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FEM2-AMBIENTE
SPIN-OFF ACCREDITATA
DALL'UNIVERSITÀ
DEGLI STUDI DI
MILANO-BICOCCA

From traditional methods to DNA barcoding:

Future perspectives in plant identification

HISTORY OF DNA TESTING AND RECENT DEVELOPMENTS

DNA BARCODING

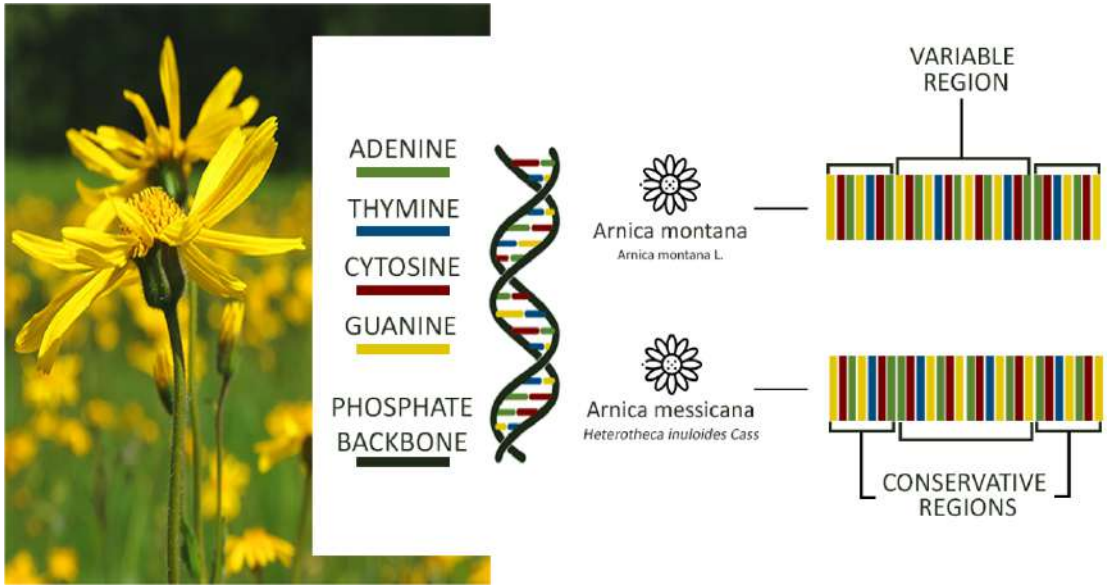
DNA barcoding was presented by Paul Hebert and his team in 2003.

Biological identifications through DNA barcodes
Paul D. N. Hebert*, Alina Cywinska, Shelley L. Balland Jeremy R. deWaardDepartment of Zoology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

SPECIES ❯ DNA ❯ BARCODE

The DNA barcoding method is based on the analysis of a DNA segment that is highly discriminative and let to identify univocally a species.

DNA BARCODING
HOW IT WORKS



DNA BARCODING
THE TECNIQUE

DNA EXTRACTION

Different extraction method based on the sample typology.

DNA AMPLIFICATION

Need to choose the most suitable marker: *COI, CytB* or other.

DNA SEQUENCING

Amplicon are sequenced using Sanger methodology.

BLAST NCBI BOLD

DATABASE COMPARING

DNA sequences were compared to public and private database obtaining species .

SPECIES IDENTIFICATION

We choose the sequence with 100% of identity

DNA BARCODING
APPLICATIONS

ANIMALS: mitochondrial region **COX-1** and eventually also other markers such as **16S rRNA** region and **Cyt-b** gene.

PLANT: **rbcL** and **matk** marker regions on the genome of the chloroplast and also other additional molecular markers as **ITS2** and **trnH-psbA**.

BACTERIA/FUNGI: some regions of the mitochondrial gene **16S rRNA**; for yeasts, fungi and algae instead, the analysis focuses on some regions of the molecular marker the **ITS**.

TYPE OF SAMPLE › DNA ISOLATION PROTOCOL

EXPECTED SPECIES › DNA MARKER

DNA BARCODING
A RELIABLE TOOL FOR QUALITY TESTING

UNIVERSAL: Similar procedure for different organism.

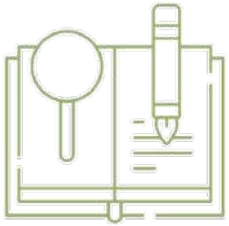
DATABASE: Wide and constantly updating.

RELIABLE: Over 16 years of research paper.

STANDARDIZED: it is currently used in USA by FDA request and GB by pharmacopeia request.

DNA BARCODING

FROM PHYLOGENESIS TO QUALITY CONTROL



2003

Phylogenesis and
taxonomical research

2019

Quality control
for industries

DNA-BASED AUTHENTICATION OF HERBAL PRODUCTS

FEM2-AMBIENTE S.R.L.

Spin-off company acknowledged by University of Milano-Bicocca

We were born in 2010 and we are a spin-off company of the University of Milano-Bicocca.

In FEM2-Ambiente (Food, Environment, ManageMent) **we value the technologies developed in university research centers and transforming them into innovative tools** to provide effective solutions to the needs of individuals and companies.

We have over than 10 years of experiences in DNA testing.

WHY?

In the food, herbal, pharmaceutical, cosmetic, etc. fields, raw materials often undergo processing (drying, freezing, etc.) and a **change in morphological characters**, which are **difficult to identify**, with relative problems in the sector.

The DNA-based techniques allow **the identification of raw materials regardless of the morphological characteristics** and the undergone processes.

DNA has unequivocal traits for every living organism, so it allows a **UNIQUE CHARACTERIZATION**.

WHY?

1) PROTECTION FROM FRAUDS/MISLABELING/MISTAKES

To buy the correct product at the correct economic value…

DNA-BASED AUTHENTICATION OF HERBAL PRODUCTS

WHY?

1) PROTECTION FROM FRAUDS/MISLABELING/MISTAKES

To buy the correct product at the correct economic value..

Cinnamomum verum vs cassia

Crocus sativus

Arnica montana vs ARNICA MESSICANA (Heterotheca inuloide)

WHY?

2) EVALUATION OF THE QUALITY AND SAFETY OF A PRODUCT

To exclude the presence of contaminants.

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To exclude the presence of contaminants.

POISONING

Aconitum, Colchicum, Veratrum, Taxus, Nerium, Digitalis, Oenanthe, Atropa belladonna, Brugmansia, Datura, Hyoscyamus, Mandragora, Solanum dulcamara,…

TOXIC (Pyrrolizidine alkaloids)

Crotalaria, Ligularia, Senecio, …

ALLERGENIC

WHY?

3) REGULATORY COMPLIANCE

To ensure the belonging to the European Belfrit list.

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Taraxacum officinale vs mongolicum

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For marketing and to communicate to consumers the quality and safety of products.

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For marketing and to communicate to consumers the quality and safety of products.

"Quality control" is becoming a common attitude of **consumers** approaching the global market with an **increasing interest for health and wellness**.

Standard consumers usually spend time **to read labels and getting information** on the products they are interested in buying.

n particular, they dedicate a special attention to the originand composition of goods.

For these reasons, FEM2-Ambiente offers to its customers the opportunity of using on genetically analysed and controlled products the brand "**Verified DNA**" with the aim to guarantee their quality and safety.

DNA-BASED AUTHENTICATION OF HERBAL PRODUCTS

WHY?

Just to recap

1) PROTECTION FROM FRAUDS/MISLABELING/MISTAKES

To buy the correct product at the correct economic value...

2) EVALUATION OF THE QUALITY AND SAFETY OF A PRODUCT

To exclude the presence of contaminants.

3) REGULATORY COMPLIANCE

To ensure the belonging to the European Belfrit list.

4) TRACEABILITY OF SUPPLY CHAIN PRODUCTION

To verify if the production process introduces contaminants into the finished product.

5) COMMUNICATION OF QUALITY

For marketing and to communicate to consumers the quality and safety of products.

NOT ONLY DNA BARCODING

NOT ONLY
DNA BARCODING

AN ANSWER TO EACH QUESTION

Which is the species?

DNA BARCODING

Which is the variety?

DNA FINGERPRINTING

Is there the species of interest in the product?

SPECIES-SPECIFIC DNA DETECTION

Which different species are in the product?

METABARCODING/NEXT-GENERATION SEQUENCING

NOT ONLY
DNA BARCODING

AN ANSWER TO EACH QUESTION

Which is the variety?

DNA FINGERPRINTING

Plant Variety Protection

Chemical profile

Plant reeders

Geographical origin

NOT ONLY
DNA BARCODING

AN ANSWER TO EACH QUESTION

Is there the species of interest in the product?

SPECIES-SPECIFIC DNA DETECTION

To verify the presence of the valuable species

To detect the contaminants

Low –cost adulterants
Unwanted metabolites
Allergenic/toxic

Advantages

Economic

Rapid

Low detection limit

NOT ONLY
DNA BARCODING

AN ANSWER TO EACH QUESTION

Which different species are in the product?

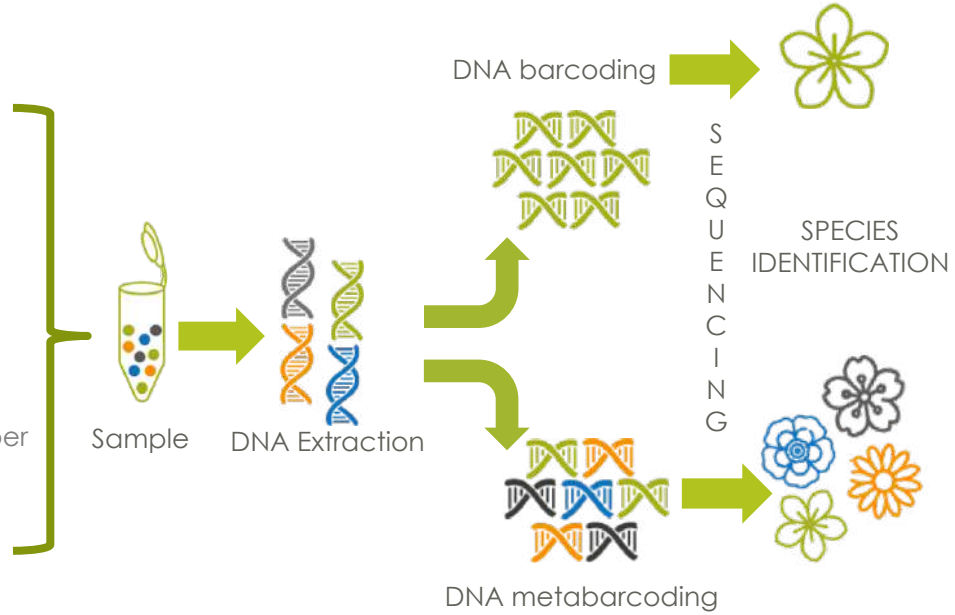
METABARCODING/NEXT-GENERATION SEQUENCING

Mixed herbal supplements

Tea & herbal tea

Spices

Oregano leaves are usually replaced by considerably cheaper leaves of *Mirthus*, *Cistus*, *Olea*, *Fragaria* and *Rhus*.



NOT ONLY
DNA BARCODING

AN ANSWER TO EACH QUESTION

Which different species are in the product?

METABARCODING/NEXT-GENERATION SEQUENCING

R&D FOR IMPROVING

Standardization
Universal DNA markers
Long timing
Expensive
Reliable databases

EPO srl

top quality botanical extracts since 1933

Dr. Violetta Insolia,
Scientific Project & Marketing Specialist

EPO srl

Established in Milan,

EPO Srl is a family company,

specialized in the production
of high quality botanical extracts

since 1933

EPO srl


Our extracts
NUTRACEUTICAL AND FOOD
PHARMACEUTICAL AND COSMETIC
FEED
AGRICULTURE
TEXTILE
CLEANSING

EPO srl

Selection of qualified suppliers

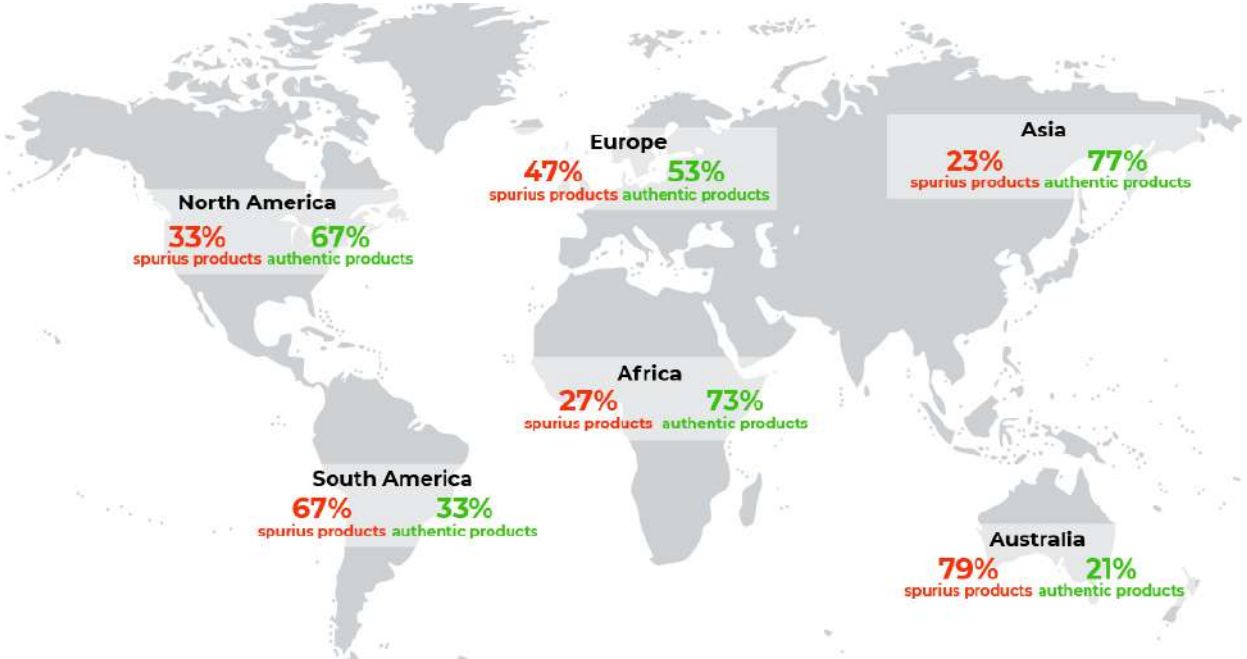
Full traceability from the field to the finished product

Manufacturing process with safe solvents and traditional methods

The extracts are carefully tested, from actives standardization to searching chemical and microbiological contaminants

DNA®
CERTIFIED EXTRACTS

EPO srl



5,957 commercial herbal products sold in 37 countries. A substantial proportion (27%) of the herbal products commercialized in the global marketplace is adulterated when compared with the claimed ingredient species.

Ichim MC. (2019). *The DNA-Based Authentication of Commercial Herbal Products Reveals Their Globally Widespread Adulteration.* Front. Pharmacol.

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Botanical species identification

Integration of multiple techniques to guarantee the botanical species

Morphological identification

Chemical characterization

DNA barcoding analysis

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Botanical species identification

Integration of multiple techniques to guarantee the botanical species

Morphological identification (sometimes it is hard due to plant similarities, part of plant and dry form)
Chemical characterization (sometimes it is not sufficient for species discrimination)
DNA barcoding analysis (not all DNA plants have been sequenced; some parts of plant could have damaged DNA)

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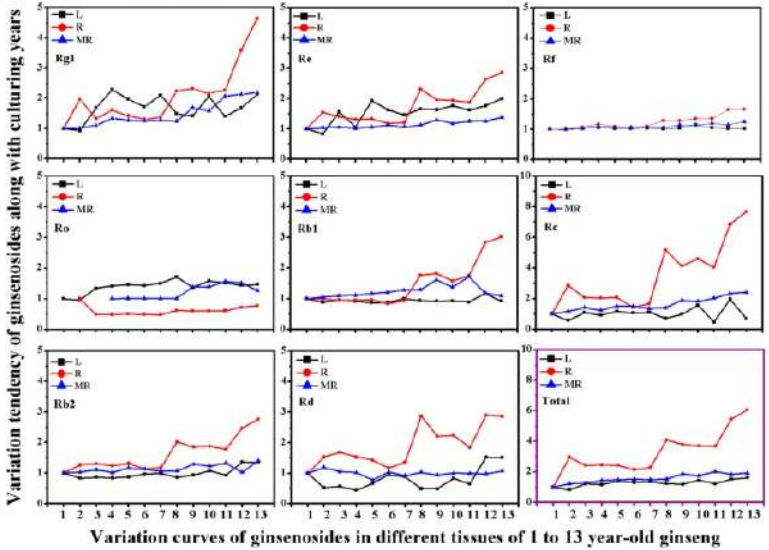
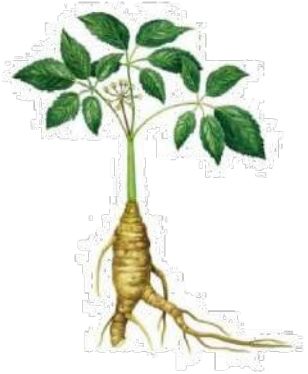
Ex. Ginseng sophistication: leaf and roots





The variation ratios of eight individual ginsenoside in **leaf (L)**, **rhizome (R)** and **main root (MR)** of 1 to 13 year-old ginseng

Zhang YC, et al., 2014. *Tissue-Specific Distribution of Ginsenosides in Different Aged Ginseng and Antioxidant Activity of Ginseng Leaf.* Molecules

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Ex. Cinnamon sophistication: safety concerns

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Ex. Fireweed harvesting difficulties

~200 species of *Epilobium* genus

only two species are admitted in food supplements in Italy by the Ministry of Health

***Epilobium angustifolium* L.**	**Onagraceae**		**folium, flos, herba**			herba: Regolarità del transito intestinale. Funzionalità del sistema digerente. Funzionalità della prostata.
***Epilobium parviflorum* Schreb.**	**Onagraceae**		**folium, flos, herba**			herba: Funzionalità della prostata.

E. angustifolium is a spontaneous plant, wild growing in Europe

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Example of DNA barcoding analysis

AATGCCGTATCGATTTGCCCAGTCAGGATCGATGCAT

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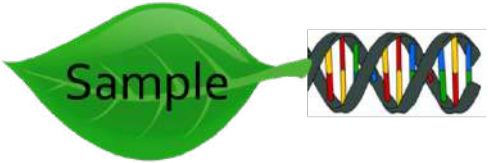

AATGCCGTATCGATTTGCCCAGTCAGGATCGATGCAT

DNA CERTIFIED EXTRACTS

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Example of DNA barcoding analysis

AATGCCGTATCGATTTGCCCAGTCAGGATCGATGCAT

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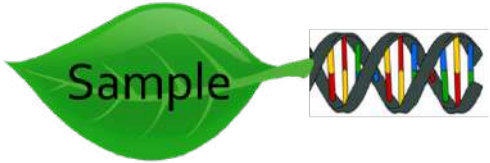

AATGCGTATCGATTTGGGGGGGGAGGATCGATGCAT

WE REJECT THE SAMPLE

EPO srl

THE DNA CERTIFIED EXTRACTS

At **EPO** the botanical identification of the raw materials is supported by the cutting-edge technique of DNA barcoding; the routinely use of it allowed **EPO** to be the first in Europe to launch a line of DNA certified extracts.
EPO allows to use the **"DNA certified extracts"** logo on the packaging of customer's finished product.
The procedure is simple and fast!
Visit www.eposrl.com to download the list of DNA certified extracts and to learn more about it.

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