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NGS and Bioinformatics for characterisation of unauthorized GMOs

Mauro Petrillo

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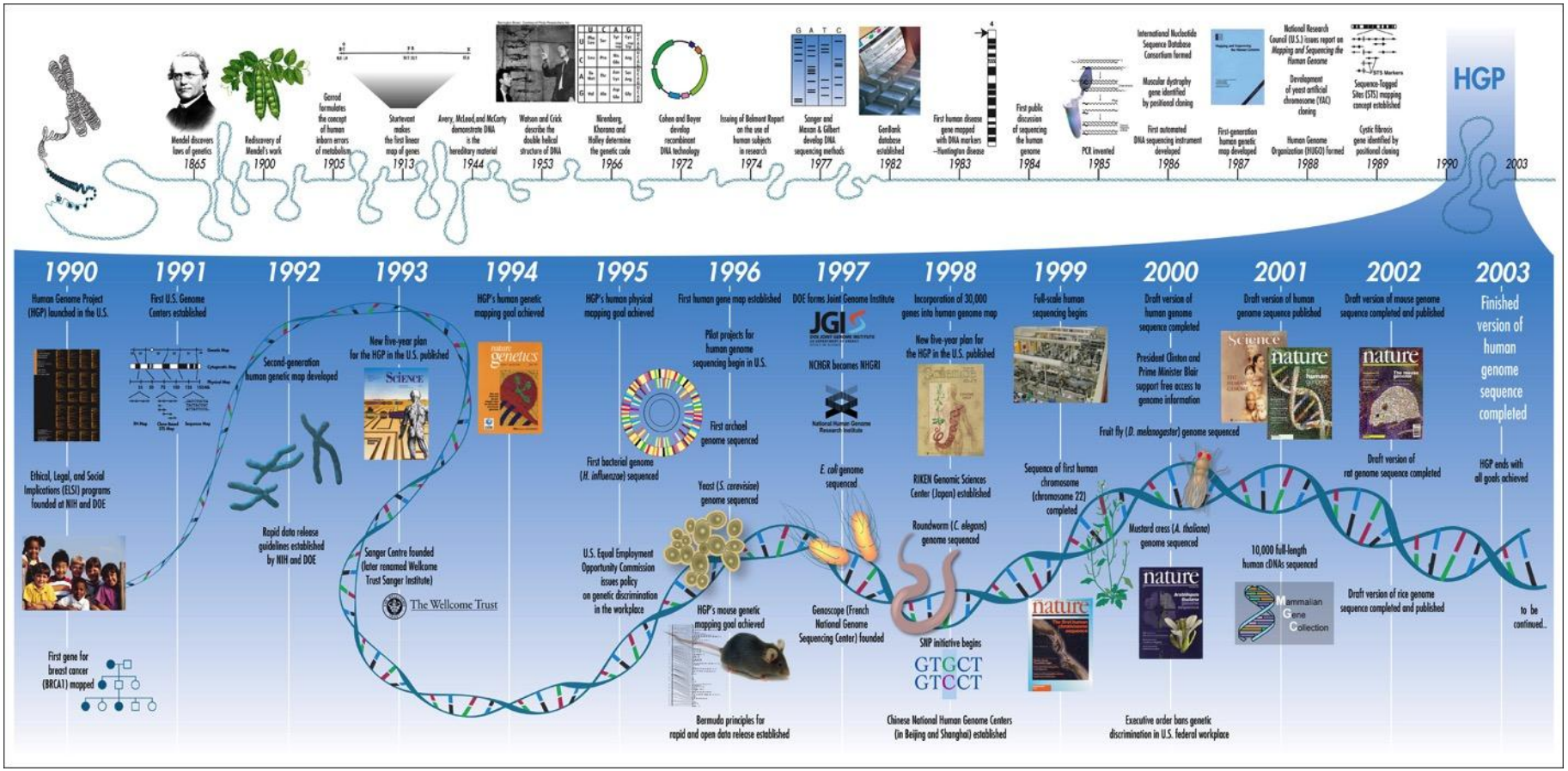
The Human genome project. What happened next?



From: Collins FS et al. ***A vision for the future of genomics research.*** Nature, 2003

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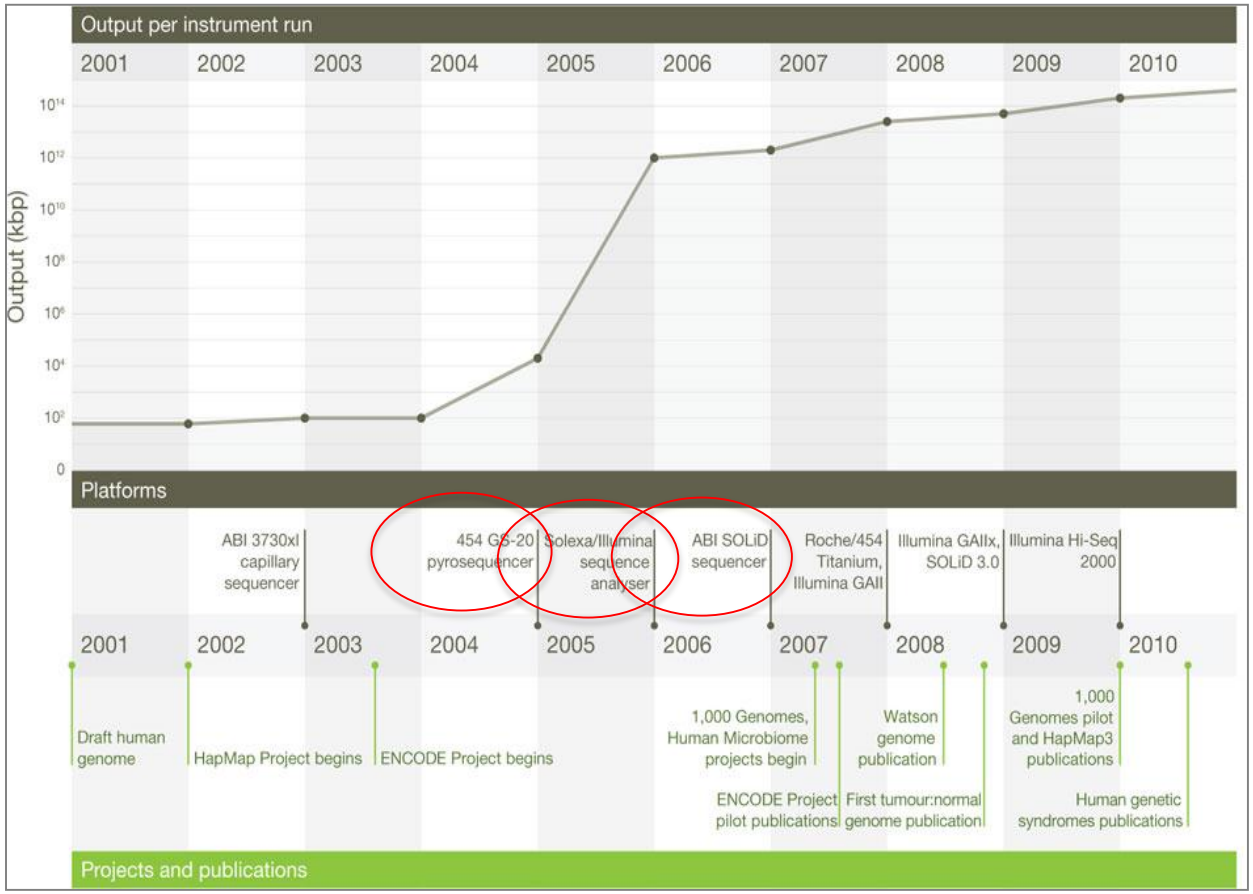
2004-2006: Advent of new sequencing technologies

Next Generation Sequencing, **NGS**



From: Mardis E. et al. ***A decade's perspective on DNA sequencing technology***. Nature, 2011

NGS = Next Generation Sequencing

Ability to read DNA:

- **in a very fast and cheap manner**
- **in every field related to DNA, i.e. related to Life Science**

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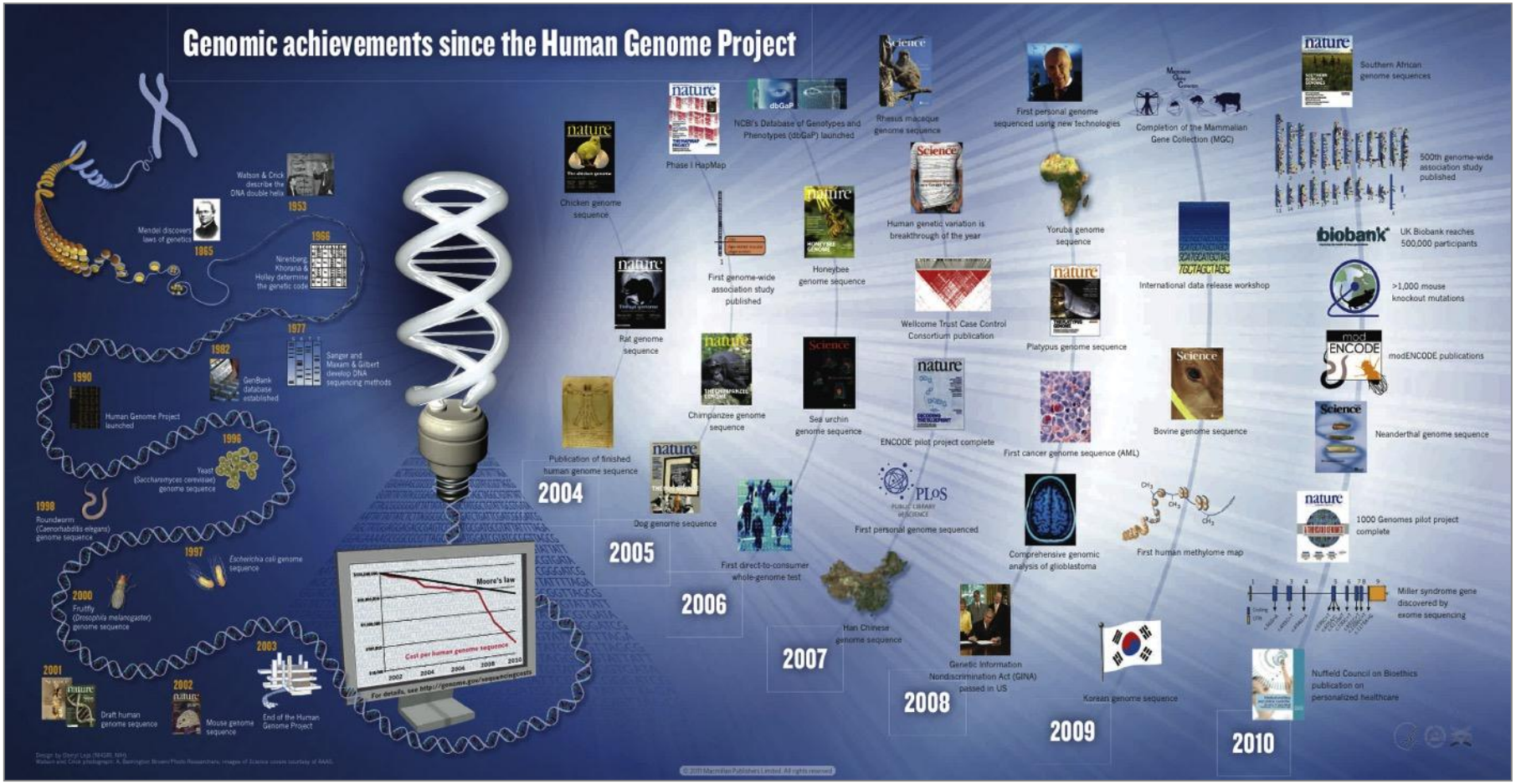
A new starting point



From: Green ED et al. ***Charting a course for genomic medicine from base pairs to bedside.*** Nature, 2011

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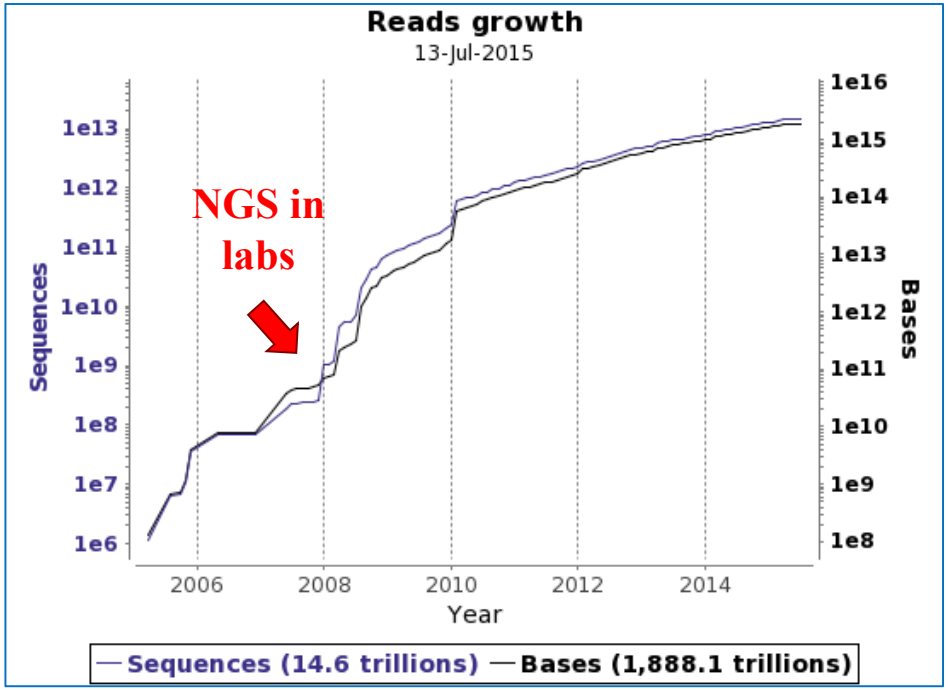
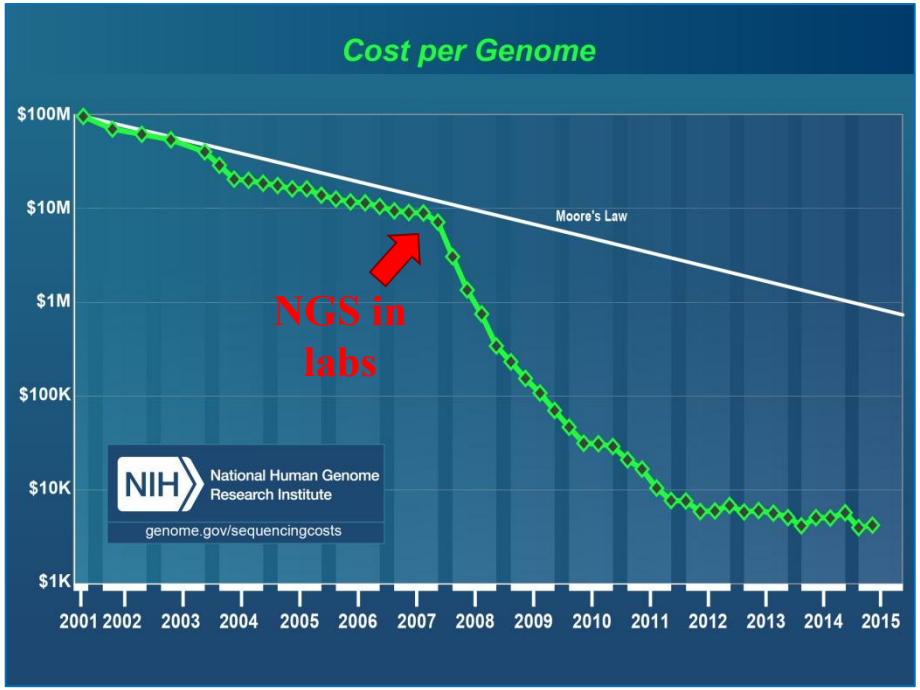
Advantages





Increased throughput

Reduction of costs

From billions to trillions!

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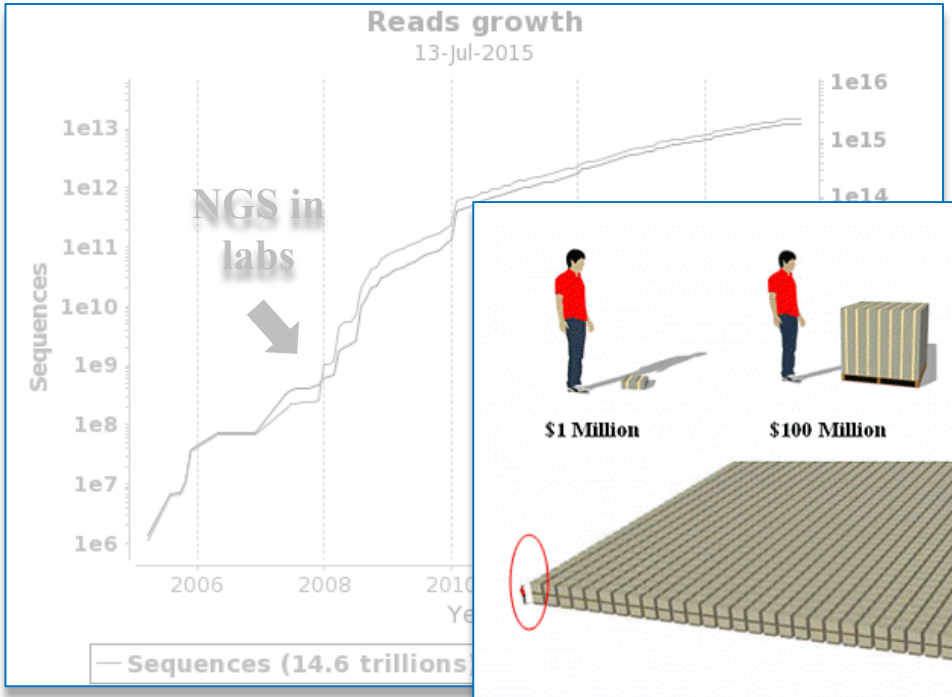
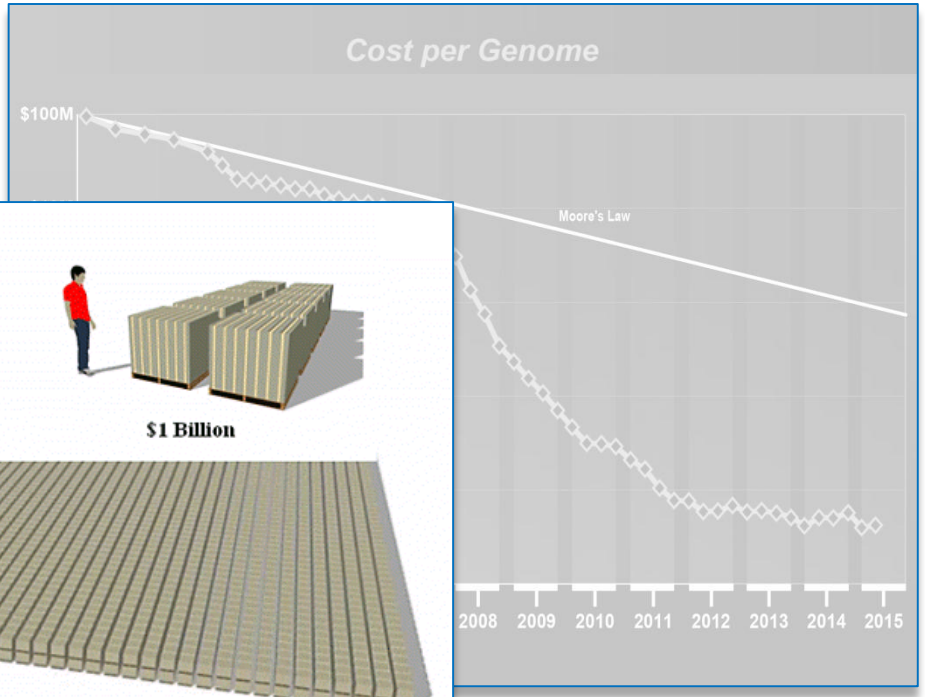
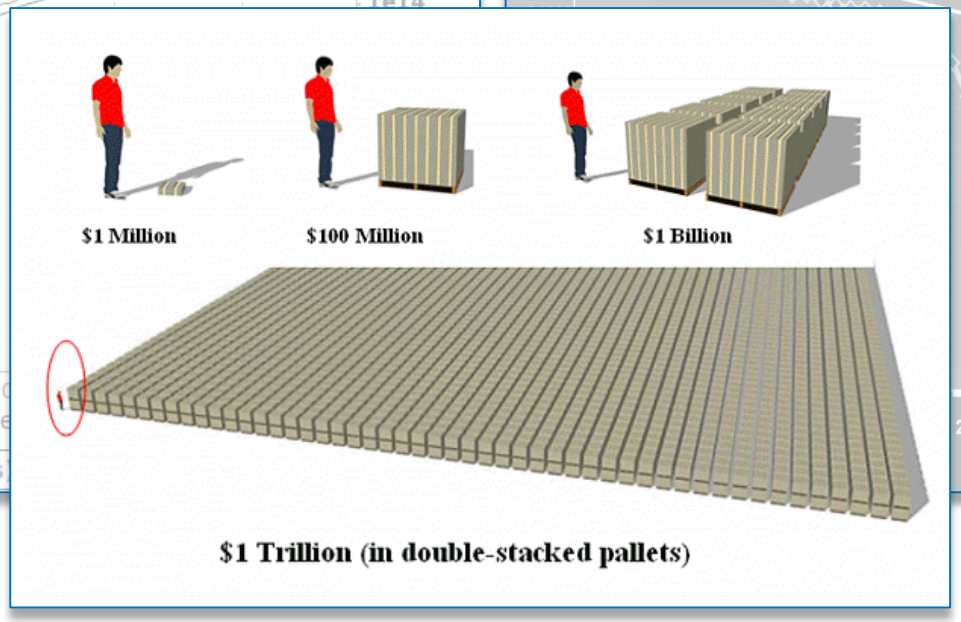
Advantages







From billions to trillions!

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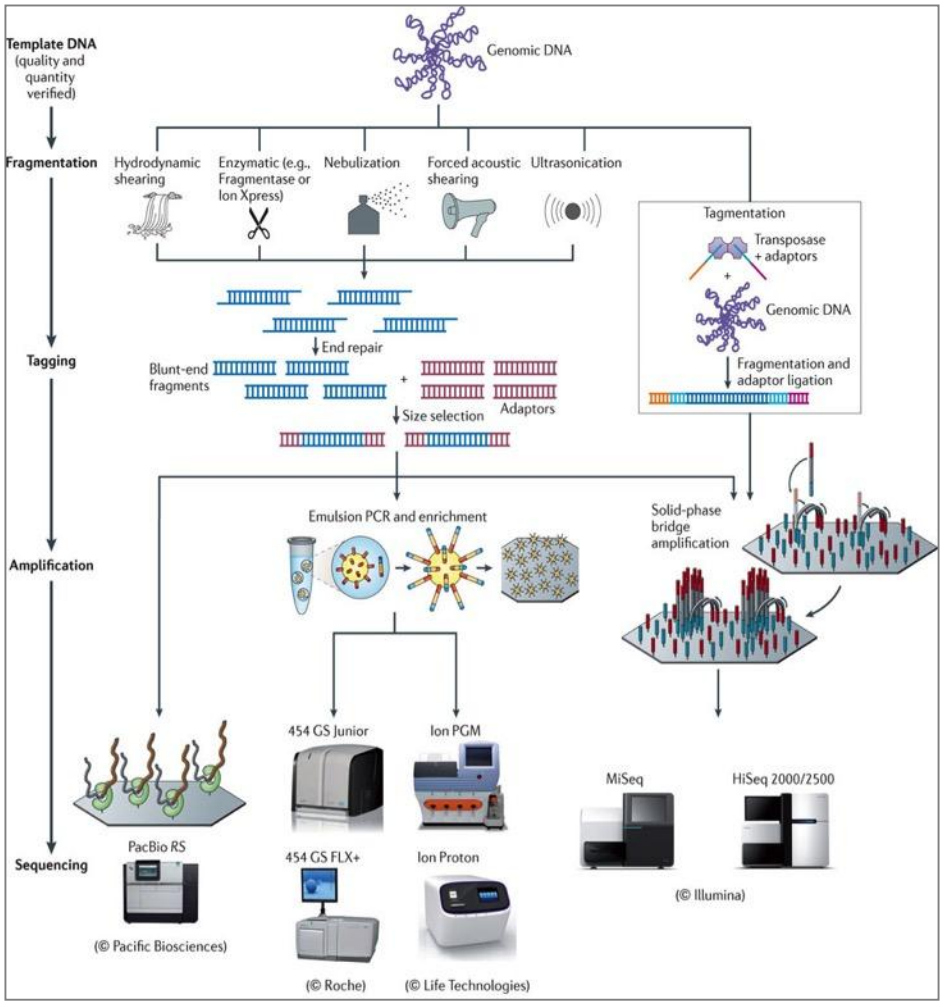
Massive parallel sequencing



From: Loman et al. ***High-throughput bacterial genome sequencing: an embarrassment of choice, a world of opportunity.*** Nature Rev Micr, 2012

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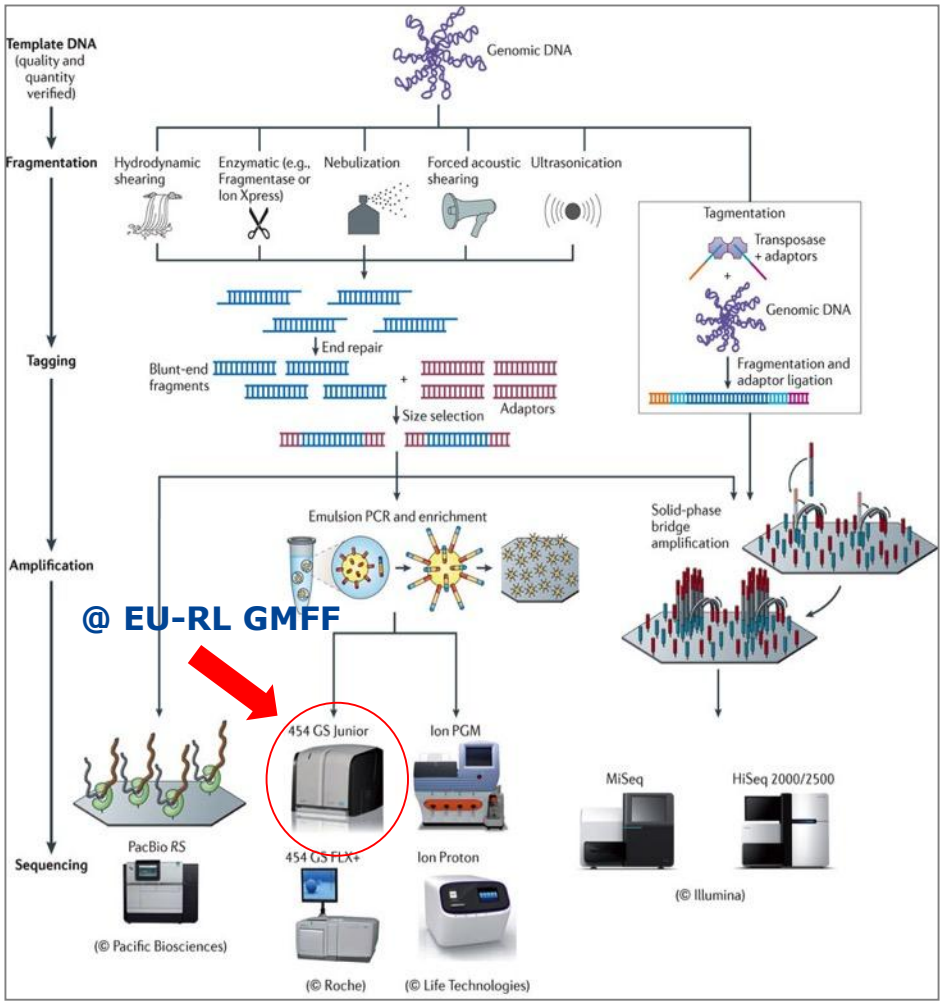
Massive parallel sequencing



From: Loman et al. ***High-throughput bacterial genome sequencing: an embarrassment of choice, a world of opportunity.*** Nature Rev Micr, 2012

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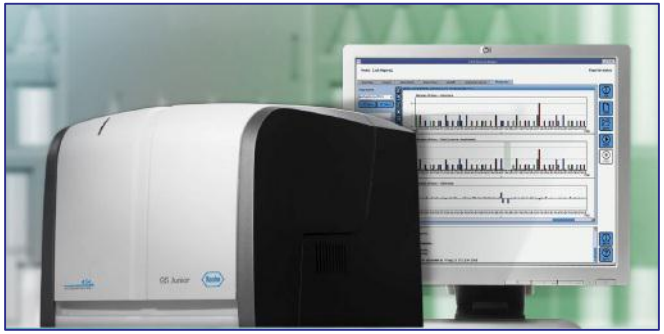
NGS @ EURL-GMFF

EURL-GMFF in 2013 acquired the Roche GS Junior, a NGS sequencer.

The New GS Junior



System Performance	
Throughput	35 million high-quality, filtered bases per run*
Run Time	10 hours sequencing 2 hours data processing
Avg. Read Length	400 bases*
Accuracy	Q20 read length of 400 bases (99% accuracy at 400 bases)
Reads per Run	100,000 shotgun, 70,000 amplicon
Sample Input	gDNA, amplicons, cDNA, or BACs depending on the application
Physical Dimensions	40 cm wide x 60 cm deep x 40 cm high (the size of a laser printer) Weight = 55 lbs.
Computing	Linux-based OS on HP desktop computer included. All software is point-and-click.

***Typical results**. *Average read length and number of reads depend on specific sample and genomic characteristics*

ONLY IN 2013?

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NGS applications in the GMO field

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NGS applications in the GMO field

Published December 12, 2012

ORIGINAL RESEARCH

The Use of Next Generation Sequencing and Junction Sequence Analysis Bioinformatics to Achieve Molecular Characterization of Crops Improved Through Modern Biotechnology

David Kovalic,* Carl Garnaat, Liang Guo, Yongpan Yan, Jeanna Groat, Andre Silvanovich, Lyle Ralston, Mingya Huang, Qing Tian, Allen Christian, Nordine Cheikh, Jerry Hjelle, Stephen Padgette, and Gary Bannon

Food Anal. Methods (2013) 6:1718–1727
DOI 10.1007/s12161-013-9673-x

Next-Generation Sequencing as a Tool for Detailed Molecular Characterisation of Genomic Insertions and Flanking Regions in Genetically Modified Plants: a Pilot Study Using a Rice Event Unauthorised in the EU

Daniela Wahler · Leif Schauser · Joachim Bendiek · Lutz Grohmann

Anal Bioanal Chem (2014) 406:2603–2611
DOI 10.1007/s00216-014-7667-1

RESEARCH PAPER

Detecting authorized and unauthorized genetically modified organisms containing *vip3A* by real-time PCR and next-generation sequencing

Chanjuan Liang · Jeroen P. van Dijk · Ingrid M. J. Scholtens · Martijn Staats · Theo W. Prins · Marleen M. Voorhuijzen · Andrea M. da Silva · Ana Carolina Maisonnave Arisi · Johan T. den Dunnen · Esther J. Kok

SCIENTIFIC REPORTS

OPEN

Characterization of GM events by insert knowledge adapted re-sequencing approaches

SUBJECT AREAS:
MOLECULAR ENGINEERING IN PLANTS
DNA RECOMBINATION
PLANT MOLECULAR BIOLOGY
NEXT-GENERATION SEQUENCING

Received
28 May 2013

Litao Yang[1]*, Congmao Wang[1]*, Arne Holst-Jensen[2], Dany Morisset[3], Yongjun Lin[4] & Dabing Zhang[1]

[1]Collaborative Innovation center for biosafety of GMOs, National Center for Molecular Characterization of GMOs, School of Life Science and Biotechnology, Shanghai Jiao Tong University. 800 Dongchuan Road, Shanghai 200240. P. R. China, [2]Norwegian Veterinary Institute. P.O.Box 750 Sentrum, 0106 Oslo, Norway, [3]Department of Biotechnology and Systems Biology, National Institute of Biology. Vecna pot 111, SI-1000 Ljubljana, Slovenia, [4]National Key Laboratory of Crop Genetic Improvement and National Centre of Plant Gene Research, Huazhong Agricultural University, Wuhan 430070, P. R. China.

Anal Bioanal Chem (2014) 406:6485–6497
DOI 10.1007/s00216-014-8077-0

REVIEW

GMO quantification: valuable experience and insights for the future

Mojca Milavec · David Dobnik · Litao Yang · Dabing Zhang · Kristina Gruden · Jana Žel

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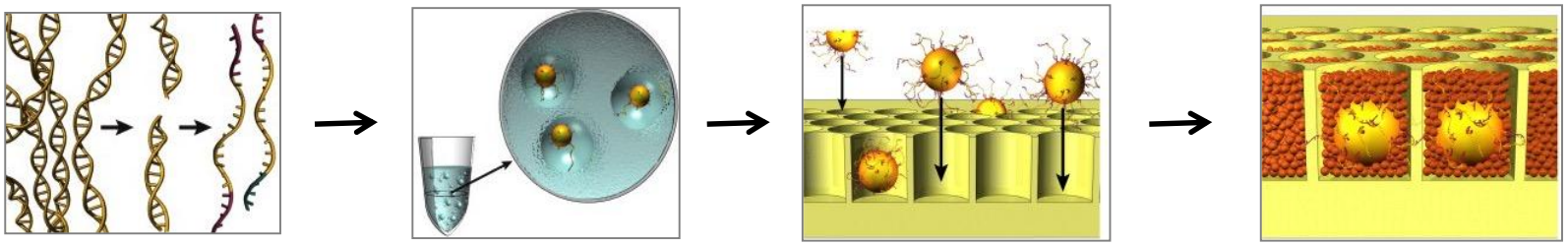
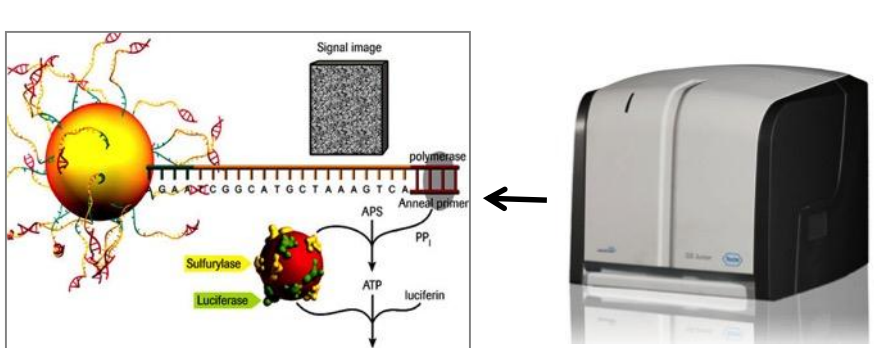
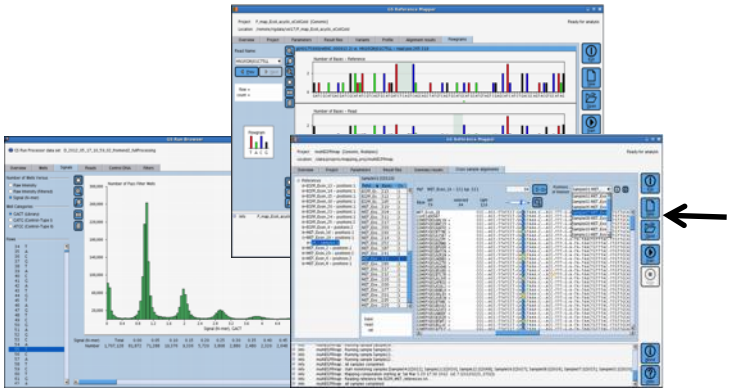
A workflow with new *actors*

Sample preparation







Data Analysis

Sequencing

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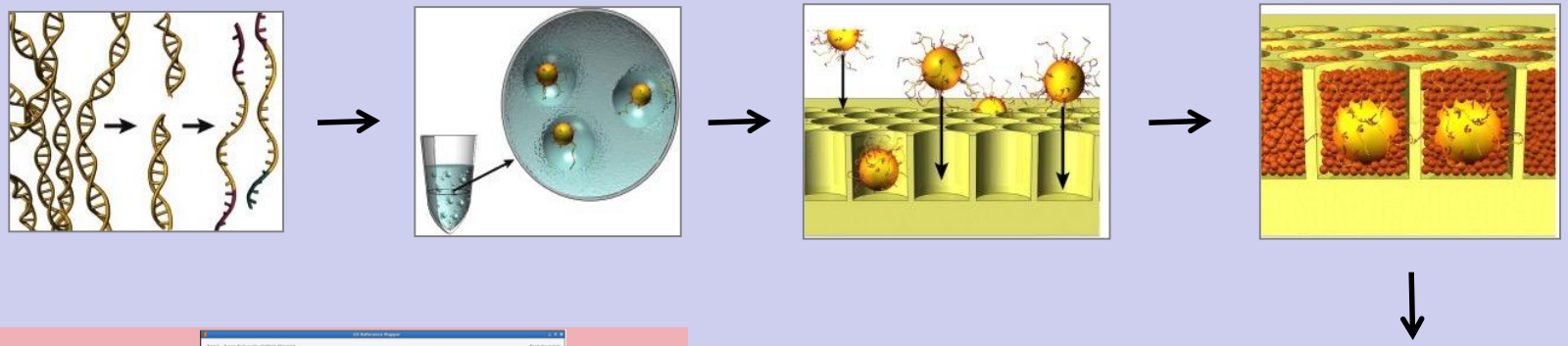
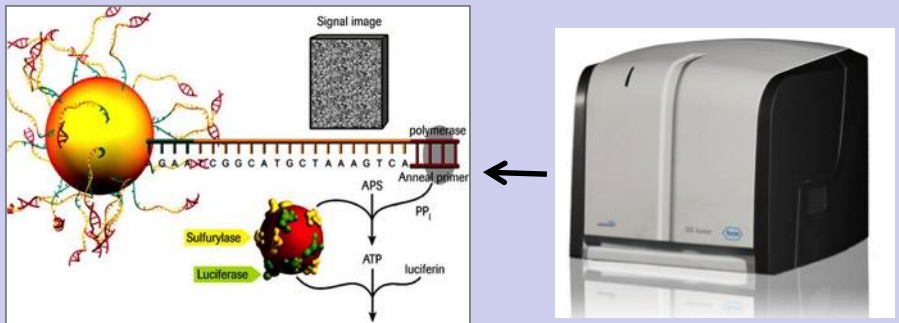
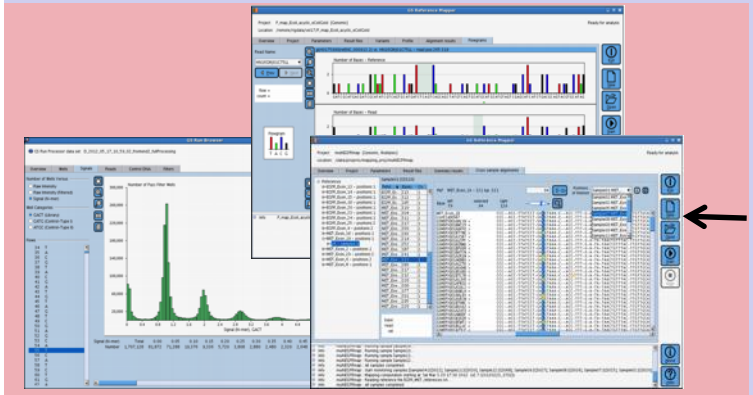
A workflow with new *actors*

Wet-lab





Bioinformatics



Wet-lab

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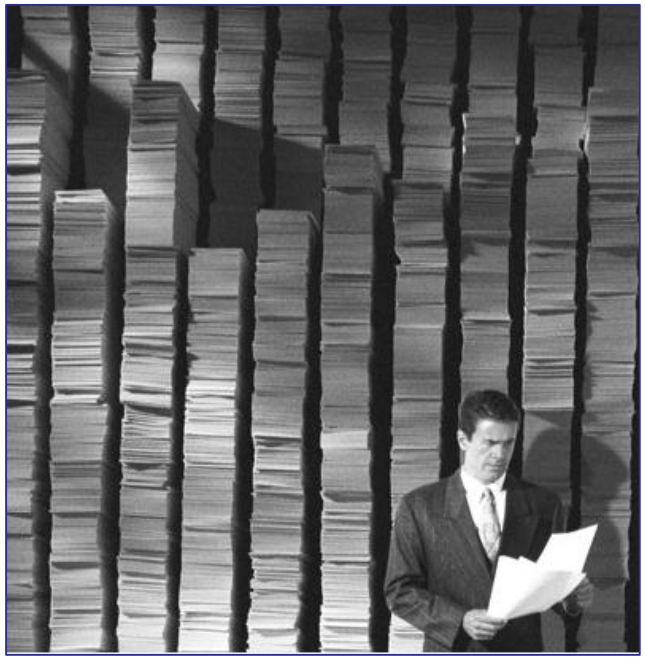
Dealing with NGS data



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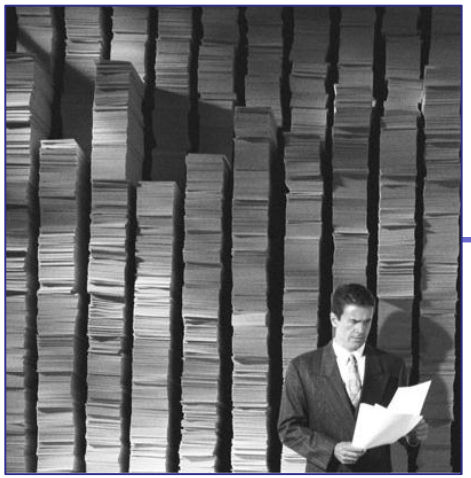
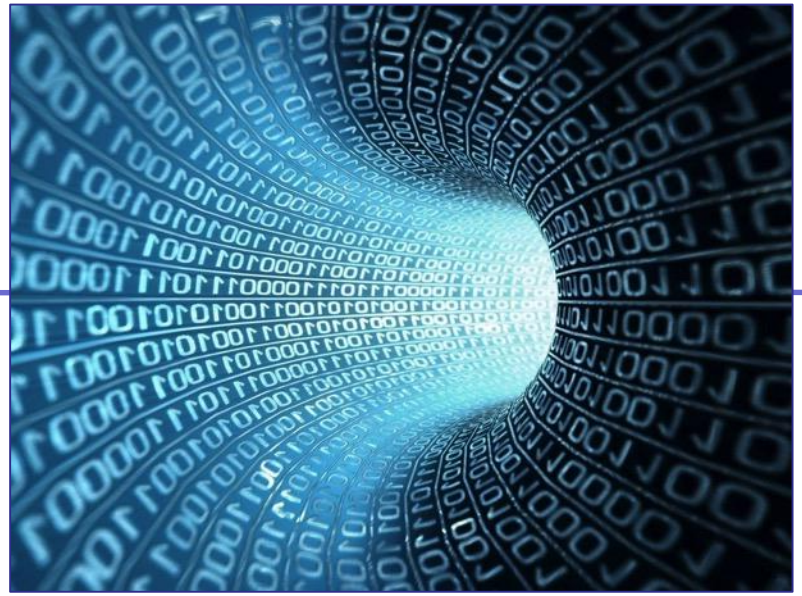
Dealing with NGS data

BIOINFORMATICS





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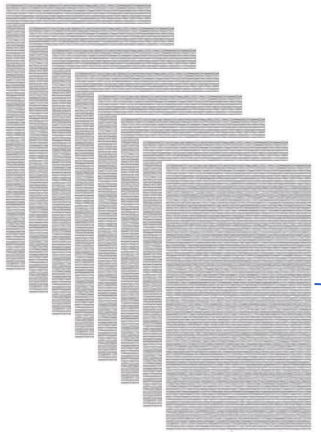
Analyses on NGS data are complex



?

?

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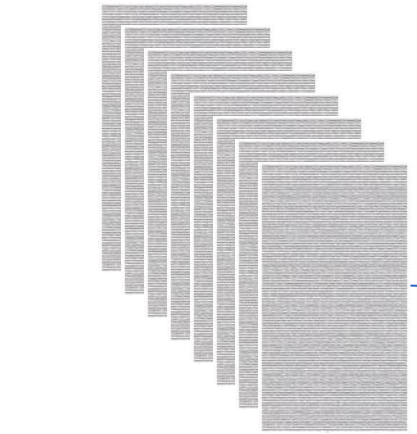
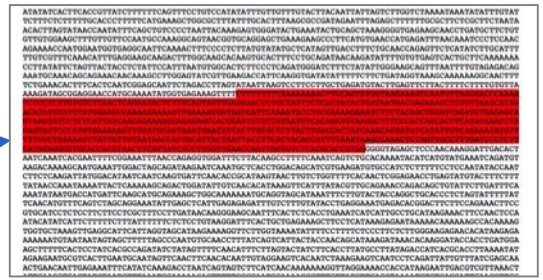
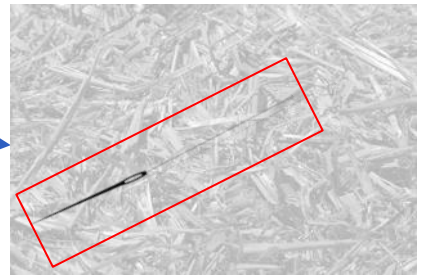
Analyses on NGS data are complex

Adequate infrastructure is needed







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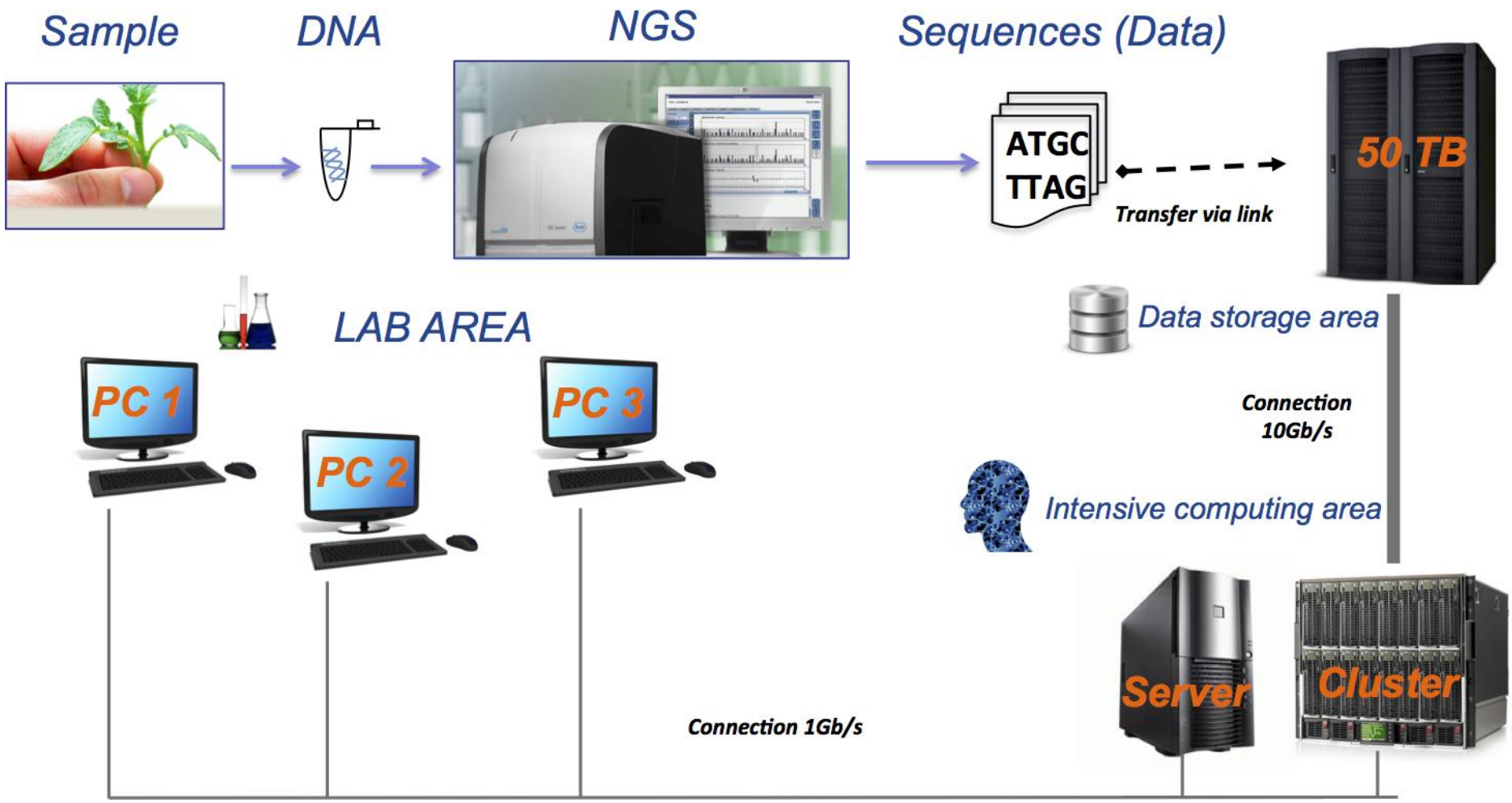
Infrastructure @ EU-RL GMFF



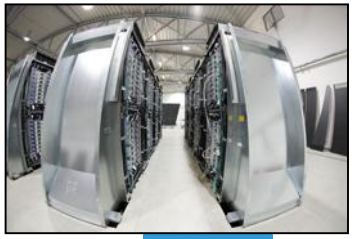
Dealing with NGS is complex

NGS

HoU

HPC

My personal
Bermuda triangle!



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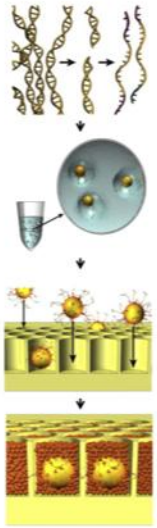
New information about GMO's

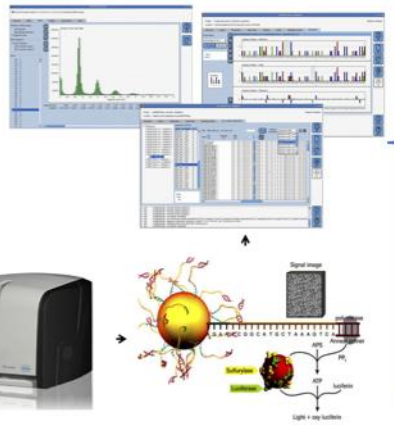
Question: *GMO ?* → **NGS SEQUENCING** → **DATA ANALYSIS** → **Answer**

Plant

or

DNA sample





NO

or

YES

NO

YES

Question: *Authorised ?*

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GM-papaya from Thailand

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GM-papaya from Thailand

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Expert warns of illegal GM Papaya on EU market

29-Jun-2012 2 comments

CERT ID

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Related topics: Financial & Industry

The food and fruit industries to be vigilant against a banned variety of genetically modified papaya coming into the EU from Thailand, say experts.

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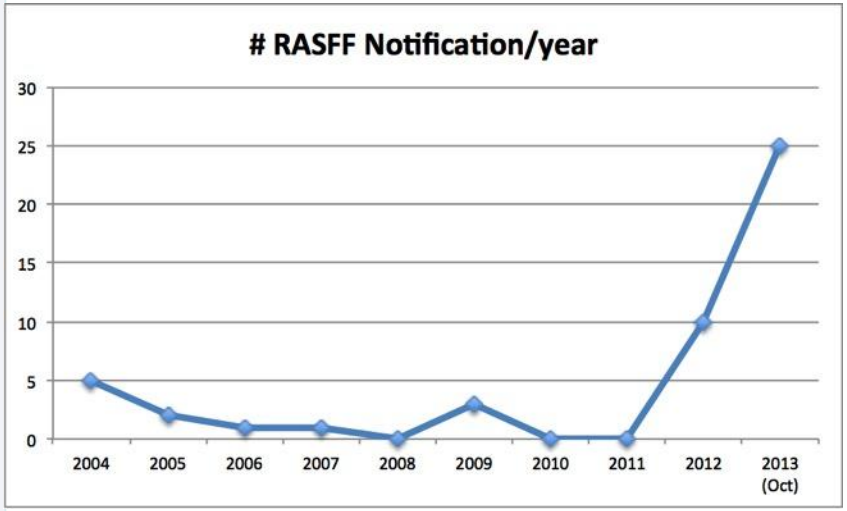
5. Cheating the taste buds: The flavour challenge of salt, sugar and fat reduction



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Why focusing on GM-papaya?

- GM papaya is not authorized in EU
- Most of detection methods are specific for GM papaya from USA
- Little information about other GM papayas
- Doubts that Thai-papaya is identical to USA papaya

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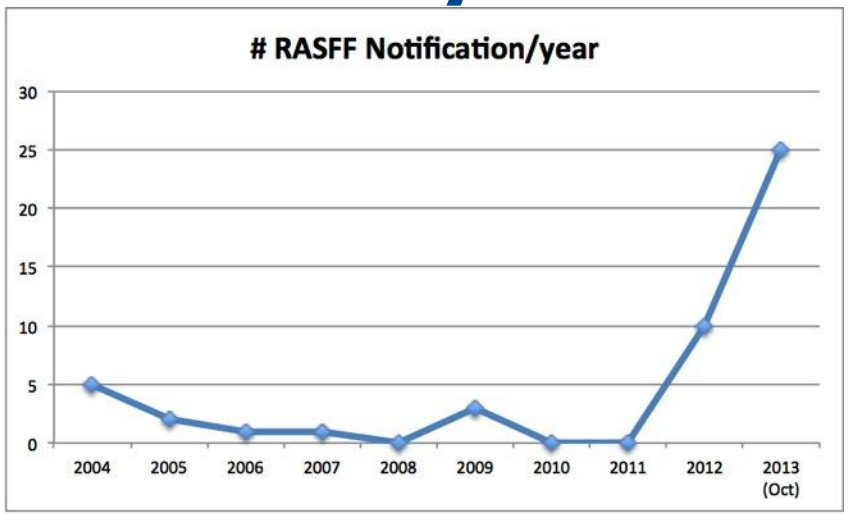
A tricky case



Samples (fresh fruit) positive to:

- **p35S**
- **NptII**
- **tNOS**

BUT

negative to known GM-papaya detection methods

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GM-papaya from Thailand

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1. Cubed yoghurt and quinoa muffins...What's cooking at Stern Wywiol's innovation centre?
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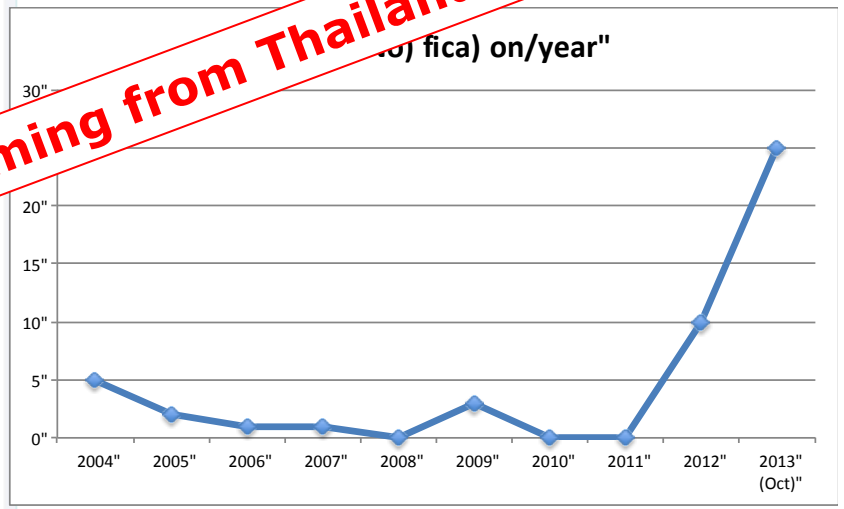
5. Cheating the taste buds: The flavour challenge of salt, sugar and fat reduction

Which GM-papaya is coming from Thailand?



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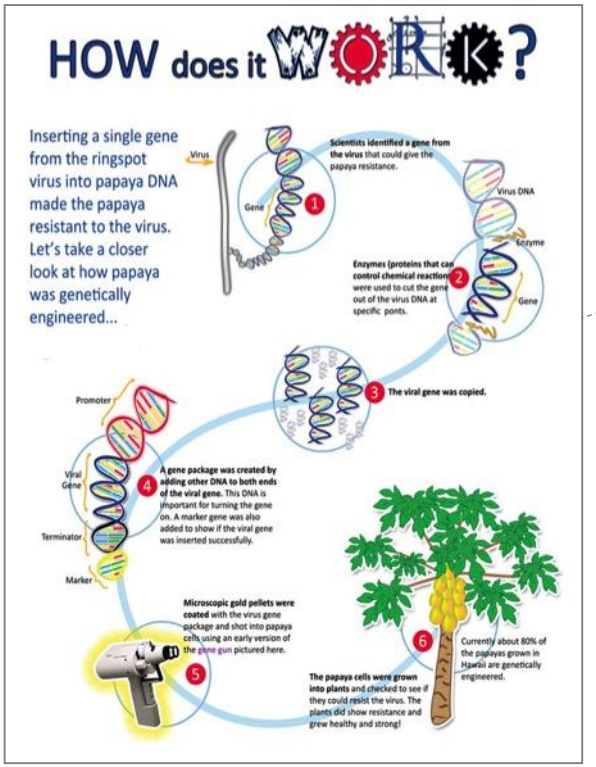
Dennis Gonsalves et al. work



particle bombardment

Hawaiian papaya: Solo type

↓ *cultivars*

Kapoho, Sunrise, Sunset, Waimanalo, Kamiya

↓

Line-55 (1991)

↓ *Homozygous F0*

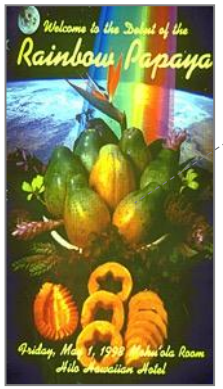
SunUp (1992)

↓ x *Kapoho*

Rainbow (1995)

↓ x *Kamiya*

Laie Gold (2002)



1998: seed distribution

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Not only USA

Global papaya production

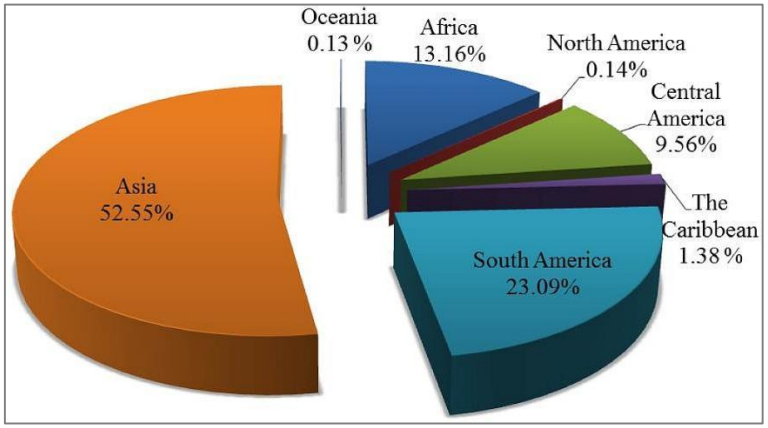

Table 2. Countries involved in developing transgenic papaya technology.

Gonsalves-associated	ACIAR group	ISAAA-led group	Independent
US (Cornell/Hawaii)	Australia	Indonesia	China
Brazil	Philippines	Malaysia	Japan
Jamaica	Malaysia	Philippines	Mexico
Thailand		Thailand	US (Florida)
Venezuela		Vietnam	US (Virgin Islands)
			US (Hawaii)

Table 3. Academic research institutions involved in papaya transgenic technology.

Grouping/ country	Academic/research institution
Gonsalves group	
United States	Cornell University and University of Hawaii
Brazil	EMBRAPA
Jamaica	University of West Indies, Biotech Centre
Thailand	Department of Agriculture
Venezuela	University of Los Andes
ACIAR group	
Australia	University of Queensland
Philippines	Institute of Plant Breeding, Crop Science Cluster, College of Agriculture (CA) University of the Philippines Los Baños (UPLB)
Malaysia	Malaysia Agricultural Research and Development Institute (MARDI)
ISAAA group	
Indonesia	Indonesian Research Institute for Agricultural Biotechnology and Genetic Resources (IABIOGRI), Agency for Agricultural Research and Development (AARD)
Malaysia	MARDI
Philippines	Institute of Plant Breeding CA UPLB
Thailand	National Center for Genetic Engineering and Biotechnology (BIOTEC) Kasetsart University
Vietnam	Institute of Biotechnology (IBT), National Centre for Natural Science and Technology
Independent groups	
China	Huazhong Agricultural University (Hubei) Zhongshan University South China Agricultural University (Guangzhou)
Japan	Japan International Research Center for Agricultural Sciences (Okinawa) National Agricultural Research Center for Hokkaido Region (Sapporo) Department of Agro-bioscience, Faculty of Agriculture, Iwate University
Mexico	CINVESTAD, Irapuato
USA	University of Florida University of Hawaii University of Virgin Islands

GM papayas

N	Year	Name	Country	Commercialized	Completed trial	Still in trial	Sequence available?
1	1998	SunUp	USA	yes			yes
2	1998	Rainbow	USA	yes			yes
3	2002	Laie Gold	USA	yes			yes
4	2004	X17-2	USA	no			no
5	2006	Huanong 1	China	yes	na	na	no
6	1999	several lines	Brazil	no			no
7	2005	several lines	Jamaica	no	yes		no
8	2004	several lines	Taiwan	no	yes		no
9	2005	several lines	Thailand	no	yes		no
10	2004	several lines	the Philippines	no		yes	no
11	2002	several lines	Malaysia	no		yes	no
12	2002	several lines	Vietnam	no		yes	no
13	2004	several lines	Venezuela	no		yes	no
14	2002	several lines	Indonesia	no		yes	no
15	2002	several lines	Australia	no		yes	no

Where to begin ???

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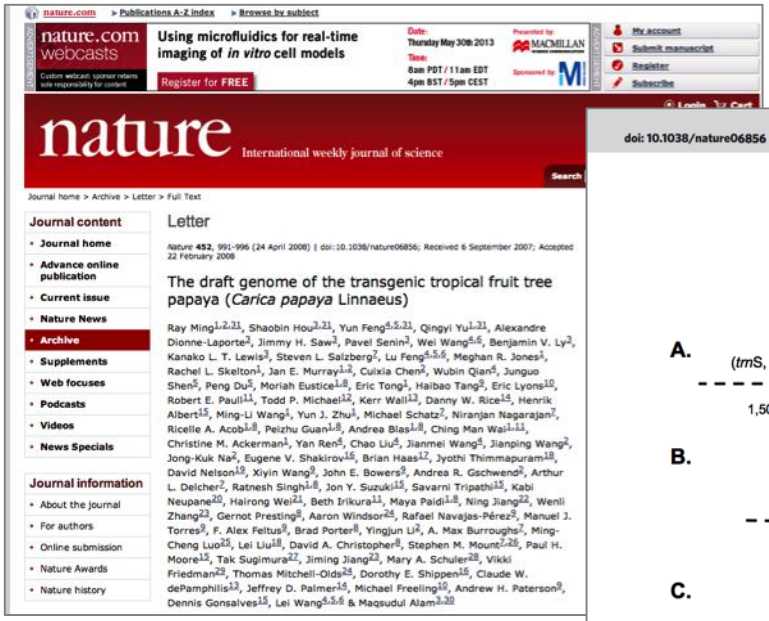
GM Papaya from USA



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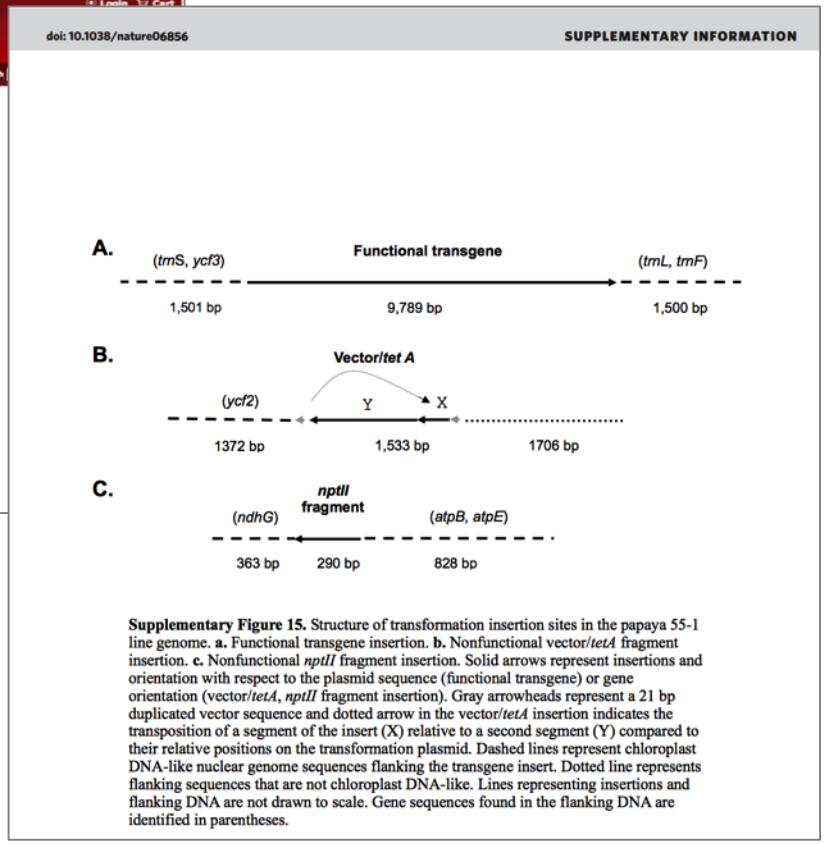
Letter

Nature **452**, 991-996 (24 April 2008) | doi:10.1038/nature06856; Received 6 September 2007; Accepted 22 February 2008

The draft genome of the transgenic tropical fruit tree papaya (*Carica papaya* Linnaeus)

Ray Ming[1,2,21], Shaobin Hou[3,21], Yun Feng[4,5,21], Qingyi Yu[1,21], Alexandre Dionne-Laporte[3], Jimmy H. Saw[3], Pavel Senin[3], Wei Wang[5,6], Benjamin V. Ly[3], Kanako L. T. Lewis[3], Steven L. Salzberg[7], Lu Feng[4,5,6], Meghan R. Jones[1], Rachel L. Skelton[1], Jan E. Murray[1,2], Cuixia Chen[2], Wubin Qian[5], Junguo Shen[5], Peng Du[5], Moriah Eustice[1,8], Eric Tong[1], Haibao Tang[9], Eric Lyons[10], Robert E. Paull[11], Todd P. Michael[12], Kerr Wall[13], Danny W. Rice[14], Henrik Albert[15], Ming-Li Wang[1], Yun J. Zhu[1], Michael Schatz[7], Niranjan Nagarajan[7], Ricelle A. Acob[1,8], Peizhu Guan[1,8], Andrea Blas[1,8], Ching Man Wai[1,11], Christine M. Ackerman[1], Yan Ren[5], Chao Liu[4], Jianmei Wang[4], Jianping Wang[2], Jong-Kuk Na[2], Eugene V. Shakirov[16], Brian Haas[17], Jyothi Thimmapuram[18], David Nelson[19], Xiyin Wang[9], John E. Bowers[9], Andrea R. Gschwend[9], Arthur L. Delcher[7], Ratnesh Singh[1,8], Jon Y. Suzuki[15], Savarni Tripathi[15], Kabi Neupane[20], Hairong Wei[21], Beth Irikura[11], Maya Paidi[1,8], Ning Jiang[22], Wenli Zhang[23], Gernot Presting[8], Aaron Windsor[24], Rafael Navajas-Pérez[8], Manuel J. Torres[8], F. Alex Feltus[8], Brad Porter[8], Yingjun Li[2], A. Max Burroughs[7], Ming-Cheng Luo[25], Lei Liu[18], David A. Christopher[8], Stephen M. Mount[7,26], Paul H. Moore[15], Tak Sugimura[27], Jiming Jiang[23], Mary A. Schuler[28], Vikki Friedman[29], Thomas Mitchell-Olds[24], Dorothy E. Shippen[16], Claude W. dePamphilis[13], Jeffrey D. Palmer[15], Michael Freeling[10], Andrew H. Paterson[9], Dennis Gonsalves[15], Lei Wang[4,5,6] & Maqsudul Alam[1,20]



doi: 10.1038/nature06856

SUPPLEMENTARY INFORMATION

A. (*trnS, ycf3*) — Functional transgene — (*trnL, trnF*)
1,501 bp — 9,789 bp — 1,500 bp

B. Vector/*tet A*
(*ycf2*) — Y — X
1372 bp — 1,533 bp — 1706 bp

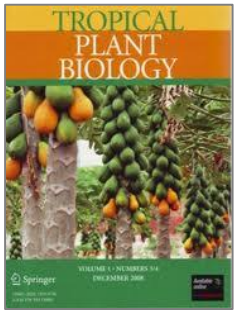
C. *nptII* fragment
(*ndhG*) — (*atpB, atpE*)
363 bp — 290 bp — 828 bp

Supplementary Figure 15. Structure of transformation insertion sites in the papaya 55-1 line genome. **a.** Functional transgene insertion. **b.** Nonfunctional vector/*tetA* fragment insertion. **c.** Nonfunctional *nptII* fragment insertion. Solid arrows represent insertions and orientation with respect to the plasmid sequence (functional transgene) or gene orientation (vector/*tetA*, *nptII* fragment insertion). Gray arrowheads represent a 21 bp duplicated vector sequence and dotted arrow in the vector/*tetA* insertion indicates the transposition of a segment of the insert (X) relative to a second segment (Y) compared to their relative positions on the transformation plasmid. Dashed lines represent chloroplast DNA-like nuclear genome sequences flanking the transgene insert. Dotted line represents flanking sequences that are not chloroplast DNA-like. Lines representing insertions and flanking DNA are not drawn to scale. Gene sequences found in the flanking DNA are identified in parentheses.

April 2008.
No GMO sequence is clearly indicated, just a figure in Supplementary Materials.

Joint Research Centre

GM Papaya from USA



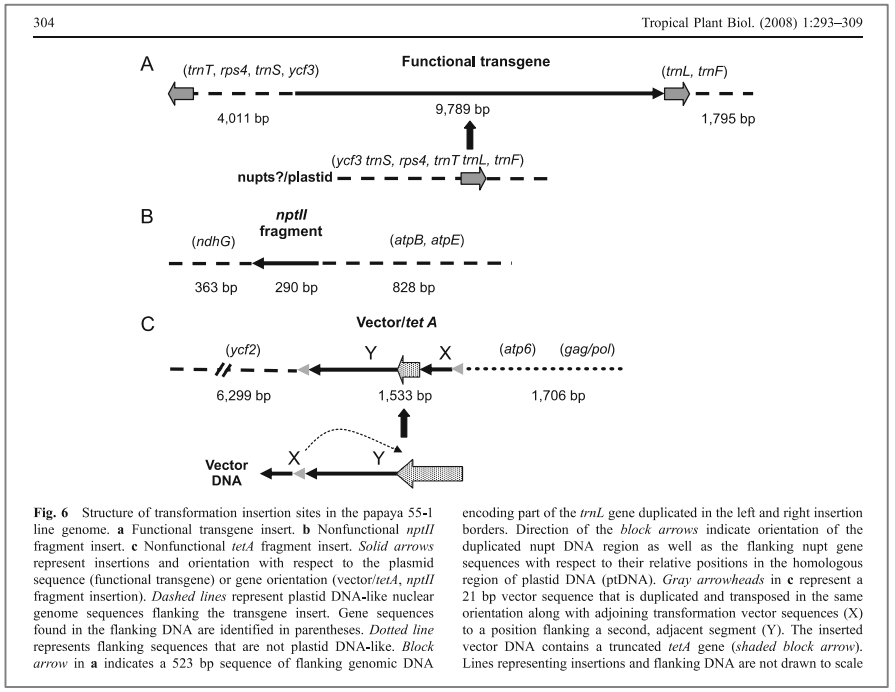
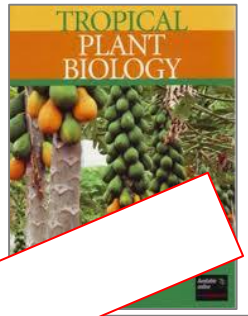


Fig. 6 Structure of transformation insertion sites in the papaya 55-1 line genome. **a** Functional transgene insert. **b** Nonfunctional *nptII* fragment insert. **c** Nonfunctional *tetA* fragment insert. *Solid arrows* represent insertions and orientation with respect to the plasmid sequence (functional transgene) or gene orientation (vector/*tetA*, *nptII* fragment insertion). *Dashed lines* represent plastid DNA-like nuclear genome sequences flanking the transgene insert. Gene sequences found in the flanking DNA are identified in parentheses. *Dotted line* represents flanking sequences that are not plastid DNA-like. *Block arrow* in **a** indicates a 523 bp sequence of flanking genomic DNA encoding part of the *trnL* gene duplicated in the left and right insertion borders. Direction of the *block arrows* indicate orientation of the duplicated nupt DNA region as well as the flanking nupt gene sequences with respect to their relative positions in the homologous region of plastid DNA (ptDNA). *Gray arrowheads* in **c** represent a 21 bp vector sequence that is duplicated and transposed in the same orientation along with adjoining transformation vector sequences (X) to a position flanking a second, adjacent segment (Y). The inserted vector DNA contains a truncated *tetA* gene (*shaded block arrow*). Lines representing insertions and flanking DNA are not drawn to scale

December 2008.
It includes sequences.

GM Papaya from USA



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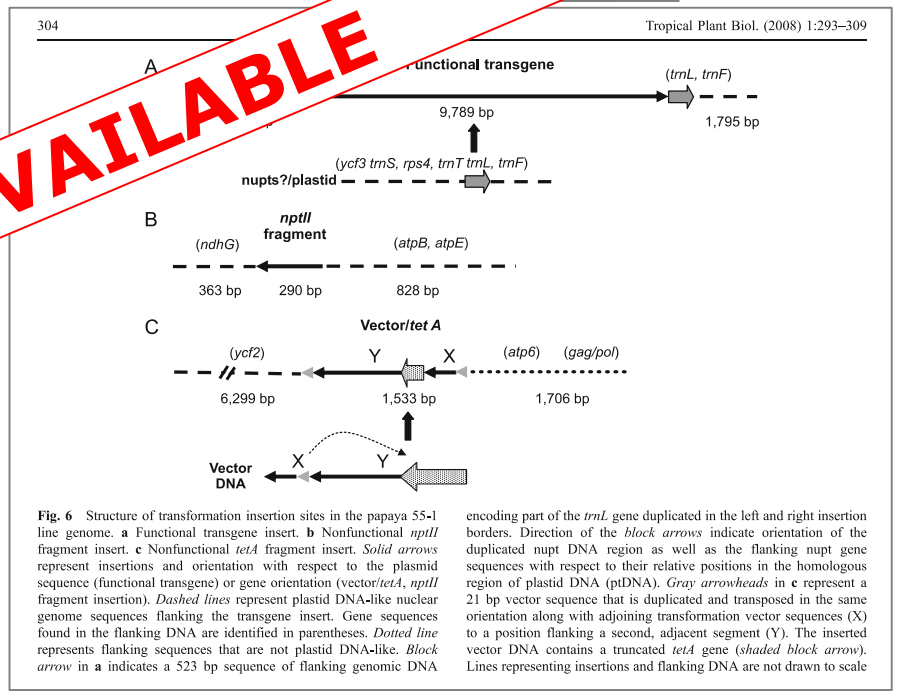


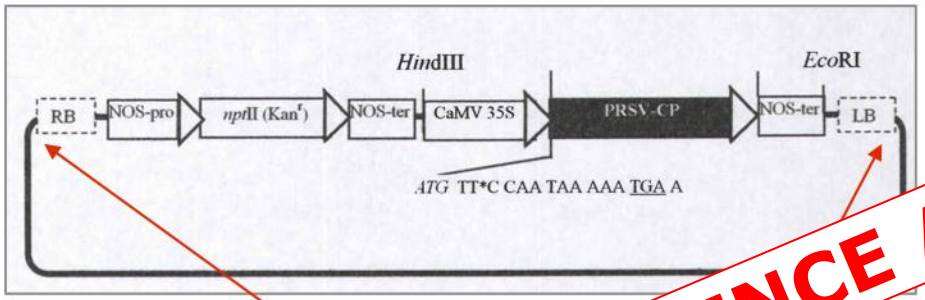
Fig. 6 Structure of transformation insertion sites in the papaya 55-1 line genome. **a** Functional transgene insert. **b** Nonfunctional *nptII* fragment insert. **c** Nonfunctional *tetA* fragment insert. *Solid arrows* represent insertions and orientation with respect to the plasmid sequence (functional transgene) or gene orientation (vector/*tetA*, *nptII* fragment insertion). *Dashed lines* represent plastid DNA-like nuclear genome sequences flanking the transgene insert. Gene sequences found in the flanking DNA are identified in parentheses. *Dotted line* represents flanking sequences that are not plastid DNA-like. *Block arrow* in **a** indicates a 523 bp sequence of flanking genomic DNA encoding part of the *trnL* gene duplicated in the left and right insertion borders. Direction of the *block arrows* indicate orientation of the duplicated nupt DNA region as well as the flanking nupt gene sequences with respect to their relative positions in the homologous region of plastid DNA (ptDNA). *Gray arrowheads* in **c** represent a 21 bp vector sequence that is duplicated and transposed in the same orientation along with adjoining transformation vector sequences (X) to a position flanking a second, adjacent segment (Y). The inserted vector DNA contains a truncated *tetA* gene (*shaded block arrow*). Lines representing insertions and flanking DNA are not drawn to scale

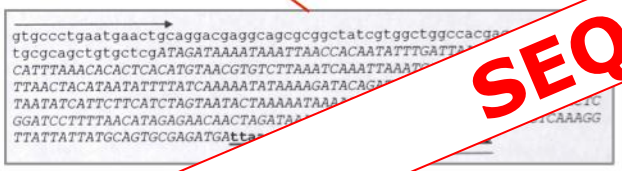
De ber 2008.
It includes sequences.

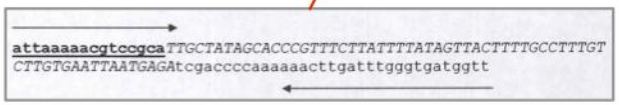
European Commission

GM Papaya X17-12 USA

Petition for Determination of Nonregulated Status for the X17-2 Line of Papaya: A *Papaya ringspot virus* – Resistant Papaya







Appendix II

Comparison Between Expected Sequence And Observed Sequence Of X17-2 T-DNA

A 4935 bp PCR fragment containing all but approxi[illegible]36 bp of the T-DNA incert from a R_5 generation plant of the X17-2 line [illegible]d sequenced, and the expected and observed sequences were compared. [illegible]9.78%.

```
miscel.          1..26
                 /[illegible]gion"
promoter         [illegible]
gene             [illegible]pII"
                 [illegible]duct="neomycin phosphotransferase II"
                 155..1810
                 /note="NOS"
                 2510..3344
                 /note="CaMV 35S"
gene             3352..4360
                 /gene="PRSV X17-2 coat protien construct"
terminator       4378..4630
                 /note="NOS"

X17-2 expected  GGTTATTATTATGCAGTGCGAGATGATTAAACTGAAGGCGGGAAACGACAATCTGATCAT 60
X17-2 observed  GGTTATTATTATGCAGTGCGAGATGATTAAACTGAAGGCGGGAAACGACAATCTGATCAT
                ************************************************************

X17-2 expected  GAGCGGAGAATTAAGGGAGTCACGTTATGACCCCCGCCGATGACGCGGGACAAGCCGTTT 120
X17-2 observed  GAGCGGAGAATTAAGGGAGTCACGTTATGACCCCCGCCGATGACGCGGGACAAGCCGTTT
                ************************************************************

X17-2 expected  TACGTTTGGAACTGACAGAACCGCAACGTTGAAGGAGCCACTCAGCCGCGGGTTTCTGGA 180
X17-2 observed  TACGTTTGGAACTGACAGAACCGCAACGTTGAAGGAGCCACTCAGCCGCGGGTTTCTGGA
                ************************************************************

X17-2 expected  GTTTAATGAGCTAAGCACATACGTCAGAAACCATTATTGCGCGTTCAAAAGTCGCCTAAG 240
X17-2 observed  GTTTAATGAGCTAAGCACATACGTCAGAAACCATTATTGCGCGTTCAAAAGTCGCCTAAG
                ************************************************************

X17-2 expected  GTCACTATCAGCTAGCAAATATTTCTTGTCAAAAATGCTCCACTGACGTTCCATAAATTC 300
X17-2 observed  GTCACTATCAGCTAGCAAATATTTCTTGTCAAAAATGCTCCACTGACGTTCCATAAATTC
                ************************************************************

X17-2 expected  CCCTCGGTATCCAATTAGAGTCTCATATTCACTCTCAATCCAAATAATCTGCACCGGATC 360
X17-2 observed  CCCTCGGTATCCAATTAGAGTCTCATATTCACTCTCAATCCAAATAATCTGCACCGGATC
                ************************************************************

X17-2 expected  TGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGT 420
X17-2 observed  TGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGT
                ************************************************************
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European Commission

GM Papaya from CHINA

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 7205–7212 7205
DOI:10.1021/jf901198x

Characterization of the Exogenous Insert and Development of Event-specific PCR Detection Methods for Genetically Modified Huanong No. 1 Papaya

Jinchao Guo,† Litao Yang,*,† Xin Liu,‡ Xiaoyan Guan,† Lingxi Jiang,† and Daring Zhang*,†,§

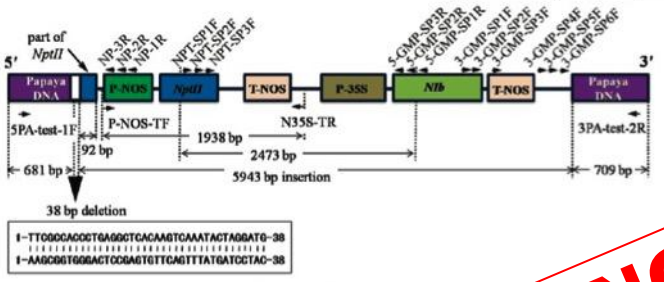

Figure 1. Schematic charaterization of the exogenous insert in GM Huanong No. 1 papaya. GM Huanong No. 1 transcription process that resulted in a 38 bp deletion. The positions of conventional PCR and TAIL-PCR

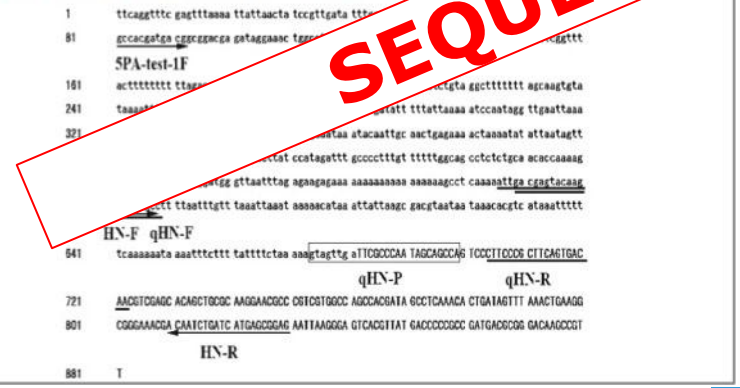

Table 3. Description of the Exogenous Insert of Huanong No. 1 and Its Flanking Regions

section	position no. of the nucleotide on the 7333 bp sequence	length (bp)	description of genetic elements	homology to Genbank no. (position of corresponding bp)
A	1–681	681	papaya genomic sequence	
B	682–773	92	sequence of the truncated *NptII* gene	AF485783 (3125–
C	774–816	43	transgenic vector sequence	AF485783
D	817–1123	307	*Agrobacterium tumifaciens* nopaline synthase (NOS) promoter	AF
E	1124–1135	12	transgenic vector sequence	
F	1136–1930	795	*neomycin phosphotransferase II* (	
G	1931–2319	389	transgenic vector sequence	4021)
H	2320–2575	256	*Agrobacterium* terminat	(4022–4277)
I	2576–3271	696		AF485783 (4278–4973)
J	3272–4006	835		AF485783 (4974–5808)
K	4007–4024			AF485783 (5809–5826)
L	4025–5726		ase (*NIb*) gene	FJ490192 (438–2039)
M	5727–5748		sequence	FJ490192 (2040–2061)
N	5749–6001		*tumifaciens* nopaline synthase (NOS) terminator	AF485783 (7727–7979)
O	6002		transgenic vector sequence	AF485783 (7980–8602)
P		709	papaya genomic sequence	

Table 1. Oligonucleotide Primers and Probes Used in This Study

PCR system	primer name	sequence (5′-3′)	target	amplicon (bp)	ref
qualitative PCR assay	5PA-test-1F	ATCTATAATGCCACGATGACG	papaya genome	1300	this work
	3PA-test-2R	AGGAAAAGAGATGGTGTGAAC			

SEQUENCE AVAILABLE

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European Commission

GM Papaya from Taiwan

Transgenic Res (2009) 18:971–986
DOI 10.1007/s11248-009-9287-7

ORIGINAL PAPER

Transgene-specific and event-specific molecular markers for characterization of transgenic papaya lines resistant to *Papaya ringspot virus*

Ming-Jen Fan · Shu Chen · Yi-Jung Kung · Ying-Huey Cheng · Huey-Jiunn Bau · Tien-Tsai Su · Shyi-Dong Yeh

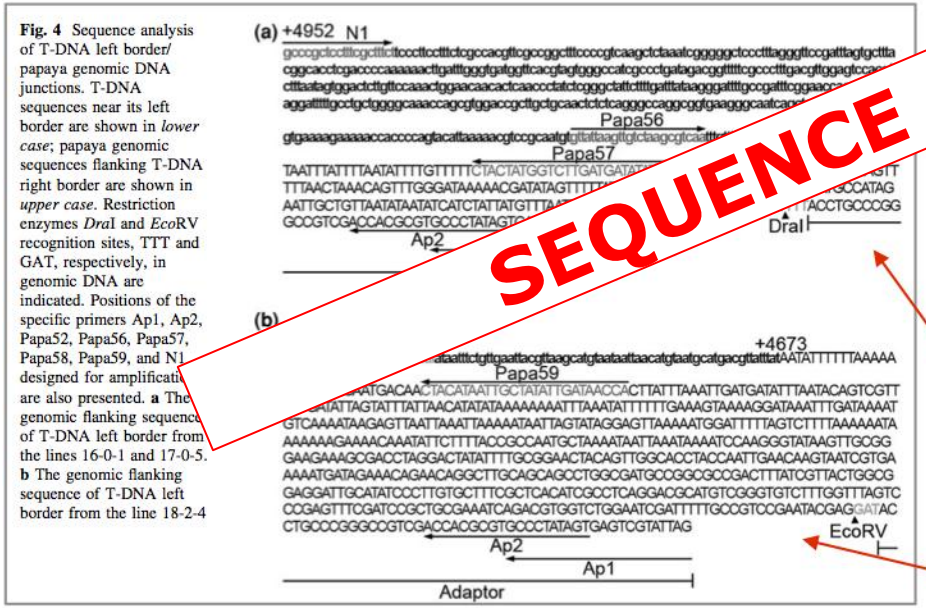

Fig. 4 Sequence analysis of T-DNA left border/ papaya genomic DNA junctions. T-DNA sequences near its left border are shown in *lower case*; papaya genomic sequences flanking T-DNA right border are shown in *upper case*. Restriction enzymes *Dra*I and *Eco*RV recognition sites, TTT and GAT, respectively, in genomic DNA are indicated. Positions of the specific primers Ap1, Ap2, Papa52, Papa56, Papa57, Papa58, Papa59, and N1 designed for amplificati are also presented. **a** The genomic flanking sequence of T-DNA left border from the lines 16-0-1 and 17-0-5. **b** The genomic flanking sequence of T-DNA left border from the line 18-2-4

US 8258282 (granted patent)

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Isolated nucleic acid molecules from transgenic papaya line 16-0-1 resistant to papaya ringspot virus and use thereof

Front Page | Full Text | PDF Version | Patent Family & Status | Sequences

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Assignees
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Filing Date

…ed Expiry Date
Dec 23, 2029

Agents
- Frenkel & Associates, PC

Application Number
12645554

Title
Isolated nucleic acid molecules from transgenic … 16-0-1 resistant to papaya ringspot virus and use thereof

Claims
1. A primer pair for a… acid sequence, which consists esse… …ng the sequence set forth in SEQ ID NO: … …g the sequence set forth in SEQ ID NO: 20…

…ed nucleic acid molecule having a right border flanking region, a left … region and a transgene sequence between the right border flanking … the left border flanking region, wherein the right border flanking region …ng at least 90% homology with the sequence set forth in SEQ ID NO: 29; the left border flanking region having at least 90% homology with the sequence set forth in SEQ ID NO: 31; and the transgene sequence having a papaya ringspot virus coat protein gene and a promoter operably linked to the papaya ringspot virus coat protein gene. Primers, probes and kit derived from the isolated nucleic acid molecule are proved to be useful for identifying the transgenic papaya line 16-0-1 in a specific, reproducible, sensitive and reliable way.

Non-Patent References
- Bau et al. Broad-spectrum resistance to differenet geographic stains of Papaya rinspot virus in coat protein gene transgenic papaya (2003) Phytopathology. 93: 112-120.
- Diffenbach et al. General tips for PCR primer design (1993) Genome Res. 3: S30-S37.
- Leoni et al. A genome walking strategy for the identification of eukaryotic nucleotide sequences adjactent to known regions (2008) Biotechniques. 44: 229-235.
- Pain et al. (GenBank Accession No. AJ928215, Jun. 10, 2005).

US 8258282 (granted patent)

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Isolated nucleic acid molecules from transgenic papaya line 16-0-1 resistant to papaya ringspot virus and use thereof

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New Search by Patent | New Search by Genbank Id | Back to Search

US8258282 DNA/RNA Sequence List

Displaying 31 - 35 of 35 sequences

Page: Previous 1 2 3 4

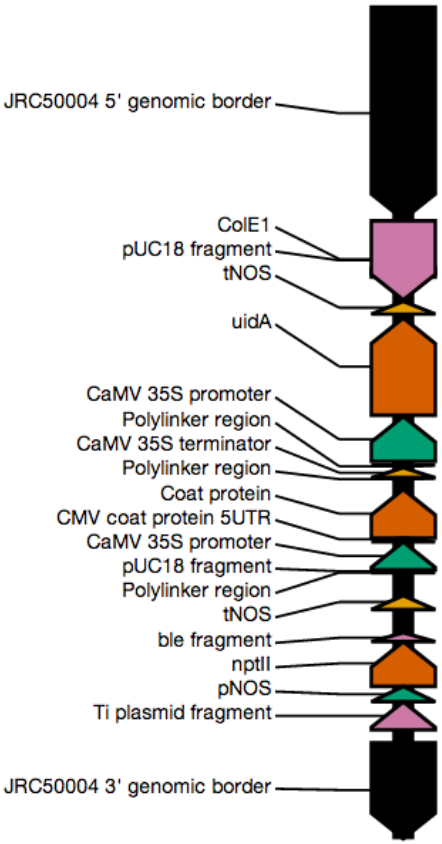
SEQ ID NO	Length	In Claims	Genbank Id	BLAST		Organism
31	237	no	407090501	patents	NCBI	N/A
32	599	no	407090502	patents	NCBI	N/A
33	5449	no	407090503	patents	NCBI	N/A
34	4666	no	407090504	patents	NCBI	N/A
35	48	no	407090505	patents	NCBI	N/A

SEQUENCE AVAILABLE

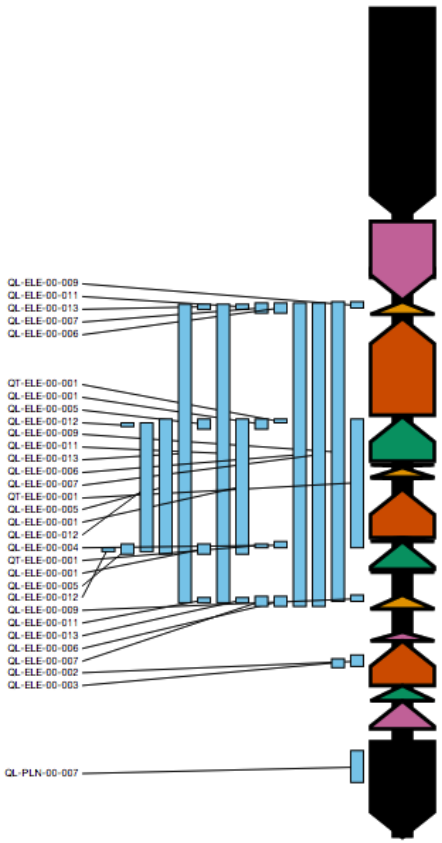
European Commission

Why the sequence is important?

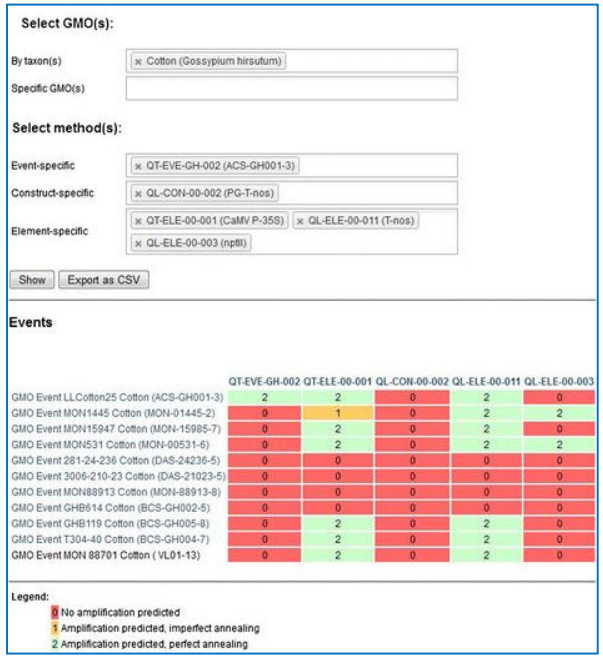
GMO elements



GMO methods



JRC GMO-Matrix



Select GMO(s):

By taxon(s): × Cotton (Gossypium hirsutum)

Specific GMO(s):

Select method(s):

Event-specific: × QT-EVE-GH-002 (ACS-GH001-3)

Construct-specific: × QL-CON-00-002 (PG-T-nos)

Element-specific: × QT-ELE-00-001 (CaMV P-35S) × QL-ELE-00-011 (T-nos) × QL-ELE-00-003 (nptII)

Show | Export as CSV

Events

	QT-EVE-GH-002	QT-ELE-00-001	QL-CON-00-002	QL-ELE-00-011	QL-ELE-00-003
GMO Event LLCotton25 Cotton (ACS-GH001-3)	2	2	0	2	0
GMO Event MON1445 Cotton (MON-01445-2)	0	1	0	2	2
GMO Event MON15947 Cotton (MON-15985-7)	0	2	0	2	0
GMO Event MON531 Cotton (MON-00531-6)	0	2	0	2	2
GMO Event 281-24-236 Cotton (DAS-24236-5)	0	0	0	0	0
GMO Event 3006-210-23 Cotton (DAS-21023-5)	0	0	0	0	0
GMO Event MON88913 Cotton (MON-88913-8)	0	0	0	0	0
GMO Event GHB614 Cotton (BCS-GH002-5)	0	0	0	0	0
GMO Event GHB119 Cotton (BCS-GH005-8)	0	2	0	2	0
GMO Event T304-40 Cotton (BCS-GH004-7)	0	2	0	2	0
GMO Event MON 88701 Cotton (VL01-13)	0	2	0	2	0

Legend:

0 No amplification predicted

1 Amplification predicted, imperfect annealing

2 Amplification predicted, perfect annealing

Joint Research Centre

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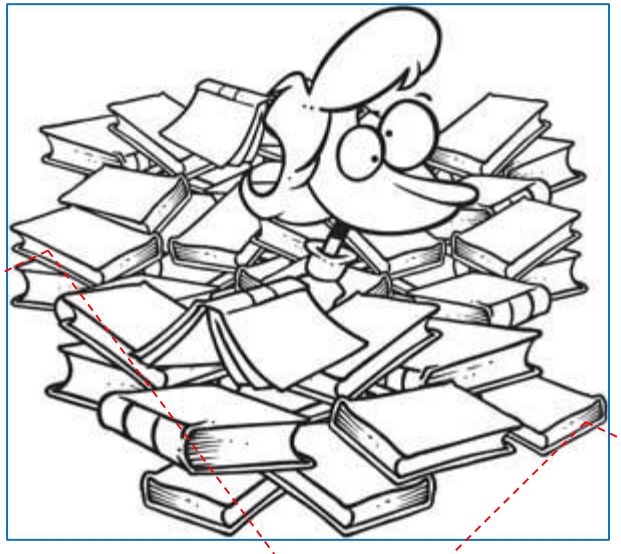
Going back to literature



Transgenic Plant Journal ©2010 Global Science Books

CP-Transgenic and non-Transgenic Approaches for the Control of Papaya Ringspot: Current Situation and Challenges

Gustavo A. Fermin[1*] • Luz T. Castro[2] • Paula F. Tennant[3]

Editor's Choice Series on the Next Generation of Biotech Crops

Forbidden Fruit: Transgenic Papaya in Thailand[1]

Sarah Nell Davidson*
Department of Plant Biology, Cornell University, Ithaca, New York 14853

Joint Research Centre

European Commission

Literature review

Table 3 Summary of the characteristics of transgenic papaya developed by various research groups.

Country	Cultivar	Construct	Transformation	Transgene copy number	Transgene expression	Resistance testing		Reference
						Greenhouse	Field	
TRANSLATABLE *cp*								
Australia	Local variety	*uidA* leader + CaMV 35S pro+ PRSVBridgeman Downs (Queensland) *cp* gene from Q/S start with stop codon in the middle of sequence	Biolistics	1- 4 reported on truncations & rearrangements of the *cp* (no correlation copy number & level of R but to level of degraded RNA in northerns)	CP not detected in ELISA and low levels of *cp* detection in northerns	Truncated 1 copy 0% R 4 copies 100% 3 copies 100% R 3 copies 15% R	Truncated 1 copy 0% R 4 copies 100%R 3 copies 80% R (15% in greenhouse)	Lines *et al.* 2002
Brazil	Sunrise solo & Sunset solo	CaMV 35S + CMV leader+ PRSV Bahia *cp* from Q/S start CaMV 35S + CMV leader+ PRSV Bahia *cp* from E/S start	Biolistics	nt	Low to high levels CP protein detected in ELISA	R_1 46% R to PRSV Cruz das Amas R_1 0% R to PRSV Bahia, HA & TH	na	Souza Junior *et al.* 2005
Florida	cv. F65 (ancestor of F65)	*uidA* leader + CaMV 35S pro+ PRSV H1K cp gene from Q/S start	*Agrobacterium*	1-> 2	*cp* not detected in northern analysis	5- 13%	nt	Davis and Ying 2004
Hawaii	Sunset solo	CMV leader + 16 aa CMV *cp* + PRSV HA 5-1 *cp* gene from Q/S start	Biolistics	1- 2 (correlation between R and sequence similarity with *cp* transgene, copy number, plant age)	Low to high levels CP protein & transcript detected in ELISA & northern analysis	55-1: R_0 NS; R1 NS but showed symptoms against PRSV Oahua 63-1: R_1 40-52% resistance against Hawaiian isolates, but 26-39% resistance against BR, JA & TH isolates	55-1: R_1 (Rainbow) & R_3 NS for 12 mo 63-1: R_0 resistant in field for 12 mo	Fitch *et al.* 1992; Tennant *et al.* 1994; Ferreira *et al.* 2002; Tennant *et al.* 2005; Souza *et al.* 2005
Jamaica	Sunrise Solo	CaMV 35S + CMV leader+ PRSV Caymanas *cp* from Q/S start	Biolistics	1-3	*cp* RNA detected in northern analysis	R_0 29-40% R	R_0 50-89%	Cai *et al.* 1999; Tennant *et al.* 2002, 2005
Taiwan	Tainung No. 2	*uidA* leader + CaMV 35S pro+ PRSV YK cp gene from Q/S start	*Agrobacterium*	1-2 (R plants had 2 copies; highly R had one copy)	*cp* transcript detected in Northern (a relationship between R and the detection of CP & *cp* transcript)	4 categories of reactions: delay & then symptoms (40%), mild mottling (70-80%), immunity, susceptible	70-80% R (no correlation between R and sequence similarity with *cp* transgene)	Bau *et al.* 2003; Tripathi *et al.* 2004
Thailand	Khak Dum	CaMV 35S + *uidA* leader+ PRSV Ratchaburi province *cp*	Biolistics	Multiple insertions with rearrangements & deletions	CP detected in western analysis in 2 of 8 lines	All lines susceptible except for one line (G2)	nt	Kertbundit *et al.* 2007; Ruanjan *et al.* 2007
Venezuela	Tailanda roja (Thailand red)	CaMV 35S + CMV leader+ PRSV EV & VE from Q/S start	*Agrobacterium*	1	CP not detected in ELISA and low levels of *cp* detection in northern analysis.	All R_0 plants with LA or EV *cp* R to PRSV LA and EV R_1 EV *cp* 7% R, 50-73% R & 60-60% R to PRSV EV, La and HA R_1 EV+LA cp 0% R, 31% R & 38% R to PRSV EV, LA and HA R_2 EV cp 22-32% R	nt	Fermin *et al.* 2004

Table 3 (Cont.)

Country	Cultivar	Construct	Transformation	Transgene copy number	Transgene expression	Resistance testing		Reference
						Greenhouse	Field	
UNTRANSLATABLE *cp*								
Brazil	Sunrise solo & Sunset solo	CaMV 35S + CMV leader+ PRSV Bahia *cp* from Q/S start	Biolistics	nt	nt	R_1 100% R to PRSV Bahia & PRSV HA, 72% R PRSV TH	na	Souza Junior *et al.* 2005
Florida	cv. F65 (ancestor of F65)	*uidA* leader + CaMV 35S pro+ PRSV H1K *cp* gene from Q/S start in antisense	*Agrobacterium*	1-2	*cp* not detected in northern analysis	12- 15%	Nt	Davis and Ying 2004
		uidA leader + CaMV 35S pro+ PRSV H1K *cp* gene from Q/S start with frame shift mutation			*cp* not detected in northern analysis	4- 42%	71- 90%	
		uidA leader + CaMV 35S pro+ PRSV H1K *cp* gene from Q/S start with 3 in frame stop			*cp* not detected in northern analysis	8-34%	12-90%	
Hawaii	Sunset solo	CaMV 35S + CMV leader+ PRSV Caymanas untranslatable *cp*	Biolistics	1-3	*cp* RNA detected in R_0 by Northern analysis	3 categories of reactions: 28 lines HA resistant (100%); 22 lines mixed R (i.e. 49% showed R & S); 33 lines S	nt	Cai *et al.* 1999; Fermin 2002
Jamaica	Sunrise Solo	CaMV 35S + CMV leader+ PRSV Caymanas untranslatable *cp*	Biolistics	nt	*cp* RNA detected in R_0 by Northern analysis	R_0 15-29% R	R_0: 10% R or delay in symptom expression; 18-66% delay & mild symptom expression R_1: 0% R, 25-100% delay, mild symptom expression	Tennant *et al.* 2002, 2005

R resistant; S susceptible; nt not tested; na not available

Transgenic Plant Journal ©2010 Global Science Books

CP-Transgenic and non-Transgenic Approaches for the Control of Papaya Ringspot: Current Situation and Challenges

Gustavo A. Fermin[1*] • Luz T. Castro[2] • Paula F. Tennant[3]

Joint Research Centre

European Commission

Transgenic Papaya in Thailand

Editor's Choice Series on the Next Generation of Biotech Crops

Forbidden Fruit: Transgenic Papaya in Thailand[1]

Sarah Nell Davidson*
Department of Plant Biology, Cornell University, Ithaca, New York 14853

PLAYERS IN THE GE PAPAYA CONTROVERSY

Farmers

"Yes, I have grown GE papaya. I received it from my brother. People told him if he ate it, he would be infertile. However, I ate the fruits from this papaya and they are delicious."—an Isaan farmer.

The Opposition

"Technology that isn't Thai isn't good for Thailand."—Natwipha Ewasakul, GE campaigner, Greenpeace Southeast Asia.

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Lines R3-319-180/1/2

Features:

- Three lines
- Coat protein of thai strain
- Sequence not available
- Unknown loci

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Are we alone?

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Are we alone?

Food Chemistry 136 (2013) 895–901

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Application of a qualitative and quantitative real-time polymerase chain reaction method for detecting genetically modified papaya line 55-1 in papaya products

Kosuke Nakamura [a], Hiroshi Akiyama [a,*], Yuki Takahashi [a,b], Tomoko Kobayashi [a], Akio Noguchi [a], Kiyomi Ohmori [c], Masaki Kasahara [d], Kazumi Kitta [e], Hiroyuki Nakazawa [b], Kazunari Kondo [a,*], Reiko Teshima [a]

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[b] Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan
[c] Chemistry Division, Kanagawa Prefectural Institute of Public Health, 1-3-1 Shimomachiya, Chigasaki, Kanagawa 253-0087, Japan
[d] Food and Agricultural Materials Inspection Center, 2-1 Shintoshin, Chuo-ku, Saitama, Saitama 330-9731, Japan
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Line-55 construct specific

Table 4
Analysis of the processed papaya products for GM papaya ingredient.

Product	Lot.	Real-time PCR Ct value (mean; $n = 2$)					
		Endogenous *Chy*	55-1 (Event-specific)	55-1 (Construct-specific)	P35S (Cis-element-specific)	T-nos (Cis-element-specific)	Unapproved GM papaya PRSV-YK (Construct-specific)
Canned	A	25.90	–	–	–	–	–
	B	30.16	–	–	–	–	–
	C	24.48	–	–	–	–	–
Pickled	A	27.68	–	–	37.63	36.54	36.47
	B	21.19	–	–	33.40	33.22	33.24
	C	21.70					
	D	–	–	–	–	–	–
Dried fruit (sulfured)	A	–	–	–	–	–	–
	B	–	–	–	–	–	–
	C	–	–	–	–	–	–
	D	–	–	–	–	–	–
	E	–	–	–	–	–	–
Dried fruit (Unsulfured)	F	24.43	–	–	–	–	–
	G	23.50	–	–	–	–	–
	H	23.20	–	–	–	–	–
	I	23.70	–	–	–	–	–
	J	22.10	–	–	–	–	–
	K	25.50	–	–	–	–	–
Papaya-leaf tea	A	22.90	33.13	33.05	24.03	24.69	26.03
	B	23.98	–	–	32.90	32.37	31.60
	C	28.60	–	–	–	–	–
Jam	A	27.20	39.72	39.61	33.57	31.72	30.32
	B	25.73	–	–	–	–	–
	C	32.98	–	–	–	–	–
	D	–	–	–	–	–	–
	E	–	–	–	–	–	–
	F	–	–	–	–	–	–
	G	–	–	–	–	–	–
Puree	A	21.96	–	–	–	–	–
	B	23.99	–	–	–	–	–
	C	23.01	–	–	–	–	–
Juice	A	24.43	–	–	–	–	–
	B	34.95	–	–	–	–	–
	C	–	–	–	–	–	–
	D	43.14	–	–	–	–	–
	E	34.40	–	–	–	–	–
Frozen dessert	A	21.30	–	–	–	–	–
	B	21.60	–	–	–	–	–

Taiwan !

1648 Communication to the Editor
Biol. Pharm. Bull. **34**(10) 1648—1651 (2011)

Identification and Detection Method for Genetically Modified Papaya Resistant to Papaya Ringspot Virus YK Strain

Kosuke NAKAMURA,[a] Hiroshi AKIYAMA,*[,a] Kiyomi OHMORI,[b] Yuki TAKAHASHI,[c] Reona TAKABATAKE,[d] Kazumi KITTA,[d] Hiroyuki NAKAZAWA,[c] Kazunari KONDO,[a] and Reiko TESHIMA[a]

European Commission

Taiwan Event TP10-4

Virology

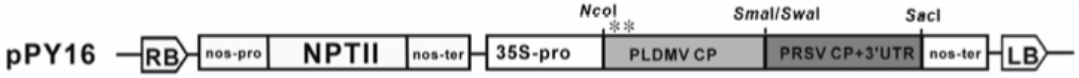
e-Xtra*

Generation of Transgenic Papaya with Double Resistance to *Papaya ringspot virus* and *Papaya leaf-distortion mosaic virus*

Yi-Jung Kung, Huey-Jiunn Bau, Yi-Ling Wu, Chiung-Huei Huang, Tsui-Miao Chen, and Shyi-Dong Yeh

First, third, fourth, fifth, and sixth authors: Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan, R.O.C.; and second author: Department of Biotechnology, Transworld Institute of Technology, Yunlin, Taiwan, R.O.C.

Accepted for publication 7 July 2009.



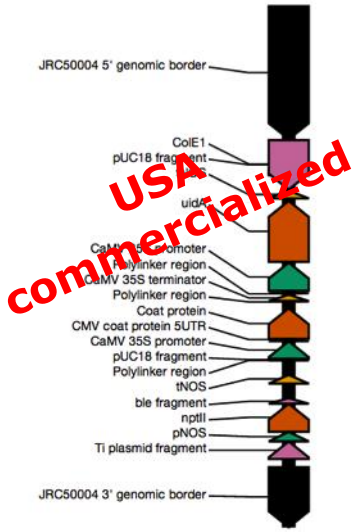
Features:

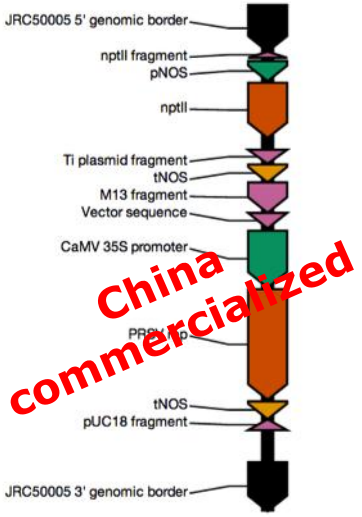
- Double resistance: PRSV + *Papaya leaf-distortion mosaic virus* (PLDMV)
- Sequence not available
- Unknown loci
- Field trials

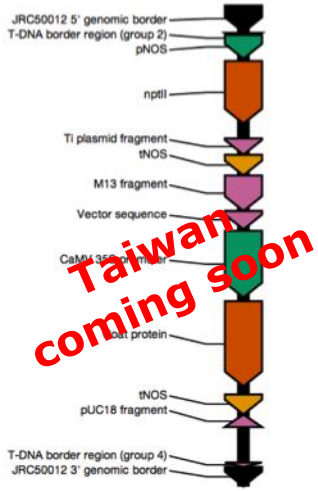
European Commission

GM-papaya state of art

Country	Reference	Gonsalves collaboration?	*Carica papaya L.* cultivar	PRSV isolate	Inserted gene(s)	Transformation method	Inserted copy numbers	Greenhouse?	Field trials?	Commericalized?	Sequence available?
Australia	Lines *et al.*, 2002	NO	local variety	PRSV AU (Queensland)	*cp*	Biolistics	1-4	YES	YES	NO	NO
Bangladesh	Azad *et al.*, 2013	NO	Shahi and Ranchi	none	only nptII and GUS	Agrobacterium	na	NO	NO	NO	NO
Brazil	Souza Junior *et al.*, 2005	YES	Sunrise, Sunset	PRSV BR (Bahia)	*cp*	Biolistics	>1	YES	YES	NO	Only cp genes
China	Guo *et al.*, 2009	NO	Huanong No.1	PRSV	*Nlb*	Agrobacterium	1	YES	YES	YES	YES*
Indonesia	Mendoza *et al.*, 2008	NO	Bangkok and Burung	PRSV (Bangor)	*cp*	Biolistics	na	na	na	NO	NO
Jamaica	Tennant *et al.*, 2005	YES	Sunrise	PRSV JA (Caimanas)	*cp*	Biolistics	1-3	YES	YES	NO	Only cp genes
Malaysia	Mendoza *et al.*, 2008	NO	Eksotika	PRSV	*Nlb* and more than 1 *cp*	Agrobacterium	na	na	na	NO	NO
Philippines	Retuta *et al.*, 2012	NO	Solo	PRSV Philippines	*Nlb* and more than 1 *cp*	Agrobacterium	na	na	na	NO	NO
Taiwan -1	Bau *et al.*, 2003	NO	Tainung No.2	PRSV YK	*cp*	Agrobacterium	1	YES	YES	NO	YES*
Taiwan -2	Kung *et al.*, 2009	NO	cv. Thailand	PRSV YK + P LDMV	more than 1 *cp*	Agrobacterium	1-3	YES	YES	NO	NO
Thailand -1	Sakuanrungsiriku *et al.*, 2005	YES	Khak Dum and Khak Nual	PRSV Thai	*cp*	Biolistics	3	YES	YES	NO	NO
Thailand -2	Kertbundit *et al.*, 2007	NO	Khak Dum	PRSV Thai	*cp*	Biolistics	>1	YES	NO	NO	NO
US (Florida)	Davis *et al.*,2004	NO	cv. F65	PRSV H1K	*cp*	Agrobacterium	>1	YES	YES	NO	YES*
US (Hawaii) -1	Fitch *at al.*, 1992	YES	Sunset	PRSV HA	*cp*	Biolistics	3	YES	YES	YES	YES
US (Hawaii) -2	Tennant *et al.*, 2005	YES	Sunset	PRSV HA	*cp*	Biolistics	>1	YES	YES	YES	NO
Venezuela	Fermin *et al.*, 2004	YES	Tailandia Roja	PRSV VE (El Vigìa) and LA (Lagunillas)	more than 1 *cp*	Agrobacterium	1	YES	NO	NO	Only cp genes
Vietnam	Mendoza *et al.*, 2008	NO	KD Thai, Tim Taiwan, Solo, Mexico and Local Lansom	PRSV Vietnam	*Nlb* and more than 1 *cp*	Agrobacterium	na	na	na	NO	NO



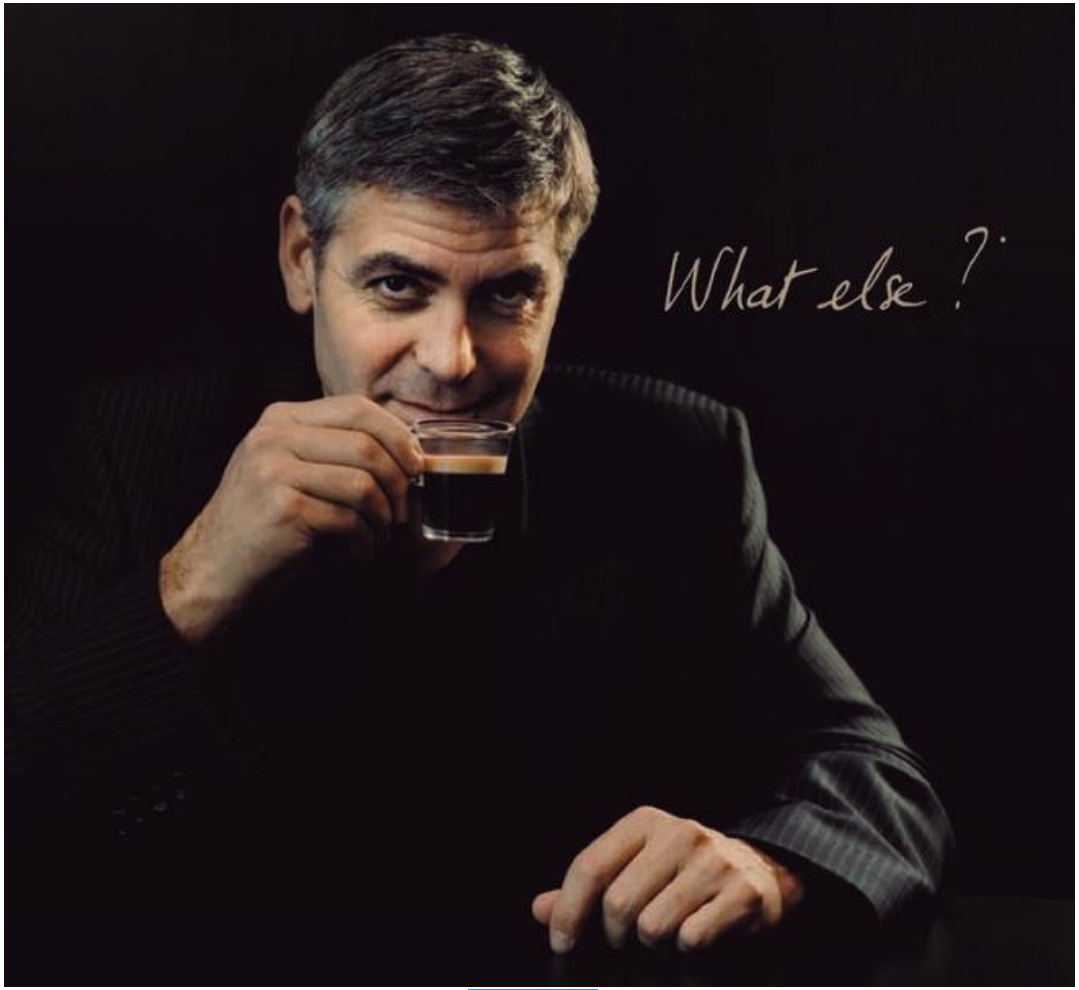




GMO Event 55-1 Rainbow papaya (CUH-CP551-8) GMO Event Huanong No.1 papaya GMO Event 18-2-4 line papaya GMO Event 16-0-1 line papaya GMO Event UFL-X17CP-6 papaya

European Commission

What else?



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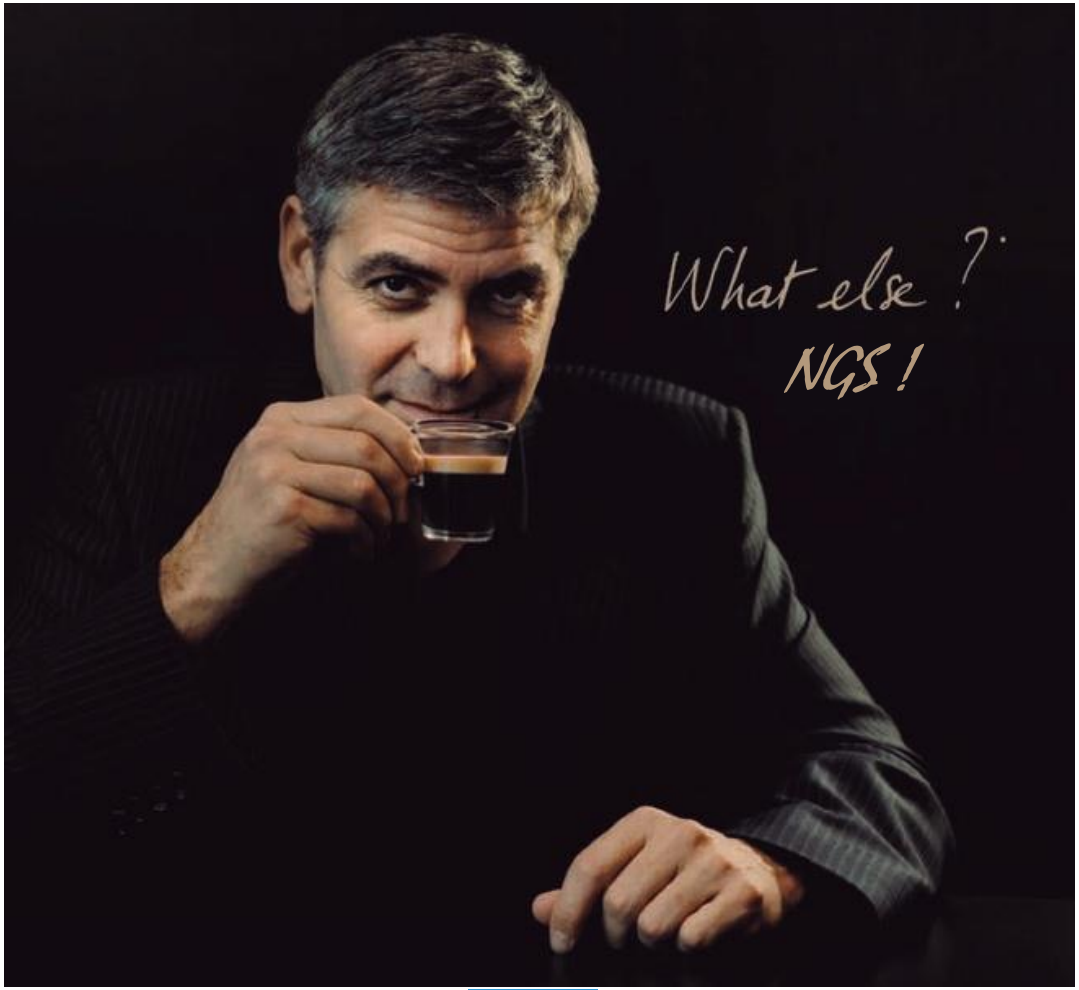
NGS!



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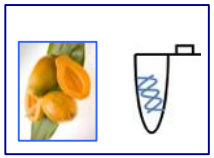
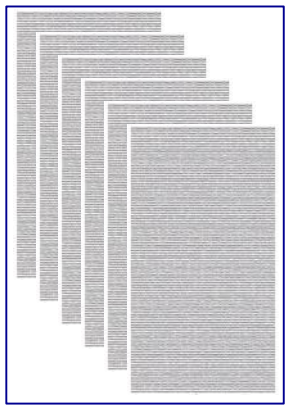
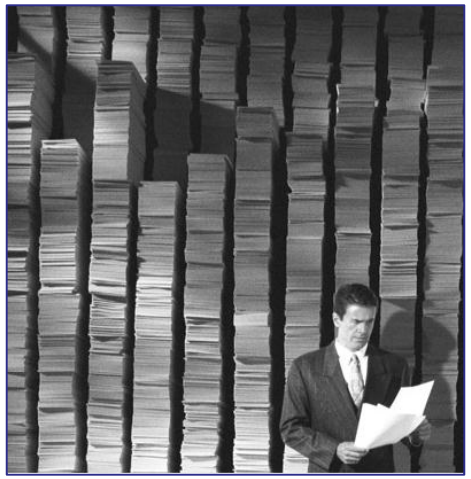
NGS brute force approach?







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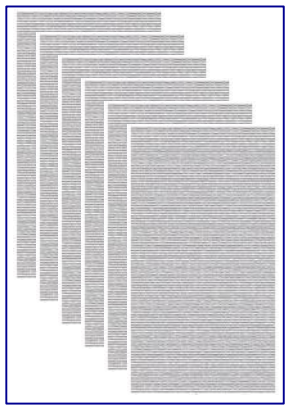
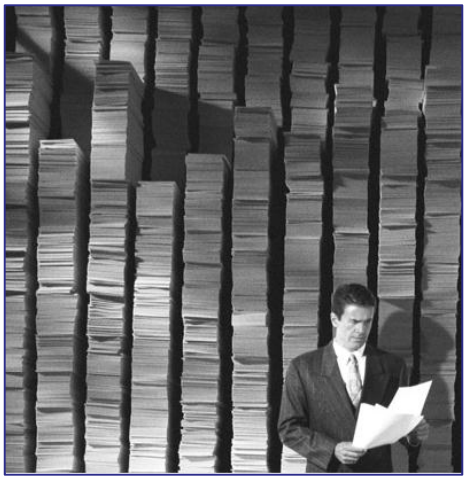
NGS brute force approach?



WHY ?



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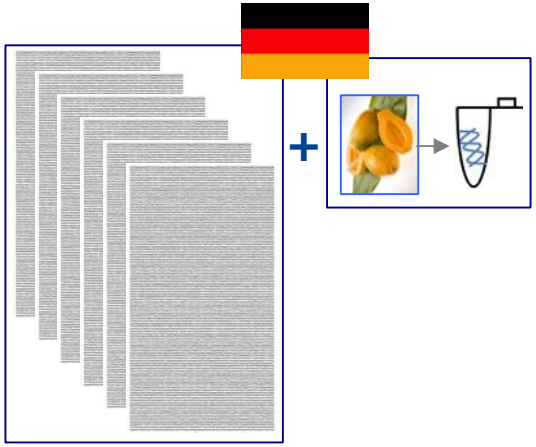
Main problems

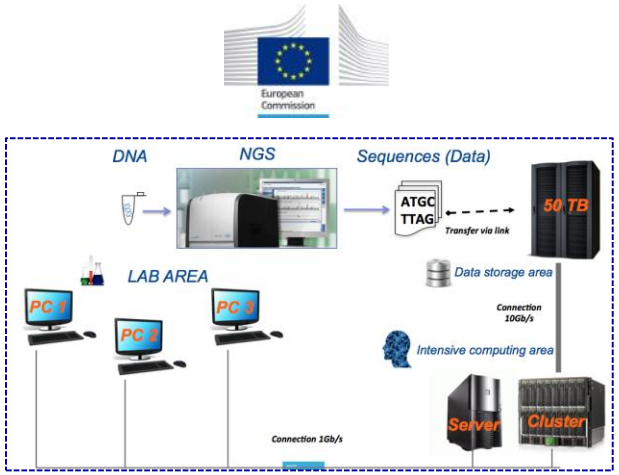
- Papaya genome is highly repeated
- Low coverage sequencing
- No genomic reference (particle bombardment)
- Multiple GM-cassette insertion site

Anyway, interesting results were found:

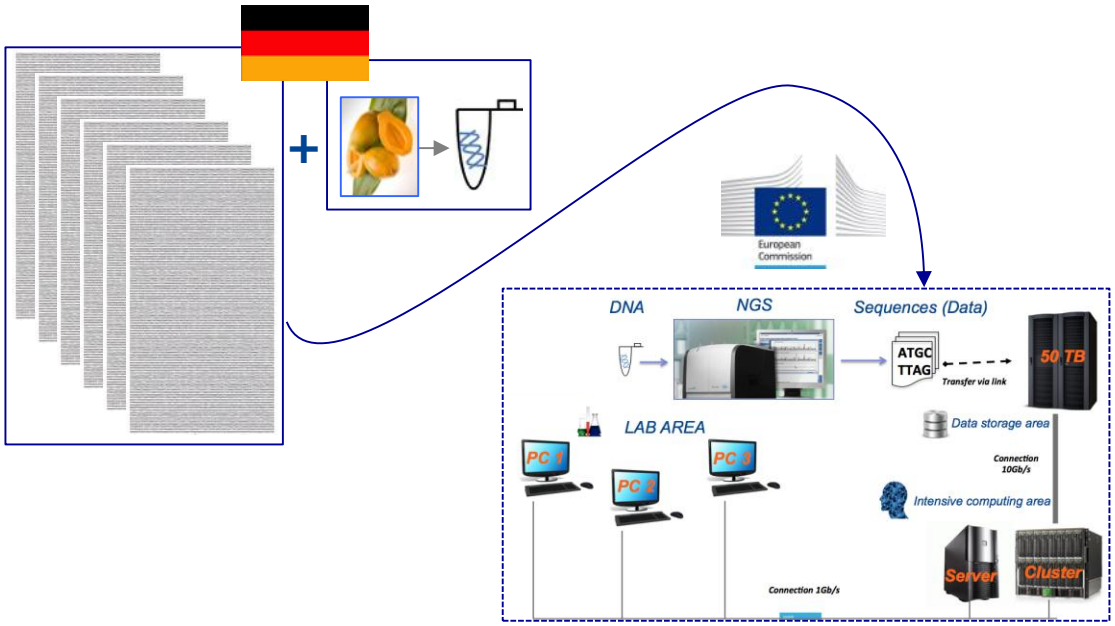
nptII **T-NOS Coat protein**

Importance of adequate infrastructure





Analyses at EU-RL GMFF



genomic	Coat protein	CaMV35S-P	nptII	T-NOS Coat protein	genomic

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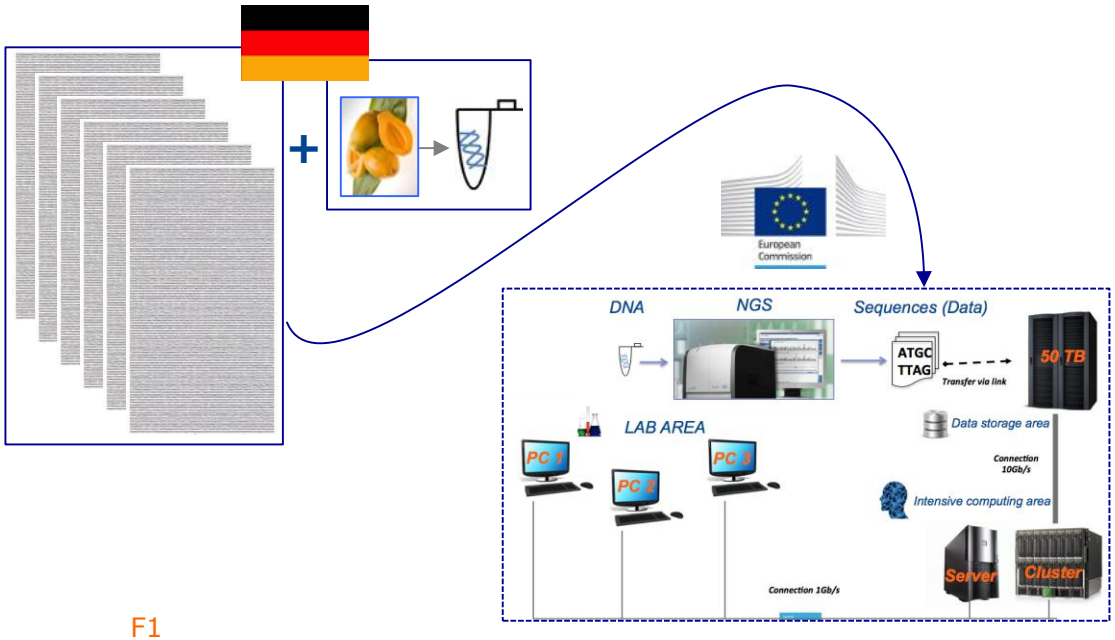
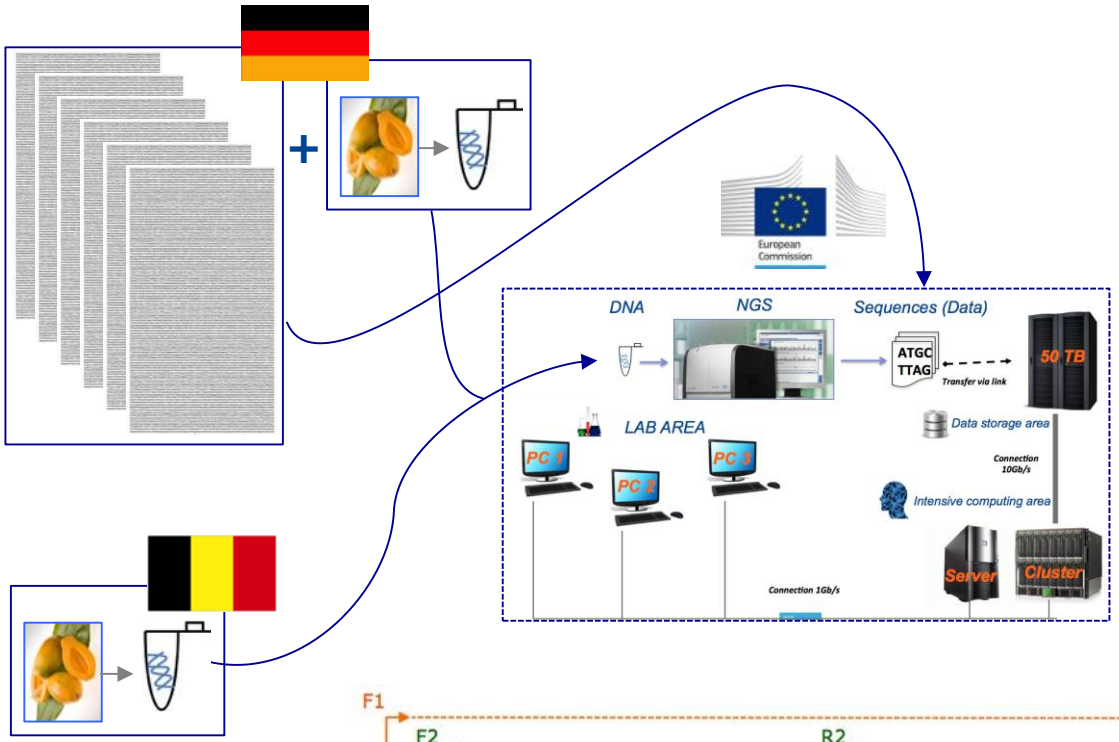


Strategy

Large amplicon target resequencing

True??

Analyses at EU-RL GMFF



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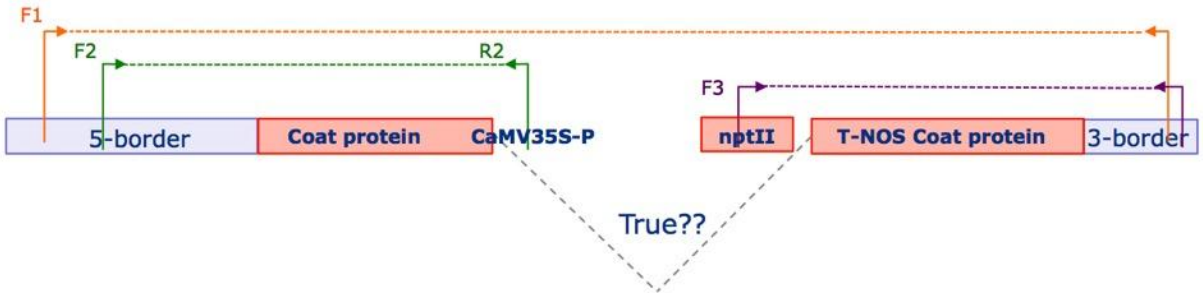

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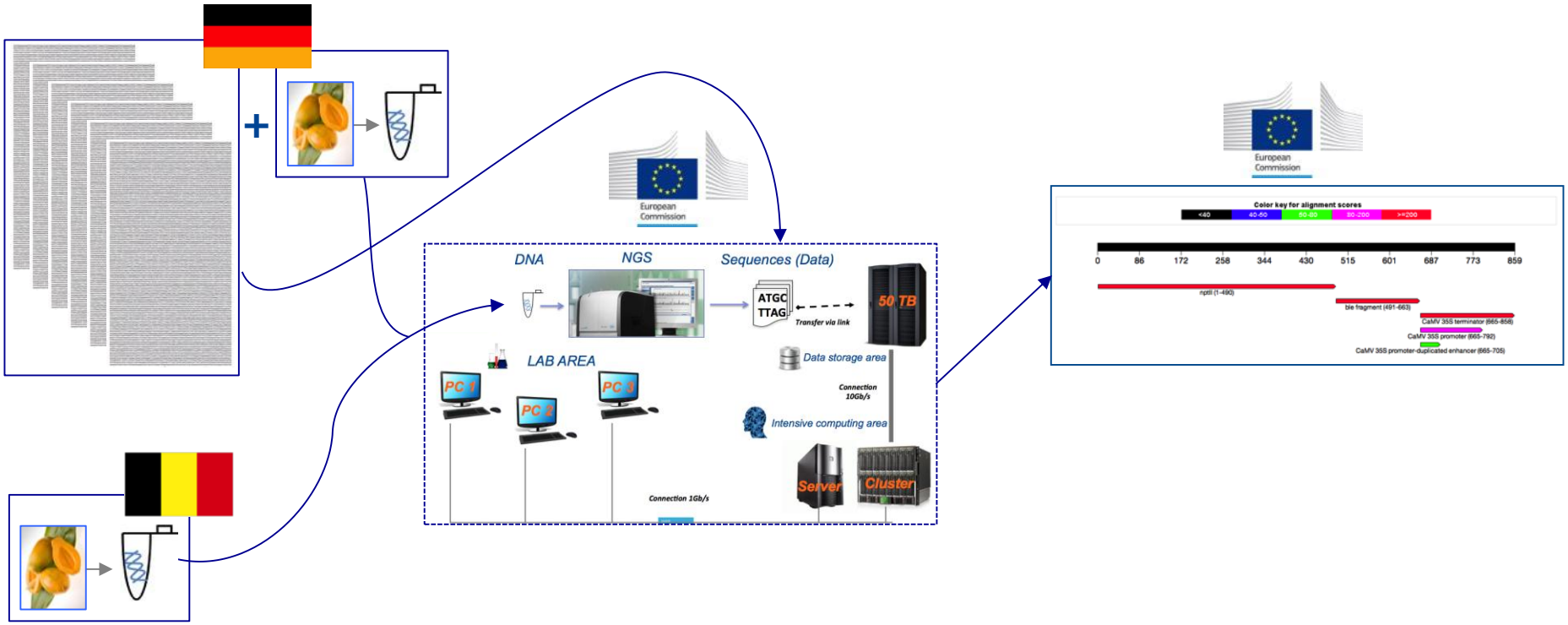

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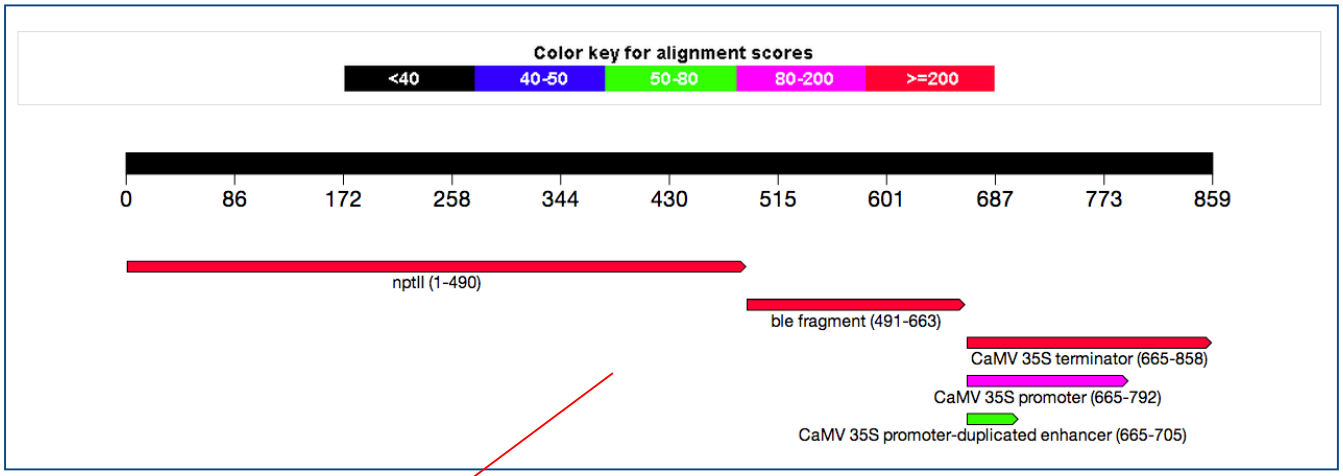

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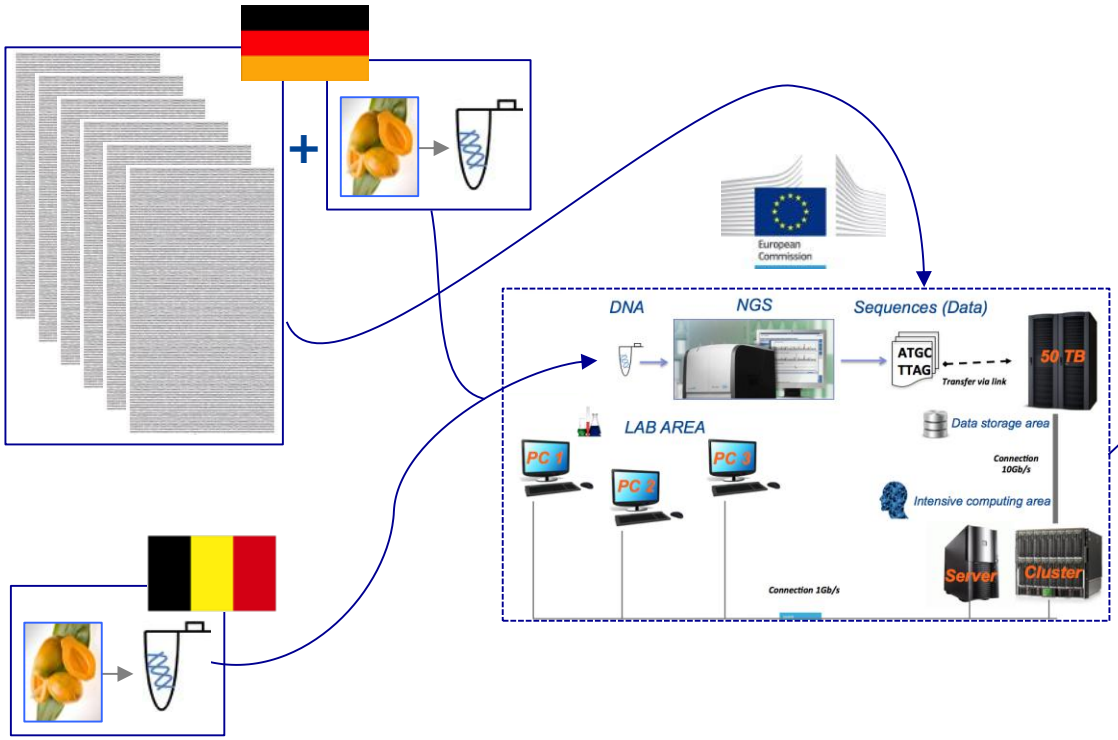
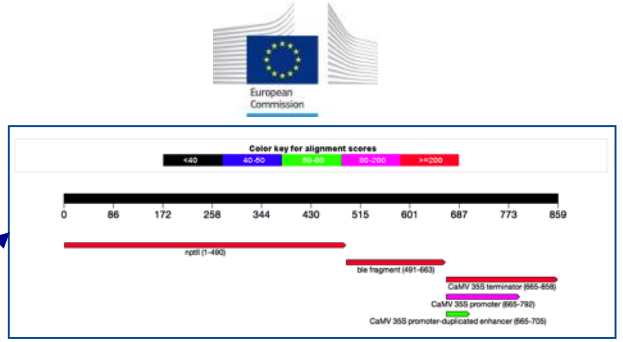
Joint Research Centre

Analyses at EU-RL GMFF

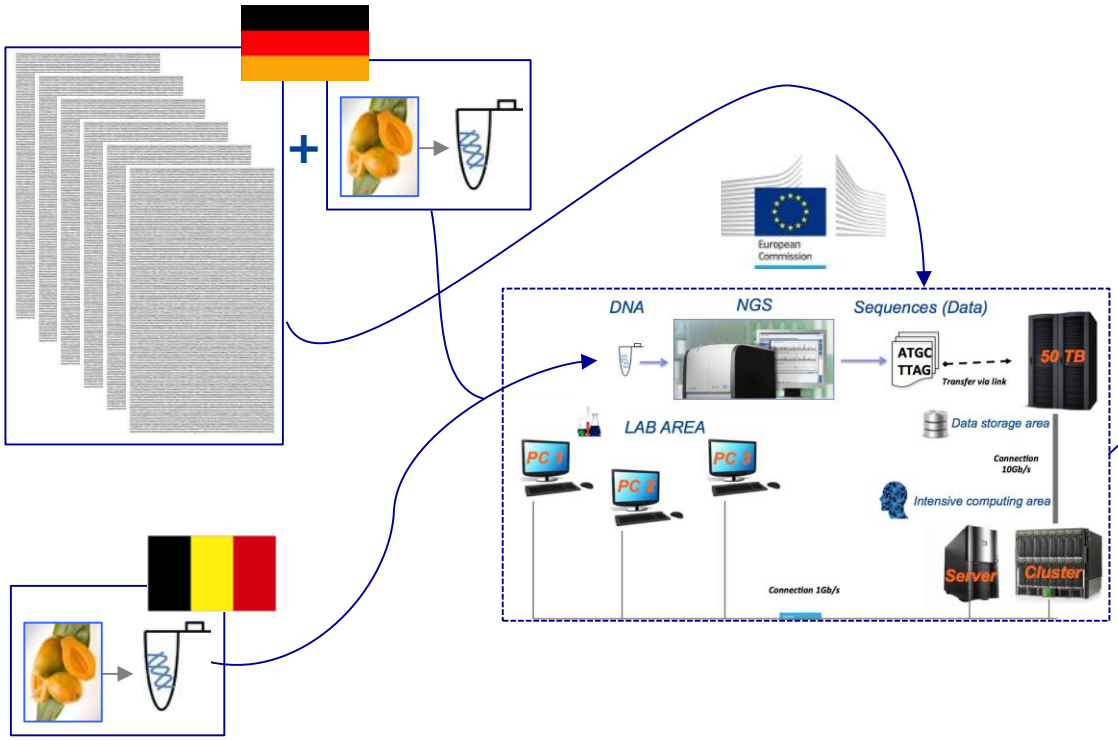


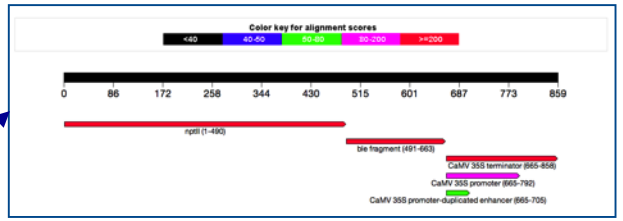


Country	Reference	Gonsalves collaboration?	*Carica papaya L.* cultivar	PRSV isolate	Inserted gene(s)	Transformation method	Inserted copy numbers	Greenhouse?	Field trials?	Commercialized?	Sequence available?
Australia	Lines *et al.*, 2002	NO	local variety	PRSV AU (Queensland)	cp	Biolistics	1-4	YES	YES	NO	NO
Bangladesh	Azad *et al.*, 2013	NO	Shahi and Ranchi	none	only nptII and GUS	Agrobacterium	na	NO	NO	NO	NO
Brazil	Souza Junior *et al.*, 2005	YES	Sunrise, Sunset	PRSV BR (Bahia)	cp	Biolistics	>1	YES	YES	NO	Only cp genes
China	Guo *et al.*, 2003	NO	Huanong No.1	PRSV	NIb	Agrobacterium	1	YES	YES	YES	YES*
Indonesia	Mendoza *et al.*, 2008	NO	Bangkok and Burung	PRSV (Bangor)	cp	Biolistics	na	na	na	NO	NO
Jamaica	Fermin *et al.*, 2006	YES	Sunrise	PRSV JA (Camuwa)	cp	Biolistics	1-3	YES	YES	NO	Only cp genes
Malaysia	Mendoza *et al.*, 2008	NO	Eksotika	PRSV	NIb and more than 1 cp	Agrobacterium	na	na	na	NO	NO
Philippines	Rebata *et al.*, 2012	NO	Solo	PRSV Philippines	NIb and more than 1 cp	Agrobacterium	na	na	na	NO	NO
Taiwan -1	Rao *et al.*, 2003	NO	Tainung No.2	PRSV YK	cp	Agrobacterium	1	YES	YES	NO	YES*
Taiwan -2	Gung *et al.*, 2009	NO	cv. Thailand	PRSV YK + P (DMV	more than 1 cp	Agrobacterium	1-5	YES	YES	NO	NO
Thailand -1	Sakwarangsritha *et al.*, 2005	YES	Khak Dum and Khak Nual	PRSV Thai	cp	Biolistics	>1	YES	YES	NO	NO
Thailand -2	Kertbundit *et al.*, 2007	NO	Khak Dum	PRSV Thai	cp	Biolistics	>1	YES	NO	NO	NO
US (Florida)	Davis *et al.*,2004	NO	cv. HOS	PRSV YLK	cp	Agrobacterium	>1	YES	YES	NO	YES*
US (Hawaii) -1	Yeh *et al.*, 1992	YES	Sunset	PRSV HA	cp	Biolistics	3	YES	YES	YES	YES
US (Hawaii) -2	Fermanti *et al.*, 2005	YES	Sunset	PRSV HA	cp	Biolistics	>1	YES	YES	YES	NO
Venezuela	Fermin *et al.*, 2004	YES	Tailandia Roja	PRSV VC (El Vigia) and AA (Lagunillas)	more than 1 cp	Agrobacterium	1	YES	NO	NO	Only cp genes
Vietnam	Mendoza *et al.*, 2008	NO	KO Thai, Tim Taiwan, Solo, Mexico and Local Lansom	PRSV Vietnam	NIb and more than 1 cp	Agrobacterium	na	na	na	NO	NO

European Commission

Analyses at EU-RL GMFF





Country	Reference	Gonsalves collaboration?	*Carica papaya L.* cultivar	PRSV isolate	Inserted gene(s)	Transformation method	Inserted copy numbers	Greenhouse?	Field trials?	Commercialized?	Sequence available?
Australia	Lines *et al.*, 2002	NO	local variety	PRSV AU (Queensland)	cp	Biolistics	1-4	YES	YES	NO	NO
Bangladesh	Asad *et al.*, 2013	NO	Shahi and Ranchi	Home	only nptII and GUS	Agrobacterium	na	NO	NO	NO	YES
Brazil	Souza Junior *et al.*, 2005	YES	Sunrise, Sunset	PRSV BR (Bahia)	cp	Biolistics	>1	YES	YES	NO	Only cp genes
China	Guo *et al.*, 2003	NO	Huanong No.1	PRSV	NIb	Agrobacterium	1	YES	YES	YES	YES*
Indonesia	Mendoza *et al.*, 2008	NO	Bangkok and Burung	PRSV (Bangor)	cp	Biolistics	na	na	na	NO	NO
Jamaica	Fermann *et al.*, 2005	YES	Sunrise	PRSV JA (Camuwa)	cp	Biolistics	1-3	YES	YES	NO	Only cp genes
Malaysia	Mendoza *et al.*, 2008	NO	Eksotika	PRSV	NIb and more than 1 cp	Agrobacterium	na	no	na	NO	NO
Philippines	Rebata *et al.*, 2012	NO	Solo	PRSV Philippines	NIb and more than 1 cp	Agrobacterium	na	na	na	NO	NO
Taiwan -1	Rao *et al.*, 2003	NO	Tainung No.2	PRSV YK	cp	Agrobacterium	1	YES	YES	NO	YES*
Taiwan -2	Gung *et al.*, 2009	NO	cv. Thailand	PRSV YK + P (DMV	more than 1 cp	Agrobacterium	1-5	YES	YES	NO	NO
Thailand -1	Sakuanrungsirikul *et al.*, 2005	YES	Khak Dum and Khak Nual	PRSV Thai	cp	Biolistics	2	YES	YES	NO	NO
Thailand -2	Kertbundit *et al.*, 2007	NO	Khak Dum	PRSV Thai	cp	Biolistics	>1	YES	NO	NO	NO
US (Florida)	Davis *et al.*,2004	NO	cv. HOS	PRSV YLH	cp	Agrobacterium	>1	YES	YES	NO	YES*
US (Hawaii) -1	Fitch *et al.*, 1992	YES	Sunset	PRSV HA	cp	Biolistics	2	YES	YES	YES	YES
US (Hawaii) -2	Fermann *et al.*, 2005	YES	Sunset	PRSV HA	cp	Biolistics	>1	YES	YES	YES	NO
Venezuela	Fermin *et al.*, 2004	YES	Tailandia Roja	PRSV VC (El Vigia) and AA (Lagunillas)	more than 1 cp	Agrobacterium	1	YES	NO	NO	Only cp genes
Vietnam	Mendoza *et al.*, 2008	NO	KO Thai, Tim Taiwan, Solo, Mexico and Local Lanson	PRSV Vietnam	NIb and more than 1 cp	Agrobacterium	na	na	na	NO	NO

Update on the development of virus-resistant papaya: Virus-resistant transgenic papaya for people in rural communities of Thailand

Food and Nutrition Bulletin, vol. 26, no. 4 (supplement) © 2005, The United Nations University.

S. Sakuanrungsirikul, N. Sarindu, V. Prasartsee, S. Chaikiatiyos, R. Siriyan, M. Sriwatanakul, P. Lekananon, C. Kitprasert, P. Boonsong, P. Kosiyachinda, G. Fermin, and D. Gonsalves

Currently banned in Thailand

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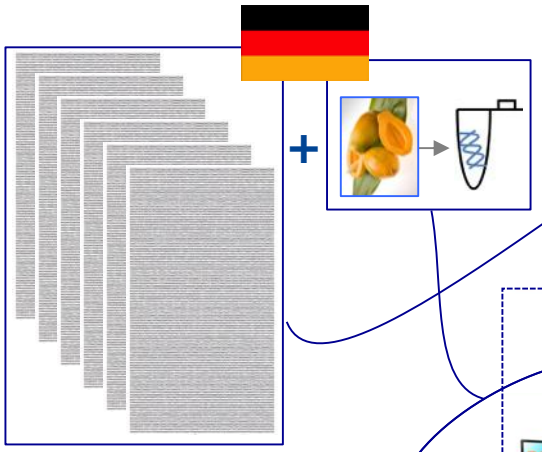
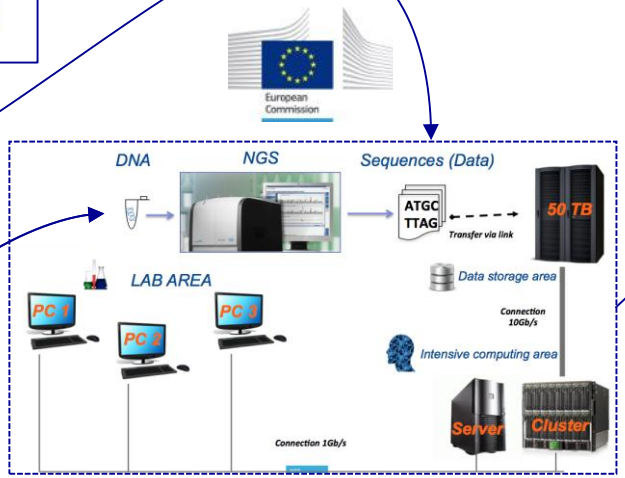
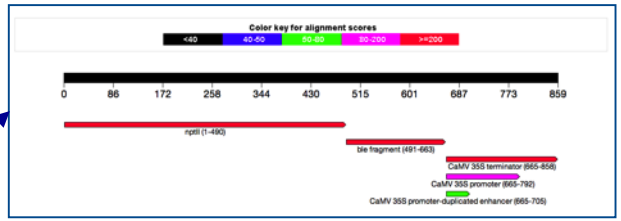
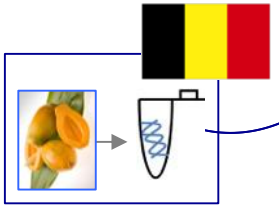
European Commission

Analyses at EU-RL GMFF









Country	Reference	Gonsalves collaboration?	*Carica papaya L.* cultivar	PRSV isolate	Inserted gene(s)	Transformation method	Inserted copy numbers	Greenhouse?	Field trials?	Commercialized?	Sequence available?
Australia	Lines *et al.*, 2002	NO	local variety	PRSV AU (Queensland)	cp	Biolistics	1-4	YES	YES	NO	NO
Bangladesh	Azad *et al.*, 2013	NO	Shahi and Ranchi	none	only nptII and GUS	Agrobacterium	na	NO	NO	NO	NO
Brazil	Souza Junior *et al.*, 2005	YES	Sunrise, Sunset	PRSV BR (Bahia)	cp	Biolistics	>1	YES	YES	NO	Only cp genes
China	Guo *et al.*, 2003	NO	Huanong No.1	PRSV	NIb	Agrobacterium	1	YES	YES	YES	YES*
Indonesia	Mendoza *et al.*, 2008	NO	Bangkok and Burung	PRSV (Bangor)	cp	Biolistics	na	na	na	NO	NO
Jamaica	Fermin *et al.*, 2005	YES	Sunrise	PRSV JA (Camirana)	cp	Biolistics	1-3	YES	YES	NO	Only cp genes
Malaysia	Mendoza *et al.*, 2008	NO	Eksotika	PRSV	NIb and more than 1 cp	Agrobacterium	na	na	na	NO	NO
Philippines	Natsuaki *et al.*, 2012	NO	Tuka	PRSV Philippines	NIb and more than 1 cp	Agrobacterium	na	na	na	NO	NO
Taiwan -1	Rao *et al.*, 2003	NO	Tainung No.2	PRSV YK	cp	Agrobacterium	1	YES	YES	NO	YES*
Taiwan -2	Kung *et al.*, 2009	NO	cv. Thailand	PRSV TK + P (DMV	more than 1 cp	Agrobacterium	1-3	YES	YES	NO	NO
Thailand -1	Sakuanrungsirikla *et al.*, 2005	YES	Khak Dum and Khak Nual	PRSV Thai	cp	Biolistics	>1	YES	YES	NO	NO
Thailand -2	Kertbundit *et al.*, 2007	NO	Khak Dum	PRSV Thai	cp	Biolistics	>1	YES	NO	NO	NO
US (Florida)	Davis *et al.*,2004	NO	cv. HOG	PRSV HC4	cp	Agrobacterium	>1	YES	YES	NO	YES*
US (Hawaii) -1	Fitch *et al.*, 1992	YES	Sunset	PRSV HA	cp	Biolistics	>1	YES	YES	YES	YES
US (Hawaii) -2	Fermin *et al.*, 2006	YES	Sunset	PRSV HA	cp	Biolistics	>1	YES	YES	YES	NO
Venezuela	Fermin *et al.*, 2004	YES	Tailandia Roja	PRSV YK (El Vigia) and AA-3 (agumBas)	more than 1 cp	Agrobacterium	1	YES	NO	NO	Only cp genes
Vietnam	Mendoza *et al.*, 2008	NO	KD Thai, Tim Taiwan, Solo, Mexico and Local Lanson	PRSV Vietnam	NIb and more than 1 cp	Agrobacterium	na	na	na	NO	NO

A new one, never sequenced before

Update on the development of virus-resistant papaya: Virus-resistant transgenic papaya for people in rural communities of Thailand

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Currently banned in Thailand

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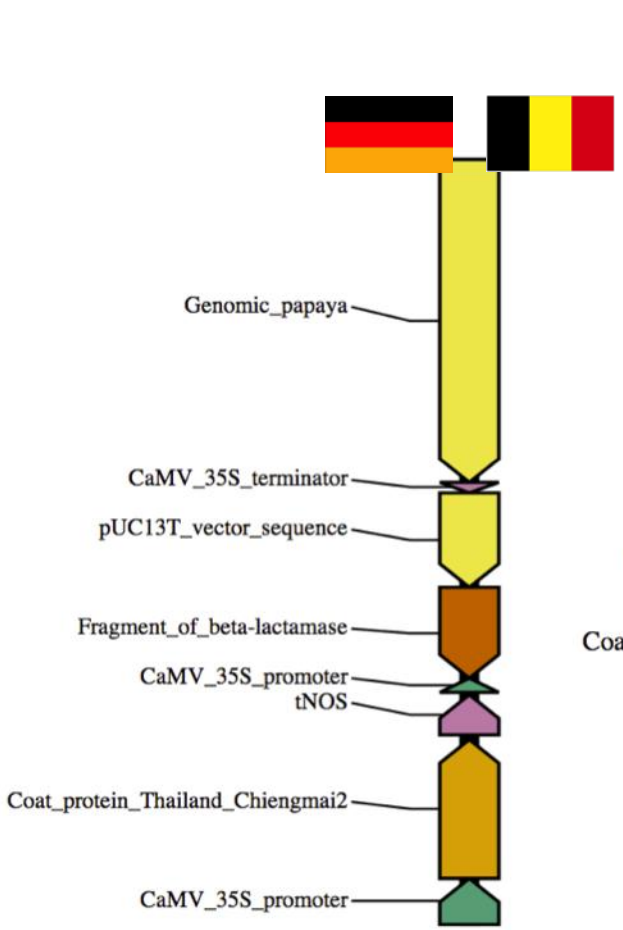
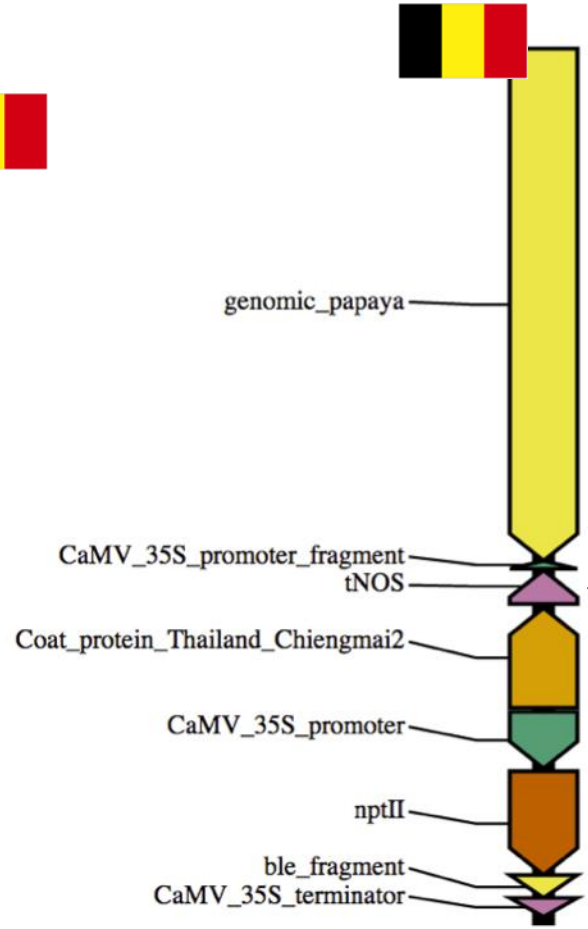
European Commission

Results



RL11

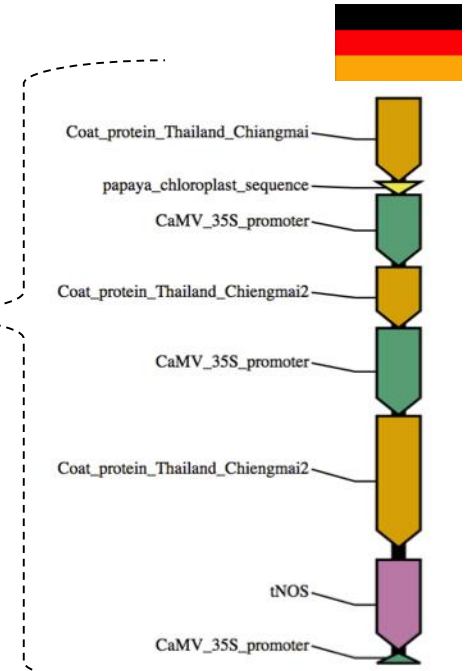
4,896bp



RL12

6,660bp

CONFIRMED!



RL12bis

1,574bp

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We are not alone

January 2014 Communication to the Editor

Identification and Detection of Genetically Modified Papaya Resistant to Papaya Ringspot Virus Strains in Thailand

Kosuke Nakamura,[a] Kazunari Kondo,*[,a]
Tomoko Kobayashi,[a] Akio Noguchi,[a] Kiyomi Ohmori,[b]
Reona Takabatake,[c] Kazumi Kitta,[c] Hiroshi Akiyama,[a]
Reiko Teshima,[a] and Tomoko Nishimaki-Mogami[a]

[a] *National Institute of Health Sciences; 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan:* [b] *Chemistry Division, Kanagawa Prefectural Institute of Public Health; 1–3–1 Shimomachiya, Chigasaki, Kanagawa 253–0087, Japan: and* [c] *National Food Research Institute; 2–1–12 Kannondai, Tsukuba, Ibaraki 305–8642, Japan.*

Received September 13, 2013; accepted October 18, 2013; advance publication released online October 29, 2013

ELSEVIER

Food Control

Volume 55, September 2015, Pages 127–132

Event-specific qualitative and quantitative detection of three genetically modified papaya events using a single standard reference molecule

Jae-Hwan Kim[a], Saet-Byul Park[a], Hyo-Jeong Roh[a], Hong-Bae Woo[a], Min-Ki Shin[b], Gui Im Moon[b], Jin-Hwan Hong[b], Dabing Zhang[c], Hae-Yeong Kim[a],

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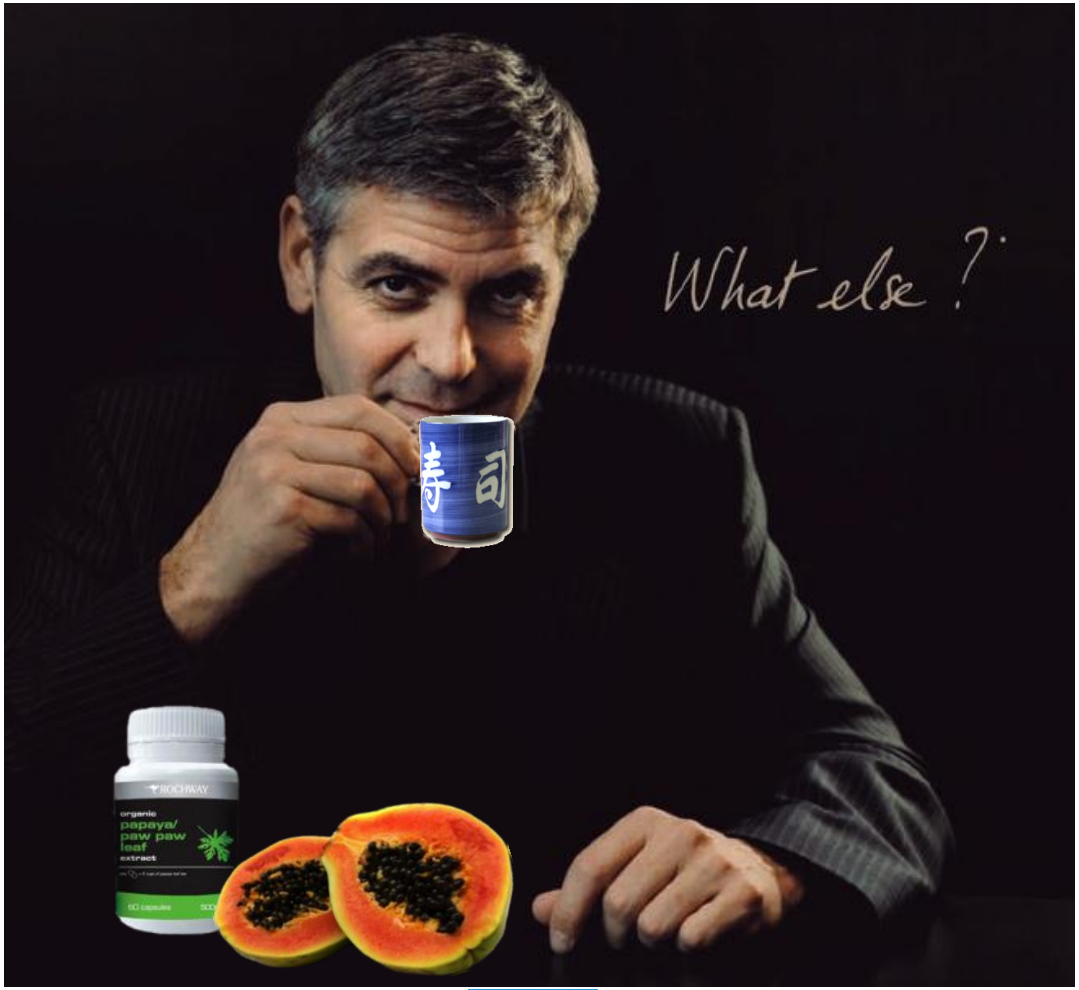
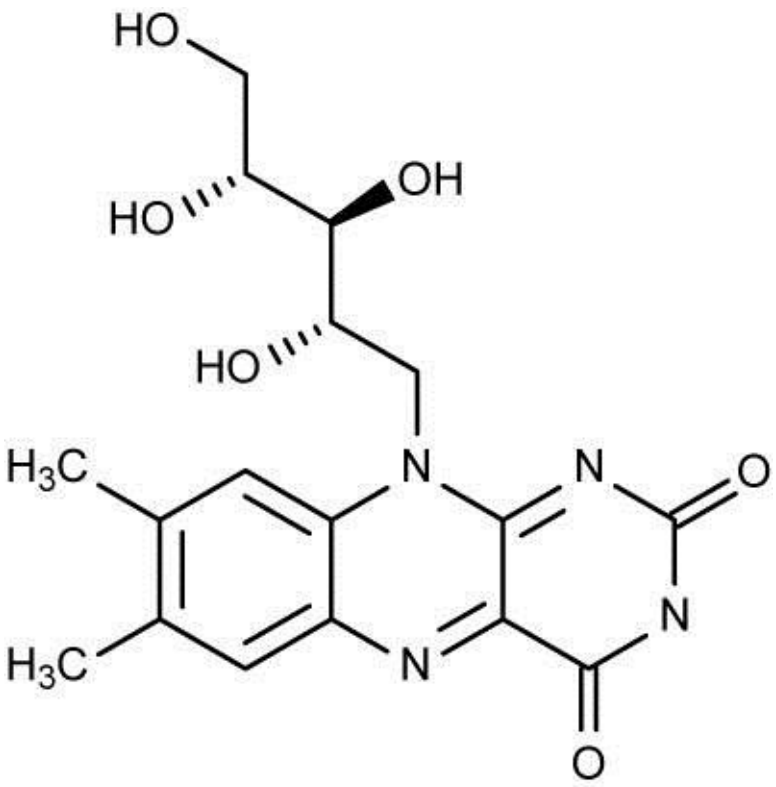
What else?



Joint Research Centre

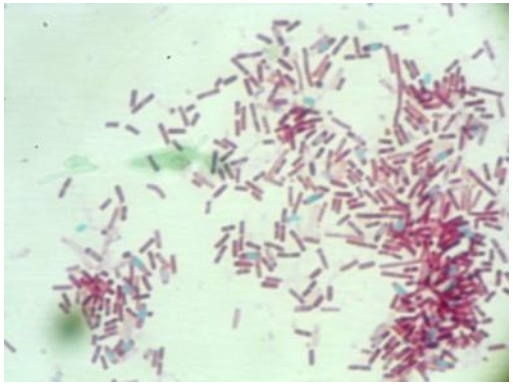
Vitamin B2 - Riboflavin

What was found in Germany ?



European Commission

Vitamin B2 – product "riboflavin feed powder 80%" from Hegno, Shanghai, China



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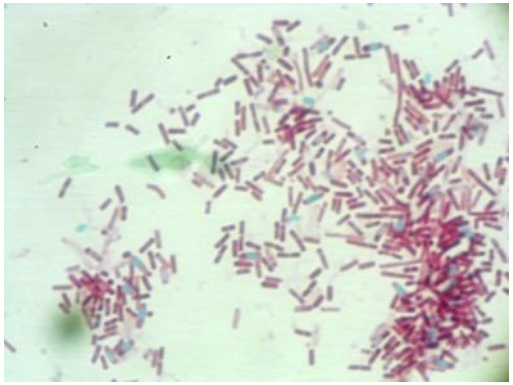
European Commission

Vitamin B2 – product "riboflavin feed powder 80%" from Hegno, Shanghai, China



Joint Research Centre

European Commission

Information received from China

Hegno

Shanghai Hegno Pharmaceuticals Co., Ltd.
ADD: Building 5, No. 1999 Zhangheng Road,
Zhangjiang High-tech Park, Shanghai 201203, China
Tel: +86 (21) 60753300 Fax: +86 (21) 60753311 www.hegno.com

致：德意志联邦共和国驻华大使馆
食品和农业参赞
蒲曼婷 女士

尊敬的蒲女士：

您好！关于 2014 年 10 月 24 号函件中涉及的问题，我司回复如下：

1. 您的理解是正确的。2012 年12 月18 日之前使用的菌种没有经过EMS突变处理 ，但是通过控制发酵时间和参数，以避免发酵过程中的孢子产生。

2. 2012年12月18日之后，没有使用过除*Bacillus subtilis* RP29::pUC19之外的菌种，同时在生产过程中不存在两种菌种同时存在的可能性。

在更换至RP29::pUC19菌种之前，已经通过碱液煮沸、清洗、高温湿热灭菌等处理手段**去除**前菌种的残留。

4. 我们目前正在根据信函中的要求收集相关信息；我们会在收集到相关信息之后立即提供。

5. 红霉素选择性标记的基因位于染色体外pMX45质粒，包括一个来自枯草芽孢杆菌的解除管制的rib操纵子

6. 在9月30日电话会议中，你们提到从最终产品中分离出核黄素生产菌株，在培养基中培养后，残留菌株可能形成孢子。根据这一现象，在10月7日上海海嘉诺回复大使馆的信函里我们推测了为什么最终产品中的残留菌株有能力产生孢子的可能原因：由于最终成品中残留的生产菌在生产过程中经过如巴氏灭菌、高温干燥等剧烈条件的处理，当这些受胁迫的生产菌根据电话会议中提到的实验被分离出来进行培养时，可能诱发某些残留在成品中的生产菌株在培养过程中产生孢子。
对于两种菌种混合可能性，请参考第2点的说明。

7. 我们没有关于使用一种特异性检测方法（PCR）明确地鉴定生产菌种的信息。

8. 没有注册过该生产菌种，并且我们没有标准物质

9. 我们没有注册过该菌种，并且对于所述菌种我们没有专属性分子PCR（聚合酶链式反应）检测方法。

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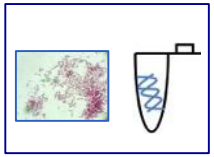
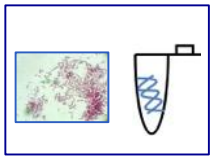
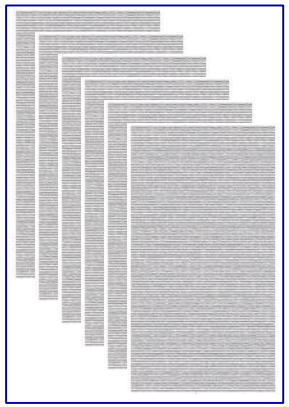
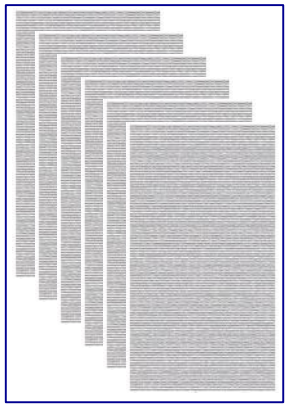
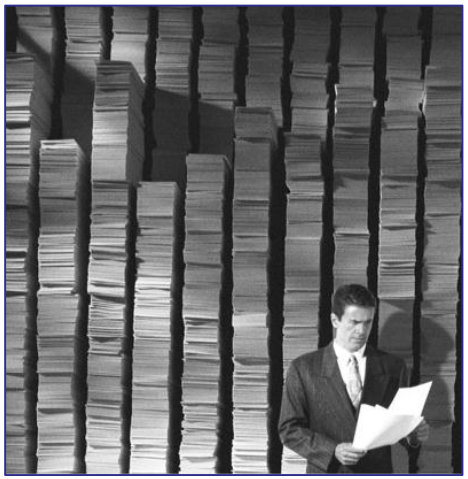
NGS brute force approach?

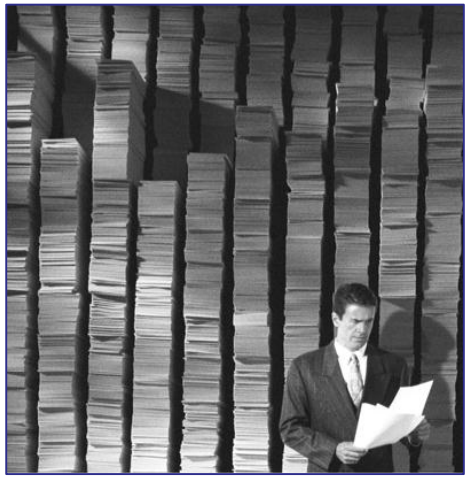












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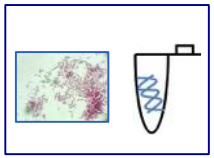
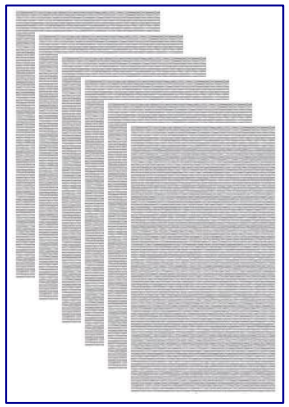
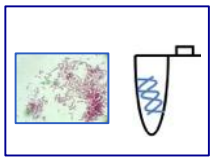
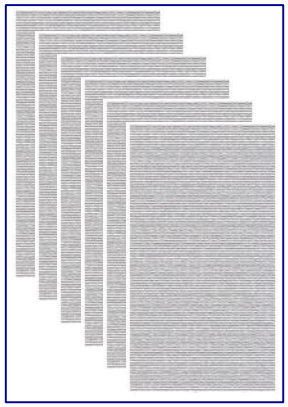
NGS brute force approach?









They came to us

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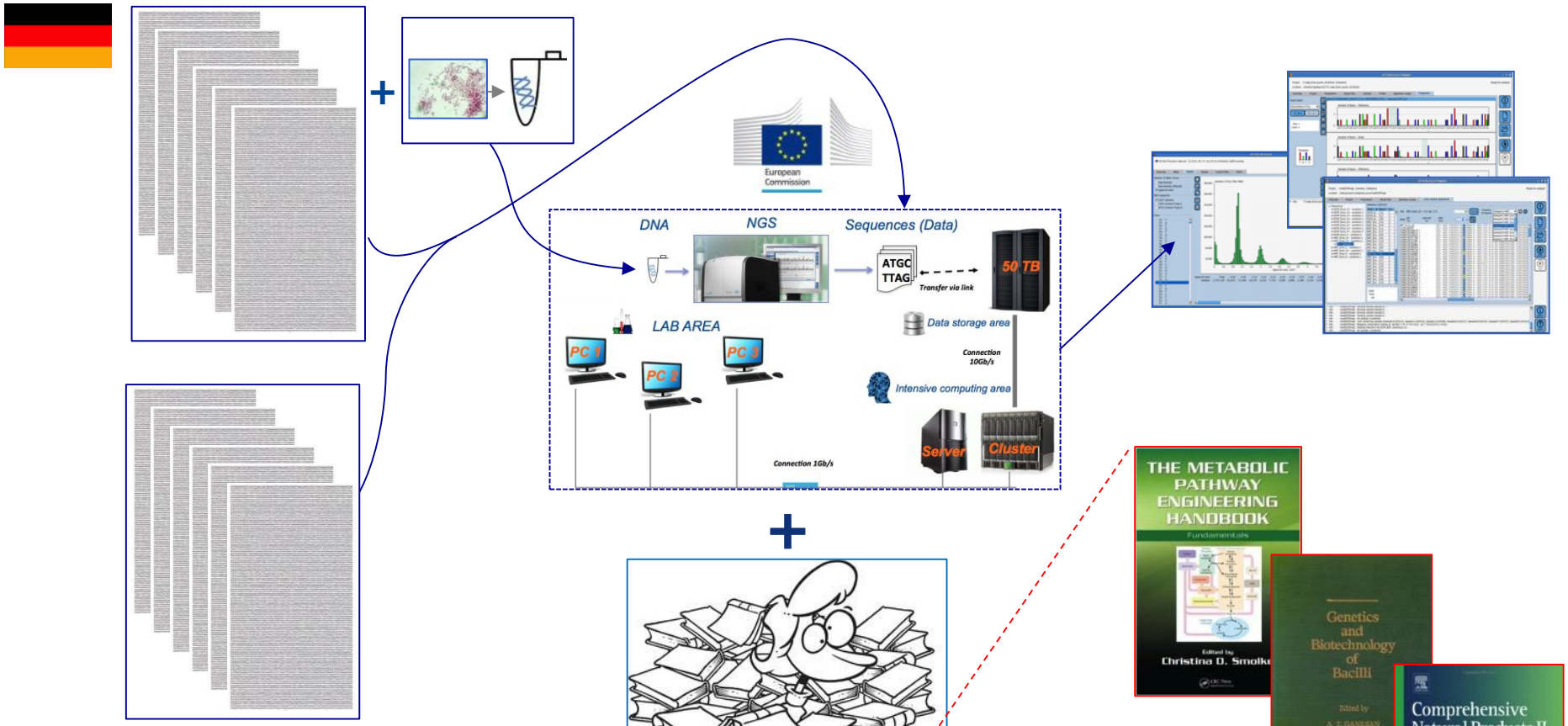
In parallel: NGS + Literature Review



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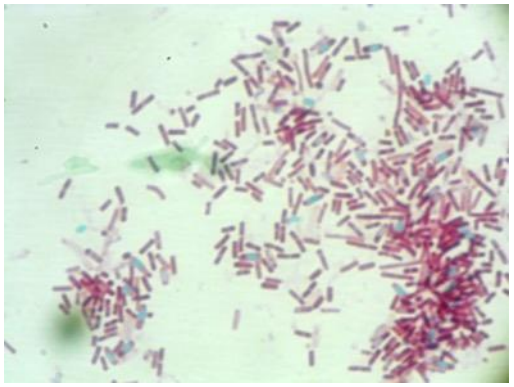
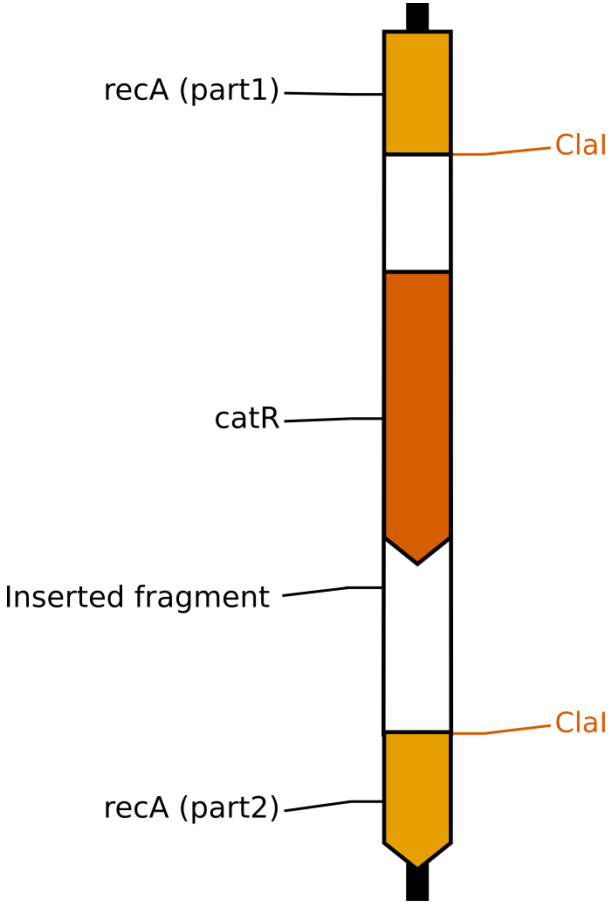
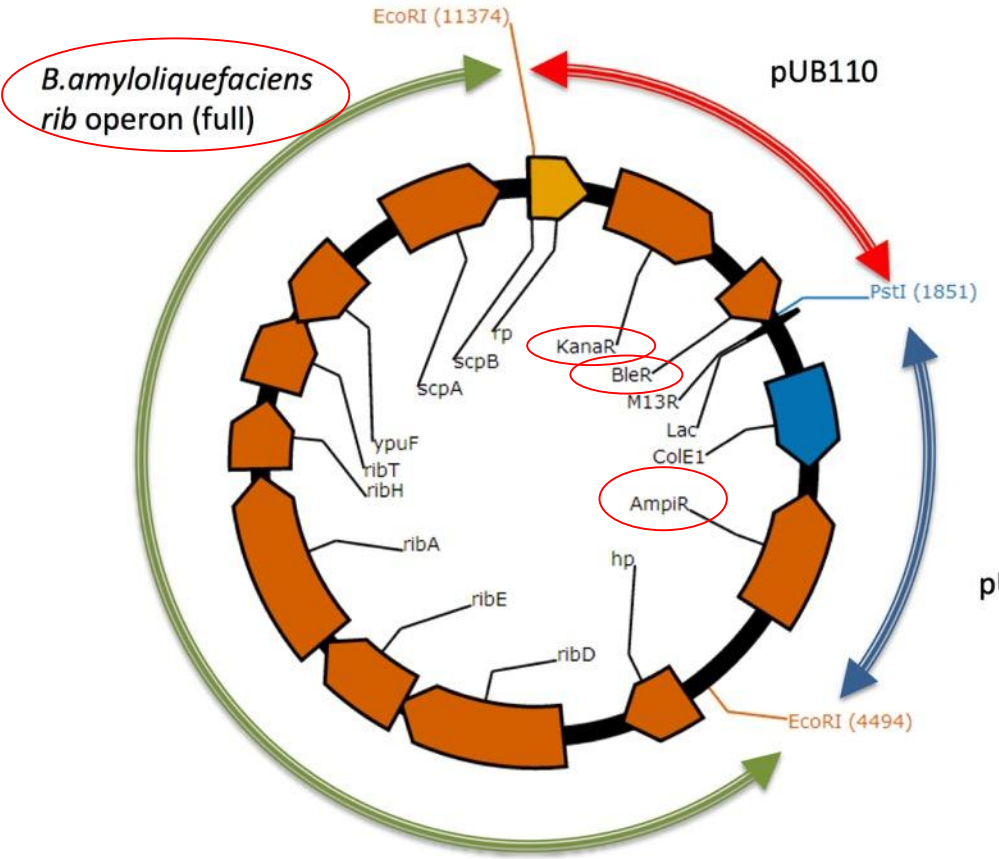
It's a GM *B. subtilis*

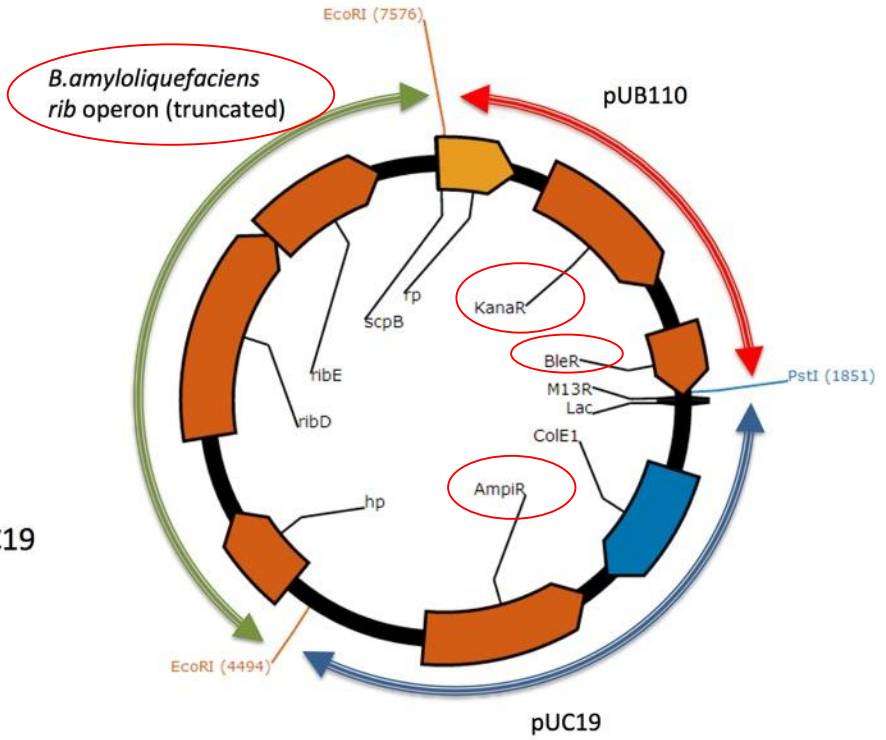




NGS analysis: a GMM with 4 plasmids, never sequenced before



pGMSub01
11,378 bp



pGMSub02
7,580 bp

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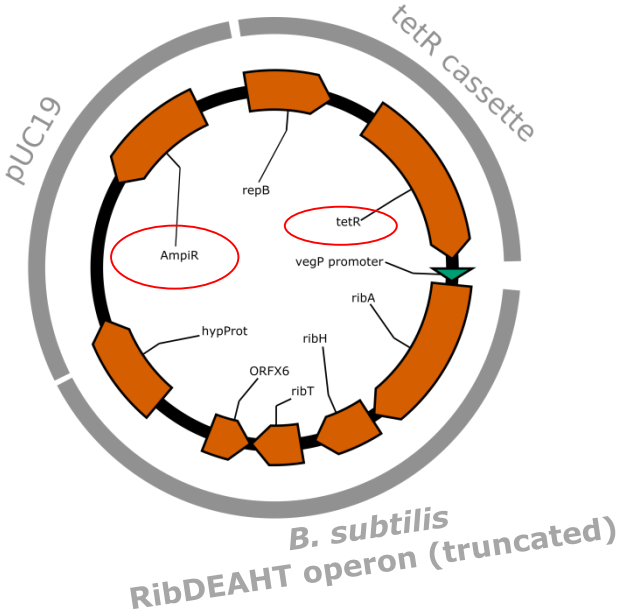
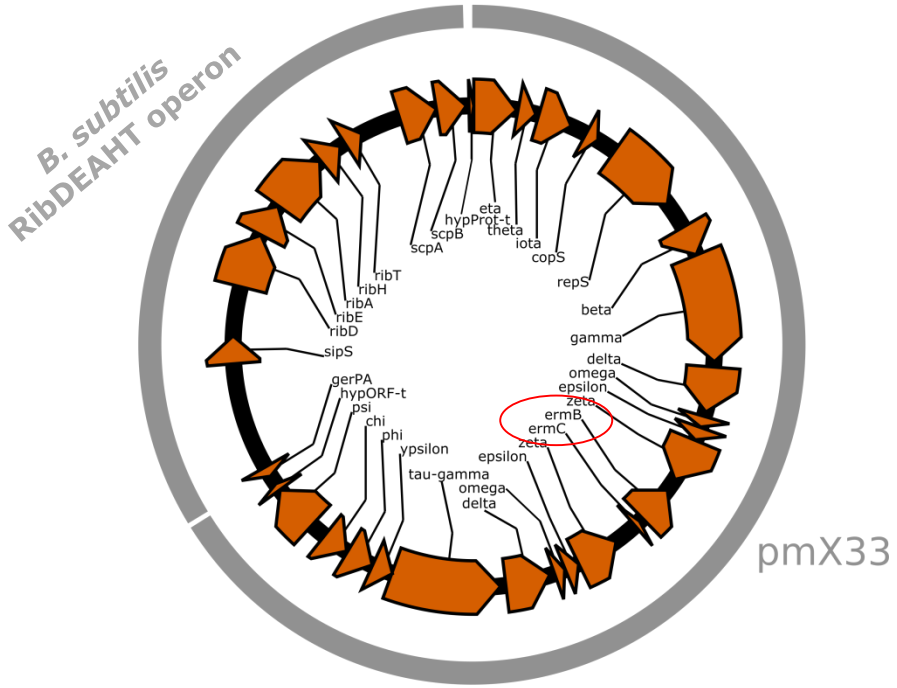
NGS analysis: a GMM with 4 plasmids, never sequenced before



pGMSub03
8,544 bp



pGMSub04
29, 760 bp

NGS analysis: a GM microbe with 4 plasmids, never sequenced before

Feature	Chinese Company	Confirmed in this work
cmR at chromosomal level into recE gene	Claimed	YES
ribC mutation on one rib operon	Claimed	YES
pMX45 with emR	Claimed	YES (pGMSub04)
pMX45 non integrated on chromosome	Claimed	YES
pUC19 with tetR	Claimed	YES (pGMSub03)
rosR at chromosomal level	Claimed	YES (in silico)
azgR at chromosomal level	Claimed	YES (in silico)
5 pUC19 copies with tetR integrated on chromosome	Claimed	NO
No sporulation	Claimed	NO
ampR resistance on 2 plasmids	Not claimed	YES (pGMSub01 and pGMSub02)
B. amyloliquefaciens ribDEAHT operon on plasmid	Not claimed	YES (pGMSub01)
B. amyloliquefaciens ribDE on plasmid	Not claimed	YES (pGMSub02)
ribDEAHT operon deleted on chromosome	Not claimed	YES
kanR resistance on plasmids	Not claimed	YES
bleR resistance on plasmids	Not claimed	YES

European Commission

Are we alone?

genomeA
Journals.ASM.org

Genome Sequence of EU-Unauthorized Genetically Modified *Bacillus subtilis* Strain 2014-3557 Overproducing Riboflavin, Isolated from a Vitamin B2 80% Feed Additive

Elodie Barbau-Piednoir,[a] Sigrid C. J. De Keersmaecker,[a] Véronique Wuyts,[a,d] Céline Gau,[b] Walter Pirovano,[c] Adalberto Costessi,[c] Patrick Philipp,[b] Nancy H. Roosens[a]

Platform Biotechnology and Molecular Biology, Scientific Institute of Public Health (WIV-ISP), Brussels, Belgium[a]; Service Commun des Laboratoires, Illkirch-Graffenstaden, France[b]; Baseclear B.V., Leiden, the Netherlands[c]; Department of Microbial and Molecular Systems, KU Leuven, Leuven, Belgium[d]

E.B.-P. and S.C.J.D.K. contributed equally.

P.P. and N.H.R. contributed equally.

This paper announces the genome sequence and annotation of the genetically modified (GM) *Bacillus subtilis* strain 2014-3557 overproducing riboflavin (vitamin B2). This GM-strain is unauthorized in the European Union. Nevertheless, it has been isolated from a lot of vitamin B2 (riboflavin) 80% feed grade imported to Europe from China.

Received 13 February 2015 **Accepted** 3 March 2015 **Published** 9 April 2015

Plasmid name	Size (bp)	Resistance genes	RibDEAHT operon	RibDEAHT origin	Similarity to known plasmids or vectors	Found in sample	Present in Barbau-Piednoir 2015?
pGMBSub01	11,378	ampR, kanR, bleR	Full	*B. amyloliquefaciens*	pUC19, pUB110	LGL, JRC, BVL	Yes, split in 5 contigs
pGMBSub02	7,580	ampR, kanR, bleR	Truncated	*B. amyloliquefaciens*	pUC19, pUB110	LGL, JRC, BVL	Yes, split in 5 contigs
pGMBSub03	8,544	ampR, tetR	Truncated	*B. subtilis*	pUC19, pLS1	LGL	Yes, split in 2 contigs
pGMBSub04	29,760	ermR	Full	*B. subtilis*	pSM19035	LGL	Yes, split in 5 contigs

The *B. subtilis* and *B. amyloliquefaciens*

Method For Preparing Riboflavin, Strains Bacillus Subtilis As Producers Of Riboflavin (variants)

Published: Sep 27, 2005 Family 4 Family Member PDF

RU 2261273 C2
Granted Patent

The full document isnt yet available to us from the patent office, but we've found a related patent (family member) to use for our images and PDF.

Abstract

FIELD: biotechnology, microbiology, vitamins. ^ SUBSTANCE: method relates to a method for preparing riboflavin by culturing the microorganism Bacillus subtilis as a producer in the nutrient medium containing rib-operon from Bacillus amyloliquefaciens, or microorganism able for utilization of glycerophosphate as a single carbon source, or eliciting the resistance against inhibition of growth by glyoxylate, and extraction of riboflavin prepared. Invention uses the following strains as producers of riboflavin: B. subtilis GM51/pMX45, B. subtilis GM41/pMX45, B. subtilis GM44/pMX45. Invention provides preparing riboflavin of the high degree of effectiveness. ^ EFFECT: improved preparing method. ^ 5 cl, 4 ex

Claims

Information currently unavailable.

Applicants

Information currently unavailable.

Inventors

Mironov A S ▸ Korol Kova N V ▸ Ehrrajs L L ▸ Semenova L Eh ▸ Perumov D A ▸ Kreneva R A ▸ Glazunov A V ▸ Akishina R I ▸ Jomantas Jurgis Antanas Vladov ▸ Abalakina E G ▸ Stojnova N V ▸ Kozlov Ju I ▸ Debabov V G

IPC Classifications

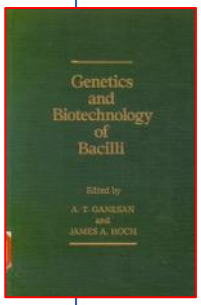
C12N1/21 ▸ C12N15/52 ▸ C12P25/00 ▸ C12R1/125

Document History

- **Publication**
  Sep 27, 2005 RU_2261273_C2
- **Application**
  Nov 15, 2002 RU_2002130592_A
- **Priority**
  Nov 15, 2002 RU_2002130592_A

Family Member Preview

These are the images from a related family member, as the full document isn't yet available to us from the patent office.



CLONING OF GENETIC MATERIAL IN BACILLI

P. M. Rabinovich
Yu. V. Yomantas
M. Ya. Haykinson
A. I. Stepanov

Institute of Genetics
and Selection of Industrial Microorganisms
Moscow, U.S.S.R.

The mechanism of genetic transformation in Bacilli (Dubnau, 1976; Canosi et al., 1978) accounts for the peculiarities of DNA cloning in Bacillus subtilis. At present, there is no doubt that the efficiency of cloning in bacilli depends on the DNA structure of the DNA vector molecule. Canosi et al. (1978) demonstrated the significance of the structure of plasmid DNA for successful transformation in bacilli. These cells are transformed with oligomeric but

CLONING IN BACILLI 305

Figure 8. A model on B. subtilis transformation with plasmide containing inverted repeats.

Figure 9. A model describing B. subtilis transformation with plasmids containing direct DNA repeats.

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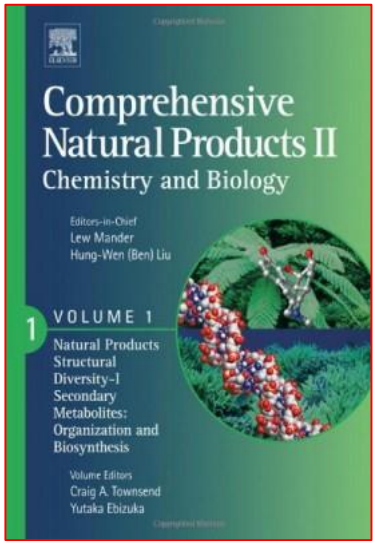
Hypothesis



pMX45 was selected for its ability to grow with 1 g/l glycerophosphate as sole carbon source in the presence of 1 g/l glyoxylate. Under small scale fed-batch fermentation conditions the strain accumulated 21 g/l riboflavin during 70 h. It is presumed that riboflavin-producing companies from China employ *B. subtilis* production strains with a genetic make up similar to that of GM41 pMX45.

Precise copy number dosing of the deregulated *rib* operon in a *B. subtilis* host strain for optimal riboflavin production was recently emphasized by a research group from the Tianjin University, Tianjin, China.[58] Seven to eight copies gave best results. Chromosomal integration via double crossover proved superior over single crossover, presumably because of stability reasons.[59]

How?

Not corresponding to what is claimed!
… **UNAUTHORIZED!**

Conclusions

1. **Through NGS it is possible to acquire new information about GMOs**

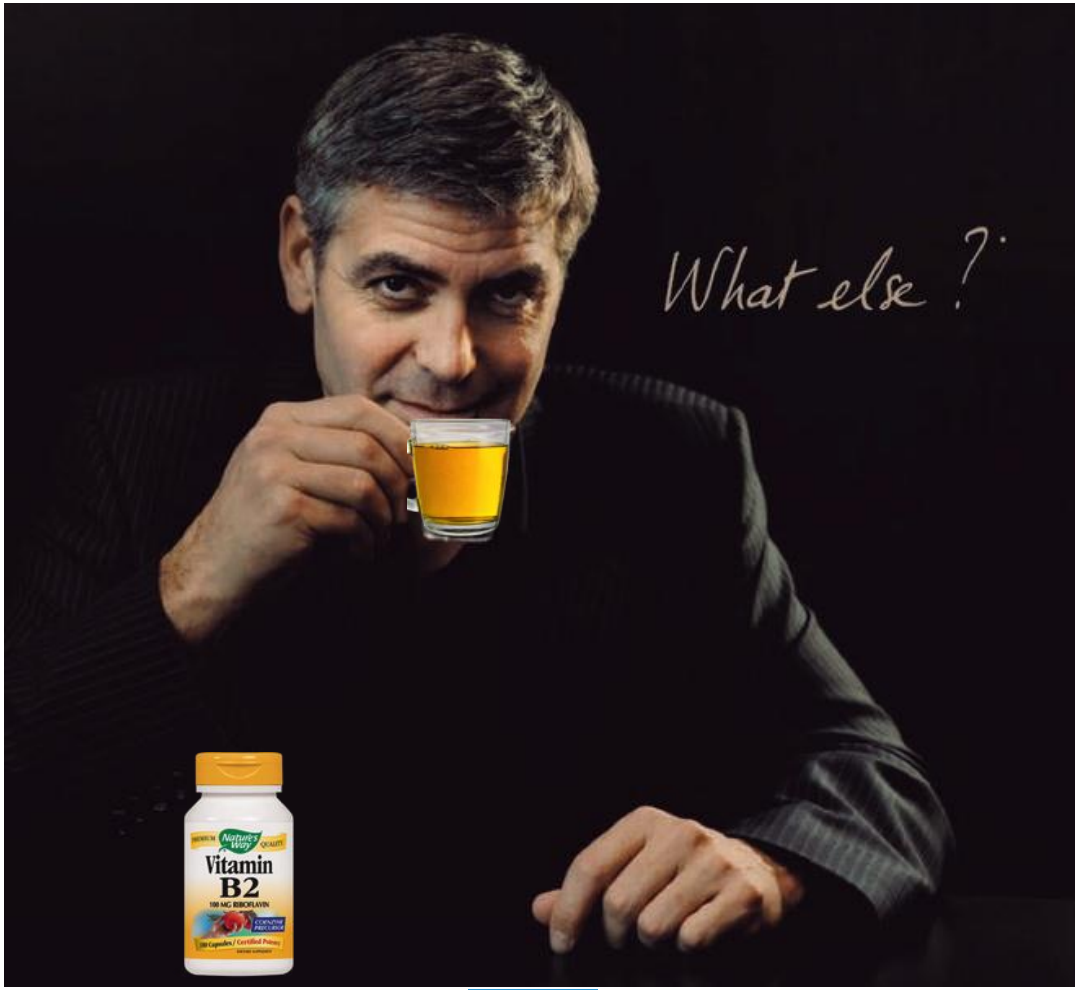
2. **Development of *ad hoc* NGS-based strategies for GMO characterization, even unauthorized**

3. **NGS as starting point for the development of new detection methods**

4. **NGS needs Bioinformatics!**

European Commission

What else?



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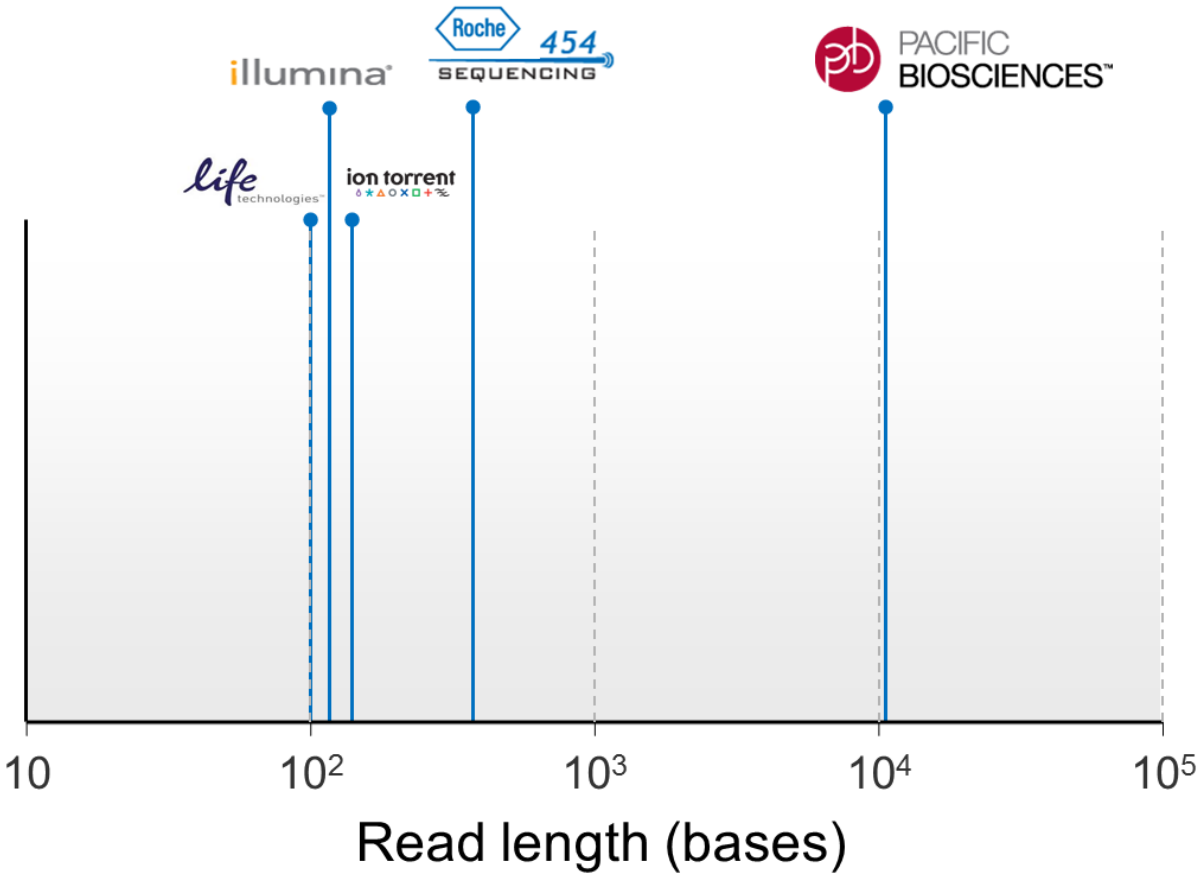
NGS is fast evolving



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Courtesy of Roche Sequencing

European Commission

We are here

1st Generation | 2nd Generation | 3rd Generation | 4th Generation

Optical / Image

454 SEQUENCING

illumina

PACIFIC BIOSCIENCES

Electronic

ion torrent

GenapSys

genia

Oxford NANOPORE Technologies

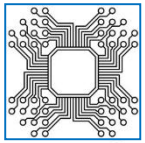
Clonal | Single Molecule

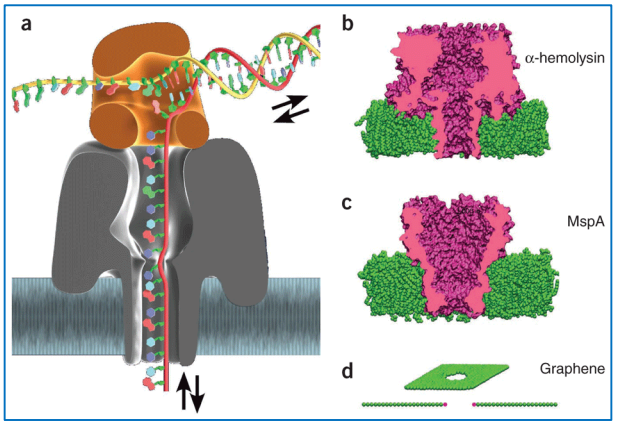
Courtesy of Roche Sequencing

European Commission

4th Generation

Single Molecule





From: Schneider GF, Dekker C. ***DNA sequencing with nanopores.*** Nat Biotechnol. 2012

Oxford NANOPORE Technologies

genia

— NOW — 2016 — 2017 →

Both are able to produce reads longer than 1Kb

genia **is claiming 1 human genome = 100$**

Oxford NANOPORE Technologies **is claiming they will switch to proteins**

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Are we ready ?

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European Commission

BIOINFORMATICS APPLICATIONS NOTE Vol. 30 no. 23 2014, pages 3399–3401
doi:10.1093/bioinformatics/btu555

Sequence analysis

Advance Access publication August 20, 2014

Poretools: a toolkit for analyzing nanopore sequence data

Nicholas J. Loman[1,*] and Aaron R. Quinlan[2,*]

[1]Institute of Microbiology and Infection, University of Birmingham, Birmingham B15 2TT, UK and [2]Department of Public Health Sciences, University of Virginia, Charlottesville 22932, VA, USA

Associate Editor: Inanc Birol

NATURE METHODS | ARTICLE

Improved data analysis for the MinION nanopore sequencer

Miten Jain, Ian T Fiddes, Karen H Miga, Hugh E Olsen, Benedict Paten & Mark Akeson

Affiliations | **Contributions** | **Corresponding authors**

Nature Methods **12**, 351–356 (2015) | doi:10.1038/nmeth.3290
Received 12 December 2014 | Accepted 20 January 2015 | Published online 16 February 2015

NATURE METHODS | BRIEF COMMUNICATION

A complete bacterial genome assembled *de novo* using only nanopore sequencing data

Nicholas J Loman, Joshua Quick & Jared T Simpson

Affiliations | **Contributions** | **Corresponding author**

Nature Methods (2015) | doi:10.1038/nmeth.3444
Received 11 March 2015 | Accepted 22 May 2015 | Published online 15 June 2015

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Thank you for your attention

Mauro

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