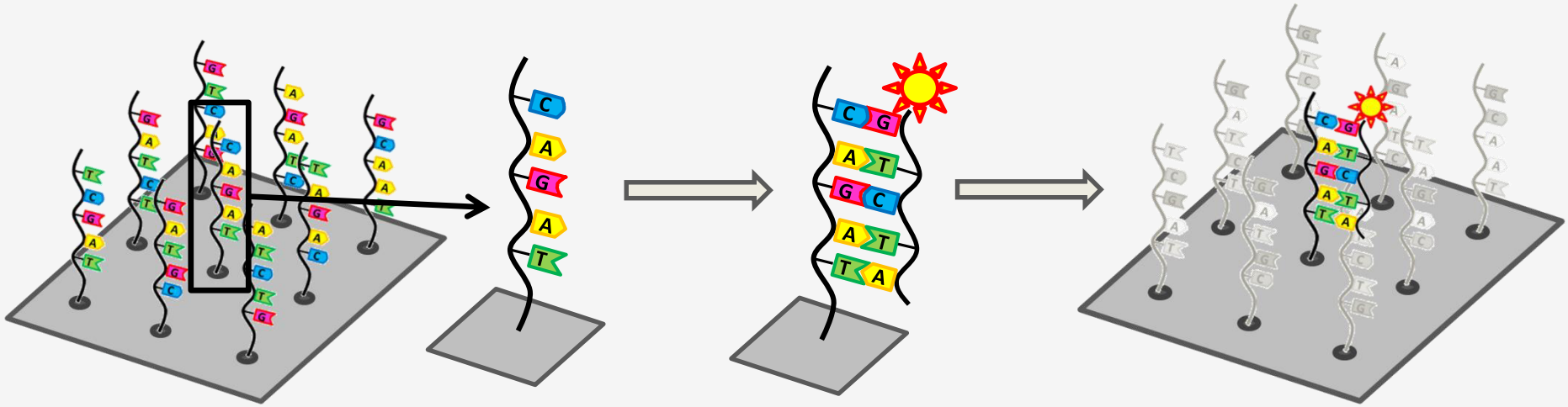
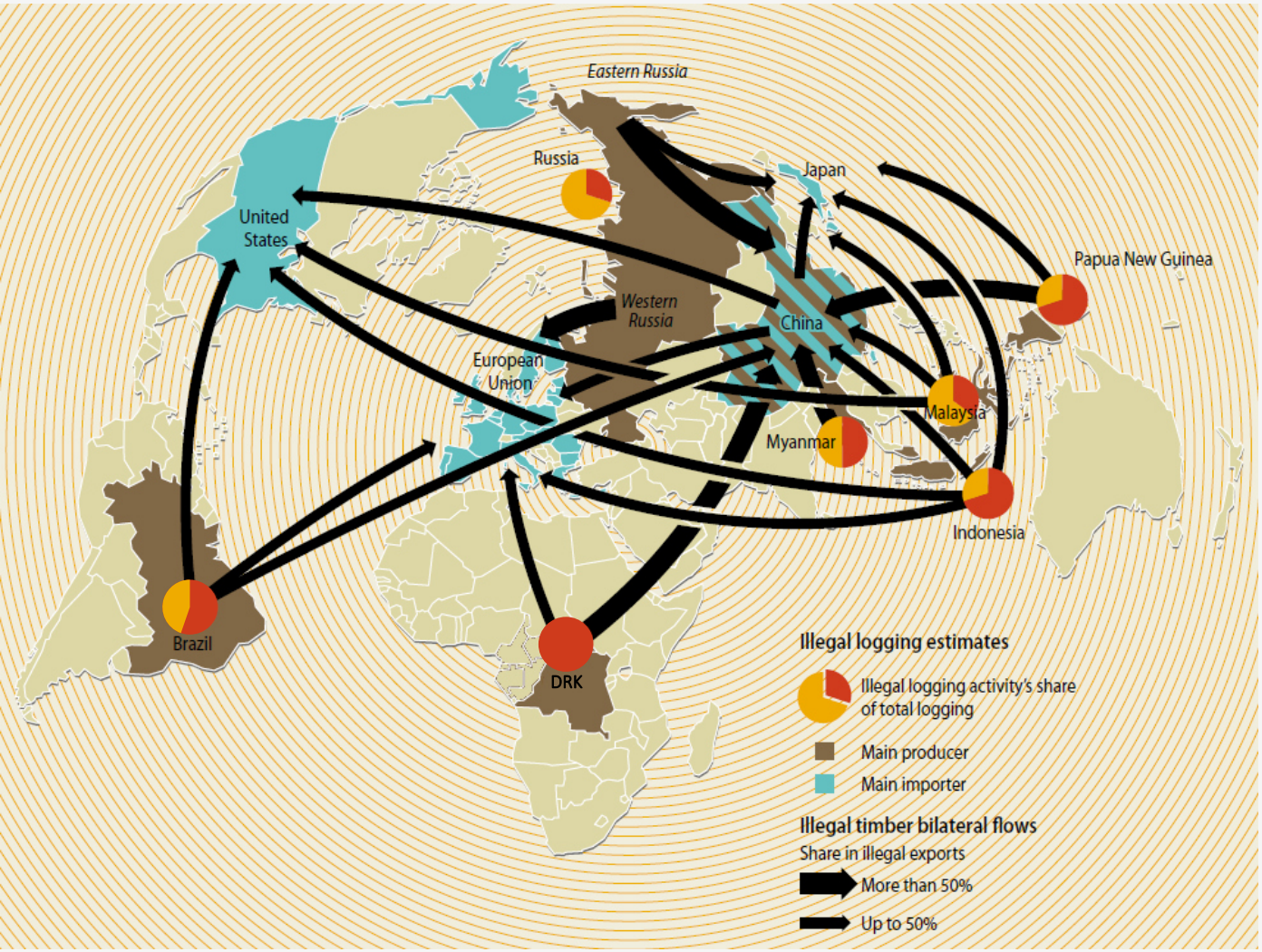
Development of a DNA macro array for the genetic identification of trade-relevant, CITES wood species and their look alikes

Hamburg, 2023



Illegal logging and trade



- 15 – 30 % of the global logging is illegal
- Sales volume of 51 – 152 bn US$ [1]
- 50 – 90 % of the national harvest [2]

⇩

- Loss of biodiversity
- No guarantee for species conservation

[1] Nellemann (2012) INTERPOL Environmental Crime Programme; INTERPOL (2021), factsheet
[2] Hoare (2015) Chatham House Report

Legal regulations in species protection

- 15 – 30 % of the global logging is illegal
- Sales volume of 51 – 152 bn US$
- 50 – 90 % of the national harvest

- Loss of biodiversity

Convention on International Trade in Endangered Species of Wild Fauna and Flora

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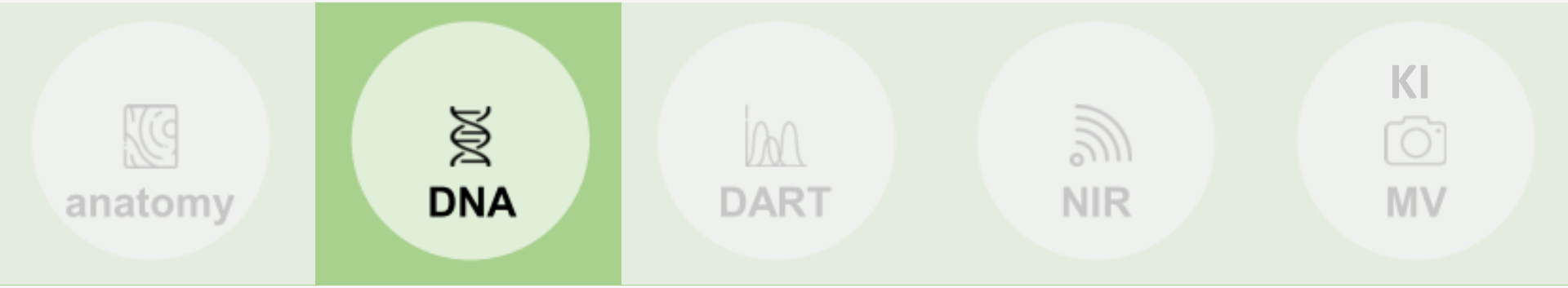
Methods for wood species identification



[1] modified

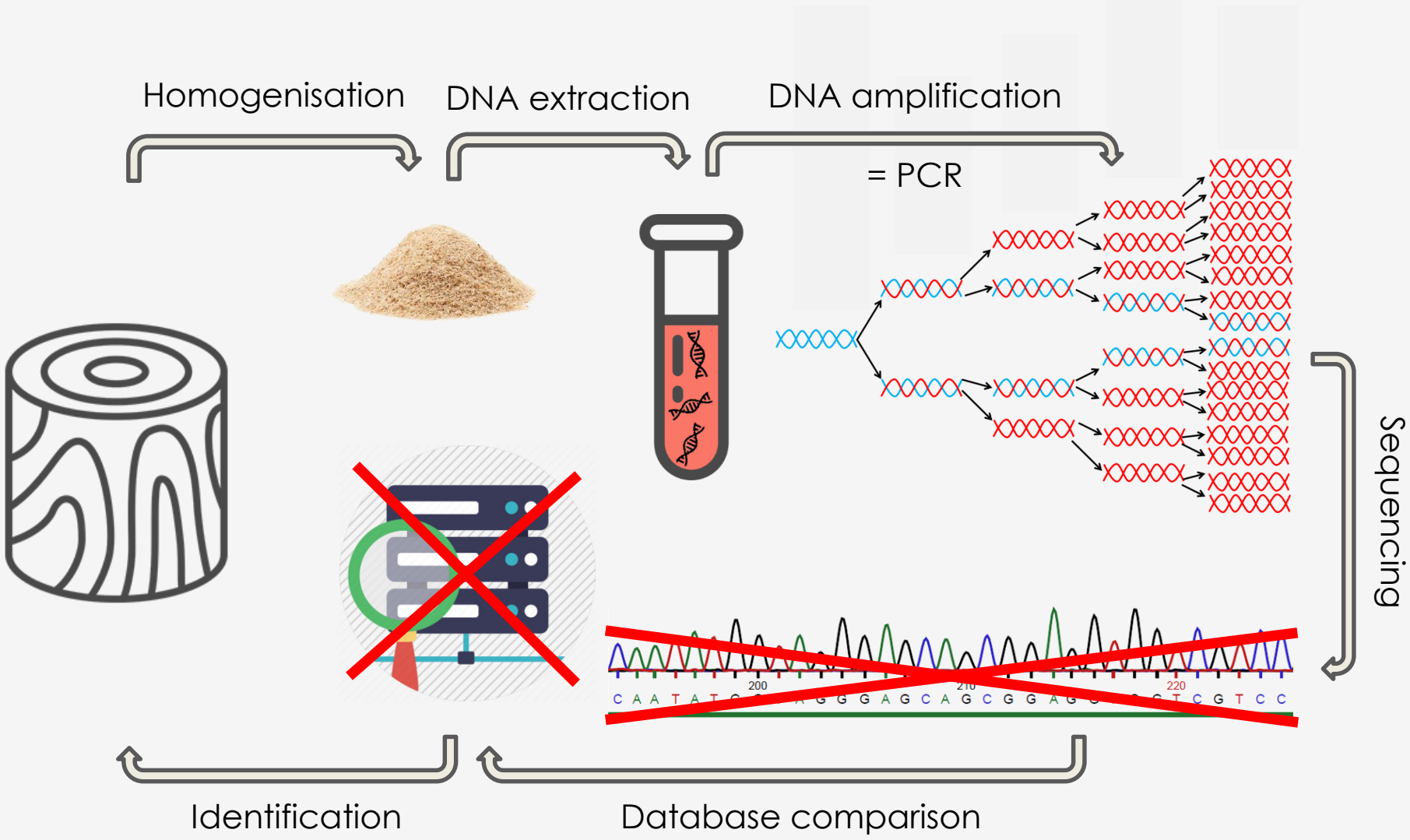
Problem

There is a lack of available, easy-to-use, low-cost tools to check the legality of a timber product. [2]

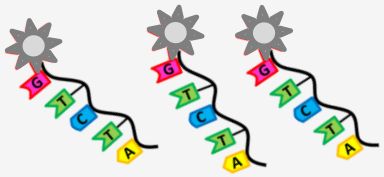
Goal

Development of a fast, cheap, genetic, low-tech tool for identifying CITES timber species without anatomical knowledge.

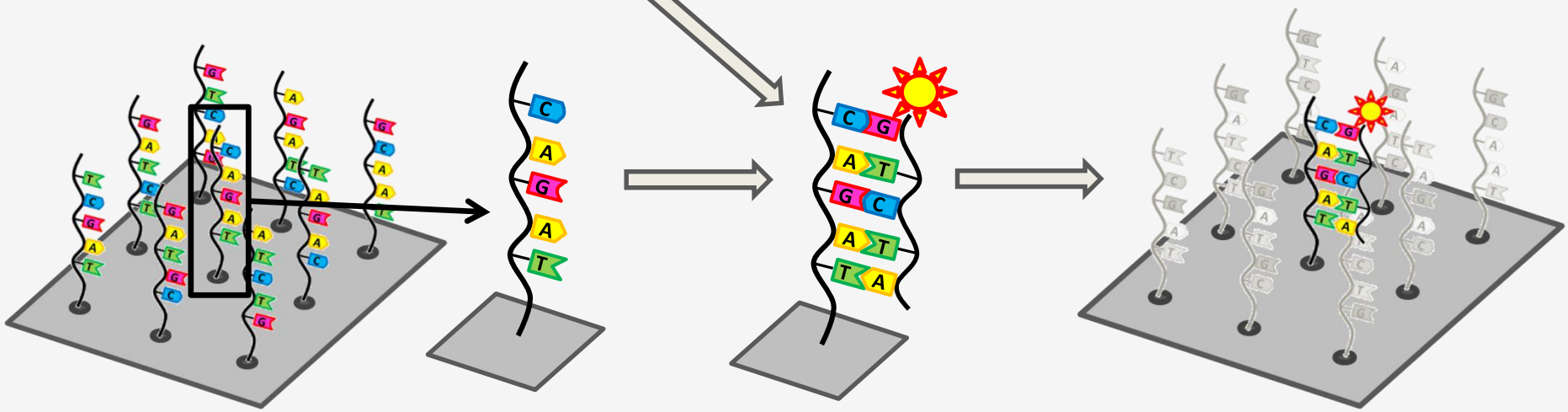
[1] Schmitz et al. (2019) „The Timber Tracking Tool Infogram."
[2] Dormontt et al. (2015); Lowe et al. (2016); Wiedenhoeft et al. (2016); Gasson et al. (2021); Low et al. (2022)


Homogenisation
DNA extraction
DNA amplification
= PCR
Sequencing
Identification
Database comparison

DIG-labelled sample DNA



Detection reaction



Macro array with oligos

Specific oligo

Hybridisation of oligo and sample

Macro array with bonded sample

- No sequencing required
- No species knowledge required
- Parallel analysis of several species within one product
- Low-tech approach

  = cost efficient

→ Application in producing countries

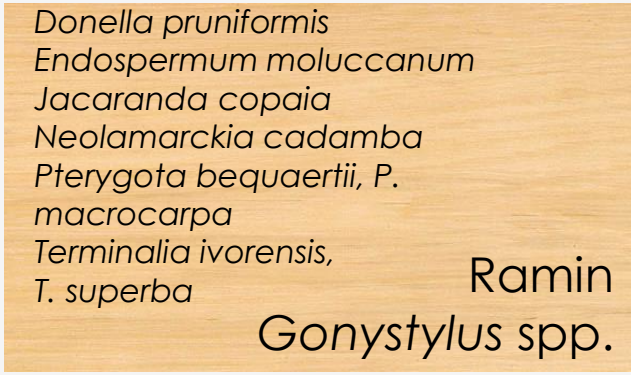
- Commercially applicable as DNA chip

- Dependent on accessible DNA
- No application for processed products such as paper or coal

Carapa guianensis, C. procera
Entandrophragma spp.
Khaya spp.
Leplaea cedrata, L. thompsonii
Sandoricum koetjape

Guibourtia arnoldiana , G. ehie
Copaifera salikounda
Hymenaea courbaril

Alstonia scholaris
Dyera costulata
Antiaris toxicaria
Brosimum alicastrum
Donella pruniformis
Endospermum moluccanum
Jacaranda copaia
Neolamarckia cadamba
Pterygota bequaertii, P. macrocarpa
Terminalia ivorensis, T. superba



Toona ciliata, T. sureni

Pterocarpus erinaceus, P. indicus, P. macrocarpus, P. soyauxii

Plectrocarpa arborea [1]
Chlorocardium rodiei
Handroanthus heptaphyllus
H. serratifolius

[1] Richter, H.G., Gembruch, K., and Koch, G. 2014 onwards. CITESwoodID. delta-intkey.com

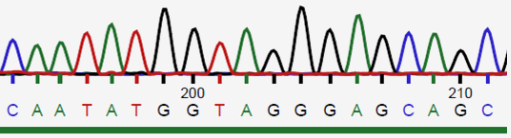
Database construction

Sampling

DNA extraction

Amplification of the Barcode

Sequencing

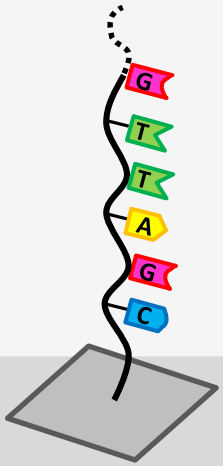


Design of the oligonucleotids

Comparison of sequences

Searching for species-specific sections

Oligo manufacture



Optimization macro array

Lievens et al. 2012

6,7h to 3,2h time reduction

Macro array tests

Pre-testing in groups

Cross-testing

Holzart / Sonde	Probe A	Probe B	Probe C	Probe D	Probe E	...
1	x					
2		x			x	
3			x			
4				x		
5				x	x	
6						
7		x				
8						
9	x					
10						
...						

richtig postive Reaktion

falsch positive Reaktion

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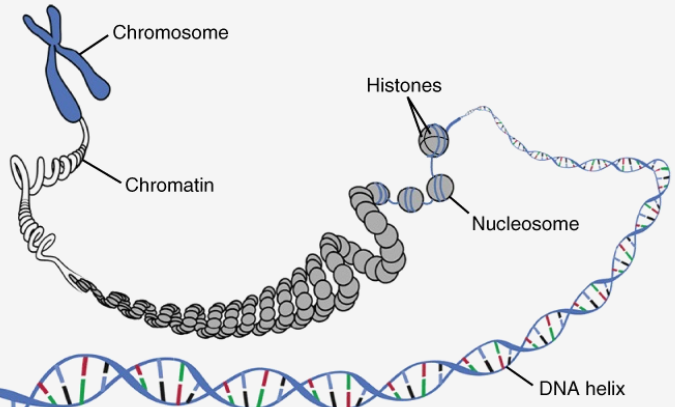
Sampling

264 samples

1 DNA extraction

DNeasy®
Plant Mini Kit

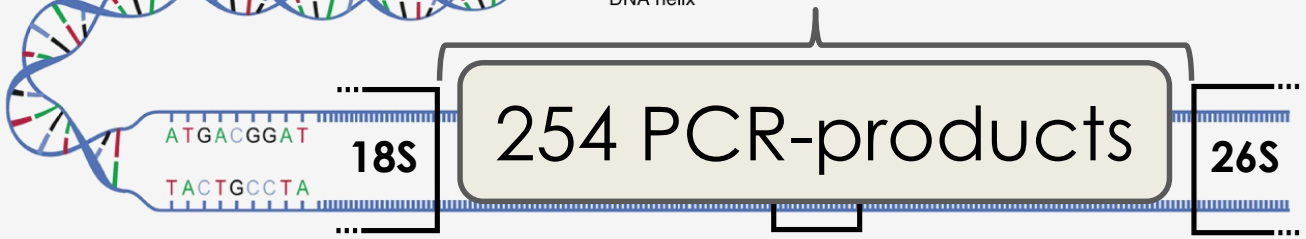
DNeasy®
mericon Food Kit
Protocol
according to
Wischnewski, 2014



2 PCR of the barcode

ITS

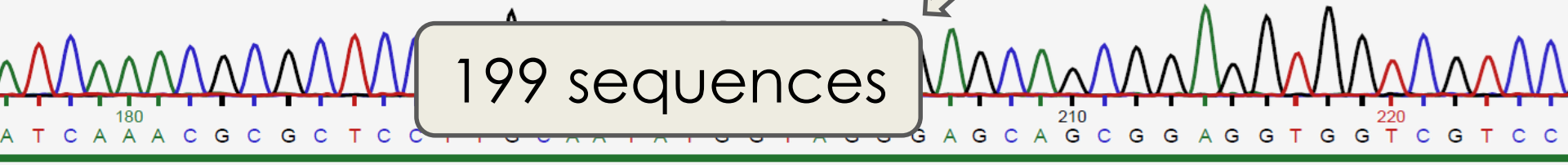
Internal
Transcribed
Spacer [1]



254 PCR-products

[2] modifiziert

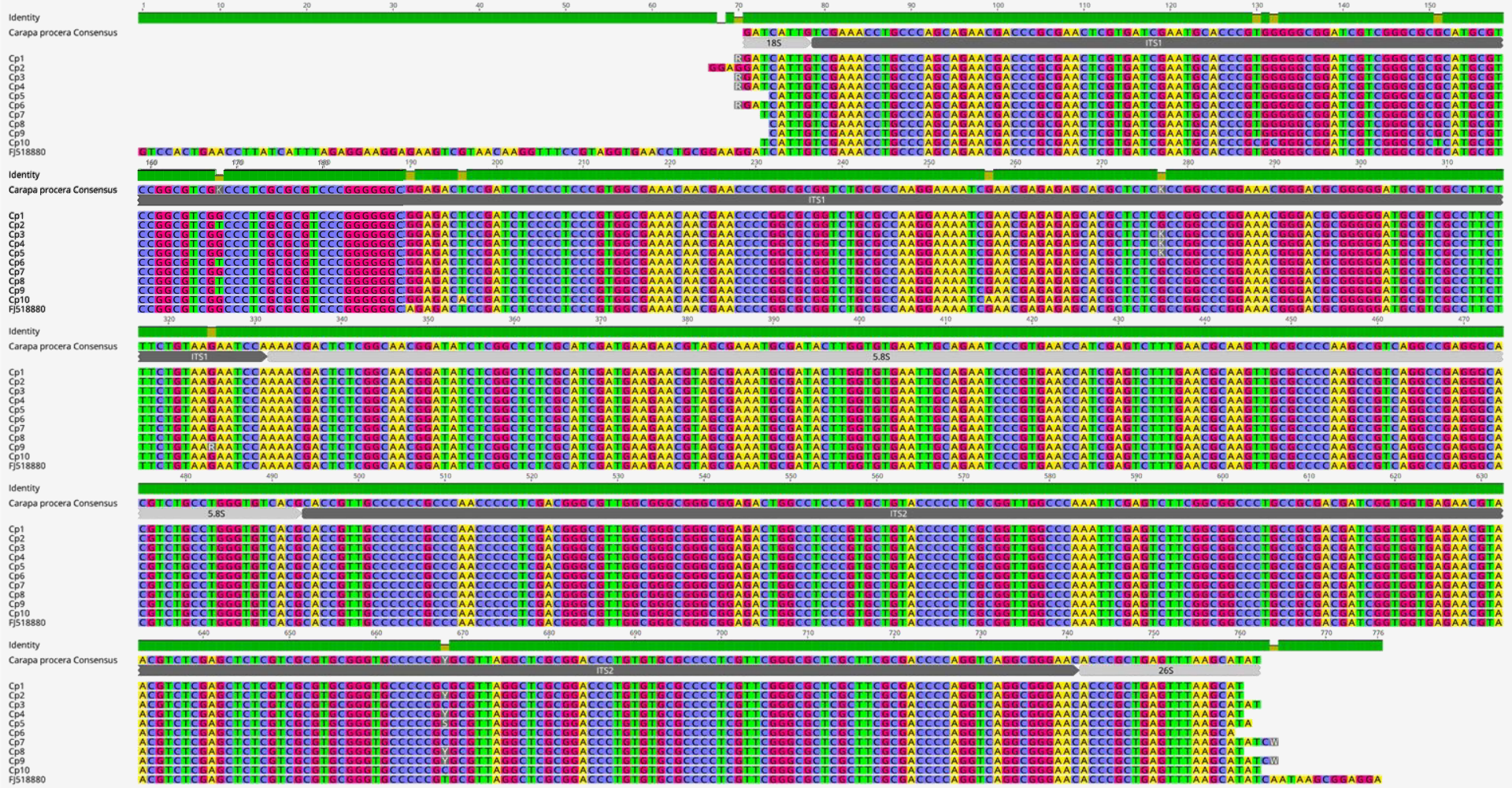
3 Sequencing



199 sequences

[1] Baldwin et al. (1995)
[2] nach CFCF 2016 – Carl Fredrik online: https://upload.wikimedia.org/wikipedia/commons/b/b4/0321_DNA_Macrostructure.jpg

Consensus sequences



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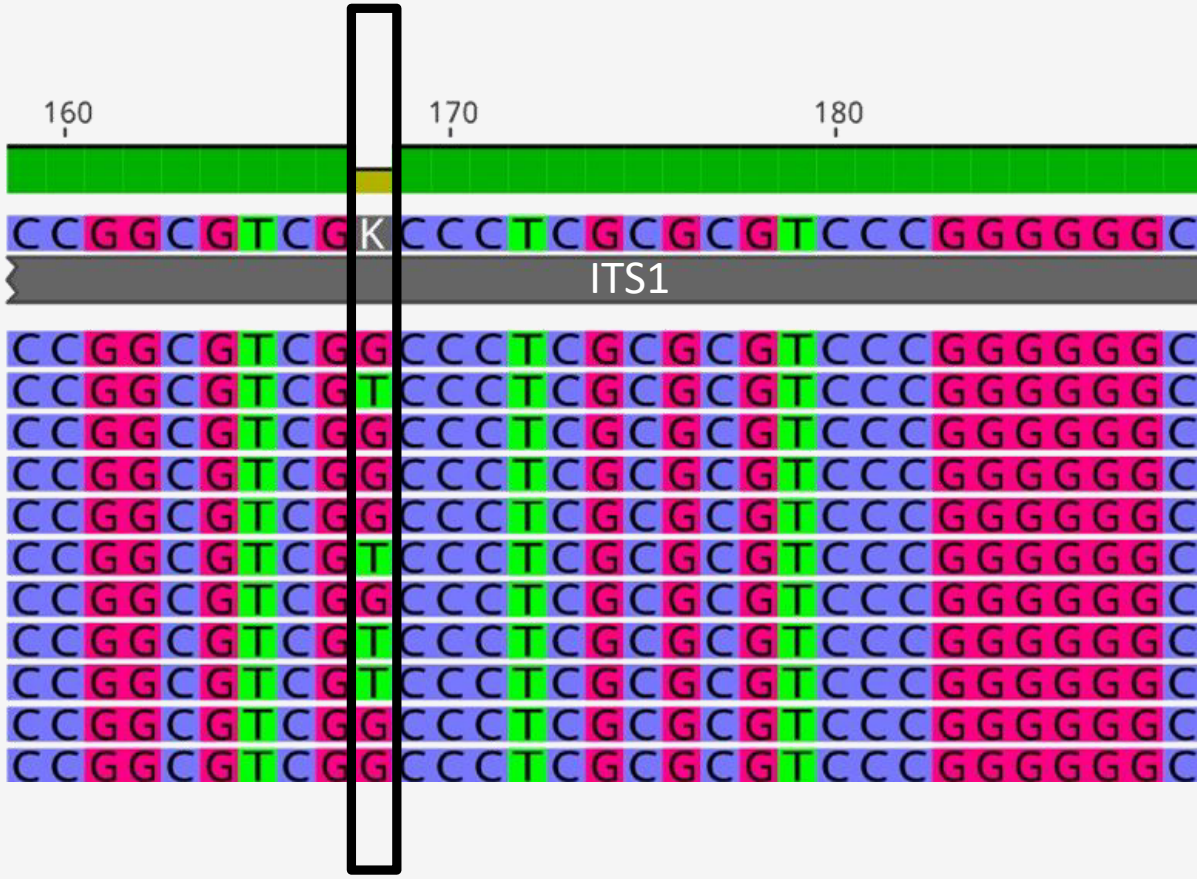
Consensus sequences



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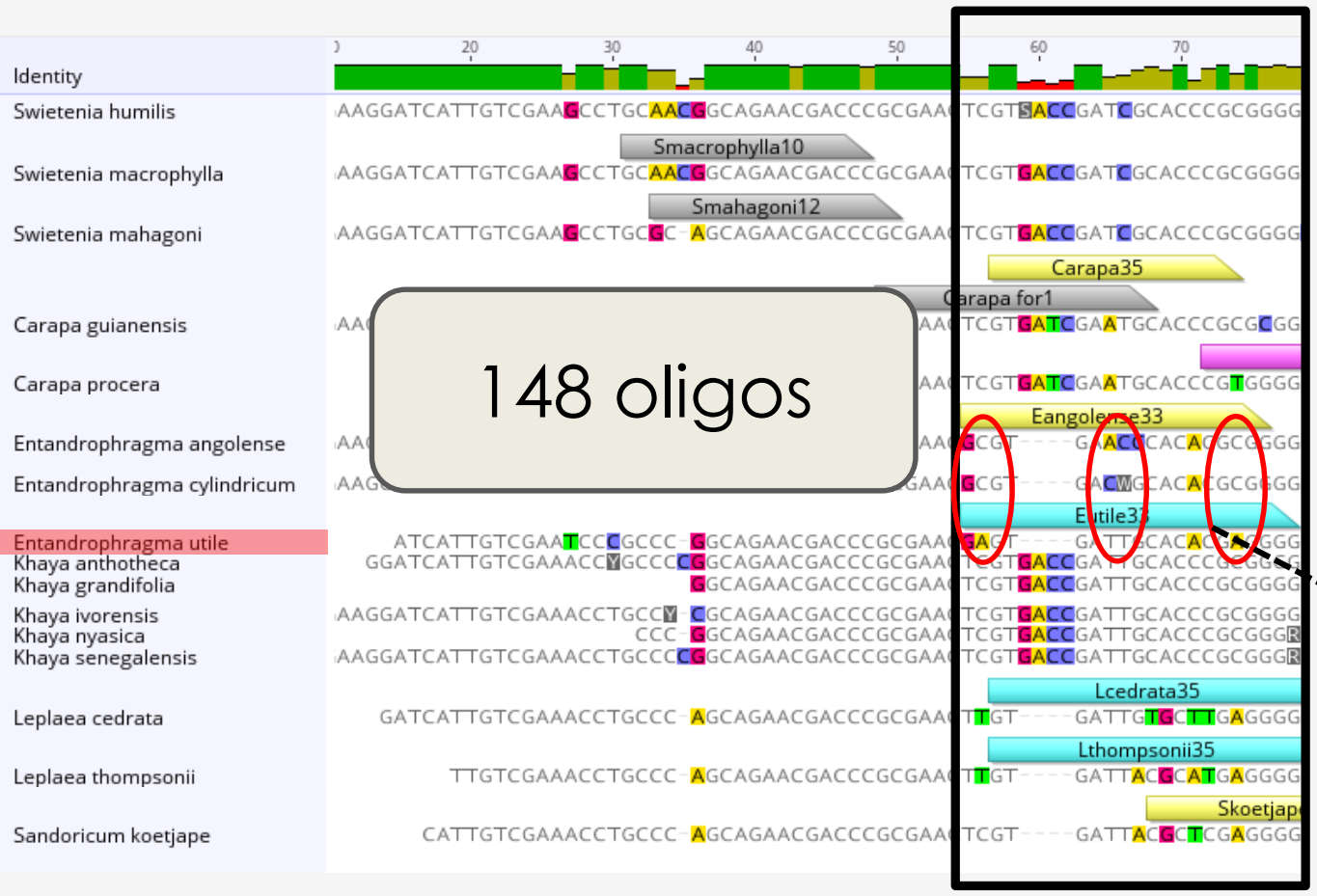
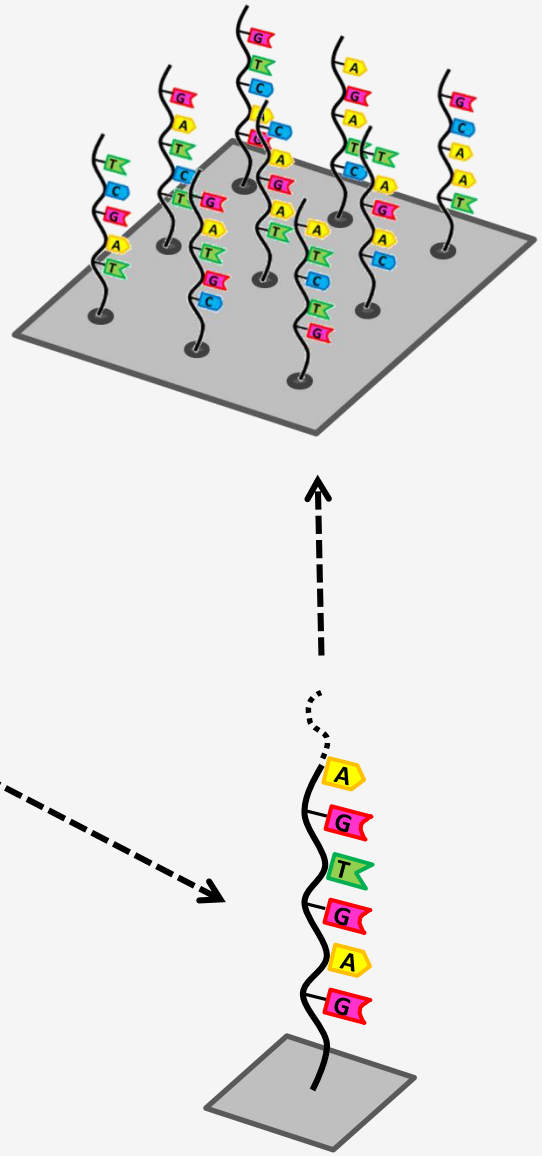
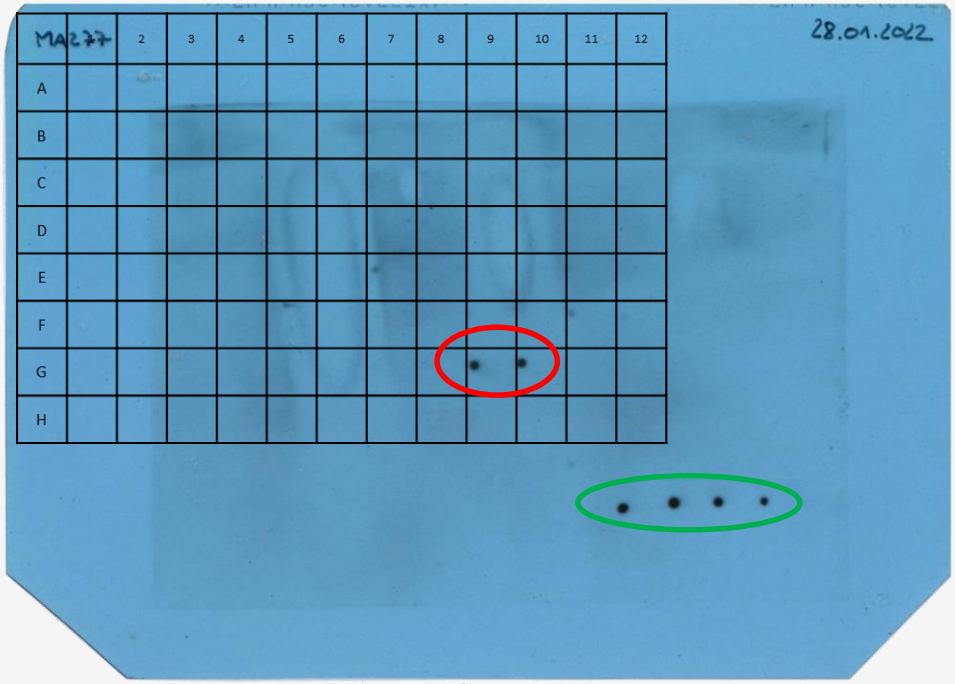
Comparison of sequences





Evaluation of the X-ray films

Scanned X-ray film

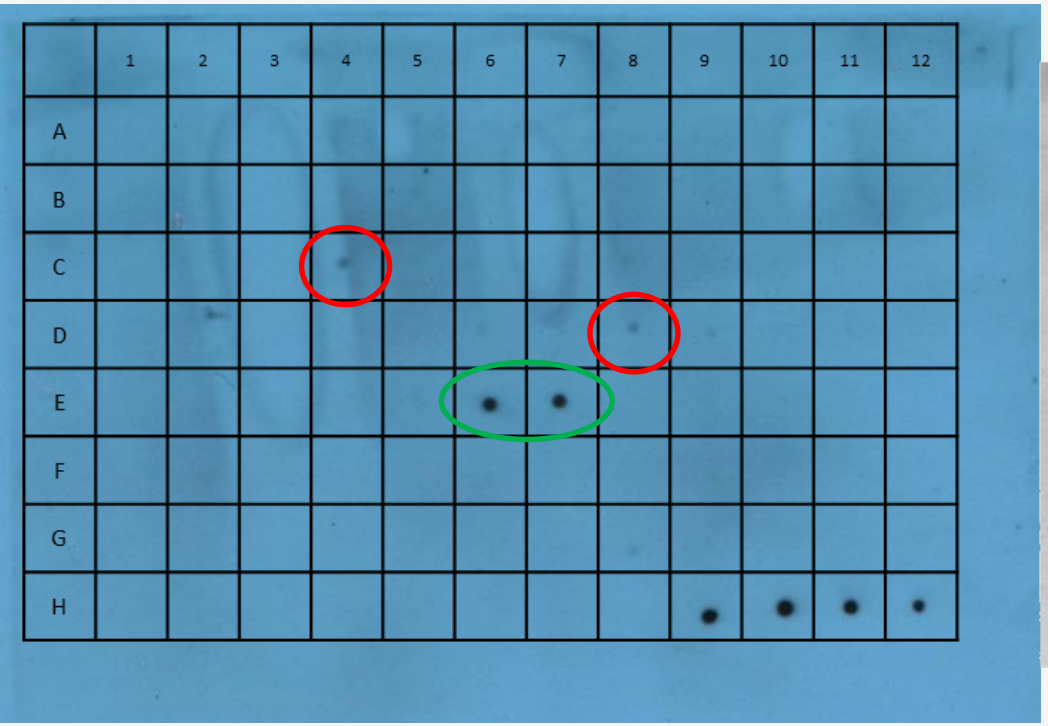


Probe grid

	1	2	3	4	5	6	7	8	9	10	11	12
A	Smahagoni181	Mahagonifor4	Smahagoni649	Smacrophylla112	Macrophylla rev1	Carapafor3	Carapafor2	Carapa rev1	Cprocera50	Ent angofor2	Ent cylfor2	Ecylindricum264
B	Eutile 86	Khayafor2	Khaya rev1	Khaya243	Kivorensis106	Lcedrata35	Lcedrata459	Lthompsonii35	Lthompsonii457	Ced fissilisfor1	Ced odorata for1	Cedrela29
C	Toona70	Gpelle221	Gtess225	Gtess579	Garnoldiana79	Garnoldiana219	Garnoldiana629	Gehie44	Csalikoundа41	Csalikoundа93	Hcourb for1	Hcourb for2
D	Perinaceus615	Psantalinus487	Psantalinus682	Pindicus487	Pindicus597	Pmacrocarpus71	Pindi macro32	Psoyauxii501	Gonystylus48	Gonystylus137	Ramin for1	Gonystylus422
E	Ramin rev1	Alstonia481	Aboonei408	Atoxicaria149	Atoxicaria553	1Balicastrum483	1Balicastrum502	Dpruniformis33	Dpruniformis512	Jcopaia28	Jcopaia510	Jcopaia589
F	Koto for1	Pbequaertii175	Pmacrocarpa26	Limba for1	Limba rev1	Tivorensis111	Tivorensis224	Tsuperba219	Guaiacum for1	Gcoulteri172	Gofficinale172	Gua officifor1
G	Gua sanct for1	Gua sanct rev1	Bulnesia for1	Bulnesia rev1	Parborea34	Chlorocardium111	Ipe for1	Handroanthus211	Ipe rev1			
H									Holz for1	Holz for2	Holz rev1	Holz rev2

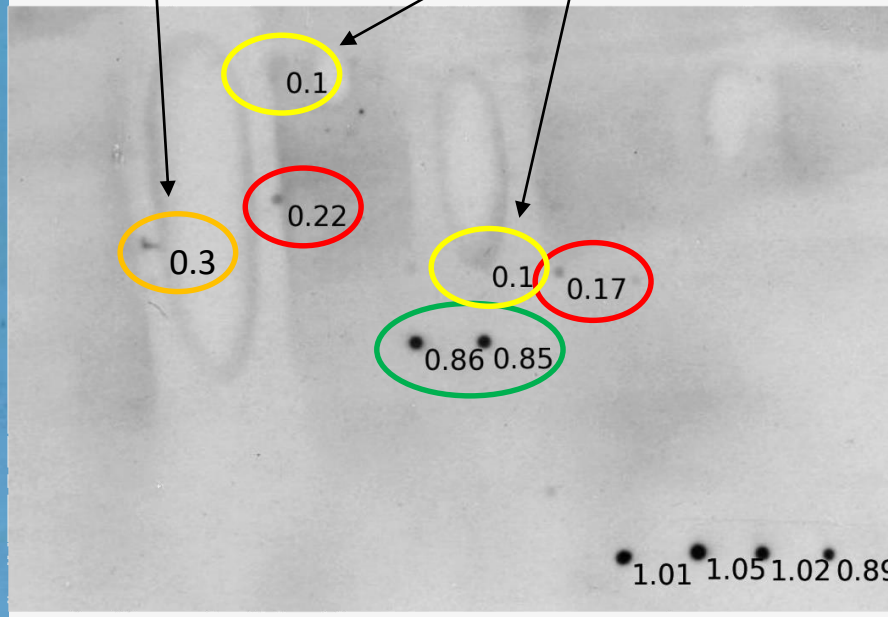
→ *Brosimum alicastrum*

Wood species: *Brosimum alicastrum*



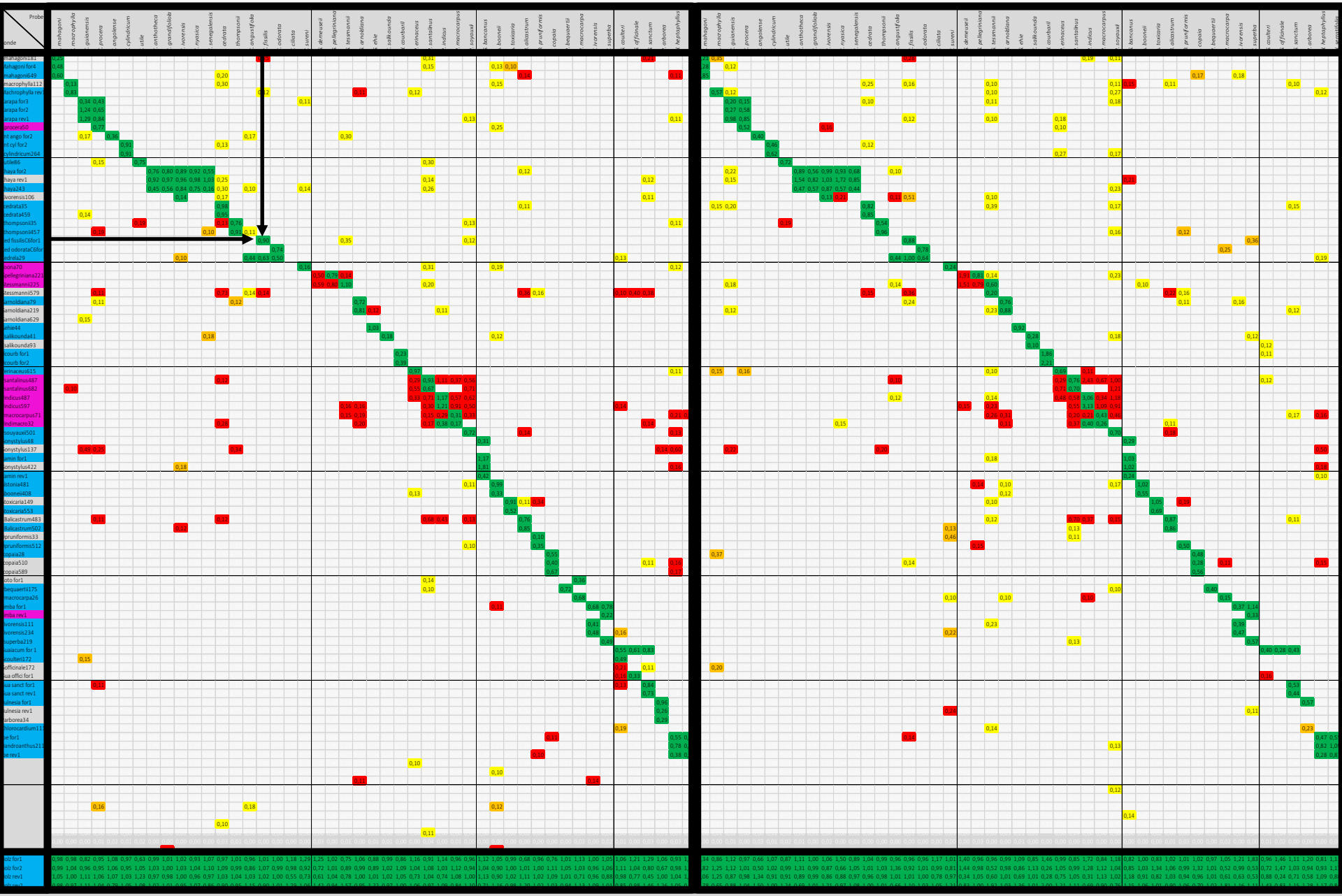
Measurement results from artefacts

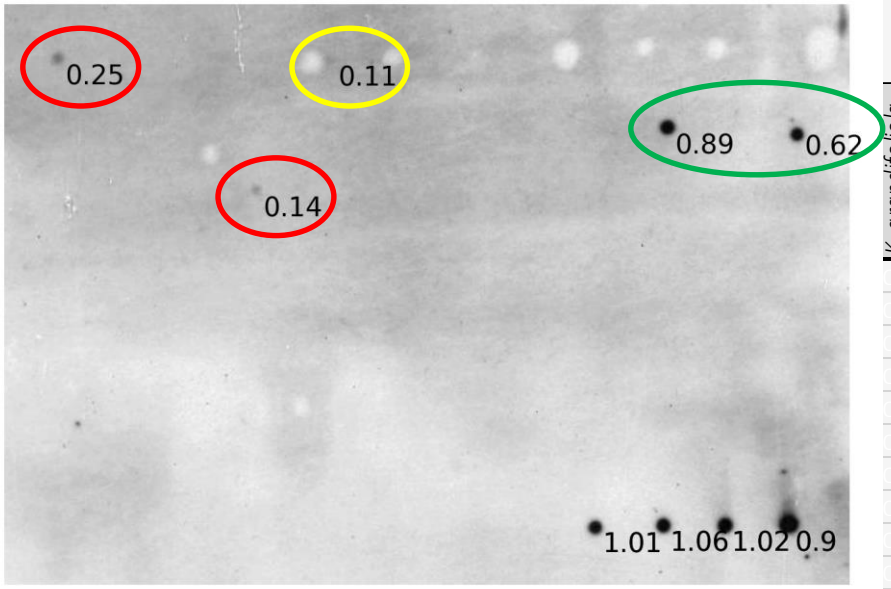
Measurement is caused by unstable background



Threshold value 0.1

→ automated signal evaluation with manual check





	K. grandifoliola	*K. ivorensis*	*K. nyasica*	*K. senegalensis*	*L. cedrata*	*L. thompsonii*	*C. angustifolia*	*C. fissilis*	*C. odorata*	*T. ciliata*	*T. sureni*
Ecylindricum264				0,91							
Eutile86		0,15		0,75							
Khaya for2					0,76 0,80 0,89 0,92 0,55						
Khaya rev1					0,92 0,97 0,96 0,98 1,03	0,25					
Khaya243					0,45 0,56 0,84 0,75 0,16	0,30	0,10				0,14
Kivorensis106				0,14		0,17					
Lcedrata35						0,98					
Lcedrata459	0,14					0,95					
Lthompsonii35				0,19		0,13	0,76				
Lthompsonii457		0,19				0,10	0,91	0,11			
Ced fissilisC6for1								0,90			
Ced odorataC6for1									0,74		
Cedrela29				0,10			0,41	0,63	0,50		
								0,25			
					0,20						
					0,30						
								0,12			
											0,11
							0,17				
					0,13						

Oligos

148 tested → 81 selected → 66 appropriate

Wood species

32 of 53 species identifiable

15 species grouped in 2 to 3 species

Sonde \ Holzart	*G. demeuseii*	*G. pellegriniana*	*G. tessmannii*	*G. arnoldiana*	*G. ehie*
Gpellegriniana221	•	•			
Gtessmannii225	•	•	•		
Garnoldiana79				•	
Gehie44					•

Interpretation of the oligo required

Sonde \ Holzart	*C. guianensis*	*C. procera*
Carapa for3	•	•
Carapa for2	•	•
Carapa rev1	•	•
Cprocera50		•

2 species no DNA

(*Alstonia scholaris, Chlorocardium rodiei*)

4 species no appropriate oligos

(*S. koetjape, D. costulata, E. moluccanum, N. cadamba*)

Goal

Development of a rapid, low-cost, genetic tool to identify CITES timber species without anatomical knowledge

Method

Marco array → time reduction of 6,7h to 3,2h

ITS as barcode region → Establishment of an internal database

Oligo design: sequences comparison → 148 developed → 66 appropriate

32 + 15 wood species identifiable or definable

Result

Identification is possible within one day

Method with low investment and running costs

No anatomical knowledge required

Outlook

Extensive validation with samples from trade necessary
Application in countries of origin

Practicability → new control method

→ Combating the illegal timber trade + Compliance with species protection

Thank you