Medicinal Plants: DNA-Based Identification of Raw and Processed Materials

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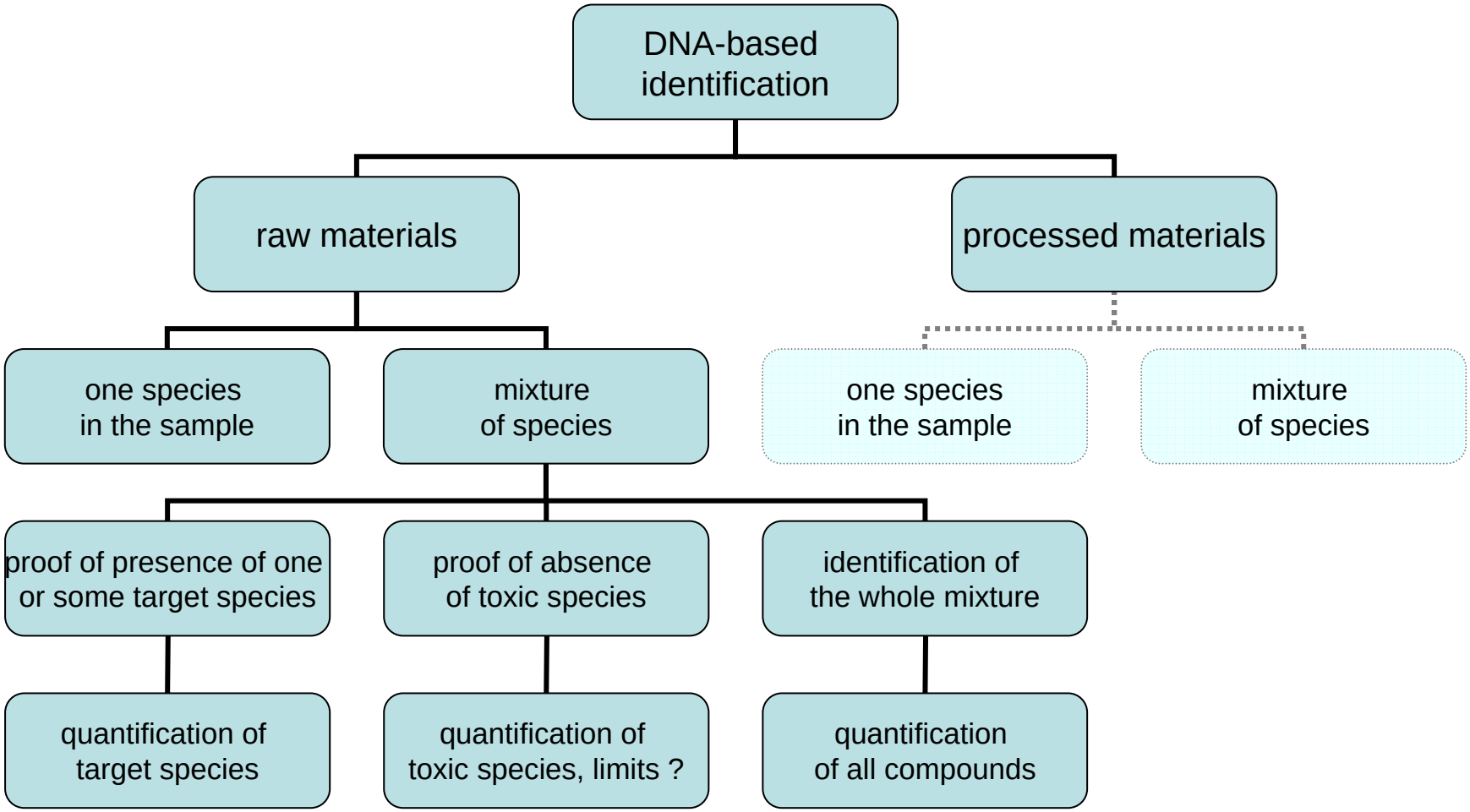
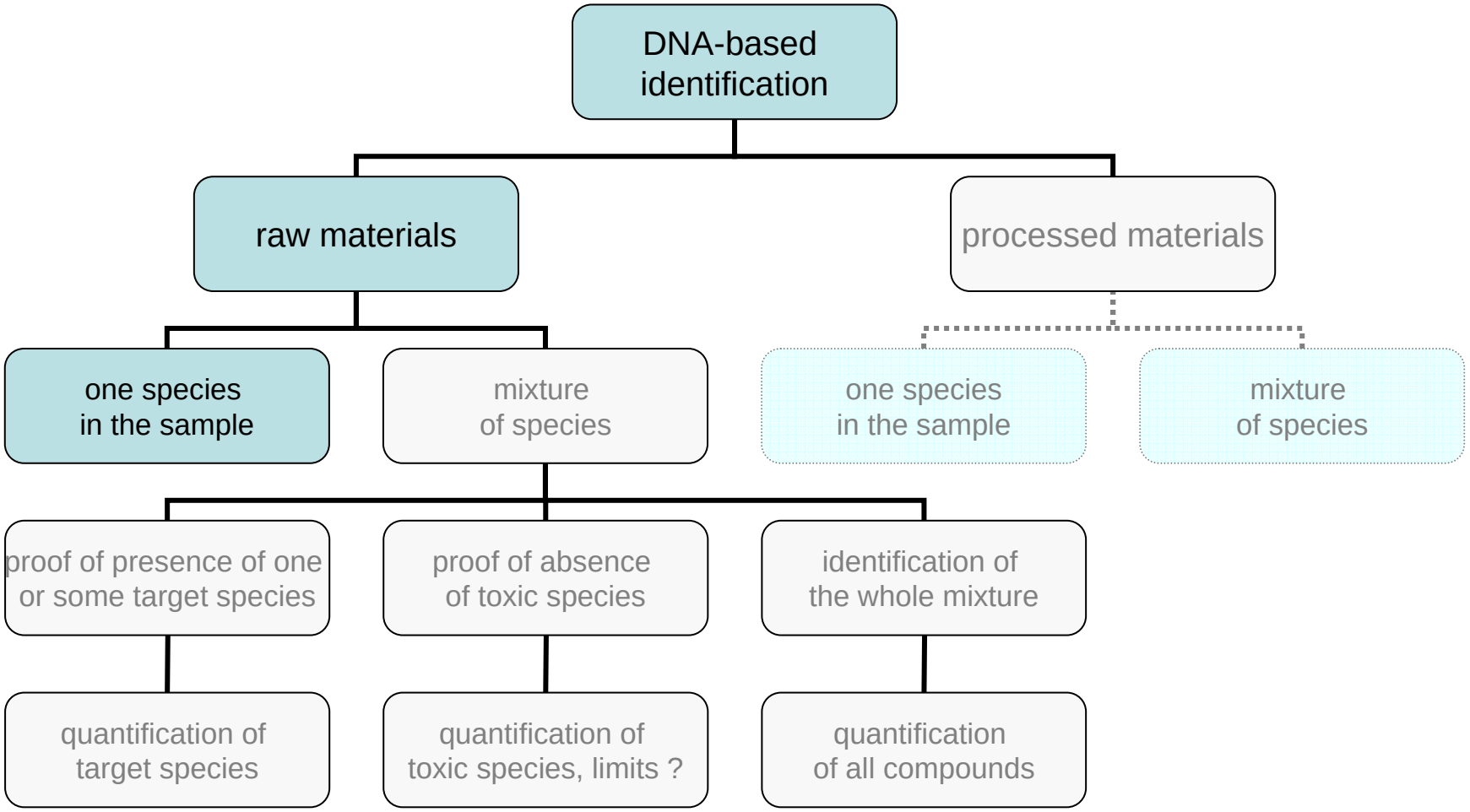
Lectures and posters at GA2007 about DNA-based methods

- **PL 009 – Sucher NJ: Chips and Qi: microchip-based authentication of traditional Chinese medicinal plants.**
- P 248 – Phonkot et al.: Antioxidant activities and DNA fingerprint of 4 varieties Lotus stamens.
- P 256 – Kersten et al.: The potential of PCR-related methods to identify medicinal plants in herbal medicinal products.
- P 610 – Xue and Li: Molecular identification of the traditional Tibetan medicinal plant *Gentianopsis paludosa* (Gentianaceae) using diagnostic PCR and PCR-RFLP based on nrDNA ITS regions.
- P 619 – Xue et al.: Molecular authenication of the traditional Chinese medicinal plant *Euphorbia humifusa* and *E. maculata.*
- P 644 – Hadian et al.: Genetic diversity of Iranian accessions of *Satureja hortensis* L. using RAPD markers.
- P 646 – Heubl et al.: A molecular approach for the discrimination of medicinal Astragali radix used in TCM.

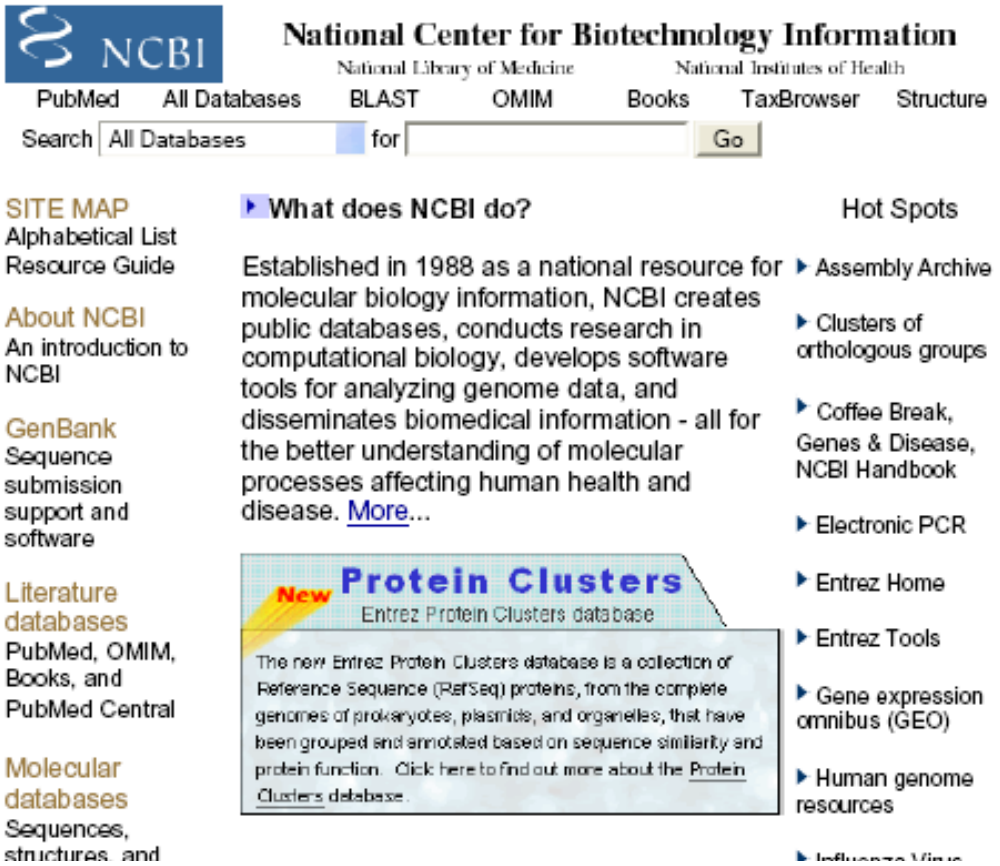

DNA-based identification
raw materials
processed materials
one species in the sample
mixture of species
one species in the sample
mixture of species
proof of presence of one or some target species
proof of absence of toxic species
identification of the whole mixture
quantification of target species
quantification of toxic species, limits ?
quantification of all compounds

I: Identifying Species (DNA-Barcoding?)



Public Database for Sequences

http://www.ncbi.nlm.nih.gov/



What is „DNA-Barcoding“?

- A single gene region (approx 600 base pairs) is sequenced and used as „barcode“.
- The same sequence will be used for all species to standardise the protocol
- An inventory of all species will be created (without accompanying morphological analysis, etc.)
- The gene sequences will be analysed with distance algorithms to assign an individual to a species.

Identification of Birds through DNA Barcodes

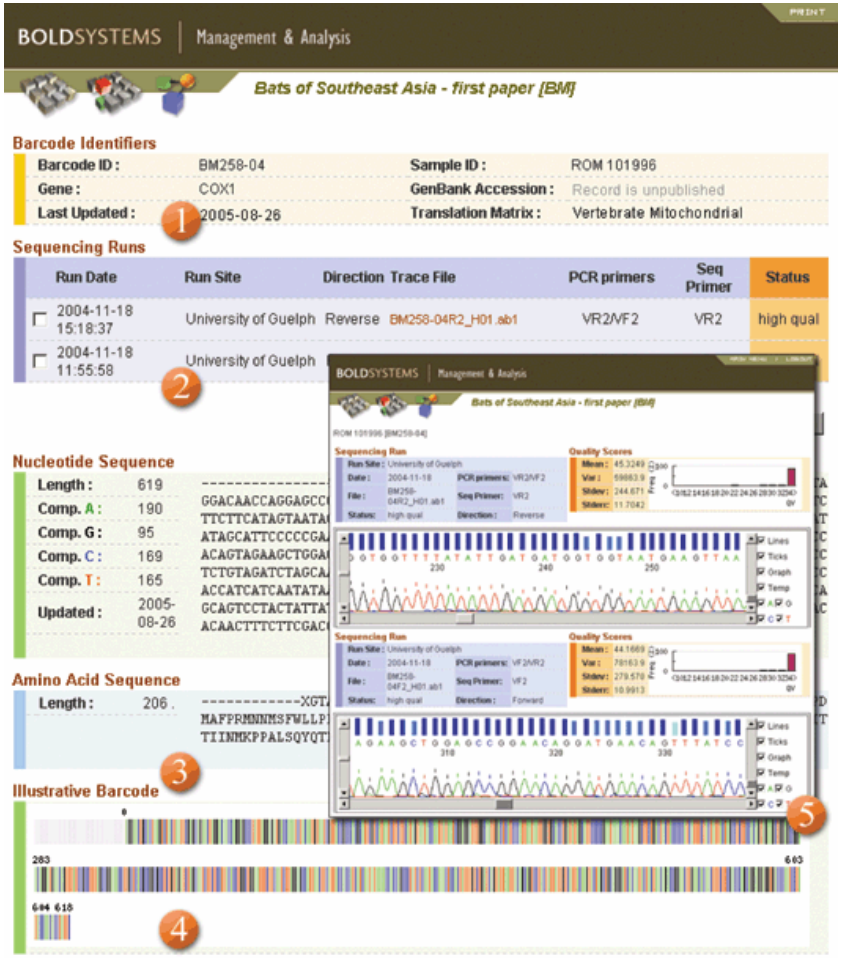
Paul D. N. Hebert[1*], Mark Y. Stoeckle[2], Tyler S. Zemlak[1], Charles M. Francis[3]

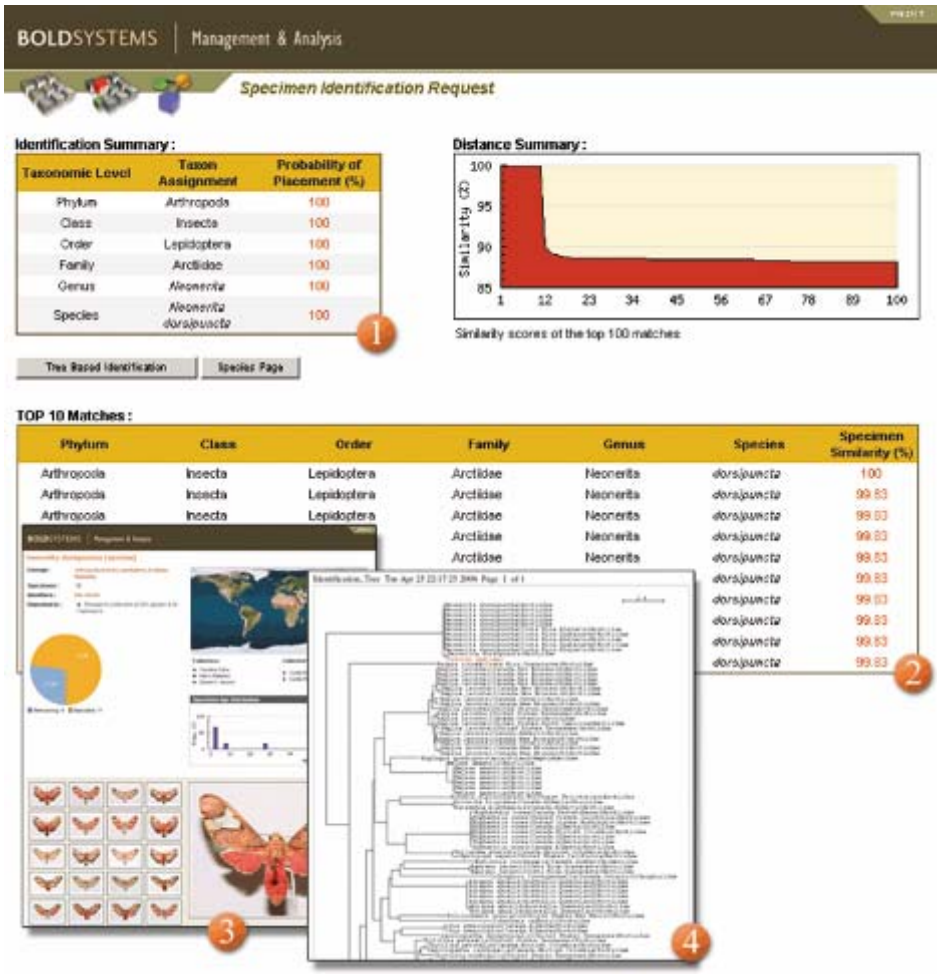
1 Department of Zoology, University of Guelph, Guelph, Ontario, Canada, 2 Program for the Human Environment, Rockefeller University, New York, New York, United States of America, 3 National Wildlife Research Centre, Canadian Wildlife Service, Ottawa, Ontario, Canada

PLoS Biol 2(10): e312 (2004).

BOLD: The Barcode of Life Data System (http://www.barcodinglife.org)

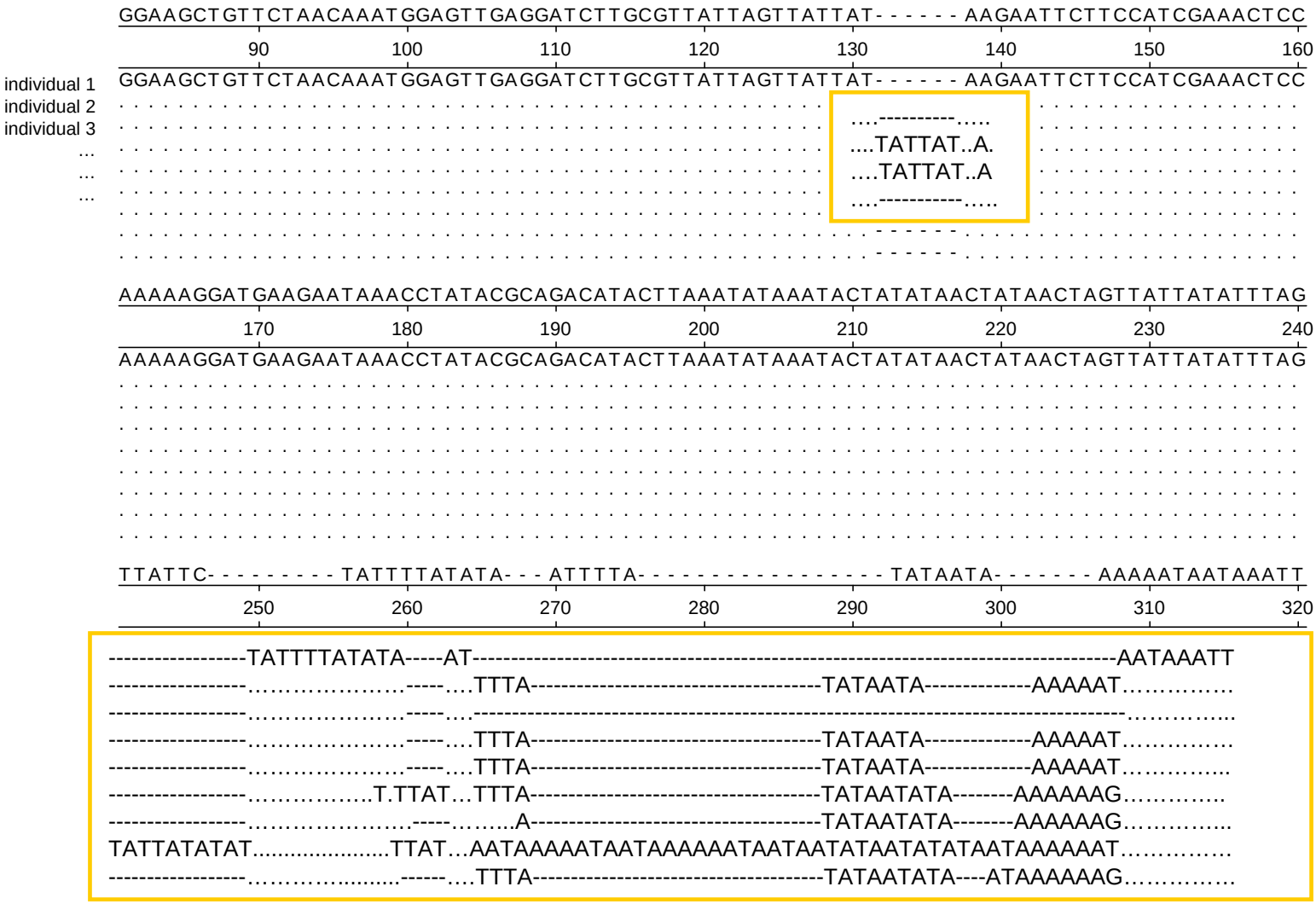
RATNASINGHAM S. and HEBERT P.D.N., Molecular Ecology Notes, 7, 355-364, 2007



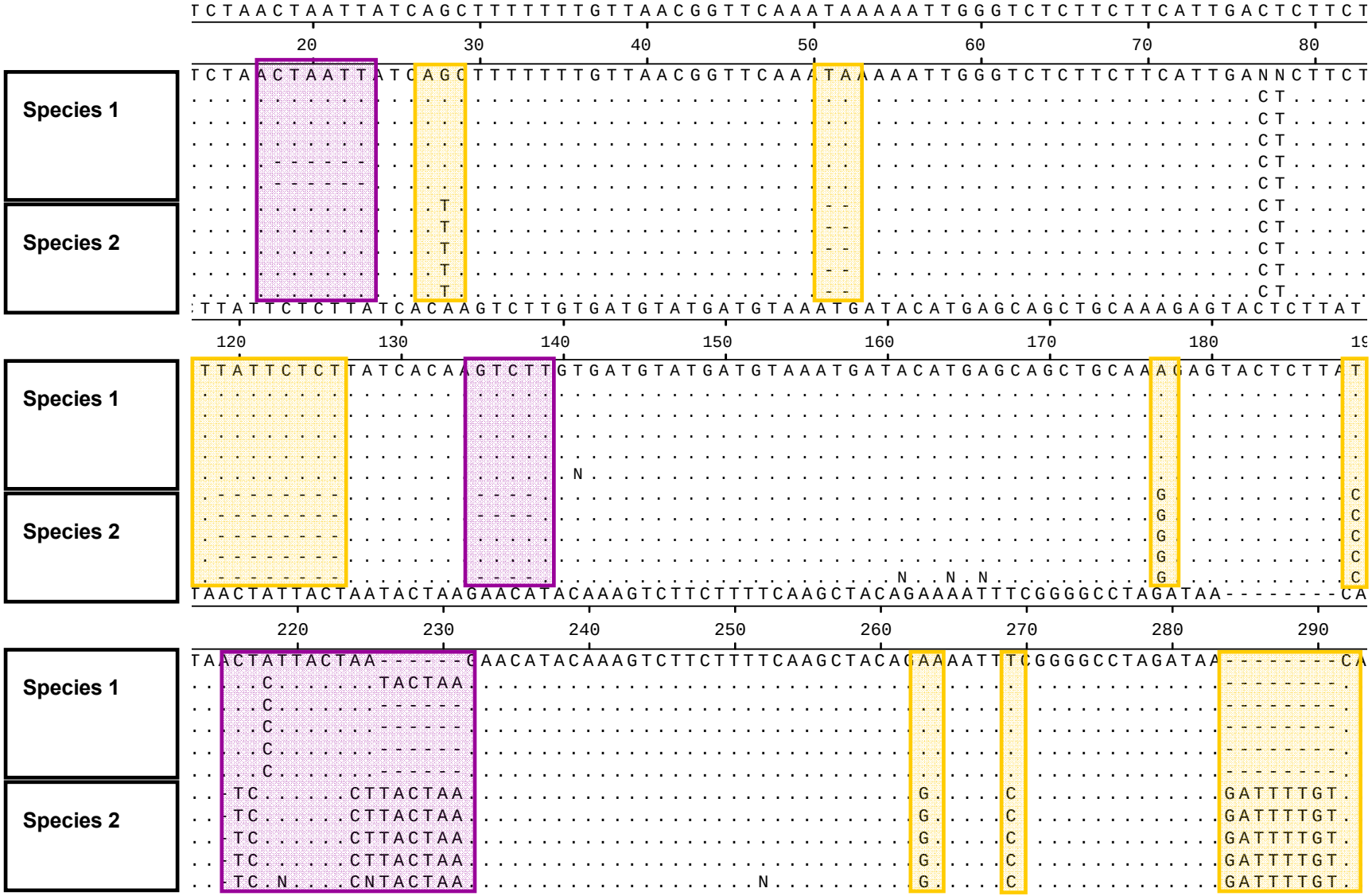


Caveat: Intraspecific Variability

example: several individuals of one species

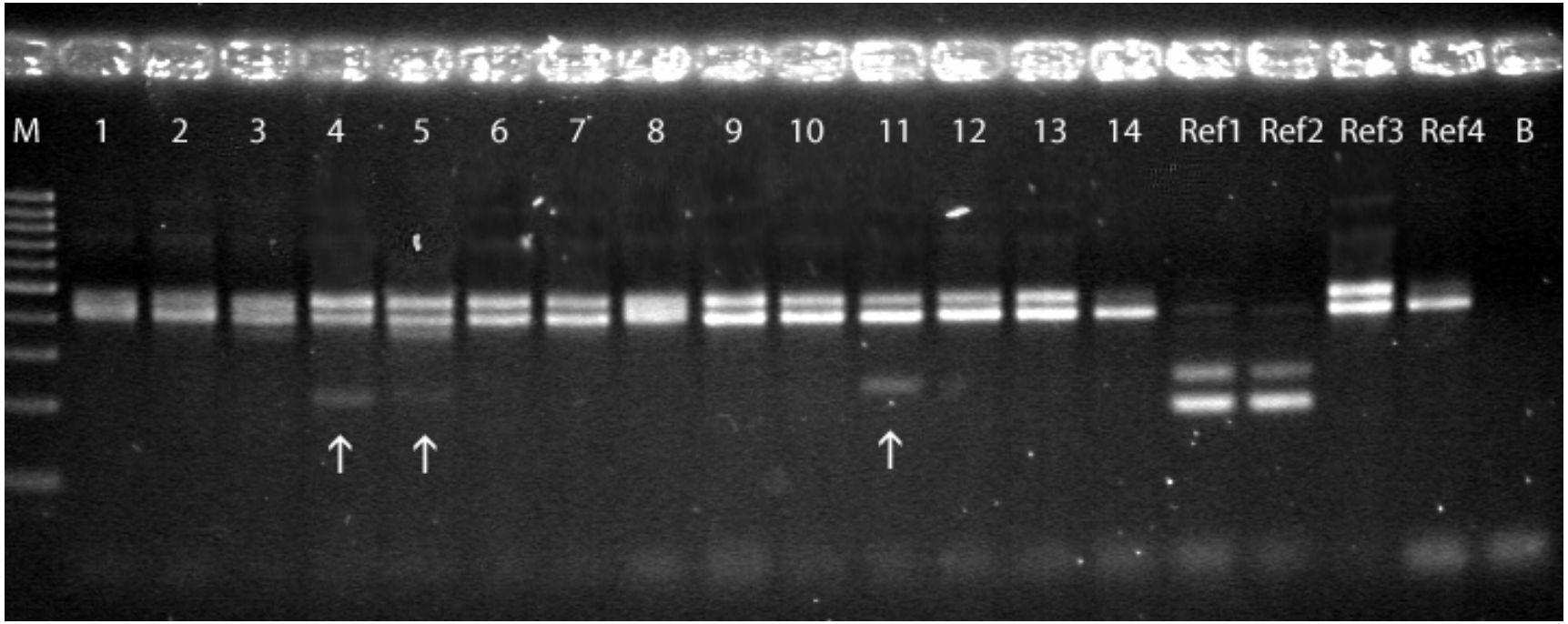


Intraspecific Variability



yellow boxes: intraspecific (usable) variability, violet boxes: interspecific variability

Subgenus-specific PCR Based on Sequence Information

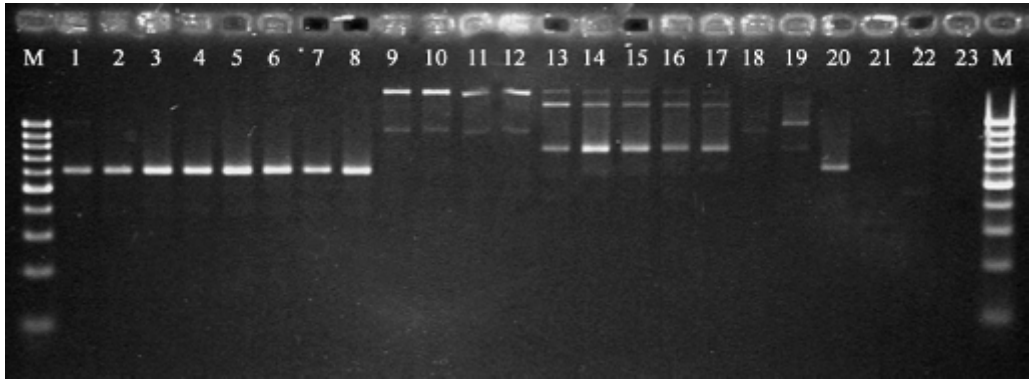


lanes 1-8: samples (drug)

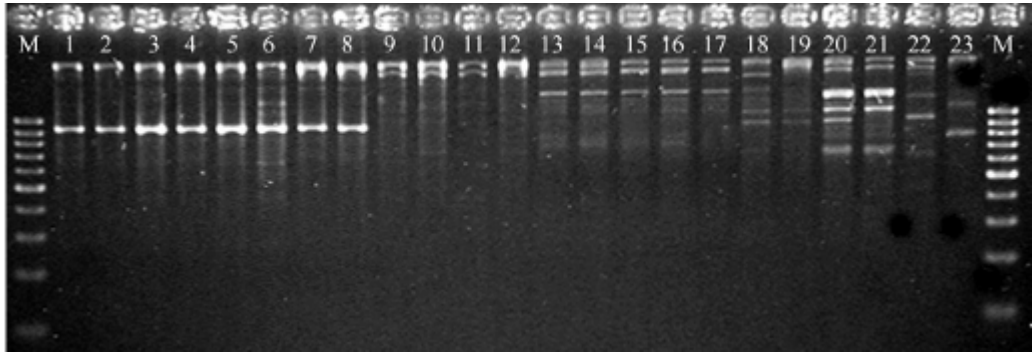
No.	Species	Subgenus
Ref1	*Species 1*	Subgenus 1
Ref2	*Species 2*	Subgenus 1
Ref3	*Species 3*	Subgenus 2
Ref4	*Species 4*	Subgenus 2

Species-specific ‚Anonymous‘ Molecular Markers
(not based on sequence information)

RAPD-Primer X:



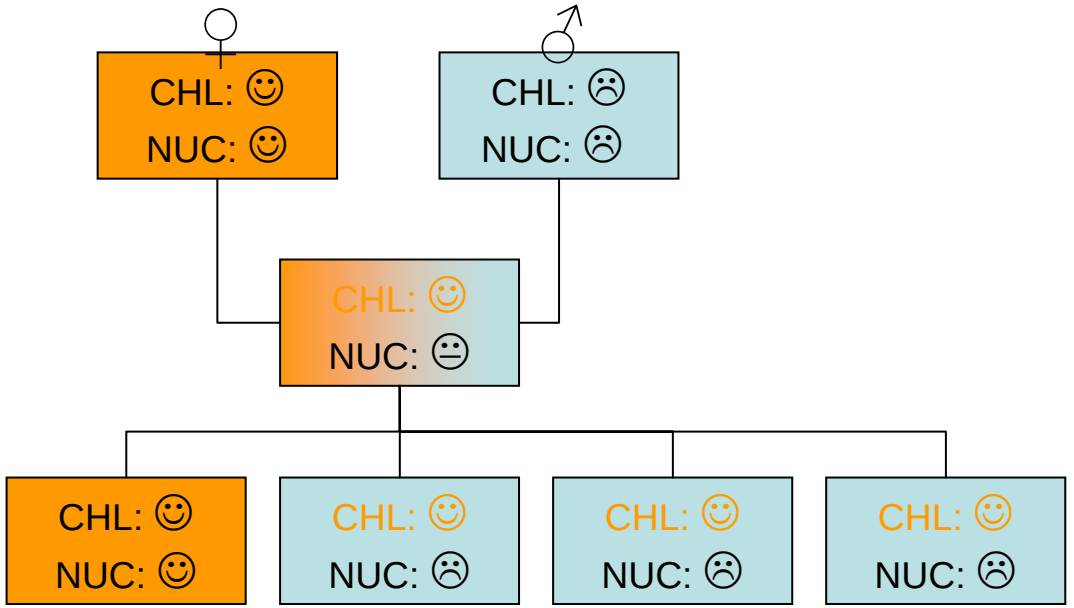
RAPD-Primer Y:



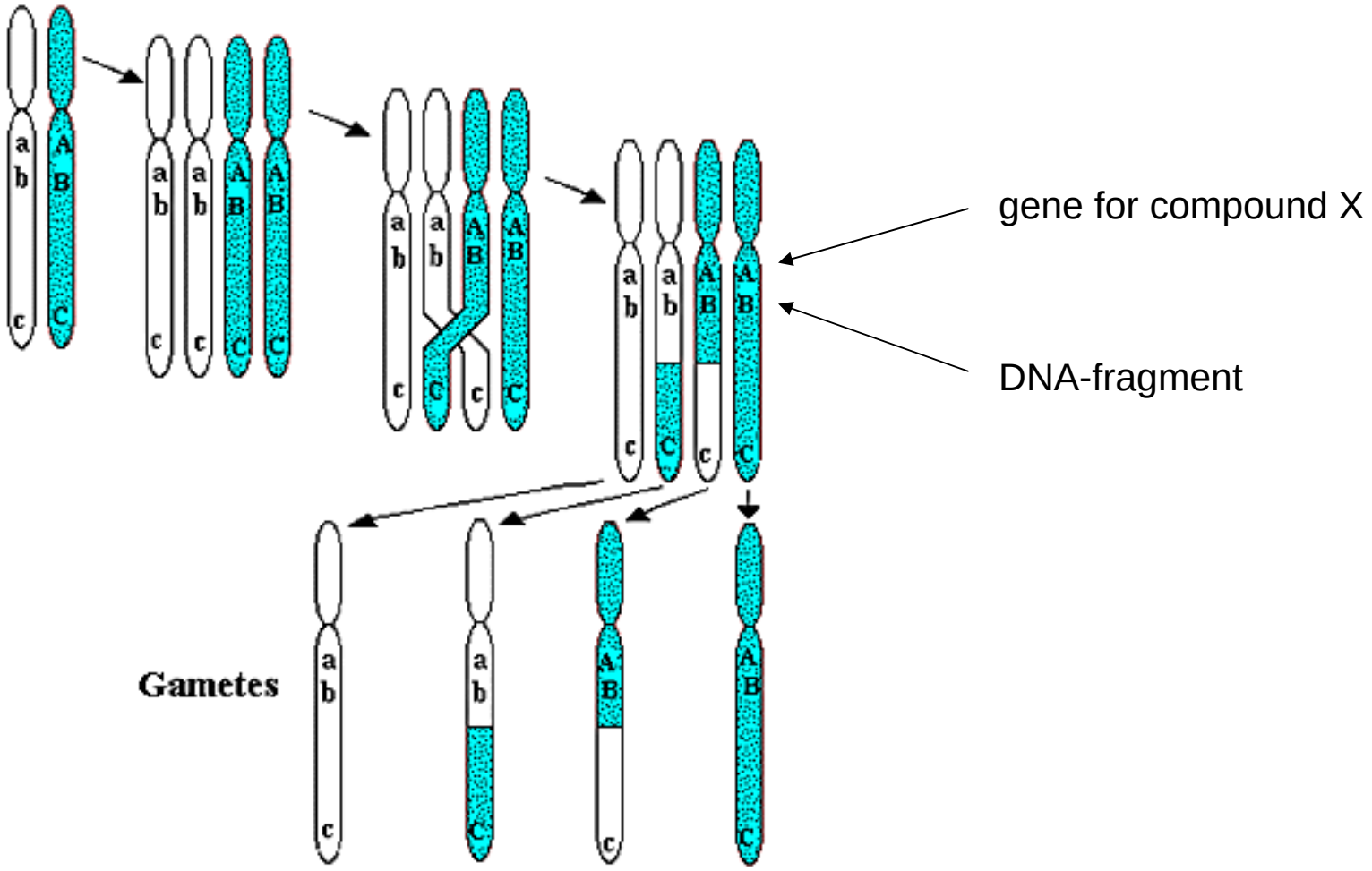
lanes 1-8: target species

Problem Hybridisations
(especially Introgression - Chloroplast vs. Nuclear Genome)

- Taxonomy is often working with chloroplast sequences
- These sequences are only of limited use in detecting hybridisations because chloroplasts are maternally inherited
- Nuclear sequence information necessary detecting recent hybridisations



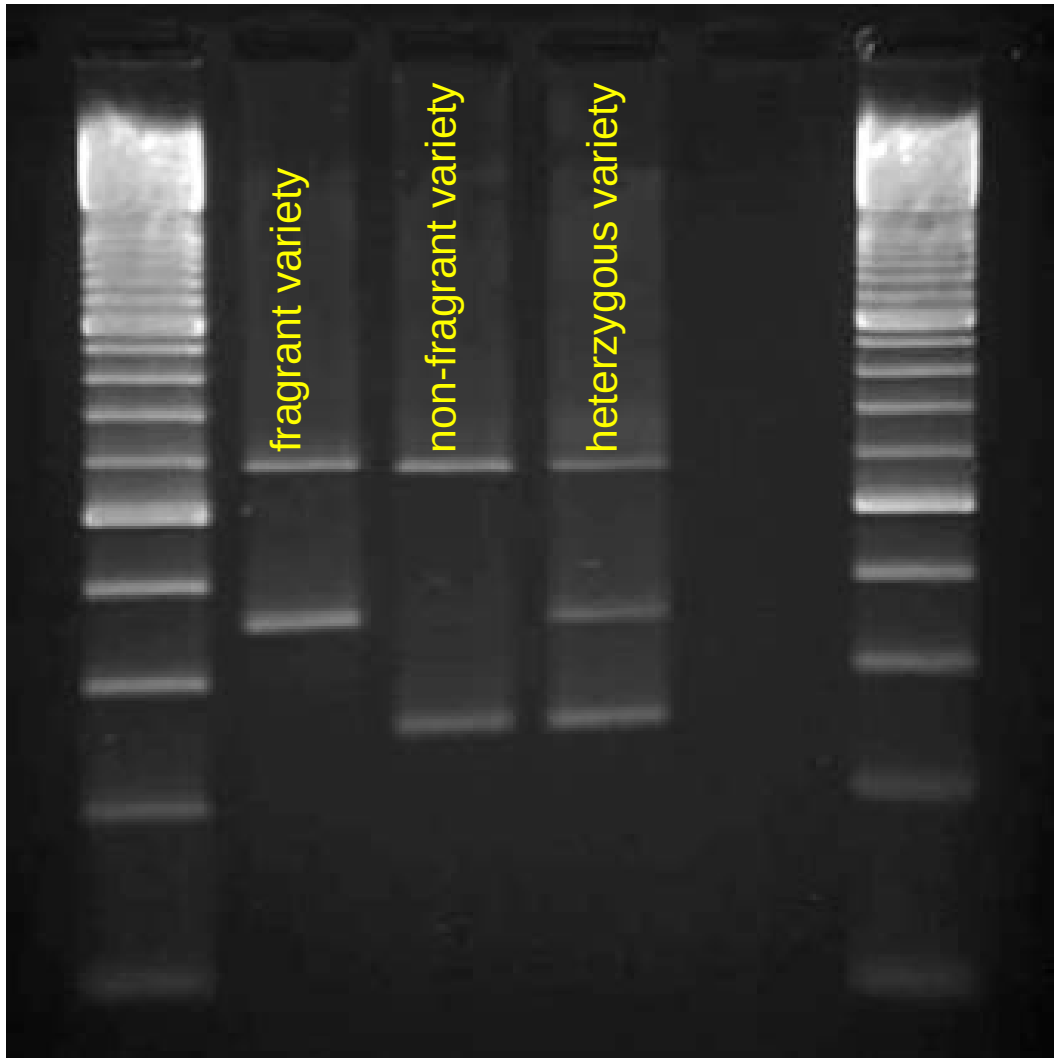
DNA-based Identification of Chemotypes (‚Molecular Markers‘)



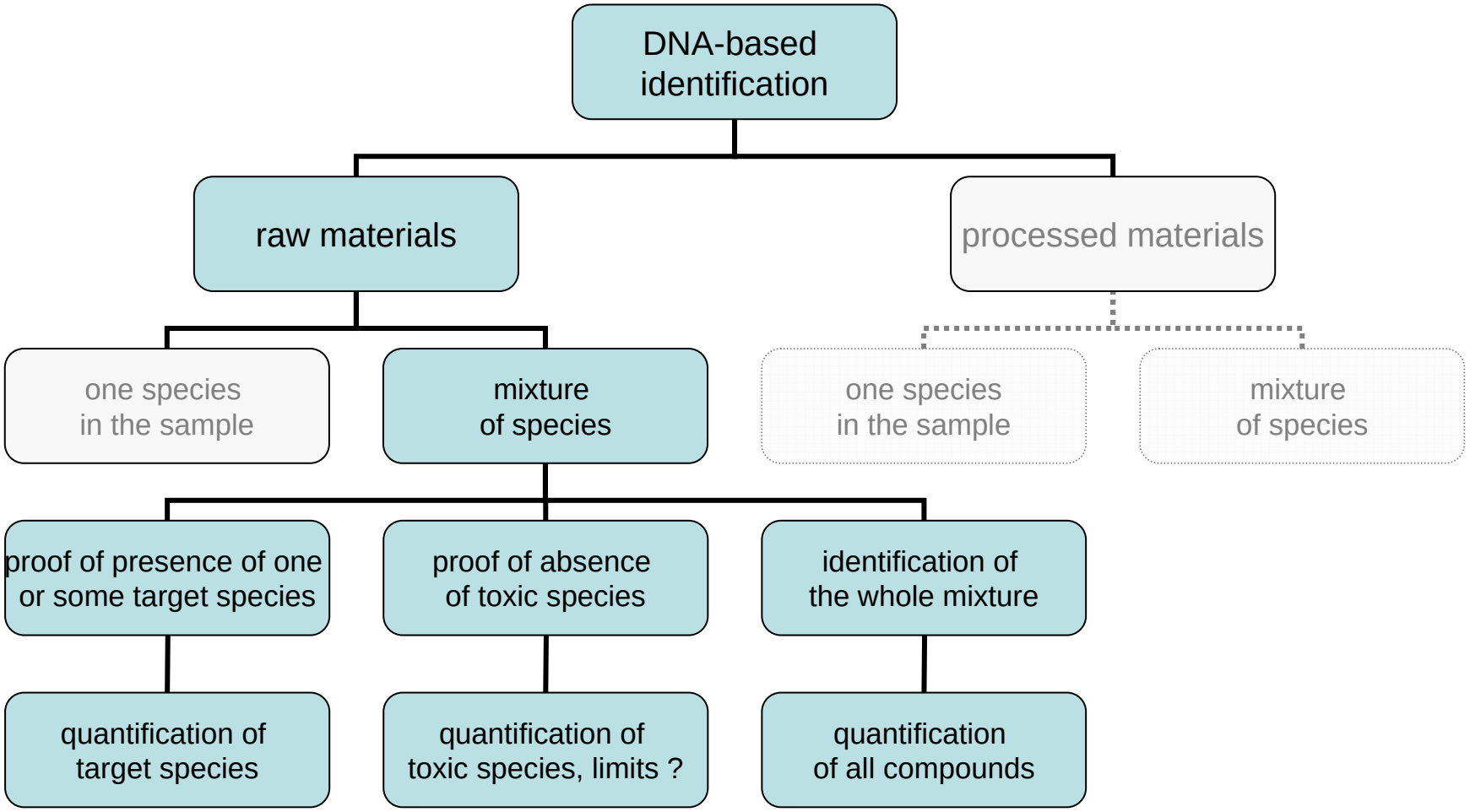
Crossing-over and recombination during meiosis

Example for Molecular Markers

Bradbury LMT et al.: A perfect marker for fragrance genotyping in rice. Molecular Breeding (2005) 16: 279–283



II: Mixtures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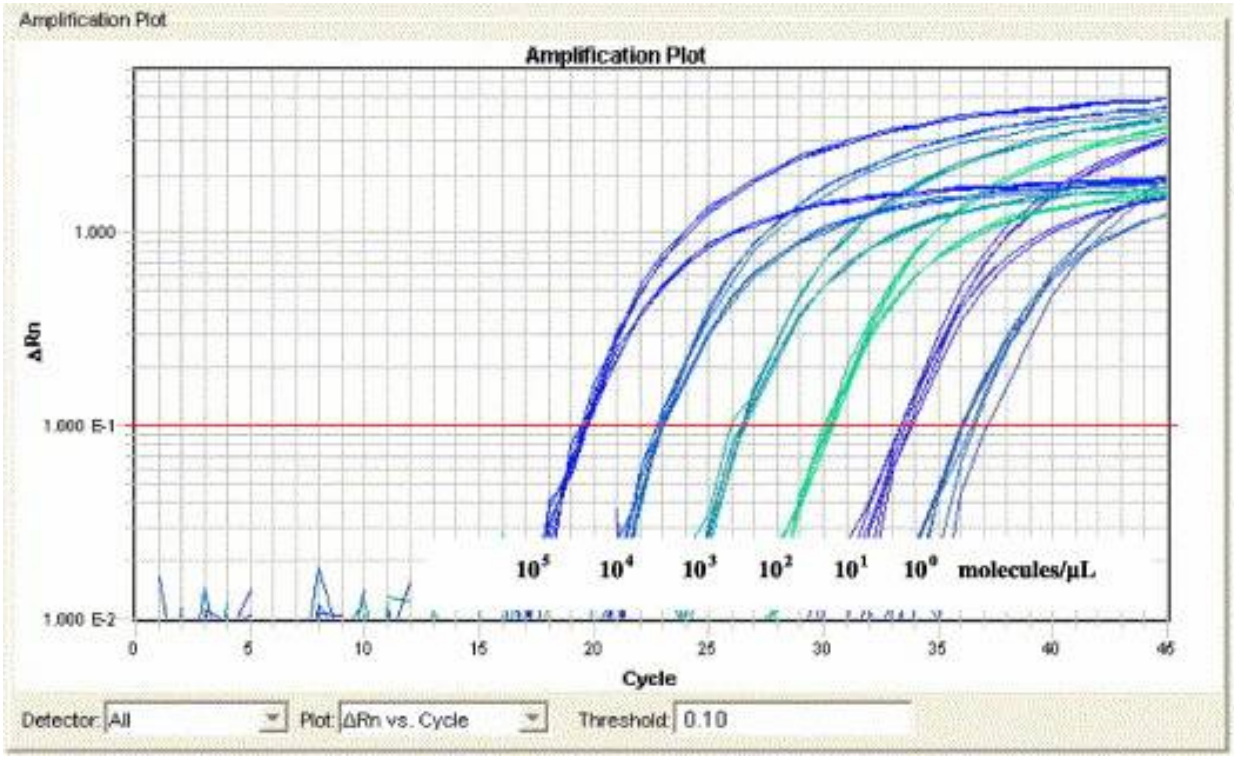
quantification of toxic species, limits ?
quantification of all compounds

qPCR (quantitative PCR)

Engel et al.: Quantification of DNA from genetically modified organisms in composite and processed foods. Trends in Food Science & Technology 17, 490-497, 2006.



GMO-specific signals
(FAM-labeled probes)

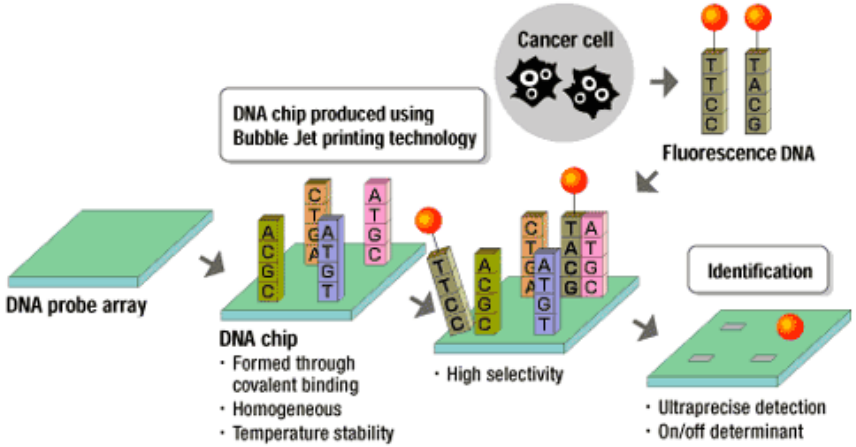
Rapeseed-specific signals
(VIC-labeled probes)

Calibration curves:

LibertyLink assay
Log Qty (recDNA) = -3.414 * C_t + 38.914
Log Qty (reference) = -3.432 * C_t + 39.335

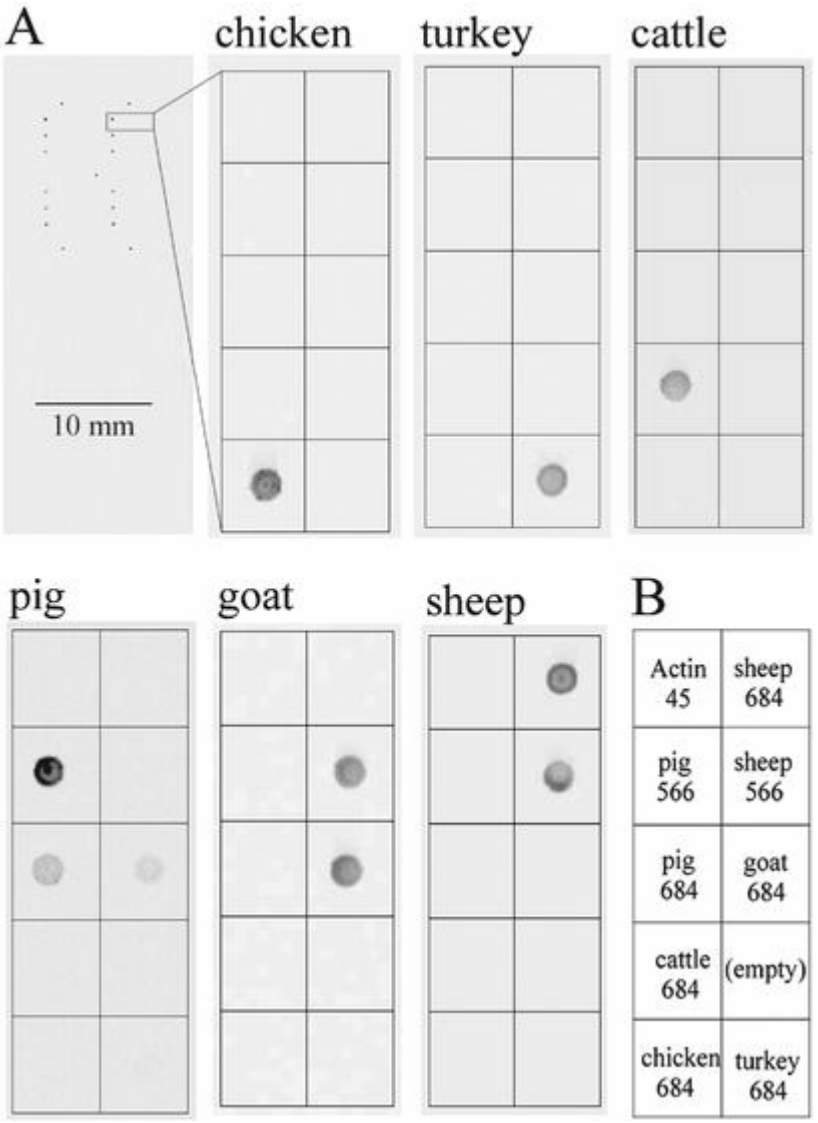
SeedLink assay
Log Qty (recDNA) = -3.492 * C_t + 39.387
Log Qty (reference) = -3.353 * C_t + 38.874

DNA-Microarrays

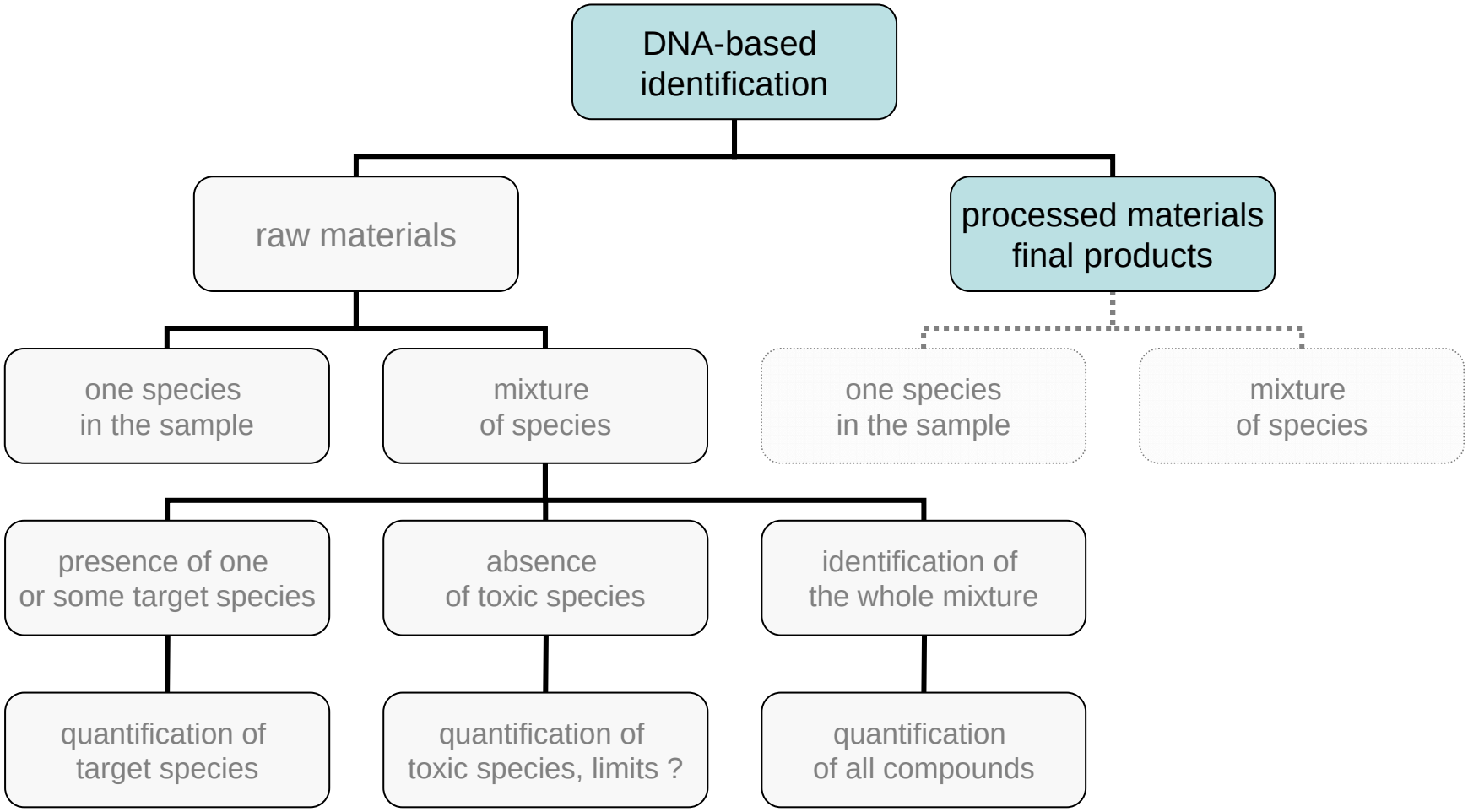


- DNA-Microarrays
  - Pharmacodynamics
  - Pharmacogenomics
  - Pharmacognosy

Example for Species Detection by DNA Microarray:
Peter et al., Eur Food Res Technol 219:286-293 (2004)



III. Processed Materials / Final Products



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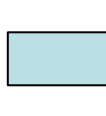
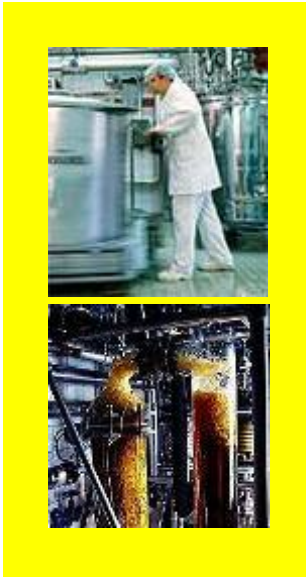


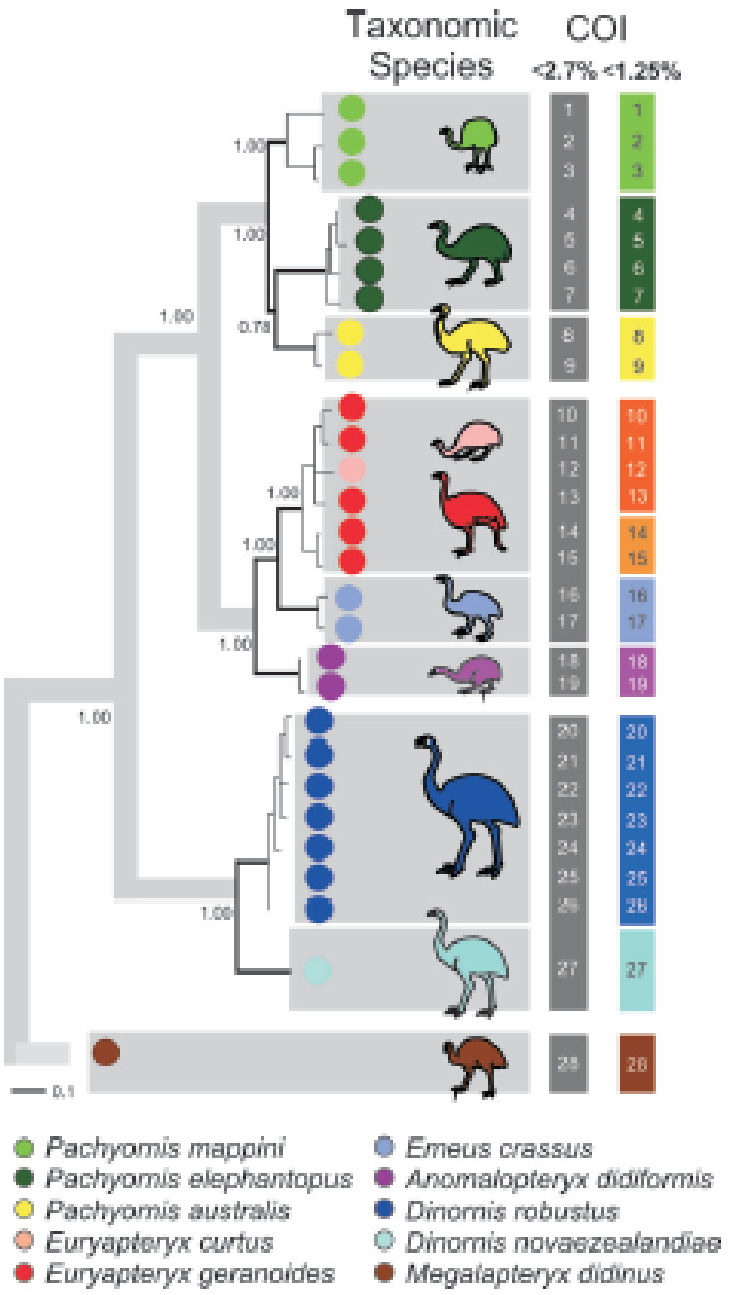


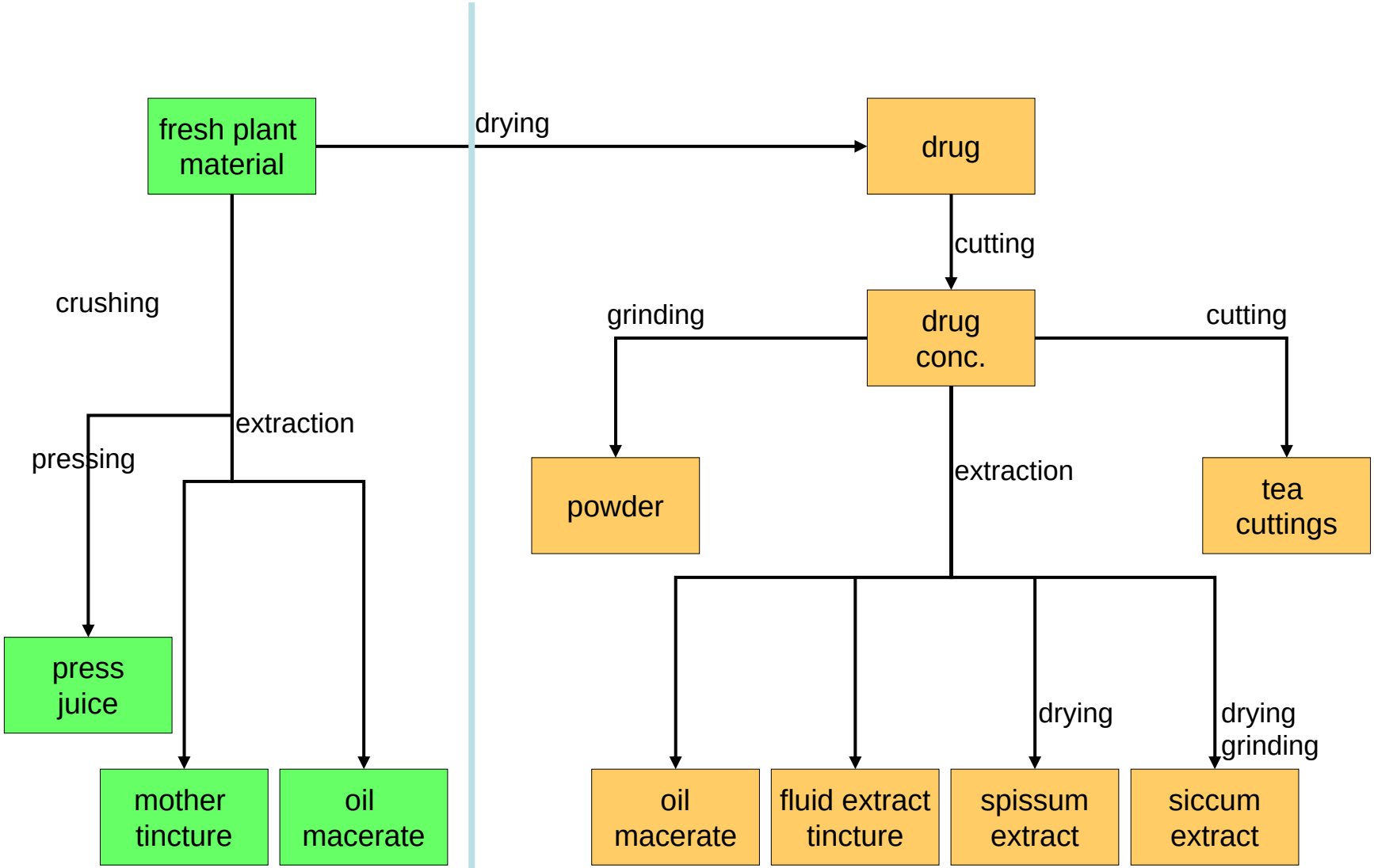
Stability of DNA
Extinct, Subfossilised Species

Is a Large-Scale DNA-Based Inventory of Ancient Life Possible?

D. M. Lambert, A. Baker, L. Huynen, O. Haddrath, P. D. N. Hebert, and C. D. Millar

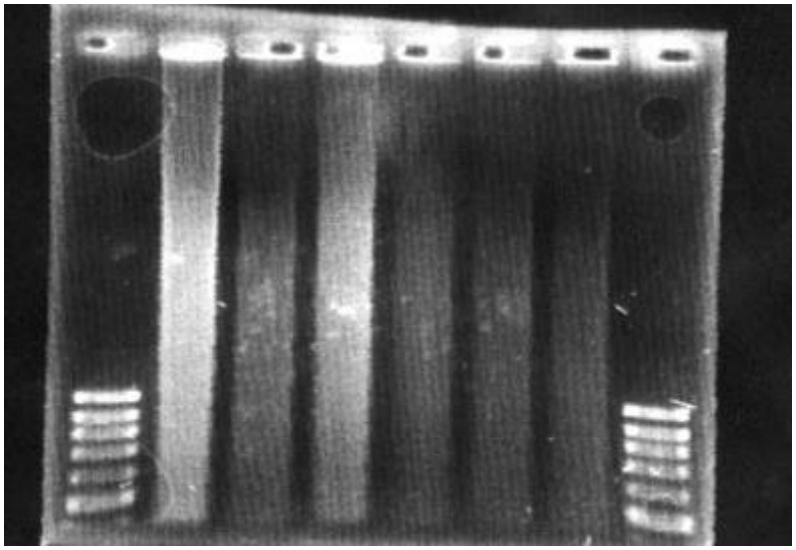
Journal of Heredity 96:279–284 (2005)




fresh plant material
drying
drug
cutting
crushing
grinding
drug conc.
cutting
extraction
pressing
powder
extraction
tea cuttings
press juice
drying
drying grinding
mother tincture
oil macerate
oil macerate
fluid extract tincture
spissum extract
siccum extract

Processed Materials: Powder

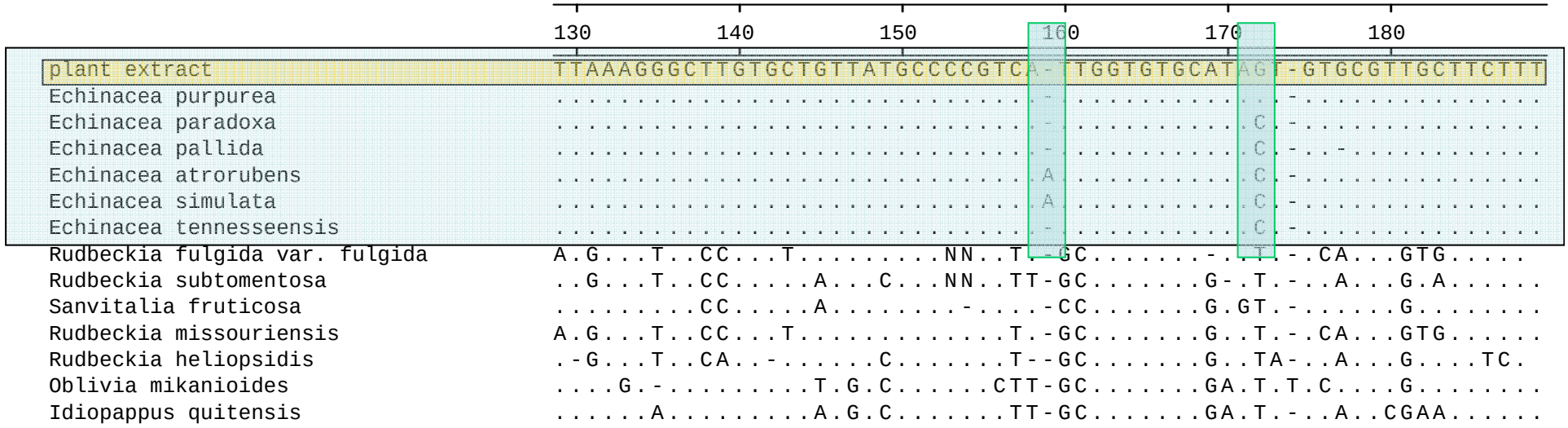
- DNA already strongly degraded, but DNA-based identification still possible



Extracts

Echinacea sp. - spissum ethanol extract

Novak et al.: DNA-Based Authentication of Plant Extracts. Food Research International 40: 388-392, 2007.

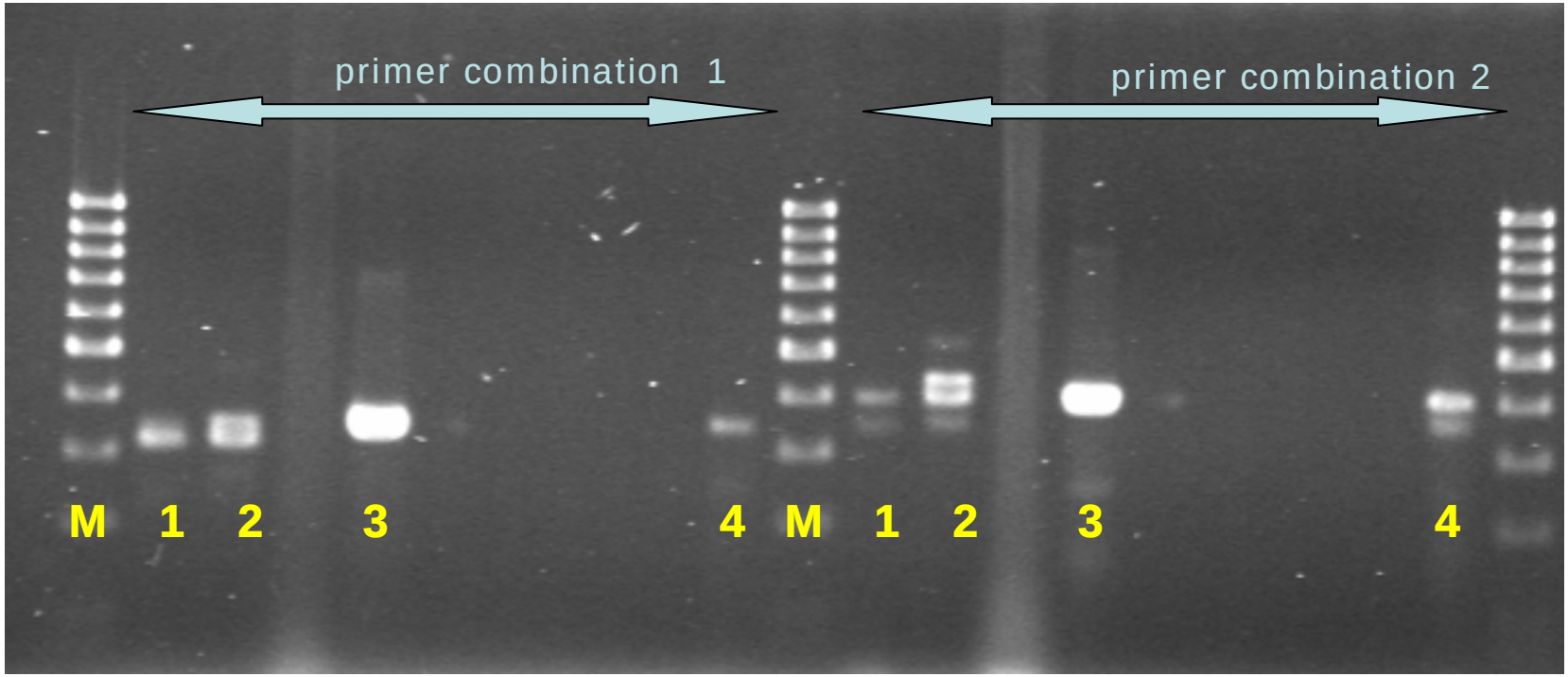


Extracts

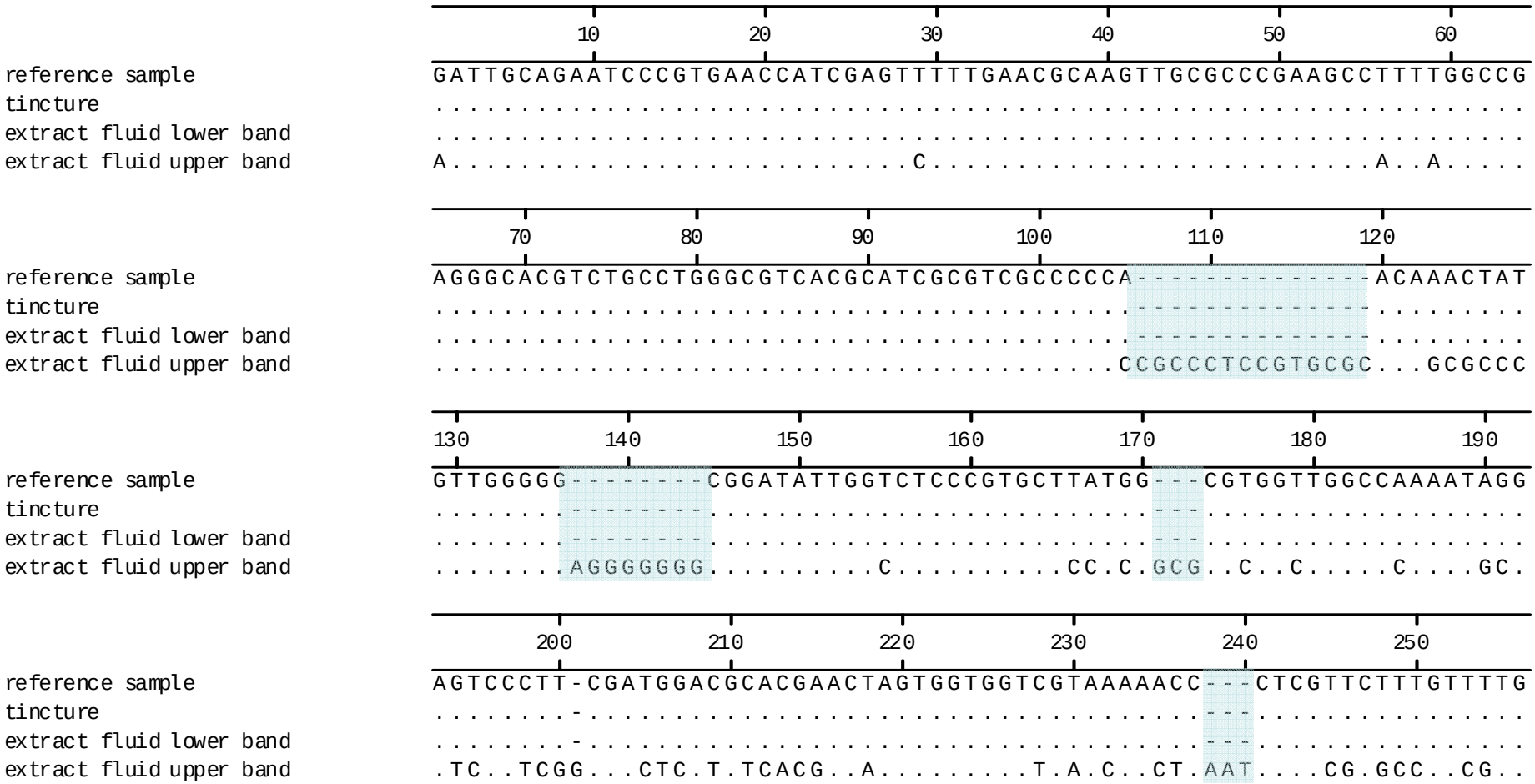
Chamomilla tincture / extract fluid / tea bag

Novak et al.: DNA-Based Authentication of Plant Extracts. Food Research International 40: 388-392, 2007.

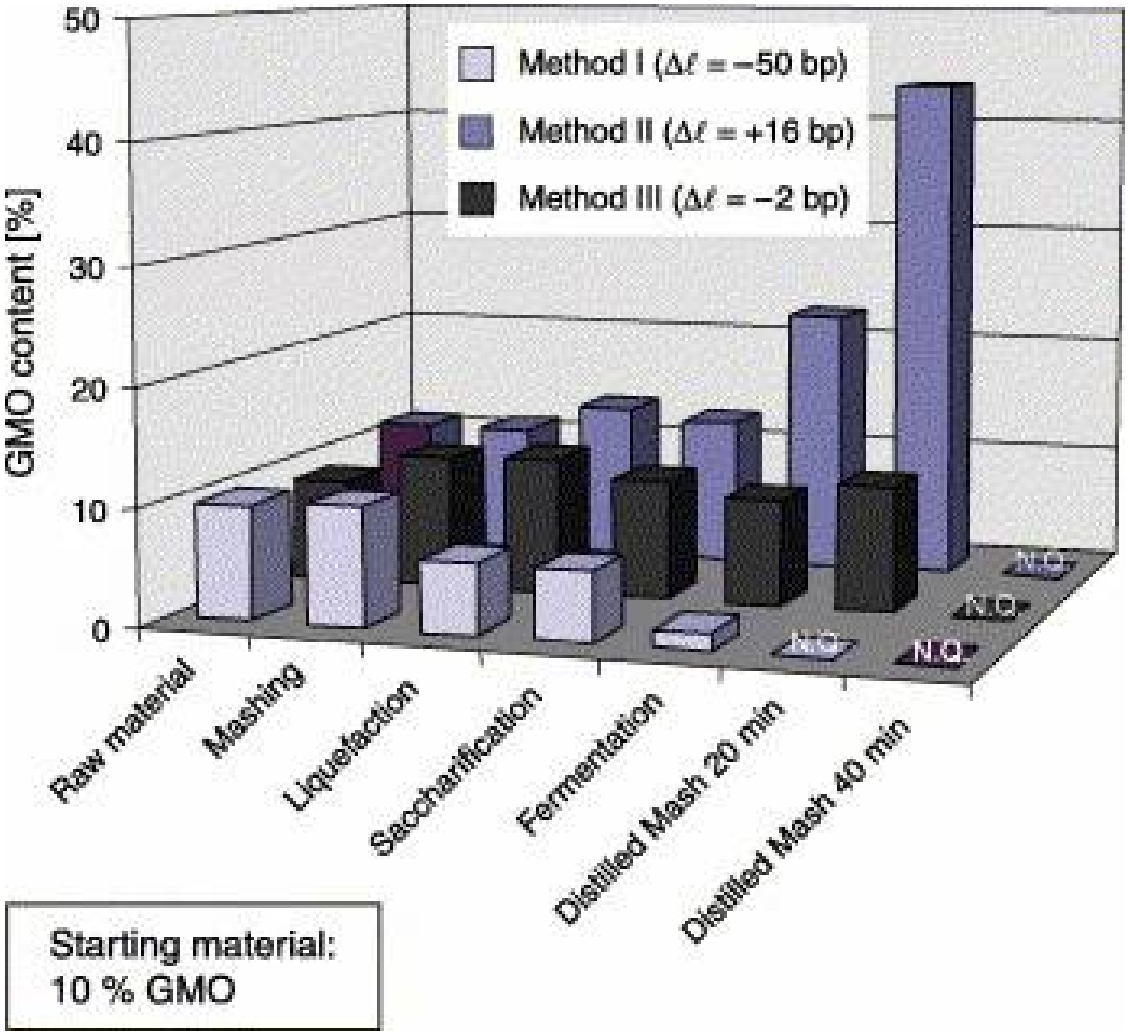
1: Chamomilla tincture
2: Chamomilla extract fluid
3: Chamomilla reference plant
4: Chamomilla teabag



Chamomilla: primer combination 2



Relative quantification of DNA from corn Bt-176 in samples from intermediate stages of the process of ethanol production; NQ, not quantifiable. (Moreano, 2005 and Moreano et al., 2005b).



We kindly appreciate Bionoricas interest in our work