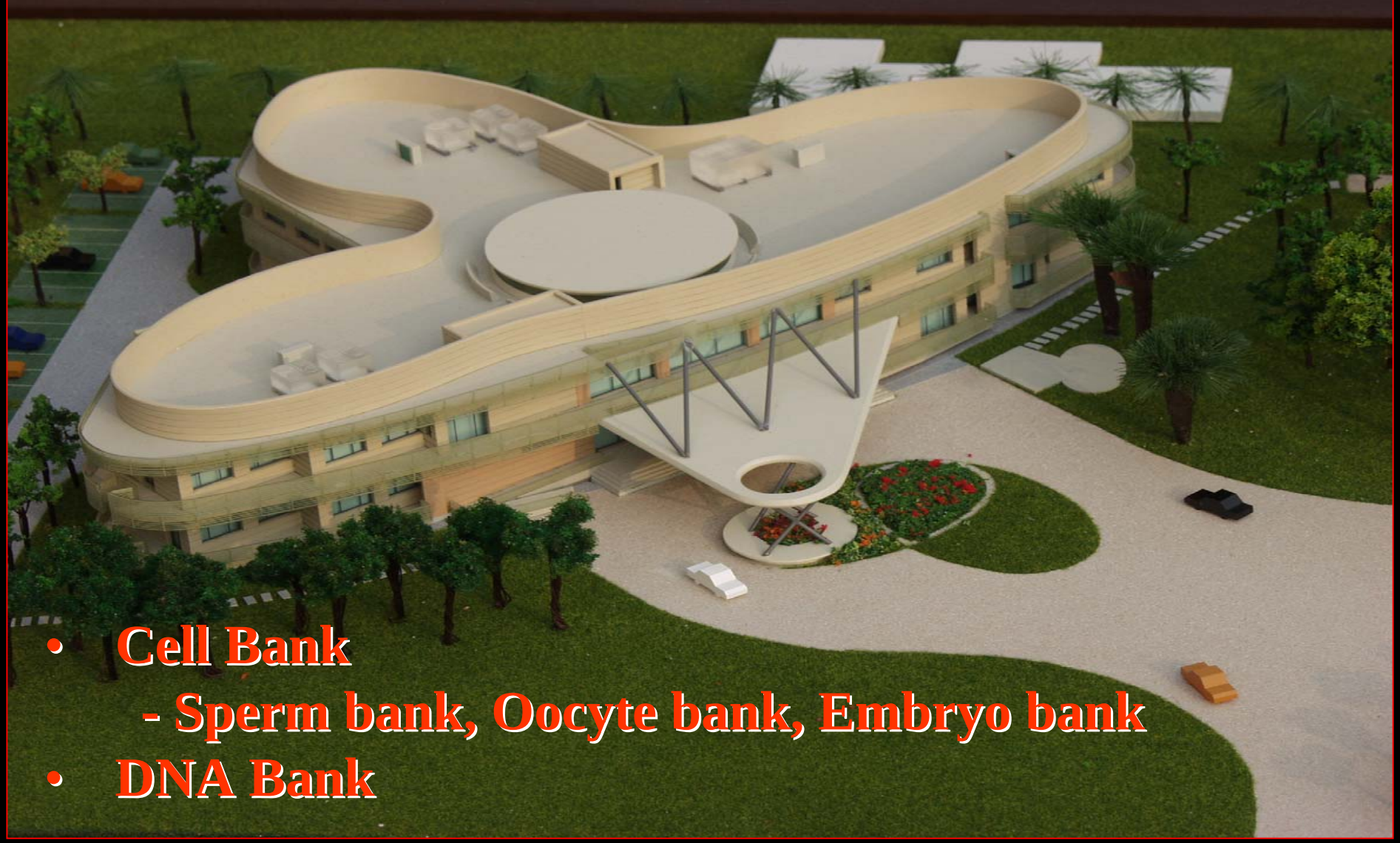
CryoFacility: 38K*17 + 24K*1

LN filling sequencer for CryoSystem





LN filling sequencer for CryoSystem



- **Cell Bank**
  - Sperm bank, Oocyte bank, Embryo bank
- **DNA Bank**

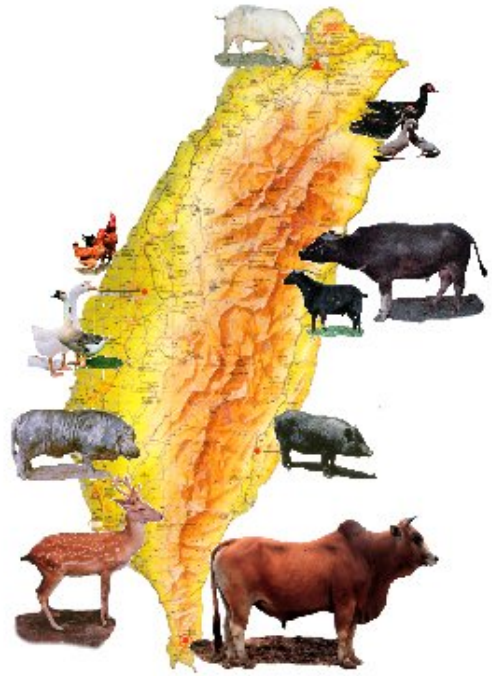
Taiwan Animal Germplasm Center

http://www.angrin.tlri.gov.tw/

DNA Samples of Farm Animals

(Breeding and Genetics Division, 2007)

Cattle	**6180**
Water Buffalo	**58**
Goat	**3127**
Sheep	**14**
Horse	**14**
Rabbit	**88**
Swine	**25129**
Chicken	**11650**
Muscovy	**151**
Duck	**689**
Hybrid Duck	**128**
Goose	**431**
Turkey	**14**
Black Swan	**135**
Quail	**50**
	47858



移地備份 Backup

➔農試所(已寄存農試所 桃園豬 20份、黃牛 31份、山羊 31份、雞 40份、鴨 27份)

(將被寄存)

畜試所➔水試所

畜試所➔中研院

Backup system of gene Cryobanks

Why?
Where?
When?
Who?
How?

Samples of Birds

Fur samples 7,737 (18 Orders, 61 Families, 354 Species)

Blood and tissue 12,700 samples

Mammal samples

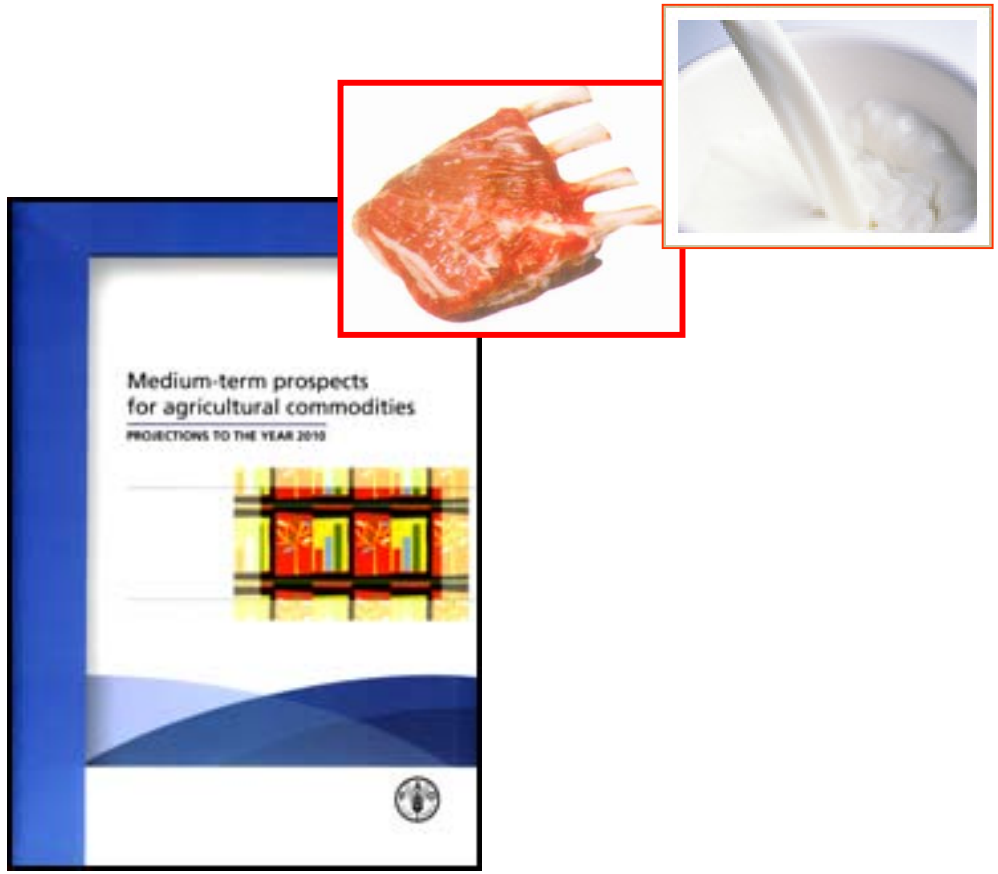
8 orders, 19 families, 67 Species 1,945 samples

Regulation for genomic breeding - Animal Industry Act Article 17

The competent authority may dispatch inspector to examine or test **the breeding flock,** breeding stock, facilities, pedigree registry and related records of breeders, and the breeders shall not evade, interfere with or object to such examination or testing. Breeding flock or breeding stock found to have contracted notified disease or have **hereditary disease** during the aforesaid examination or testing shall be **banned** from breeding. The aforesaid inspectors shall present their identification when carrying out their duty.

	Pig	Cow	Goat	Chicken	Duck	Goose	Deer	Rabbit
Gene	Hal-1843 ESR HFABP-H HFABP-ad IGF2-In3 IGF2-In7 D5FAD D-Loop SW18 SW1943 SW2588 PRLR	BLAD DUMP CITR CVM XY GH mtDNA	G6S IGF2 mtDNA	HSP70 PRLR PRL ESR mtDNA ZW	PRL ESR ZW mtDNA	PRL ESR ZW mtDNA	IGF2 XY D5FAD mtDNA	GPI PGD XY IGF2 mtDNA

DNA in food chain



FAO糧食預估白皮書

Origin assurance

- market to farm

乳 Milk

DNA

Meat 肉

Egg 蛋

Landrace

Lanyu

Duroc

Taoyuan

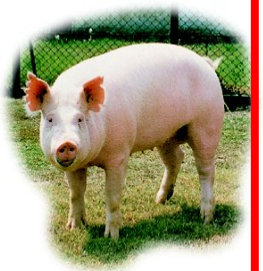
Lee-Sung

Inbreeding

Spotty

Mitsae

TLRI Black



Yorkshire

Meishing

Hampshire

Berkshire

LY

LYD Hog

Pig Breeding

行政院農業委員會畜產試驗所
Livestock Research Institute
Council of Agriculture, Executive Yuan

黃牛

Meat Cattle

聖達

布拉曼

肉用品種

夏洛利

乳用品種

Milk

荷蘭乳牛

役用品種

Draft Buffalo

水牛(無法與上列品種生育後代)

DNA Test on Frozen Bull Semen

Genetic Defect

Uridine monophosphate synthase (DUMPS)

Bovine leukocyte adhesion deficiency (BLAD)

Citrullinemia (Citr)

Semen sources

USA

Canada

Japan

Holland

行政院農業委員會畜產試驗所
Livestock Research Institute
Council of Agriculture, Executive Yuan

Meat Goat

肉用品種

波爾

土山羊

努比亞

黑色波爾

吉安山羊

Milk Goat

乳用品種

撒能

阿爾拜因

吐根堡

毛用品種

Fur Sheep

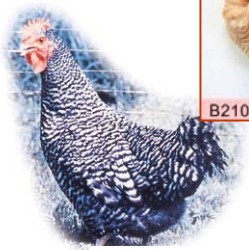
巴貝多綿羊

考利黛綿羊

行政院農業委員會畜產試驗所
Livestock Research Institute
Council of Agriculture, Executive Yuan

蛋用品種

Chicken
雞



Turkey
火雞

Goose
鵝

来亨雞

菜鴨

Meat

Egg

Duck & Muscovy

北京鴨

番鴨(與上列鴨品種
生育後代無法再繁殖)

BioUtilization

Conservation

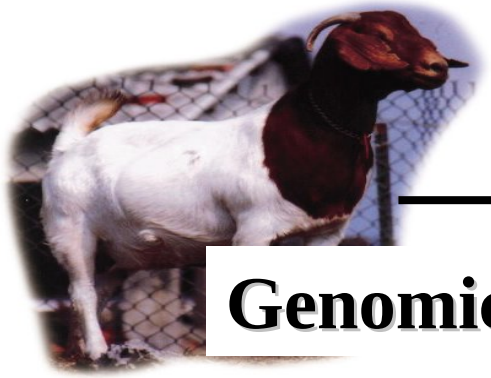
Species

Biodiversity

Selection

Demand

Value add

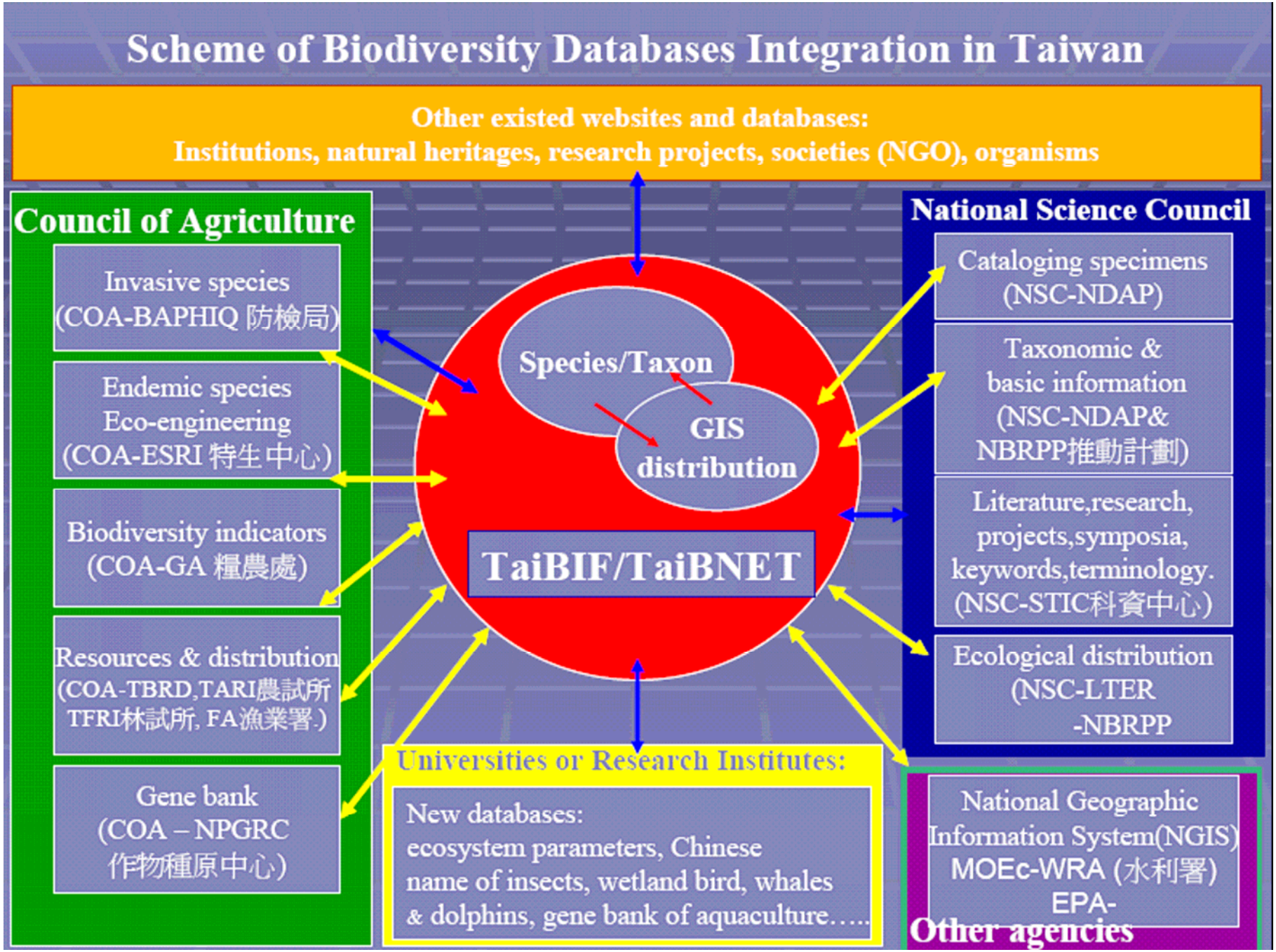


Export

Tracking

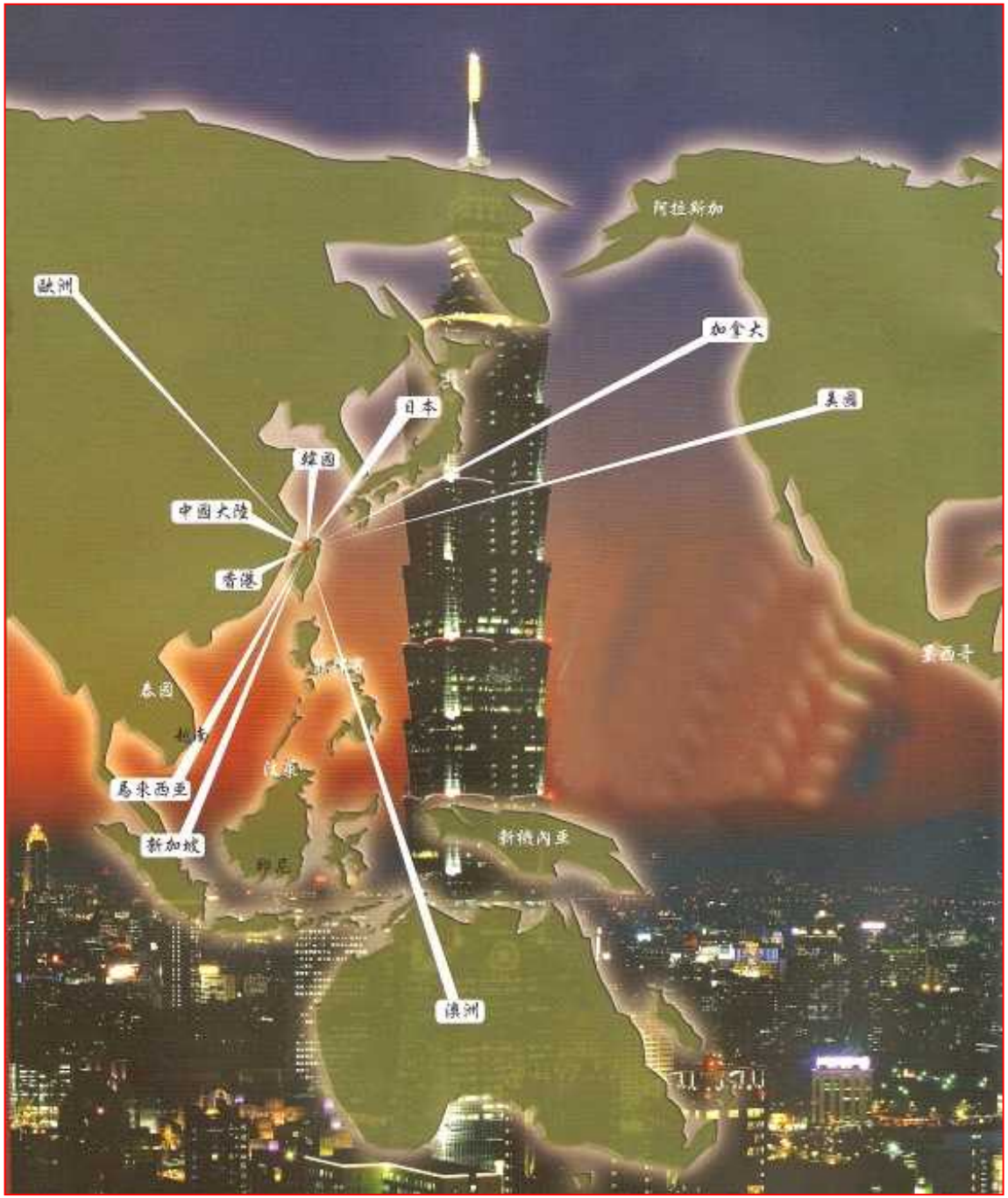
Genomic breeding

- **Species** verification
- **New breed/line**
- **Genetic** improvement



Source: Dr. Kwang-Tsao Shao, Academia Sinica, Taiwan

Global cooperation on Backup System for various gene cryobanks under conditions of …..



Thanks!

Livestock Research Institute
Taiwan