BIOTECHNOLOGY
Academic year: 2025/26

Literature:

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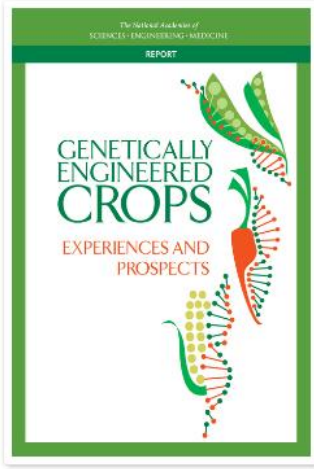
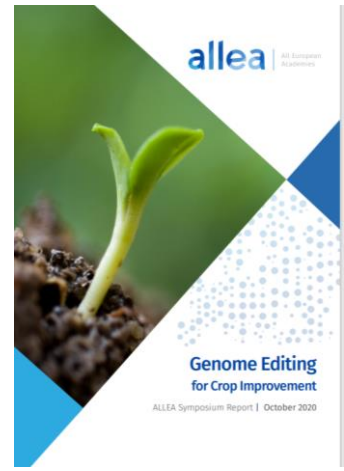
https://www.nap.edu/read/23395/chapter/1





https://allea.org/wp-content/uploads/2020/10/ALLEA_Gen_Editing_Crop_2020.pdf

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Plant	Beneficial trait	Genome-editing technique	Research study
Traits related to improved food/feed quality			
Alfalfa	Reduced lignin content	TALEN	APHIS* database[47]
Canola	Improved fatty acid composition	CRISPR-Cas	Okuzaki *et al.*, 2018[82]
Peanut	Improved fatty acid content	TALEN	Wen *et al.*, 2018[83]
Rice	Increased amylose content	CRISPR-Cas	Sun *et al.*, 2017[84]
Tomato	Increased lycopene content	CRISPR-Cas	Li *et al.*, 2018[85]
Wheat	Increased fibre content	TALEN	APHIS* database[47]
Wheat	Reduced gluten content	CRISPR-Cas	Sánchez-León *et al.*, 2017[88]
Soybean	Improved oil quality	TALEN	Haun *et al.*, 2014[87] Demorest *et al.*, 2016[88] APHIS* database[47]
Sage	Reduced phenolic acid content	CRISPR-Cas	Zhou *et al.*, 2018[89]
Maize	Improved starch production	CRISPR-Cas	APHIS* database[47]
Lettuce	Increased vitamin C content	CRISPR-Cas	Zhang *et al.*, 2018[90]
Traits related to reduced crop losses, pesticide use or water consumption			
Cacao	Resistance to Phytophthora tropicalis	CRISPR-Cas	Fister *et al.*, 2018[91]
Cucumber	Broad resistance to viruses	CRISPR-Cas	Chandrasekaran *et al.*, 2016[92]
Grapefruit	Resistance to citrus canker	CRISPR-Cas	Jia *et al.*, 2015[93] Jia *et al.*, 2017[94]
Orange	Resistance to citrus canker	CRISPR-Cas	Peng *et al.*, 2017[95]
Grapevine	Resistance to Botrytis cinerea	CRISPR-Cas	Wang *et al.*, 2018[96]
Tomato	Broad resistance to bacterial infections	CRISPR-Cas	de Toledo Thomazella *et al.*, 2016[97]
Wheat	Resistance to powdery mildew	TALEN/CRISPR-Cas	Wang *et al.*, 2014[79] Zhang *et al.*, 2017[98] APHIS* database[47]
Soybean	Drought and salt tolerance	CRISPR-Cas	APHIS* database[47]
Maize	Drought tolerance	CRISPR-Cas	Njuguna *et al.*, 2017[99]
Potato	Resistance to Potato Virus Y (PVY)	CRISPR-Cas	Zhan *et al.*, 2019[100]
Traits related to agronomic importance			
Rice	Increased seed weight	CRISPR-Cas	Li *et al.*, 2016[101]
Canola	Increased shatter resistance and seeds number per husk	CRISPR-Cas	Braatz *et al.*, 2017[102] Yang *et al.*, 2018[103]
Lettuce	Germination at high temperature	CRISPR-Cas	Bertier *et al.*, 2018[104]
Wheat	Increased grain weight	CRISPR-Cas	Wang *et al.*, 2018[105]
Potato	Improved cold storage and processing traits	TALEN	Clasen *et al.*, 2015[80]
Tomato	Increased fruit size	CRISPR-Cas	Rodríguez-Leal *et al.*, 2017[106]

Genome Editing for Crop Improvement
ALLEA Symposium Report | October 2020
https://allea.org/wp-content/uploads/2020/10/ALLEA_Gen_Editing_Crop_2020.pdf

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Adopted: 19 June 2024
DOI: 10.2903/j.efsa.2024.8894

SCIENTIFIC OPINION

efsa JOURNAL

Scientific opinion on the ANSES analysis of Annex I of the EC proposal COM (2023) 411 (EFSA-Q-2024-00178)

EFSA Panel on Genetically Modified Organisms (GMO) | Ewen Mullins | Jean-Louis Bresson | Tamas Dalmay | Ian Crawford Dewhurst | Michelle M. Epstein | Leslie George Firbank | Philippe Guerche | Jan Hejatko | Francisco Javier Moreno | Hanspeter Naegeli | Fabien Nogué | Nils Rostoks | Jose Juan Sanchez Serrano | Giovanni Savoini | Eve Veromann | Fabio Veronesi | Josep Casacuberta | Ana Afonso | Paolo Lenzi | Nikoletta Papadopoulou | Tommaso Raffaello

Correspondence: nif@efsa.europa.eu

Abstract
EFSA was asked by the European Parliament to provide a scientific opinion on the analysis by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) of Annex I of the European Commission proposal for a regulation 'on plants obtained by certain new genomic techniques (NGTs) and their food and feed, and amending regulation (EU) 2017/625'. The Panel on genetically modified organisms (GMO) assessed the opinion published by ANSES, which focuses on (i) the need to clarify the definitions and scope, (ii) the scientific basis for the equivalence criteria and (iii) the need to take potential risks from category 1 NGT plants into account. The EFSA GMO Panel considered the ANSES analysis and comments on various terms used in the criteria in Annex I of the European Commission proposal and discussed definitions based on previous EFSA GMO Panel opinions. The EFSA GMO Panel concluded that the available scientific literature shows that plants containing the types and numbers of genetic modifications used as criteria to identify category 1 NGT plants in the European Commission proposal do exist as the result of spontaneous mutations or random mutagenesis. Therefore, it is scientifically justified to consider category 1 NGT plants as equivalent to conventionally bred plants with respect to the similarity of genetic modifications and the similarity of potential risks. The EFSA GMO Panel did not identify any additional hazards and risks associated with the use of NGTs compared to conventional breeding techniques in its previous Opinions.

KEYWORDS
genetically modified plants, genome editing, new genomic techniques, risk assessment

https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2024.8894

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Assessment:

- Seminar paper and presentation 10 %
- Colloquium 20 %
- Exam 70 %

Attendance at laboratory exercises is mandatory. The laboratory notebook must be submitted before taking the colloquium.

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What is biotechnology/ plant biotechnology?

The term Biotechnology was coined by Karl Ereky, a Hungarian engineer in 1919. The origin of biotechnology can be traced back to prehistoric times when microorganisms were already used for processes like fermentation, formation of yoghurt and cheese from milk,…

However, biotechnology got a boost in the 1970's with the discovery of restriction enzymes which led to the development of a variety of gene technologies and is thus considered to be the greatest scientific revolution of this century.

Some definitions:
The application of science and engineering in the direct or indirect use of living organisms, or parts or products of living organisms, in their natural or modified form.

The Convention on Biological Diversity defined biotechnology as "any technology application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use."

"Plant biotechnology describes a precise process in which scientific techniques are used to develop useful and beneficial plants'' (According to the Council for Biotechnology Information).

MODERN BIOTECHNOLOGY RELIES ON ADVANCES IN MOLECULAR BIOLOGY AND COMPUTER TECHNOLOGY
We think of biotechnology as modern because of recent advances in molecular biology and genetic engineering

Plant biotechnology

- Plant tissue cultures
- Genetic engineering

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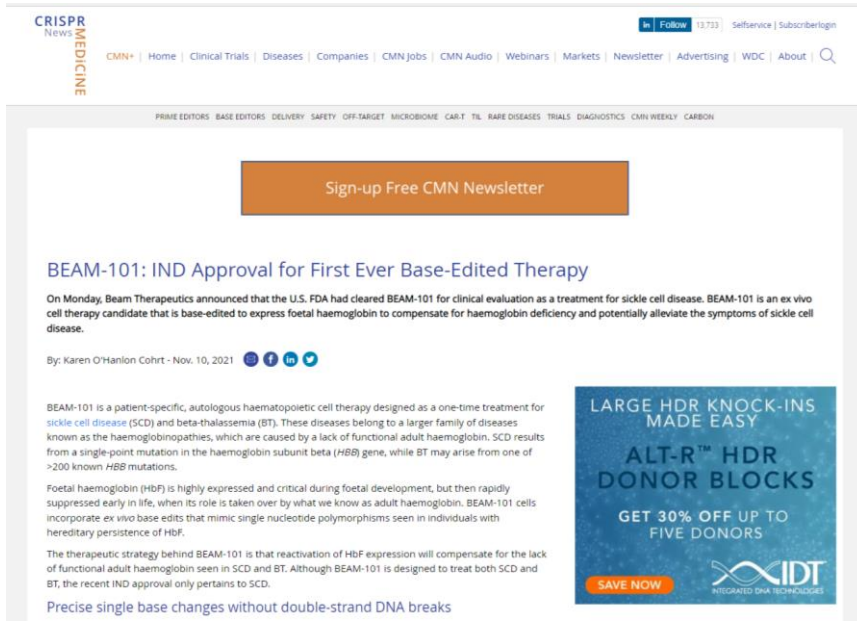

News: BEAM-101: IND Approval for First Ever Base-Edited Therapy - CRISPR Medicine (crisprmedicinenews.com)

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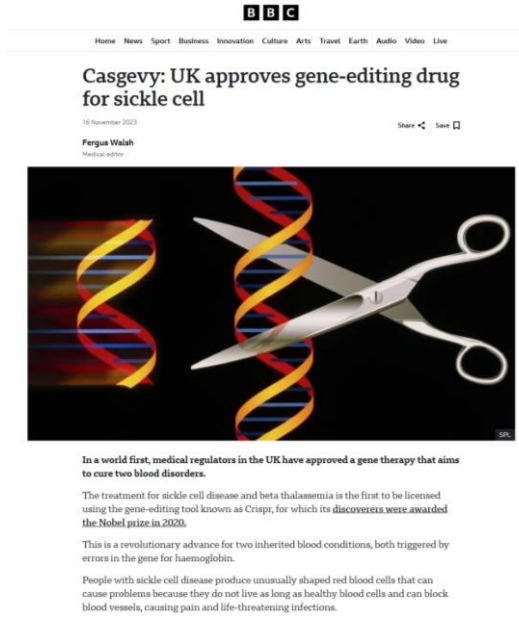

https://www.bbc.com/news/health-67435266

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Syllabus:

History of biotechnology

Recombinant DNA techniques

Genome editing methods (site-specific endonucleases CRISPR-Cas, TALEN, ZFN, meganucleases, oligonucleotide directed mutagenesis based on homology recombination process)

Methods for indirect / direct transfer of DNA / ribonucleoprotein complex in the plant or animal cell (transgenic plants, genome edited plants / animals)

Examples of plant traits, developed with genetic engineering (e.g. resistance to herbicides, viruses and fungal diseases, frost, drought, improvement of food quality,…)

Gene therapy

Legislation on the field of biotechnology

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Science considers that breeding of new varieties has contributed most to the dramatic increase in yield from the same cultivated area, which is at least five times that of a century ago.

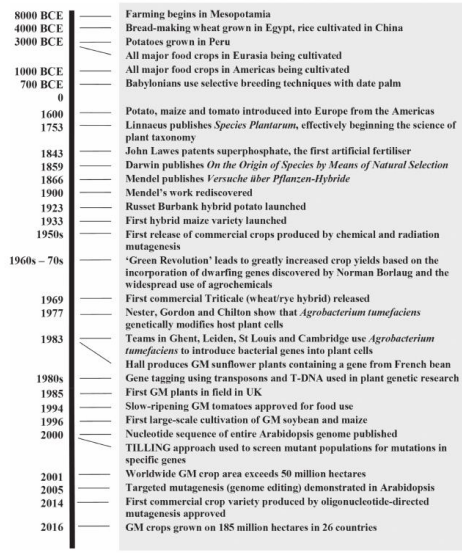

Figure 1.4. Timeline: 10,000 years of farming and plant breeding.

Halford G. N. 2018. Crop Biotechnology: Genetic Modification and Genome Editing

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Journal List › Proc Natl Acad Sci U S A › v.102(6); 2005 Feb 8 › PMC548540

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PNAS
Proceedings of the National Academy of Sciences of the United States of America

Proc Natl Acad Sci U S A. 2005 Feb 8; 102(6): 2232–2237.
Published online 2005 Jan 26. doi: 10.1073/pnas.0409339102
Plant Biology

PMCID: PMC548540
PMID: 15677315

Targeted mutagenesis using zinc-finger nucleases in *Arabidopsis*

Alan Lloyd,* Christopher L. Plaisier,* Dana Carroll,† and Gary N. Drews*‡

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ABSTRACT

Go to:

Targeted mutagenesis is an essential tool of reverse genetics that could be used experimentally to investigate basic plant biology or modify crop plants for improvement of important agricultural traits. Although targeted mutagenesis is routine in several model organisms including yeast and mouse, efficient and widely usable methods to generate targeted modifications in plant genes are not currently available. In this study we investigated the efficacy of a targeted-mutagenesis approach based on zinc-finger nucleases (ZFNs). In this procedure, ZFNs are used to generate double-strand breaks at specific genomic sites, and subsequent repair produces mutations at the break site. To determine whether ZFNs can cleave and induce mutations at specific sites within higher plant genomes, we introduced a construct carrying both a ZFN gene, driven by a heat-shock promoter, and its target into the *Arabidopsis* genome. Induction of ZFN expression by heat shock during seedling development resulted in mutations at the ZFN recognition sequence at frequencies as high as 0.2 mutations per target. Of 106 ZFN-induced mutations characterized, 83 (78%) were simple deletions of 1–52 bp (median of 4 bp), 14 (13%) were simple insertions of 1–4 bp, and 9 (8%) were deletions accompanied by insertions. In 10% of induced individuals, mutants were present in the subsequent generation, thus demonstrating efficient transmission of the ZFN-induced mutations. These data indicate that ZFNs can form the basis of a highly efficient method for targeted mutagenesis of plant genes.

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Maize breeding
Maize was domesticated from its wild relative teosinte.

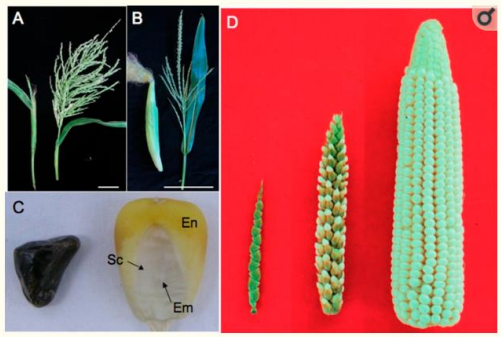

Figure 1.
Teosinte compared to maize.

(**A**) A teosinte female inflorescence (left), which arises as a secondary branch from tillers, and tassel (right). (**B**) An ear (left) and tassel (right) of maize. Size bar in **A** and **B** is 10 cm. (**C**) Teosinte kernel (left) and maize kernel (right). The teosinte kernel is hidden by hardened glumes (see Glossary). The maize kernel is exposed and reveals the endosperm (En) and embryo (Em). The embryo is surrounded by the scutellum (Sc), the nutritive tissue of the cotyledon. (**D**) A comparison of teosinte on the left, maize on the right and the F1 of maize and teosinte in the middle. Image credits: (**D**) John Doebley, Department of Genetics, University of Wisconsin–Madison; all other images, Sarah Hake.

DOI: http://dx.doi.org/10.7554/eLife.05861.002

Hake, S., & Ross-Ibarra, J. (2015). Genetic, evolutionary and plant breeding insights from the domestication of maize. *eLife, 4*, e05861. https://doi.org/10.7554/eLife.05861

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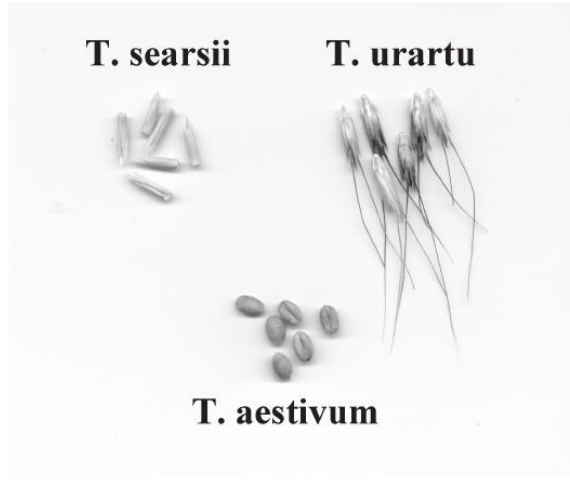

Figure 1.5. Grain of bread wheat (*Triticum aestivum*) compared with that of two wild relatives, *Triticum searsii* and *Triticum urartu*. Bread wheat is a product of thousands of years of selective breeding.

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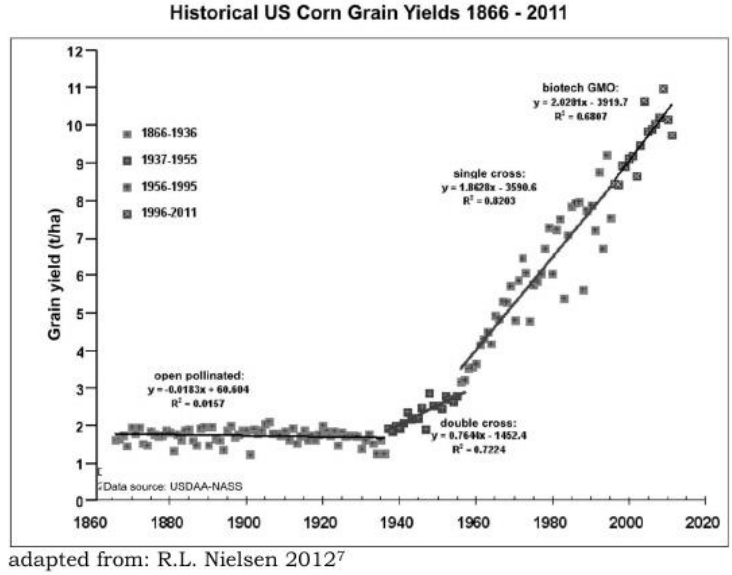

adapted from: R.L. Nielsen 2012[7]

Alkalaj and Bohanec. 2015. Yes to GMO.

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LIFE BOX 1.1. NORMAN E. BORLAUG

Norman E. Borlaug (1914–2009) Nobel Laureate, Nobel Peace Prize, 1970; Recipient of the Congressional Gold Medal, 2007.

Norman Borlaug. Courtesy of Norman Borlaug.

When Borlaug was born in 1914, the world's population was 1.6 billion. During his lifetime, population has increased four times, to 6.5 billion. Borlaug is often asked, "How many more people can the Earth feed?" His usual response: "I think the Earth can feed 10 billion people, IF, and this is a big IF, we can continue to use chemical fertilizer and there is public support for the relatively new genetic engineering research in addition to conventional research."

Stewart, C. N. (2016). *Plant Biotechnology and Genetics: Principles, Techniques, and Applications*: Wiley.

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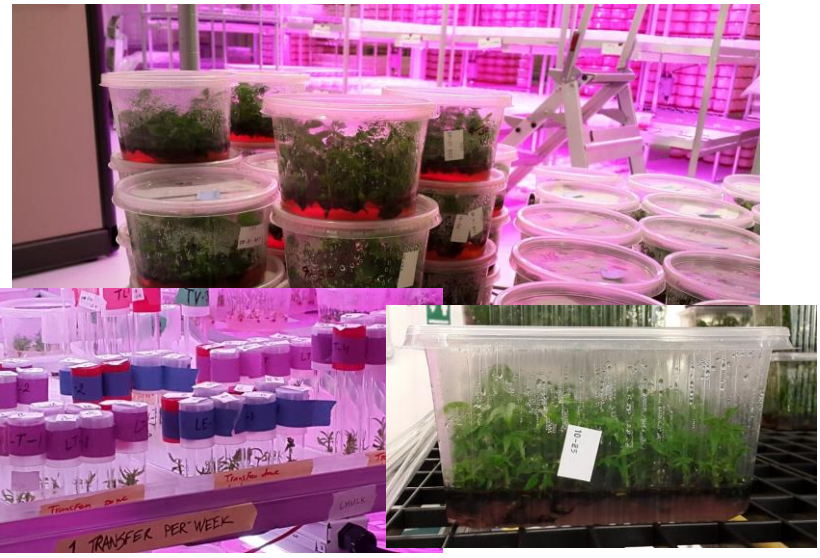
Plant tissue cultures



Term "plant tissue culture", broadly refers to the in vitro cultivation of all plant parts, whether a single cell, a tissue or an organ, on nutrient medium under aseptic conditions.

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Development of plant tissue culture techniques

<u>First attempts to cultivate differentiated cells and to demonstrate totipotency of plant cells.</u>

Gottlieb Haberlandt (1902) developed the concept of in vitro cell culture. He was the first to culture isolated, fully differentiated cells in a nutrient medium containing glucose, peptone, and Knop's salt solution. Haberlandt realized that asepsis was necessary when culture media are enriched with organic substances metabolised by microorganisms. In his cultures, free from microcontamination, cells were able to synthesize starch as well as increase in size and survived for several weeks. However, Haberlandt failed in his goal to induce these cells to divide. Despite drawbacks, he made several predictions about the requirements for cell division under experimental conditions in 1902, which have been confirmed with the passage of time. Haberlandt is thus regarded **as the father of tissue culture**.

GOTTLIEB HABERLANDT (1854-1945)

Bhojwani & Dantu, 2013

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1904 Hannig – excised nearly mature embryos of some crucifers (*Raphanus sativus, R. landra, R. caudatus, and Cochlearia donica*) and successfully grew them to maturity on mineral salts and sugar solution.

1922 Kotte in Robbins were successful in the establishment of excised plant root tips in vitro (they showed that it is possible to establish tissue culture with a meristem)

1925 Laibach – **Embryo culture has since become a useful tool** in the hands of plant breeders to obtain rare hybrids which otherwise fail due to **post-zygotic sexual incompatibility**

PHILIP R. WHITE (1901-1968)

1934 White – pioneering work of growing excised roots of tomato in vitro for periods of time without theoretical limits (experimental beginning of tissue cultures) (some tissue cultures were maintained with subculturing till 1968)

1935 – Snow demonstrated that indole acetic acid (IAA – a growth substance discovered by Went in 1926) stimulated cambial activity.

ROGER J. GAUTHERET (1910-1997)

1937 – 1939 - fundamental discovery that vitamins B1 (thiamine) and B (pyridoxine) were the root growth factors

1939 Gautheret – initiation of plant tissue culture from carrot root cambium using IAA and vitamins B (tiamin B1 and pyrodoxine B6)

Bhojwani & Dantu, 2013

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1939 - White in Nobécourt –) reported the establishment of callus cultures from tumor tissue of the hybrid *Nicotiana gauca* x *Nicotiana langsdorffii*

1955 – Skoog – discovery of kinetine in a yeast extract (cytokinin)

1957 - Skoog in Miller – an experiment demonstrating the role of different plant regulators concentrations in organogenesis

1965 - An important breakthrough was achieved in 1965 when Vasil and Hildebrandt observed that colonies arising from cloning of isolated cells of the hybrid Nicotiana glutinosa x N. tabacum regenerated plantlets.

PIERRE NOBÉCOURT
(1895-1961)

FOLKE SKOOG
(1908-2001)

TOSHIO MURASHIGE
(Born 1930)

Bhojwani & Dantu, 2013

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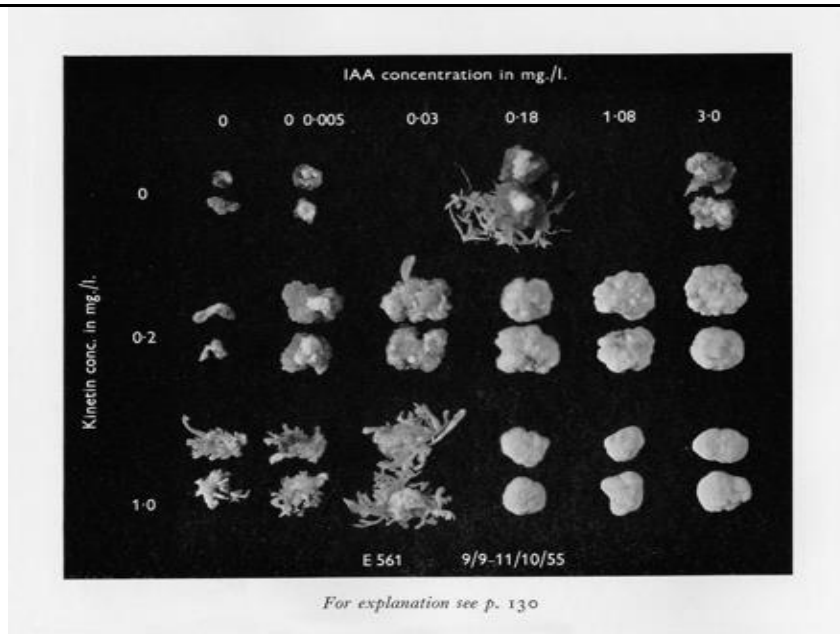

Results of an Skoog and Miller (1957) experiment showing the interaction between various concentrations of IAA and Kin in promoting shoot formation, root formation, or callus.

Trigiano, R. N., & Gray, D. J. (2016). *Plant Tissue Culture, Development, and Biotechnology*: CRC Press.

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1960 Cocking – Protoplasts released by cell wall degrading enzymes have been prepared from many plant tissues.

1964 Guha and Maheshwari – Another landmark in the development of Plant tissue culture has been the discovery of haploids, when Guha and Maheshwari (1964) obtained haploid embryos from *Datura innoxia*

Backs-Hüsemann and Reinert (1970) achieved embryo formation from an isolated single cell of carrot.

1972 Cocking – protoplasts fusion (somatic hybridization)

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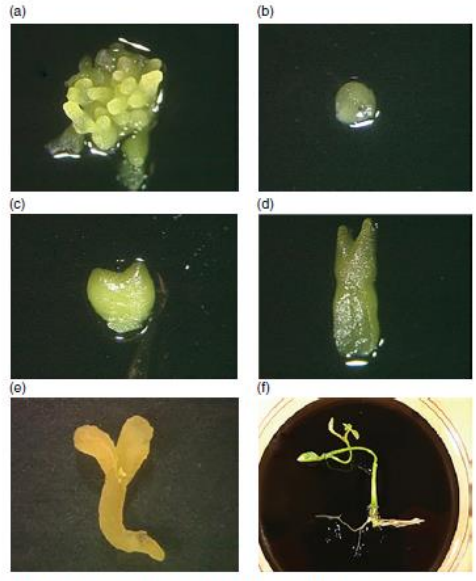

Figure 5.11. Somatic embryogenesis system showing the sequential developmental stages: (a) a cluster of globular somatic embryos, (b) a globular embryo, (c) a heart-shaped embryo, (d) a torpedo-shaped embryo, (e) a mature embryo with cotyledons, and (f) a plantlet from a germinated embryo.

Stewart, C. N. (2016). *Plant Biotechnology and Genetics: Principles, Techniques, and Applications*: Wiley.

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A Brief History of the Development of Recombinant DNA Technology

In 1966, Bernard Weiss and Charles Richardson, working at Johns Hopkins University, isolated **DNA ligase**, an enzyme that 'glues' two ends of DNA together.

Discovery of **the restriction enzymes** (Paul Berg, 1968)

1972 -Paul Berg (Stanford University) – reported that he had constructed a DNA molecule by cutting viral and bacterial DNA molecules with restriction enzymes and then recombining them

Construction of recombinant plasmid *in vitro* (Cohen et al., 1973)

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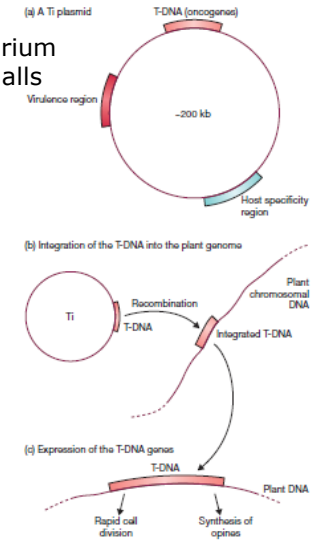
1974 – discovery that Ti plasmid of a bacterium *Agrobacterium tumefaciens* causes crown galls

1977
Chilton in sod. – successful integration of Ti plasmid into the plant DNA - *A. tumefaciens* became one of the most reliable and widely used means of transferring foreign DNA (DNA from a different organism) into plants.

1977 – Maxam and Gilbert – method for DNA sequencing

1977 – Frederick Sanger – method for DNA sequencing



The Ti plasmid and its integration into the plant chromosomal DNA after A. tumefaciens infection. (Vir: Brown, T.A., 2010)

24

1980 – Commercial production of human insulin through genetic engineering in bacterial cells

1980 – Restriction fragment length polymorphism (RFLP) technique developed.

1984 - Horsch et al. – transformation of tobacco with *Agrobacterium* ; transgenic plants developed (bacterial antibiotic resistance genes)

1986 – TMV virus-resistant tobacco and tomato transgenic plants developed using cDNA of coat protein gene of TMV (Powell-Abel et al.).

1986 – The discovery of polymerase chain reaction (Mullis et al.).

1987 – Development of biolistic gene transfer method for plant transformation (Sanford et al., Klein et al.).

1987 – Bt gene from bacterium (*Bacillus thuringiensis*) isolated (Barton et al.).

1990 – Random amplified polymorphic DNA (RAPD) technique developed (Williams et al., Welsh and McClelland).

1995 – DNA finger printing by amplified fragment length polymorphism (AFLP) technique developed (Vos et al.).

1997 – Sequencing of *E. coli* genome (Blattner et al.).

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Figure 2.1. Timeline of developments in genetics and recombinant DNA technology.

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- 1985 – USA approved the first open-air field trials of transgenic plants
- 1988 – company Calgene got the approval for field experiments with tomato FLAVR SAVR
- -1993 - the first whole plant was commercialized and grown unregulated in the field, a virus-resistant tobacco in China
- 1994 – first transgenic food crop, Flavr Savr tomato on the market
- 1996 First large-scale cultivation of GM HR soybean and maize
- 1996 GSO were grown on 1,66 mil. hectares

Plant structural genomics was revolutionized by completion of the whole genome sequencing of *Arabidopsis thaliana* in 2000, rice (*Oryza sativa* ssp. *Japonica*)

2004 First generation Golden rice field trial. Golden rice was developed as a fortified food to be used in areas where there is a shortage of dietary vitamin A

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First/second generation of GM plants

Production of GE crops in 2015 (National Academies of Sciences & Medicine, 2016):

- About 12 percent (179.7 million of 1.5 billion hectares) of global crop land produced GE crops in 2015
- The United States produced 10 crops with GE varieties, followed by Canada with four. GE maize, soybean, and cotton were grown in many countries, whereas GE varieties of alfalfa, apple, poplar, potato, squash, and eggplant were grown in just one country each. Over 70 million of the 179.7 million hectares producing GE crops were in the United States.6 GE crops produced in Brazil, Argentina, India, and Canada accounted for another 91.3 million hectares. The remaining 17.5 million hectares were spread among 23 countries.

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TABLE 3-1 Genetically Engineered Traits Deregulated and Approved for Field Release in the United States as of 2015

Crop	Crop Scientific Name	Trait	Year Approved	Developer
Alfalfa	*Medicago sativa*	Glyphosate HR[a,b]	2005	Monsanto & Forage Genetics
		Reduced Lignin	2014	Monsanto & Forage Genetics
Apple	*Malus domestica*	Nonbrowning	2015	Okanagan Specialty Fruits
Canola	*Brassica napus*/ *Brassica rapa*	Oil Profile Altered[a]	1994	Calgene
		Glufosinate HR	1998	AgrEvo
		Glyphosate HR	1999	Monsanto
Cichory	*Cichorium intybus*	Male Sterility[c]	1997	Bejo
Cotton	*Gossypium hirsutum*	Bromoxynil HR[c]	1994	Calgene
		Bt IR[d]	1995	Monsanto
		Glyphosate HR	1995	Monsanto
		Sulfonylurea HR	1996	DuPont
		Glufosinate HR	2003	Aventis
		Dicamba HR	2015	Monsanto
		2,4-D HR	2015	Dow
Flax	*Linum usitatissimum*	Tolerant to Soil Residues of Sulfonylurea Herbicide[e]	1999	University of Saskatchewan
Maize	*Zea mays*	Glufosinate HR	1995	AgrEvo
		Bt IR	1995	Ciba Seeds
		Male Sterility[a]	1996	Plant Genetic Systems
		Glyphosate HR	1997	Monsanto
		Increased Lysine[c]	2006	Monsanto
		Imidazolinone HR[c]	2009	Pioneer
		Alpha-Amylase	2011	Syngenta
		Drought Tolerance	2011	Monsanto
		ACCase[g] HR	2014	Dow
		2,4-D HR	2014	Dow
		Increased Ear Biomass	2015	Monsanto
Papaya	*Carica papaya*	Ring Spot Virus VR[f]	1996	Cornell University, University of Hawaii, USDA Agricultural Research Service
Plum	*Prunus domestica*	Plum Pox VR[c]	2007	USDA Agricultural Research Service
Potato	*Solanum tuberosum*	*Bt* IR[c]	1995	Monsanto
		Potato Leafroll VR[c]	1998	Monsanto
		Potato Virus Y VR[c]	1999	Monsanto
		Low Acrylamide	2014	Simplot Plant Sciences
		Nonbrowning	2014	Simplot Plant Sciences
		Resistance to Late Blight Pathogen	2015	Simplot Plant Sciences
Rice	*Oryza sativa*	Glufosinate HR	1999	AgrEvo
Rose	*Rosa* spp.	Altered Flower Color	2011	Florigene
Squash	*Cucurbita pepo*	Zucchini Yellow VR	1994	Upjohn
		Watermelon Mosaic VR	1994	Upjohn
		Cucumber Mosaic VR	1996	Asgrow
Soybean	*Glycine max*	Glyphosate HR	1994	Monsanto
		Glufosinate HR	1996	AgrEvo
		High Oleic Oil	1997	DuPont
		Acetolactate Synthase HR[c]	2008	Pioneer
		Bt IR[c]	2011	Monsanto
		Improved Fatty Acid Profile[a]	2011	Monsanto
		Stearidonic Acid Produced[a]	2012	Monsanto
		Isoxaflutole HR[c]	2013	Bayer and M.S. Technologies
		Increased Yield[a]	2013	Monsanto
		Imidazolinone HR	2014	BASF
		2,4-D HR	2014	Dow
		HPPD[f] HR[c]	2014	Bayer/Syngenta
		Dicamba HR	2015	Monsanto
Sugar beet	*Beta vulgaris*	Glufosinate HR[c]	1998	AgrEvo
		Glyphosate HR	1998	Novartis & Monsanto
Tobacco	*Nicotiana tabacum*	Reduced nicotine[a]	2002	Vector
Tomato	*Solanum lycopersicum*	Fruit Ripening Altered[a]	1992	Calgene
		Fruit Polygalacturonase Level Decreased[a]	1995	Zeneca & Petoseed
		Bt IR[c]	1998	Monsanto

NOTE: The table identifies the first time a trait was deregulated for a specific crop in the United States. Some deregulated trait–crop combinations have never been used in commercial production.

[a] HR = herbicide resistance.
[b] Returned to regulated status in 2007; returned to deregulated status in 2011.
[c] Trait–crop combination not in production in 2015.
[d] IR = insect resistance (different *Bacillus thuringiensis* Cry genes inserted to encode proteins that kill specific species).
[e] Acetyl CoA Carboxylase inhibitor herbicide.
[f] VR = virus resistance.
[g] 4-Hydroxyphenylpyruvate dioxygenase inhibitor herbicide.

DATA SOURCE: USDA–APHIS Petitions for Determination of Nonregulated Status. Available at http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml. Accessed December 20, 2015.

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Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors

Eugenio Butelli[1], Lucilla Titta[2], Marco Giorgio[2], Hans-Peter Mock[3], Andrea Matros[3], Silke Peterek[3], Elio G W M Schijlen[4], Robert D Hall[5], Arnaud G Bovy[4], Jie Luo[1] & Cathie Martin[1]

Dietary consumption of anthocyanins, a class of pigments produced by higher plants, has been associated with protection against a broad range of human diseases. However, anthocyanin levels in the most commonly eaten fruits and vegetables may be inadequate to confer optimal benefits. When we expressed two transcription factors from snapdragon in tomato, the fruit of the plants accumulated anthocyanins at levels substantially higher than previously reported for efforts to engineer anthocyanin accumulation in tomato and at concentrations comparable to the anthocyanin levels found in blackberries and blueberries. Expression of the two transgenes enhanced the hydrophilic antioxidant capacity of tomato fruit threefold and resulted in fruit with intense purple coloration in both peel and flesh. In a pilot test, cancer-susceptible *Trp53*$^{-/-}$ mice fed a diet supplemented with the high-anthocyanin tomatoes showed a significant extension of life span.

Maddie Hall
@maddiehalla

Yesterday was a historic day in plant biotech: a purple tomato engineered with high antioxidants was approved by @USDA @BigPurpleTomato helps prevent cardiovascular disease and fight cancer in humans. This approval under new regulation ushers in a new era for plant synbio!

12:42 AM · Sep 10, 2022 · Twitter Web App

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Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors

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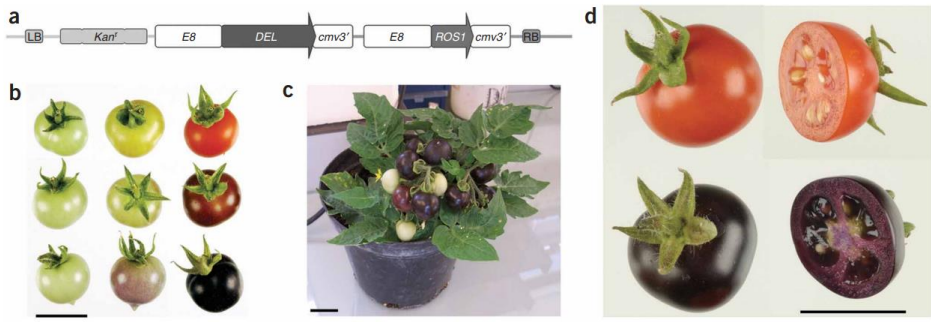
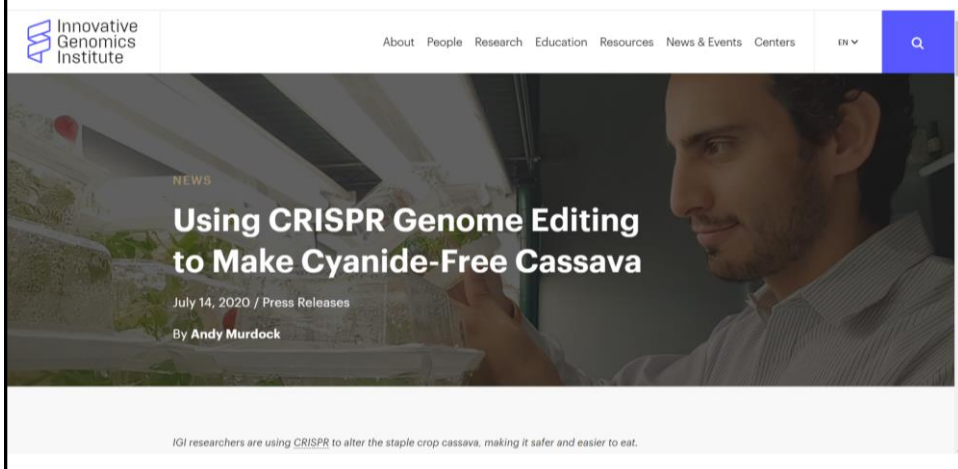


Figure 1 Fruit-specific phenotypes of T1 generation tomatoes (cv. MicroTom) expressing both *Del* and *Ros1* under the control of the *E8* promoter. (**a**) Map of T-DNA region of the binary vector used for transformation. LB, left T-DNA border region; RB, right T-DNA border region; Kan^r, *nptII* gene conferring kanamycin resistance under the control of the *nos* promoter; cmv3′, terminator region of cauliflower mosaic virus. (**b**) Phenotypic analysis of wild-type (upper row), *Del/Ros1*C (middle) and *Del/Ros1*N (lower) tomato fruit harvested at the green (left column), breaker (middle) and red (right) ripening stages. (**c**) *Del/Ros1*N tomato plant showing fruit at different stages of ripening. (**d**) Whole and cross-section of ripe wild-type and *Del/Ros1*N tomato fruit. All scale bars, 2 cm.

31



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Gene structure

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Opposite DNA strands can serve as template for RNA

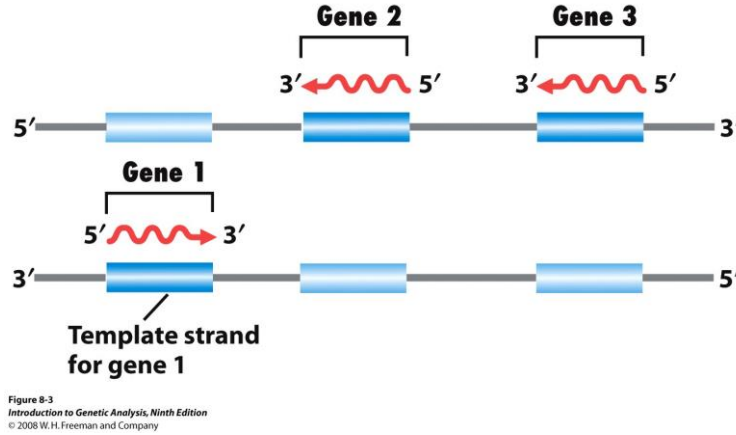

Figure 8-3
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In the chromosome overall, both DNA strands are used as templates, *but, in any one gene, only one strand is used,* and, in that gene, it is always the same strand, starting at the 3 ′ end of the template gene.

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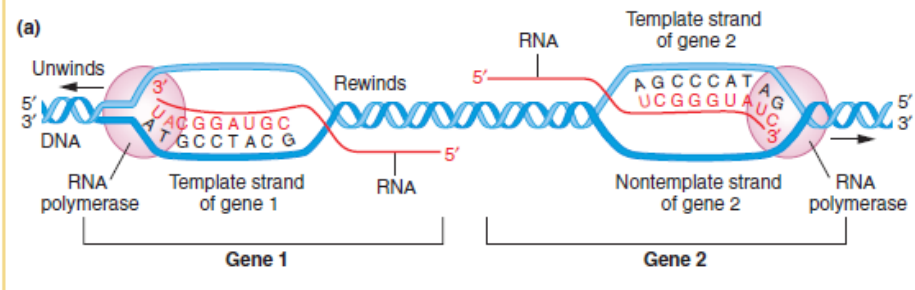
Overview of transcription



Griffiths, A. J. F., Wessler, S. R., Carroll, S. B., & Doebley, J. (2015). *An Introduction to Genetic Analysis*: Macmillan Learning.

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Transcription is asymmetrical: only one strand of the DNA of a gene is used as a template for transcription. This strand is in the 3 ′-to-5 ′ orientation, and RNA is synthesized in the 5 ′-to-3 ′ direction.

Sequence of mRNA is identical to nontemplate strand of DNA, except that the T's are replaced by U's.

For this reason, the nontemplate strand of the DNA is referred to as the **coding strand.**

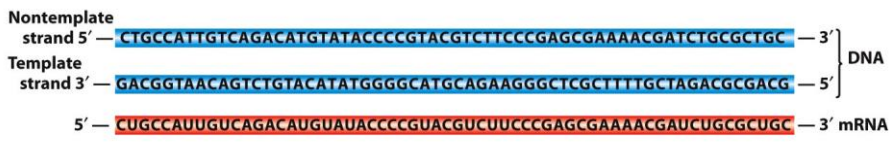

Figure 8-6
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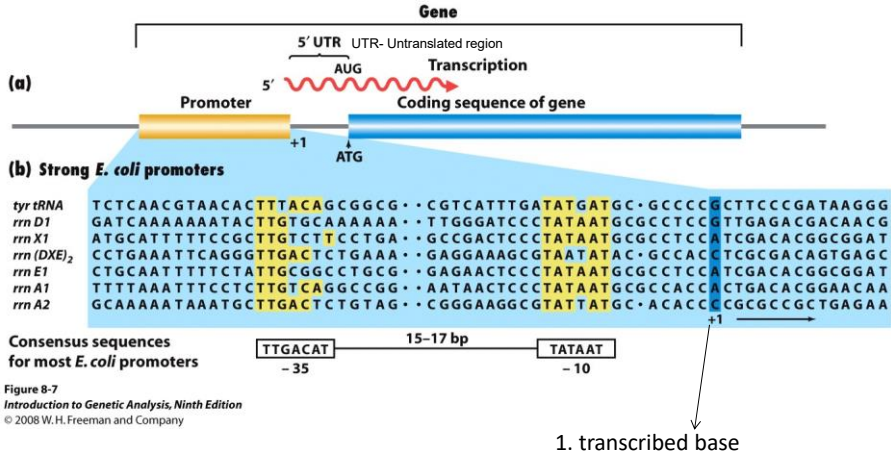
Promoter sequences in *E. coli*.



Consensus sequence = in agreement with most sequences

Promoters have regions of similar sequences – as indicated by the yellow shading in seven different promoter sequences.

Griffiths, A. J. F., Wessler, S. R., Carroll, S. B., & Doebley, J. (2015). *An Introduction to Genetic Analysis*: Macmillan Learning.

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Transcription in Eukaryotes

Three different polymerases do the transcription in eukaryotes:

TABLE 12.1 FUNCTIONS OF THE THREE EUKARYOTIC NUCLEAR RNA POLYMERASES

Polymerase	Types of RNA
RNA polymerase I	28S, 5.8S, and 18S rRNAs
RNA polymerase II	mRNA, Sm family of snRNAs, some snoRNAs, siRNA*, miRNA, piRNA, lncRNA
RNA polymerase III	tRNAs, 5S rRNA, Lsm family of snRNAs, some snoRNAs, 7SL RNA, 7SK RNA

*In plants, some siRNAs are transcribed by RNA polymerase IV or V.

Brown, T. A. (2018). *Genomes 4*: CRC Press.

38

Processing 5‘ and 3‘ ends

• When the nascent RNA first emerges from RNA polymerase II, a special structure, called a **cap**, is added to the 5’ end by several proteins that interact with the CTD. The cap consists of a 7-methylguanosine residue linked to the transcript by three phosphate groups.

• CAP has two functions:
1. It protects the RNA from degradation when transported to the site of translation.
2. It is required for translation of the mRNA.

RNA elongation continues until the conserved sequence AAUAAA or AUUAAA is reached, marking the 3’ end of the transcript. An enzyme recognizes that sequence and cuts off the end of the RNA approximately 20 bases farther down. To this cut end, **a stretch of 150 to 200 adenine nucleotides** called a **poly(A) tail** is added. Hence, the AAUAAA sequence of the mRNA from protein-encoding genes is called a *polyadenylation signal.*

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The primary division is between coding RNA and noncoding RNA. Coding RNA is made up of just one class of molecule, the messenger RNAs (mRNAs), which are transcripts of protein-coding genes and hence are translated into protein.

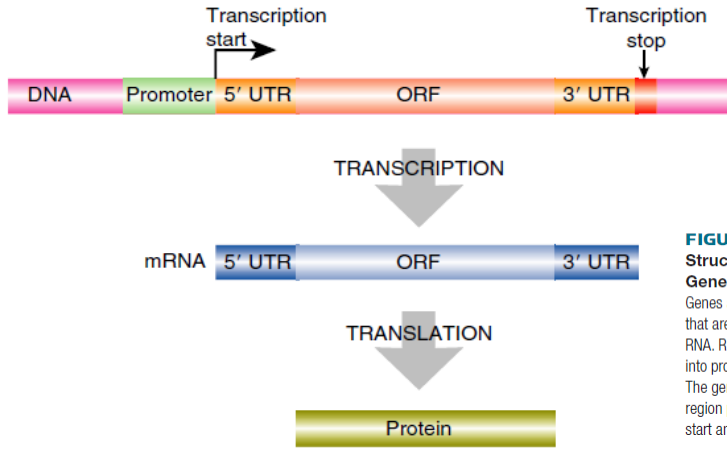

FIGURE 2.2 The Structure of a Typical Gene
Genes are regions of DNA that are transcribed to give RNA. RNA can be translated into protein or used directly. The gene has a promoter region plus transcriptional start and stop points that

Clark, D. P., & Pazdernik, N. J. (2015). *Biotechnology*: Elsevier Science.

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Parts of machinery for gene expression regulation

- Trans-acting regulatory proteins

  1) RNA polymerase II complex and GTF,

  2) TF that bind to cis-acting regulatory DNA sequences called enhancers.

- Cis-acting regulatory DNA sequences **promoter-proximal elements, enhancers**

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Promoter-proximal elements precede the promoter of a eukaryotic gene

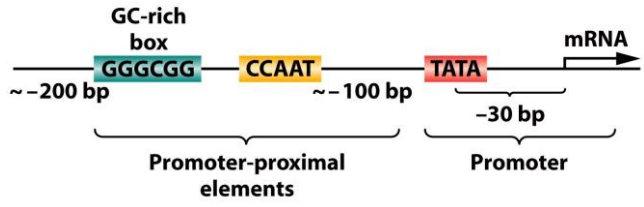

Figure 11-3
Introduction to Genetic Analysis, Ninth Edition
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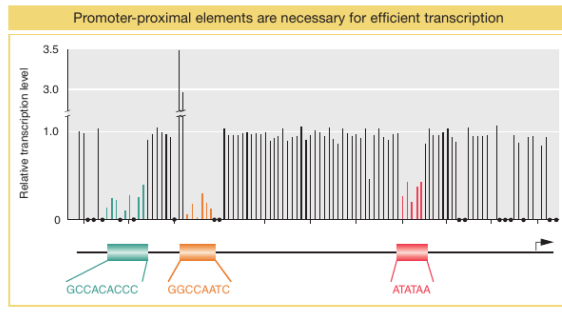

Griffiths, A. J. F., Wessler, S. R., Carroll, S. B., & Doebley, J. (2015). *An Introduction to Genetic Analysis*: Macmillan Learning

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Mechanism of *Lac* operon

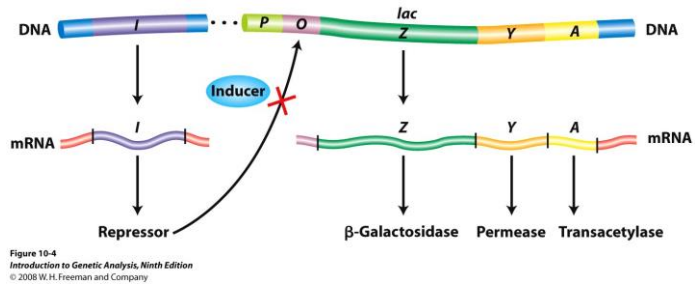

Figure 10-4
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Simplified model of *Lac* operon. Coordinated expression of *Z, Y* and *A* genes is under **the negative control** of the product of the *I* gene, the repressor. When the **inducer binds the repressor**, the operon is fully expressed.

Lac represor – with DNA binding site can bind to operator (O) and prevents transcription by RNA polymerase, whereas an allosteric site binds allolactose (which acts as an inducer of the *lac* operon) or analogs of lactose (useful experimentally).

When repressor undergoes to allosteric transition, it loses affinity for the operator.

The lacZYA genes are transcribed as a polycistronic message.

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Lac structural genes

- Enzymes:

1) permease: transports lactose into the cell (gene *Z*);

2) β-galactosidase: modifies lactose into allolactose and cleaves the lactose molecule into glucose and galactose (gene *Y*).

3) Transacetylase: not required for lactose metabolism (gene *A*)

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Regulatory components of the *lac* system

Key regulatory components:

1. a gene encoding a transcription regulatory protein
- Gene *I* (gene for *Lac* repressor)

2. two binding sites on DNA
- *Lac* promoter site (P) (the site where RNA polymerase binds)
- *Lac* operator site (O): site on the DNA to which the Lac repressor binds. It is located between the promoter and the *Z* gene.

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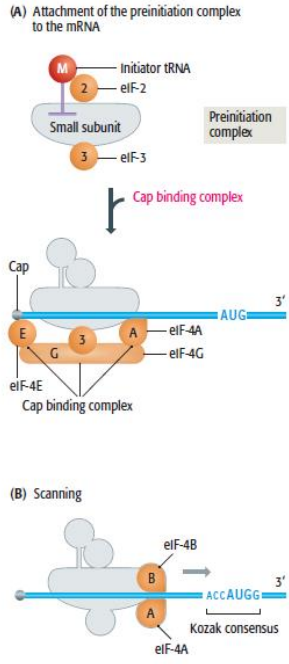

Figure 13.16 Initiation of translation in eukaryotes. (A) Assembly of the preinitiation complex and its attachment to the mRNA. For clarity, several proteins whose precise roles are not understood have been omitted. The overall configuration of the complex is not known. (B) The preinitiation complex scans along the mRNA until it reaches the initiation codon, which is recognizable because it is located within the Kozak consensus sequence. Scanning is aided by eIF-4A and eIF-4B, which are thought to have helicase activity. It is probable that eIF-3 remains attached to the preinitiation complex during scanning, as shown here. It is not clear whether eIF-4E and eIF-4G also remain attached at this stage. Note that scanning is an energy-dependent process that requires hydrolysis of ATP. Abbreviation: M, methionine.

The consensus nucleotide sequence surrounding the AUG start codon in dicotyledonous plants (dicots) is aaA(A/C)aAUGGCu; while in monocotyledonous plants(monocots), it is c(a/c)(A/G)(A/C)cAUGGCG.

Brown. 2007. Genomes 3.

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Techniques of recombinant DNA technology

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BASIC TECHNIQUES

- DNA isolation
- Restriction enzymes

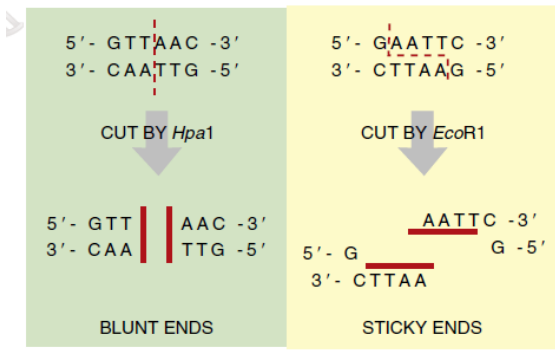

FIGURE 3.2
Type II Restriction Enzymes—Blunt versus Sticky Ends

Clark, D. P., & Pazdernik, N. J. (2015). *Biotechnology*: Elsevier Science.

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Spletni naslov do REBASE podatkovne zbirke:
http://rebase.neb.com/rebase/rebase.html

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Using two restriction enzymes with different recognition sequences, one can combine two DNA molecules in a predetermined orientation

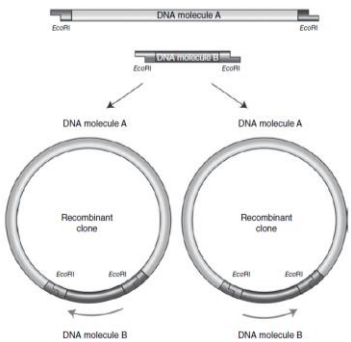


Figure 8.4. DNA fragments produced with a single *Eco*RI restriction enzyme give rise to compatible protruding termini that can anneal in either orientation, bringing together the 5' phosphate and the 3' hydroxyl residues on each strand. This allows DNA ligase to catalyze the formation of phosphodiester bonds, joining the two molecules together.

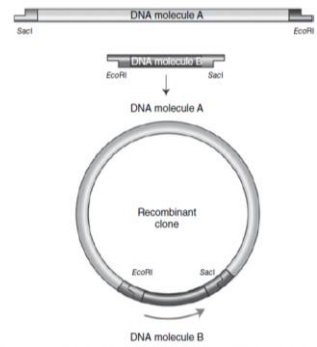
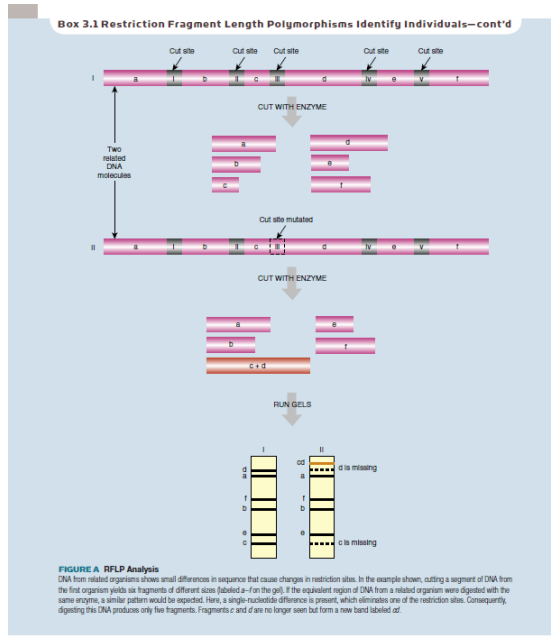


Figure 8.5. DNA fragments produced with two restriction enzymes, *Eco*RI and *Sac*I, give rise to fragments with protruding termini that can anneal in only one orientation with respect to one another, forcing the two molecules to combine in one direction only.

Stewart, C. N. (2016). *Plant Biotechnology and Genetics: Principles, Techniques, and Applications*: Wiley.

50

Primer uporabe RFLP molekulskih markerjev



Clark, D. P., & Pazdernik, N. J. (2015). *Biotechnology*: Elsevier Science.

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Other enzymes

- DNA ligase
  - e.g. T4 DNA ligase (bacteriophage T4, it requires ATP): catalyzes the formation of a phosphodiester bond
- kinase – T4 polynucleotide kinase – catalyzes the transfer of phosphate of ATP to a 5' terminus of DNA or RNA
- Alkaline phosphatase – catalyzes the removal of 5' phosphate groups from the DNA and thus modify the termini of DNA (by treatment with alkaline phosphatase, both recircularization and plasmid dimer formation are prevented because ligase cannot join the ends)
- DNA polymerase
- Terminal transferase – can add oligodeoxynucleotide tails to the 3 ends of DNA duplexes. Thus, it provides the means by which the homopolymeric extensions can be synthesized, known as homopolymeric tailing.

- Exsonuclease (it removes nucleotides in a direction 5`-3` or 3`-5)
- Recombinases!!!

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Linkers and adapters

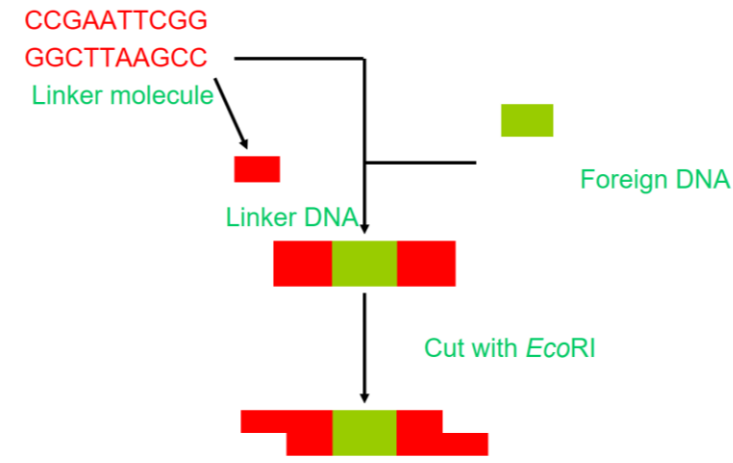

Fig. 15.9: The use of linker for construction of DNA fragments with cohesive termini.

Chawla, H. S. (2009). *Introduction To Plant Biotechnology, 3/E* (3 ed.): Oxford & IBH Publishing Company Pvt.

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Linkers and adapters

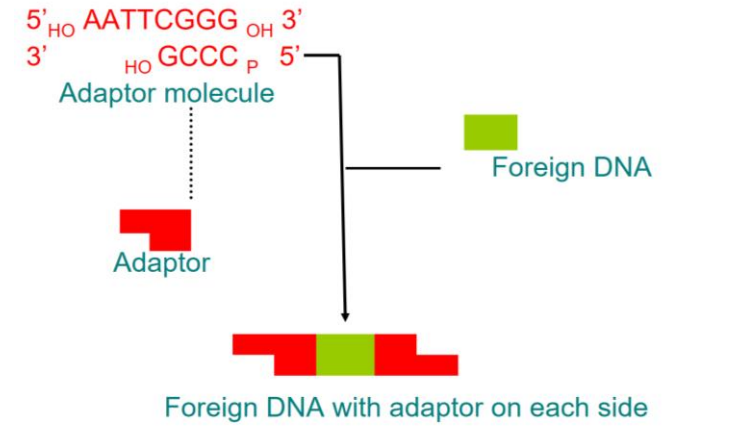

Fig. 15.10: The use of adaptors for construction of DNA fragments with cohesive termini.

Chawla, H. S. (2009). *Introduction To Plant Biotechnology, 3/E* (3 ed.): Oxford & IBH Publishing Company Pvt.

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Methods for detection of nucleic acids:
- gel electrophoresis
- capillary sequencing machine

Methods for detection of specific nucleic acids:
- Southern blot (method for detection of DNA)
- Northern blot (method for detection of RNA)

Methods for amplification and quantification of DNA/RNA
- PCR
- Q-PCR
- digital PCR

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Other….

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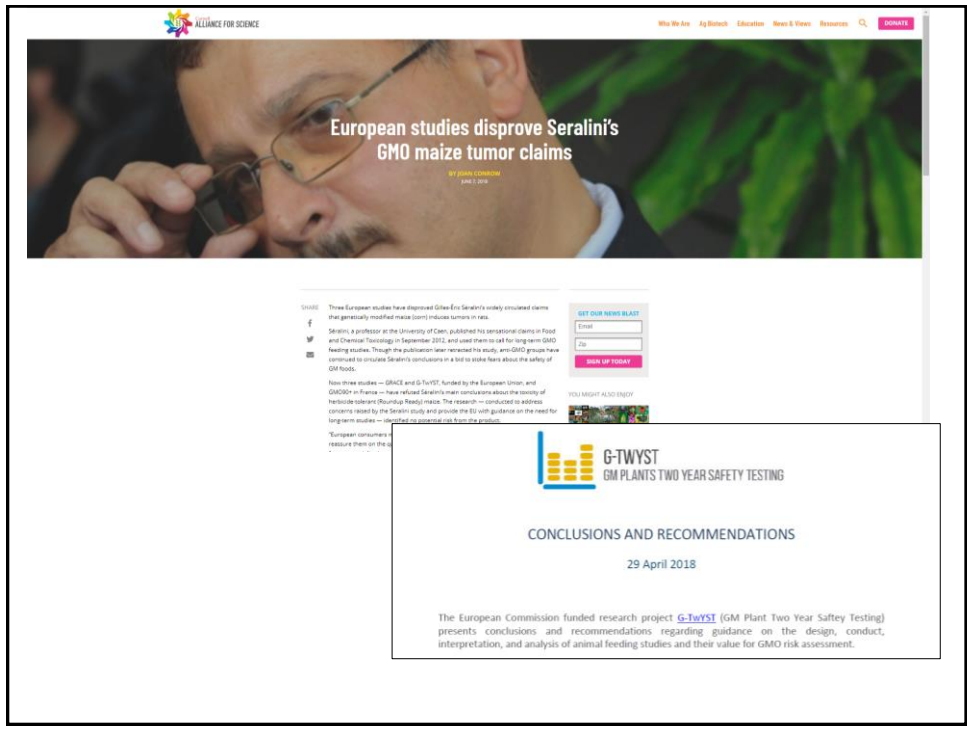

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https://crispr-gene-editing-regs-tracker.geneticliteracyproject.org/

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