基因體工程技術於農產相關產業之應用現況與發展

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DNA sequencing, genetic profiling, genetic mapping, recombinant DNA technology, structural and functional analysis of genome

RNA sequencing, expression profiling, transcriptional regulation

Protein identification, quantification, post-translational modification

Element profiling, biochemical regulation, element interactions

Study metabolite profiles, metabolic intermediates, hormones and other signaling molecules

Genomics — Transcriptomics — Proteomics — Omics — Inomics — Metabolomics — Phenomics

Evaluation of morphological, biochemical and physical traits, establish link between genetic, epigenetic and environmental factors

Systems Biology

2

Deshmukh et al. (2014) Frontiers in Plant Science 5: 244

The Connectivity of Biological Studies

Genomics

Metabolomics

Bioinformatics
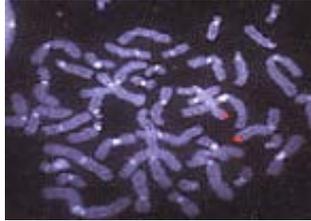
Gene Expression

Systems Biology

Transcriptomics
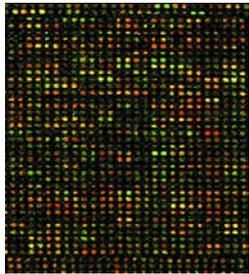
Protein Expression

Proteomics

基因體研究 Genomics

Genomic DNA → DNA Sequencing → Sequence Assembly → Gene Annotation

Chr5:1464000..1500000

http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/

基因體研究 Genomics
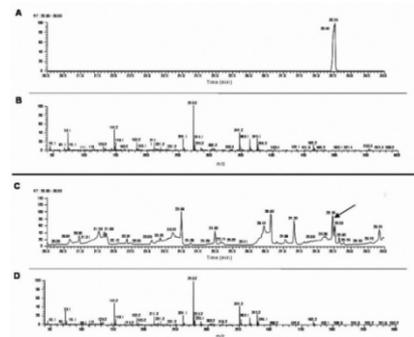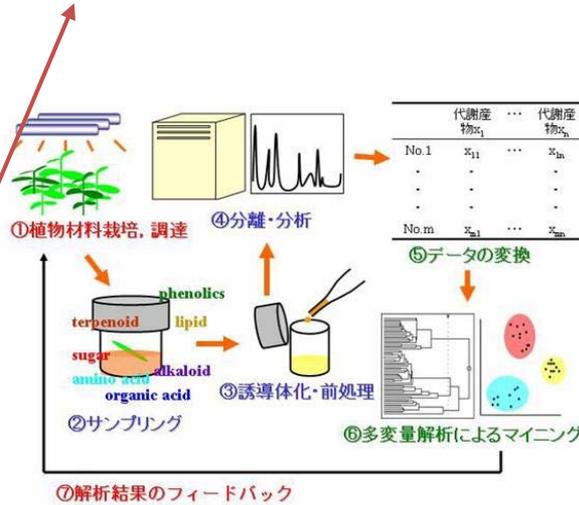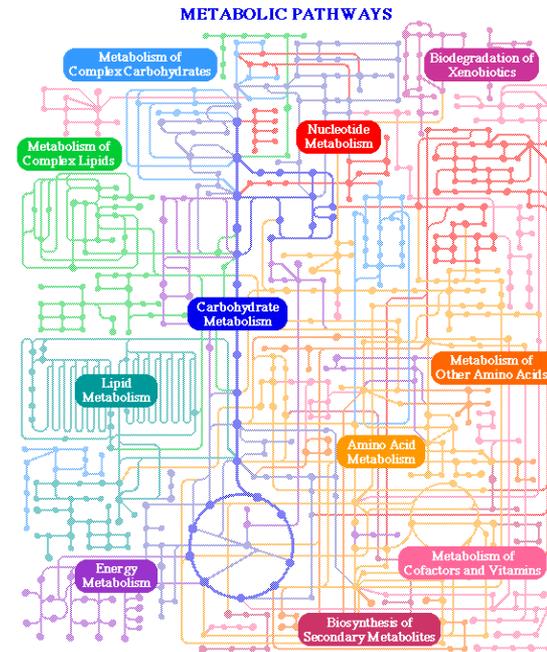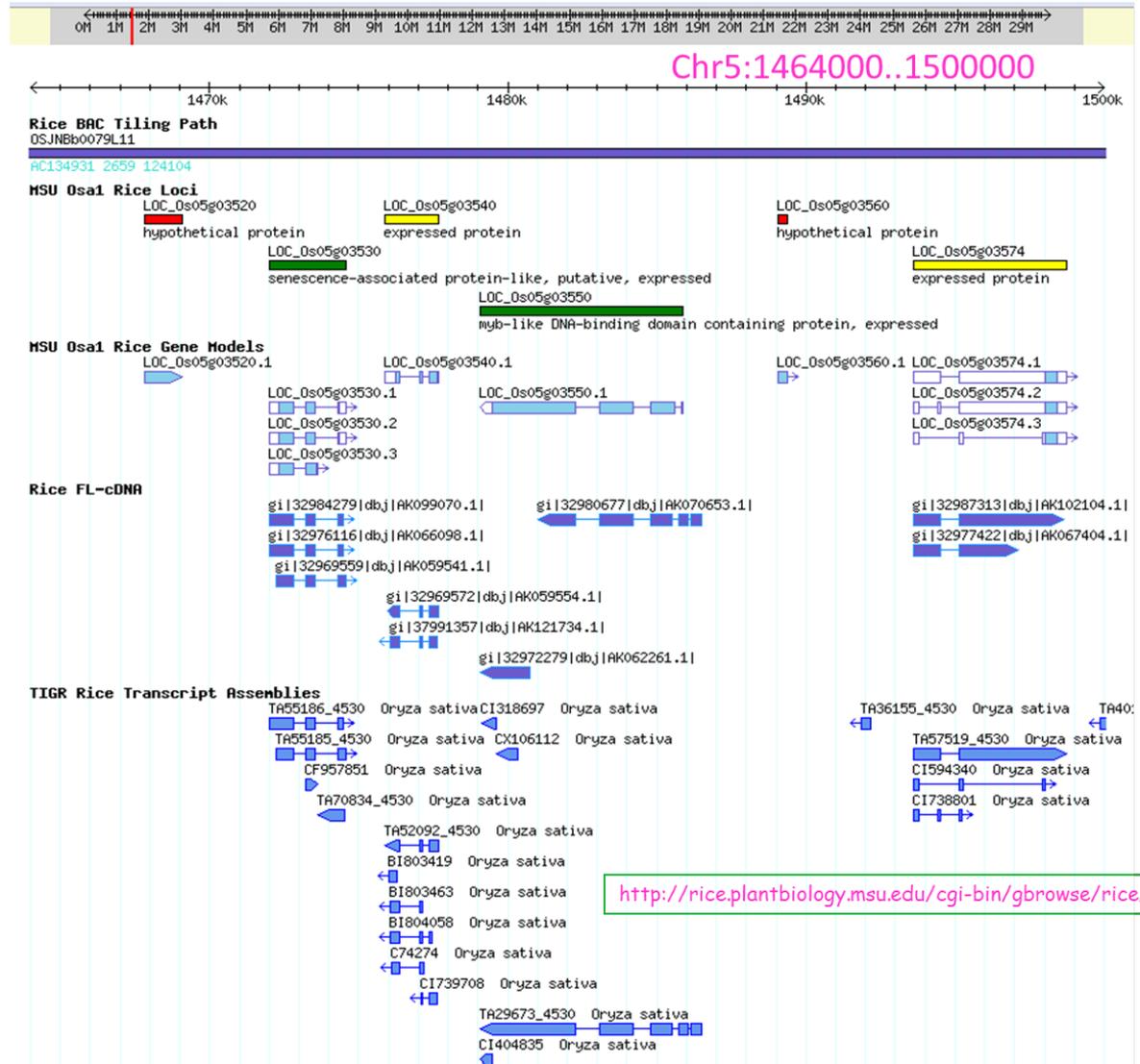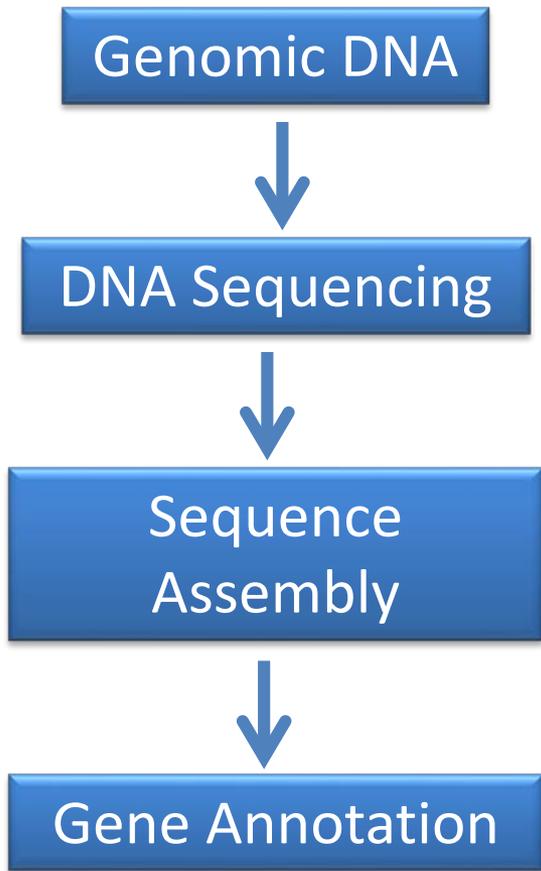
DATA NOTE **Open Access**

The 3,000 rice genomes project

The 3,000 rice genomes project[1,2,3*†]

Abstract

Background: Rice, *Oryza sativa* L., is the staple food for half the world's population. By 2030, the production of rice must increase by at least 25% in order to keep up with global population growth and demand. Accelerated genetic gains in rice improvement are needed to mitigate the effects of climate change and loss of arable land, as well as to ensure a stable global food supply.

Findings: We resequenced a core collection of 3,000 rice accessions from 89 countries. All 3,000 genomes had an average sequencing depth of 14x, with average genome coverages and mapping rates of 94.0% and 92.5%, respectively. From our sequencing efforts, approximately 18.9 million single nucleotide polymorphisms (SNPs) in rice were discovered when aligned to the reference genome of the temperate *japonica* variety, Nipponbare. Phylogenetic analyses based on SNP data confirmed differentiation of the *O. sativa* gene pool into 5 varietal groups – *indica*, aus/boro, basmati/sadri, tropical *japonica* and temperate *japonica*.

Conclusions: Here, we report an international resequencing effort of 3,000 rice genomes. This data serves as a foundation for large-scale discovery of novel alleles for important rice phenotypes using various bioinformatics and/or genetic approaches. It also serves to understand the genomic diversity within *O. sativa* at a higher level of detail. With the release of the sequencing data, the project calls for the global rice community to take advantage of this data as a foundation for establishing a global, public rice genetic/genomic database and information platform for advancing rice breeding technology for future rice improvement.

Keywords: *Oryza sativa*, Genetic resources, Genome diversity, Sequence variants, Next generation sequencing
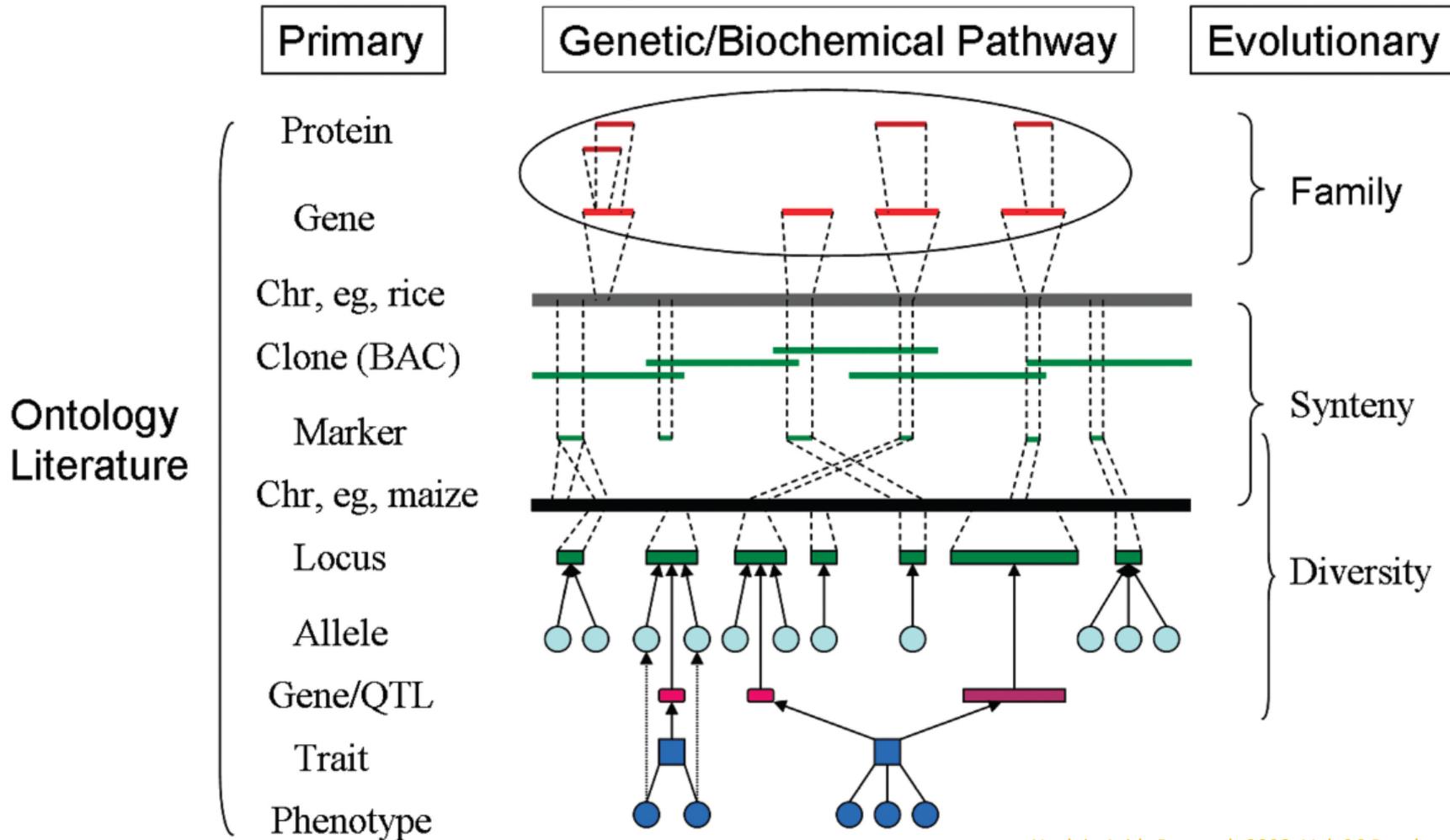
全球種子產值持續上升
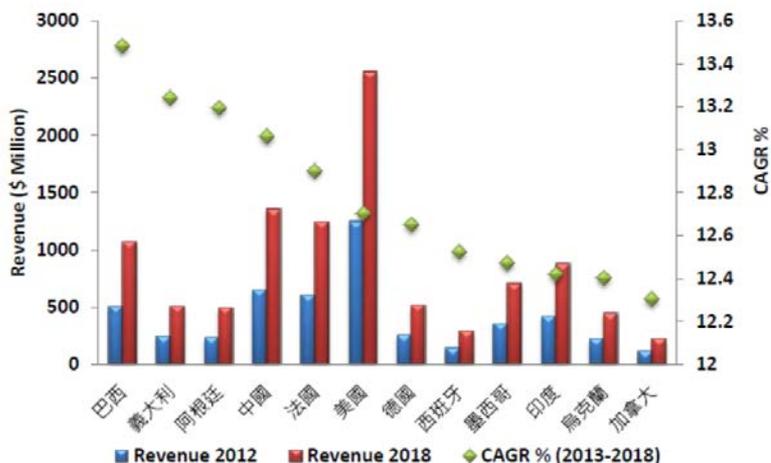
種子產業帶動下游產業鏈

(資料來源：2014 Fruit & Vegetable seed market)

圖 1. 全球蔬菜種子主要市場發展趨勢
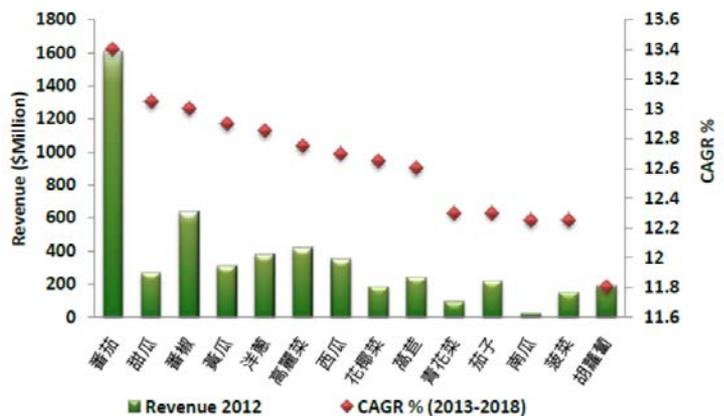
(資料來源：2014 Fruit & Vegetable seed market)

圖 2. 全球主要蔬菜種子收益與發展

丁川翊　全球蔬菜種子市場之動態與我國競爭力簡析

Figure 1.1 Importance in EU agricultural chain

- Seeds: 6.8 billion euros
- Ex-farm agricultural products: 70 billion euros
- Processed agricultural products: 700 billion
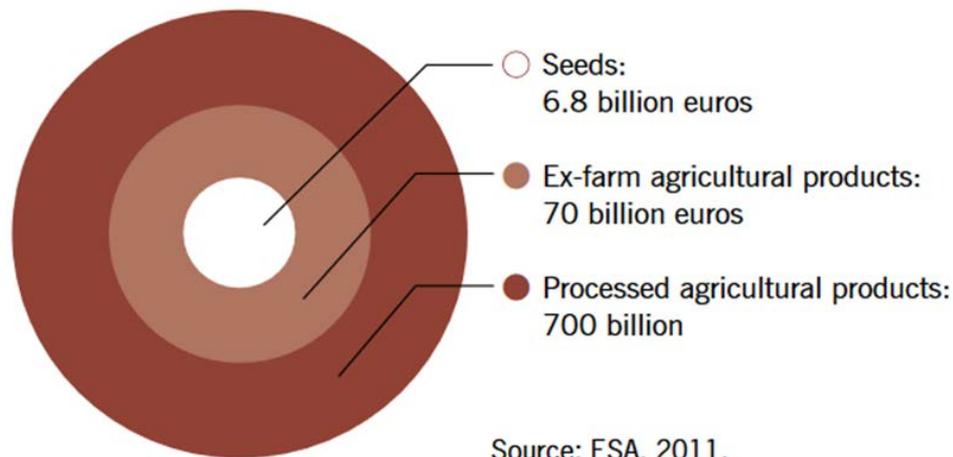
Source: ESA, 2011.

轉譯農學Translational Agriculture
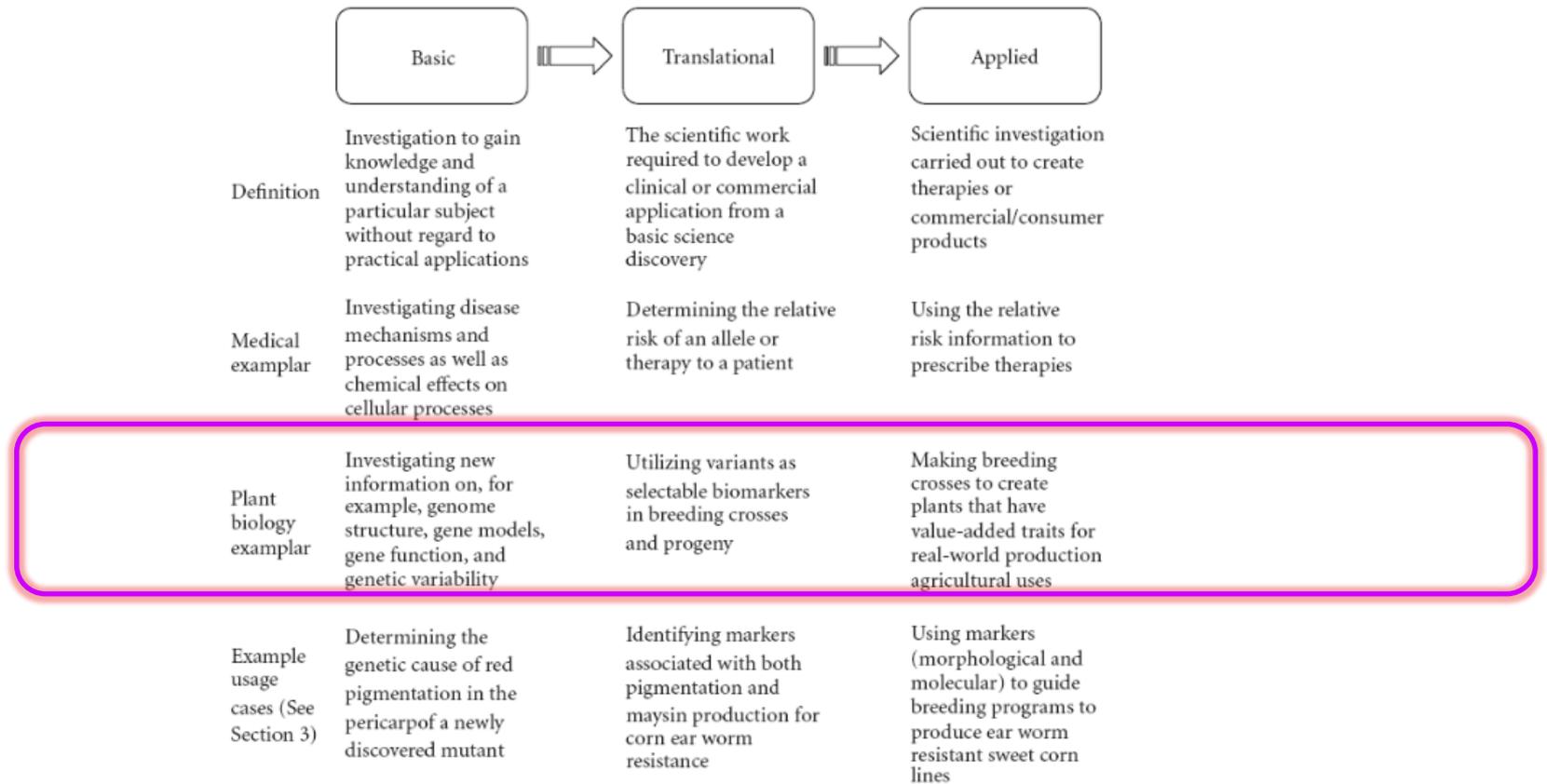
FIGURE 3: Three types of biological research. Research can be divided into three categories: basic, translational, and applied. Outcomes from basic research feed into translational predictions, and developed uses for these findings constitute the basis for developing real-world applications that benefit humanity and the world. Listed after the flow of research are definitions for each research type as well as medical and plant biological models for how the different divisions are interrelated. Also shown are overviews of the example usage cases presented in Section 3.

Lawrence et al. (2008) Int J Plant Genomics. 2008:496957

8

轉譯農學 Translational Agriculture
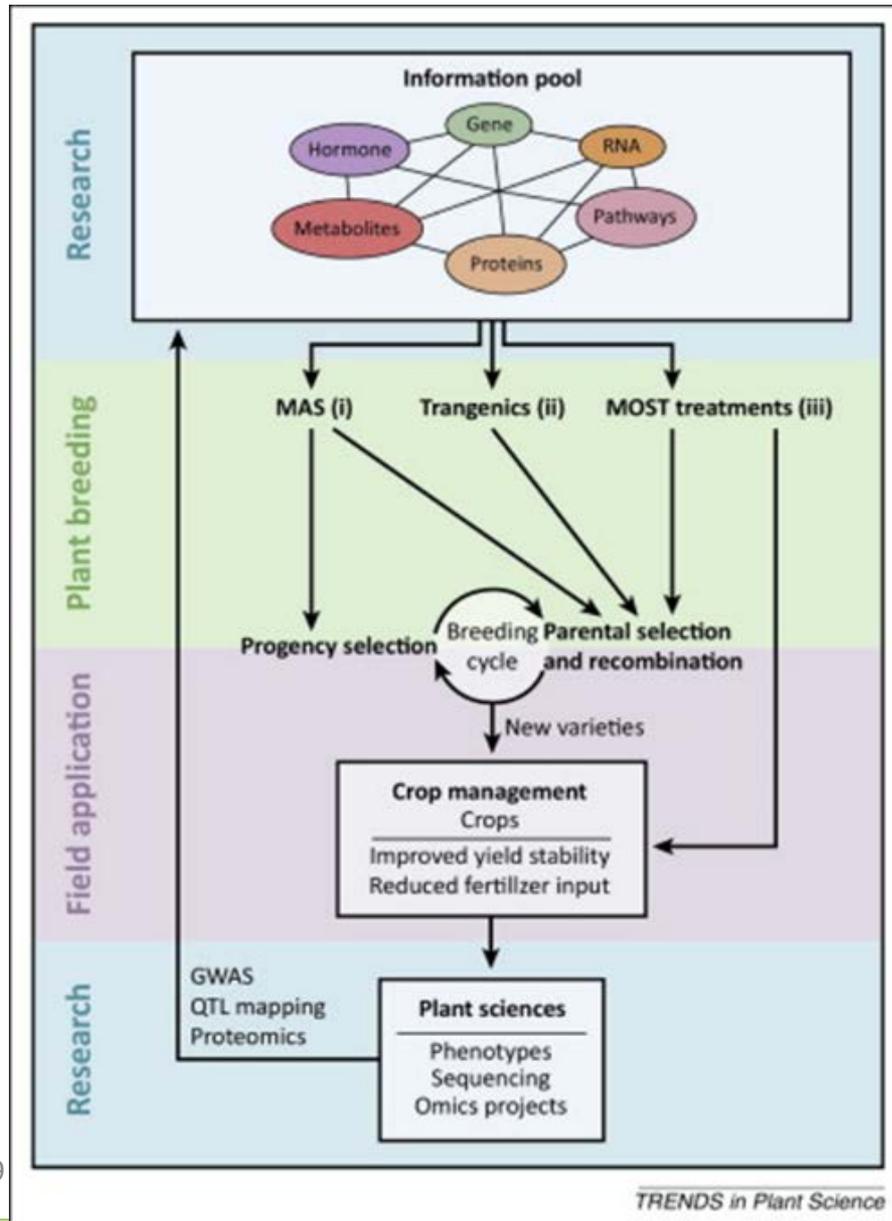
Figure 1. Three ways to utilize detailed molecular genetic information for crop improvement. Phenotypes generated from the field, high-throughput sequencing techniques, and various -omics approaches generate vast amounts of valuable data in the information pool for crop improvement. Traditionally, this detailed molecular genomic information is exploited by marker-aided selection (MAS) (i) and transgenic approaches (ii) for producing better new varieties. MAS can be used for both parental and progeny selection, whereas transgenic methods provide opportunities for introducing novel beneficial traits. Molecular strengthening (MOST) treatments (iii) as a third outlet can facilitate the breeding process (i.e., flowering time regulation or controlled crosses). MOST treatments can also be applied directly in the field for crop management, for reduced fertilizer input, or improved yield stability due to increased biotic and/or abiotic resistance. Abbreviations: GWAS, genome-wide association studies; QTL, quantitative trait loci.

9

MILESTONES IN PLANT BREEDING

馴化

CROP DOMESTICATION
Farmers select the best wild species to create crops

10,000 BC

Domestication of wheat

雜交育種

PLANT BREEDING BASED ON CROSS BREEDING
Development of improved varieties by combining good characteristics from two parents

1865

Mendel's laws
Gregor Mendel describes the inheritance of traits from one generation to the next. His laws become the core of classical genetics

HYBRID BREEDING
Crossing two genetically different individuals to develop better performing hybrid

More vigorous hybrid corn

1926

MUTAGENESIS
Developing new genetic diversity by exposing crop plants to chemical agents or radiation

Blast-resistant rice

1940

1953

Understanding the structure of DNA
James Watson and Francis Crick identify the double helix of DNA

GMO
Introducing foreign genes into the DNA of a plant

Insect-resistant cotton

1994

MARKER-ASSISTED SELECTION
Locating desirable traits in a plant for efficient selection and breeding

Barley resistant to yellow dwarf virus

2000

TARGETED BREEDING
Using modern tools such as genome editing for more targeted breeding

Waxy corn

now · future

PLANT BREEDING BASED ON GENETIC INFORMATION
Development of improved varieties by working directly with the DNA

基因資訊

FACTS

For **10,000** years, farmers and breeders have been developing and improving crops

For **150** years, plant scientists and breeders have improved plant breeding on a scientific basis

Today, farmers feed at least **10** times more people using the same amount of land as 100 years ago

By 2050, we will need **50%** more food to feed a population of 11 billion

ISF International Seed Federation
Seed is Life

轉譯農學Translational Agriculture
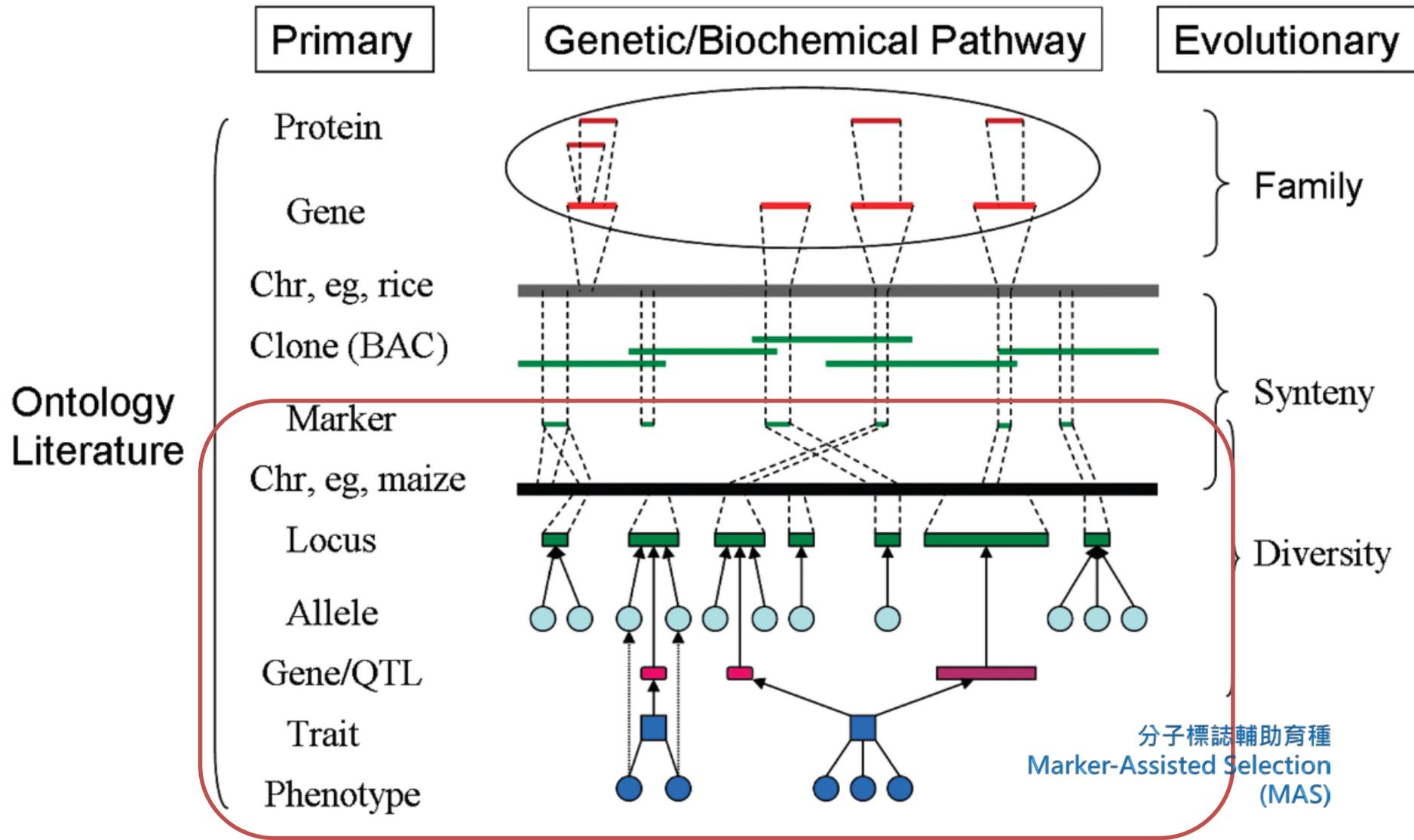
分子標誌 Marker
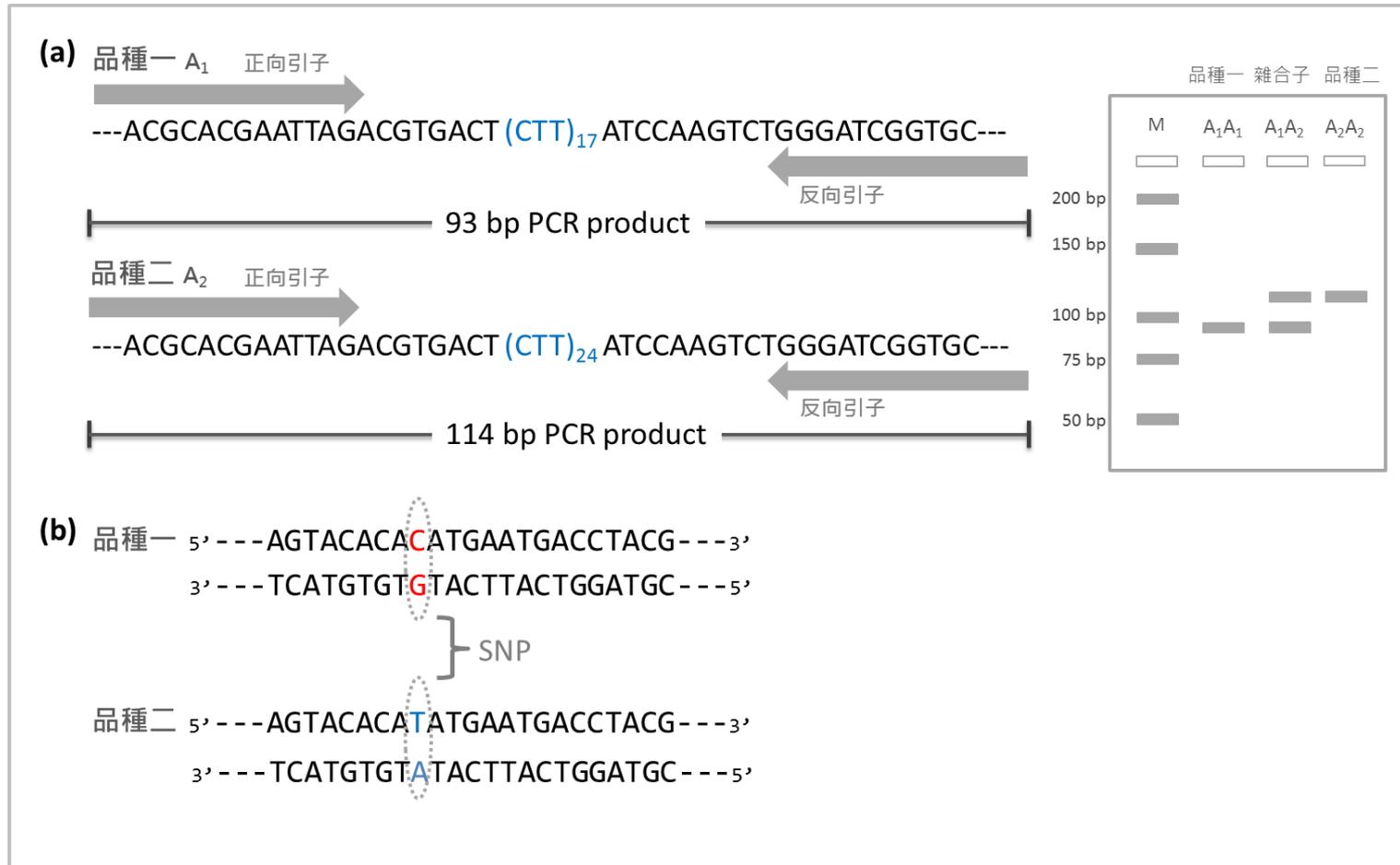
圖1.4.3、簡單序列重複與單一核苷酸多型性分子標誌。

郭舒孟、林彥蓉 (2018) 第一篇、第四章。基因體科技於農業及能源之發展

分子標誌輔助育種
Marker-Assisted Selection (MAS)
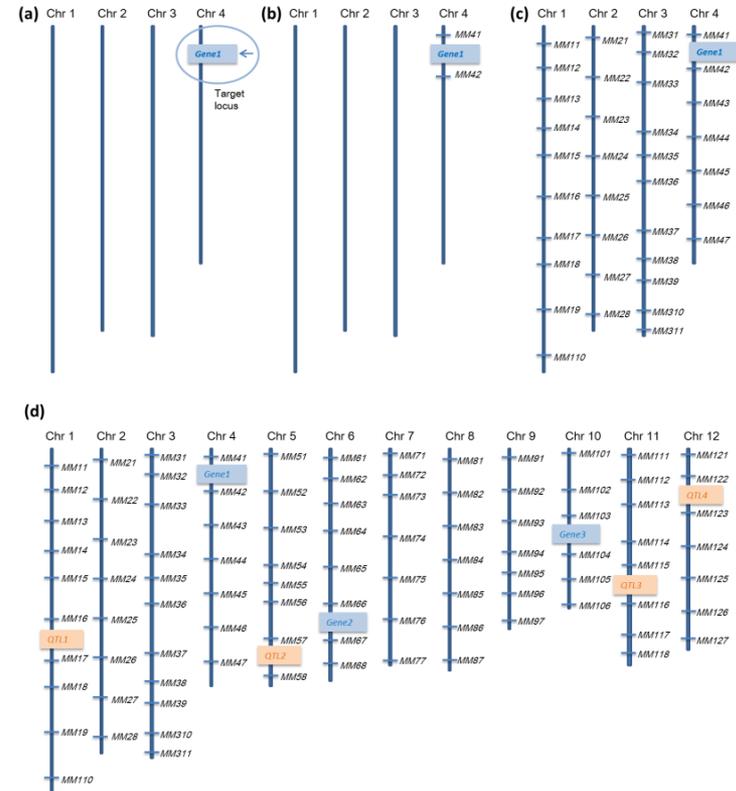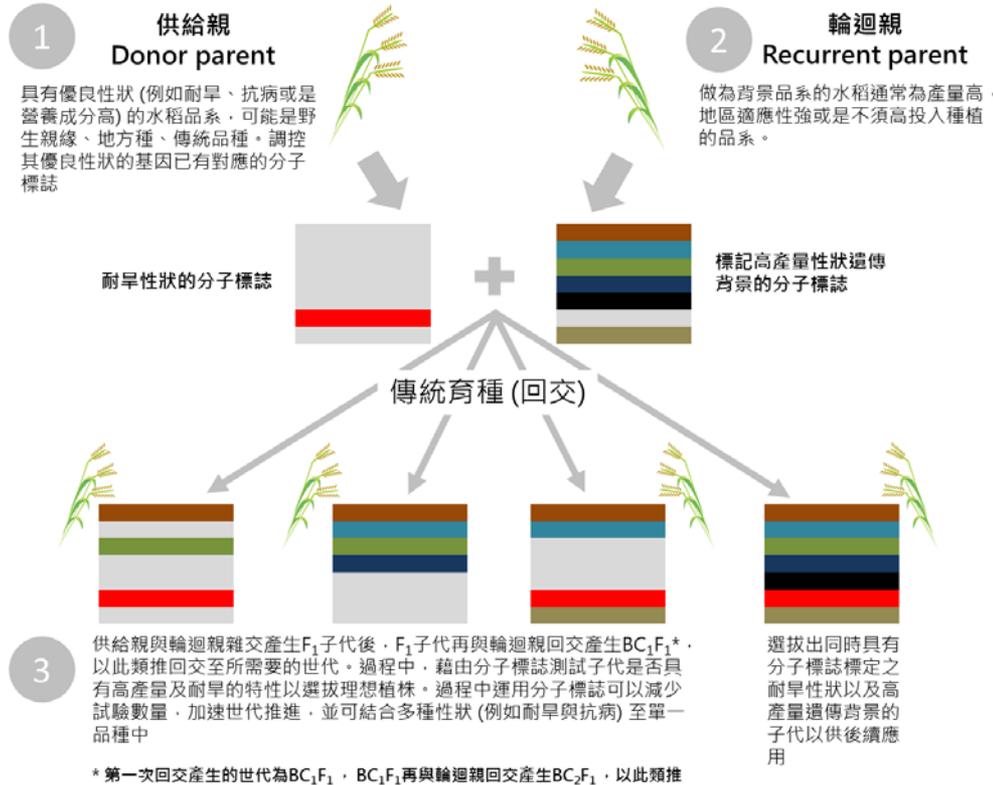
➤ 前景選拔: 針對標的基因做選拔
　　→ 提升標的基因的導入的育種效率
➤ 背景選拔: 依分佈在全基因體的分子標誌做選拔
　　→提升恢復輪迴親（優良親）之基因的育種效率

SMART BREEDING: THE NEXT GENERATION

MARKER ASSISTED SELECTION: A BIOTECHNOLOGY FOR PLANT BREEDING WITHOUT GENETIC ENGINEERING

October 2014

GREENPEACE

Table 1: Crop-trait combinations identified to have been realised in variety development by MAS

Trait	Crops in which varieties has been developed by MAS
Biotic stress resistance	
Insect resistance	maize, rice, wheat
Fungal resistance	barley, bean, chilli, lettuce, pearl millet, rice, soybean, tomato, wheat
Bacteria resistance	bean, lettuce, rice
Virus resistance	barley, bean, cassava, tomato, wheat, lettuce
Nematode resistance	barley, peanut, potato, soybean
Parasite resistance	sorghum
Abiotic stress resistance	
Acid soil tolerance	barley, rice
Drought tolerance	maize, rice
Salt tolerance	rice
Flood tolerance	rice
Quality	
High protein grain	wheat
High-quality protein	maize
Cooking quality	rice
High glucoraphanin	broccoli
Malting quality	barley
Oil quality	peanut, soybean
Low cadmium grain	wheat
Yield	
High yield	rice, soybean, tomato

Table 2: Examples of MAS-derived varieties released by the private sector

Variety/ Trade Mark	Trait	Country	Year	Company
Barley				
Litmus	Acid soil tolerance	Australia	2013	Syngenta[1]
Broccoli				
Beneforte	High glucoraphanin	USA	2011	Seminis[2]
Maize				
Sunrise	Insect resistance	Germany	2010	Saaten Union[3]
Agrisure Artesian*	Drought tolerance	USA	2011	Syngenta[4]
Optimum AQUAmax*	Drought tolerance	USA	2011	Pioneer[5]
Potato				
Figaro	Disease resistance	Germany	2012	SaKa Pflanzenzucht[3]
Soybean				
Vistive*	Low-linolenic acid	USA	2005	Monsanto[6]
Y Series*, **	Yield	USA	2009	Pioneer[7]
T Series*, ***	Yield	USA	2013	Pioneer[8]
Wheat				
Expresso	Disease resistance	USA	2006	Westbred[9]
Blanca Grande 515	Disease resistance	USA	2010	Resource Seeds[9]
Summit 515	Disease resistance	USA	2011	Syngenta[9]
New Dirkwin	Disease resistance	USA	2013	Baglietto Seed Company[9]
Westmore****	Grain potein	USA	2007	Arizona Plant Breeders[9]
SY Tyra	Insect resistance	USA	2011	Syngenta[10]

*: Varieties are stacked with transgenes conferring herbicide tolerance and/or insect resistance; **: Y Series includes 32 varieties; ***: T Series includes 39 varieties; ****: Westmore is a durum wheat variety.

References: 1: Paynter (2014); 2: Mithen (2012); 3: Brumlop & Finckh (2011); 4: Fithian & Martin (2012); 5: Warner (2012); 6: Monsanto (2004); 7: Pioneer (2008); 8: Pioneer (2013b); 9: Jackson (2011); 10: TCAP (2013).

> "Already routinely applied in the private sector breeding companies, such as the multinational companies, Monsanto, Pioneer Hi-Bred and Syngenta, MAS is yet to take hold in public crop improvement programmes mostly on account of high set-up costs and intellectual property rights restrictions."
>
> – Mba et al. 2012

臺灣研究單位分子標誌輔助選育的應用實例

作物	性狀	單位	出處
單一性狀			
水稻	開花時間	臺灣大學、臺南區農業改良場	陳等人 (2010)；陳等人 (2012)；劉 (2017)
	耐鹽	農業試驗所、嘉義大學、臺灣大學	郭等人 (2013)；郭等人(2015)；連等人 (2016)；溫等人 (2016)；
	耐旱	嘉義大學、農業試驗所、臺灣大學	林等人 (2014)；郭等人 (2015)
	抗白葉枯病	嘉義大學、農業試驗所、臺灣大學、中興大學、臺中區農業改良場	Yap et al. (2016)；張等人 (2016)；曾 (2012)；蕭 (2017)；洪 (2016)張 (2015)；楊 (2013)
	米質	臺灣大學、桃園區改良場、臺東區農業改良場、臺南區農業改良場	郭等人 (2014)
	產量	農業試驗所、臺灣大學	賴等人 (2016)
青花菜	開花時間	臺灣大學、農友種苗公司	Lin et al. (2018)
西瓜	抗果斑性	臺灣大學、農友種苗公司	胡凱康等人 (未發表)
番椒	雄不稔性	臺灣大學、農友種苗公司	胡凱康等人 (未發表)
番茄	抗晚疫病	臺灣大學、農友種苗公司	陳凱儀等人 (未發表)
堆砌性狀			
水稻	耐旱、耐鹽	農業試驗所、嘉義大學、臺灣大學	廖等人 (2018)
	高營養米	農業試驗所、臺灣大學、嘉義大學	吳永培、林彥蓉等人 (未發表)
	氮素利用效率	臺灣大學	張孟基 (研究進行中)

郭舒孟、林彥蓉 (2018) 第一篇、第四章。基因體科技於農業及能源之發展

分子標誌輔助育種之成功實例

✳ 已知的基因 ✳ 探勘新的基因 ✳ 堆砌數個基因 ✳ 高通量基因型分析
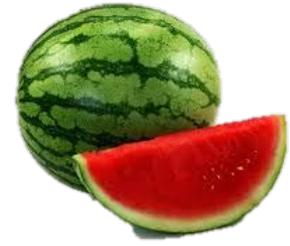
粳稻臺南16號	早生性青花菜	高營養米	西瓜抗性基因育種
林彥蓉	林彥蓉	林彥蓉	胡凱康
陳榮坤 、羅正宗 臺南區農業改良場	農友種苗公司	吳永培 農業試驗所嘉義分所	農友種苗公司

臺南16號

分子標誌輔助回交育種 導入臺農67號抽穗期基因至越光
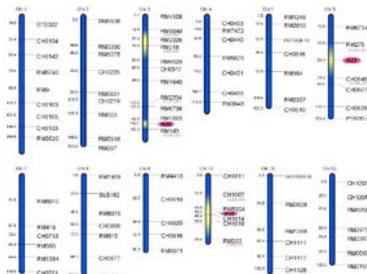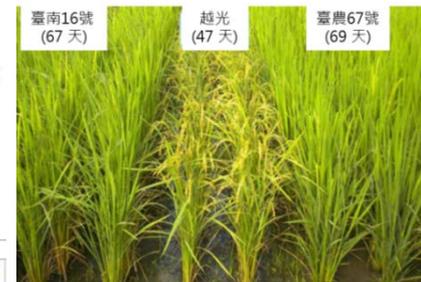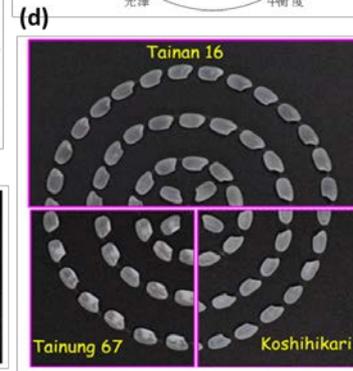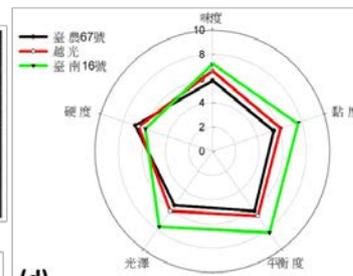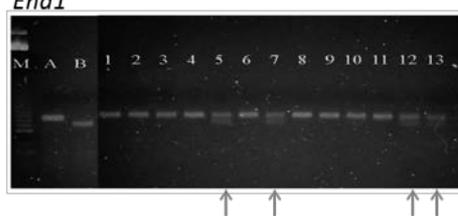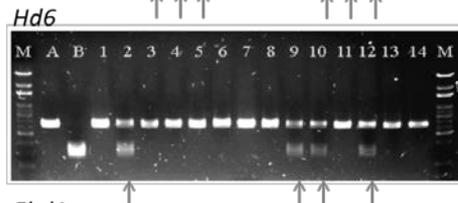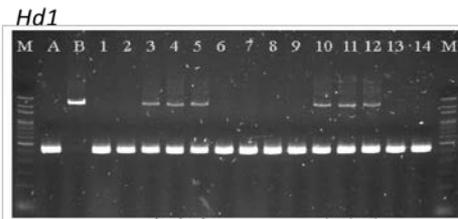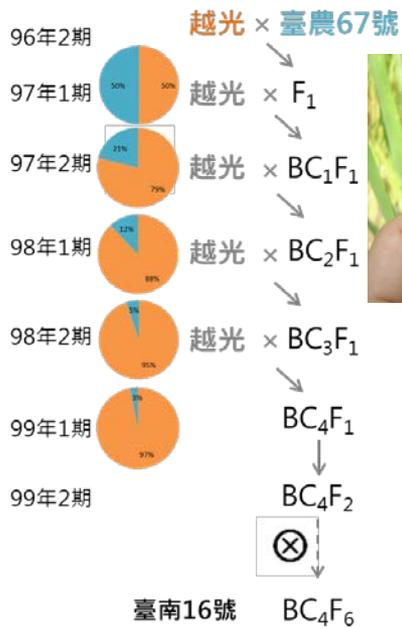
郭舒孟、林彥蓉 (2018) 第一篇、第四章。基因體科技於農業及能源之發展

早生青花菜育種（農友種苗公司）
分子標誌輔助回交育種

2015/05/05

BC$_3$，輪迴親本回復率 > 94%　　優良親本　　早生親本

BLM98-25 × BLM98-29
↓
F$_1$　⊗
↓
BLM98-25 × F$_2$
↘
BLM98-25 × BC$_1$F$_1$
↘
BLM98-25 × BC$_2$F$_1$
↘
BC$_3$F$_1$
↓　⊗
BC$_3$F$_2$
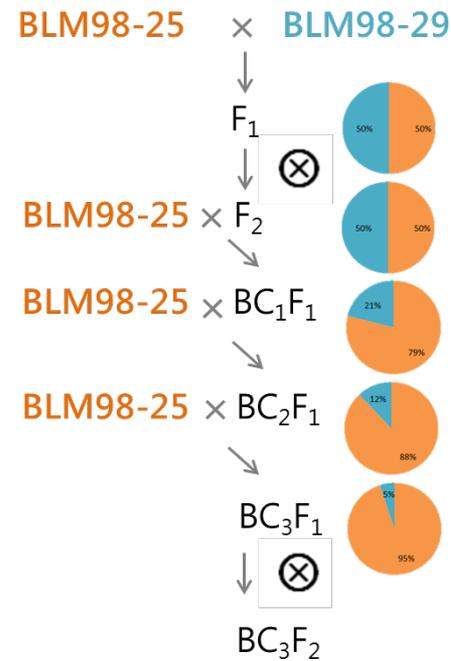
基因堆疊 Gene Pyramiding

① 將 *ge2* 基因導入紫香糯米

98年2期

嘉農育922401
(紫香糯米) × 臺農78號
(巨胚米)

↓

F₁

↓

BC₁F₁

與嘉農育922401回交
- 前景選拔：巨胚基因 *ge2*
- 背景選拔：嘉農育922401遺傳背景

BC₂F₁

↓

BC₃F₁

↓

⊗

↓

BC₃F₃

↓

102年2期 **巨胚紫香糯米** BC₃F₄:₅

② 將 *gr1* 基因導入已具 *ge2* 基因之紫香糯米

BC₂F₁ × 臺農76號
(黃金米) 100年1期

前景選拔：黃米基因 *gr1*
背景選拔：嘉農育922401遺傳背景

↓

CoF₁

與嘉農育922401回交
- 前景選拔：黃米基因 *gr1* 與巨胚基因 *ge2*
- 背景選拔：嘉農育922401遺傳背景

CoBC₁F₁

↓

CoBC₂F₁

↓

CoBC₃F₁

↓

⊗

↓

CoBC₃F₄ **巨胚、黃金米、紫香糯米** 103年2期

農業試驗所嘉義分所 吳永培博士

(A) *gr1*、*ge2* linkage marker

(B) *gr1* functional marker

(C) *ge2* functional marker

Fig. 4 The markers of *gr1* and *ge2* genes used for foreground selection. (A) The genotypes of *gr1* and *ge2* genes linkage markers in a backcross population. (B) The genotypes of *gr1* gene functional markers in a backcross population. (C) The genotypes of *ge2* gene functional markers in a backcross population.

高通量基因型分析

✓ SNP（單一核苷酸多型性）基因型分析
✓ 自動化基因型分析
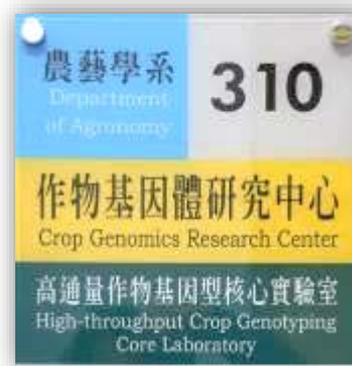
96 x 96

512 RAM

4 小時

西瓜抗病育種（農友種苗公司）
分子標誌輔助回交育種

背景選拔分子標誌平台

晶片影像

資料點整理

一片晶片完成
9216資料點

優良親本 × 抗 BFB 野生種原

優良親本 × F₁ 自交 F₂

回交一代BC₁F₁

利用分子標至輔助回交選種　利用外表型選種

分子標誌前景選拔

對照組BC₄F₁ (僅作接種與外表型挑選回交植株)

栽培選定 F₂分離族群；完成基因型分析；連鎖圖譜建立；抗病 QTL 定位

BC₁F₁自交產生BC₁F₂分離族群抗病接種驗證

BC₂F₁

前景&背景選拔

BC₃F₁ 分子標誌挑選高回復率植株

BC₃F₁

自交固定BC₃F₃將目標基因導入優良系統

BC₄F₁果實性狀表現仍分離，顯示從外觀挑選回交植株的效率比不上分子標誌輔助回交選種
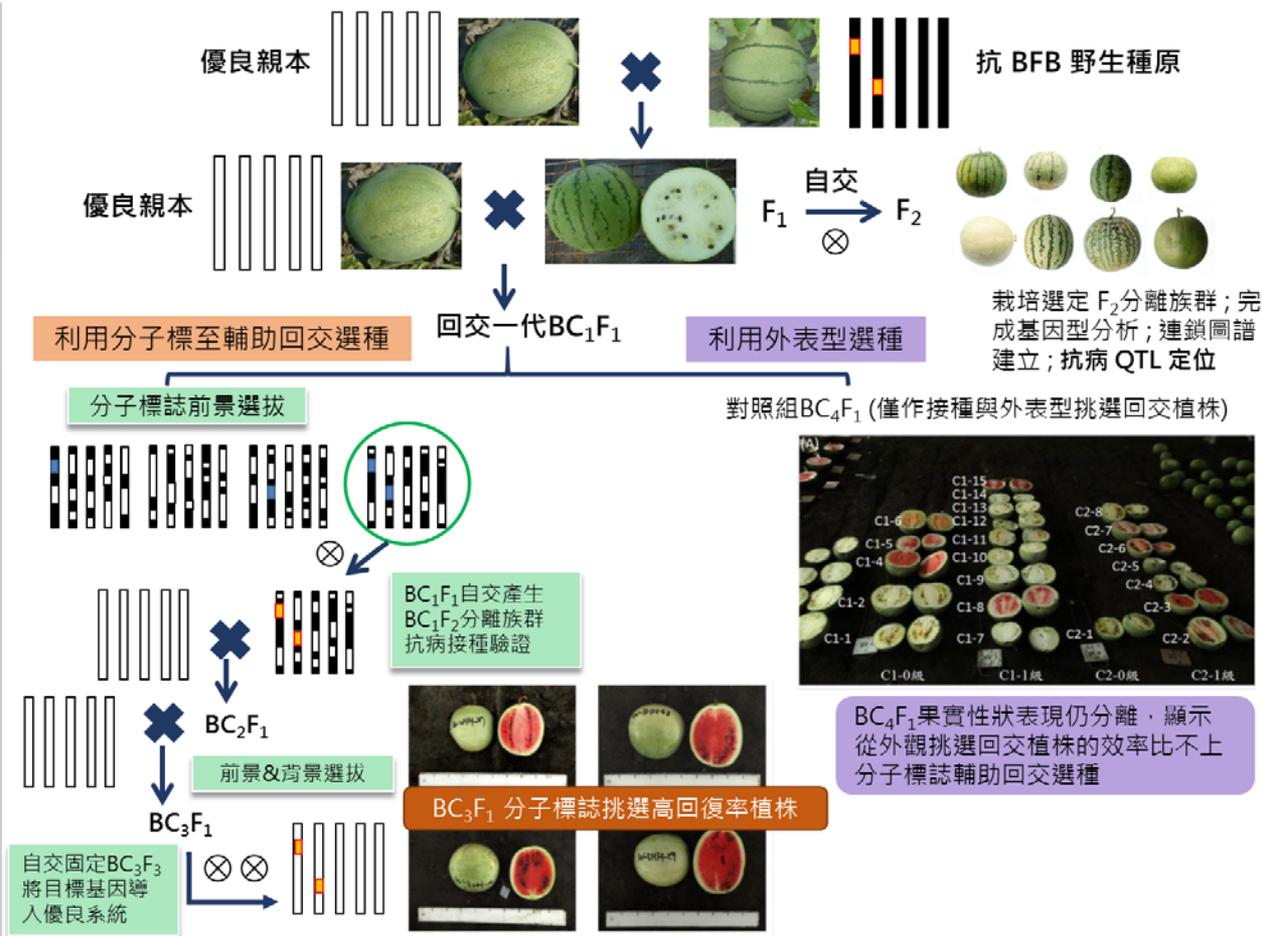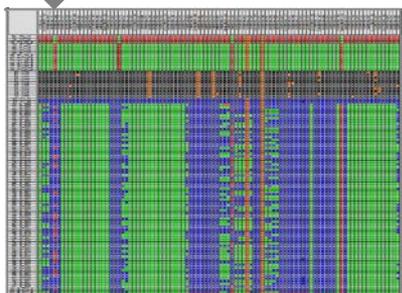
臺大農藝系胡凱康博士

✳ 次世代農業基因體產業應用
  ➢ 基因體庫建立與鑑定技術擴大產業應用
  ➢ 農魚畜分子標幟育種應用，提高育種效率

✳ 高通量基因型分析
  ➢ GBS (genotype-by-synthesis)
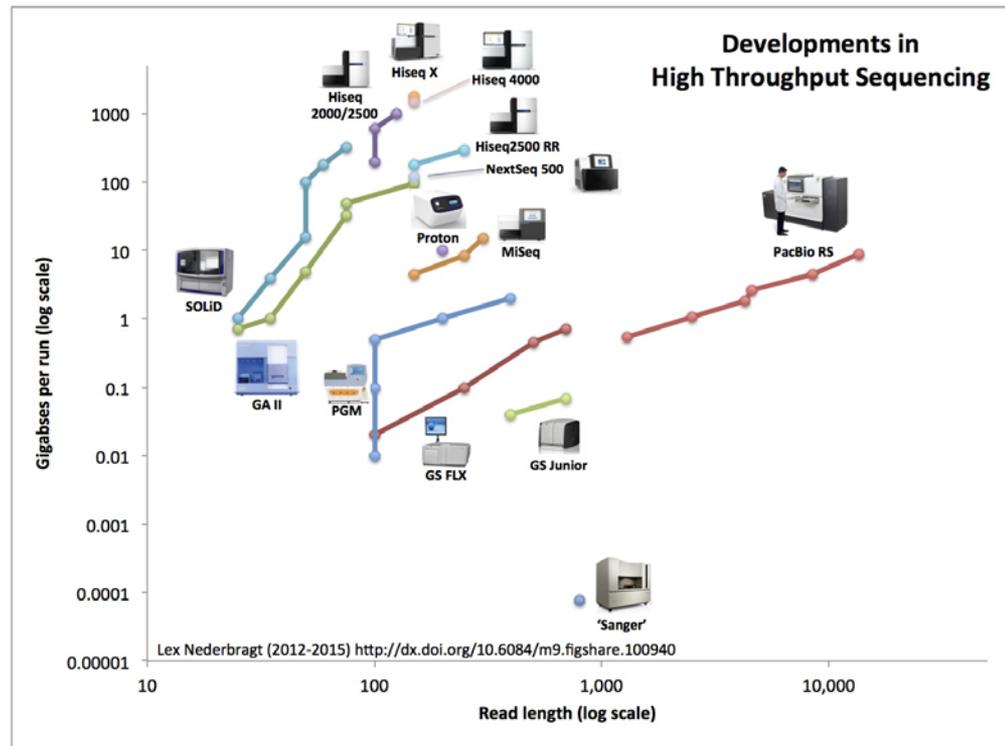
聖鯛水產科技
Sheng-Diao Aquatic Technolog
屏東新埤

Genotype-by-Synthesis (GBS)

Trends in Genetics 2013 29, 190-196
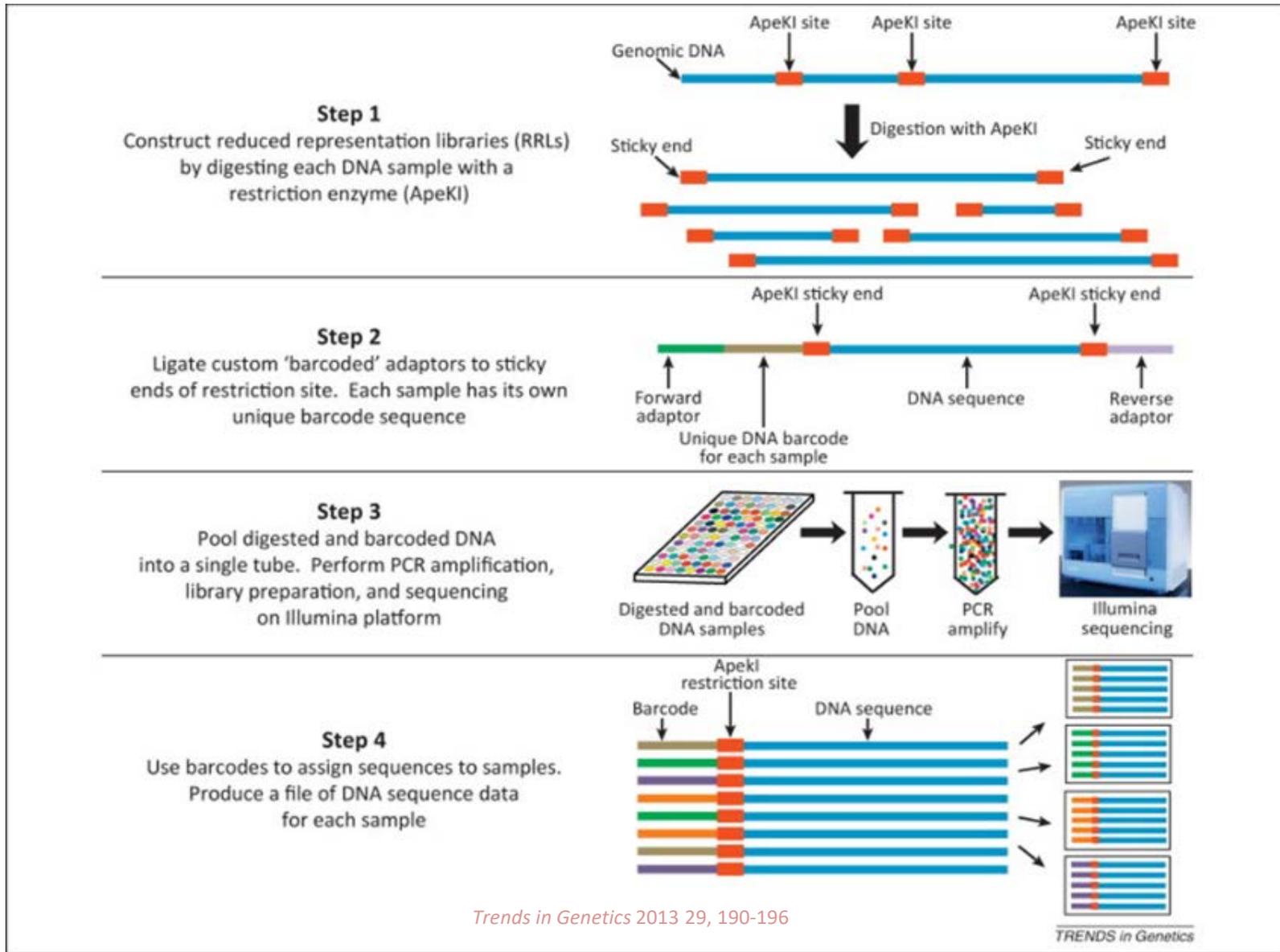
Genotype-by-Synthesis (GBS)

Table 1. A technical comparison of current genotyping methods using next-generation sequencing of multiplex barcoded libraries. Adapted from Wang et al. (2012). Flavors of genotyping using next-generation sequencing of multiplex DNA-barcoded reduced-representation libraries.

Method	Random shearing	Size selection	Fragment size	Enzymes[†]	Multiplexing level[‡]	Analysis tool(s)	Reference
Multiplex shotgun genotyping	No	Yes	Size selected	MseI	96 (up to 384)	Burrows-Wheeler alignment tool	Andolfatto et al., 2011
Restriction association DNA sequencing (RAD-seq)	Yes	Yes	Size selected	SbfI EcoRI	96	Custom Perl scripts	Baird et al., 2008
Double digest RAD-seq	No	Yes	Size selected	EcoRI and MspI	48[§]	MUSCLE[¶]	Peterson et al., 2012
2b-restriction association DNA	No	No	33–36 bp	BsaXI[#]	NA[††]	Custom Perl scripts	Wang et al., 2012
Genotyping-by-sequencing	No	No	<350 bp	ApeKI[‡‡]	48 (up to 384)	TASSEL[§§]	Elshire et al., 2011
Genotyping-by-sequencing — two enzyme	No	No	<350 bp	PstI and MspI	48 (up to 384)	TASSEL	Poland et al., 2012a
Sequence-based genotyping	No	Yes	Size selected	EcoRI and MseI PstI and TaqI	32	Burrows-Wheeler alignment tool and unified genotyper	Truong et al., 2012
Restriction enzyme sequence comparative analysis	No	Yes	Size selected	MseI NlaIII	NA[¶¶]	Burrows-Wheeler alignment tool and Samtools	Monson-Miller et al., 2012

林彥蓉
陳凱儀
胡凱康
董致韡

[†]All of these approaches can use different enzymes. Shown are the enzyme(s) used in the initial study.

[‡]All of these methods have the possibility to increase the number of multiplexed samples using additional unique barcodes. The multiplex level as reported in the reference paper. Given in parenthesis are subsequent increases.

[§]Combinatorial barcoding is possible, placing a barcode on each end of the DNA fragment. Using a set of 48 adapter P1 barcodes and × 12 polymerase chain reaction (PCR) 2 indices it is possible to uniquely label 576 individuals (48 [adapter P1 barcodes] × 12 [PCR2 indices]). This method would require paired-end sequencing.

[¶]MUSCLE, multiple sequence comparison by log-expectation.

[#]Uses type IIB restriction endonucleases.

[††]NA, not applicable.

[‡‡]Has been successfully applied to using PstI and HindIII (E. Buckler and R. Elshire, personal communication, 2012).

[§§]TASSEL, trait analysis by association, evolution, and linkage.

[¶¶]96-plexing reported but unpublished.

Genotype-by-Synthesis (GBS)

Multiplexed Shotgun Genotyping (MSG)

DNA sample
Digestion
(HinP1I)

Adaptor ligation
(96 barcodes)

3. Final structure looks like below (colors also consistent with oligo sequences):

RE site

Pooled DNA

Barcodes | Insert sequence

Size selection
(250-550bp)
PCR amplification

Illumina sequencing
(single end-100bp, 1 flow cell)
Approx. 200 million reads

Bioinformatics analysis
(TASSEL 5)

Demultiplexing

Alignment

SNP calling

Andolfatto *et al.*, 2011

• Analysis software: Tassel 5.0 GBS Pipeline & Bowtie 2

Genotype-by-Synthesis (GBS)

**192 _Brassica oleracea_ inbred lines (Known-you seed Co.)**
93 Cauliflower (_B. oleracea_ var. _botrytis_)
86 Borccoli (_B. oleracea_ var. _italica_)
13 kale derived (_B. oleracea_ var. _alboglabra_)

Sequencing summary

Reads	Library 1	Library 2
Total Sequences	114,573,049	105,469,099
Ambiguous Barcodes	95,51,640	11,702,628
Low Quality	113,048	498,119
Ambiguous RAD-Tag	1,007,920	1,185,880
Retained Reads	103,900,441	92,082,472
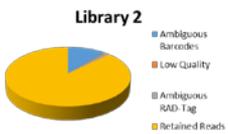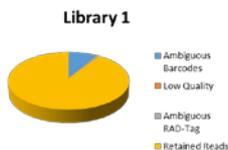
	Tag count per individual
Average	1,020,744
Lowest (GWAS205)	90,325
Highest (GWAS095)	3,020,847

SNP sites	40,018
Filtered SNP	10,439
Proportion Missing	3.56%
Proportion Heterozygous	2.98%

QTLs conferring Curding Time by GWAS

A SNP Set for Variety Identification and F1 seed purity

LGC SNPline

高通量基因型分析
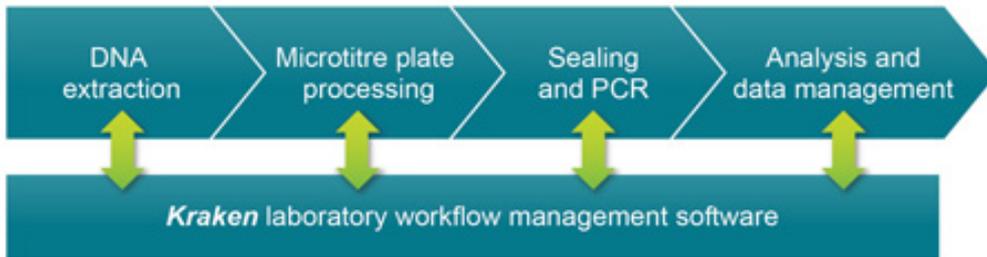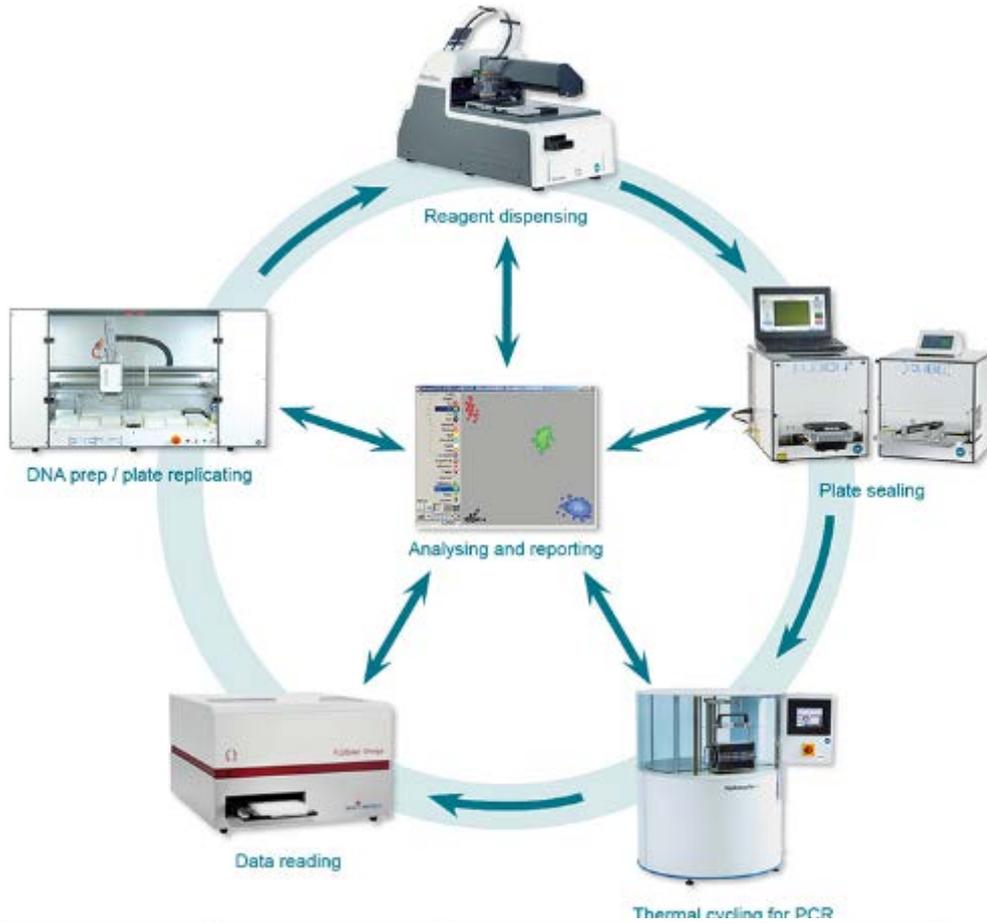
- **SNPline Lite** for lower throughput applications (up to **20,000 data points** per day; 96 & 384-well plates only)

- **SNPline XL** for higher throughput applications (up to **200,000 data points** per day; 96, 384, and 1536-well plates).

https://www.lgcgroup.com/products/genotyping-instruments/snpline/snpline-workflow/#.Wa17K8gjFPY

基因工程技術於農業相關產業之應用現況與發展 (2018)
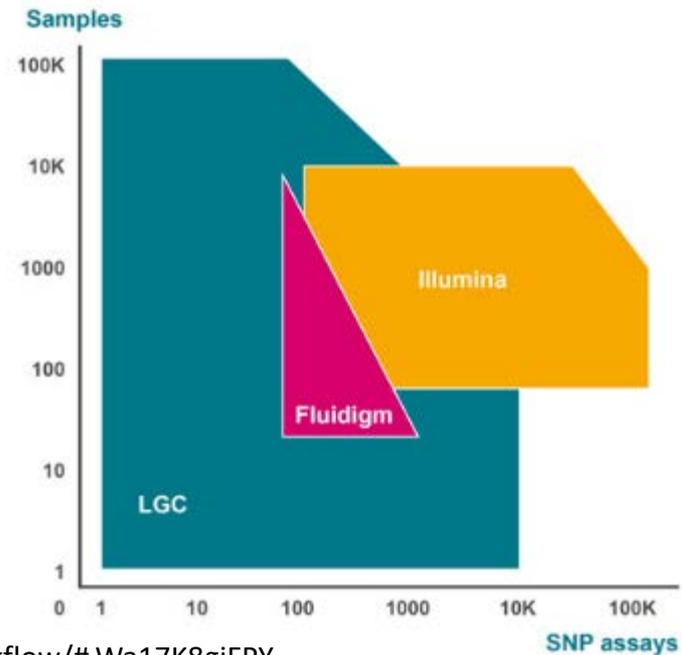
農友種苗公司分子輔助選育系統建立

SNPline - Components

96/384/1536 Replikator/K-pette — Meridian Dispenser — Kube — Fusion³

Kraken software — End-point Analyser and stacker — Hydrocycler
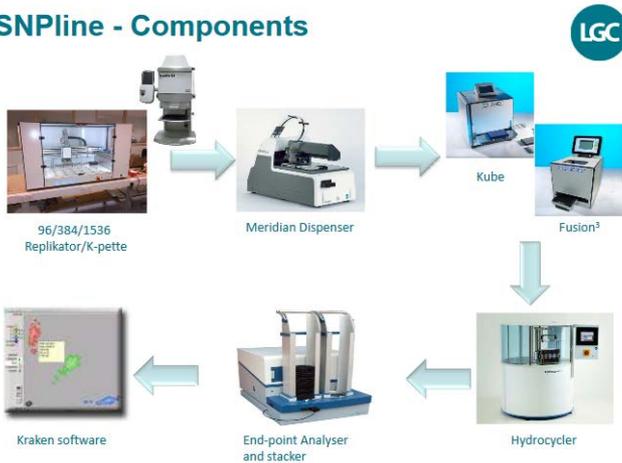
1536 plates
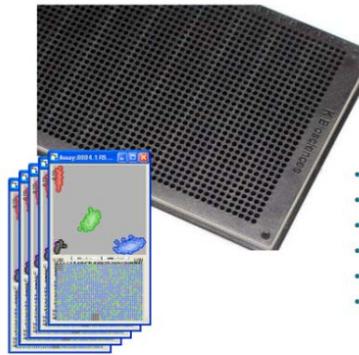
- Proprietary plates
- Automation friendly
- Designed for laser sealing
- 250nl – 7µl well volumes
- Excellent heat transfer
- Reduce automation demands
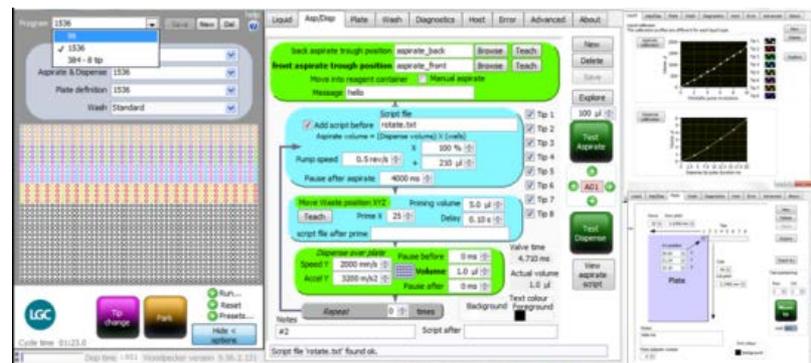
轉譯農學Translational Agriculture

基因編輯 Gene Editing
基因體編輯 Genome Editing

基因編輯 Gene Editing
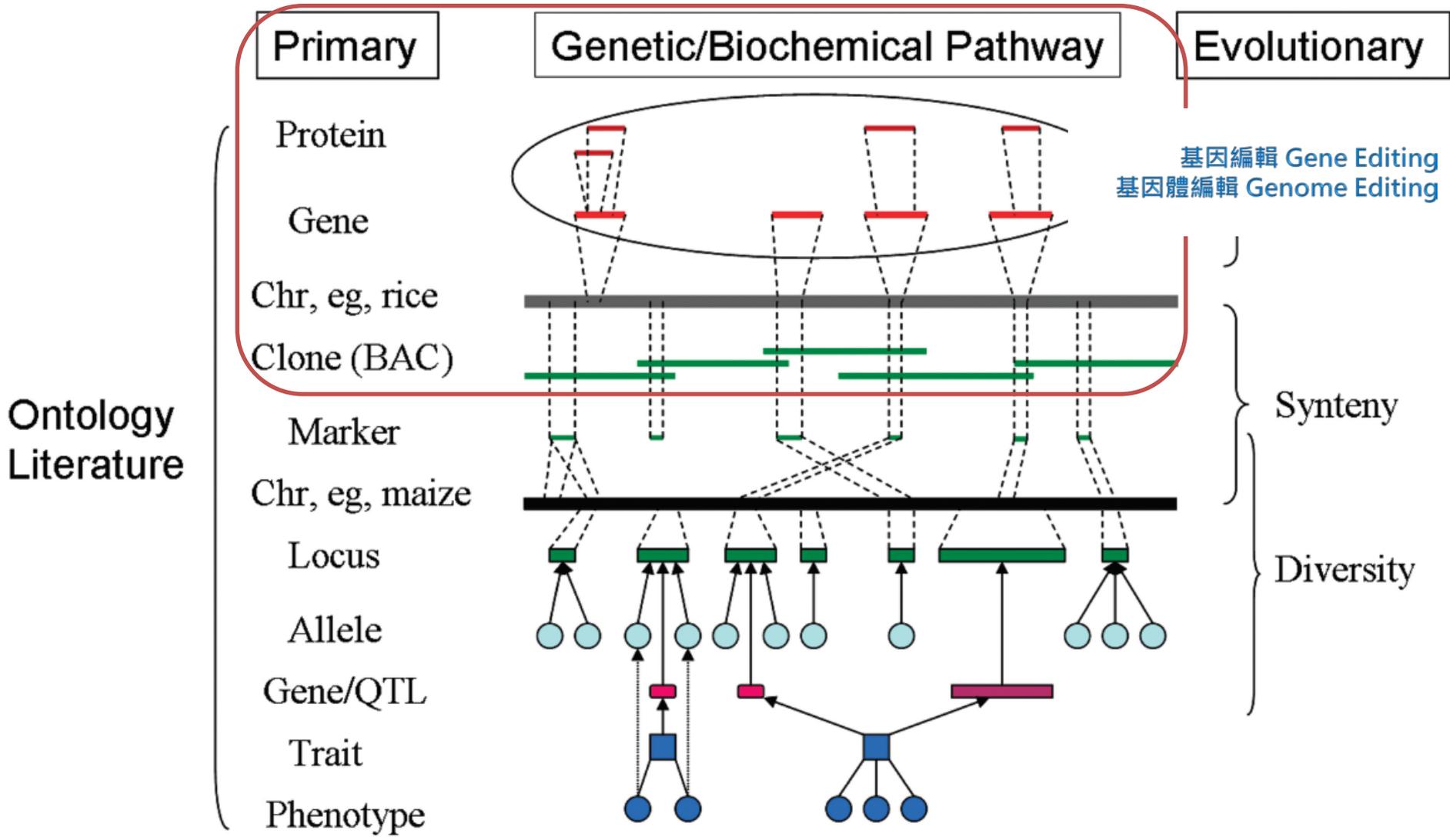
ZFN /TALEN/ CRISPR-Cas9
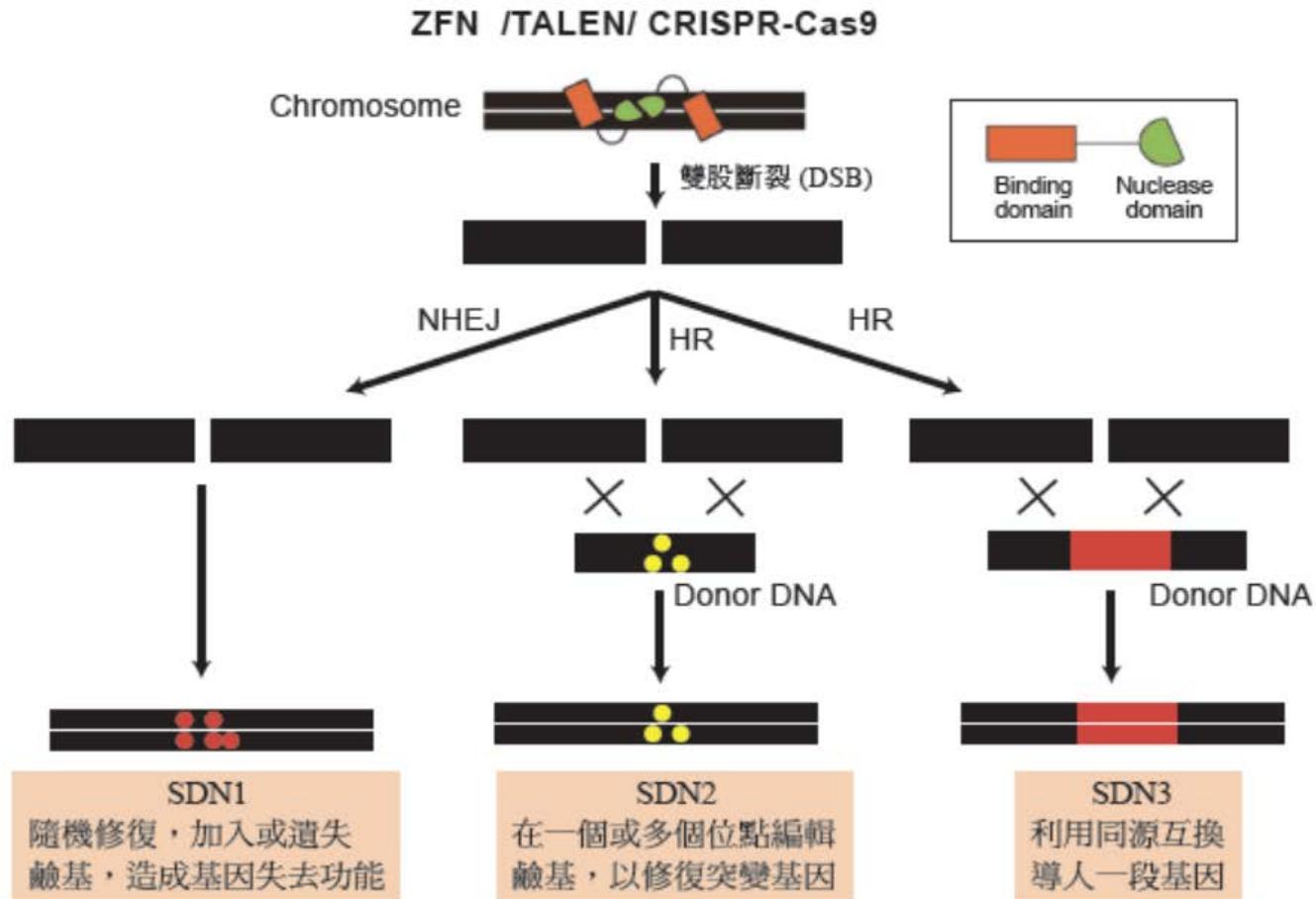
圖1. ZFN、TALEN、CRISPR/Cas9等三種基因編輯方法修補斷裂DNA機制。可客製化定點斷裂的DNA經過修補後,產生鹼基缺失、表達框架位移或嵌入一段相類似的DNA,最終造成基因功能喪失、基因序列修復、或基因嵌入。SDN (Site-directed nuclease) 表示標的序列專一性核酸酶,NHEJ (Non-homologous end joining) 表示非同源互換端點黏合系統,HR (Homologous recombination) 表示雙股同源互換DNA修補系統修復。

洪傳揚 (2018)作物、環境與生物資訊 15:101-115

基因編輯 Gene Editing

表 2. 三種主要基因編輯系統的比較。

|	ZFN	TALEN I	CRISPR/Cas9
與目標 DNA 識別方式	蛋白質-DNA 相互作用	蛋白質-DNA 相互作用	DNA-RNA 鹼基配對和蛋白質-DNA 相互作用
識別目標序列區域	鋅指結構區域	雙氨基酸殘基 RVD 重覆單元	sgRNA
切割酵素	FokI 內切酶	Fokl 內切酶	Cas9 內切酶
辨識序列長度	單個 ZFN:9-18 bp 每對 ZFN: 18-36 bp	單個 TALEN:14-20 bp 每對 TALEN: 28-40 bp	22 bp
操作難易度	難; 需大量蛋白質工程	中等; 需複雜的分子選殖方法	容易; 使用標準選殖過程
多重編輯能力	低	低	高
優點	平台成熟、效率高於同源重組	設計較 ZFN 簡單、特異性高	精確、脫靶率低、細胞毒性低、便宜簡便
缺點	設計依賴上下游序列、脫靶率高、有細胞毒性	具有細胞毒性、組裝過程繁複，需要大量定序工作	目標序列前面無 PAM 則不能精準切割

洪傳揚 (2018)作物、環境與生物資訊 15:101-115

基因編輯
Gene Editing

FIGURE 2. A schematic diagram representing the execution of CRISPR/Cas9 based system in date palm genome editing. The most vulnerable target sites in the desired gene(s) are selected specifically using online available web sourcing to design primers for complementary 20-nucleotides. The target specific sgRNA and Cas9 cassettes are constructed either in a single binary vector or separate expression vectors. These cassettes are then co-transformed *in vivo* into the plant cells employing a suitable transformation method. Following the putative transformation, the mutated cells are screened an analyzed for target-specific mutations using reporter genes, endonucleases, polyacrylamide gel electrophoreses, or high throughput sequencing techniques. The successfully transformed cells are then selected for further downstream applications and analysis.

Sattar et al. (2017) Front. Plant Sci. 8: 1469

基因體科技於農業及能源之發展中技社

❋ **第二篇：基因體工程技術於農林漁畜之國內外應用現況與未來技術**

➢ **基因編輯技術在植物之應用**
  ✓ 臺灣大學農業化學系 洪傳揚教授

➢ **基因編輯技術在畜產動物之應用與發展**
  ✓ 農業科技研究院動物科技研究所 杜清富研究員

➢ **基因編輯技術在農業微生物之應用與發展**
  ✓ 臺灣大學植物病理與微生物學系 沈偉強系主任

➢ **基因編輯益生菌於畜牧生產之應用**
  ✓ 臺灣大學生物科技研究所 劉嘉睿所長

➢ **基因編輯技術在漁業之應用與發展**
  ✓ 中央研究院細胞與個體生物研究所 吳金洌客座講座

基因編輯 Gene Editing
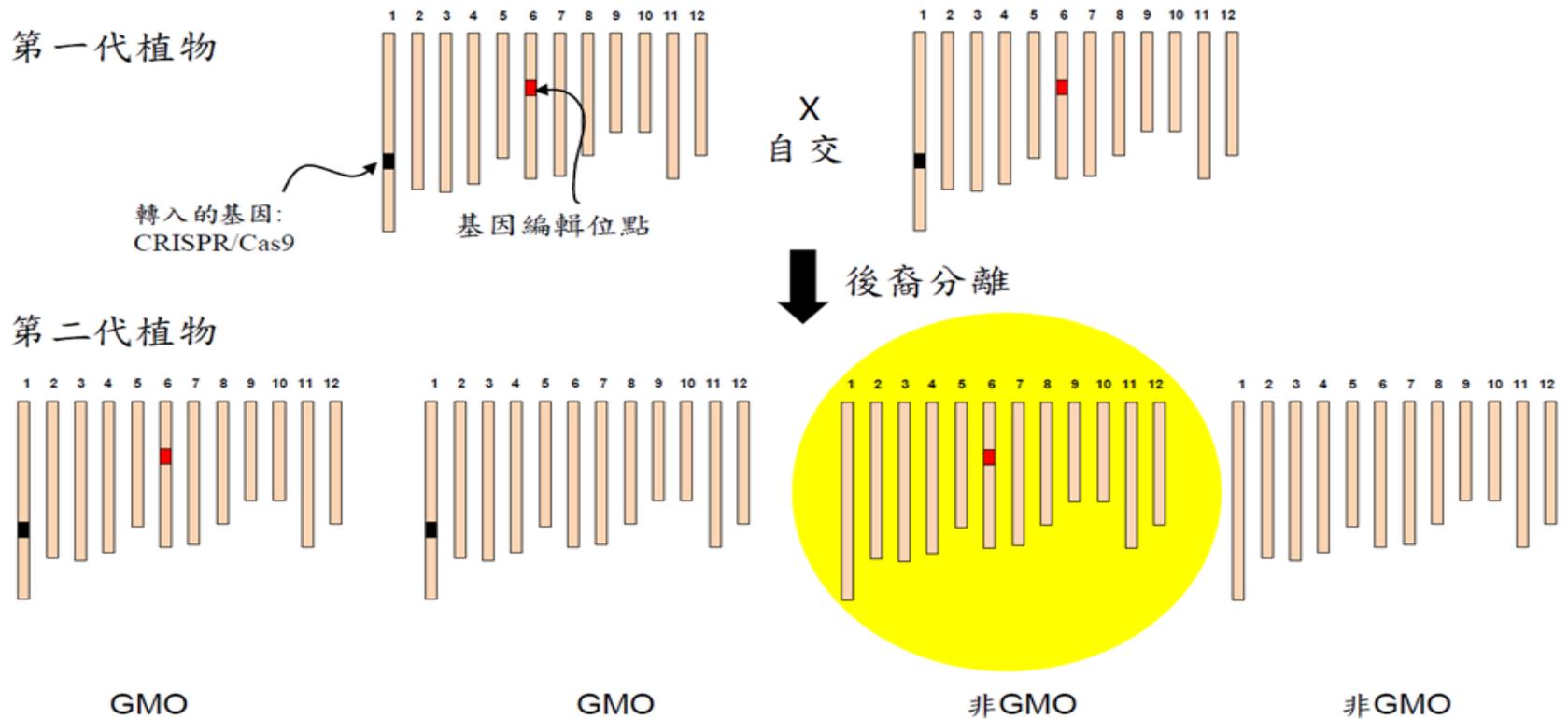
圖2.1.1 不含有轉基因的基因編輯作物之獲得。
藉由穩定性基因轉殖，將基因組編輯的元件送入植物細胞核中以進行
基因編輯，經後代分離後，即可篩選得到非基因轉殖的GE plants。

表2.1.2、近年應用CRISPR/Cas9基因編輯技術進行主要作物之改良

作物	受編輯基因(敲除)	改良性狀	引用文獻
水稻	*RGG2*	穀粒變大提高產量	[55]
水稻	*IPA1, Gn1a,GS3*	增加產量	[54]
水稻	*DEP1*	增加直立性及產量	[56]
水稻	*GW2、GW5、TGW6*	增加粒種	[54]
水稻	*Waxy*	育成糯性稻米	[59]
水稻	*BEIIb*	增加直鏈性澱粉	[60]
水稻	*csa*	光敏核雄不孕	[62]
水稻	*TMS5*	溫敏核雄不孕	[61]
水稻	*C287*	抗除草劑imazamox	[65]
水稻	*OsERF922*	抗稻熱病	[66]
水稻	*DERF1, PMS3*	提高抗旱性	[67]
水稻	*AOX1a, AOX1b, AOX1c, BEL*	抗多種非生物性逆境	[68]
水稻	*Nramp5*	降低鎘累積	[69]
小麥	*α-gliadin gene*	降低Gluten	[63]
小麥	*TaGW2*	增加粒重提高產量	[70]
小麥	*TaGASR7*	增加穀粒種	[41]
小麥	*TaMLO*	抗白粉病	[22]
小麥	*TaDEP1*	植株變矮	[41]
玉米	*Waxy*	育成糯性玉米	[64]
玉米	*AGROS8*	增加缺水時產量	[71]
大豆	*GmFT2a*	延緩開花	[72]
甘蔗	*ScGluA1*	黑穗病	[73]

洪傳揚 (2018) 第二篇、第一章。
基因體科技於農業及能源之發展
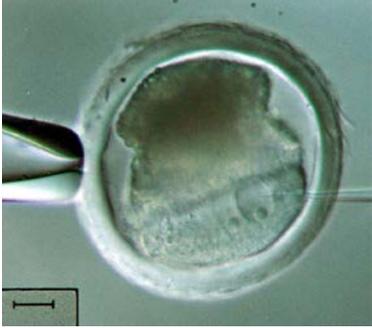
資料來源：杜清富 攝。
圖 2.2.1. 豬受精卵原核顯微注射。

基因編輯 Gene Editing
一動物

✸ 改善畜產生產效率及產品品質
　➢ 以**ZFN**剔除免疫球蛋白基因之乳牛
　➢ 以**ZFN**剔除豬隻**myostatin (MSTN)** 基因之雙肌 **(double muscle)** 豬隻

✸ 增加生物安全性
　➢ 培育抗病種畜
　　✓ 豬呼吸暨生殖道症候群病毒 **(porcine respiratory reproductive syndrome virus, PRRSV)**
　➢ 人畜共同傳染病之防治
　　✓ 感染牛隻為*Mycobacterium bovis*，不過*M. bovis*也會感染人類

✸ 改善動物福祉：無牛角奶牛

✸ 在生物醫學之應用
　➢ 實驗用模式動物
　➢ 提高生物相容性醫材及移植組織器官

✸ 應用誘發型幹細進行囊胚代償產製客製化移植組織或器官

杜清富 (2018) 第二篇、第二章。
基因體科技於農業及能源之發展

基因工程技術於農業相關產業之應用現況與發展 (2018)　　　　　　　　　臺灣大學農藝學系 林彥蓉

表2.5.1 基因編輯技術CRISPR / Cas9於水生生物之運用

物種 (Species)	目標基因 (Genes)	功能 (Functions)	年代 (Years)
吳郭魚(Nile tilapia)	抗穆勒氏管荷爾蒙基因(Anti-Müllerian hormone)	生殖調控	2015
	性腺體細胞衍生因子(Gonadal soma-derived factor)	生殖調控	2016
	類固醇轉錄因子(Steroidogenic factor-1)	生殖調控	2016
	Nanos2和Nanos3基因	不孕技術	2014
	真核轉譯延長因子(Eukaryotic elongation factor 1 alpha, eEF1A)	不孕技術	2017
大西洋鮭(Atlantic salmon)	Dead end (Dnd)基因	不孕技術	2016
鯰魚(Channel catfish)	肌肉生長抑制素(Myostatin)	生長促進	2017
	TICAM 1(Toll-interleukin 1 receptor domain-containing adapter molecule)和RBL (Rhamnose-binding lectin)基因	免疫及病害控制	2018
鯉魚(Common carp)	肌肉生長抑制素(Myostatin)	生長促進	2016
	TLR22(Toll-like receptor 22)基因	免疫及病害控制	2016
珊瑚(Coral)	成纖維細胞生長因子(Fibroblast growth factor 1a)、綠螢光蛋白(Green fluorescent protein (GFP)和紅螢光蛋白(Red fluorescent protein)	環境適應	2017
鱂魚(Atlantic killifish)	多環芳香烴受體(AHR2)基因	環境適應	2014
斑馬魚(Zebrafish)	突觸融合蛋白結合蛋白1(STXBP1)	人類癲癇疾病之動物模式	2016
	ATP6V1H基因	人類骨質疏鬆疾病之動物模式	2017
	Rb1基因	人類癌症之動物模式	2018

李雅雯 黃信傑 吳金洌(2018) 第二篇、第五章。基因體科技於農業及能源之發展

臺灣大學農藝學系 林彥蓉

表2.3.1、CRISPR/Cas9基因編輯技術應用絲狀真菌的研究

物種 (Species)	篩選標記	sgRNA/Cas9 啟動子	突變效率(%)	年份
麴菌 (*Aspergillus*)	PyrG, areB, hph, ble	gpdA/tef1		2017
米麴菌 (*Aspergillus oryzae*)	PyrG	AmyB/AmyB	10～20	2016
煙麴菌 (*Aspergillus fumigatus*)	hph, ble	SNR52/tef1	25～53	2015
煙麴菌 (*Aspergillus fumigatus*)	Pyr4, hph	U6/gpdA	95～100	2016
煙麴菌 (*Aspergillus fumigatus*)	PyrG	gpdA/Tet[ON]		2017
碳麴菌 (*Aspergillus carbonarius*)	hph	gpdA/tef1	27	2017
黑麴菌 (*Aspergillus niger*)	PyrG, hph	gpdA/tef1	37.5-100	2016
黑麴菌 (*Aspergillus niger*)	PyrG			
互生鏈隔孢菌 (*Alternaria alternata*)	Pyr4, hph	gpdA/tef1		2017
靈芝 (*Ganoderma lucidum*)	ura3	T7/gpdA		2017
灰蓋似鬼傘 (*Coprinopsis cinerea*)	hph	U6/CcDED1	21	2017
玉米黑穗菌 (*Ustilago maydis*)	ip	U6/ Otef (modified tef1)	70	2016
瑞氏木霉 (*Trichoderma reesei*)	ura5	T7/ cbh1, pdc	100	2015
稻熱病菌 (*Pyricularia oryzae*)	Bar	U6; TrpC/tef1	36.1 - 80.5	2015
橘色麵包黴 (*Neuropora crassa*)	Bar	SNR52/TrpC		2015
產黃青黴菌 (*Penicillum chrysogenum*)	amds	T7/xinA	100	2016
大豆疫病菌 (*Phytophthora sojae*)	G418	U6/ Ham34		2016
嗜熱毀絲黴菌 (*Myceliophthora thermophila*)	Bar	U6/tef1	15-95	2017
白殭菌 (*Beauveria bassiana*)	GFP, ura5, bar	in vitro transcribe/gpdA		2017
竹黃 (*Shiraia bambusicola*)	hph	U6/tef1		2017

沈偉強 (2018) 第二篇、第三章。
基因體科技於農業及能源之發展

表2.2.3 CRISPR/Cas基因工程技術應用於益生菌的相關研究

| 菌種	改造之目的	參考文獻
Bacillus subtilis	Site-specific mutation, gene insertion/deletion and transcriptional modulation	[80]
	Knockdown essential genes	[81]
Enterococcus faecalis	Plasmid targeting	[82]
Lactobacillus casei	Site-specific mutation, gene insertion and deletion	[77]
Lactobacillus gasseri	Function of native CRISPR	[83]
Lactobacillus reuteri	Site-directed mutagenesis	[76]
Streptococcus thermophilus	DNA targeting	[84]

劉嘉睿(2018) 第二篇、第四章。
基因體科技於農業及能源之發展

New Breeding Era

- ✓ **Resistance to biotic stresses**
- ✓ **Tolerance to abiotic stresses**
- ✓ **Yield**
- ✓ **Quality**
- ✓ **Shift production**
- ✓ **Multiple-dimensional usages**
- ✓ **Sustainable agriculture**

Phenotypic Selection	Controlled Trials	Genomic Selection	Designed Genomes
• First 10k years • Testing species diversity	• Quantitative Genetics • Exp. design • Statistics • Harvesters	• Marker assisted breeding • Whole genome prediction	• Causative nucleotides • Editing • Physiology
1.0	2.0	3.0	4.0

Ed Buckler 2018 Plant and Animal Genome Conference

✹ **Unveil useful genes from natural germplasm**
- ➢ **Forward genetics, reverse genetics**
- ➢ **Basic research**

✹ **Breeding**
- ➢ **Allelic variation**
  - ✓ **GE, MAS**
- ➢ **Genes resided in wild relatives or exotic lines**
  - ✓ **MAS, GE (?)**

SCIENTIFIC REPORTS

OPEN | Subtropical adaptation of a temperate plant (*Brassica oleracea* var. *italica*) utilizes non-vernalization-responsive QTLs

Received: 9 April 2018
Accepted: 28 August 2018
Published online: 11 September 2018
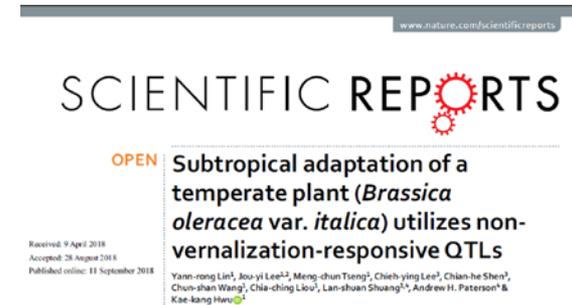
分子輔助育種 - 蓬萊米 臺南16號
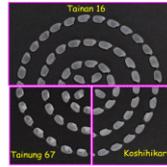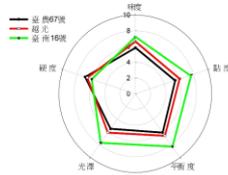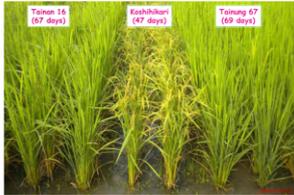
2012年5月28日 品種命名
2013年8月20日 品種權

✓ 臺南16號的育成,從一開始的雜交至品種命名僅5年半,而一般傳統育種則需8~10年,是以,分子輔助選拔大幅提高育種之效率。

✓ 臺南16號的成熟期屬於中晚熟品種,與一般常見的品種雷同,適於臺灣一年兩期的耕作。

✓ 相較於日本越光米在臺灣種植的產量與米質而言,臺南16號的產量在二期作可高達近三倍,擁有較優良米粒外觀、烹煮和食味品質。

分子輔助育種 -
臺南 16 號/ 鹿鳴米
輔導農業產的升級

慶祝臺大88米壽 鹿鳴米榮獲2016全國名米產地總冠軍

禮盒 (0.6公斤 X3):
880元/盒

袋裝 (1公斤):
180元

小包裝 (300公克):
75元

林彥蓉