http://hpt.faperta.ugm.ac.id/suputa/

Entomologi Molekuler (PCR, Sequensing DNA “Blast Gen Bank”, Tanaman Transgenik)

Kuliah Entomologi Dasar

http://www.faperta.ugm.ac.id/perlintan2005/puta.htm

PCR

Polymerase Chain Reaction

Teknik atau metode perbanyakan DNA secara enzimatik melalui reaksi berantai polimerase tanpa menggunakan organisme

Polymerase chain reaction (PCR)

Proses Mendapatkan Basa DNA Lalat Buah

- **Ekstraksi DNA akan mendapatkan Genomic DNA**
- **Elektroforesis Genomic DNA untuk memastikan ekstraksi DNAnya berhasil**
- **PCR dalam rangka melipat-gandakan DNA yang dikehendaki berdasar pada primer**
- **Purifikasi PCR product untuk membersihkan DNA hasil PCR dari komponen lain (sisa primer, dNTP, Taq polymerase, $MgCl_2$, dll.)**
- **Check kualitas dan kuantitas DNA**
- **Sequencing DNA**

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Proses Mendapatkan Basa DNA Lalat Buah

- **Ekstraksi DNA akan mendapatkan Genomic DNA**

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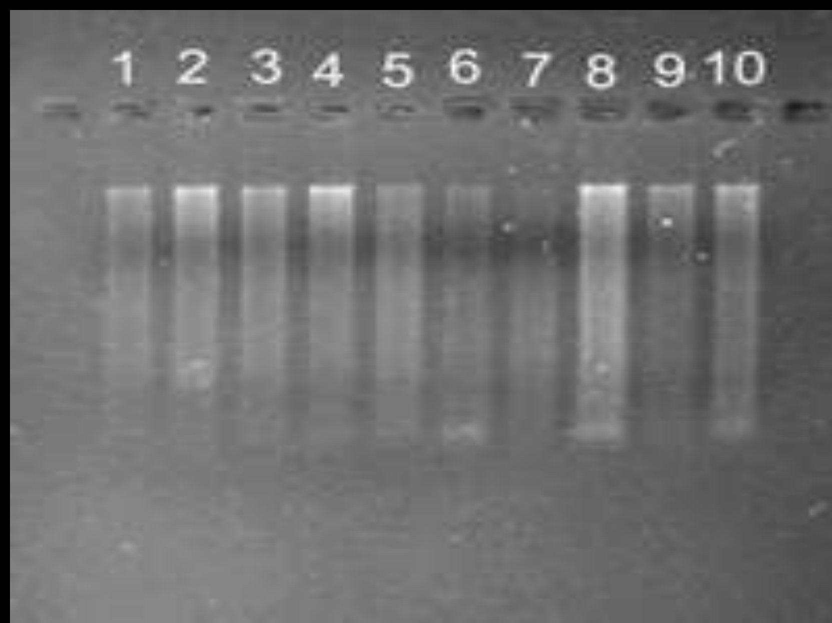
Proses Mendapatkan Basa DNA Lalat Buah

- **Elektroforesis Genomic DNA untuk memastikan ekstraksi DNAnya berhasil**



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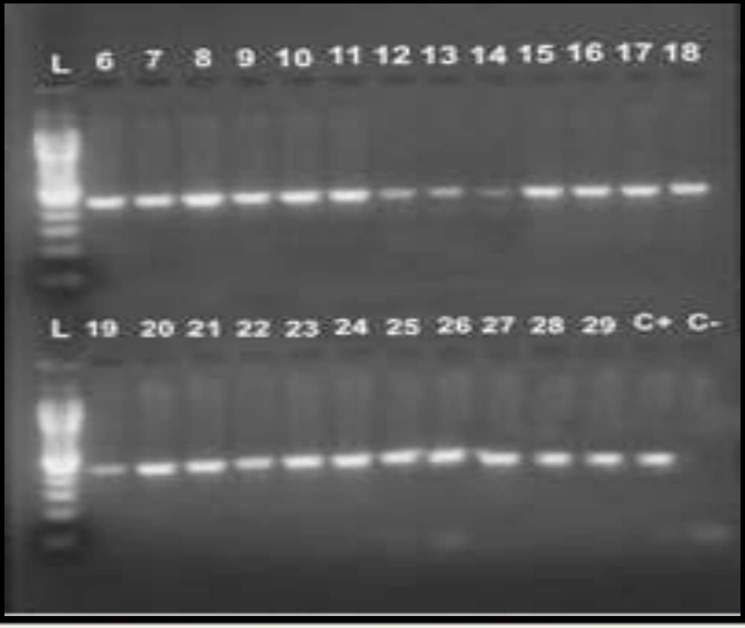
Proses Mendapatkan Basa DNA Lalat Buah

- **PCR dalam rangka melipat-gandakan DNA yang dikehendaki berdasar pada primer**



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Proses Mendapatkan Basa DNA Lalat Buah

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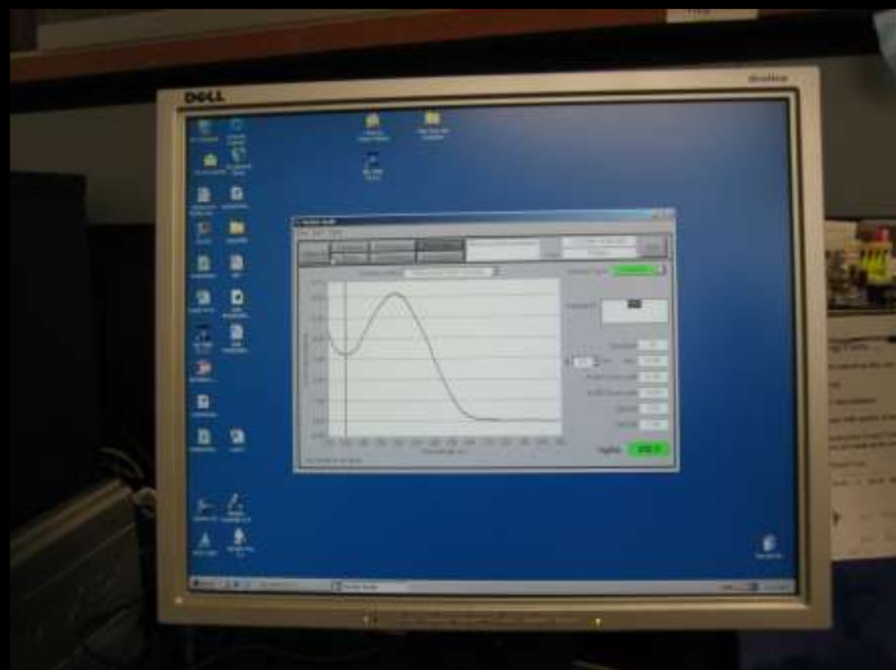
Proses Mendapatkan Basa DNA Lalat Buah

- **Check kualitas dan kuantitas DNA**



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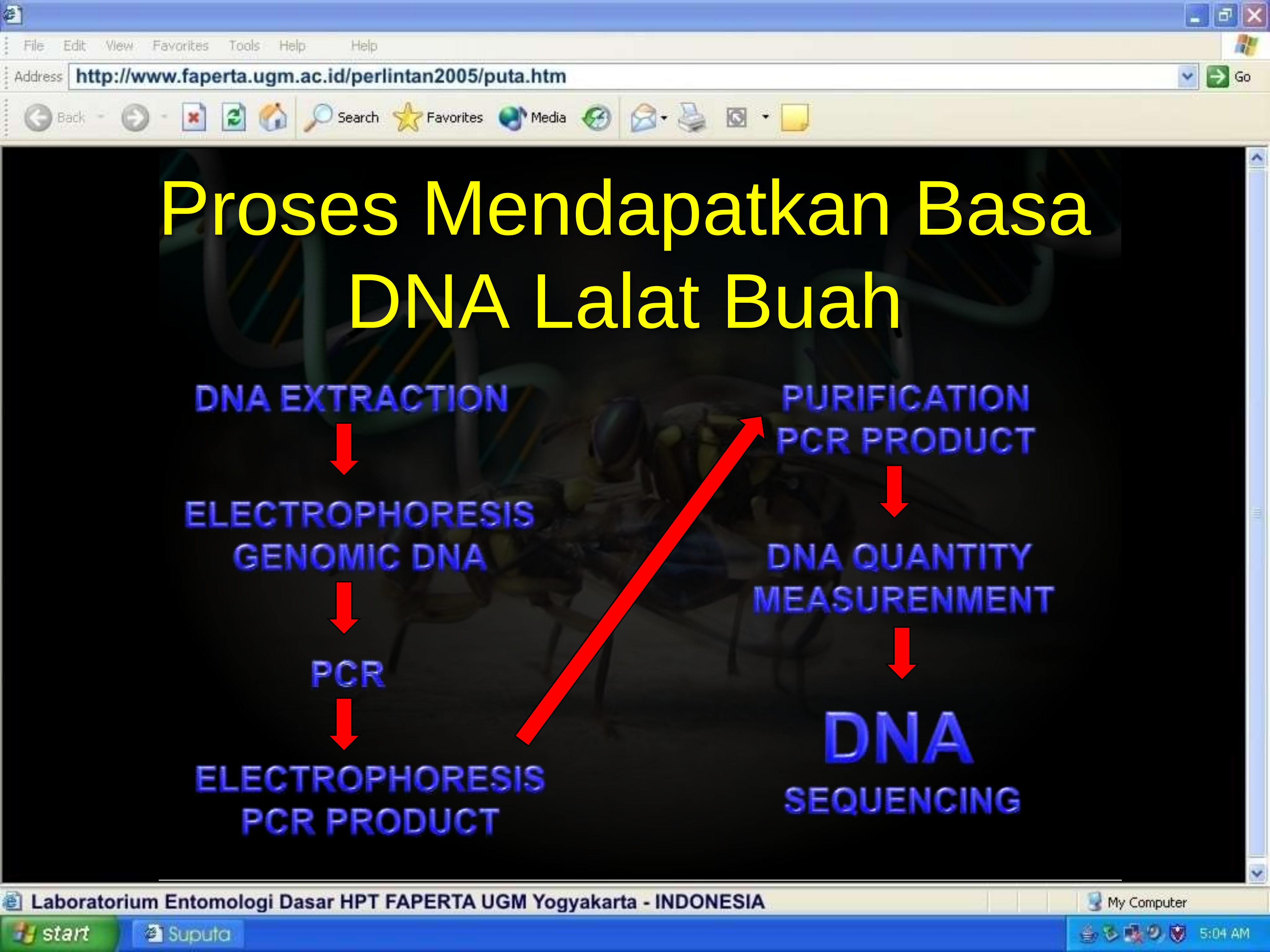
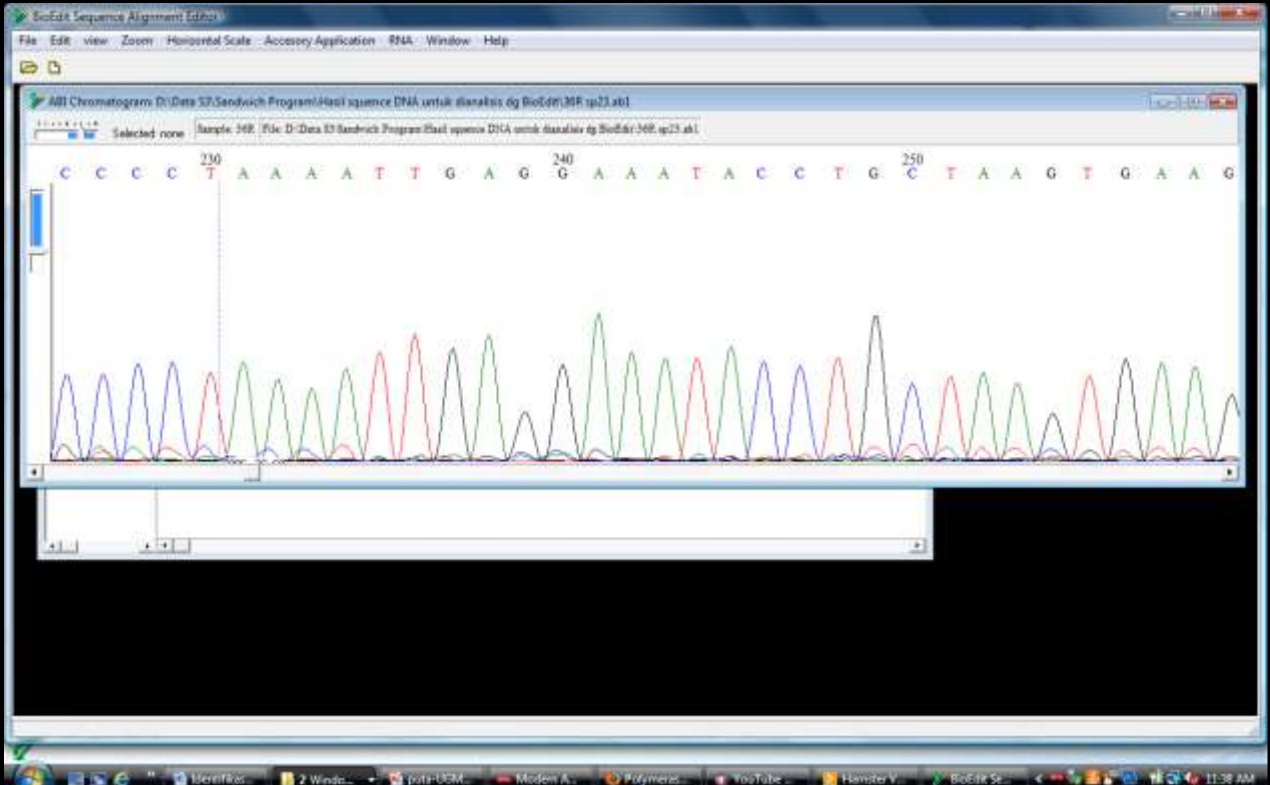
Proses Mendapatkan Basa DNA Lalat Buah



Urutan Proses Mendapatkan Urutan Basa DNA

- **Sequencing DNA**



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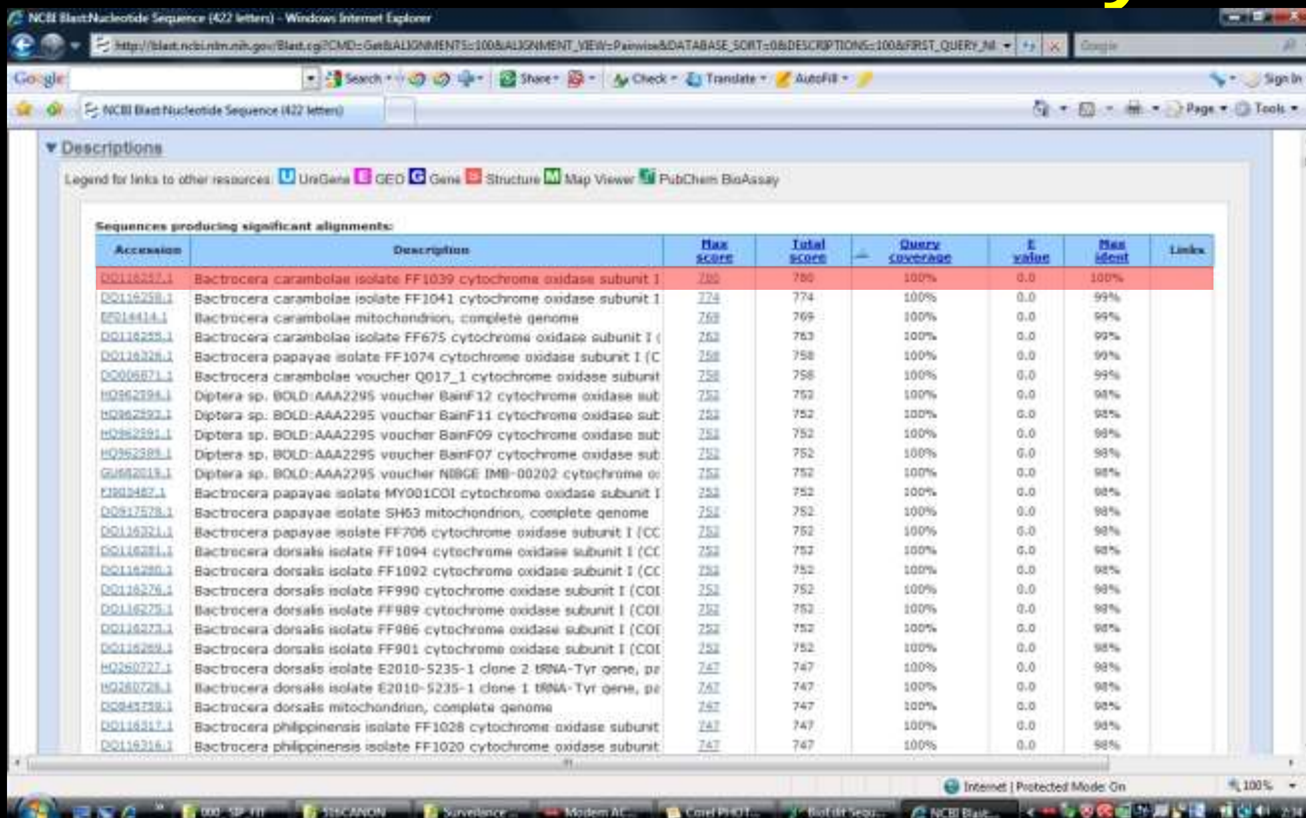
Identifikasi Lalat Buah berdasarkan DNAnya



Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
DQ116257.1	Bactrocera carambolae isolate FF1039 cytochrome oxidase subunit I	780	780	100%	0.0	100%	
DQ116258.1	Bactrocera carambolae isolate FF1041 cytochrome oxidase subunit I	774	774	100%	0.0	99%	
EF014414.1	Bactrocera carambolae mitochondrion, complete genome	769	769	100%	0.0	99%	
DQ116255.1	Bactrocera carambolae isolate FF675 cytochrome oxidase subunit I (	763	763	100%	0.0	99%	
DQ116326.1	Bactrocera papayae isolate FF1074 cytochrome oxidase subunit I (C	758	758	100%	0.0	99%	
DQ006871.1	Bactrocera carambolae voucher Q017_1 cytochrome oxidase subunit	758	758	100%	0.0	99%	
HQ962394.1	Diptera sp. BOLD:AAA2295 voucher BainF12 cytochrome oxidase sub	752	752	100%	0.0	98%	
HQ962392.1	Diptera sp. BOLD:AAA2295 voucher BainF11 cytochrome oxidase sub	752	752	100%	0.0	98%	
HQ962391.1	Diptera sp. BOLD:AAA2295 voucher BainF09 cytochrome oxidase sub	752	752	100%	0.0	98%	
HQ962389.1	Diptera sp. BOLD:AAA2295 voucher BainF07 cytochrome oxidase sub	752	752	100%	0.0	98%	
GU682019.1	Diptera sp. BOLD:AAA2295 voucher NIBGE IMB-00202 cytochrome o	752	752	100%	0.0	98%	
FJ903487.1	Bactrocera papayae isolate MY001COI cytochrome oxidase subunit I	752	752	100%	0.0	98%	
DQ917578.1	Bactrocera papayae isolate SH63 mitochondrion, complete genome	752	752	100%	0.0	98%	
DQ116321.1	Bactrocera papayae isolate FF706 cytochrome oxidase subunit I (CC	752	752	100%	0.0	98%	
DQ116281.1	Bactrocera dorsalis isolate FF1094 cytochrome oxidase subunit I (CC	752	752	100%	0.0	98%	
DQ116280.1	Bactrocera dorsalis isolate FF1092 cytochrome oxidase subunit I (CC	752	752	100%	0.0	98%	
DQ116276.1	Bactrocera dorsalis isolate FF990 cytochrome oxidase subunit I (COI	752	752	100%	0.0	98%	
DQ116275.1	Bactrocera dorsalis isolate FF989 cytochrome oxidase subunit I (COI	752	752	100%	0.0	98%	
DQ116273.1	Bactrocera dorsalis isolate FF986 cytochrome oxidase subunit I (COI	752	752	100%	0.0	98%	
DQ116269.1	Bactrocera dorsalis isolate FF901 cytochrome oxidase subunit I (COI	752	752	100%	0.0	98%	
HQ260727.1	Bactrocera dorsalis isolate E2010-5235-1 clone 2 tRNA-Tyr gene, pa	747	747	100%	0.0	98%	
HQ260728.1	Bactrocera dorsalis isolate E2010-5235-1 clone 1 tRNA-Tyr gene, pa	747	747	100%	0.0	98%	
DQ845759.1	Bactrocera dorsalis mitochondrion, complete genome	747	747	100%	0.0	98%	
DQ116317.1	Bactrocera philippinensis isolate FF1028 cytochrome oxidase subunit	747	747	100%	0.0	98%	
DQ116316.1	Bactrocera philippinensis isolate FF1020 cytochrome oxidase subunit	747	747	100%	0.0	98%	

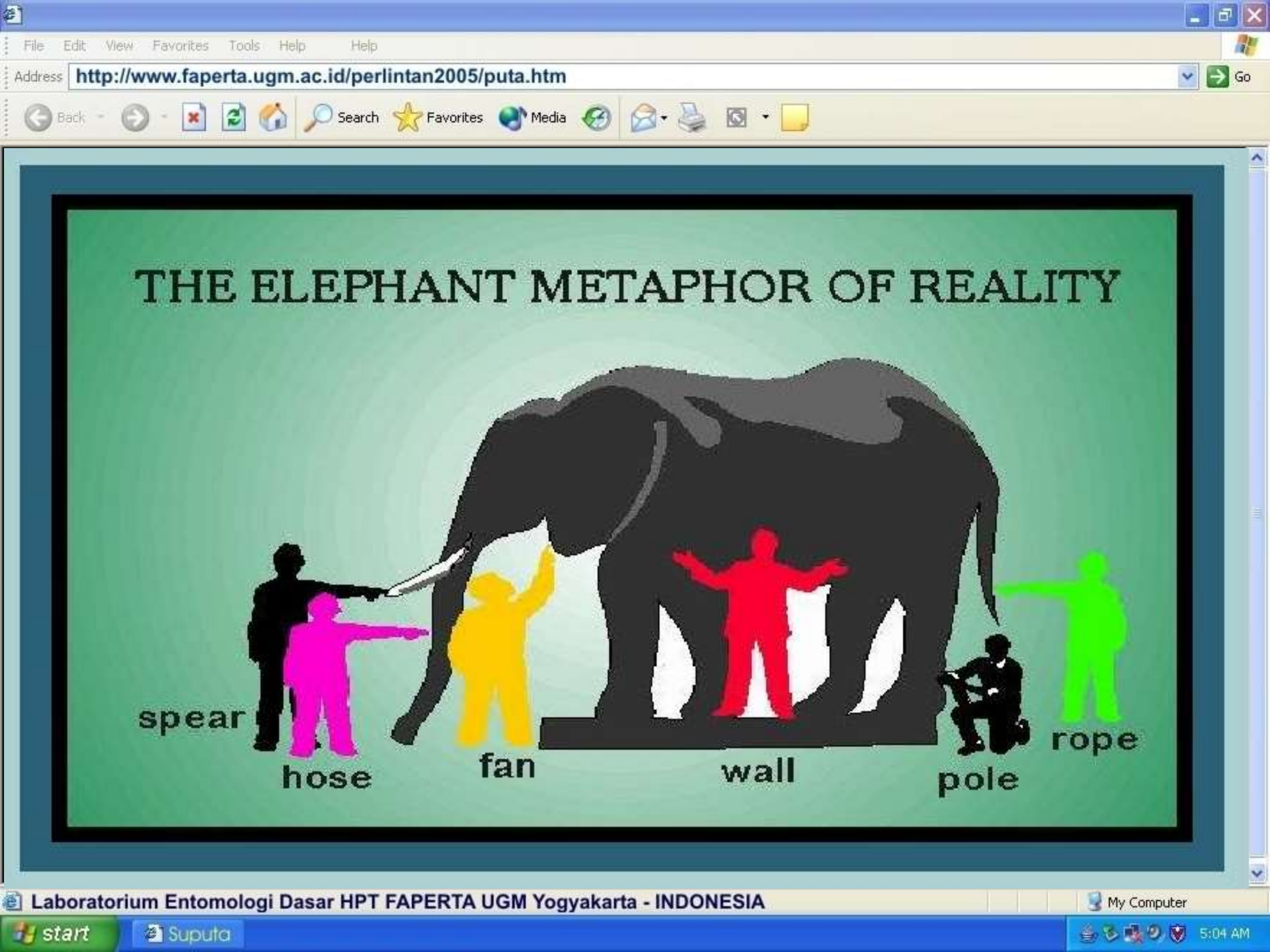
GMOs

Genetically Modified Organisms

By

SUPUTA

JURUSAN HAMA & PENYAKIT TUMBUHAN
FAKULTAS PERTANIAN
UNIVERSITAS GADJAH MADA


http://www.faperta.ugm.ac.id/perlintan2005/puta.htm
THE ELEPHANT METAPHOR OF REALITY
spear
hose
fan
wall
pole
rope
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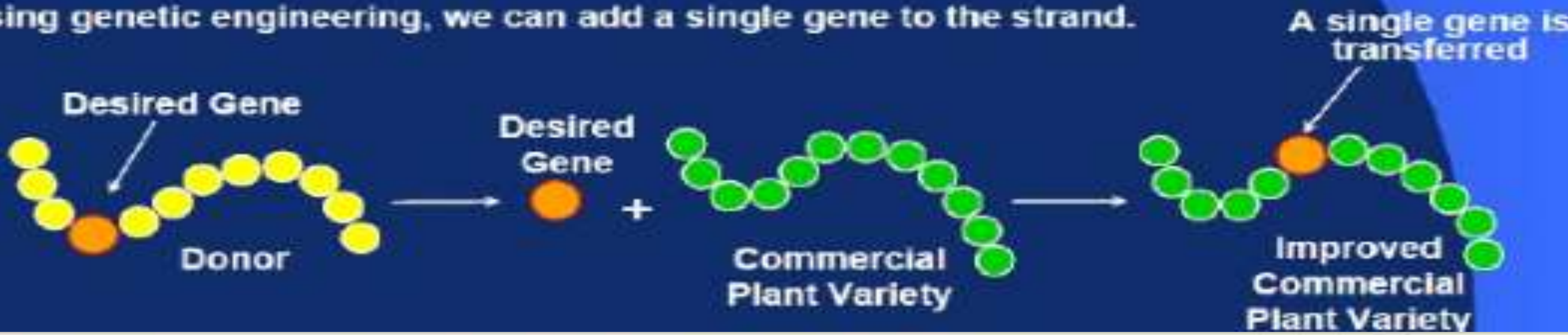
Genetic Engineering is an Extension of Traditional Plant Breeding

TRADITIONAL PLANT BREEDING

DNA is a strand of genes, much like a strand of pearls. Traditional plant breeding combines many genes at once.

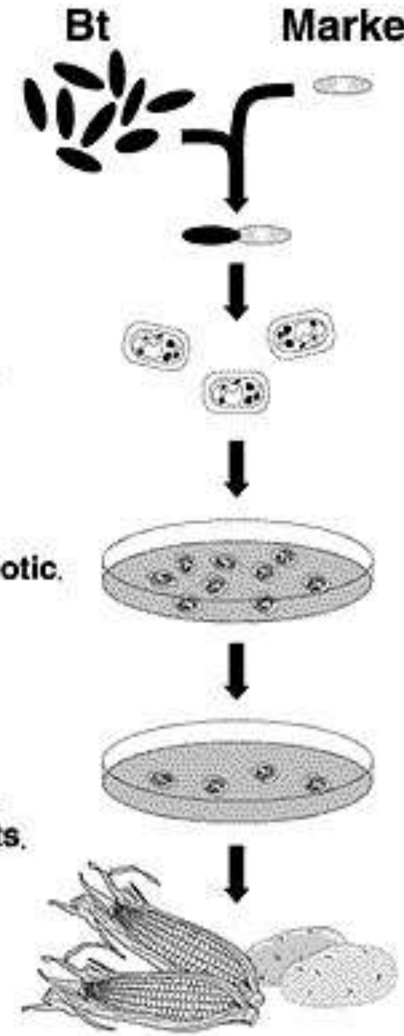
GENETIC ENGINEERING

Using genetic engineering, we can add a single gene to the strand.



INTRODUCTION

1. The Bt gene that produces the desired lethal protein is joined to a marker gene for antibiotic resistance.
2. Bt gene + marker is inserted into plant cells.
3. Plant cells are grown in the presence of antibiotic.
4. Cells that carry the Bt gene + antibiotic resistance gene survive and are grown into plants.



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INTRODUCTION

Mechanism of *Agrobacterium*

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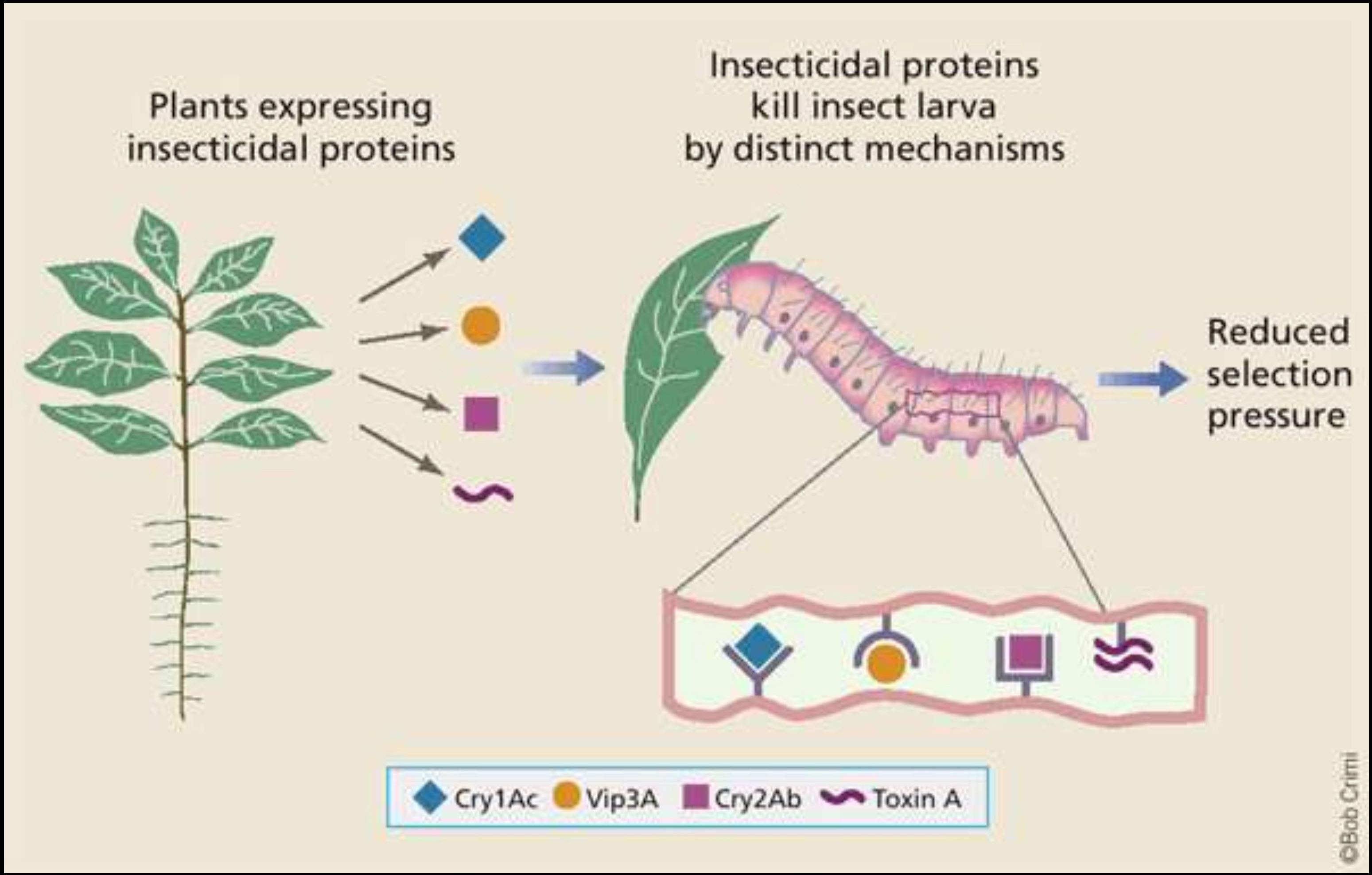
INSECT KILLED BY Bt



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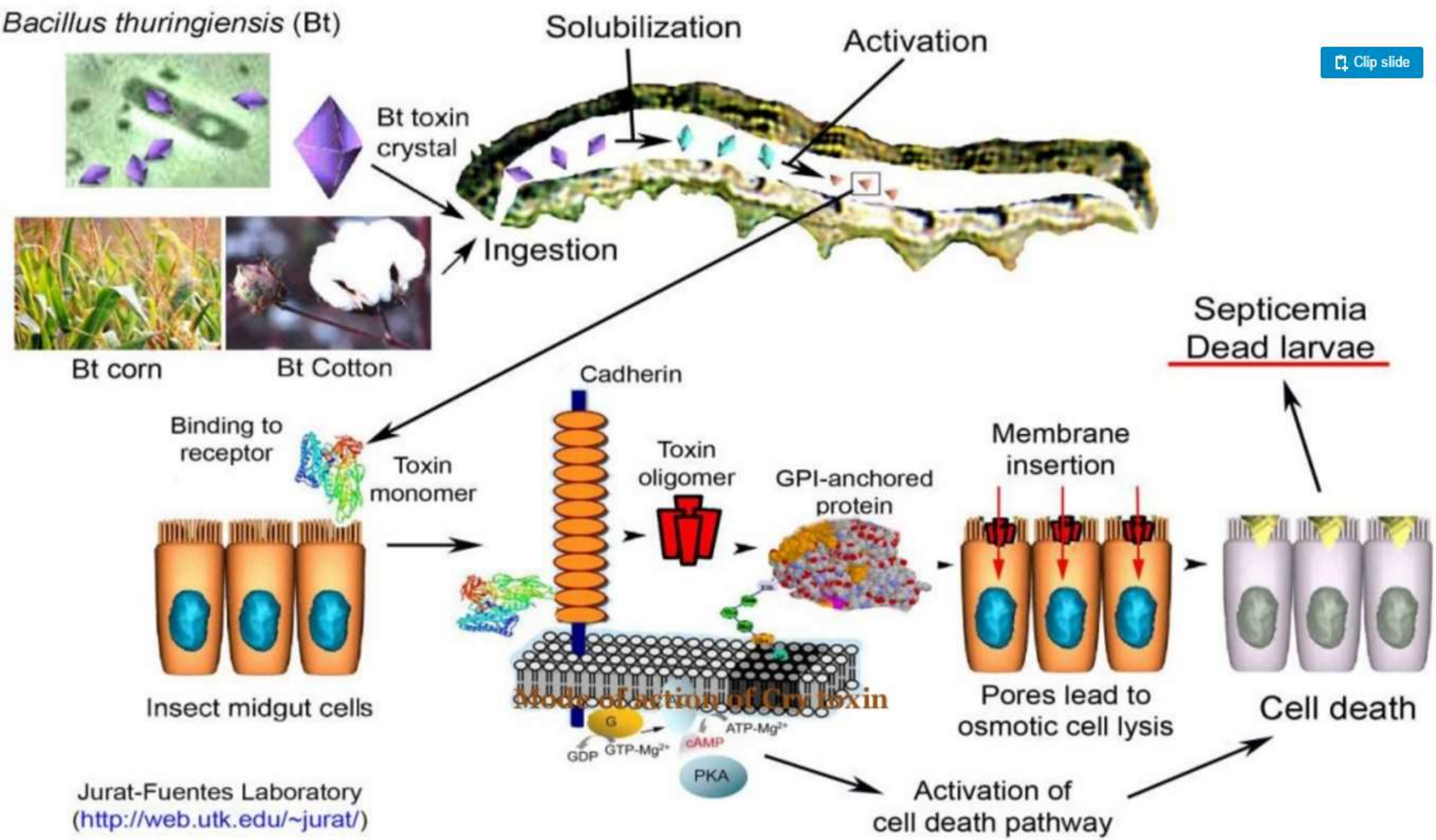

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Selective action of *Bt* in insects

Ingestion (occurs while feeding plant tissues)

Solubilization (Alkalinity)

Activation (pH >9.5)

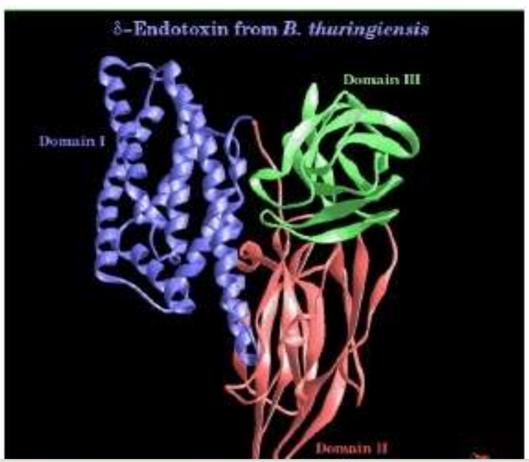
Binding (Specific receptor)

Insertion (Damage gut wall)

Pore formation

Cell lyses

D e a t



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Crystal protein of *Bacillus thuringiensis* and their specificity

Crystal proteins	Order(s) specific
Cry-I	Lepidoptera
Cry-II	Lepidoptera & Diptera
Cry-III	Coleoptera
Cry-IV	Diptera
Cry-V	Lepidoptera & Coleoptera

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Transgenic plants expressing foreign gene for insect resistance

Crop	Foreign gene	Origin of gene	Target Insect Pest (s)
Cotton	*Cry1Ab, Cry1Ac, Cry2Ab*	*Bacillus thuringiensis*	*Helicoverpa zea* (Boddie) *Spodoptera exigua* (Hubner) *Trichoplusia ni* (Hubner)
Brinjal	*CryIIIb*	*B. thuringiensis*	*Leptinotarsa decemlineata* (say)
Maize	*Cry1Ab*	*B. thuringiensis*	*Ostrinia nubilalis* (Hubner)
Rice	*Corn cystatin (cc)*	Corn	*Sitophilus zeamais* (Motschulsky)
	Pin 2	Potato	*Chilo suppressalis* (Walker)
	CpTi	Cowpea	*C. suppressalis*
	Cry1Ab	*B. thuringiensis*	*C. suppressalis, Cnaphalocrosis medinalis* (Guenee), *Scirpophaga incertulas* (Walker)

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Contd..

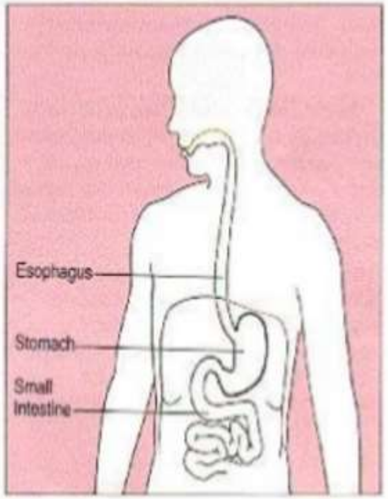
Crop	Foreign gene	Origin of gene	Target Insect Pest (s)
Potato	*Cry1Ab*	*B. thuringiensis*	*Phthorimaea operculella* (Zeller)
	Oryza cystatin 1 (oc1)	Rice	*L. decemlineata*
Sugarcane	*Cry1Ab*	*B. thuringiensis*	*Diatraea sachharalis* (Fabricius)
Tobacco	*Cry1Ab*	*B. thuringiensis*	*Heliothis virescens* (Fabricius)
	α-ai	Pea	*Tenebrio molitor* (Linnaeus)
	CpTi	Cowpea	*H. virescens, Manduca sexta* (L.)
Tomato	*Cry1Ac*	*B. thuringiensis*	*M.Sexta*
	B.t. (k)	*B.thuringiensis*	*H.zea, M.sexta, Keifera lycopersicella* (Walsingham)

Comparison of breeding and transgenic technology

Breeding	Transgenic Technology
Exchange of genes within a species	No barrier
Gene of interest with flanking sequence transferred	Only Gene of Interest
Simple Technology	Intensive Technology

Bt has no toxic impact in higher animals (HUMAN) because of…..

- Acidic stomach
- Very low pH (∽1.5 in humans)
- Absence of required receptors

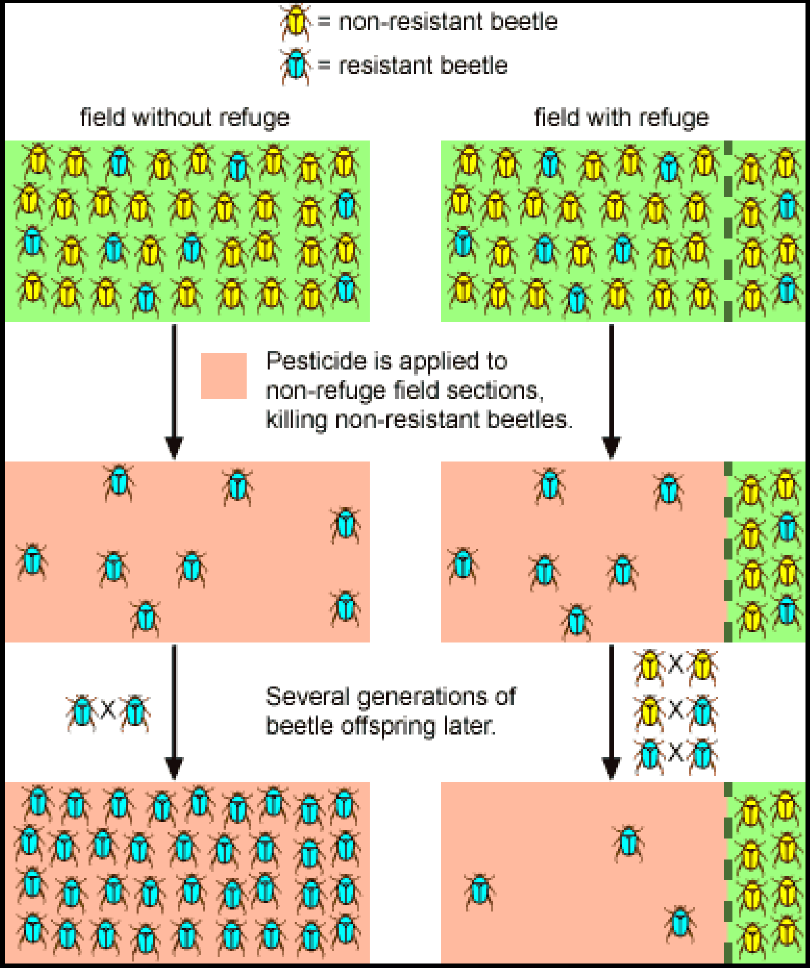


Bt is safe to non-target organisms-HUMAN

http://www.faperta.ugm.ac.id/perlintan2005/puta.htm

http://evolution.berkeley.edu/evolibrary/article/agriculture_04

THE PROBLEM IS INSECT RESISTANT



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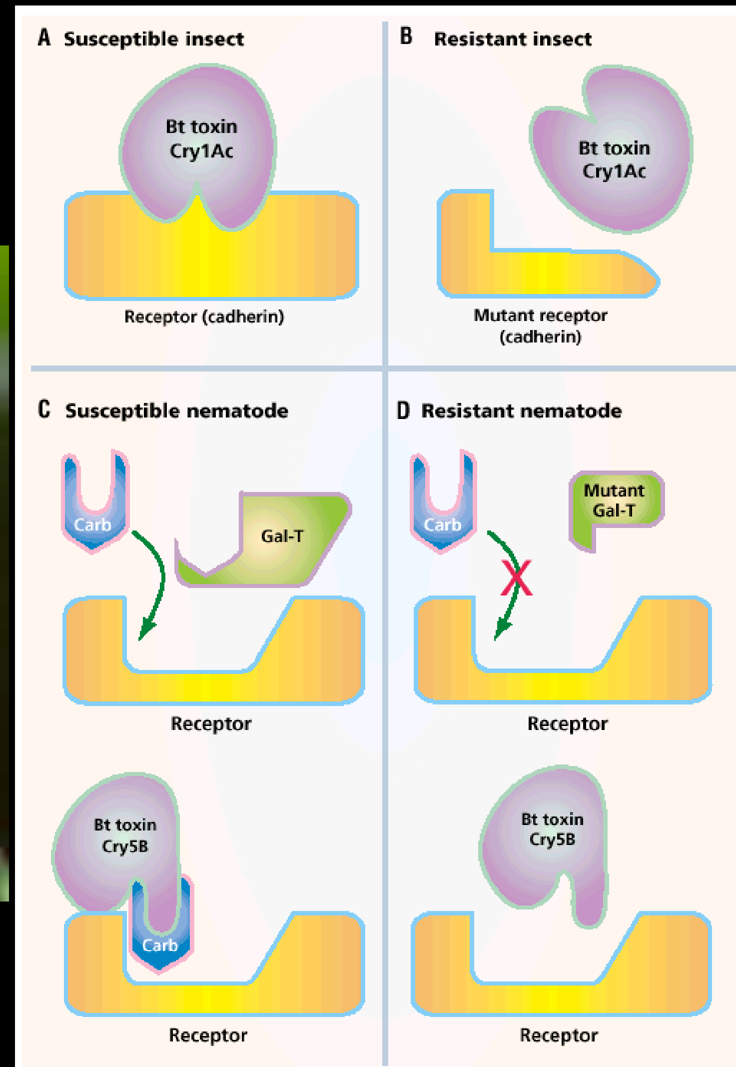
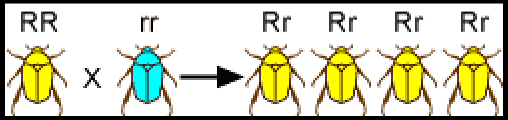
INSECT RESISTANT TO Bt



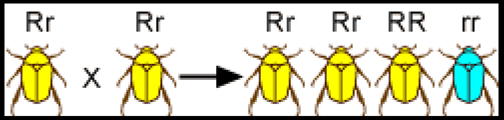
SOLVE THE PROBLEM

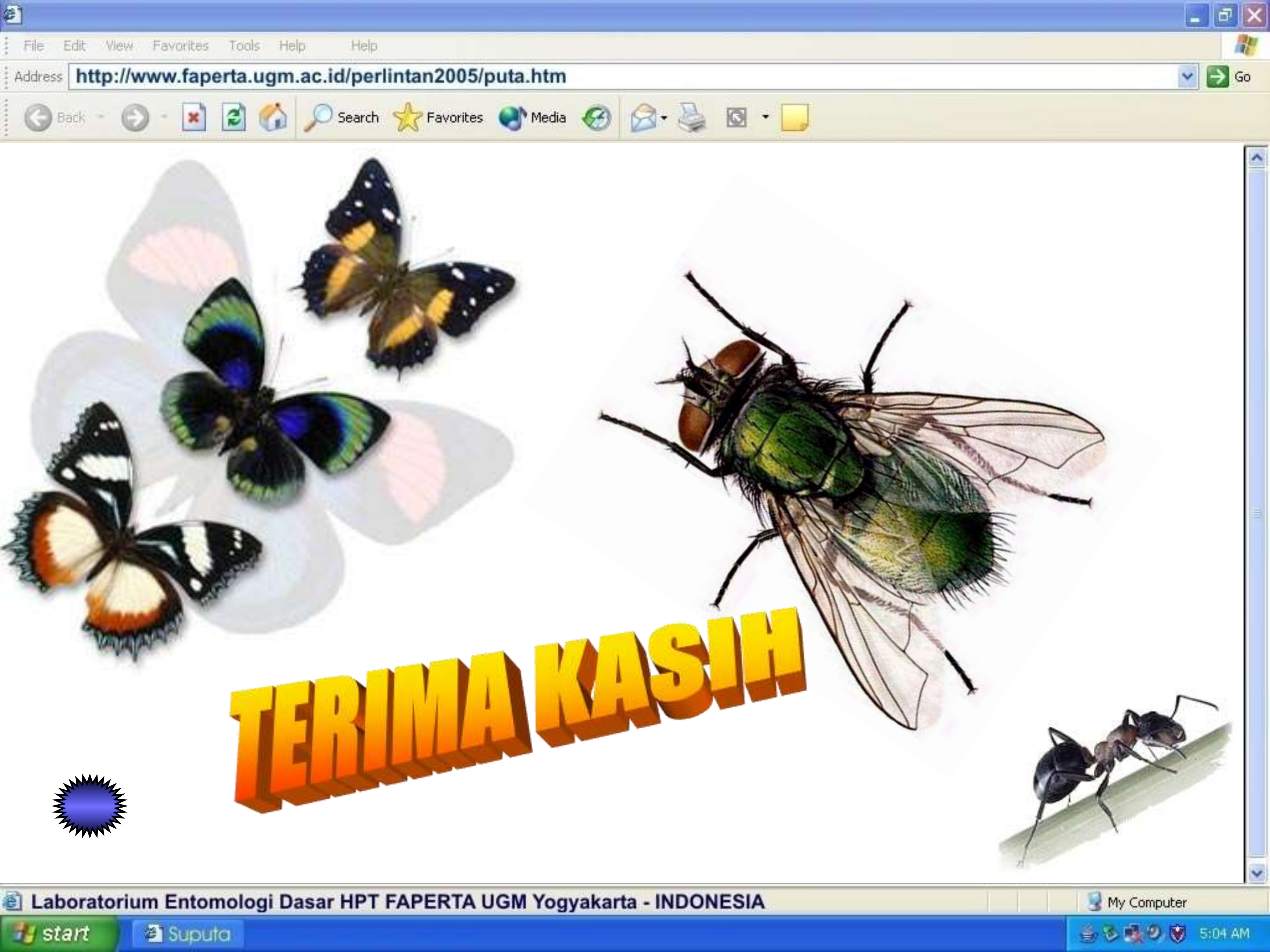
Refugia → the non-resistant insects survive. The allele for Bt resistance happens to be recessive = that means that the resistant allele can be masked by the dominant non-resistant allele.

So if a resistant insect (rr) surviving in the Bt-producing field mates with a non-resistant insect (RR) surviving in the refuge, all of their offspring will be non-resistant (Rr).



When two heterozygous pests mate, only one in four offspring (on average) will be homozygous recessive (rr) and therefore resistant to the pesticide.

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