How GMOs Are Really Made:

Simple Precision or Messy Complexity?

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Fundamentals in Food and Agriculture

Why does it matter?

1. GMO Risks to Human or Livestock Health or to the Environment arise from:

    - ✧The inserted DNA itself (gene cassette)
    - ✧The methods used to insert the gene cassette into the plant genome

2. Both can give rise to harmful unintended effects

3. These are not being adequately taken into account by regulators

4. Key Myth of Precision

- What is a GMO?
- How do you make a GMO?
- Why do GMOs not behave as expected?

What is a GMO?

(Genetically Modified Organism)

(g) “Living modified organism” means any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology;

--- Legal definition of a LMO (=GMO) of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity, Montreal, 2000 (in force since 1993). The Convention is the main international instrument for addressing biodiversity issues.

Cartagena Protocol: https://www.cbd.int/doc/legal/cartagena-protocol-en.pdf

What is a GMO?
(Genetically Modified Organism)

An organism with one or more traits that have been created and introduced using genetic engineering techniques.

A few examples of Commercialized GMO Plants

Herbicide Resistance Traits	Bt Pesticide Traits	Other Traits
Roundup Ready soybean Liberty Link Rice Liberty Link Cotton	YieldGard Mon810 Bt Maize Bollgard Bt cotton	Artic Apple SunUp Papaya CZW-3 Squash

Genes: "DNA makes RNA makes Protein"

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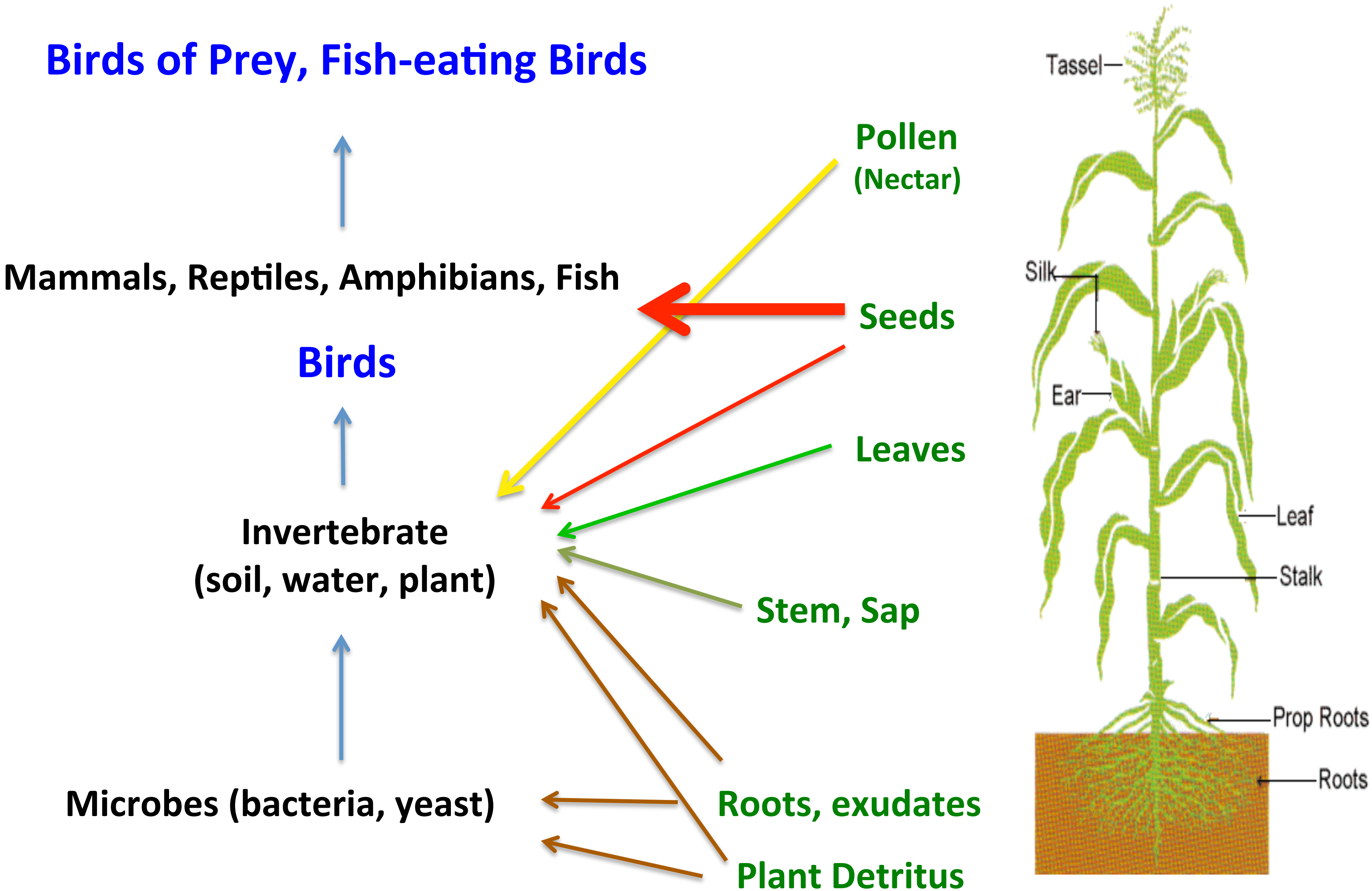
Birds and the GMO Food Web



Midwestern GMO Maize Fields

- 2015: 400 million acres GMOs worldwide
- Most Bt corn and Herbicide Resistant Soybean
- ¾ of those acres were in US, Brazil and Argentina

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Roundup Ready Soybeans in the Amazon: Before and After

How to Make a GMO

1. Prepare a gene cassette

2. Transfer the gene cassette into the plant genome

3. Select a successful GMO for commercialization

1. Prepare
a DNA gene cassette

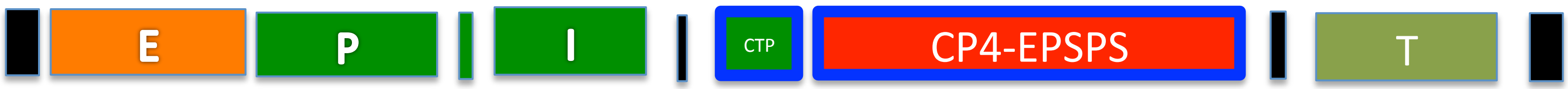
- Identify DNA that specifies the protein that results in the desired trait

- Identify regulatory sequences (eg. promoter, terminator, protein targeting sequences)

- Cut and paste them together using recombinant DNA technology

- Repeat if needed

DNA Gene-Cassette Used to Create RR2Yield's Herbicide Resistance Trait



- Intervening DNA
- Figwort Mosaic Virus Enhancer (E)
- *A. thaliana* Promoter (P)
- *A. thaliana* 5' Untranslated Leader (L)
- *A. thaliana* Intron (I)
- *A. thaliana* Chloroplast Transit Peptide (CTP)
- ***Agrobacterium* Herbicide-Resistance Gene (CP4-EPSPS)**
- *P. sativum* 3' Untranslated Terminator (T)

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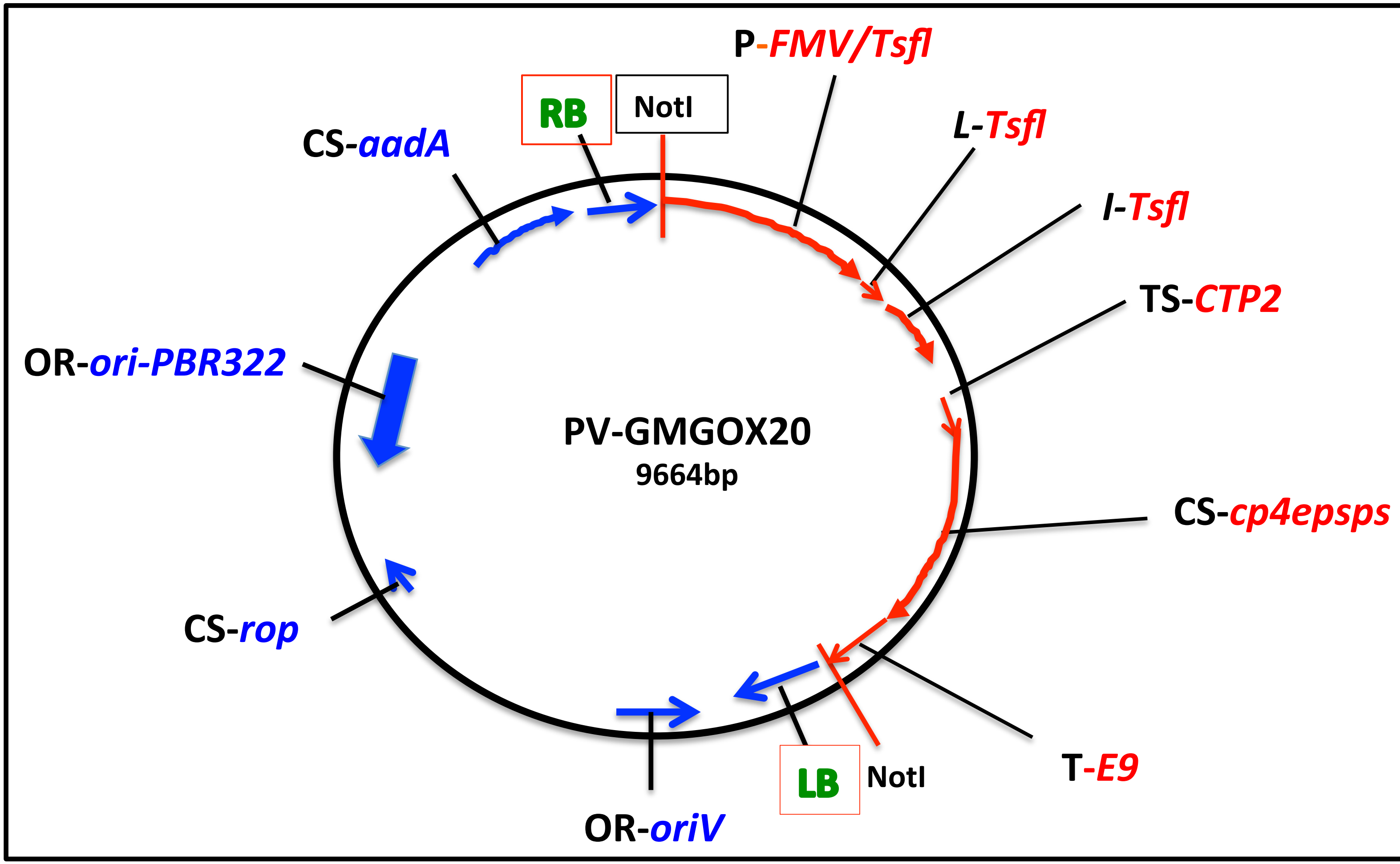
Plasmid Vector and Gene Cassette



P=promoter, L=leader, I=intron, TS=Target sequence, CS=coding sequence, T=3'non-translated transcript term. Seq. and poly A signal, OR=origin of replication

Data from Petition for the Determination of Nonregulated Status for Roundup Ready2YieldTM Soybean MON 89788 (2006) submitted to USDA by Monsanto.

Recap: Gene Cassettes are Complex

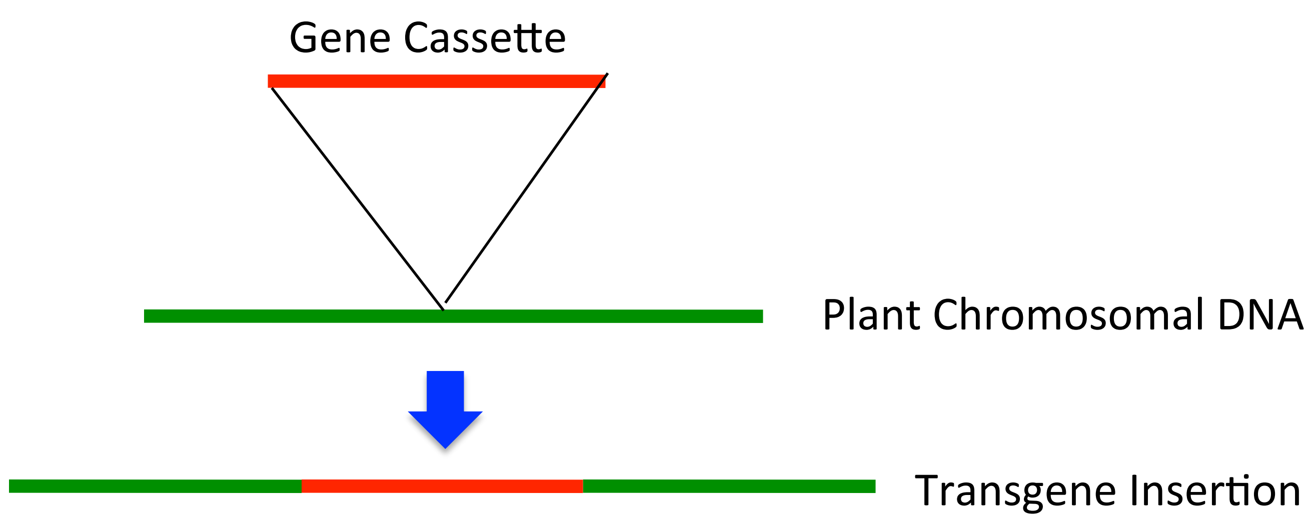
- Gene cassettes usually mix DNA from highly diverse organisms (e.g viruses, bacteria, fungi) or different plant species

- "Promoters" and "terminators" are just names and are poorly understood
  - Viral Promoters specify proteins
  - Terminators that don't terminate

Podevin, Nancy, and Patrick Du Jardin. "Possible consequences of the overlap between the CaMV 35S promoter regions in plant transformation vectors used and the viral gene VI in transgenic plants." *GM crops & food* 3.4 (2012): 296-300.

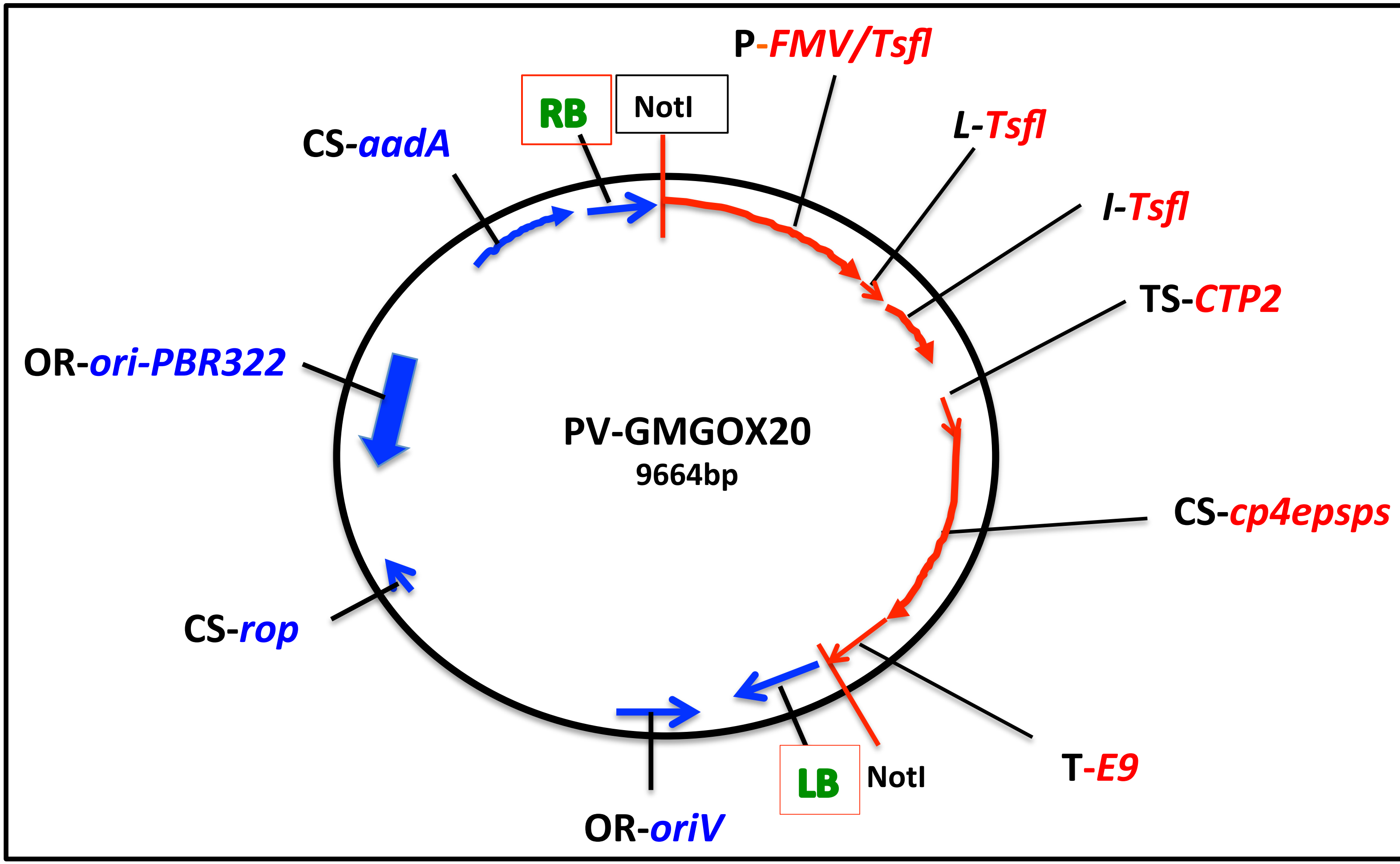
Rang, Andreas, Bettina Linke, and Bärbel Jansen. "Detection of RNA variants transcribed from the transgene in Roundup Ready soybean." European Food Research and Technology 220.3-4 (2005): 438-443.

2. Transfer gene cassette DNA into the plant genome



Agrobacterium infection
or
A Gene Gun

Plasmid Vector and Gene Cassette for *Agrobacterium*-mediated DNA Transfer



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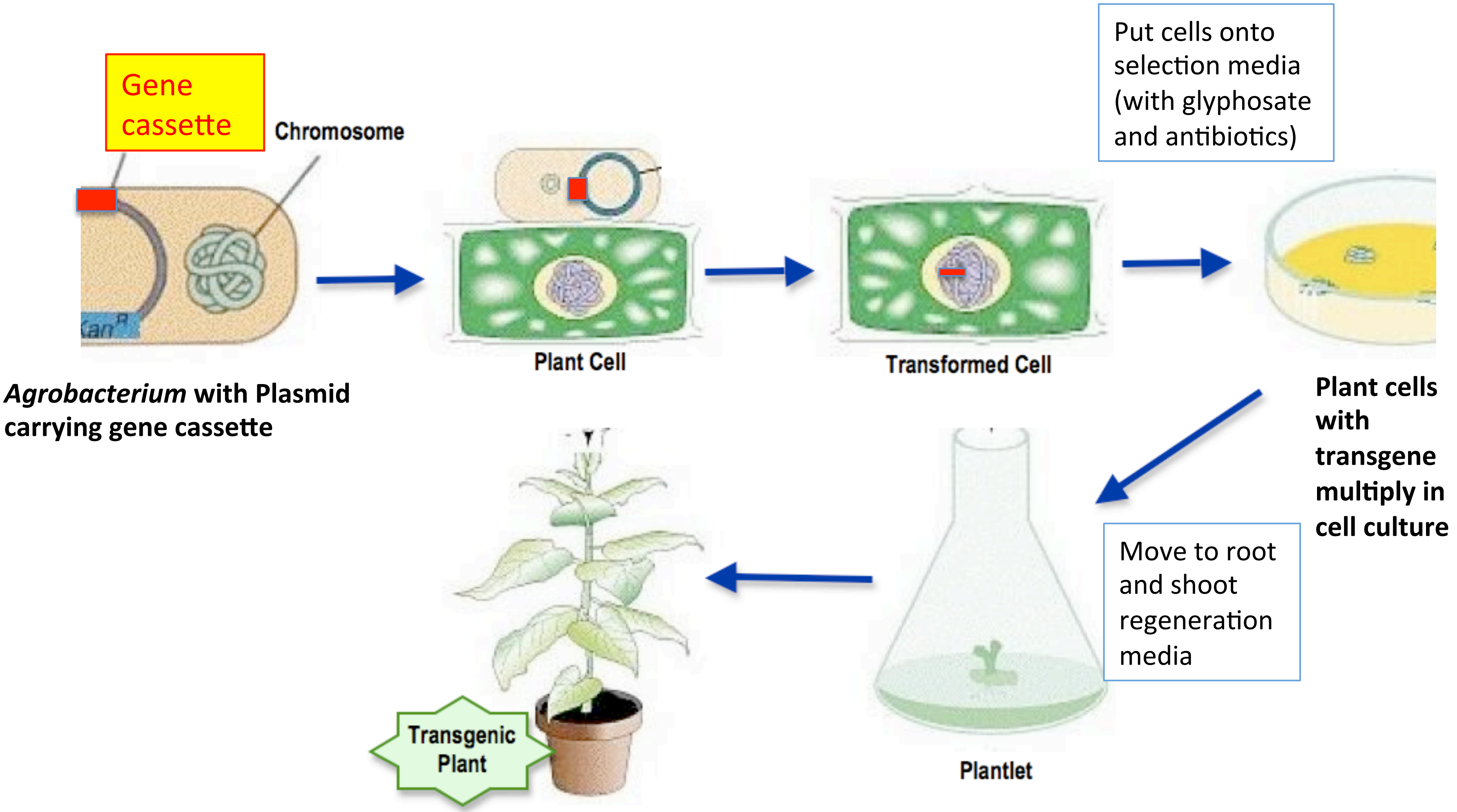
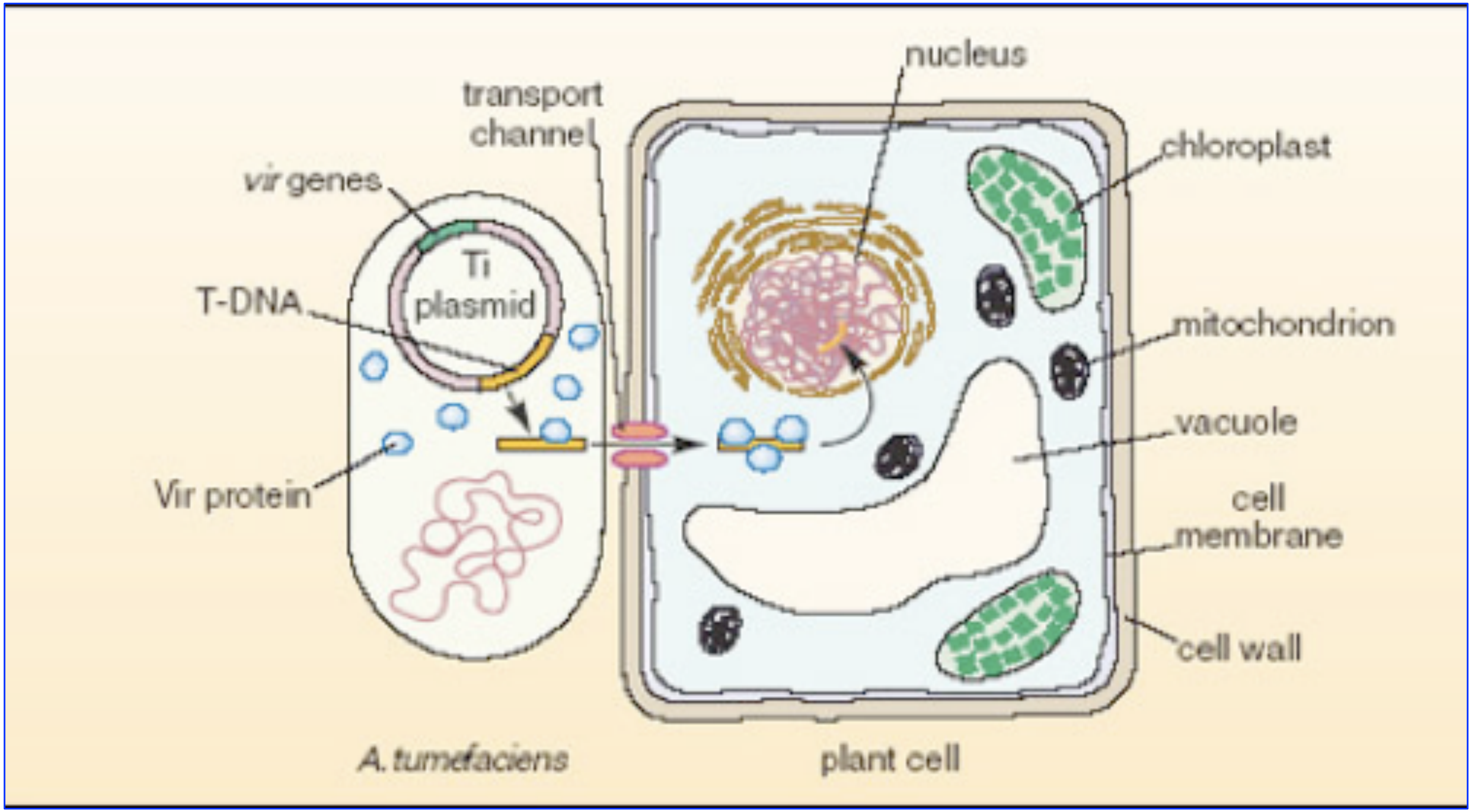
Agrobacterium-mediated DNA Transfer



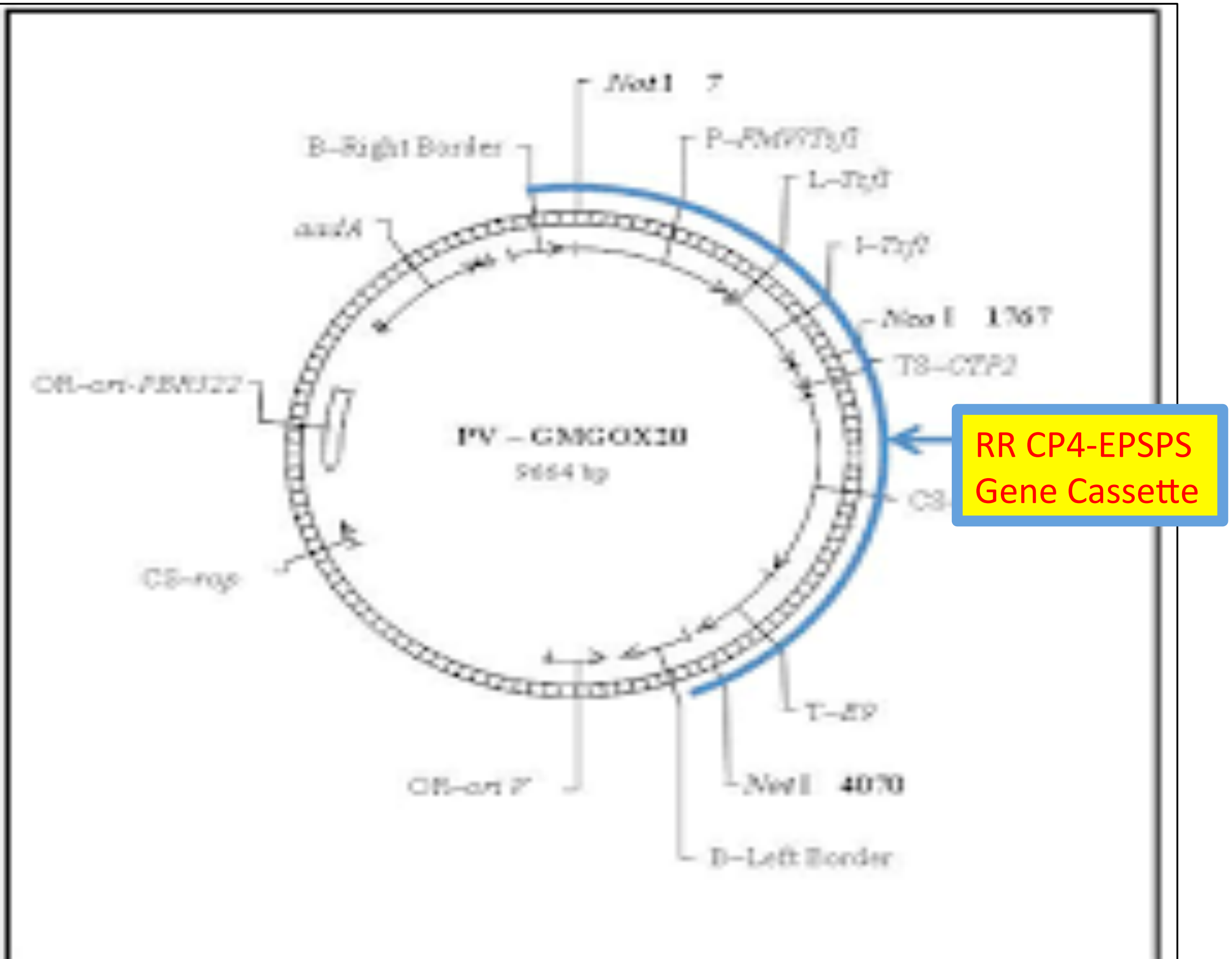
http://2010.igem.org/Team:Nevada/Agrobacterium_Transformations

Agro Gene Cassette Transfer Process "Up Close"



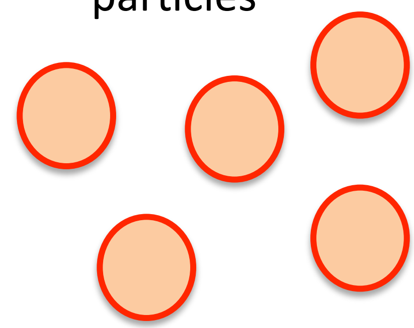
http://2010.igem.org/Team:Nevada/Agrobacterium_Transformations

Gene Gun-mediated DNA Transfer (Particle Bombardment)



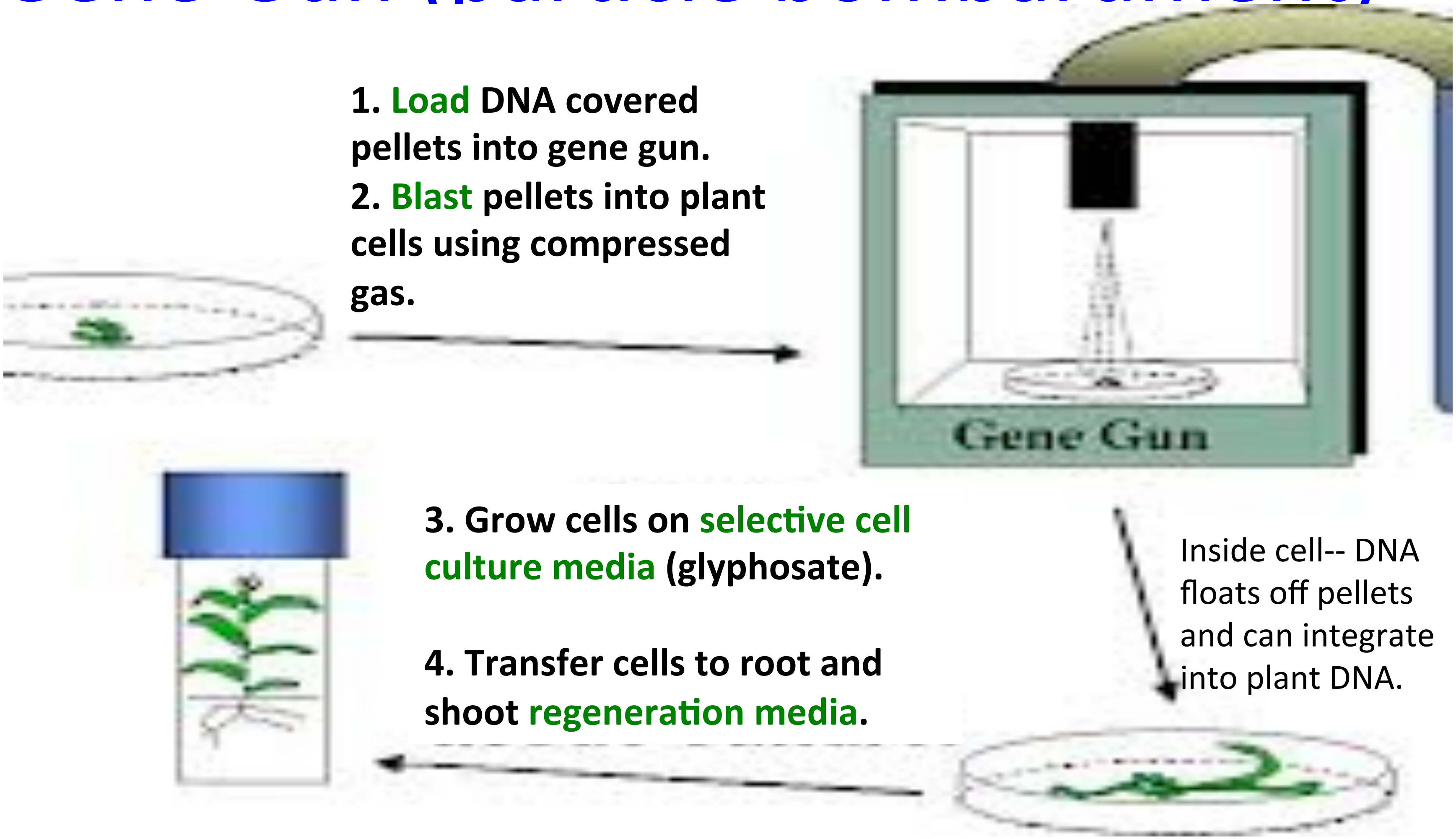
1. **Isolate** large amounts of gene cassette DNA

2. **Coat** DNA onto tiny gold or tungsten particles



3. **Shoot** particles into plant cells using a Gene Gun

Gene Gun (particle bombardment)



1. Load DNA covered pellets into gene gun.
2. Blast pellets into plant cells using compressed gas.

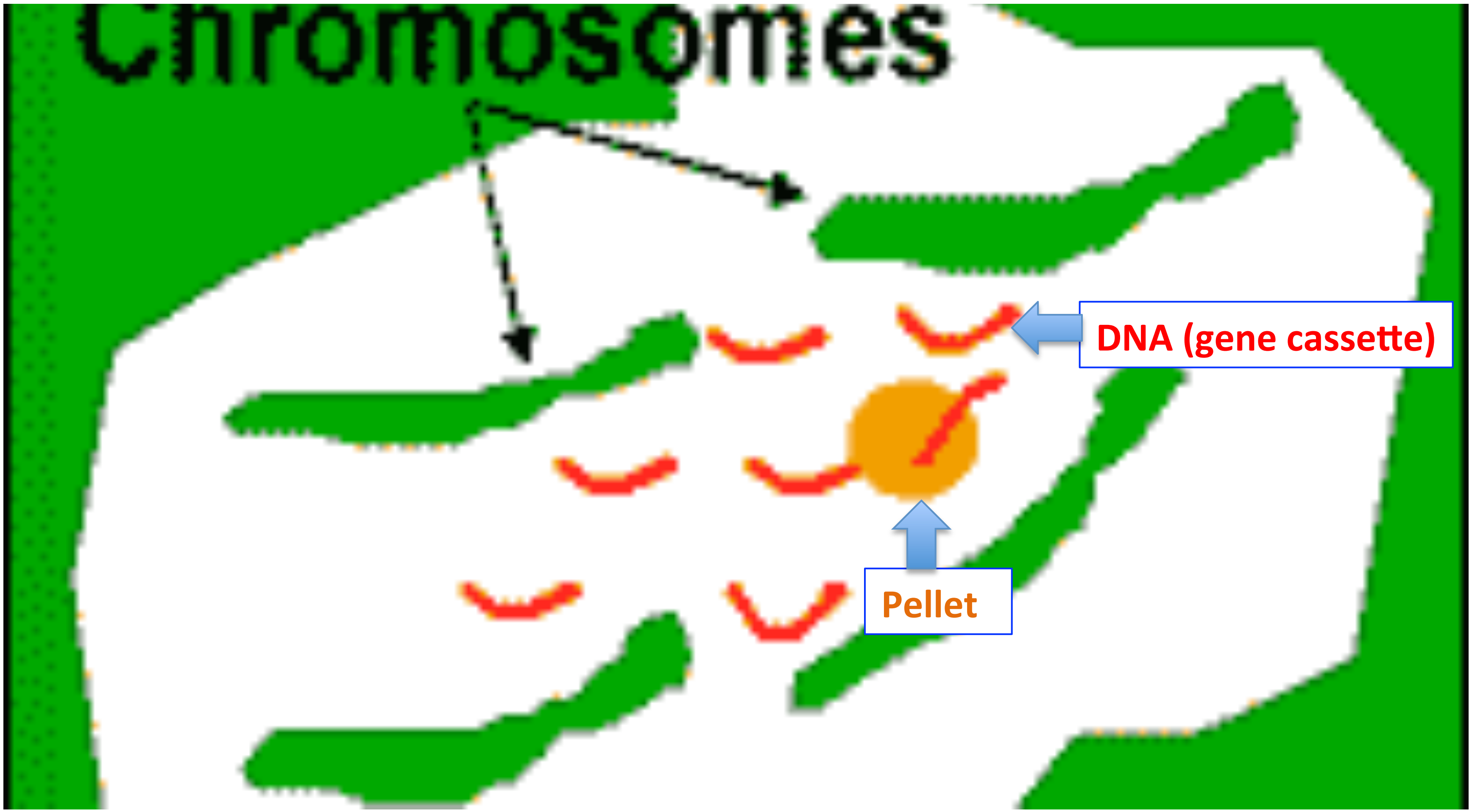
Inside cell-- DNA floats off pellets and can integrate into plant DNA.

3. Grow cells on selective cell culture media (glyphosate).

4. Transfer cells to root and shoot regeneration media.

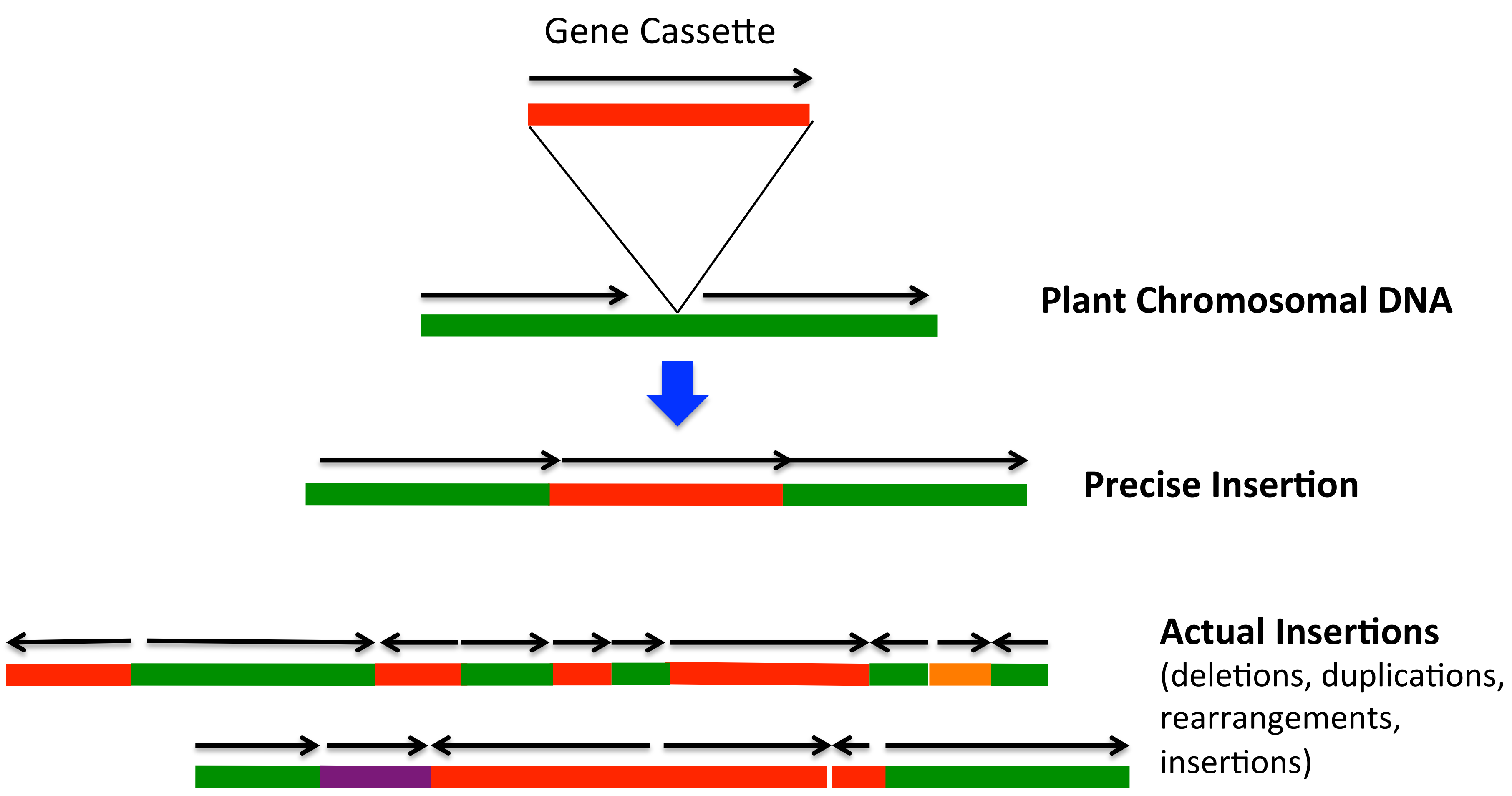
http://passel.unl.edu/pages/informationmodule.php?idinformationmodule=958077244&topicorder=3&maxto=7

Inside Cell Nucleus after Gene Gun Bombardment



http://passel.unl.edu/pages/informationmodule.php?idinformationmodule=958077244&topicorder=3&maxto=7 (on 23/09/16)

Examples of Gene Cassette Insertion Sites



Wilson, Allison K., Jonathan R. Latham, and Ricarda A. Steinbrecher. "**Transformation-induced mutations in transgenic plants: Analysis and biosafety implications**." Biotechnology and Genetic Engineering Reviews 23.1 (2006): 209-238.

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Recap: Gene Transfer is Complex and Messy

- Can't choose site of transgene insertion
  - *Agrobacterium* insertion used by researchers as a mutagen to disrupt genes intentionally

- Can't predict or prevent damage at site of transgene insertion

- Numerous Genome-wide mutations
  - Superfluous DNA insertion around the genome
  - Tissue culture, *Agro* infection, gene gun – mutagenic

What does this mean for the resulting GMOs -- and the Biotech Companies?

3. Select a GMO for commercialization

Selecting a Glyphosate Resistant GMO Wheat for Commercialization

Table 1 Large-scale transformation efficiencies for the *Agrobacterium* and biolistic transformation methods using immature embryos of wheat precultured for 4 days and glyphosate selection

Method	Number of constructs	Number of explants	Number of transgenic plants[a]	TE%[b]
Agrobacterium-mediated	30	81,253	3,354	4.4
Microparticle bombardment	17	17,729	5,97	3.4

[a] Independent transgenic event
[b] TE%, The average transformation efficiency from the total number of transgenic events divided by the total number of explants transformed

A. Infect/bombard many independent "explants" with gene cassette

(explants = e.g. pieces of leaf, stem or embryo)

(Total for Monsanto's GMO wheat: >98,800)

B. Select many independent candidate GMO plants

(Total for Monsanto's GMO wheat: >4000)

C. Carry out several generations of testing and selection

(for candidates with good GMO trait expression and acceptable crop characteristics)

Hu, T., et al. "Agrobacterium-mediated large-scale transformation of wheat (Triticum aestivum L.) using glyphosate selection." *Plant cell reports* 21.10 (2003): 1010-1019.

Select
a GMO for commercialization

Glyphosate Resistant GMO Wheat: Four Generations of Selection

Table 3 The number of transgenic events derived from *Agrobacterium* transformation and biolistic transformation of wheat and the percentage advanced by evaluation at different generations (*ae* acid equivalent)

Generation	*Agrobacterium* transformation		Biolistic transformation		Criteria for advancement
	Number of events	Percentage	Number of events	Percentage	
Number of T_0	773	100	597	100	Survival at 0.02 m*M* glyphosate in the medium
T_0 to T_1	131	17	110	18	Survival at 1.68 kg ae ha^{-1} Roundup Ultra
T_1 to T_2	19	2.5	16	2.6	>80% fertility on 3.36 kg ae ha^{-1} Roundup Ultra with single locus
T_2 to T_3	11	1.4	7	1.2	Homozygosity, single-copy insert, agronomic performance
T_3 to T_4	6	0.8	1	0.2	Clean molecular background, comprehensive agronomic performance

1. Selection for "normal" looking candidate plants with good GMO trait expression

(>1300 T_0 selected for further testing/~2700 T_0 discarded)

2. More intensive selection for good GMO trait expression

(241 T_1 selected for further testing/~1060 T_1 discarded)

3. Even more intensive selection for good GMO trait expression AND >80% fertility

(35 selected for further testing/ 206 T_2 discarded)

4. One copy of gene cassette inserted, Homozygous, good agronomic performance

(17 T_3 selected for further testing/ 18 T_3 discarded)

5. Molecular analysis, further agronomic tests

(7 T_4 selected / 10 T_4 discarded)

1 candidate (#33391) was chosen as a Roundup Ready wheat commercial candidate.

Does Selection Ensure GMOs Behave As Expected?

NO

Numerous unexpected effects have been documented in commercial lines

e.g. RR 2 Yield Soybean is 5% shorter than the non-GMO parent

Unintended Consequences Are Important

Commercial Transgenic Events	Unintended Consequence	Reference
RR Soybean (Glyphosate^R) 40-3-2 **RR Rice (Glufosinate^R)** LLRice601 LLRice06 LLRice62	**7-11% Yield Decrease** **(> 210 Million Bushels Lost pa from 3 Billion pa US Soybean Crop)** **3-7% Yield Decrease**	USDA 93-258-01p Elmore et al. 2001 Nelson et al. 2002 USDA 06-234-01p USDA 98-329-01p USDA 06-234-01p
Bt+ Paymaster Cotton 1560 BG **Bt+ Atlantic Newleaf Potato**	**Loss of Nematode Resistance** **Loss of Golden Nematode Resistance**	Colyer et al. 2000 Brodie 2003
Squash CZW-3 Virus Resistance	▪**Iron 87% of control** ▪**Fat 50% of control** ▪**Beta-Carotene 1.5% of control** ▪**Vitamin A increased 2-fold** ▪**Sodium increased 4-fold**	USDA 95-352-01p

Documented Unintended Effects of Genetic Engineering with Health or Agronomic Implications

Harmful or Potentially Harmful Unintended Effects	Some Commercial Examples from the Scientific Literature (references available)
Altered Nutrition (*nutrients:* e.g. vitamins, minerals, antioxidants or *antinutrients*: e.g. lectins, protease inhibitors)	• Squash (Virus-R): e.g. 67.6 times less Beta Carotene • Maize (and other commercial Bt crops): higher lignin
Increased Toxicity (novel or increased toxins or allergens, high pesticide residues)	• Soybeans now routinely contain extreme levels of Roundup and AMPA (its breakdown product) • Bt maize: systemic expression of (Bt toxin +neonic) + additional sprays • Maize (Bt) Mon810: newly expressed allergen
Non-target effects (organisms in bird food chain are harmed due to unintended effects of genetically engineered crop)	• Maize (Bt) harmful effects on earthworm, beetles • Various GMO crops used in animal feeding studies show harmful effects (Krimsky review 2015)
Agronomic effects (Can lead to higher pesticide or herbicide use by farmers)	• Bt Cotton and Bt potato: lost nematode resistance • Bt Maize increased susceptibility to aphids and decreased mycorrhizal levels • RR2Yield Soybean: decreased height • RR Soybean: decreased yield, Manganese deficiency

Why are GMOs Especially Prone to Harmful UEs?

GE Technique or Trait	Risk (well-documented – refs. available)
Plant transformation: *Agrobacterium* infection or Gene Gun; random transgene insertion; cell culture	All are highly mutagenic procedures
Transferred genes are often composed of DNA from other species (bacterial, viral, fungal)	Different mRNA or protein processing can alter the transgene product's toxicity, allergenicity or behavior
Viral promoters are widely used	1. High expression leads to imbalances (eg. Altered protein profile) 2. Viral promoters encode viral proteins (potential toxins)
RNAi technology: Suppress expression of genes in crop plant or in targeted "pest" organism	1. Imprecise (off-target effects) 2. The ds-RNAs produced are PAMPS known to stimulate vertebrate immune responses
Herbicide tolerance and Pesticidal traits are most common	1. Increased exposure of consumers to herbicide and pesticide toxins 2. Result in altered composition (biochemistry) of the GE plant

Conclusion

1. The methods used to make a GMO are imprecise, unpredictable and highly mutagenic.

2. Commercial GMOs are highly prone to showing unintended effects.

3. These risks are ignored by GMO safety regulators and have urgent implications for
    - Food safety
    - Crop failure
    - Biodiversity loss

For more examples of unintended effects of GMOs collected from the scientific literature see:
Nature Institute Website *http://natureinstitute.org/nontarget/report_class.php*

https://bioscienceresource.org/

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Ithaca Event: Join NOFA-NY to Create a New York Organic Action Plan on April 7, 2016

By admin | Published: March 14, 2016

Welcome

The Bioscience Resource Project provides scientific and intellectual resources for a healthy future. It publishes Independent Science News, a media service devoted to food and agriculture, and their impacts on health and the environment. It also offers resources for scientists and educators and internships and training for students. Through its innovative scientific journalism and original biosafety review articles, the project provides unique and revealing perspectives on issues that are fundamental to the survival of people and the planet. The project does not accept advertising or corporate funding and is a non-profit 501(c)3 organization. It is completely dependent on individual donations.

We invite you to join the Project as a contributor or a donor.

Quote of the Day

> *With respect to phenomena like mass extinction, somebody might say-- why worry about it because in a geological perspective mass extinctions aren't so bad, they wipe out some things and then 10 million years down the road we get new and interesting objects. But I tell you mass extinctions are really awful for folks caught in the midst of them.*