高中生命科學研究人才培育

植物學特論： 植物的生殖與育種

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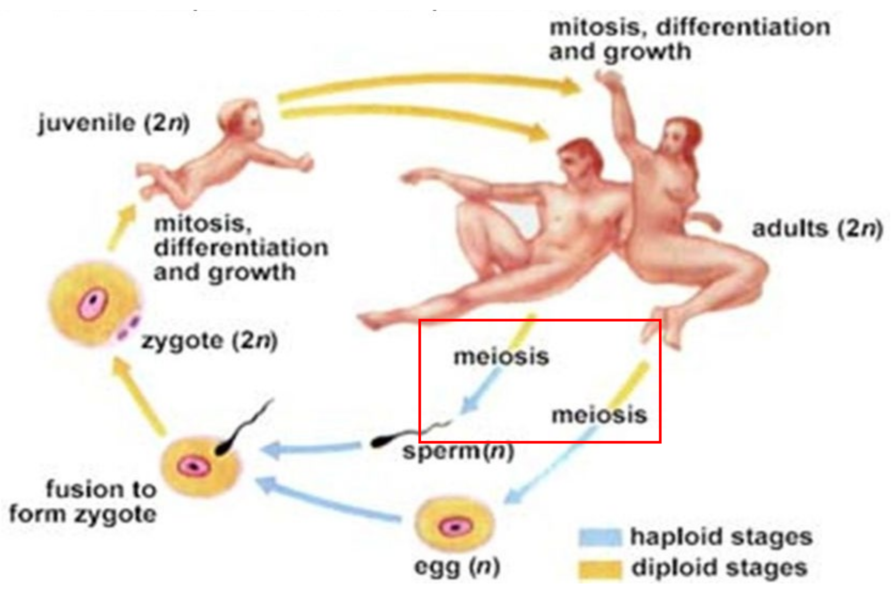
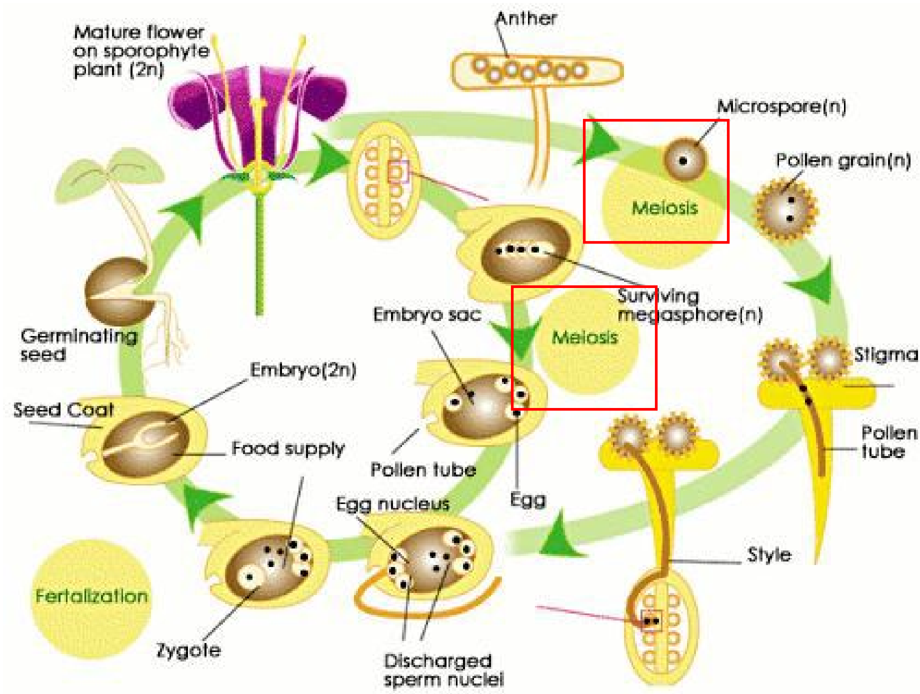
植物的生殖:

- 世代交替
- 減數分裂
- 玉米的種化
- 育種方法
- 基因改造/編輯

動植物的有性生殖



mitosis, differentiation and growth
juvenile (2n)
mitosis, differentiation and growth
adults (2n)
zygote (2n)
meiosis
meiosis
sperm(n)
fusion to form zygote
egg (n)
haploid stages
diploid stages

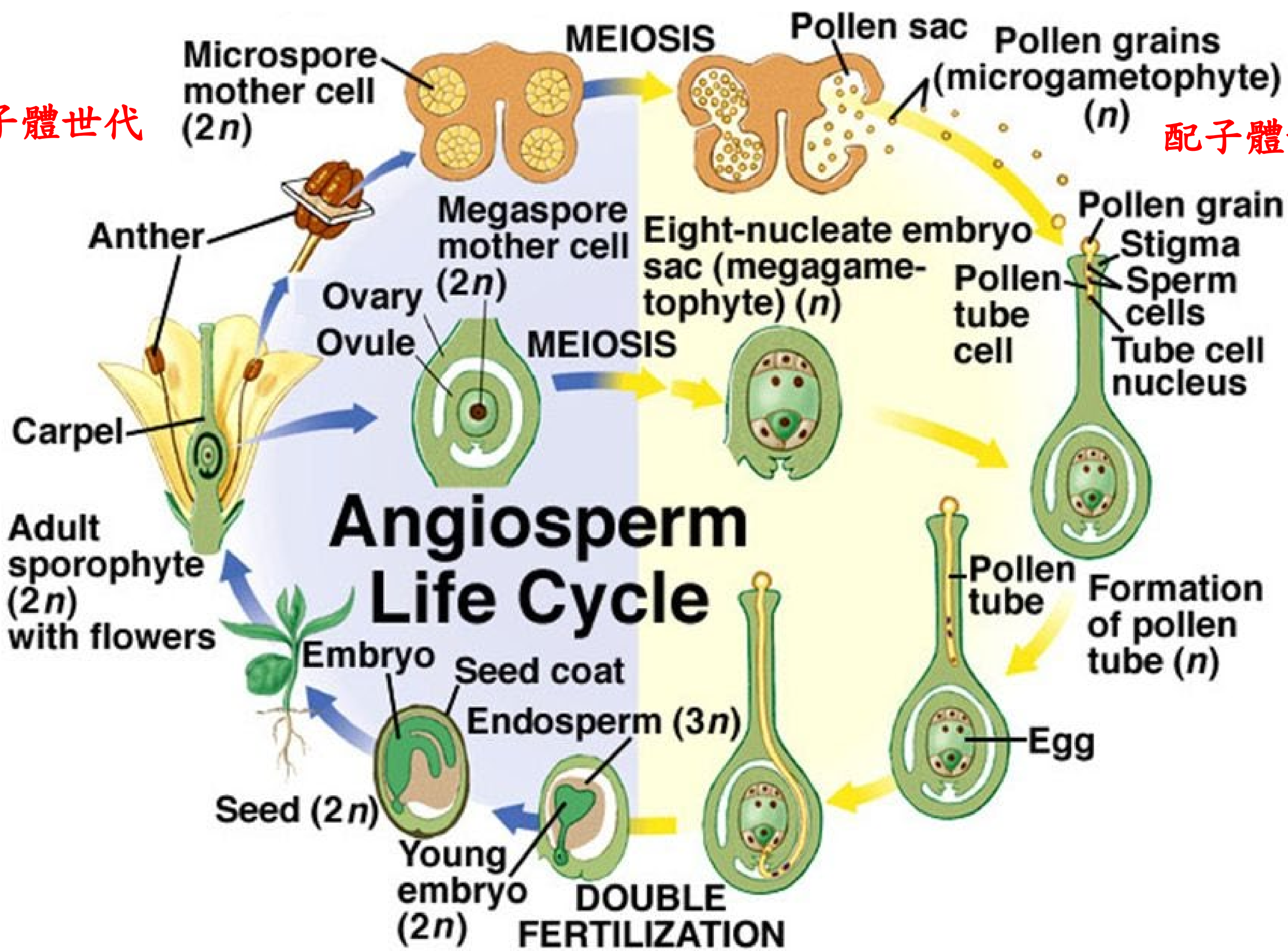


Mature flower on sporophyte plant (2n)
Anther
Microspore(n)
Pollen grain(n)
Meiosis
Surviving megasphore(n)
Meiosis
Embryo sac
Stigma
Germinating seed
Embryo(2n)
Seed Coat
Food supply
Pollen tube
Pollen tube
Egg nucleus
Egg
Style
Fertalization
Zygote
Discharged sperm nuclei

植物的世代交替

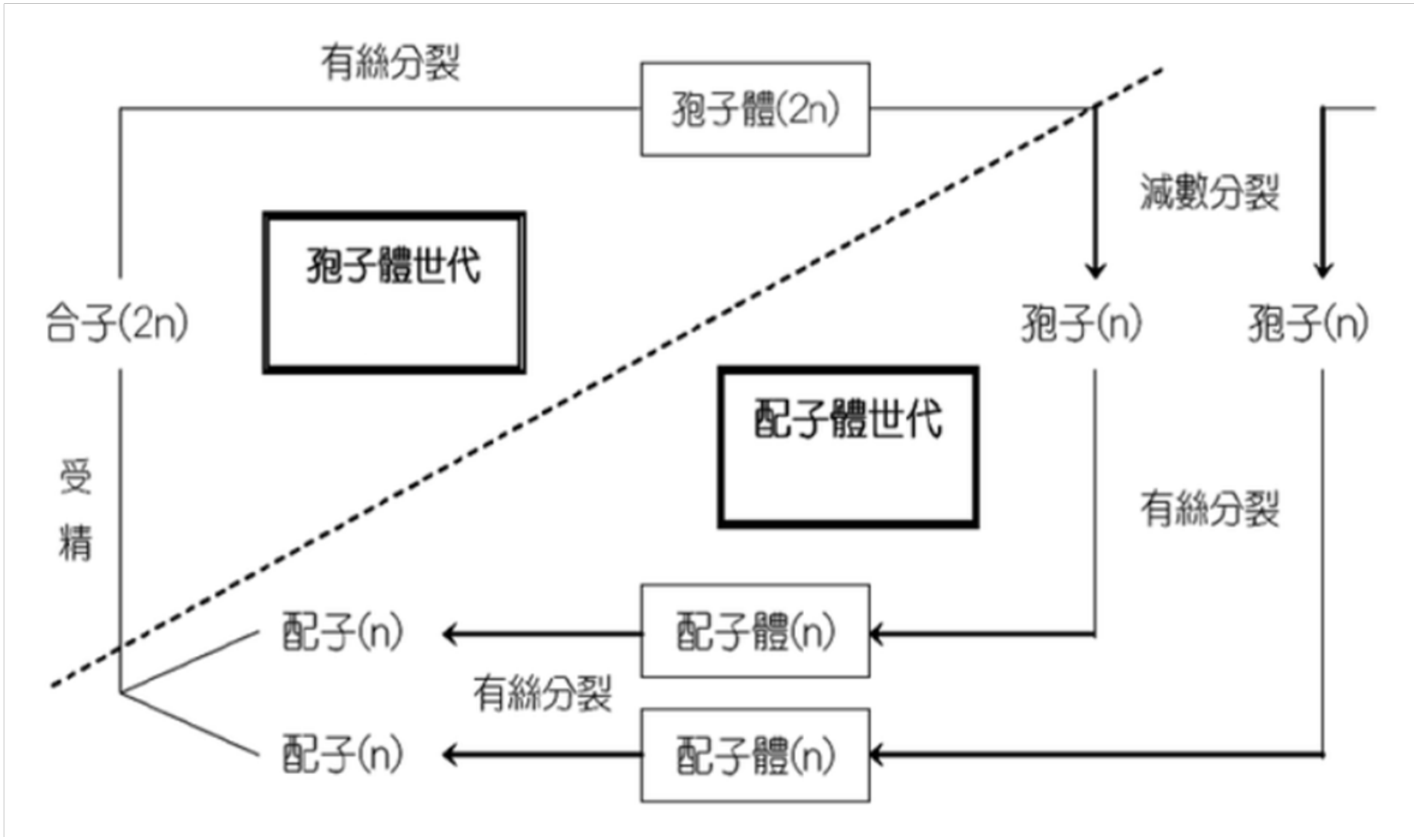
Life Cycle of Flowering Plants

配子體世代



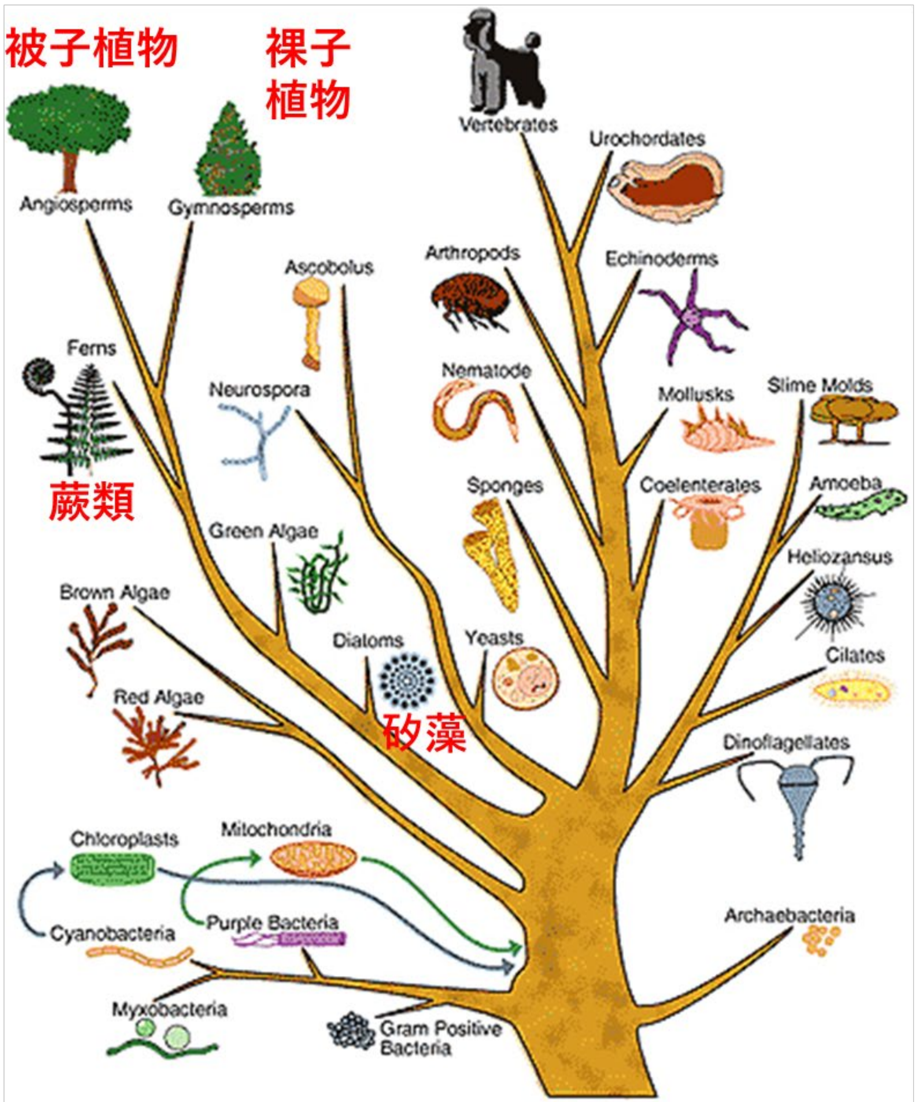
配子體世代

植物的世代交替


有絲分裂
孢子體(2n)
減數分裂
孢子體世代
合子(2n)
孢子(n)
孢子(n)
配子體世代
受精
有絲分裂
配子(n)
配子體(n)
有絲分裂
配子(n)
配子體(n)

為何麼植物的有性生殖要這麼麻煩?

陸生植物

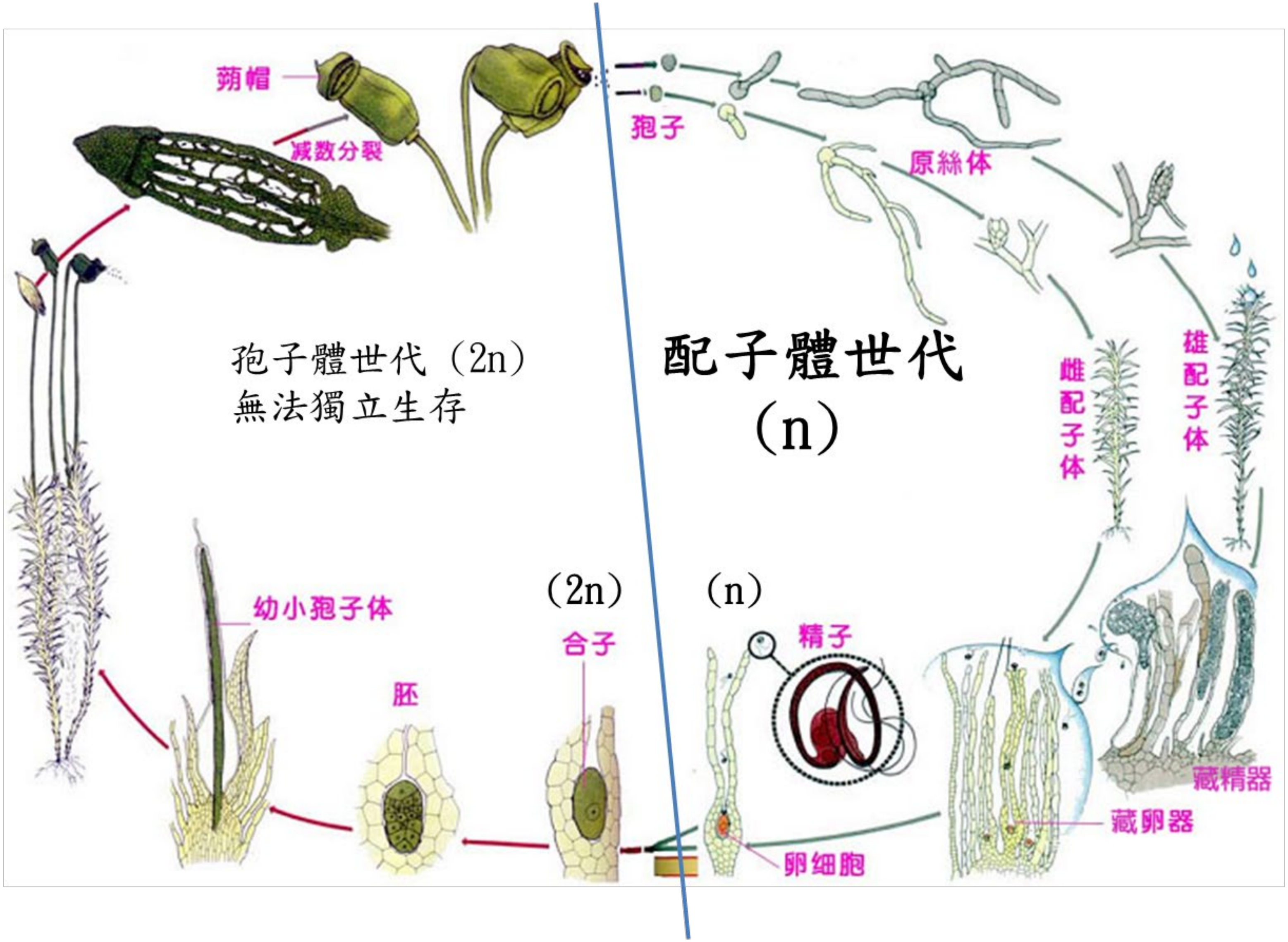


植物離開水生活
需要面臨的挑戰??

1. 水分蒸散
2. 地心引力
3. 有性生殖

蘚苔類世代交替

https://youtu.be/jcWYAnmm-QE



蕨類植物的世代交替

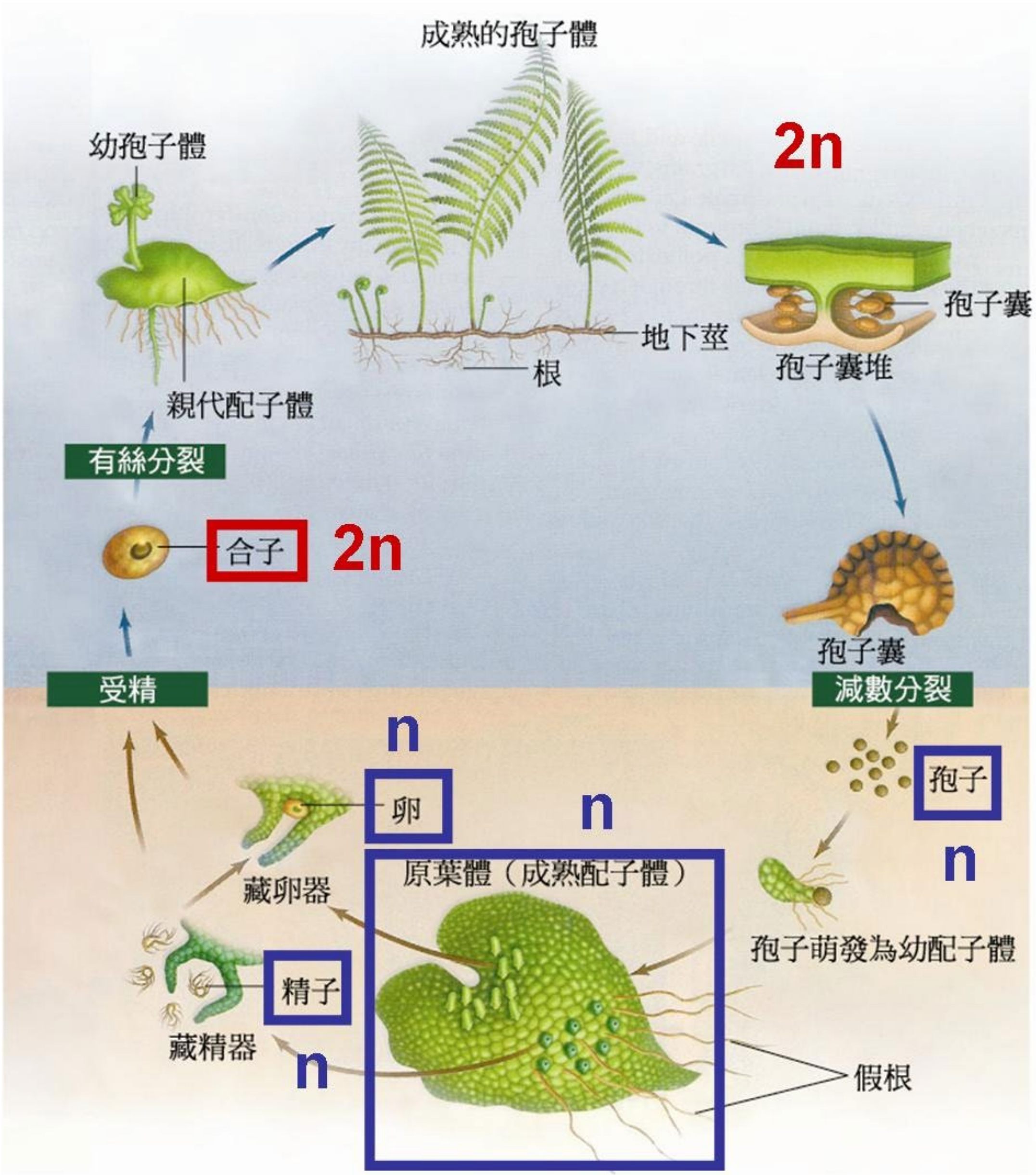
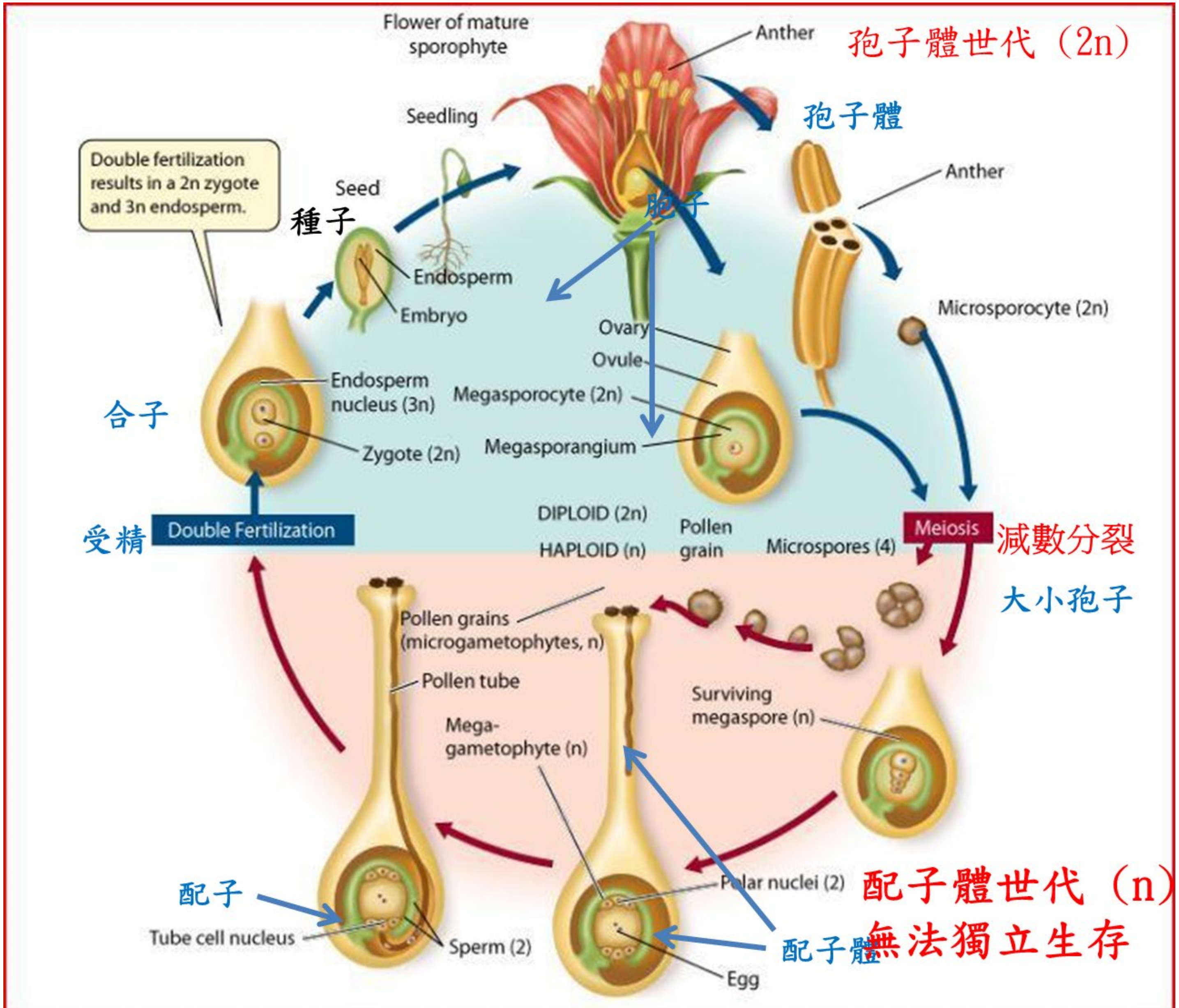


圖 3-16　蕨類植物的生活史

被子植物的世代交替



Flower of mature sporophyte
Anther
孢子體世代（2n）
孢子體
Seedling
Double fertilization results in a 2n zygote and 3n endosperm.
Seed
種子
胞子
Anther
Endosperm
Embryo
Microsporocyte (2n)
Ovary
Ovule
合子
Endosperm nucleus (3n)
Megasporocyte (2n)
Megasporangium
Zygote (2n)
DIPLOID (2n)
HAPLOID (n)
Pollen grain
Microspores (4)
受精
Double Fertilization
Meiosis
減數分裂
大小孢子
Pollen grains (microgametophytes, n)
Pollen tube
Surviving megaspore (n)
Mega-gametophyte (n)
Polar nuclei (2)
配子體世代（n）
無法獨立生存
配子
Tube cell nucleus
Sperm (2)
配子體
Egg

世代交替演化的可能原因

1. 有性生殖增加遺傳多樣性，有利於族群演化

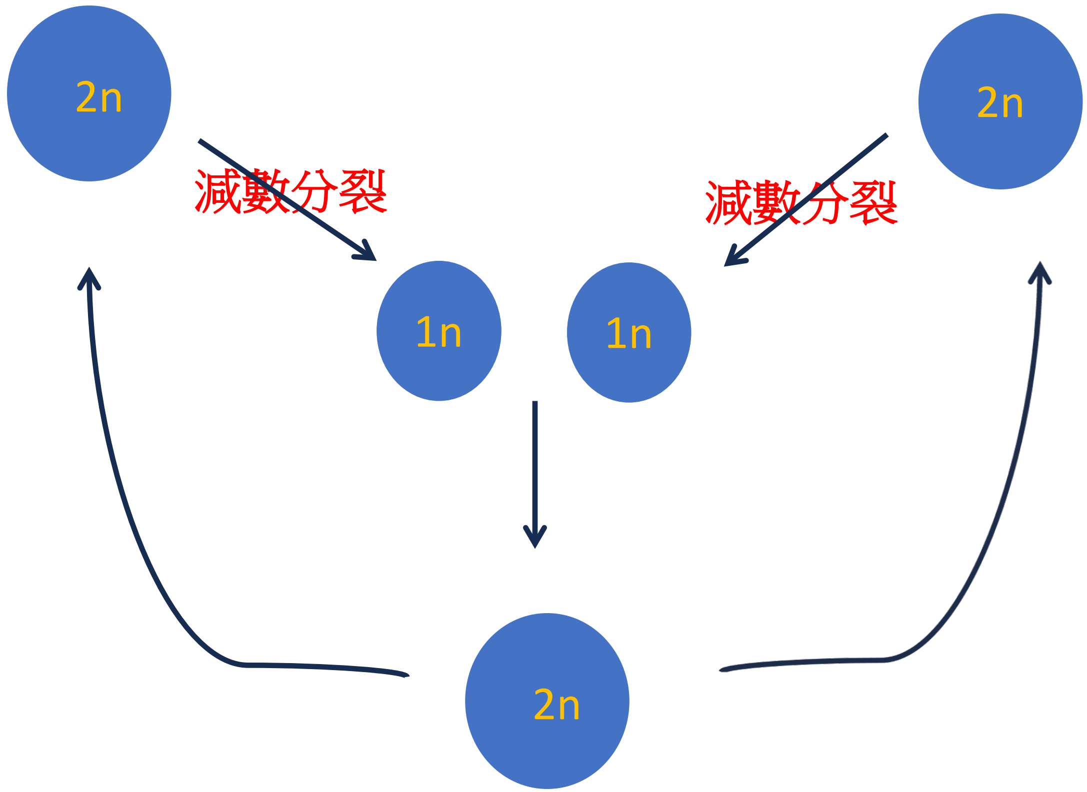
2. 植物不能動，無法主動找其他個體進行有性生殖

3. 植物需要擴大生存空間

4. 隨著演化，目前的高等植物(開花植物)配子體(1n)世代越來越式微

有性生殖

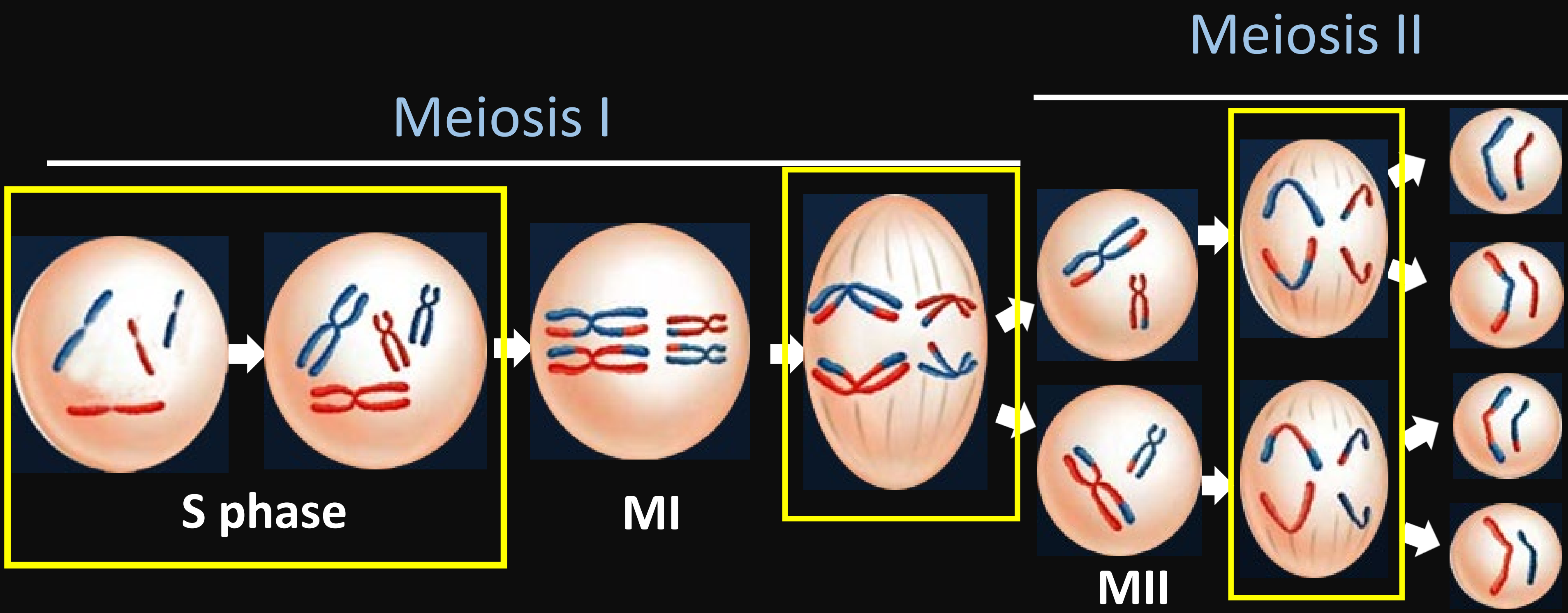
生命起源為無性生殖
單倍體（1n）



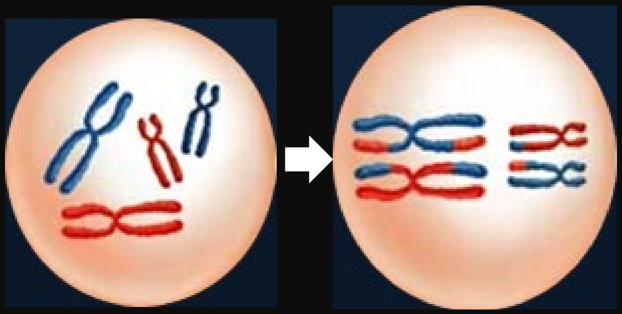
減數分裂

—染色體數目減半並獨立分配
—基因重組(染色體互換)

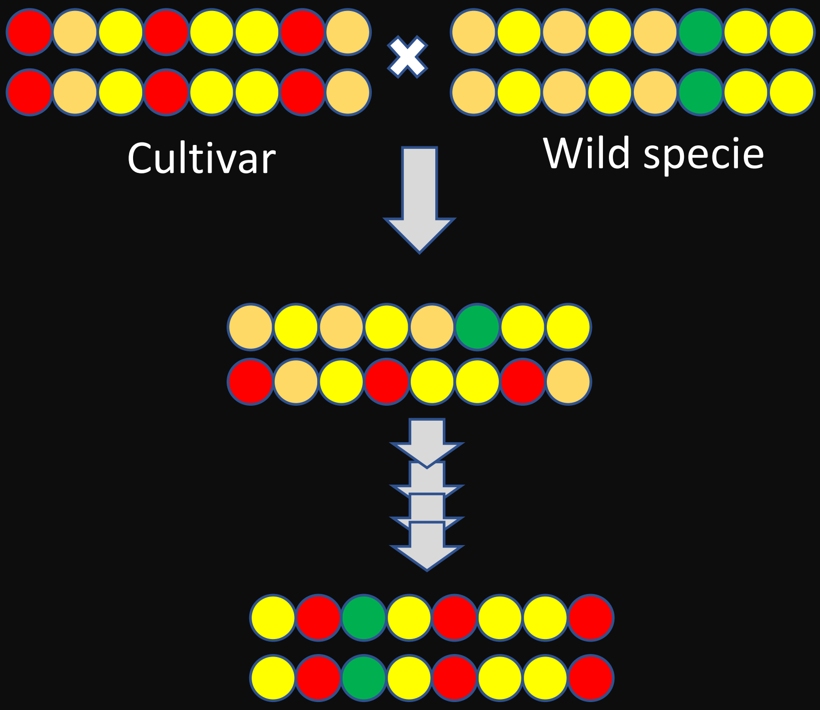
Meiosis



The mystery:
Homologous pairing and recombination



Basis of breeding

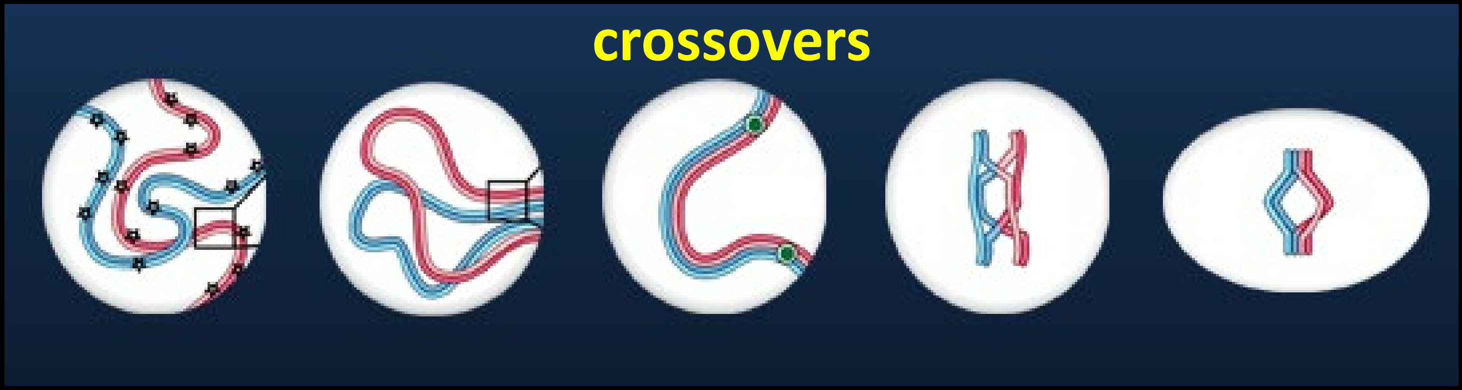


不管是同源染色體正確分離或是互換，

都要先配對

怎麼配????

Major processes of meiotic prophase I



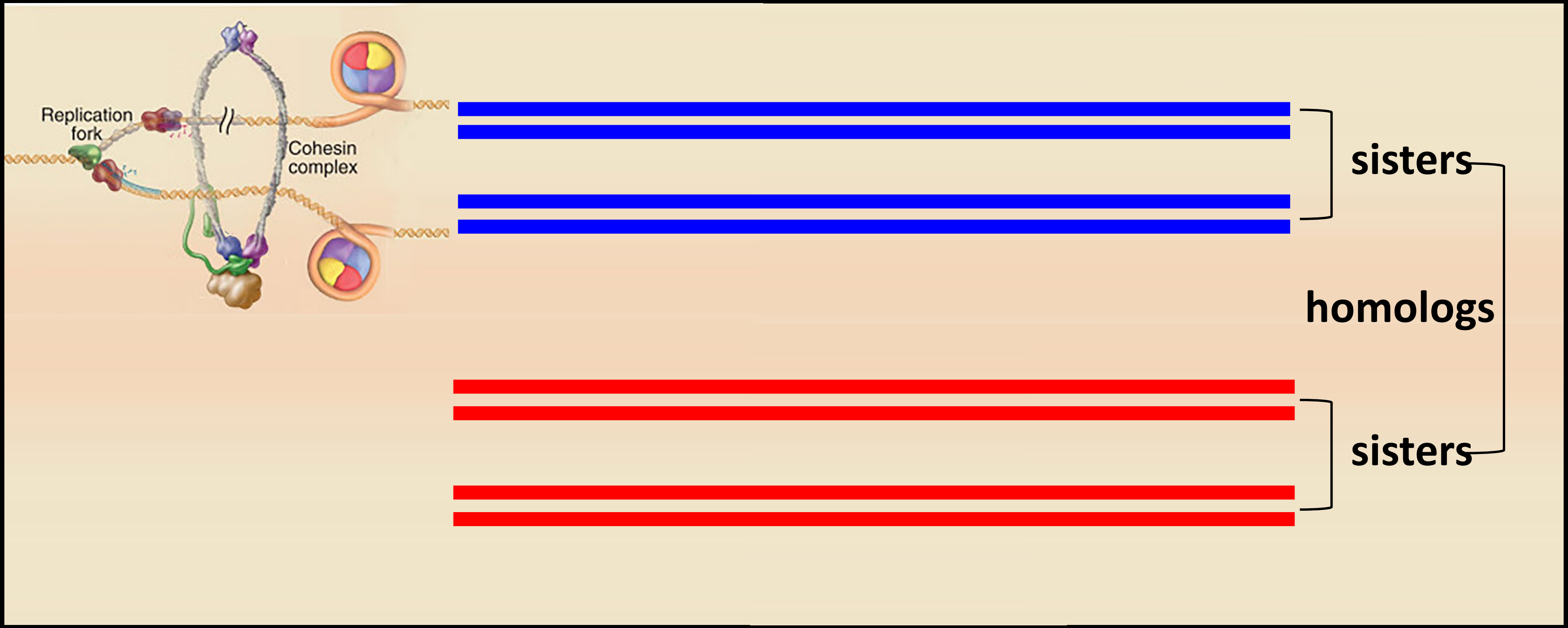
Leptotene Zygotene Pachytene Diplotene Diakinesis

recombination

synapsis

pairing

The DSB repair pathway



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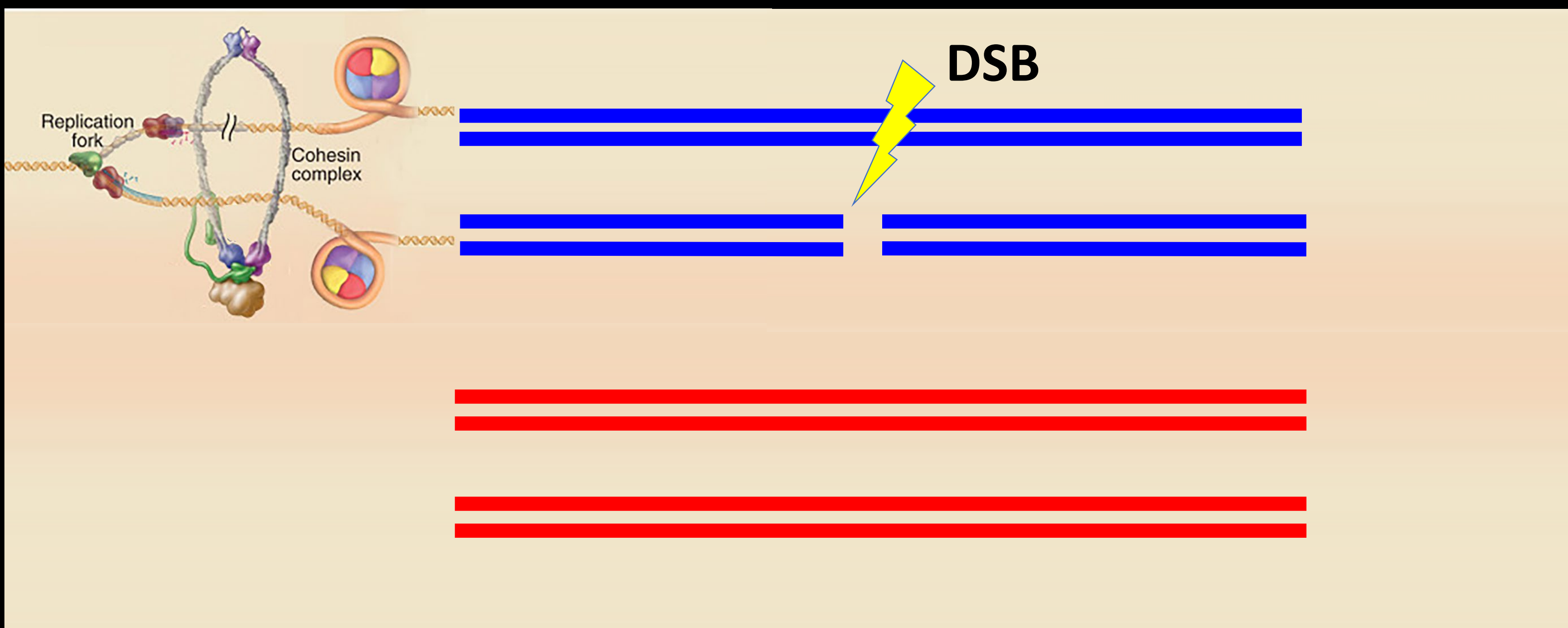

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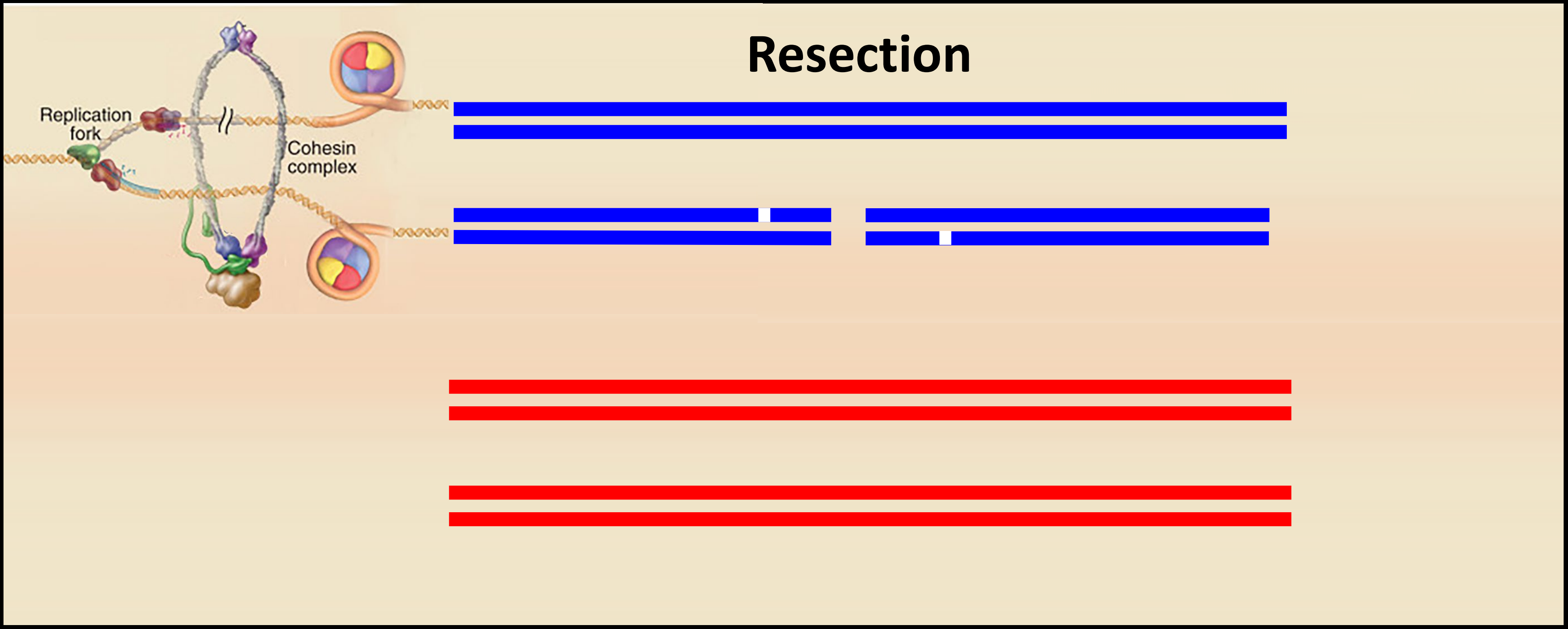

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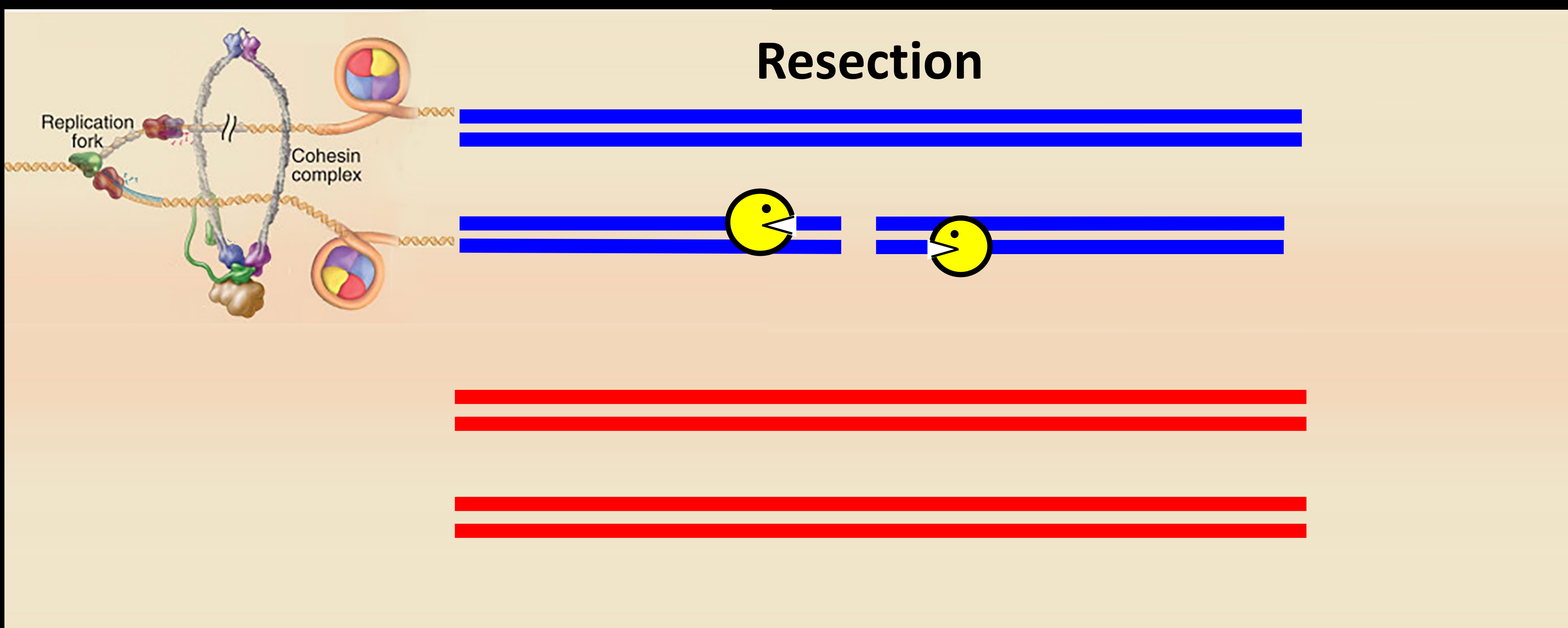

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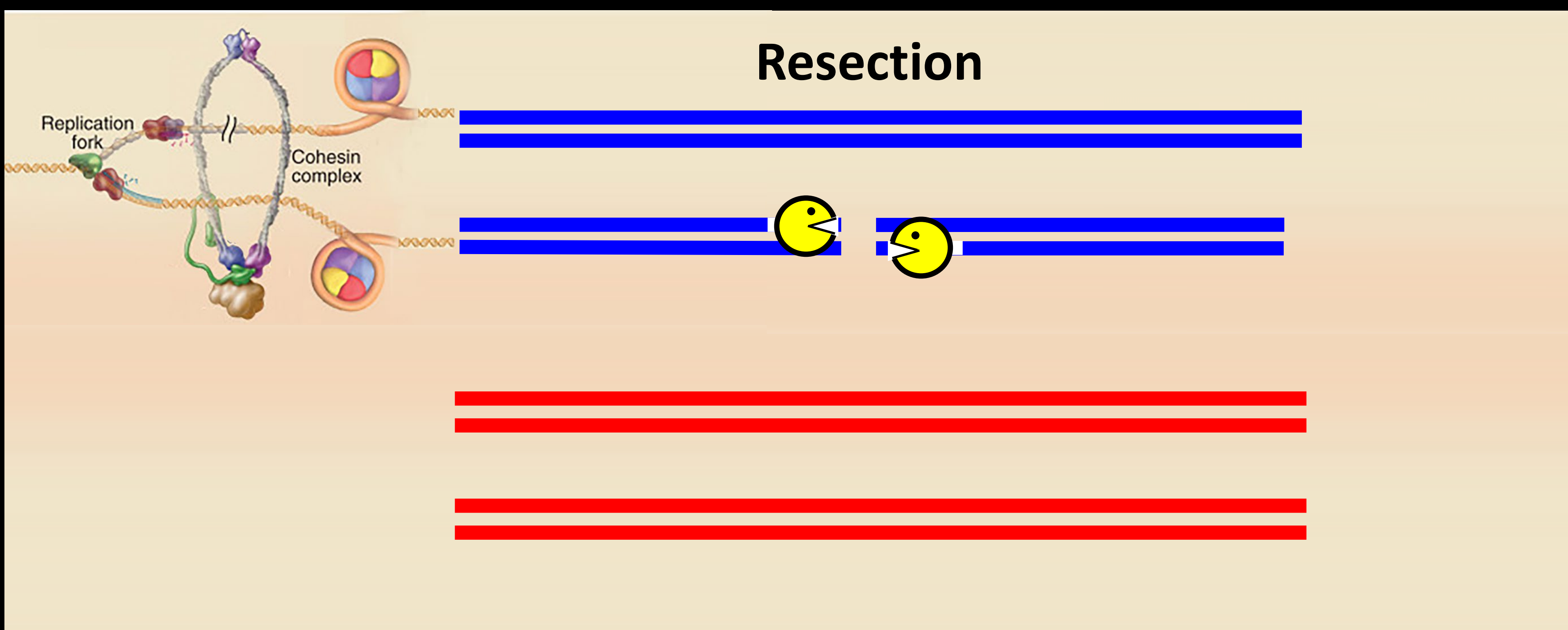

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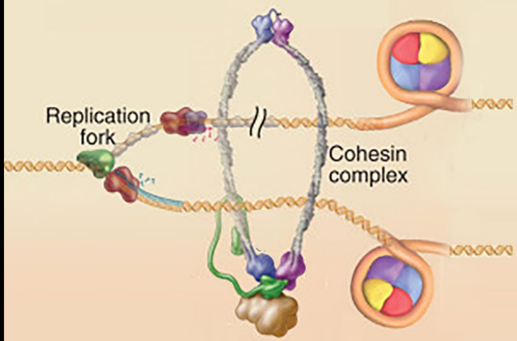
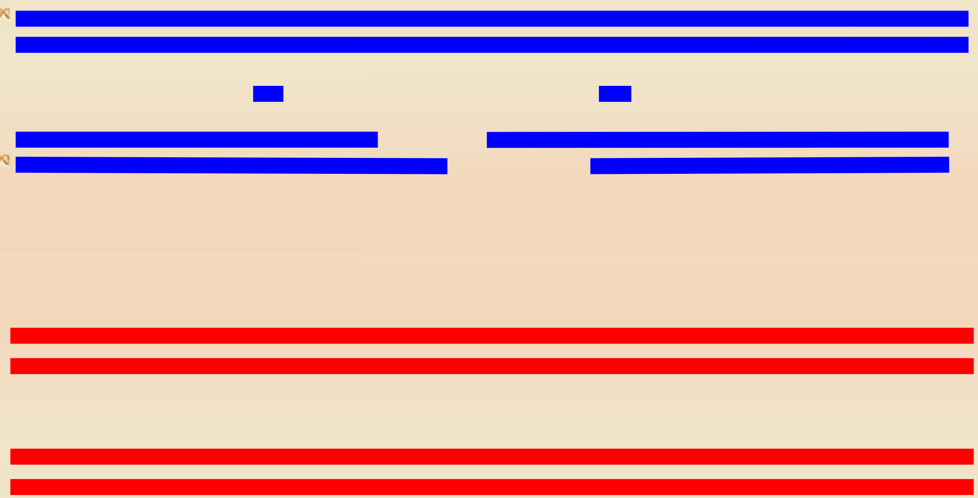


Resection



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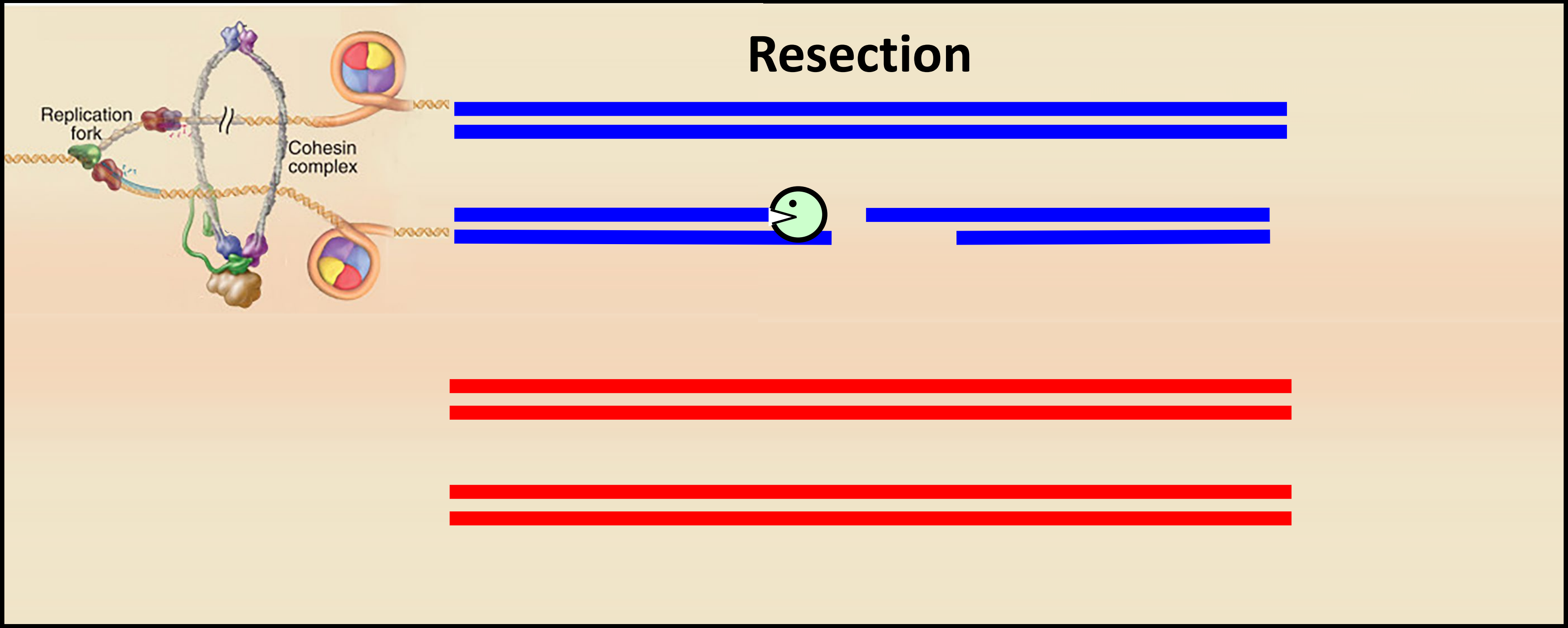

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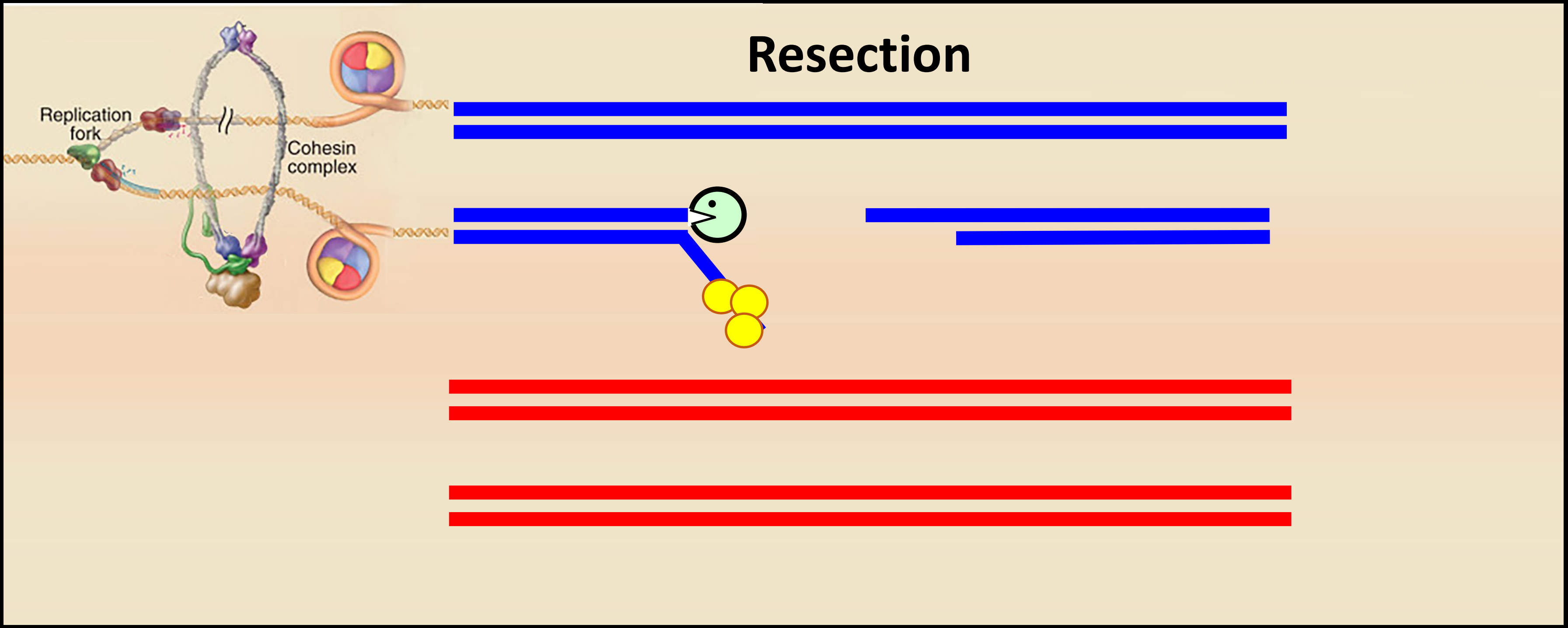

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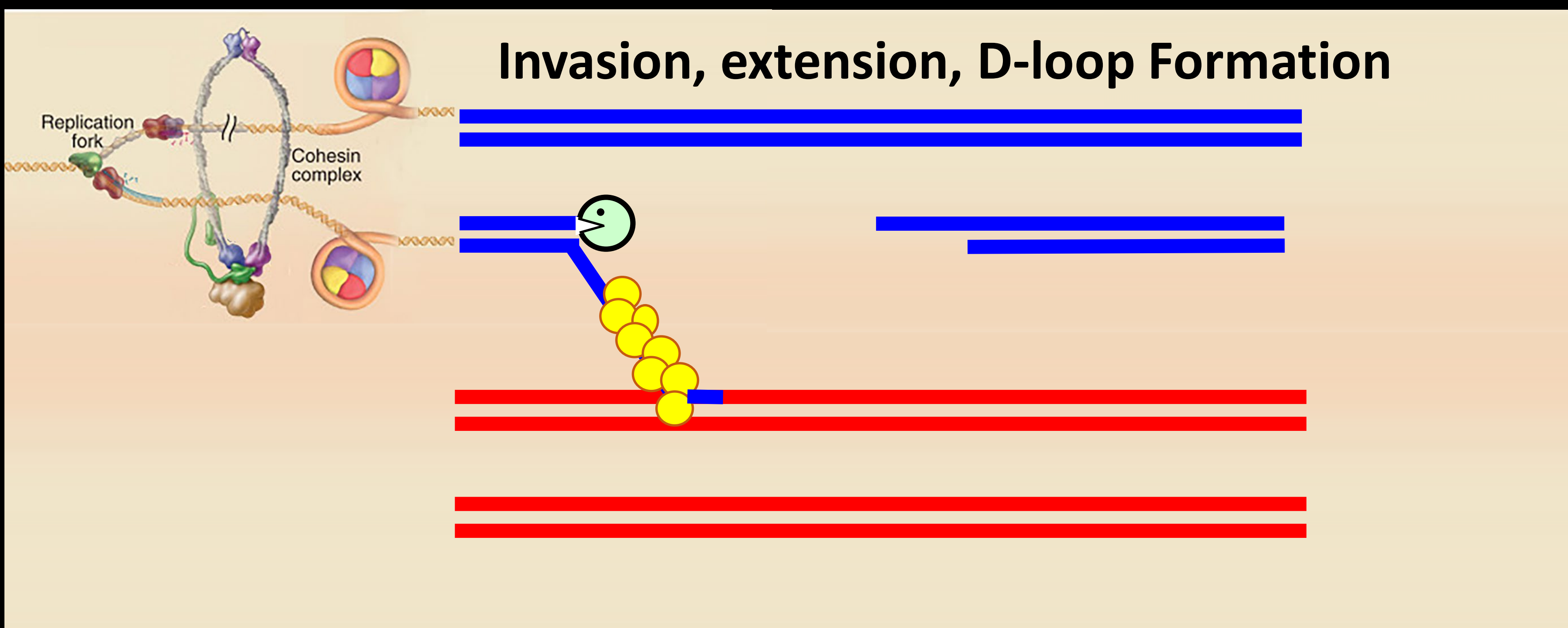

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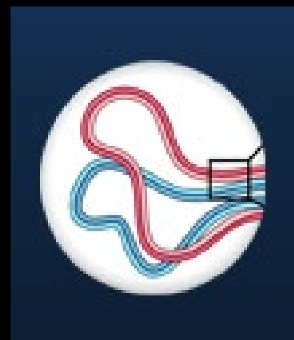

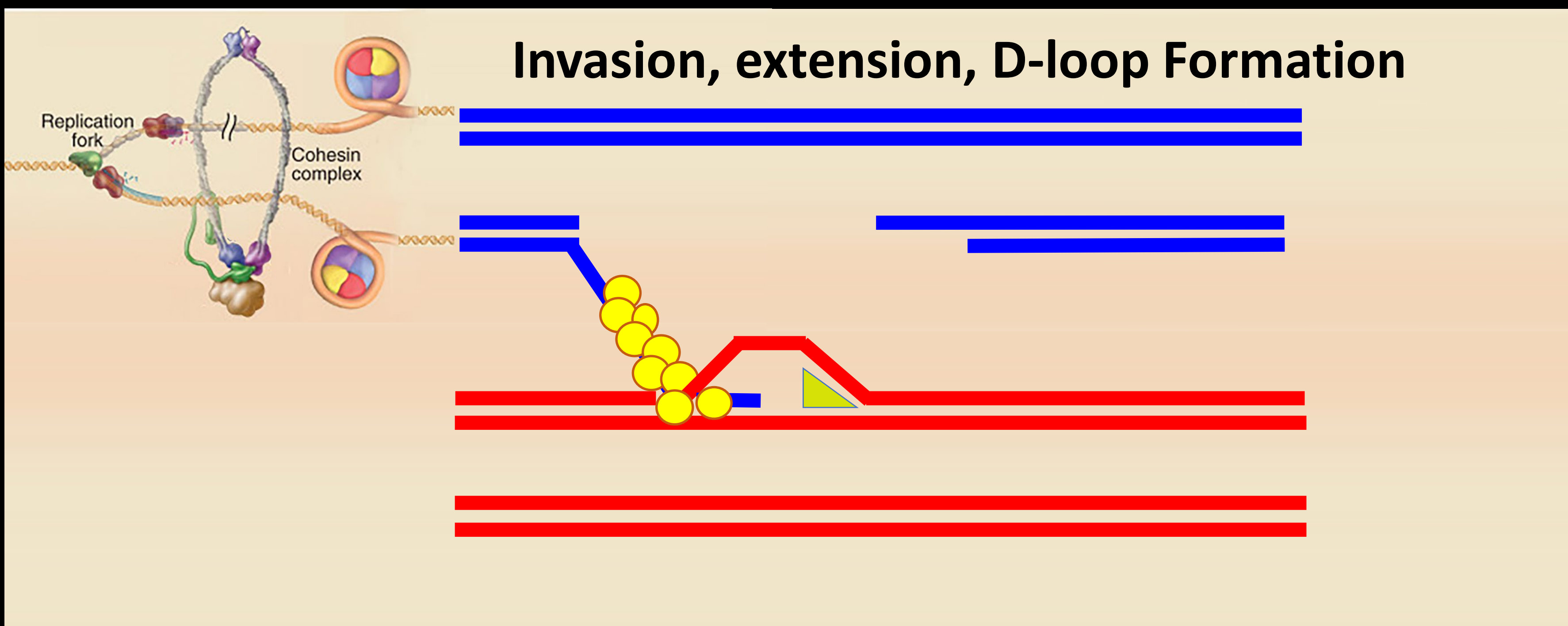

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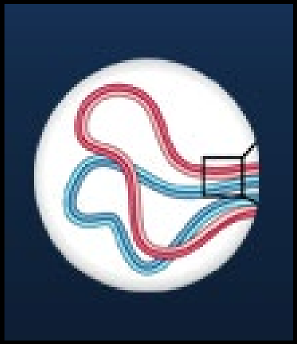
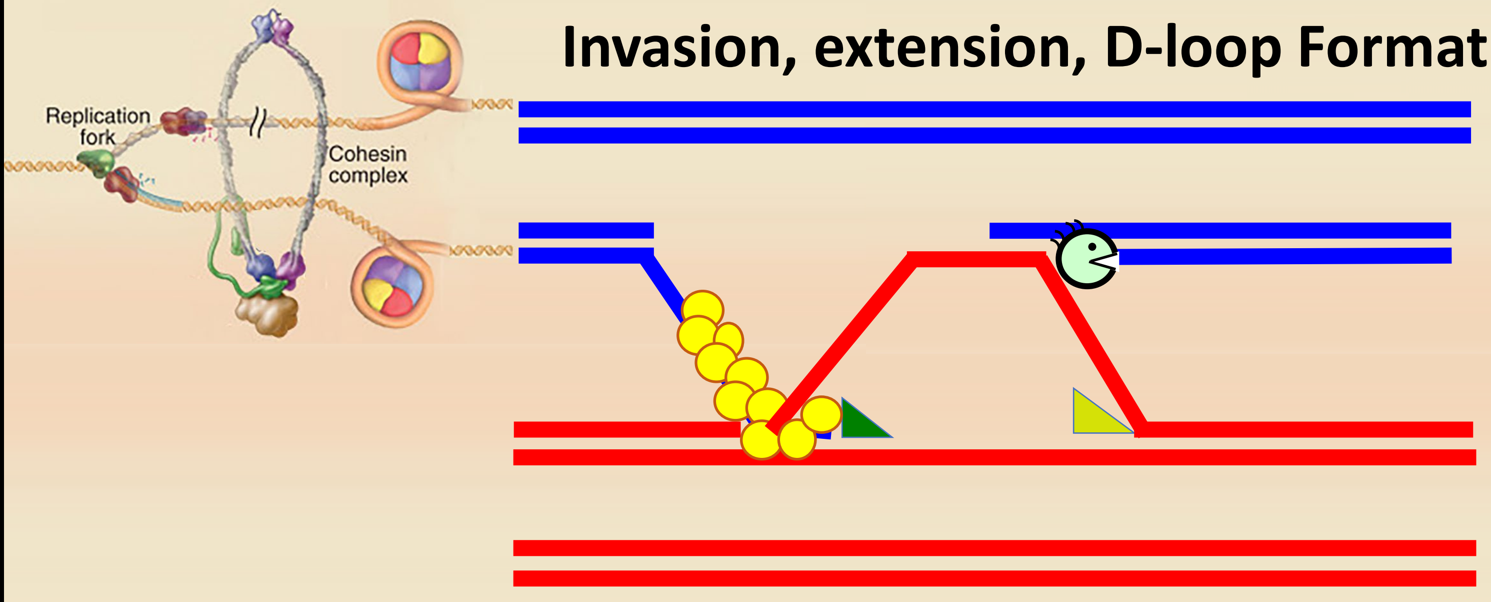


Invasion, extension, D-loop Formation



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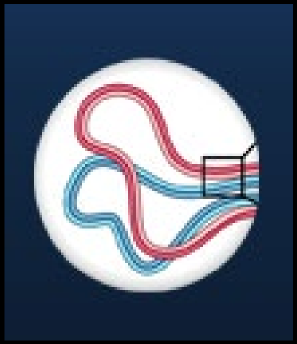
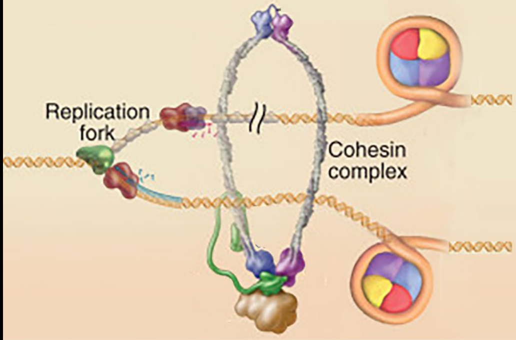
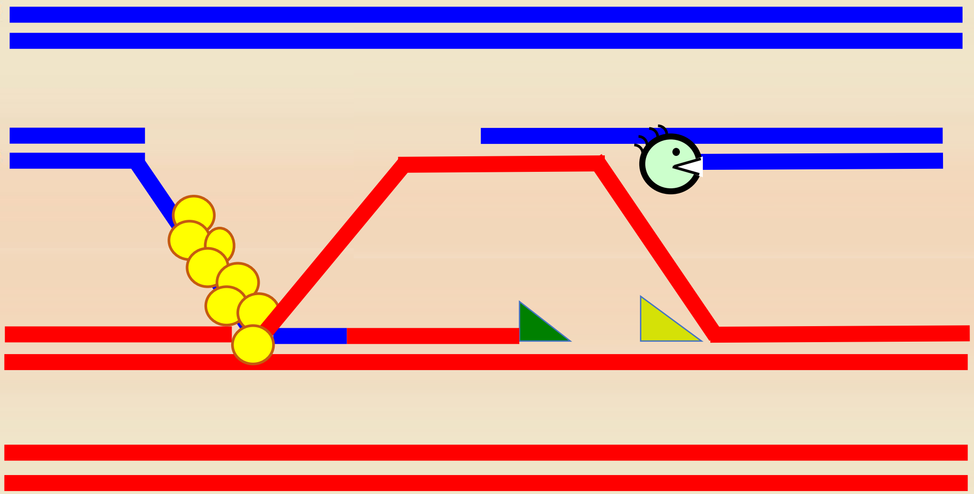




2nd strand annealing



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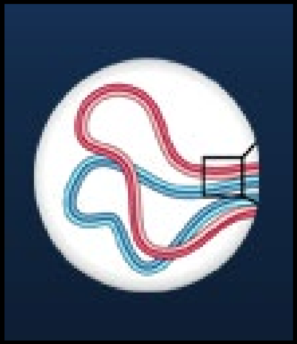
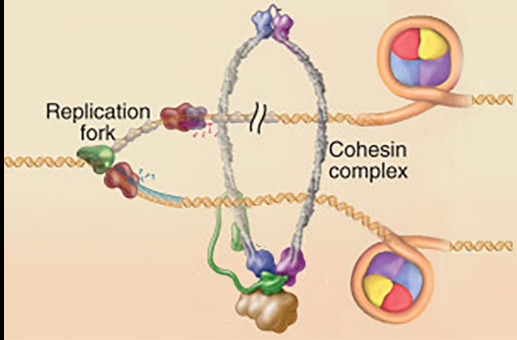
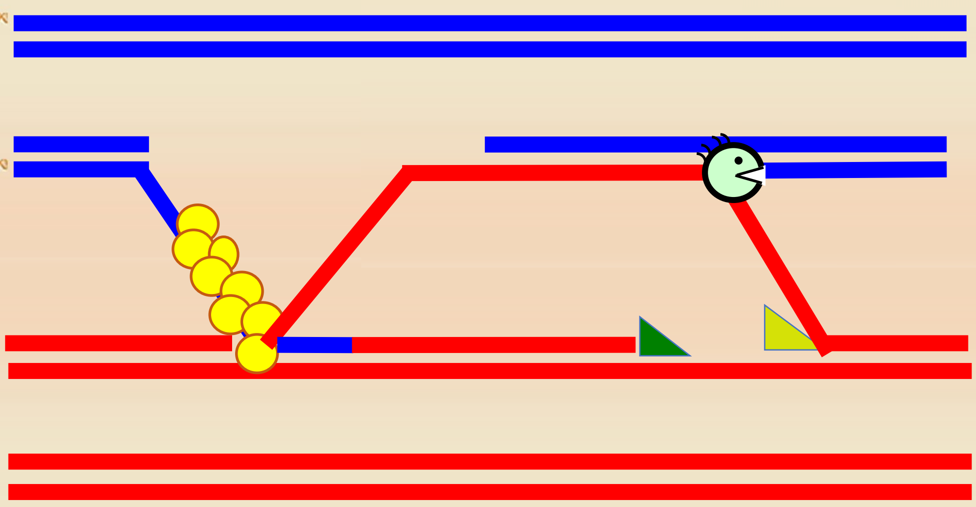




2nd strand annealing



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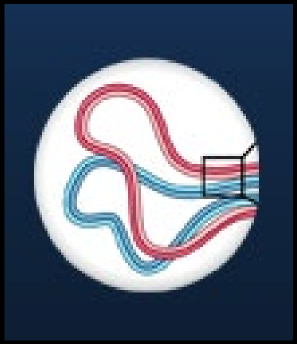
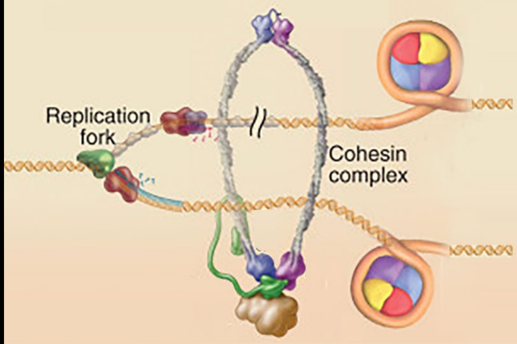
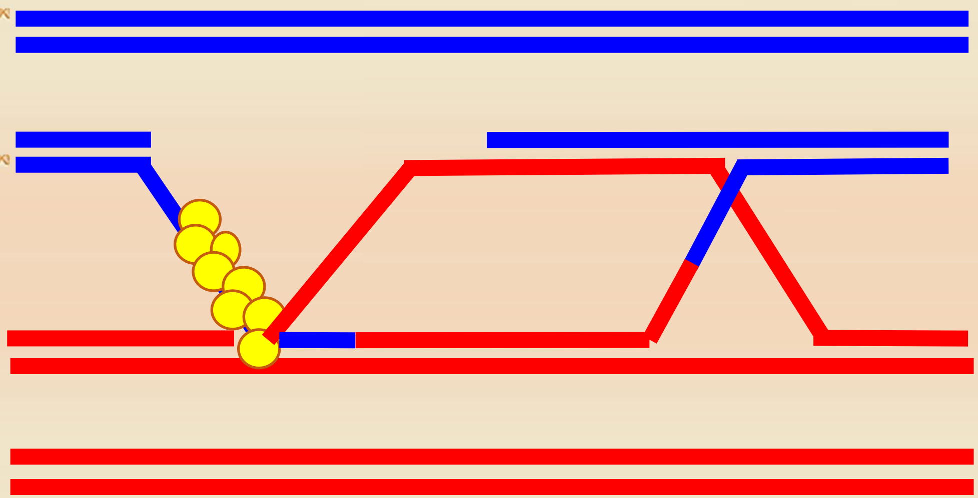




2nd strand annealing



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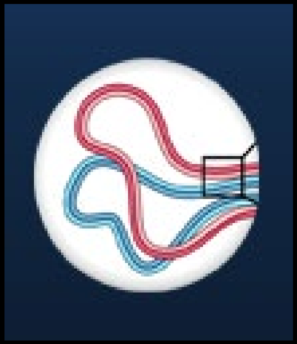
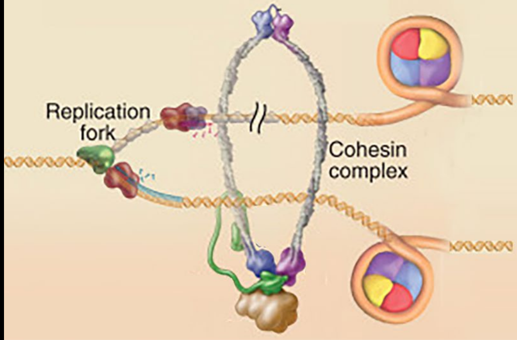
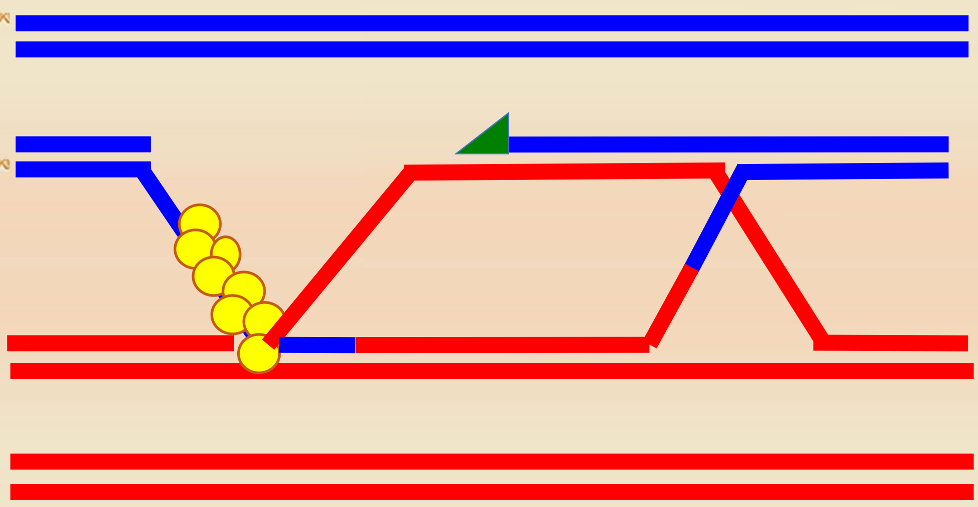
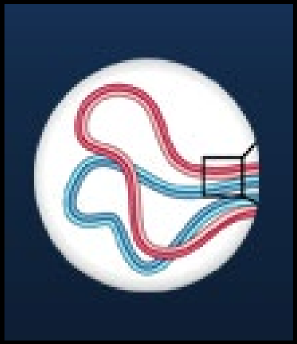


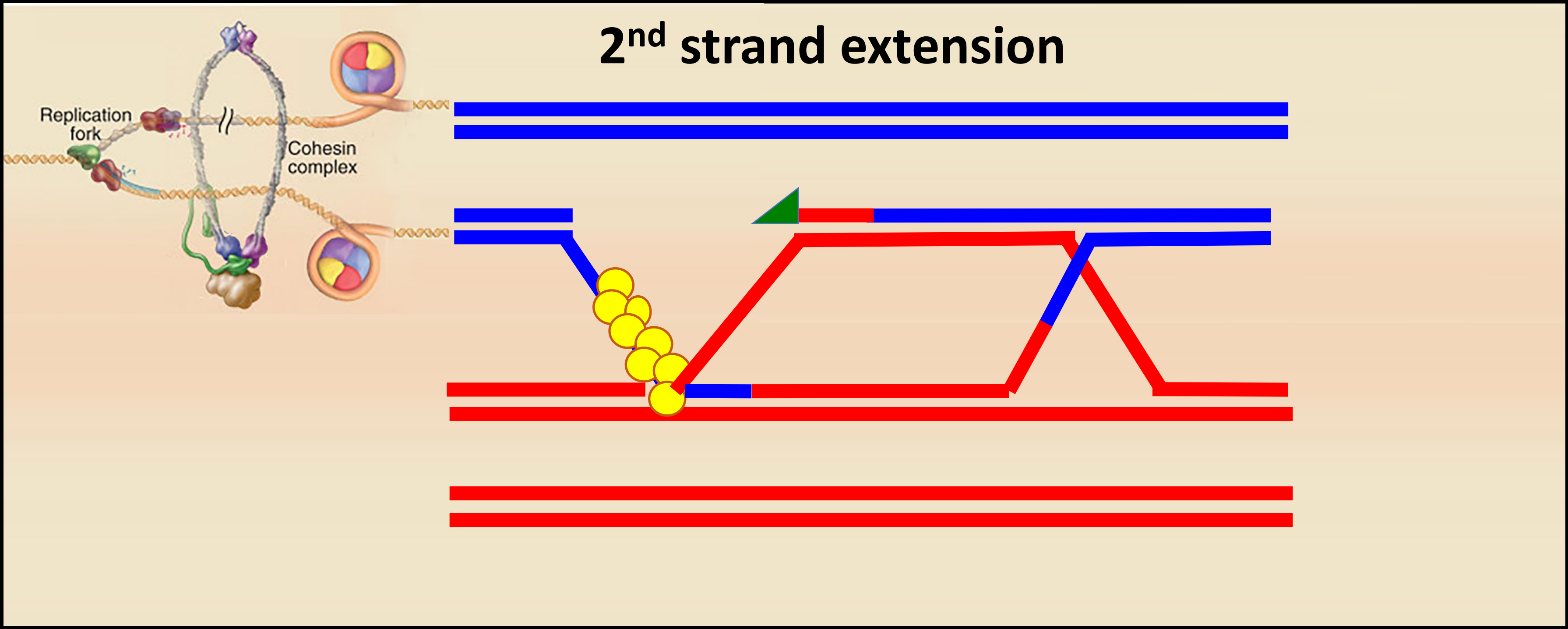


2nd strand extension



The DSB repair pathway





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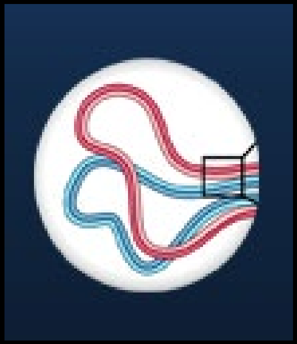

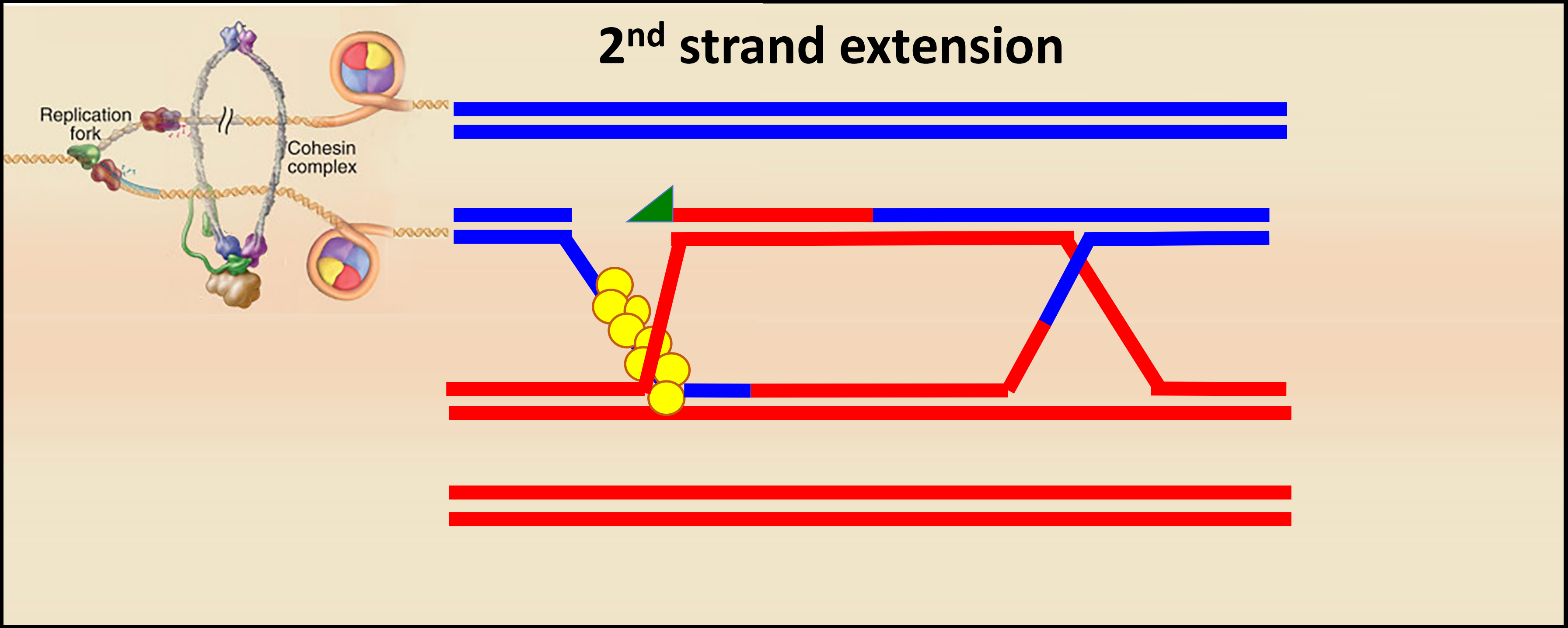

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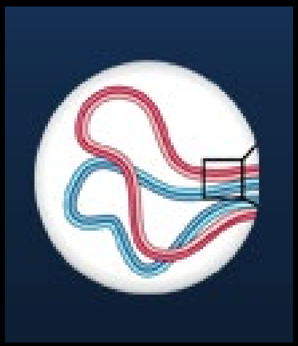
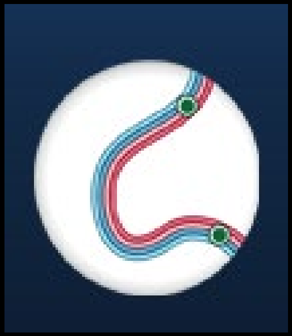
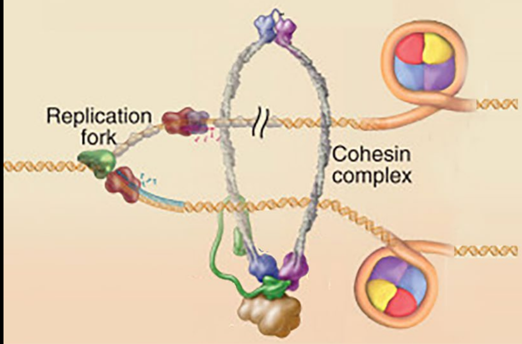
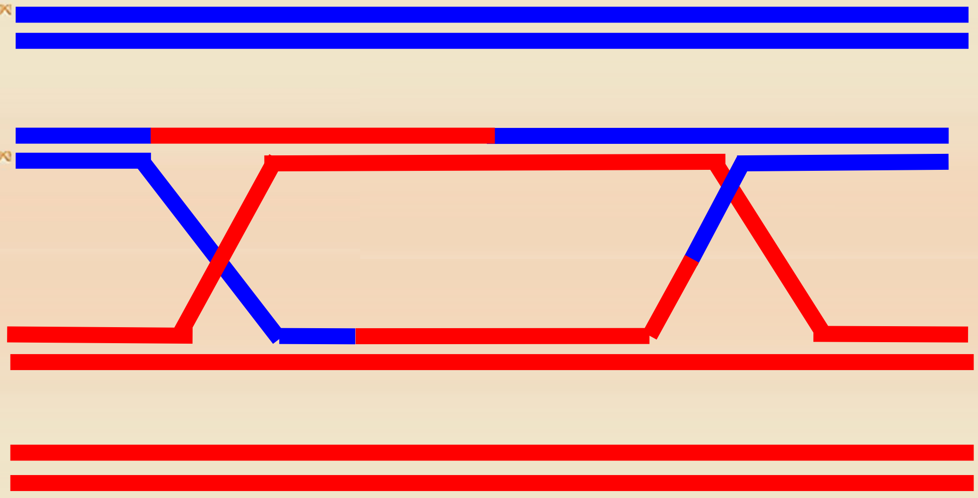


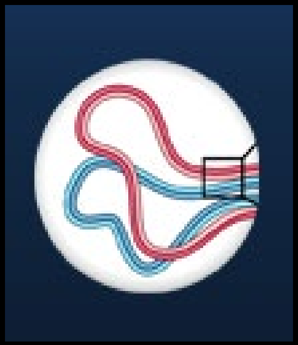


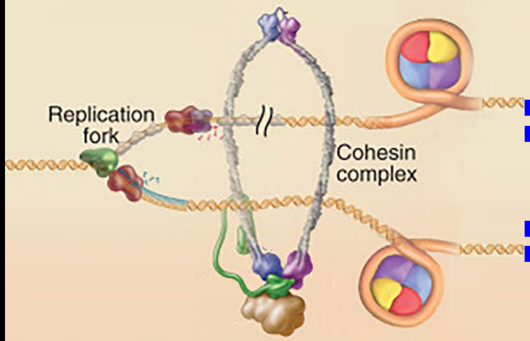


Double Holliday junction

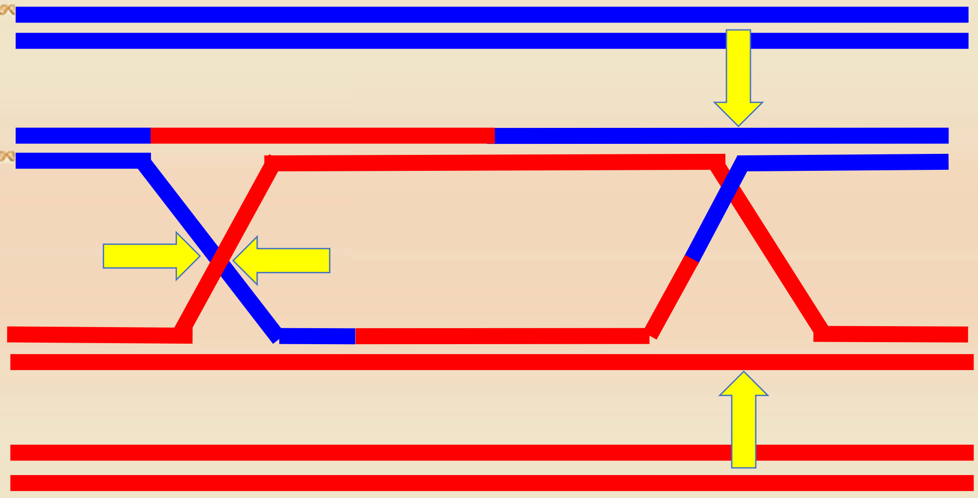


The DSB repair pathway

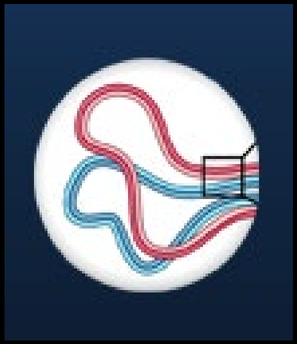


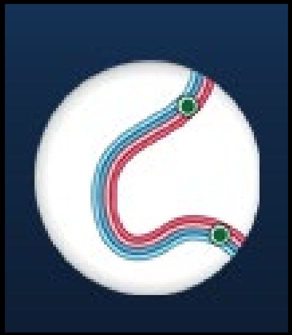


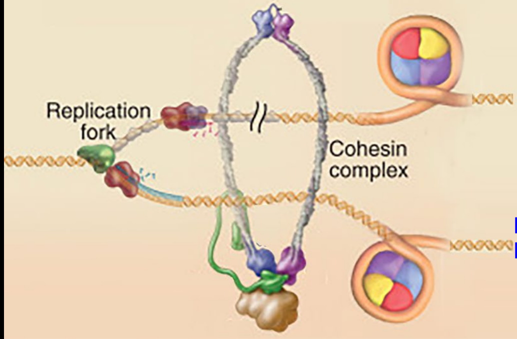
Double Holliday junction resolution

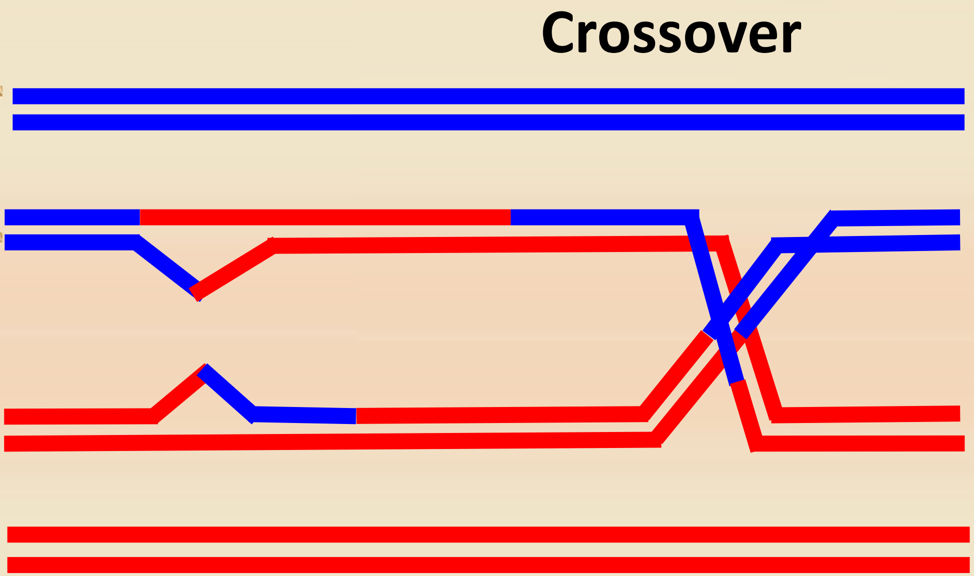


The DSB repair pathway

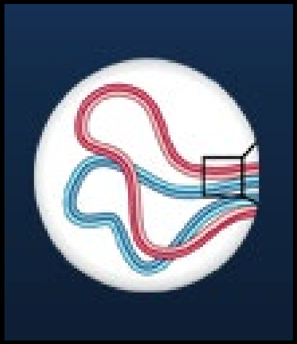


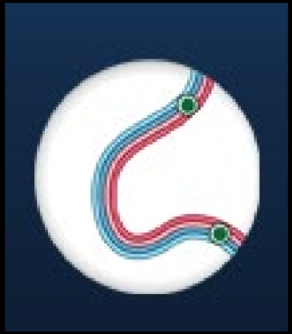


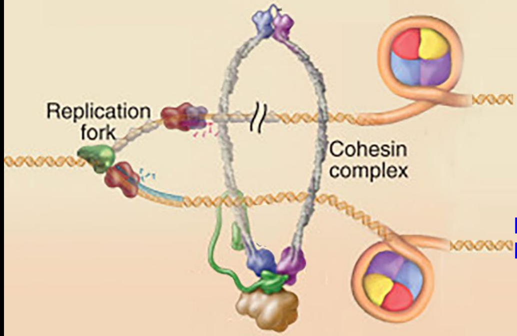




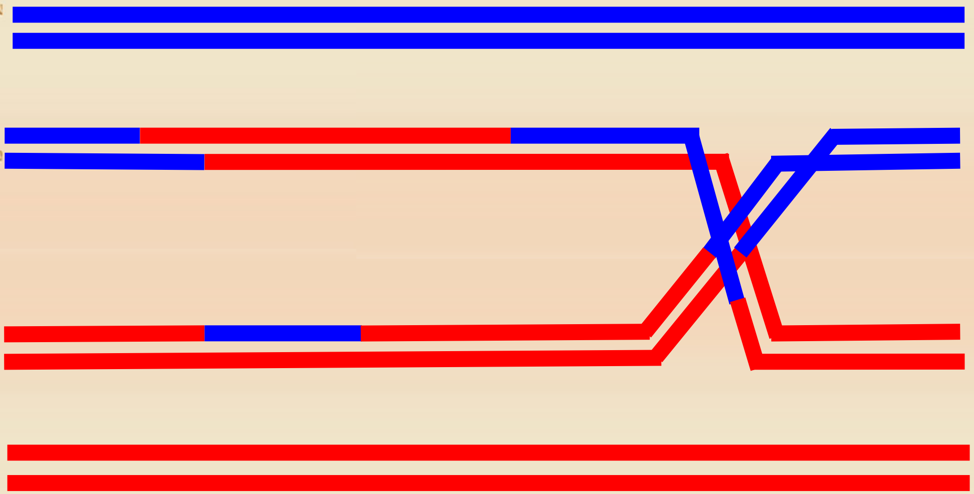
The DSB repair pathway



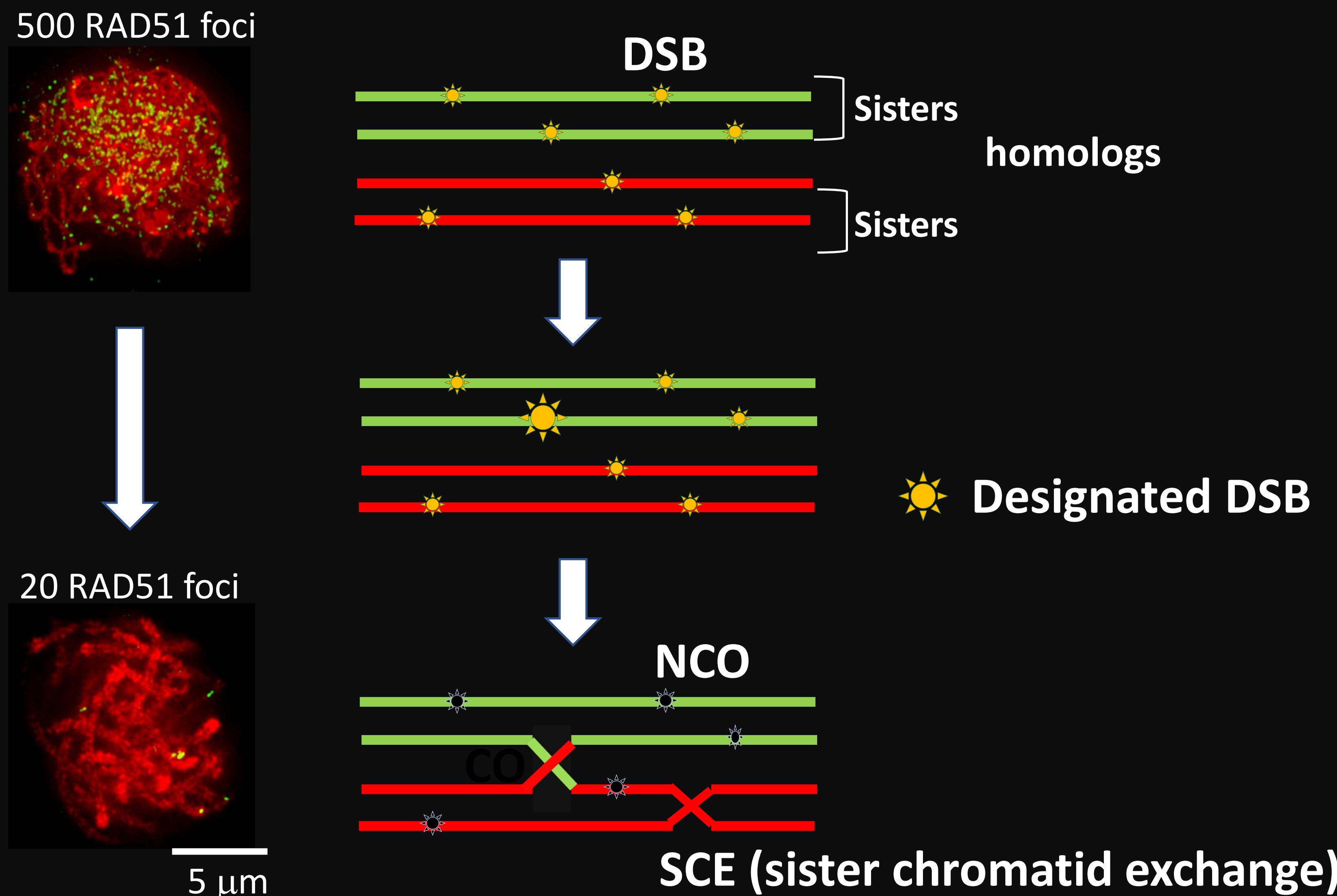




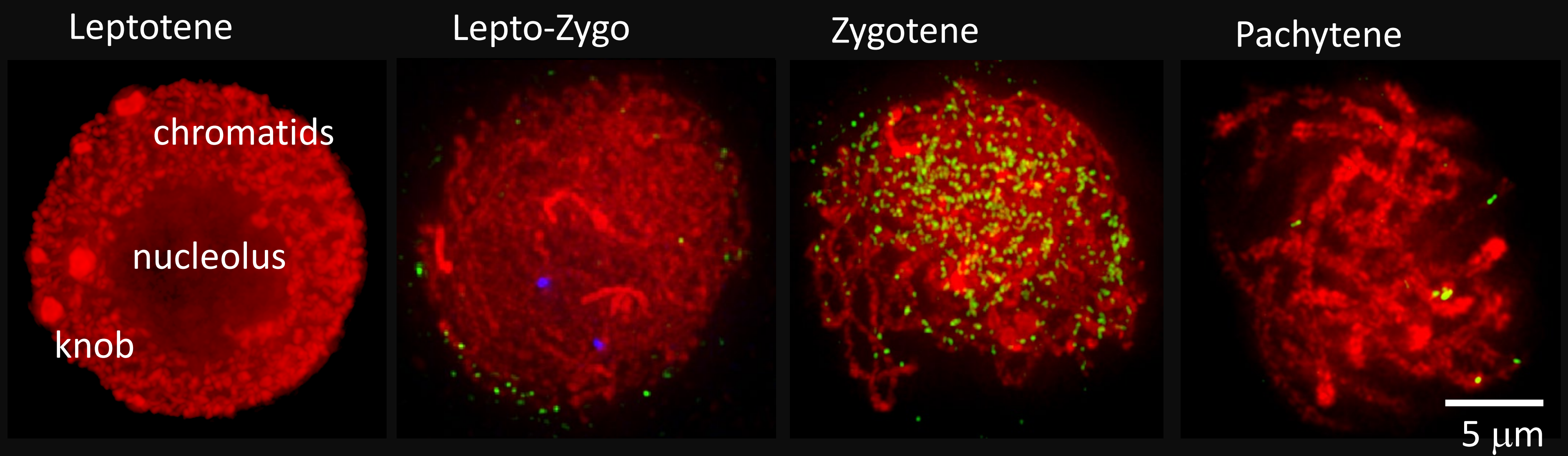
Crossover



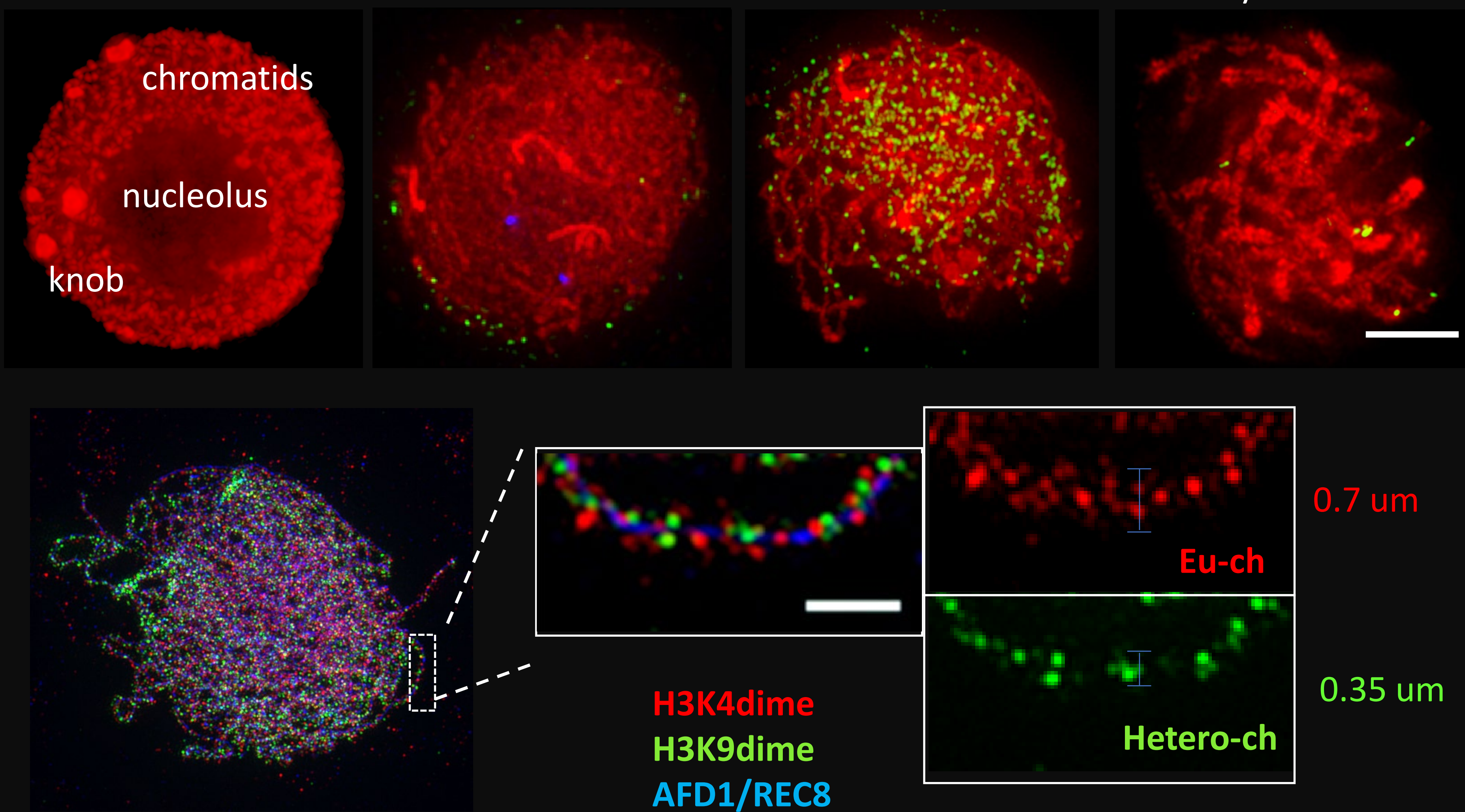
From DSBs to COs with interference



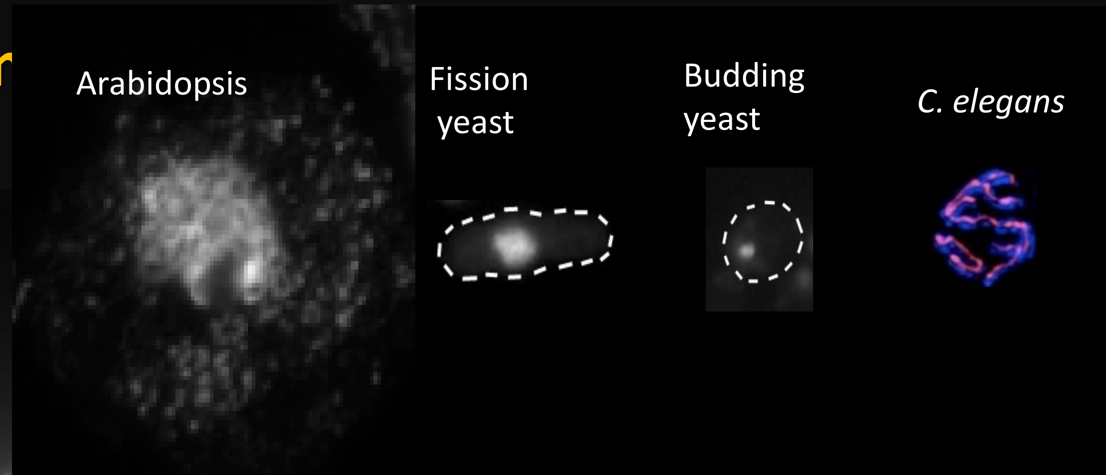
Male Meiosis in Maize

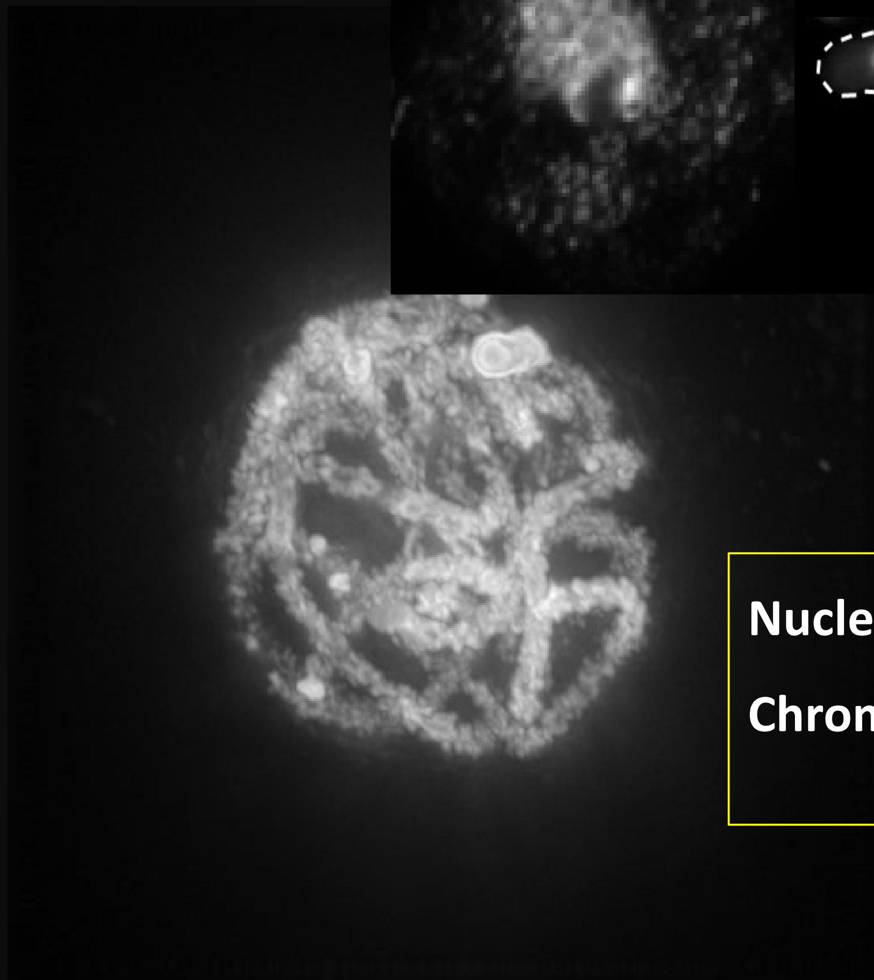


Male Meiosis in Maize



Maize meiotic chrom



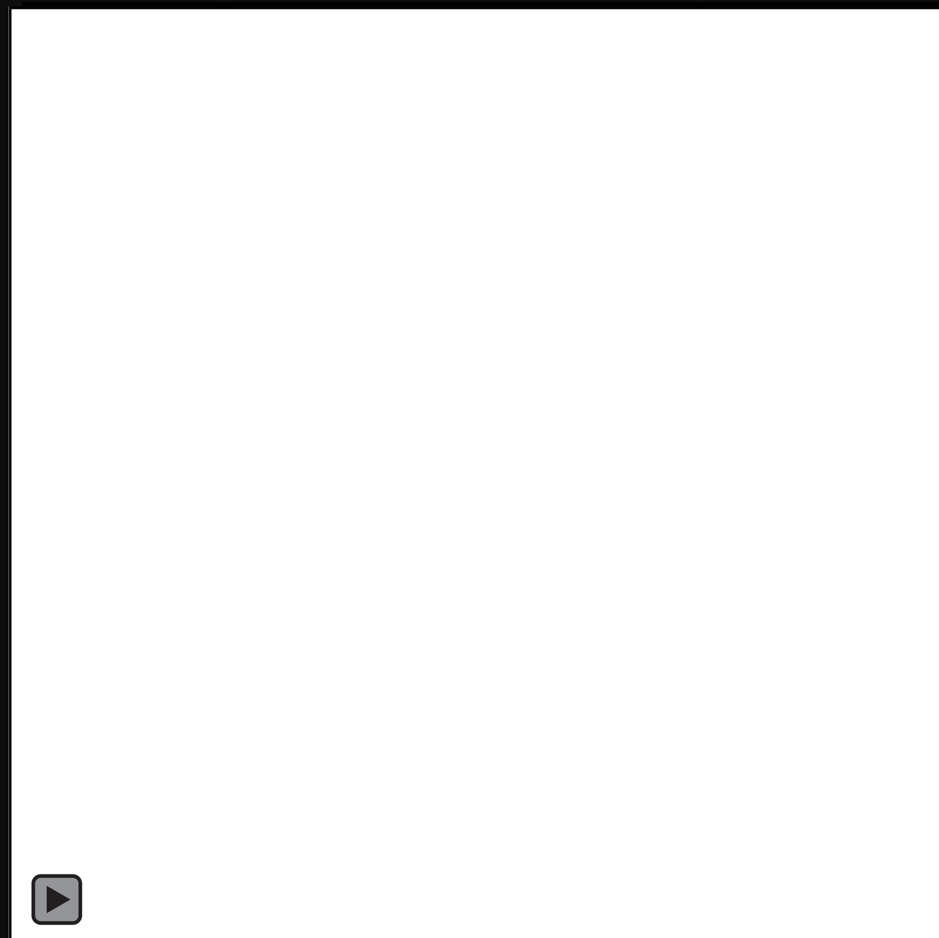


Nucleus diameter: 25 µm

Chromosome width:1 µm

Taken by Deconvolution Microscope

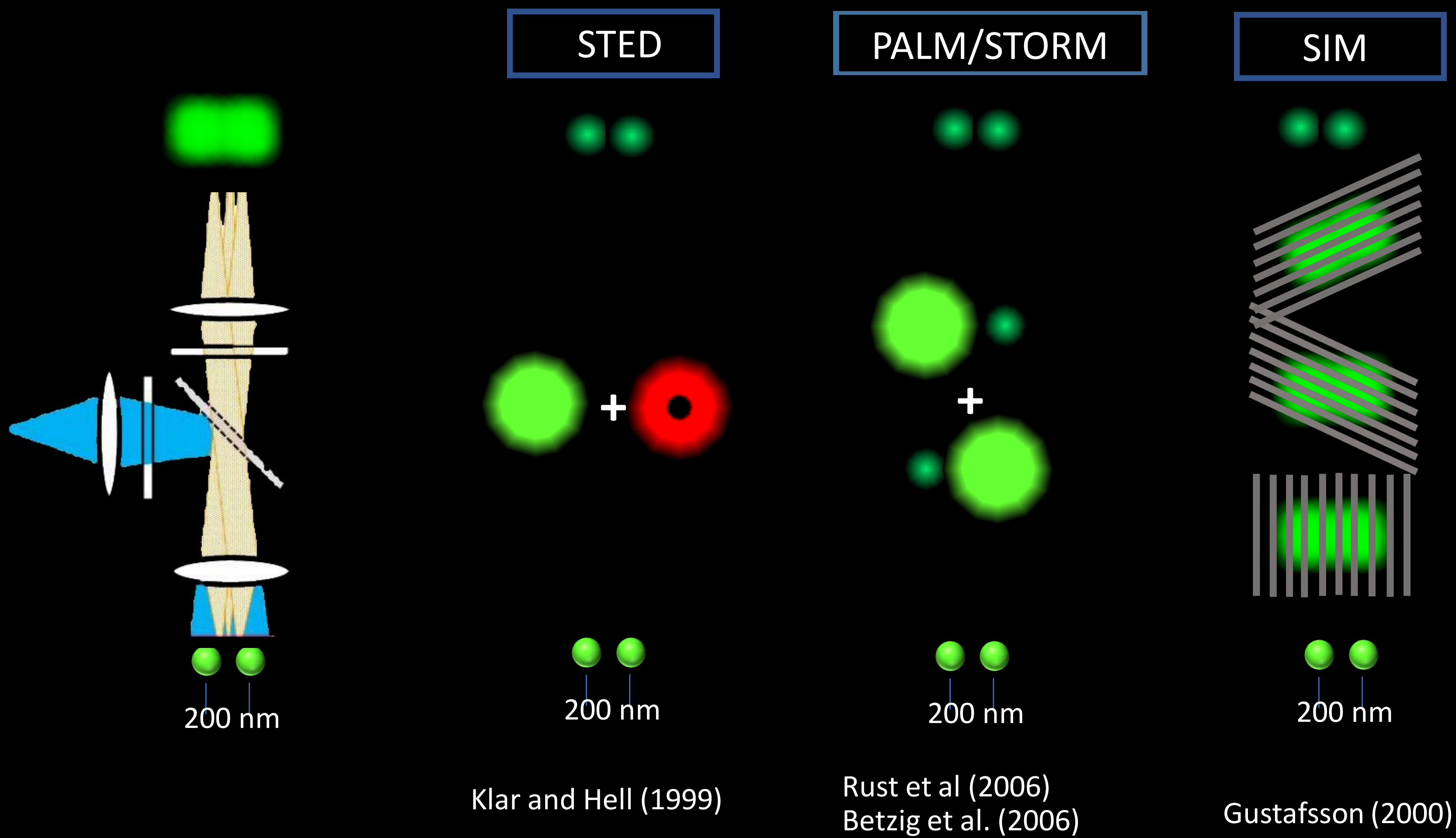
Maize meiocytes are amenable to super-resolution microscopy



AFD1/REC8 antibody

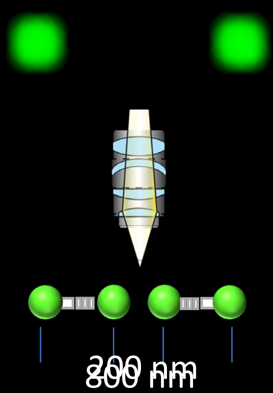
Included in the textbook *"Molecular Biology of the Cell"*, 6th edition by Alberts et al. 2015

Resolution limit and super-resolution microscopy



Expansion Microscopy (ExM)

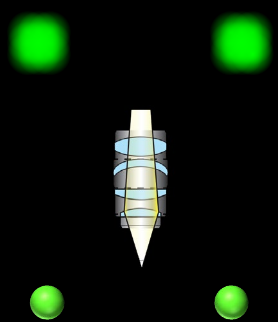
Make samples physically BIGGER

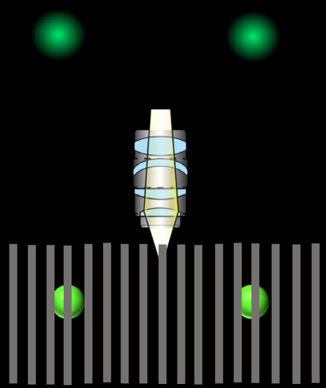


Ed Boyden
Chen et al (2015)

Expansion Microscopy (ExM)

By 3D-SIM





Resolution improves ~ 8 folds.

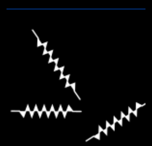
Ed Boyden
Chen et al (2015)

Expansion Microscopy (ExM)

Use of polyelectrolyte hydrogel

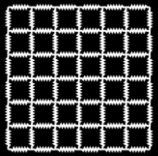
4X expansion in X, Y and Z = 64X in volume

Monomers

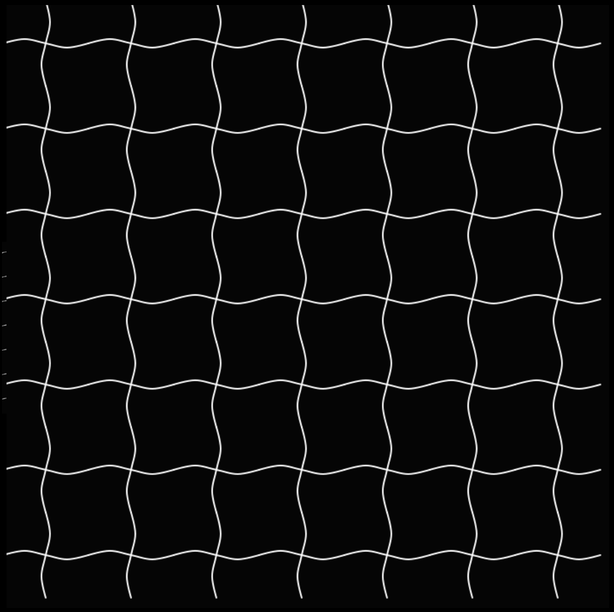


TEMED
APS

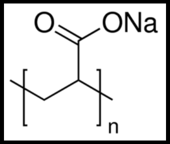
Polymer network



WATER



Sodium acrylate
Acrylamide



negatively charged carboxylic groups (羧酸)

Expansion Microscopy (ExM)

ProExM (Nature Biotech, 2016)

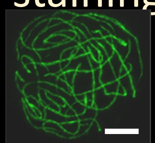
Expansion

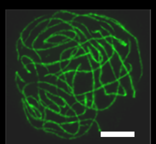
Ab staining

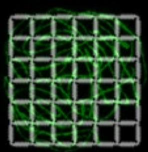
Anchoring

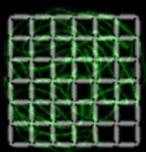
Gelation vs Cross-linking

Mechanical homogenization







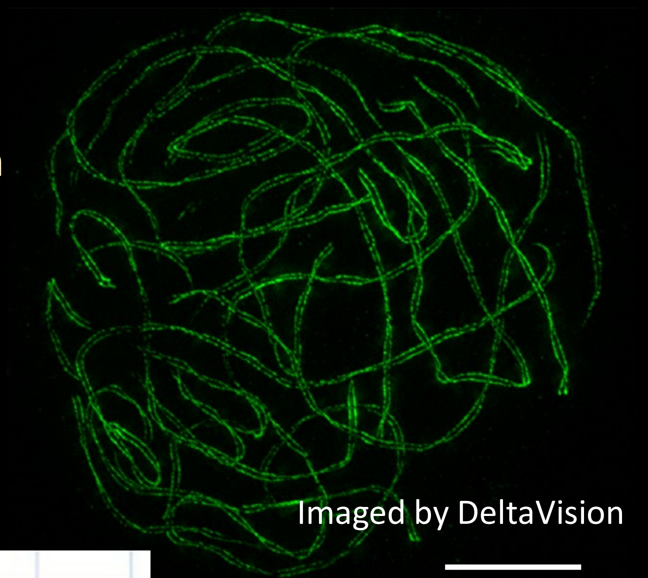


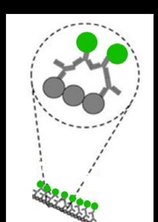
DSY2/RED1

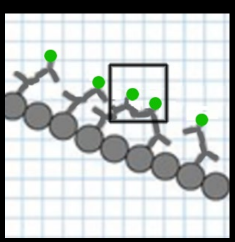
acryloyl-X SE (AcX)

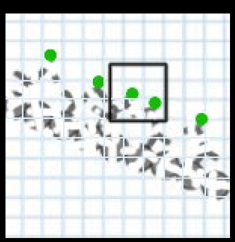
Hydrogel matrix

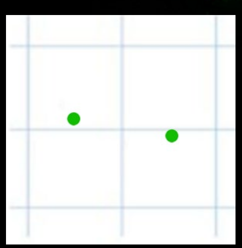
Proteinase K





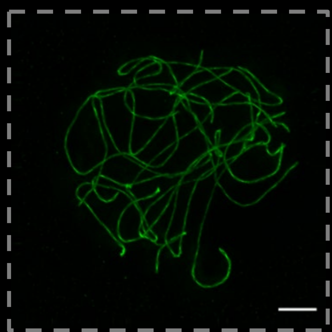




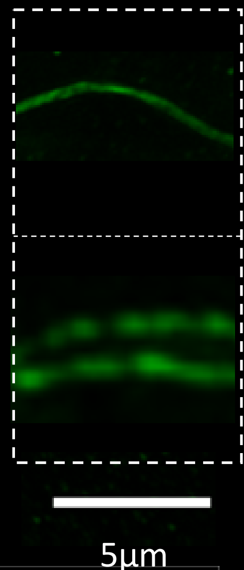


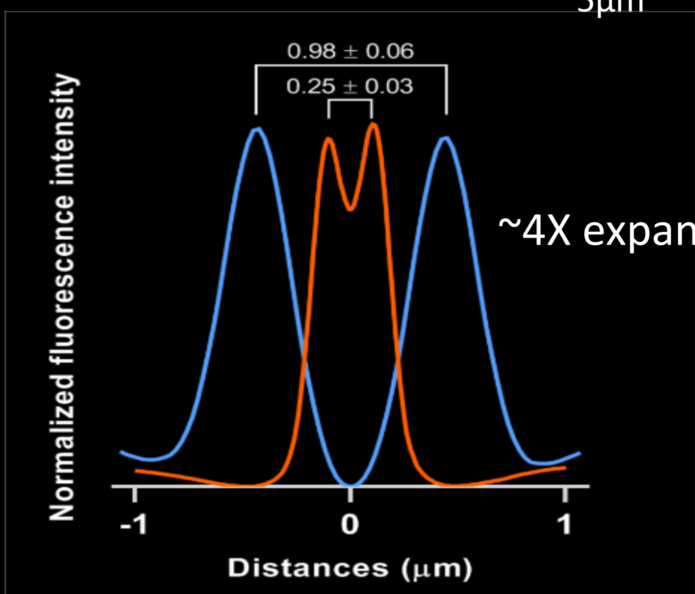
ExM of maize meiocytes

Deltavision



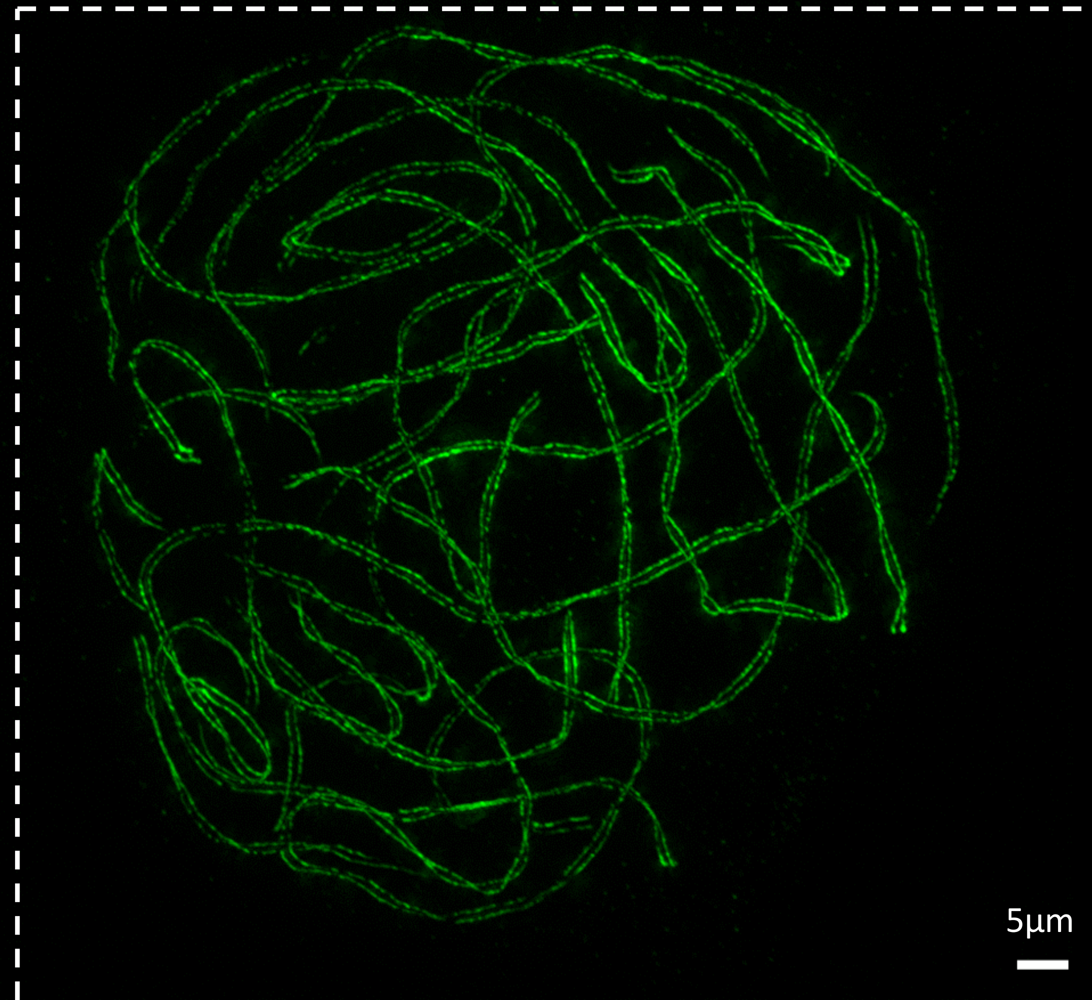
DSY2



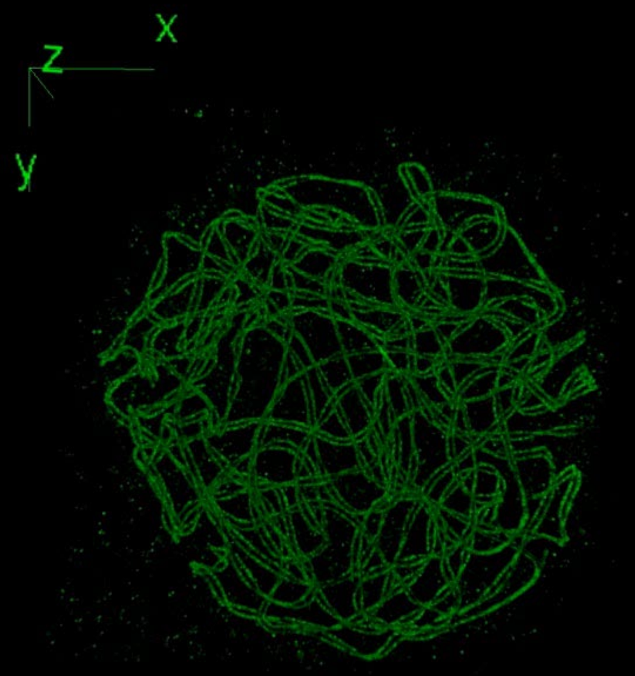


~4X expansion

ExM + Deltavision

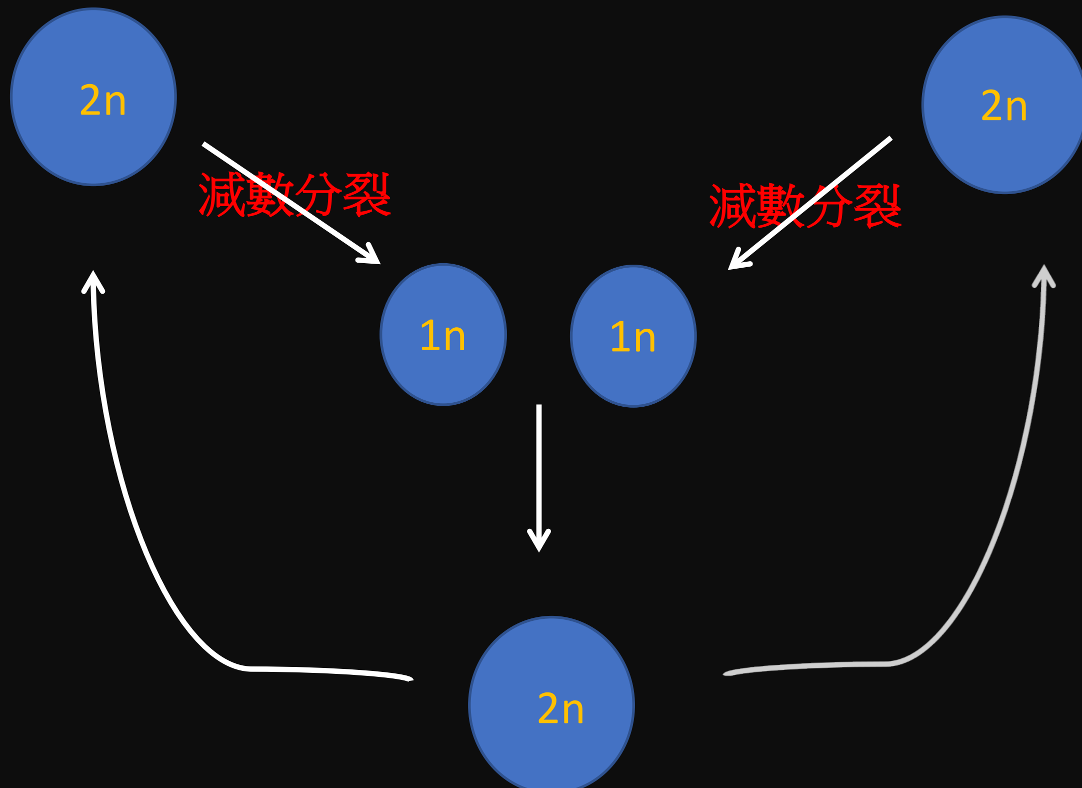


Expansion Microscopy (ExM)



生命起源為
無性生殖
單倍體（1n）

有性生殖

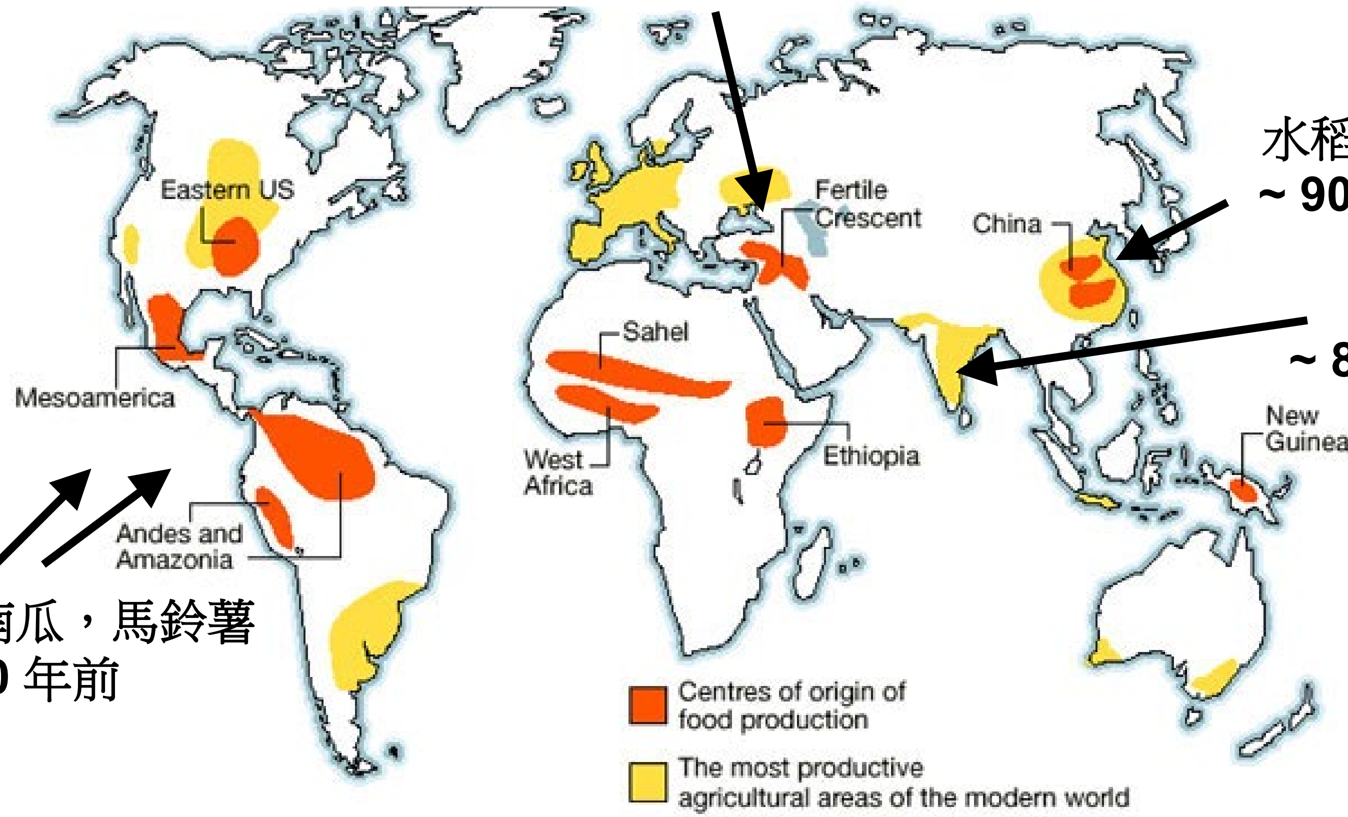


人類已經開始育種/馴化工程數千年

小麥，大麥，碗豆
~ 13,000年前

水稻，大豆
~ 9000年前

水稻
~ 8500年前



玉米，南瓜，馬鈴薯
~ 10,000 年前

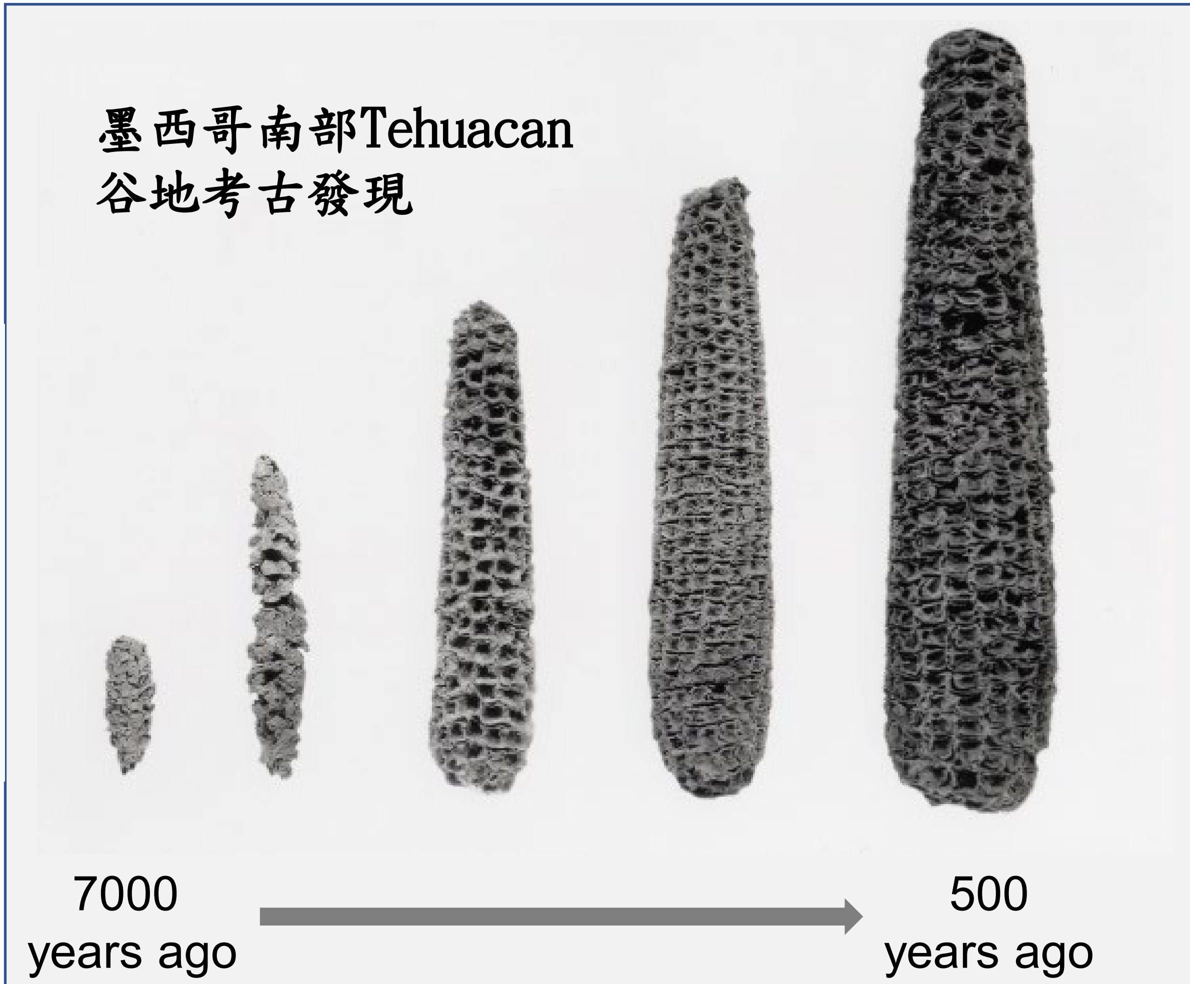
玉米的種化

玉米的馴化

大芻草　　　　　　現代玉米

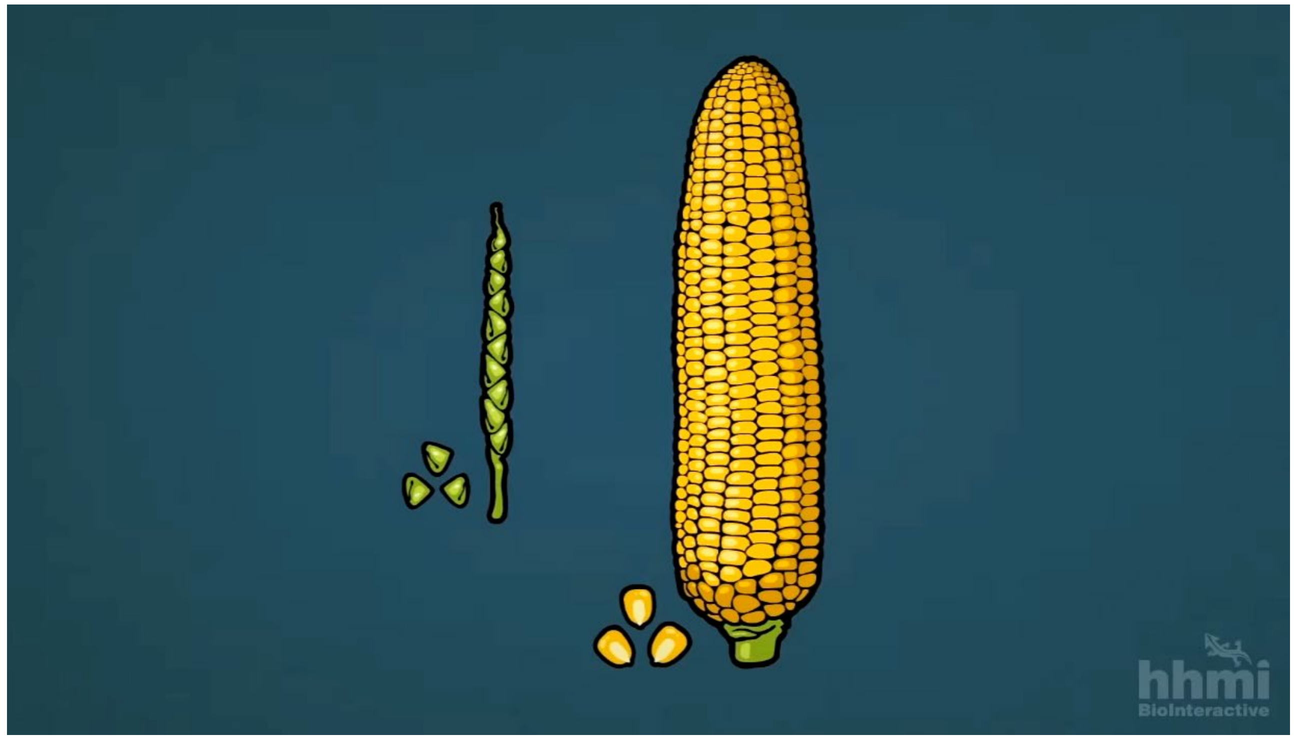
馴化的力量


Map of the Tehuacán Valley matorral



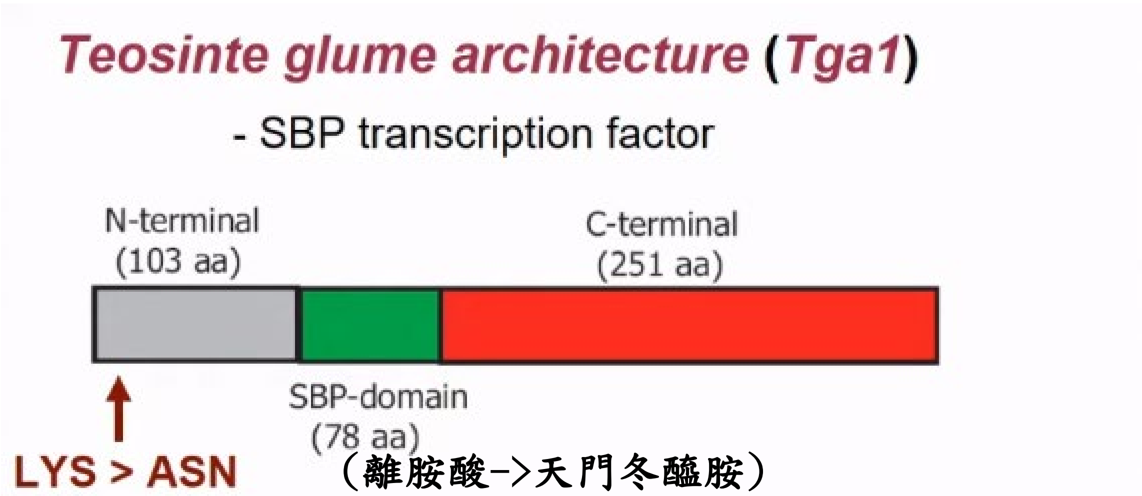
從大芻草到玉米：基因改變了多少

George Beadle (1903-1989)

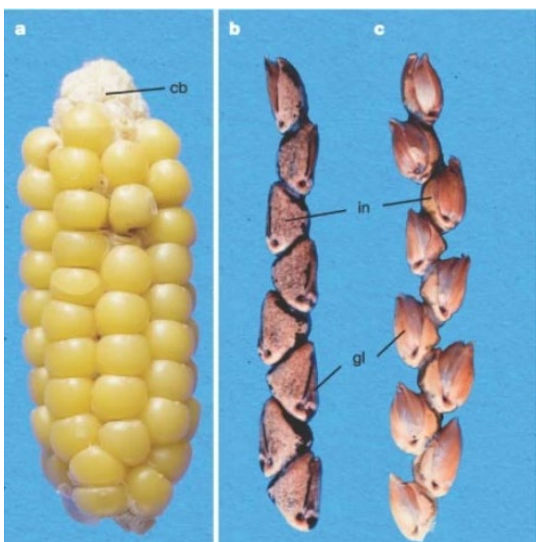


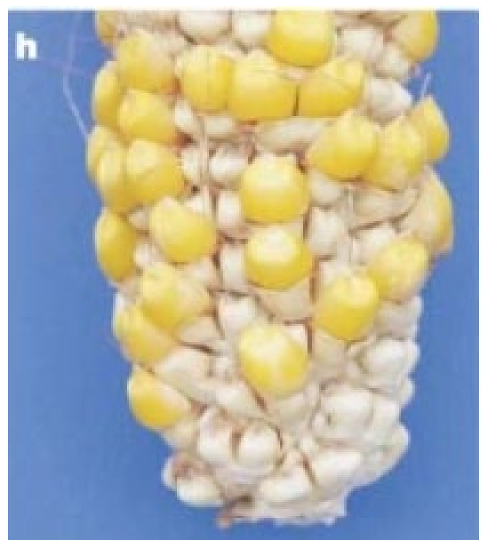
HHMI BioInteractive Video

基因*Tga1* 控制部分種子硬殼的性狀



tga1 *Tga1*



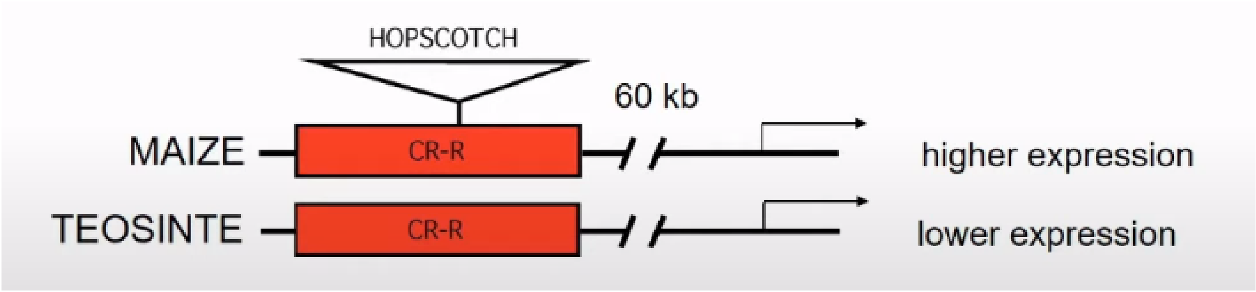


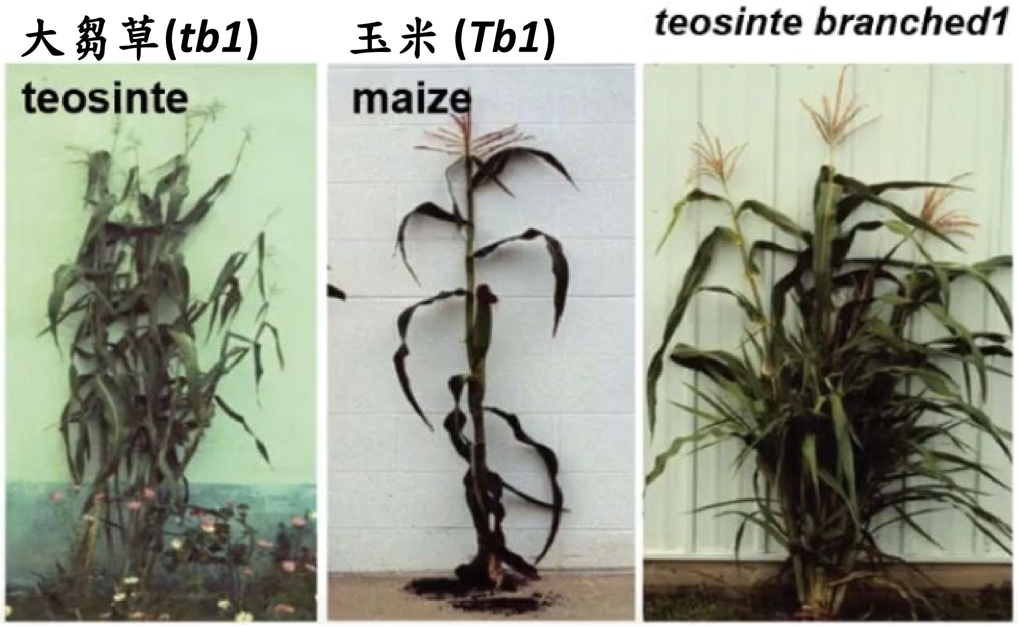
(Doebley's group, 2005, Nature)

基因*Tb1* 控制植株分糵

Teosinte branched1 (tb1)

TCP transcription factor

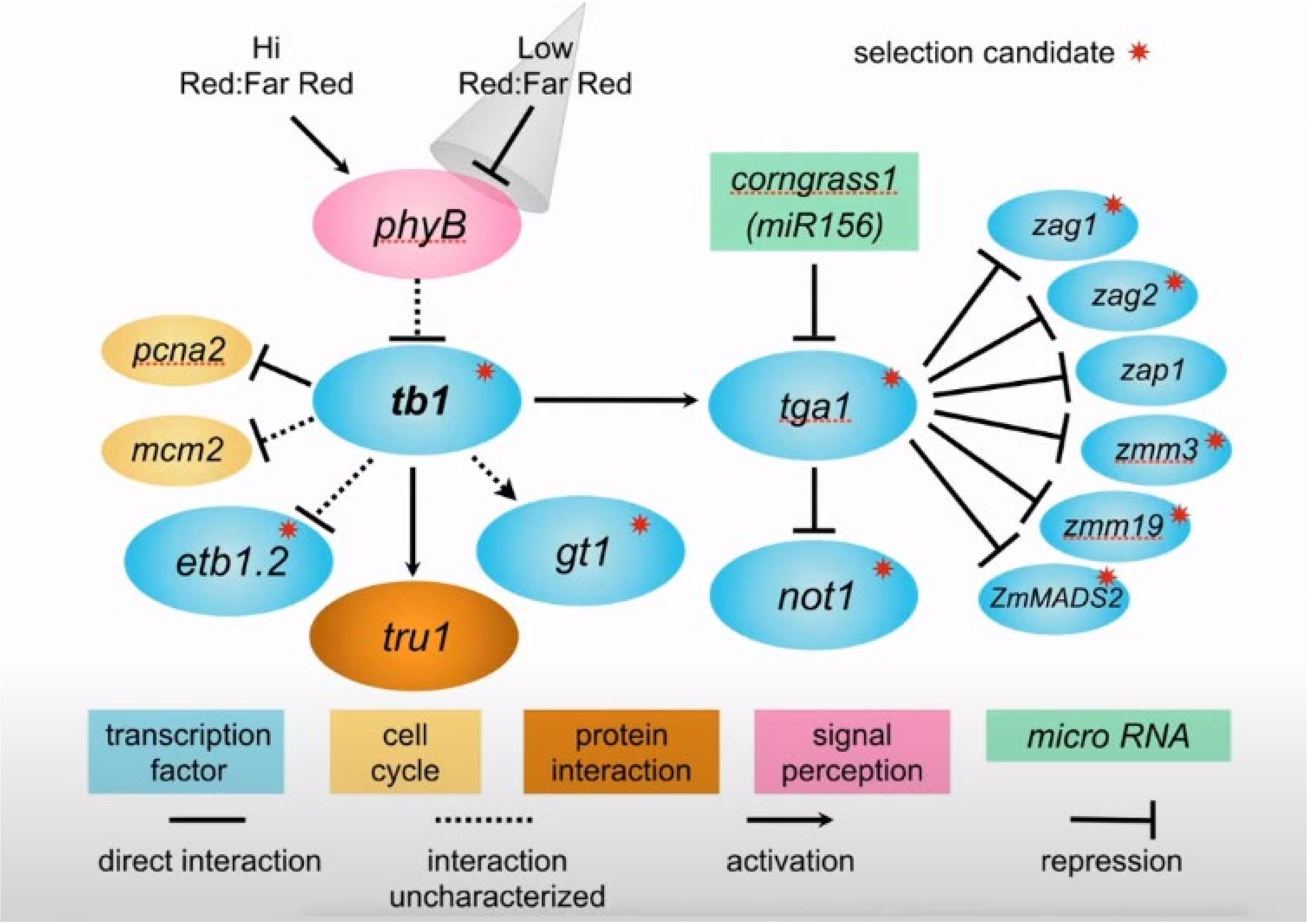




玉米帶有大芻草
tb1 基因版本

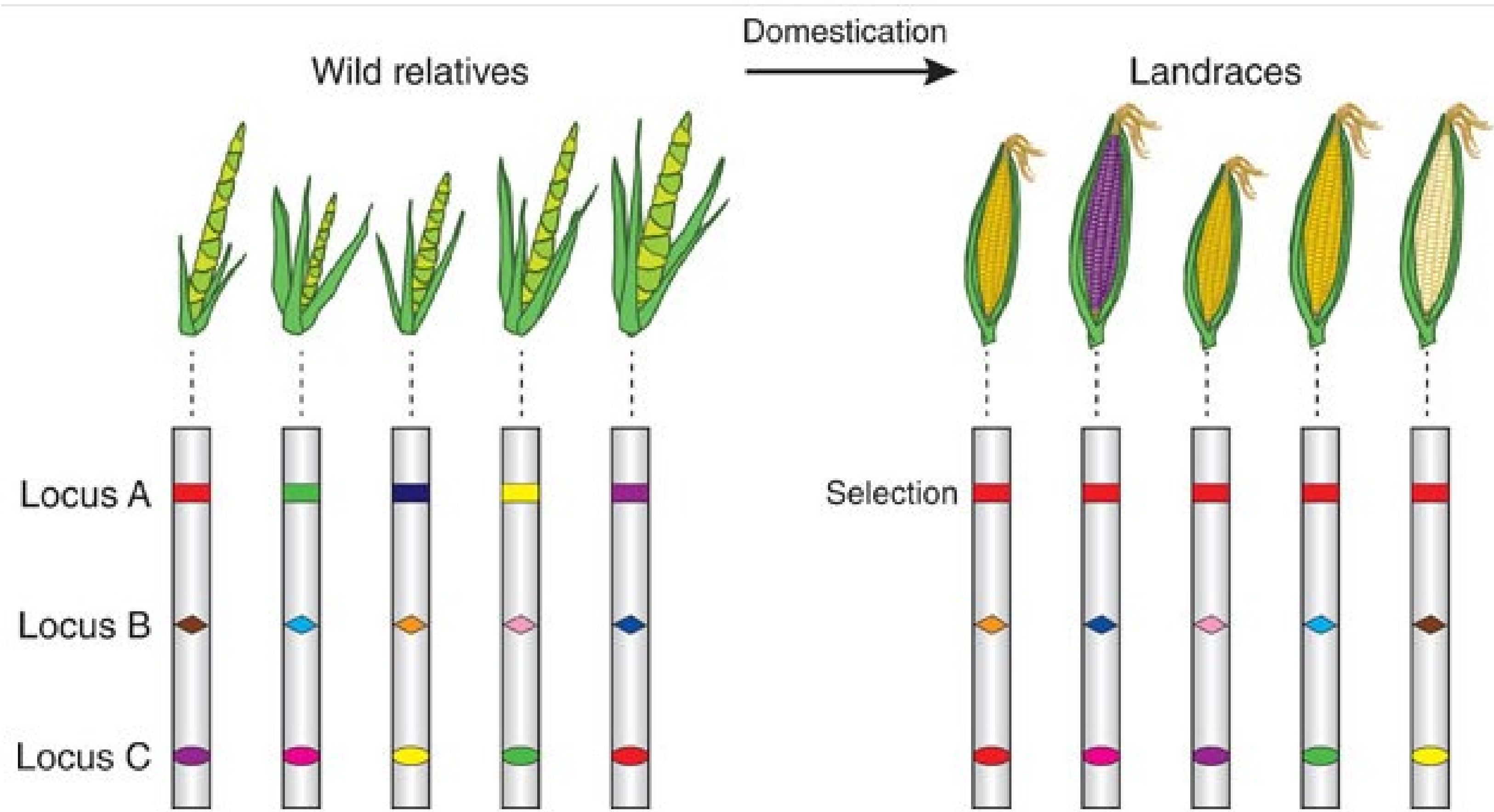
(Doebley et al., 1995. Genetics)

玉米馴化基因調控網路

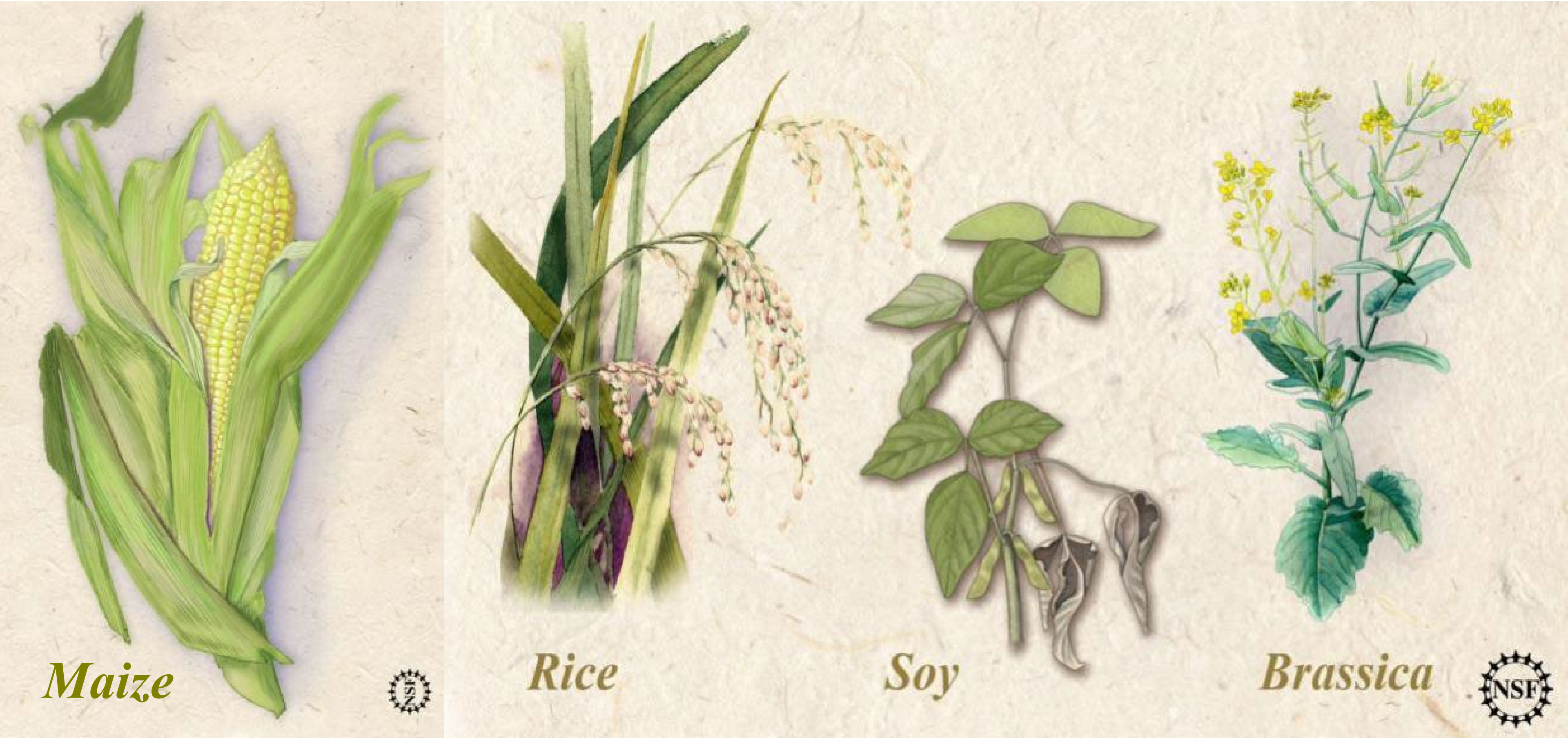


(Trends in Genetics, 2019)

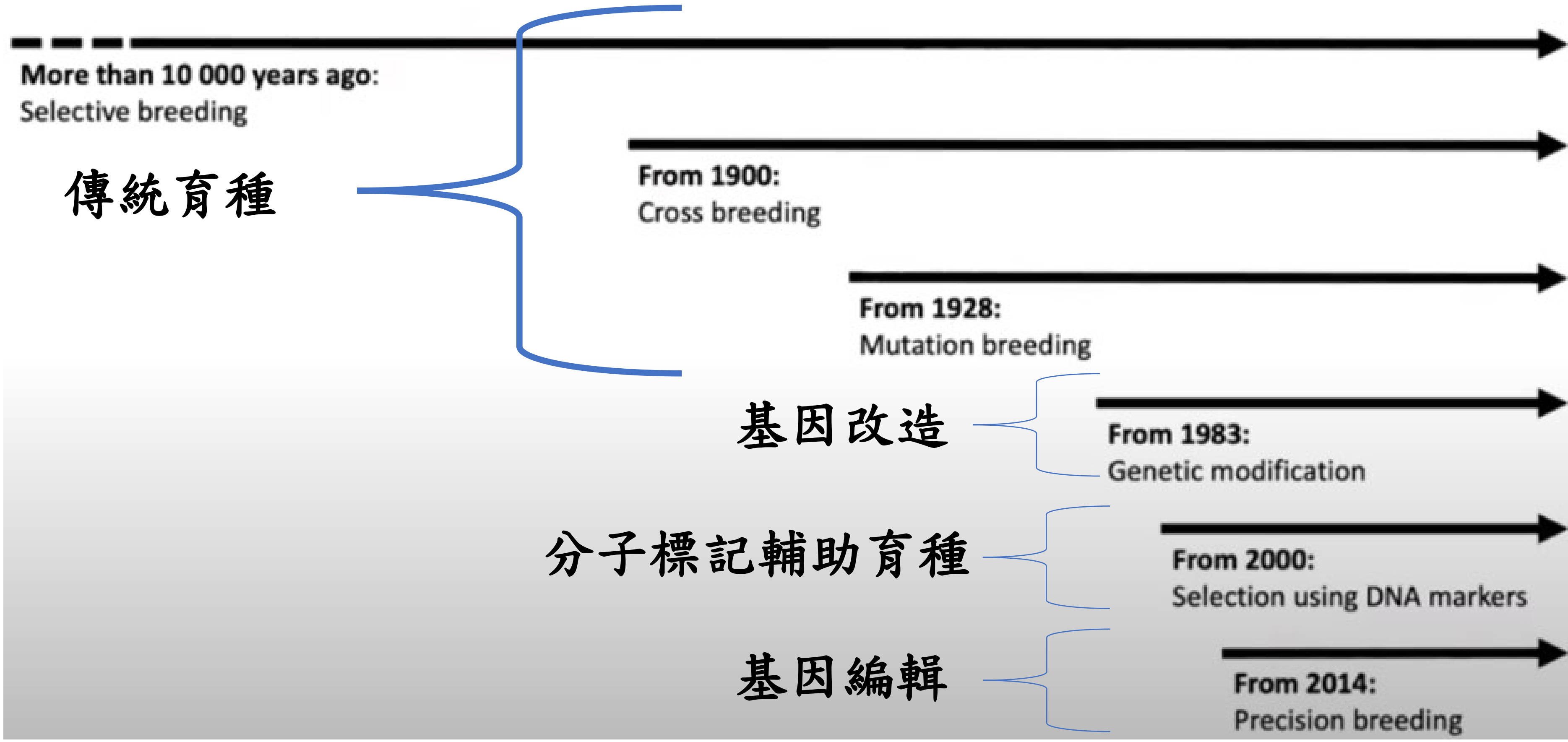
利用天然突變，再人為選拔



作物育種

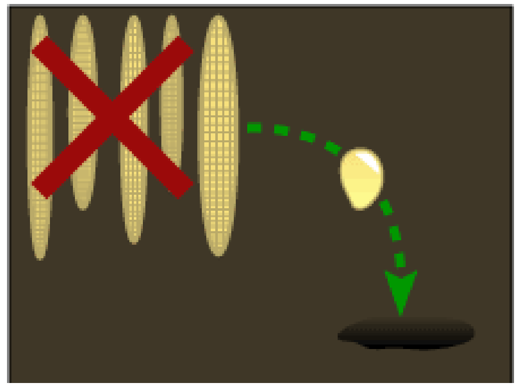


育種的方法隨著人類演化

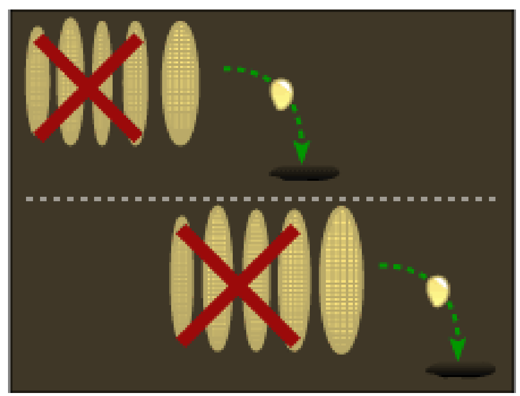


育種/馴化:透過突變，篩選特定性狀

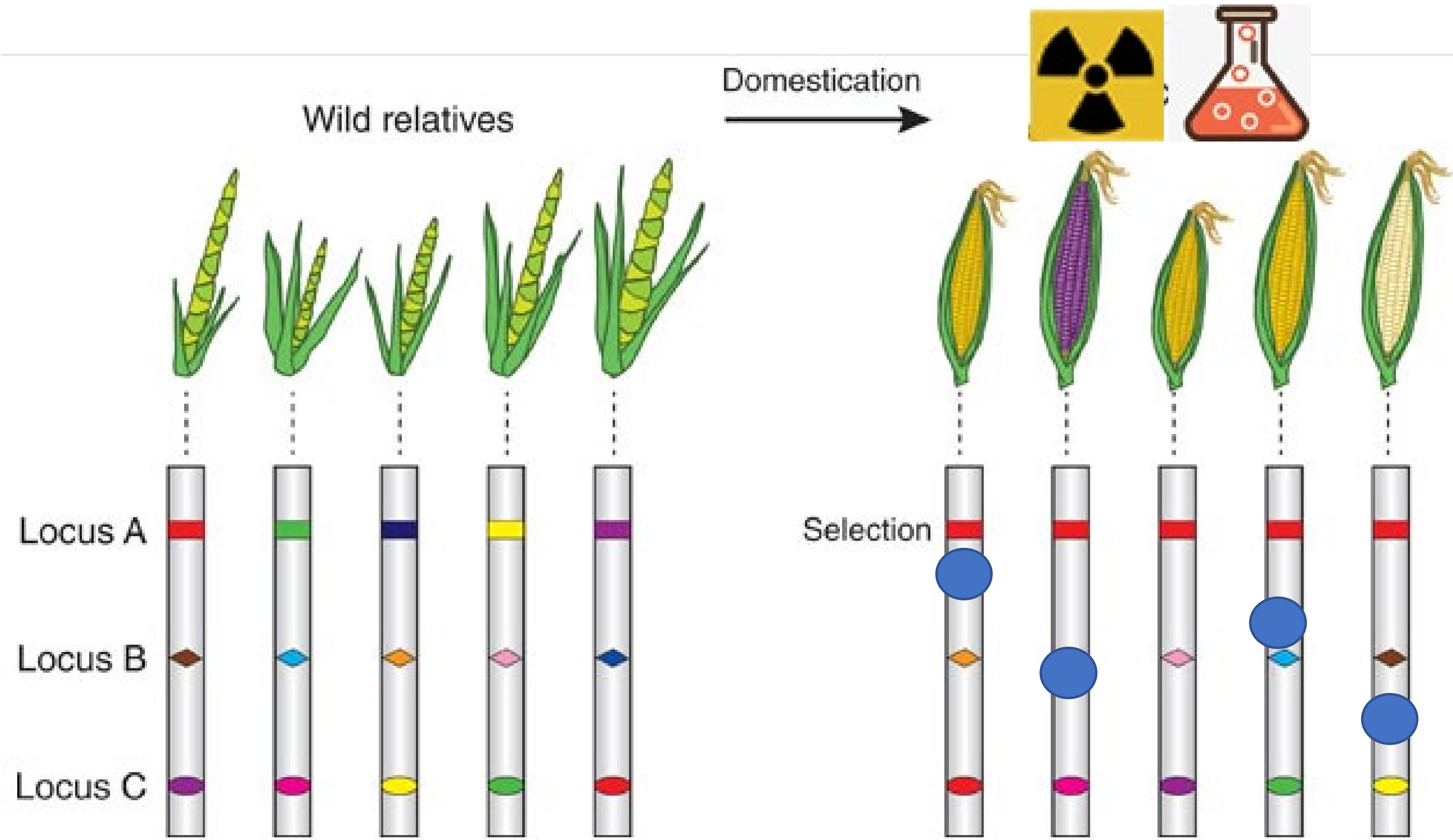
自然突變(多樣性)



種植”好”的植物產生的種子增加”好的基因”在後代中的代表性



人為增加突變機會，產生基因型多樣性



分子標記輔助育種

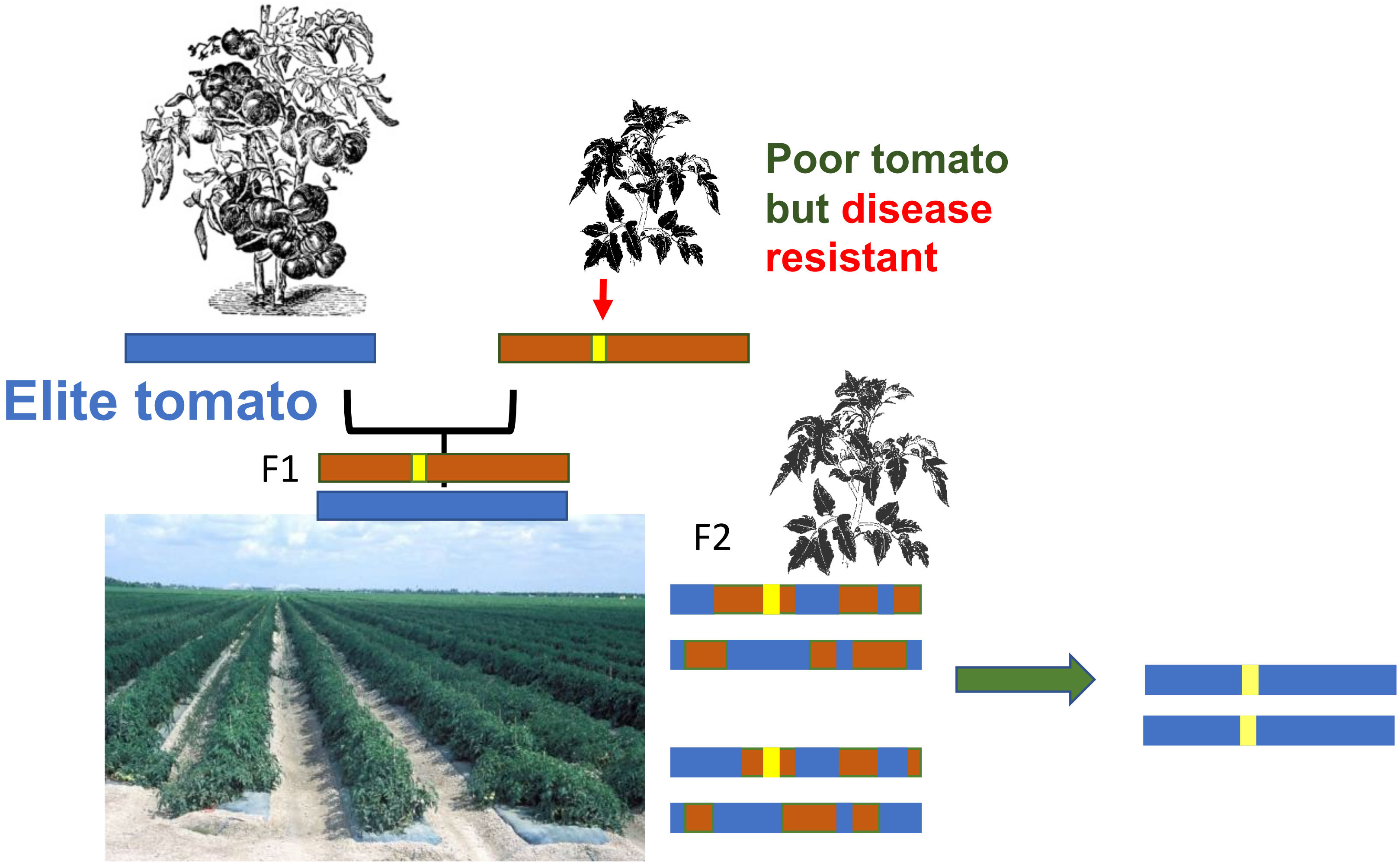
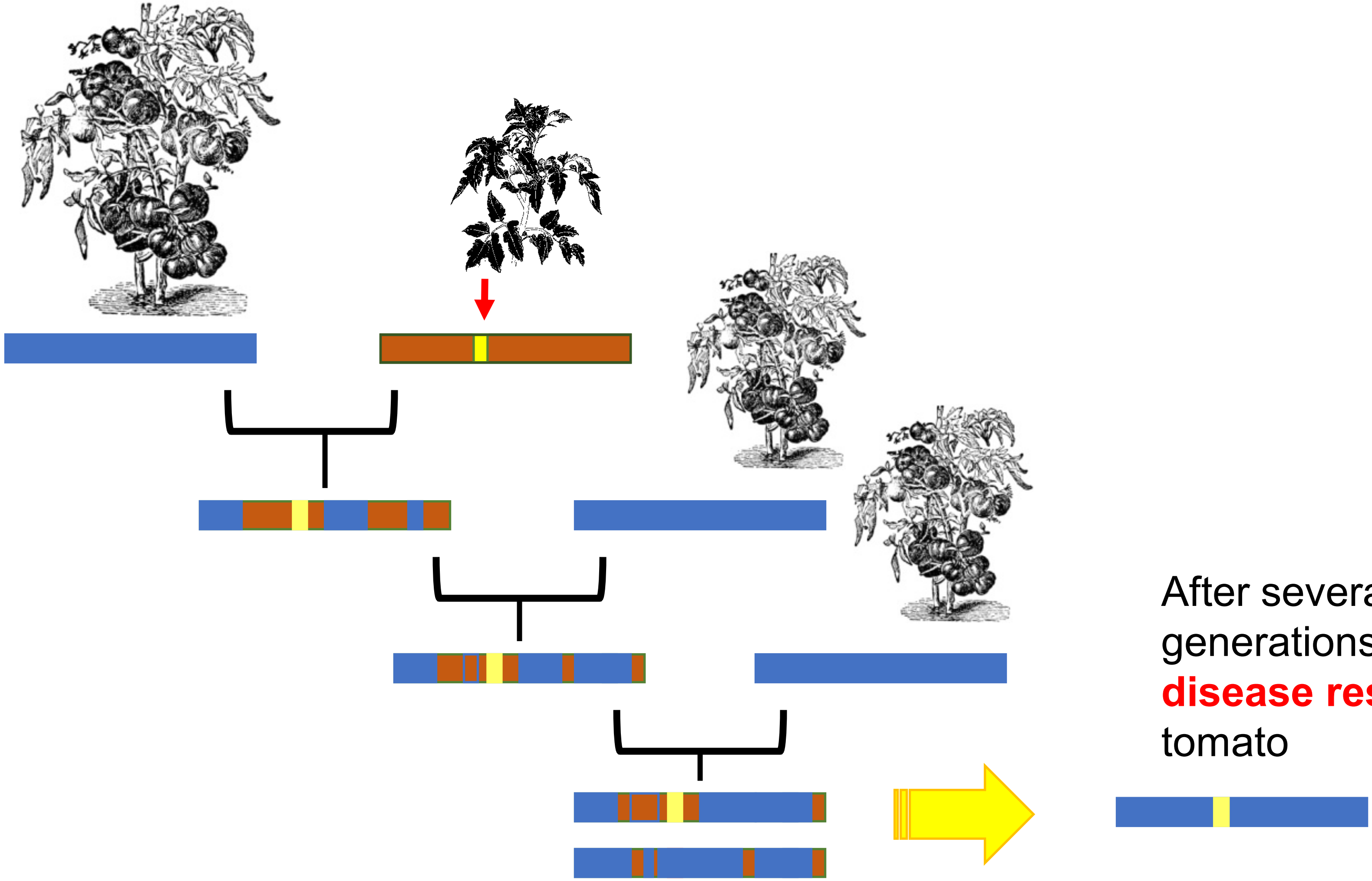


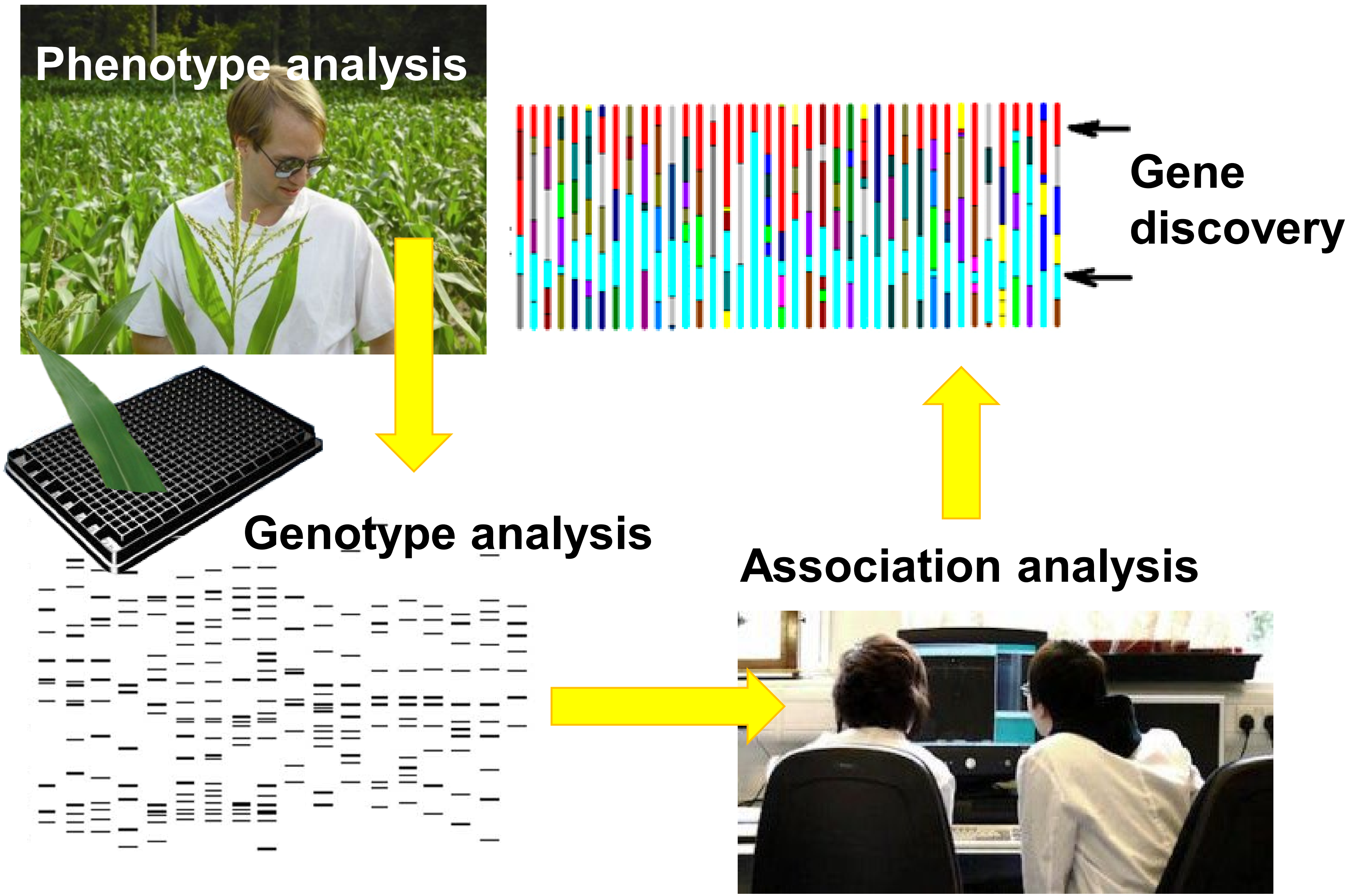
Photo by Stephen Ausmus USDA

分子標記輔助育種

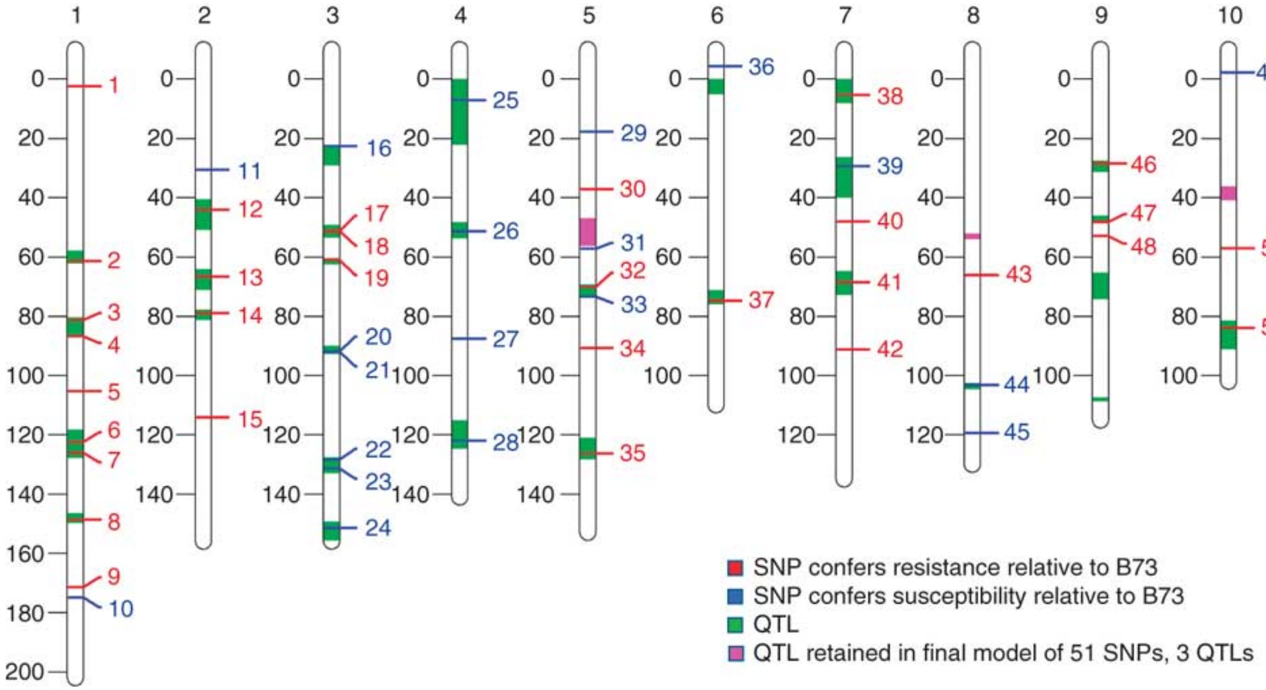


After several generations, **elite**, **disease resistant** tomato

全基因組關聯分析加快育種效率



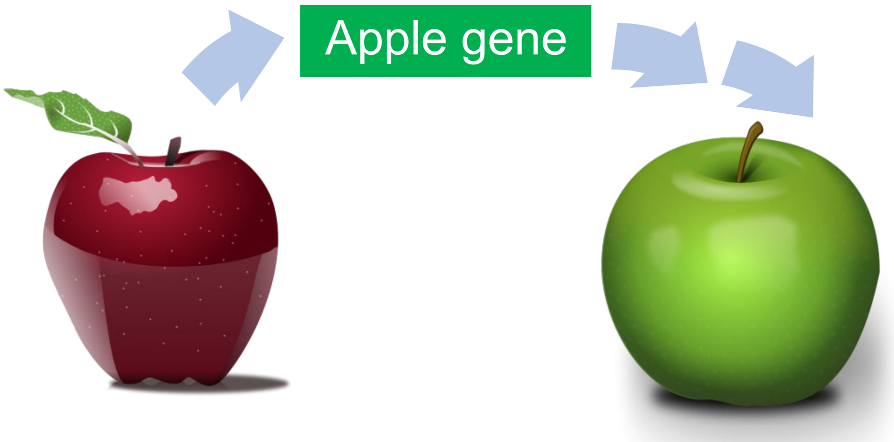
全基因組關聯分析快速找到抗病基因



Similar studies have led to the identification of genes contributing to other agronomically important traits including drought tolerance

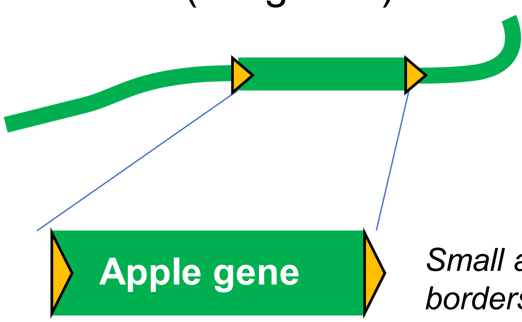
 Kump, K.L., Bradbury, P.J., Wisser, R.J., Buckler, E.S., Belcher, A.R., Oropeza-Rosas, M.A., Zwonitzer, J.C., Kresovich, S., McMullen, M.D., Ware, D., Balint-Kurti, P.J., and Holland, J.B. (2011). Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. Nat Genet 43: 163-168.

基因轉殖：
同源基改 vs 異種基改



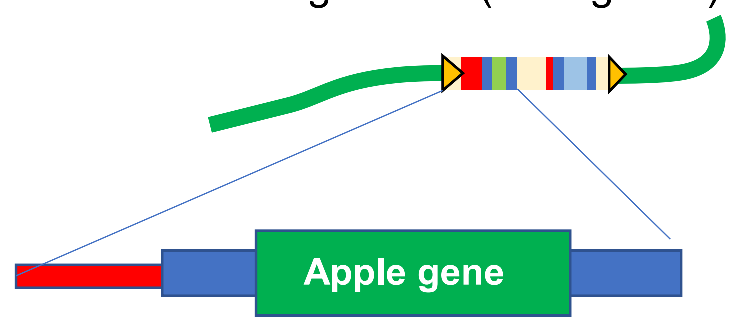
Some traits can be modified by the introduction of a cisgene – a gene from the same or closely-related species

That might mean that little foreign DNA is introduced (“cisgenic”)



Small amounts of DNA from T-DNA borders may be incorporated

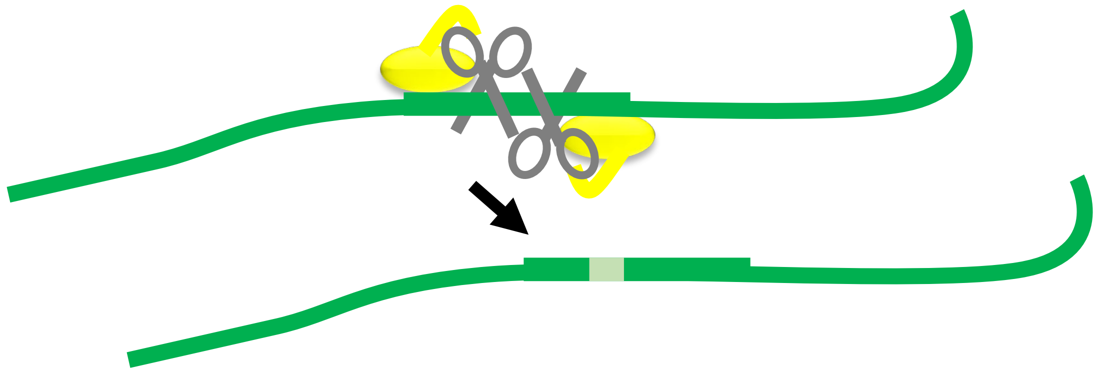
Or, bacterial and viral DNA may be included, but no protein-coding regions from other organisms (“intragenic”)

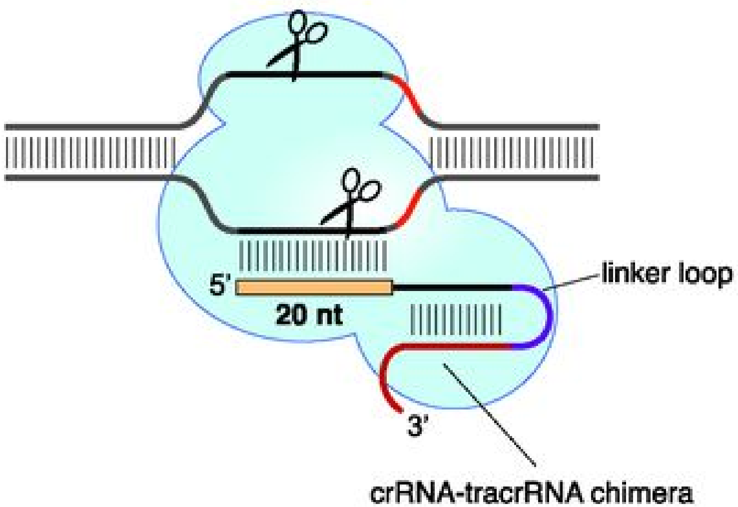


Schouten, H.J., Krens, F.A. and Jacobsen, E. (2006). Cisgenic plants are similar to traditionally bred plants. EMBO Rep. 7: 750-753.

基因編輯

Zinc-finger nucleases (ZFNs) and **transcription activator-like effector nucleases (TALENs)** are proteins that can produce double-strand DNA breaks that when repaired introduce site-specific mutations or insertions

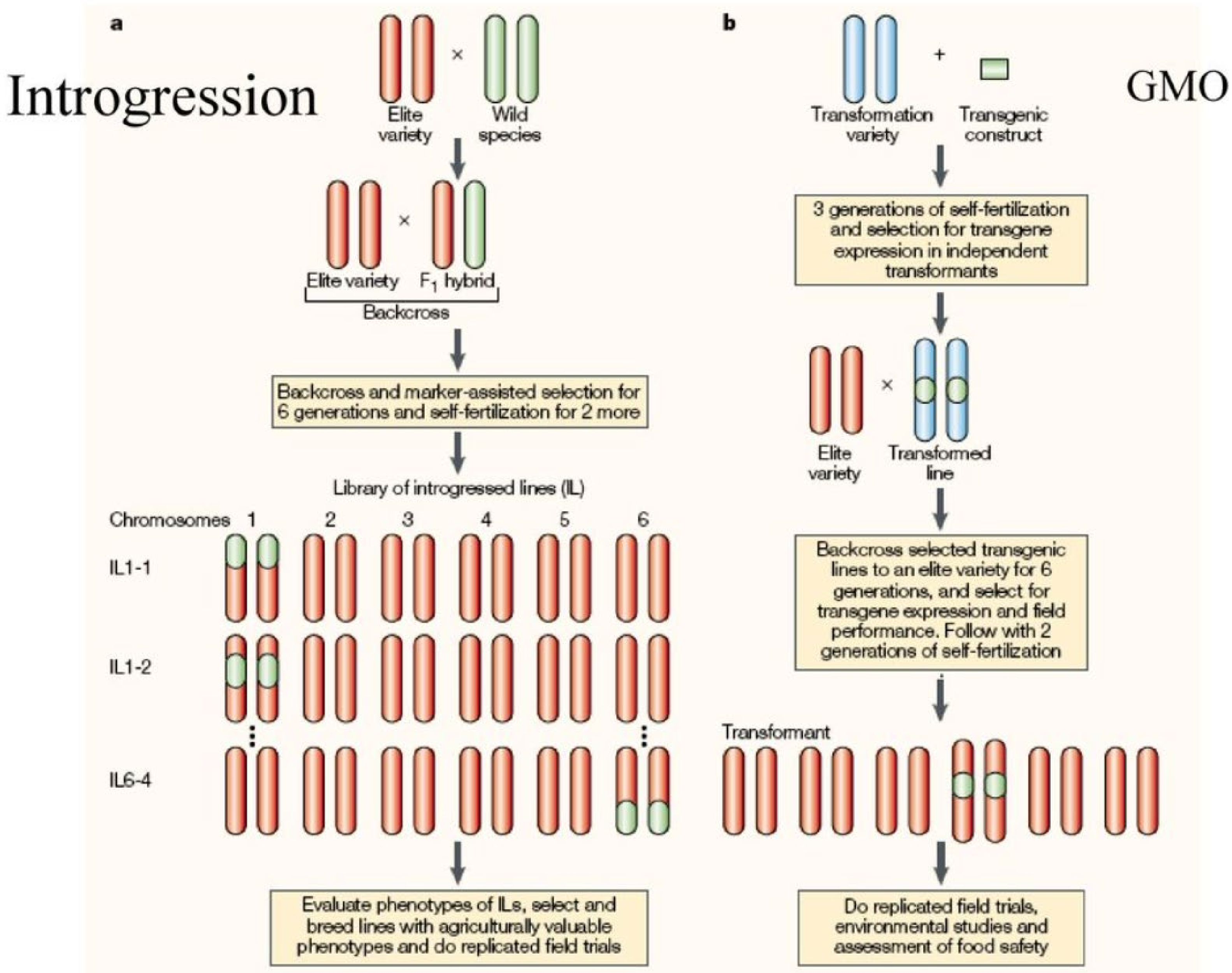




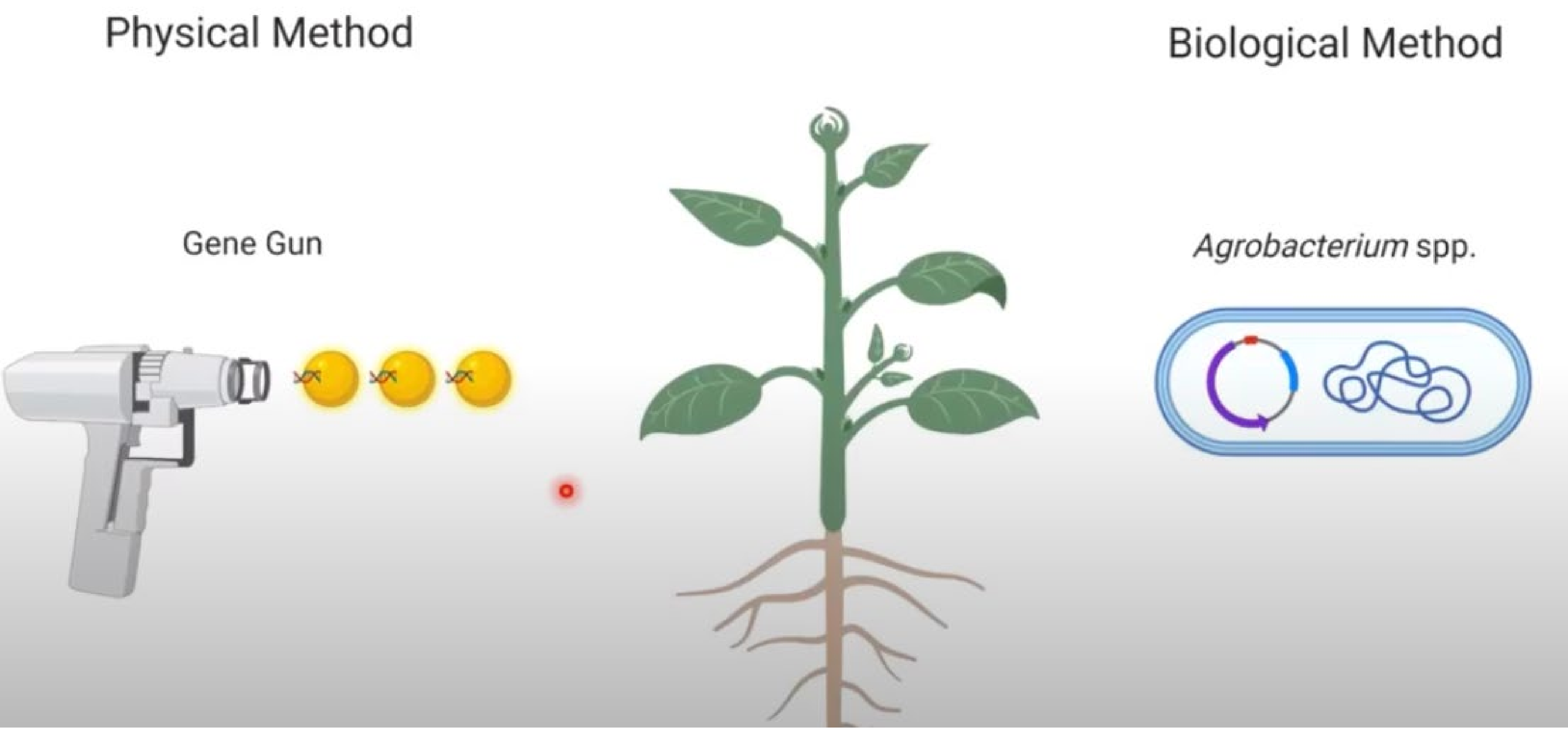
The **clustered regularly interspaced short palindromic repeats (CRISPR) / CRISPR-associated (Cas)** system uses RNAs to target nucleases to specific sites; when repaired, site-specific mutations or insertions are introduced

From Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A. and Charpentier, E. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science. 337: 816-821 with permission from AAAS; see also Bhaya, D., Davison, M. and Barrangou, R. (2011). CRISPR-Cas Systems in Bacteria and Archaea: Versatile small RNAs for adaptive defense and regulation. Annu. Rev. Genet. 45: 273-297.

遺傳導入還是基因轉殖或是基因編輯

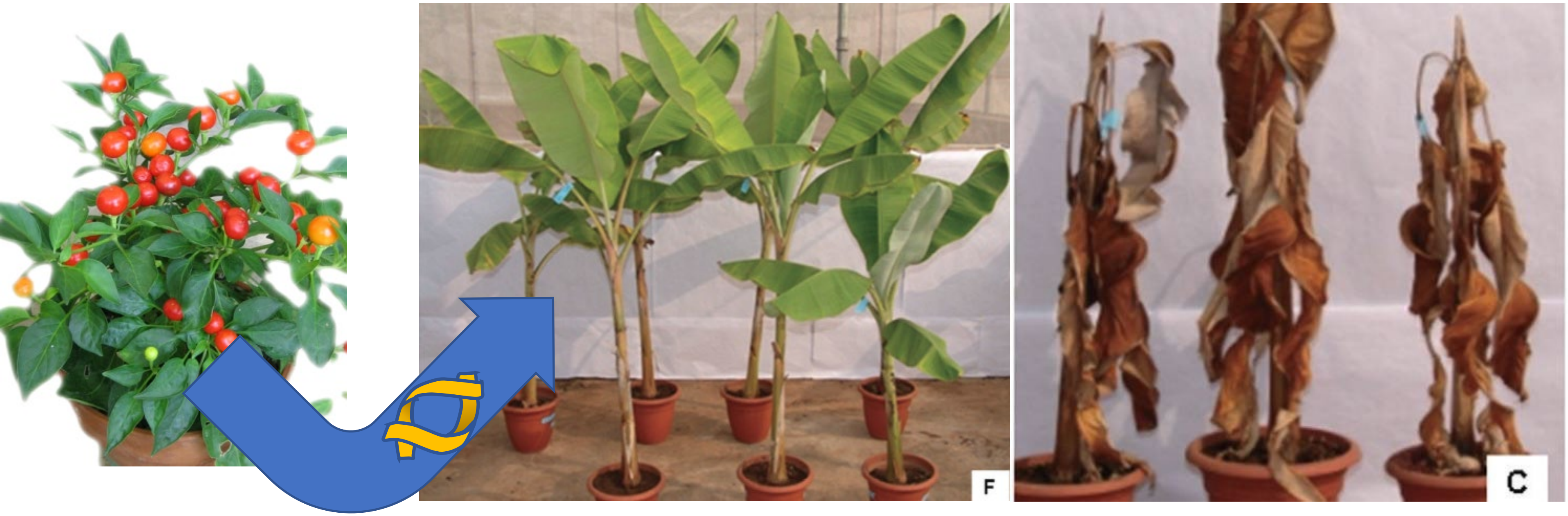


基因轉殖方法



Physical Method
Biological Method
Gene Gun
Agrobacterium spp.

基因轉殖(基改)香蕉



Resistant **Susceptible**

Banana bacterial wilt (*Xanthomonas campestris* pv. *musacearum*) is destroying plants in eastern Africa. Transgenic plants carrying a resistance gene from pepper are resistant to the disease

Tripathi, L., Mwaka, H., Tripathi, J.N., and Tushemereirwe, W.K. (2010). Expression of sweet pepper Hrap gene in banana enhances resistance to Xanthomonas campestris pv. musacearum. Molecular Plant Pathology 11: 721-731.

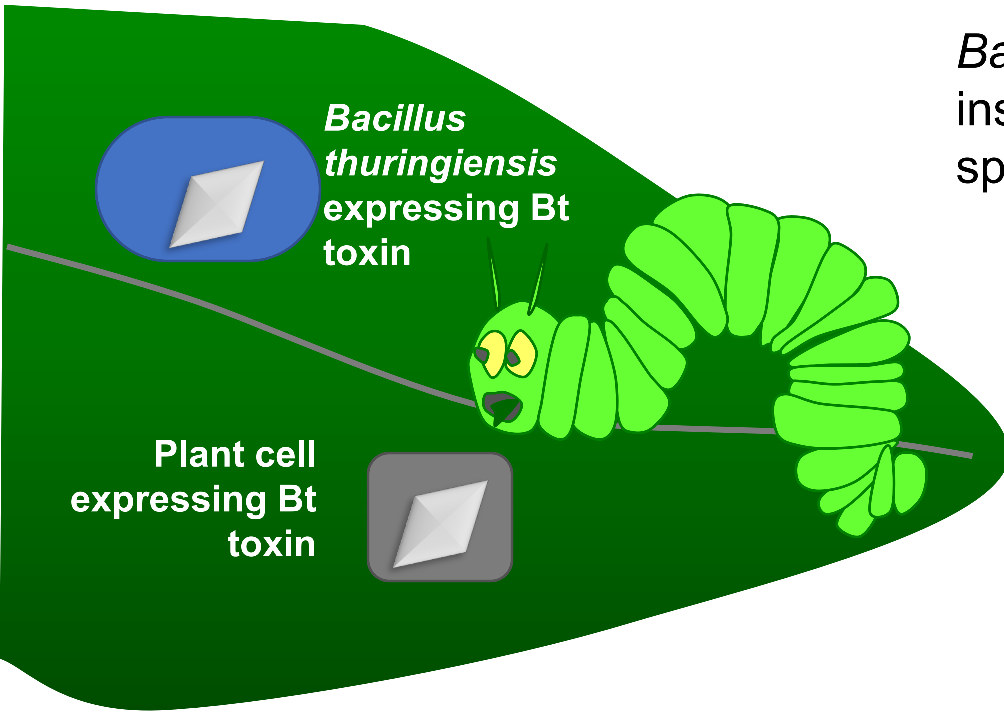
基因轉殖(基改)花生

Wild-type peanut plant

Peanut plant expressing the *Bt* gene

Photo by Herb Pilcher USDA

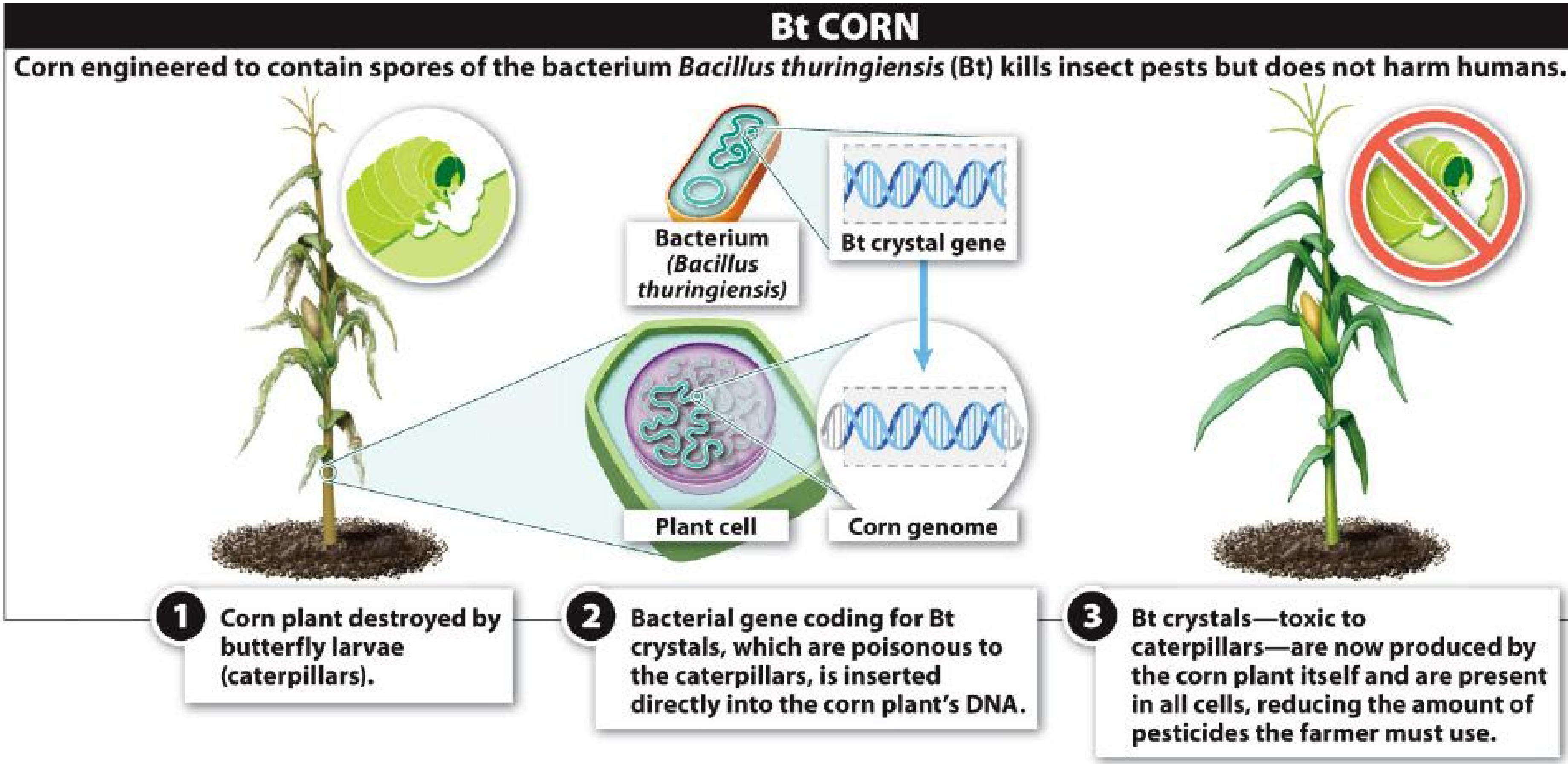
Bacillus thuringiensis (Bt) 細菌的基因對抗毛毛蟲



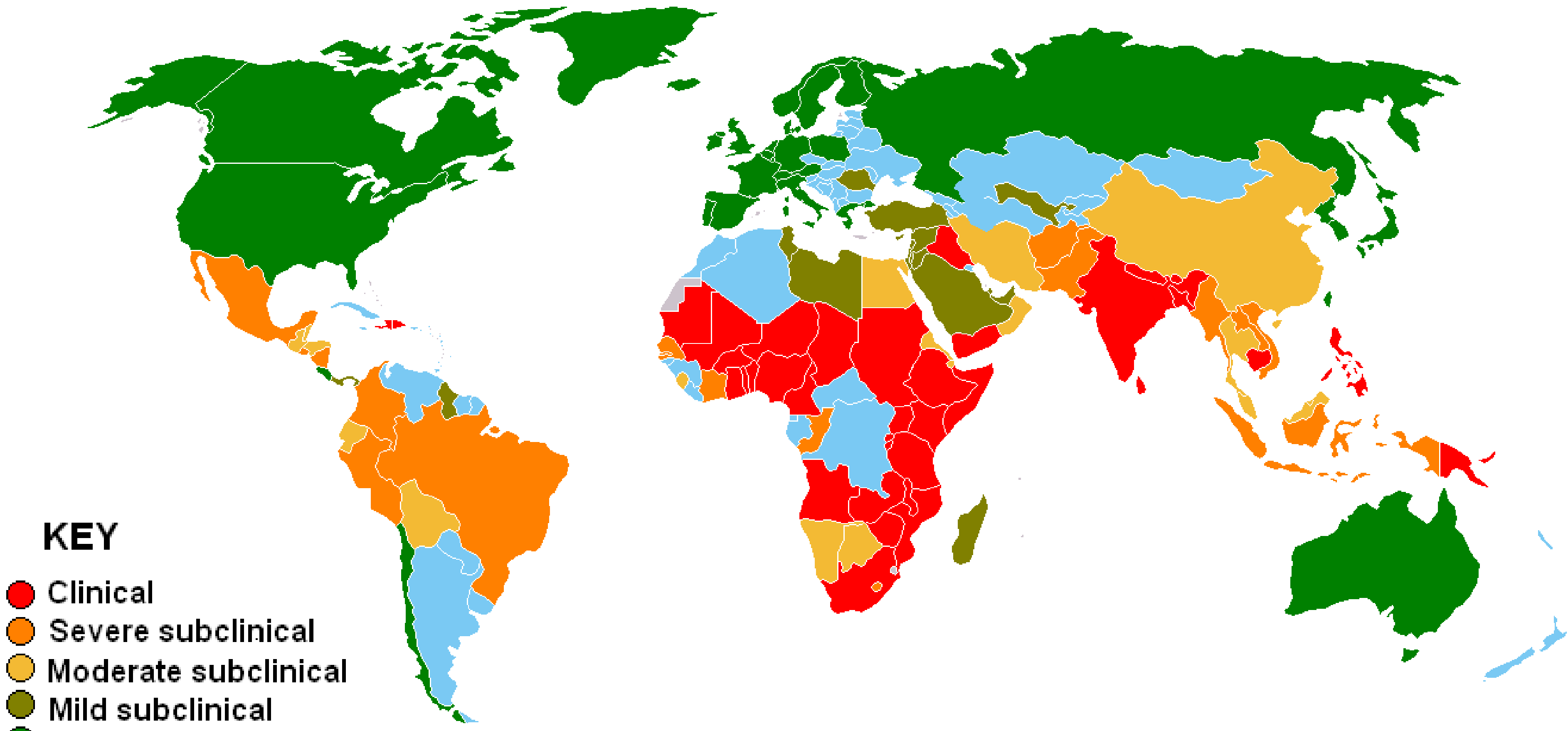
Bacillus thuringiensis expressing insecticidal Bt toxin can be sprayed onto plants

Or the plants can be engineered to express the *Bt* gene coding for Bt toxin

Bacillus thuringiensis (Bt) 細菌的基因對抗毛毛蟲



基改植物對抗維他命A缺乏症

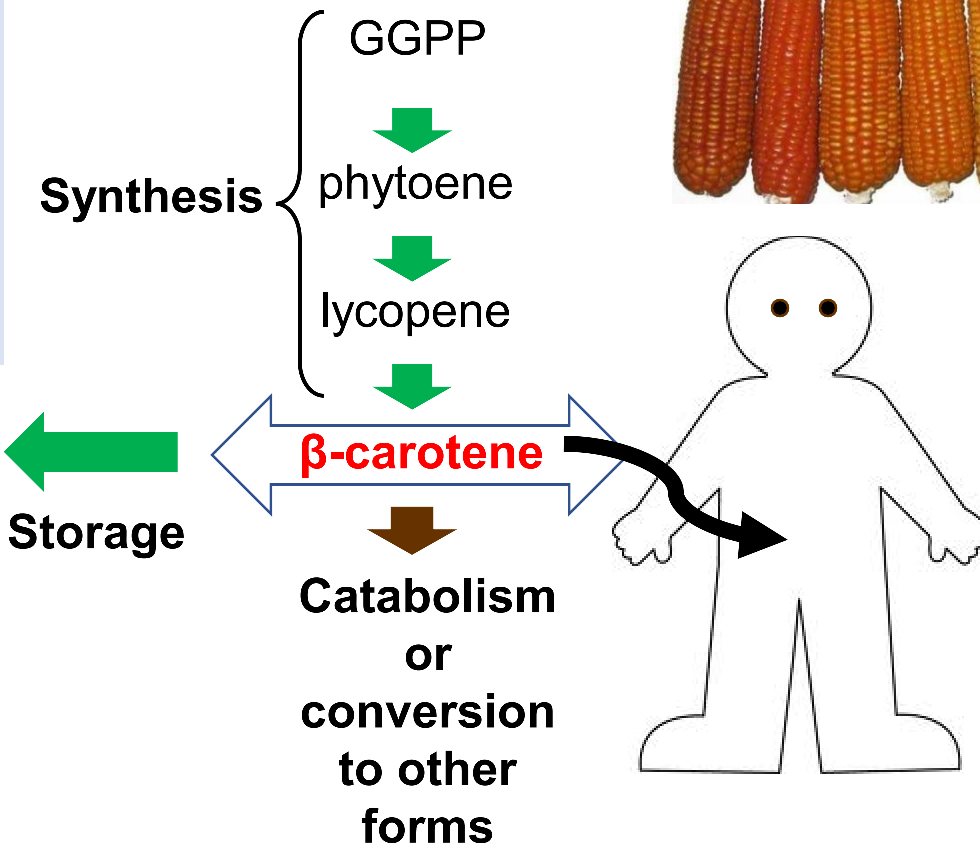


Vitamin A deficiency is a leading cause of blindness

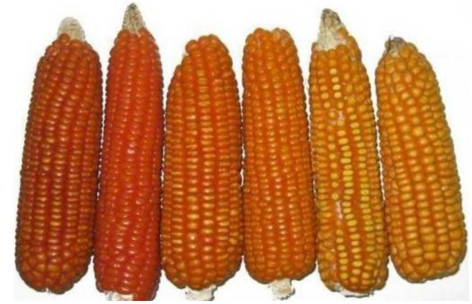
Image sources: Petaholmes based on WHO data;

基改作物產生β-胡蘿蔔素

To increase beta-carotene levels in plants, you need **more synthesis, more storage** or **less catabolism**

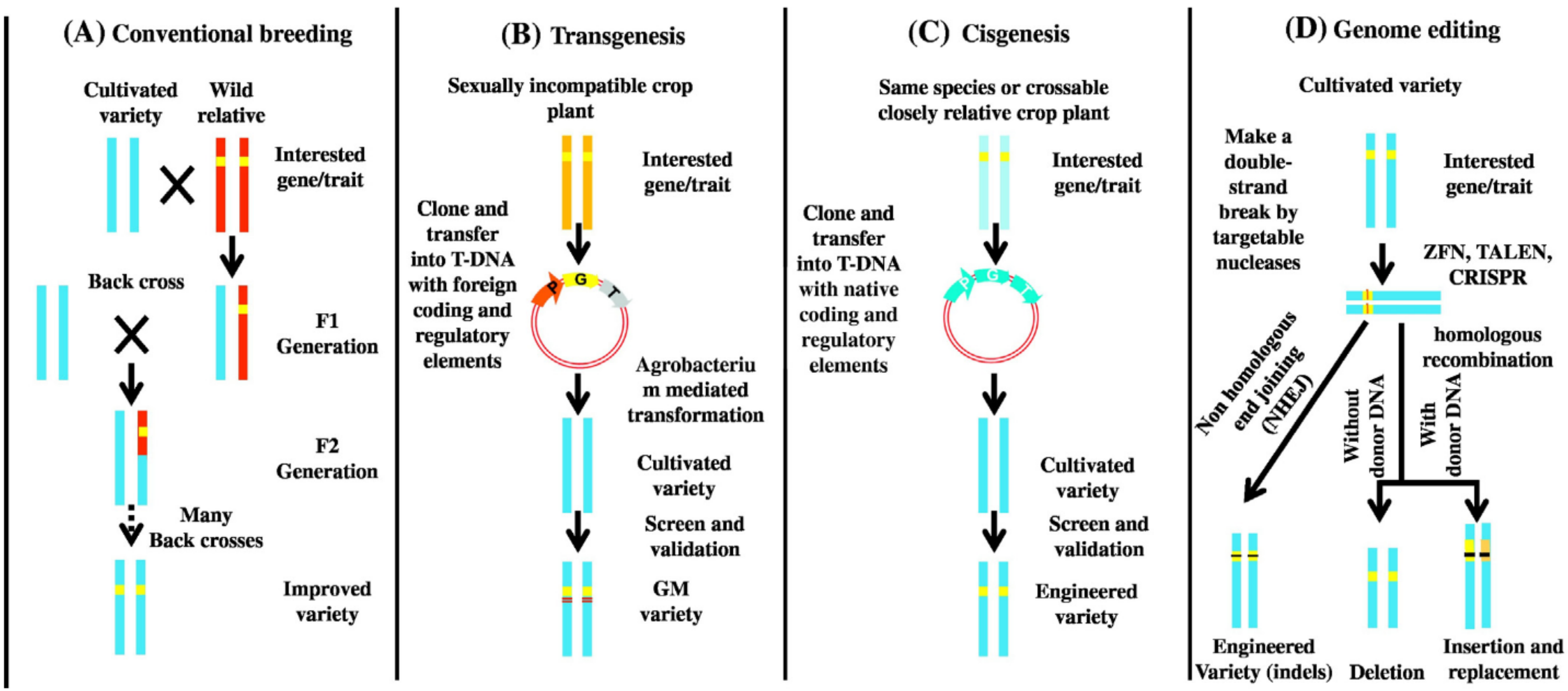


Chromoplasts – organelles that store carotenoids



育種過程都會經過有性生殖

6 R. Li et al. / Biotechnology Advances xxx (2017) xxx–xxx



What risk assessments are performed on GM crops?

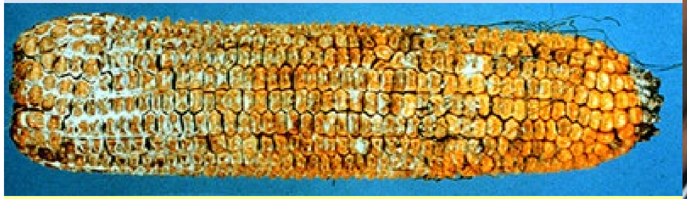
Before release into the environment, GM crops are subject to risk-assessment and risk-management measures to evaluate:

- Risks to human health (including toxicity and allergenicity)
- Risks of evolution of resistance in target pathogens or pests
- Risks to non-target organisms
- Risks from movement of transgenes

Are GM crops safe to eat?

YES

All GM plants are subject to extensive testing and regulatory oversight and no detrimental health effects have been identified



Bt corn is less prone contamination by fungi which produce toxins linked to cancer and birth defects

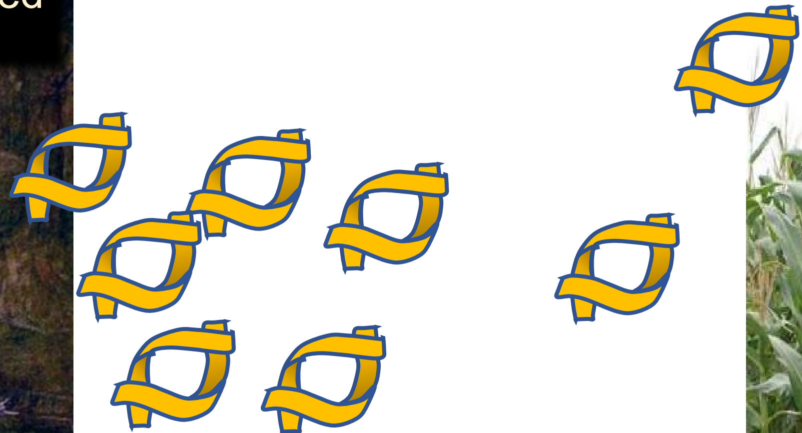
GM biofortification can ensure that *all* children get adequate levels of protein, vitamins and mineral nutrients.

GM is a safe and beneficial tool in the quest to sustainably feed the growing population

Photo credit: Neil Palmer/ CIAT

Will genes from GMOs contaminate wild populations?

John William Waterhouse: Pandora - 1896



Pollen can move DNA between plants. To minimize this possibility, GM crops have to be grown prescribed distances away from closely related plants. Technological methods to reduce this risk are being developed.

Will anti-insecticidal genes harm unintended targets?

The evidence shows that the planting of GE crops has largely resulted in less adverse or equivalent effects on the farm environment compared with the conventional non-GE systems that GE crops replaced (National Academies 2010)

Image credit jons2

Scientists worldwide endorse GM as an important tool for breeding

"Both genetic improvement and better crop management are vital and both should be resourced in parallel." - 2009

American Society of Plant Biologists

Cultivating a better future through plant biology research

"The ASPB believes strongly that, with continued responsible regulation and oversight, GE will bring many significant health and environmental benefits to the world and its people." - 2006