THE ABS CAPACITY DEVELOPMENT INITIATIVE

L'INITIATIVE DE RENFORCEMENT DES CAPACITES POUR L'APA

Contribution of DSI in the development of commercial applications

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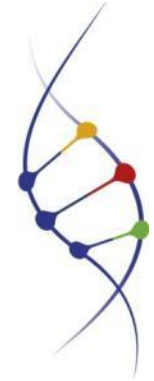
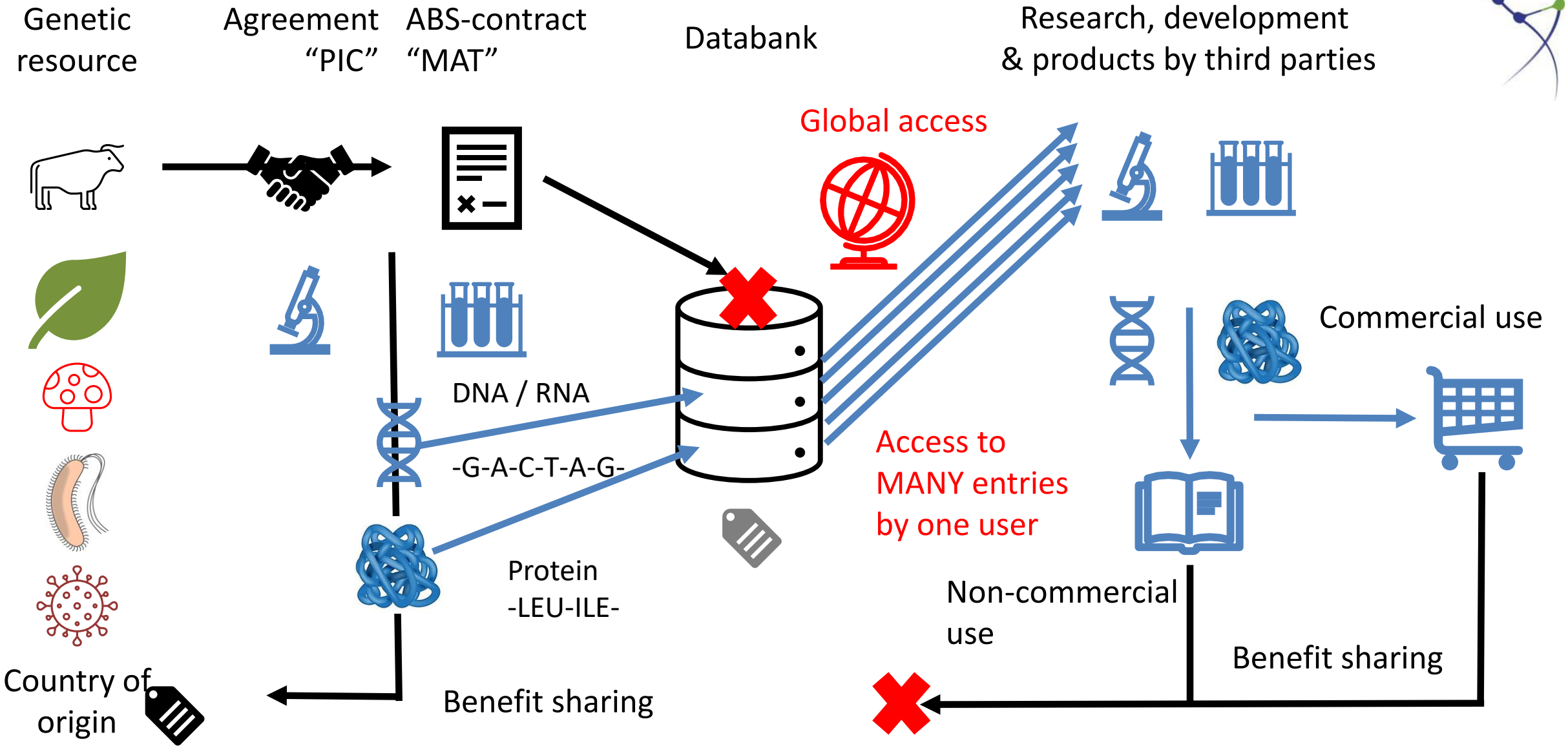
Topics of this presentation

1) Benefit sharing under the bilateral model in the two phases of access to and use of DSI
2) Intellectual Property (IP) relevant to DSI
3) Exploring the use of DSI in exemplary inventions and products
   - Food: Production of vanillin in transgenic yeast
   - Perfume: Synthesis of isoprenoid precursors in transgenic tobacco / yeast
   - Medicine: Stereospecific enzymatic synthesis of Sitaglitin

1) DSI – Third Party (Commercial) Use – Benefit sharing





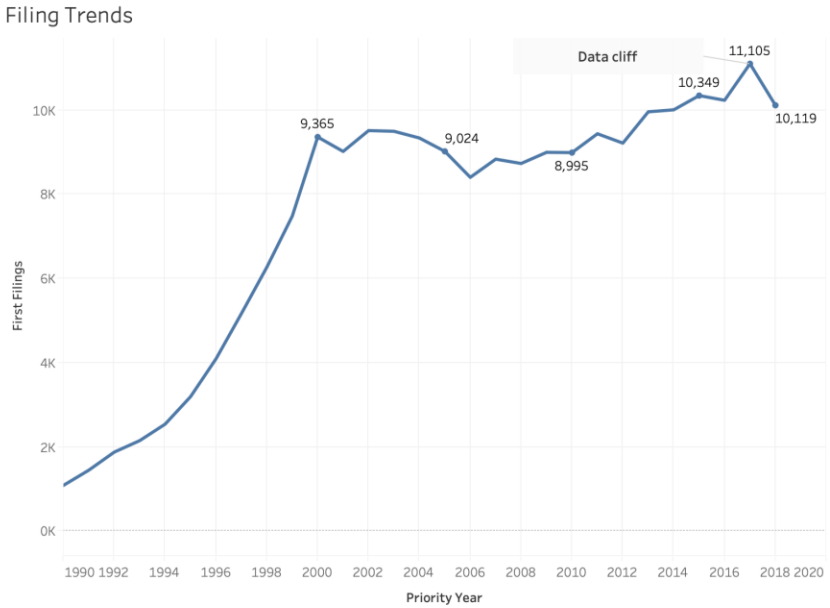
2) Intellectual Property (IP) relevant to DSI

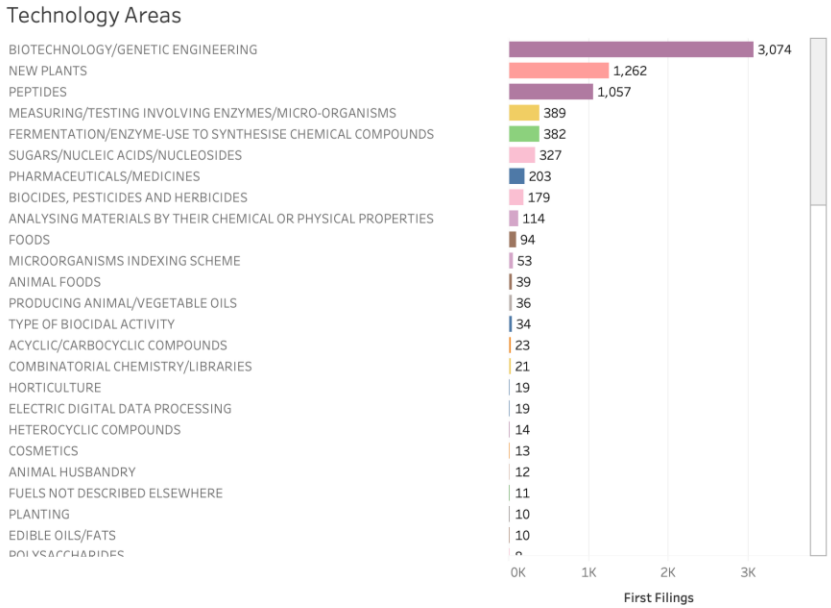
- Academic credit and recognition (obligation to deposit data to get funding)

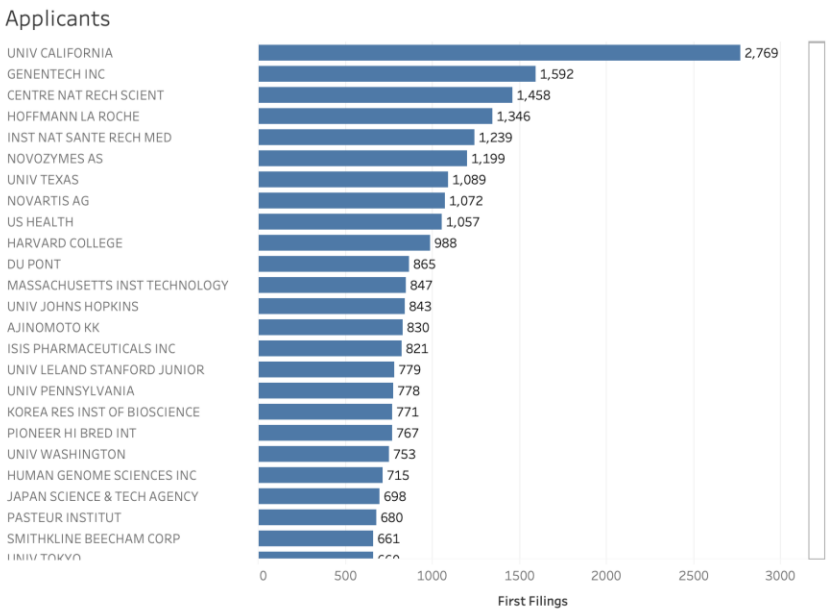
Senjuti Saha, PhD | সেঁজুতি সাহা
Jun 2
We are frequently contacted by HIC researchers to share data we generated from our studies for their meta-analysis. We spend days compiling, entering data for multi-million$ studies. No funds, benefits, usu no authorship, but many emails, high demands. Normal in #globalhealth?

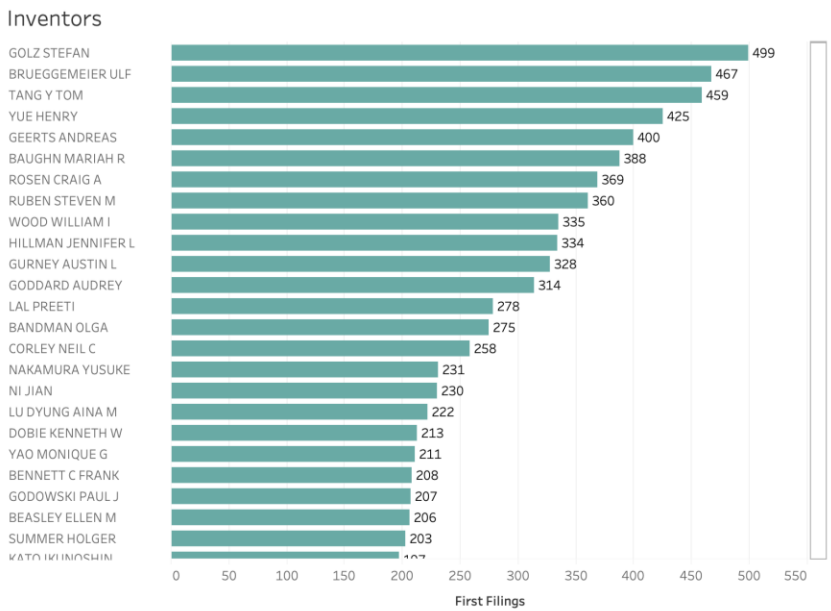
- Creative Commons approaches
- Terms and Conditions for access to open public databases (e.g. GISAID, GBIF downloads)
- Proprietary “curated” databases – T&C and subscription fee
- Plant Breeders’ Rights (breeder’s exemption vs trait patents)
- Patents – patent pools, patent thickets, standard-essential patents, patents on tools, licencing, …
- Trade secrets (proprietary sequences evaluated against downloaded open access data)
- Traditional Knowledge associated with sequenced GR (“embodied aTK”)

DSI in patent applications









Analysis provided by
Dr. Paul Oldham, UK

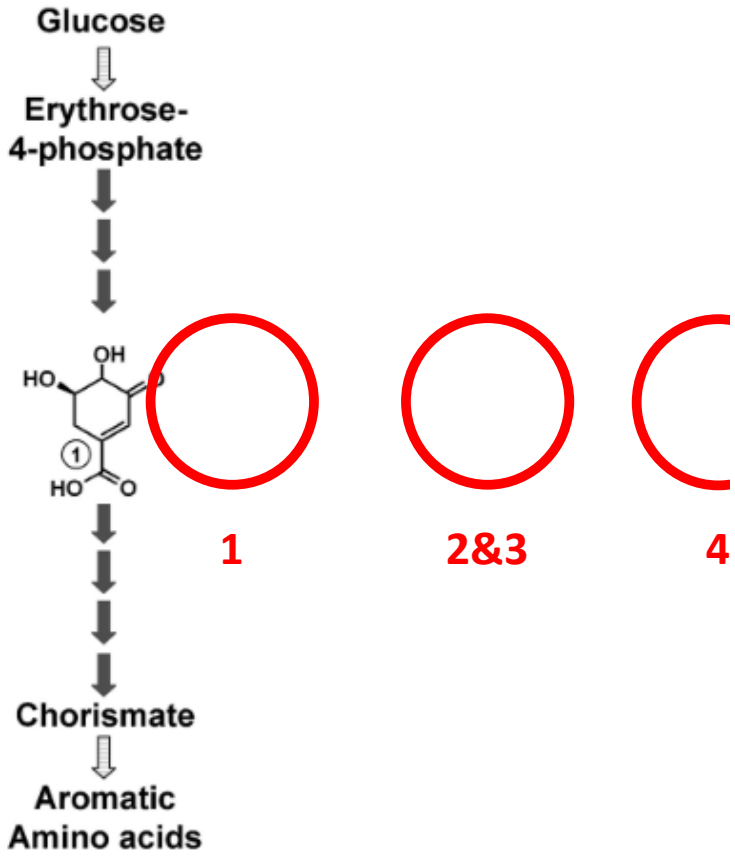
3) Contribution of DSI for a product on the market

Example 1: Vanillin-producing recombinant yeast of the Swiss company Evolva

- Prices for vanilla beans are high, with a peak of 600 USD in 2018, the demand for natural / organic vanilla is increasing
- The vanilla-growing regions (Madagascar, Indonesia, Mexico, Papua New Guinea and others) face various challenges to secure constant supply and quality
- Synthetic vanillin (from e.g. synthetic guaiacol or lignin) dominates the market with appr. 99% global volume share
- Global vanillin market is appr. 500 Mill USD (2019) and is fairly fragmented
- Several companies produce vanillin by fermentation or recombinant microorganisms ("Synbio"), in 2014, the Swiss company Evolva started to market Vanillin produced by a recombinant yeast with 5 new genes / enzymes
- ABS Initiative started an analysis of the role and contribution of DSI in this case

Evolva's de novo synthesis of vanillin in baker's yeast

a) Hansen et al. (2009) Applied and Environmental Microbiology 75 (9): 2765-74
b) Brochado et al. (2010) Microbial Cell Factories 9 (1): 84-98



1. Dehydroshikimic acid

8. Vanillin β-*D*-glucoside

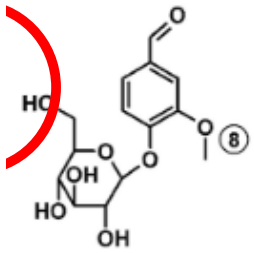


FIG. 1. Biosynthetic scheme for de novo biosynthesis of vanillin in *Schizosaccharomyces pombe* and outline of the different vanillin catabolites and metabolic side products observed in different yeast strains and constructs. Gray arrows, primary metabolic reactions in yeast; black arrows, enzyme reactions introduced by metabolic engineering; diagonally striped arrows, undesired inherent yeast metabolic reactions.

Analysis of patent WO 2015 / 121379 A2 and scientific publications

Questions: Which DSI is mentioned? What is the contribution to the invention? Which DSI and genetic material was used in engineering the production strain?

- Patent documents on inventions in the field of synthetic biology usually mention several DSI, they are regarded as important for gaining information on how DSI is used in commercial R&D
- Our analysis includes two main categories of DSI in patent documents:
  1) DSI involved in analysing genetic material used for the invention
  2) DSI involved in synthesising genes used for the invention
- When discussing benefit sharing in the context of the bilateral approach of the NP, it is also important to gain information about the country of origin of the GR from which the DSI was generated
- Two scientific publications by Evolva inform in detail about the transformation process and the DSI / genes used for engineering the yeast strains

Step 1: 3-Dehydroshikimate dehydratase & DSI

DSI mentioned in "Detailed Description of the Invention – Recombinant Hosts" of the patent			
GenBank-Accession	**Origin of Protein Sequence**	**Organism**	**Origin of Organism**
CAD60599	submitted by French institutions 2003	*Podospora pauciseta* (also *anserina*) fungus on dung	no information France ?
XP_001905369.1	Espagne et al. 2008	*Podospora pauciseta* (also *anserina*) fungus on dung, strain S mat+ (Rizet et al. 1952)	no information France ?
XP_761560	sequence deleted in GenBank	*Ustilago maydis* fungus causing corn smut	---
ABG93191.1	submitted by Canadian institution 2006	*Rhodococcus jostii* bacterium	no information Canada ?
AAC37159.1	Barbe et al. 2004	*Acinetobacter baylyi* ADP1 bacterium, mutant of strain BD4 (Taylor & Juni 1961)	Soil, USA ?
XM_001392464	automatic computa-tional analysis using gene prediction 2011	*Aspergillus niger* fungus causing black mold, strain CBS 513.88	2007 from Spain

Step 2: Aryl carboxylic acid reductase and DSI

DSI mentioned in "Detailed Description of the Invention – Recombinant Hosts" of the patent			
Accession	**Origin of DNA- / protein sequence**	**Organism**	**Origin of Organism**
AY495697 (in detailed descript-tion of the Invention – recombinant hosts)	He et al. 2004	*Nocardia iowensis* bacteria strain NRRL 5646 (Martin et al. 1977)	soil from USA
XP_955820 (in example section)	Galagan et al. 2003	*Neurospora crassa* fungus causing red bread mold strain 74-OR23-1VA (Mylyk et al. 1974)	mutant from decade-old laboratory strains

Contribution of DSI to vanillin-producing Baker‘s yeast

New gene / enzyme	Source of new gene	Role of DSI
Step 1: 3DSD [a]	isolation from *Podospora pauciseta* Origin: ?	Sequence CAD60599 (*P. pauciseta*) used for characterising new protein/gene Origin: ?
Step 2: ACAR [a]	**codon-optimised synthesis based on sequence from *Nocardia iowensis***	**Sequence AY495697 (*N. iowensis*) used as template for gene synthesis** Origin: USA
Step 3: PPTase [b]	isolation from *Escherichia coli* Origin: ?	Sequence NZ_ABHP01000004 (*E. coli* O157:H7 str. EC4113) used characterising new gene Origin: ?
Step 4: OMT [a]	**codon-optimised synthesis based on sequence from *Homo sapiens***	**Sequence NM_000754 (*H. sapiens*) used as template for gene synthesis** Origin: ?, 10 submissions since 1991
Step 5: UGT [a]	isolation from *Arabidopsis thaliana* Origin: Property of Evolva	Sequence NM_126067 (*A. thaliana*) used for characterising the new gene Origin: Colombia ecotype, derived from plants accessed in Germany, 4 submissions since 2000

a) Hansen et al. (2009), b) Brochado et al. (2010)

Conclusions

- 25 DNA and protein sequences mentioned in the patent were analysed in GenBank
- In 19 cases, the sequence information was uploaded more than 10 years ago
- In 5 cases, information on the country of origin of the respective genetic resource was available (Spain, USA, Japan, Germany, Italy)
- The DSI mentioned in the patent was used as reference to describe the new genes in different recombinant hosts
- The publications show that
  3 new genes were cloned from genetic resources
  - 3DSD from *Podospora pauciseta*, PPTase from *Escherichia coli*, UGT from *Arabidopsis thaliana* (property of Evolva)
  2 new genes were synthesised based on DSI accessed in databanks
  - ACAR from *Nocardia iowensis* (GR accessed in USA 1977), OMT from *Homo sapiens*
- It is likely that more DSI was used during the R&D process

3) Contribution of DSI for a product on the market

Example 2: Clearwood – perfume - Firmenich

- **“Driving Game-Changing Technology** - Committed to breakthrough innovation, we launched Clearwood™ the very first ingredient of our new family of sustainable molecules from our white biotechnology platform. Totally renewable and highly cost-effective, this very first ingredient of its kind was born from the intuition of our perfumers, over 10 years of internal Research and Development as well as our partnership with Amyris.“ Firmenich, Firnews 2014
- A scientific publications from 2006 described a way to produce the scent molecules in transgenic tobacco
- PTC-patent document filed in 2011 and granted e.g. in the U.S. in 2015 describes a way to produce mainly two scent molecules in transgenic yeast
- In cooperation with Amyris (USA) providing transgenic yeast production technologies, Clearwood has been introduced into the market in 2014

Contribution of DSI to synthesis of isoprenoid precursors in transgenic tobacco (R&D phase)

New gene / enzyme	Source of new gene	Role of DSI
Patchoulol synthase [a]	Synthetic gene by PCR	Sequence AY508730 (*Pogostemon cablin)* used as template **Uploaded:** 2003 **Origin:** Nursery Jardin des Senteurs, Neuchâtel, Switzerland [b]
Farnesyl diphosphate synthase [a]	Synthetic gene by PCR	Sequence P08836 (*Gallus gallus*) used as template **Uploaded:** 1988, 2 more uploads **Origin:** Chicken liver [c]
Transit peptid gene [a]	Synthetic gene by PCR	Propably sequence AC007591 (*Arabidopsis thaliana)* used as template **Uploaded:** 1999 **Origin:** Colombia ecotype, derived from German collection

a) Wu et al. 2006, b) Deguerry et al. 2006, c) Tarshis et al. 1994

Contribution of DSI to synthesis of isoprenoid precursors in transgenic tobacco (R&D phase)

New gene / enzyme	Source of new gene	Role of DSI
Amorpha-4, 11-diene synthase [a]	Synthetic gene by PCR	Sequence AF138959 (*Artemisia annua)* used as template **Uploaded:** 1999 **Origin:** ? [b]
Limonen synthase [a]	Isolated from lemon fruits	Sequence AF514289 (*Citrus limon* (L.) Burm) used for characterising the new gene **Uploaded:** 2002 **Origin:** nursery in Sicily, Italy [c]
DNA gyrase subunit A [a]	Isolated from *Arabidopsis thaliana*	Sequence ATH17376 (*Bordetella pertussis)* used for characterising the new gene **Uploaded:** 2016 **Origin:** 469 assembled *B. pertussis* genomes of the U.S. Centers for Disease Control and Prevention's collection [d]

a) Wu et al. 2006, b) Mercke et al. 2000, c) Lücker et al 2002, d) Weigand et al. 2019

Contribution of DSI to synthesis of isoprenoid precursors in transgenic yeast (production phase)

New gene / enzyme	Source of new gene	Role of DSI
Sesquiterpene synthase [a]	Isolated from *Valeriana jatamansi* (syn. *Valeriana walichii*) bought at B & T World Seeds, France	Sequence AAD02269 (*Solanum tuberosum)* used for characterising new gene **Uploaded:** 1998 **Origin:** Botany and Plant Pathology, Michigan State University, USA [b] Automatically predicted sequence XP_002321642, derived from NC_037299.1 (*Populus trichocarpa),* used to characterise new gene [c] **Uploaded:** ? **Origin:** *P. trichocarpa* clone Nisqually1, Nisqually River, Washington, USA Sequence AY508730 (see Slide 14) used to characterise new gene [d]

a) Schalk & Deguerry 2011, b) Direct upload, no publication, c) Tuskan et al. 2006, d) Deguery et al 2006

3) Contribution of DSI for a product on the market

Example 3: Sitgaliptin - drug - Merck

- Some organic molecule have a similar atomic composition but differ in their spatial configuration: so-called stereo-isomers as e.g. L- and D-aminoacids
- Some drugs contain ingredients which are only active in one of the two possible forms, their stereospecific chemical production poses challenges
- Enzymes are able to perform stereospecific reactions, in this case an enzyme has been changed in 27 amino acid to achieve >99.95% purity of the stereospecific active ingredient Sitgaliptin
- Januvia/Januvet, a diabetes drug from Merck, with its active ingredient Sitgaliptin increases insulin secretion by inhibiting dipeptidyl peptidase 4. It is the 95th most-prescribed drug with appr. 10,000,000 prescriptions
- Merck's sales of diabetes type 2 medication Januvia/Januvet were 1,35 billion USD in 2019

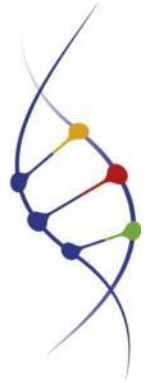
Voigt 2020, Kane 2020, Johnson 2018

Contribution of DSI to stereospecific enzymatic synthesis of Sitaglitin

New gene / enzyme	Source of new gene	Role of DSI
(R) – Transaminase [a)]	Computational enzyme design for higher reactivity and specifity for the production of the active ingredient	Sequence AB638718 (R)-enantioselective transaminase gene [c)] served as template for protein aminoacid sequences **Uploaded:** 2011 **Origin:** Arthrobacter sp. KNK168 Accession FERM BP-5228 in Nite Patent Microorganisms Depositary Soil sample from Japan [b)]

a) Savile et al. 2010, Savile et al. 2012
b) Iwasaki et al. 2003, Yamada et al. 2007
c) Iwasaki et al. 2012

The issues and challenges



- Advances in biological sciences / synthetic biology support using DSI for commercial R&D and production for previously biodiversity-sourced products
- The use of DSI could replace the use of biological material
- Due to different interpretations of the CBD / NP and varying provisions in national ABS legislation and ABS contracts, the first users of DSI would not be always obliged to share the benefits of utilisation of DSI
- The architecture of databanks for DSI does not allow uploading of ABS contracts and connecting the third party use of DSI to contractual benefit sharing obligations
- The scientific practice of using thousands and more sequences of DSI in one R&D project by third party users would make compliance to a potentially large number of ABS contracts very difficult
- Many sequences in DSI databanks do not have a country tag, which would make benefit sharing with the original provider of the GR impossible