Plumeria DNA: What's related to what?

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Plumerias

- 7-8 *Plumeria* spp., subspecies (Woodson, 1938)
- Native to New World Tropics
- Variety of colors, scents, sizes

Plumeria in Hawaii

In Hawaii, Plumeria cultivars contribute to the floriculture, landscape, and tourist industries.

Many of the cultivars suffer from pests & diseases, thereby diminishing flower yields and rendering plants unmarketable or unfit for export.

Thus, a Plumeria breeding program will help to overcome these problems, while simultaneously creating novel Plumeria cultivars for floriculture industry & “Plumies”.

Genetic Relationships

Information from molecular studies can allow us to:

- Develop better breeding (or understanding)
- Introduce horticulturally important traits from *Plumeria* spp. to CVs

STUDIES IN THE APOCYNACEAE. VIII

AN EVALUATION OF THE GENERA PLUMERIA L. AND HIMATANTHUS WILLD.

ROBERT E. WOODSON, JR. *Research Assistant, Missouri Botanical Garden, Assistant Professor in the Henry Shaw School of Botany of Washington University*

Annals Missouri Botanical Garden Vol. 25:189-224, 1938

1. *Plumeria inodora*
2. *Plumeria pudica*
3. *Plumeria rubra* (and forms)
4. *Plumeria subsessilis*
5. *Plumeria obtusa* (and varieties)
6. *Plumeria filifolia*
7. *Plumeria alba*

Criley, 2006

4. PLUMERIA OBTUSA L. Sp. Pl. ed. 1. **1**: 210. 1753; A. DC. in DC. Prodr. **8**: 392. 1844; Britton, Bull. Torrey Bot. Club. **42**: 505. 1915.

var. **typica.**

Plumeria Tenorii Gasp. Oss. Piant. Ort. Boccad. p. 20. 1833; A. DC. in DC. Prodr. **8**: 391. 1844.

Plumieria obtusa L. β. *parviflora* Griseb. Mem. Amer. Acad. II. **8**: 519. 1862.

Plumieria obtusa L. γ. *laevis* Griseb. Mem. Amer. Acad. II. **8**: 519. 1862.

Plumeria clusioides Griseb. Cat. Pl. Cub. 171. 1866; Britton, Bull. Torrey Bot. Club **42**: 504. 1915.

Plumieria emarginata Griseb. Cat. Pl. Cub. 171. 1866; Britton, Bull. Torrey Bot. Club **42**: 505. 1915.

Plumieria clusioides Griseb. var. *parviflora* Maza, Ann. Soc. Esp. Hist. Nat. **23**: 273. 1895.

Plumieria Krugii Urb. Symb. Ant. **1**: 387. 1900.

Plumieria bahamensis Urb. Symb. Ant. **1**: 387. 1900.

Plumieria portoricensis Urb. Symb. Ant. **1**: 387. 1900.

Plumieria Marchii Urb. Symb. Ant. **3**: 334. 1902.

Plumiera inaguensis Britton, Bull. N. Y. Bot. Gard. **3**: 448.

Morphological traits

1. Leaf shape
2. Leaf tip
3. Leaf margins & veination
4. Flower shape
5. Flower petal tip
6. Flower petal orientation
7. Etc. …

Some problems with morphology…

Himatanthus sp.

Himatanthus odorata
Photo by Luc Vannoorbeeck

Himatanthus phagedaenicus

Himatanthus seed pod

Criley, 2006

Morphological Variation on a plant

Criley, 2006

- 3 leaves that came from the same tree

Morphological variation of seedlings

Criley, 2006

Plumeria stenopetala

- Variation in leaf shapes
- Variation in leaf size
- Variation in venation pattern

Same species?

Criley, 2006

P. subsessilis

Criley, 2006

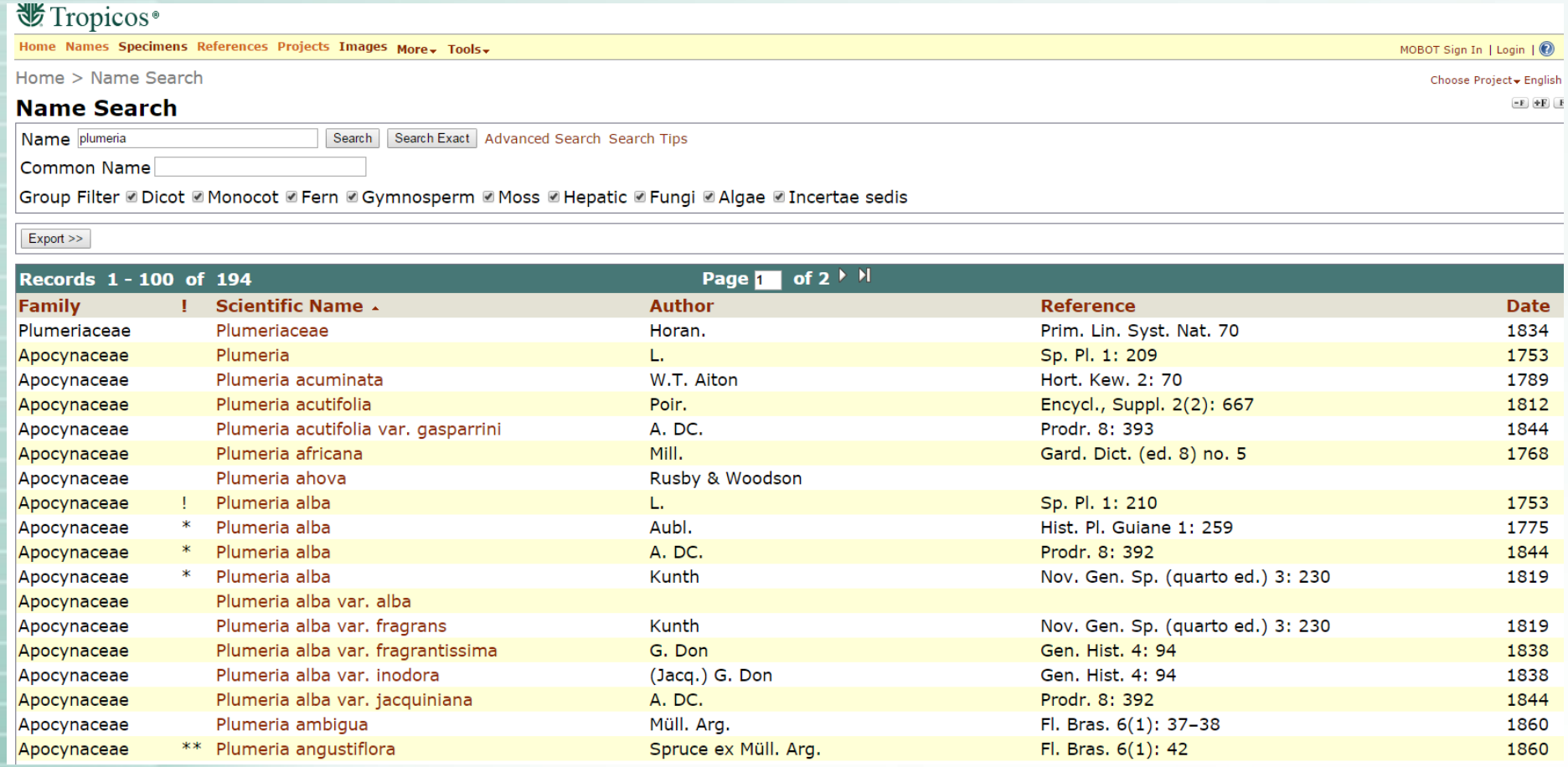
Plumeria sp. ‘Isabella’

Herbarium specimens

Someone called this *Plumeria obtusa*.

http://sweetgum.nybg.org/science/vh/specimen_details.php?irn=1207994

http://www.tropicos.org/



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Name Search

Name plumeria [Search] [Search Exact] Advanced Search Search Tips

Common Name

Group Filter ☑ Dicot ☑ Monocot ☑ Fern ☑ Gymnosperm ☑ Moss ☑ Hepatic ☑ Fungi ☑ Algae ☑ Incertae sedis

[Export >>]

Records 1 - 100 of 194 — Page 1 of 2

Family	!	Scientific Name	Author	Reference	Date
Plumeriaceae		Plumeriaceae	Horan.	Prim. Lin. Syst. Nat. 70	1834
Apocynaceae		Plumeria	L.	Sp. Pl. 1: 209	1753
Apocynaceae		Plumeria acuminata	W.T. Aiton	Hort. Kew. 2: 70	1789
Apocynaceae		Plumeria acutifolia	Poir.	Encycl., Suppl. 2(2): 667	1812
Apocynaceae		Plumeria acutifolia var. gasparrini	A. DC.	Prodr. 8: 393	1844
Apocynaceae		Plumeria africana	Mill.	Gard. Dict. (ed. 8) no. 5	1768
Apocynaceae		Plumeria ahova	Rusby & Woodson		
Apocynaceae	!	Plumeria alba	L.	Sp. Pl. 1: 210	1753
Apocynaceae	*	Plumeria alba	Aubl.	Hist. Pl. Guiane 1: 259	1775
Apocynaceae	*	Plumeria alba	A. DC.	Prodr. 8: 392	1844
Apocynaceae	*	Plumeria alba	Kunth	Nov. Gen. Sp. (quarto ed.) 3: 230	1819
Apocynaceae		Plumeria alba var. alba			
Apocynaceae		Plumeria alba var. fragrans	Kunth	Nov. Gen. Sp. (quarto ed.) 3: 230	1819
Apocynaceae		Plumeria alba var. fragrantissima	G. Don	Gen. Hist. 4: 94	1838
Apocynaceae		Plumeria alba var. inodora	(Jacq.) G. Don	Gen. Hist. 4: 94	1838
Apocynaceae		Plumeria alba var. jacquiniana	A. DC.	Prodr. 8: 392	1844
Apocynaceae		Plumeria ambigua	Müll. Arg.	Fl. Bras. 6(1): 37–38	1860
Apocynaceae	**	Plumeria angustiflora	Spruce ex Müll. Arg.	Fl. Bras. 6(1): 42	1860

! = Legitimate name, *=Illegitimate, **=Invalid

What about DNA???

- Recent studies have employed the use of intergenic spacer (IGS) regions to distinguish species and assess genetic relatedness

Objectives

Identify IGS regions that distinguish Plumeria species and assess genetic relationships.

“Who’s who & What’s related to what?”

Phase I: Screening for potential use

Phase II: Add more samples (taxa)

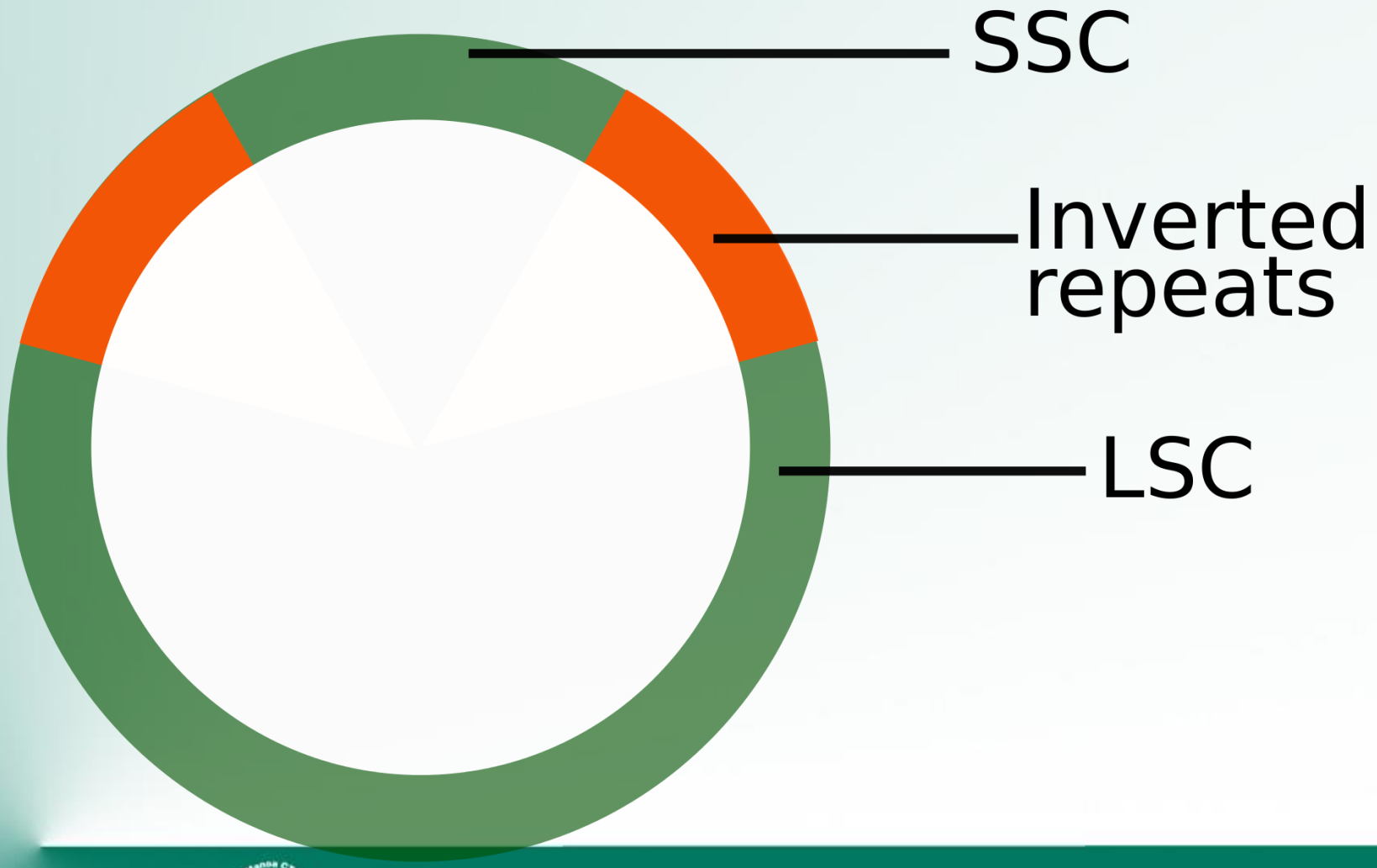
Phase III: Add morphological traits

Background

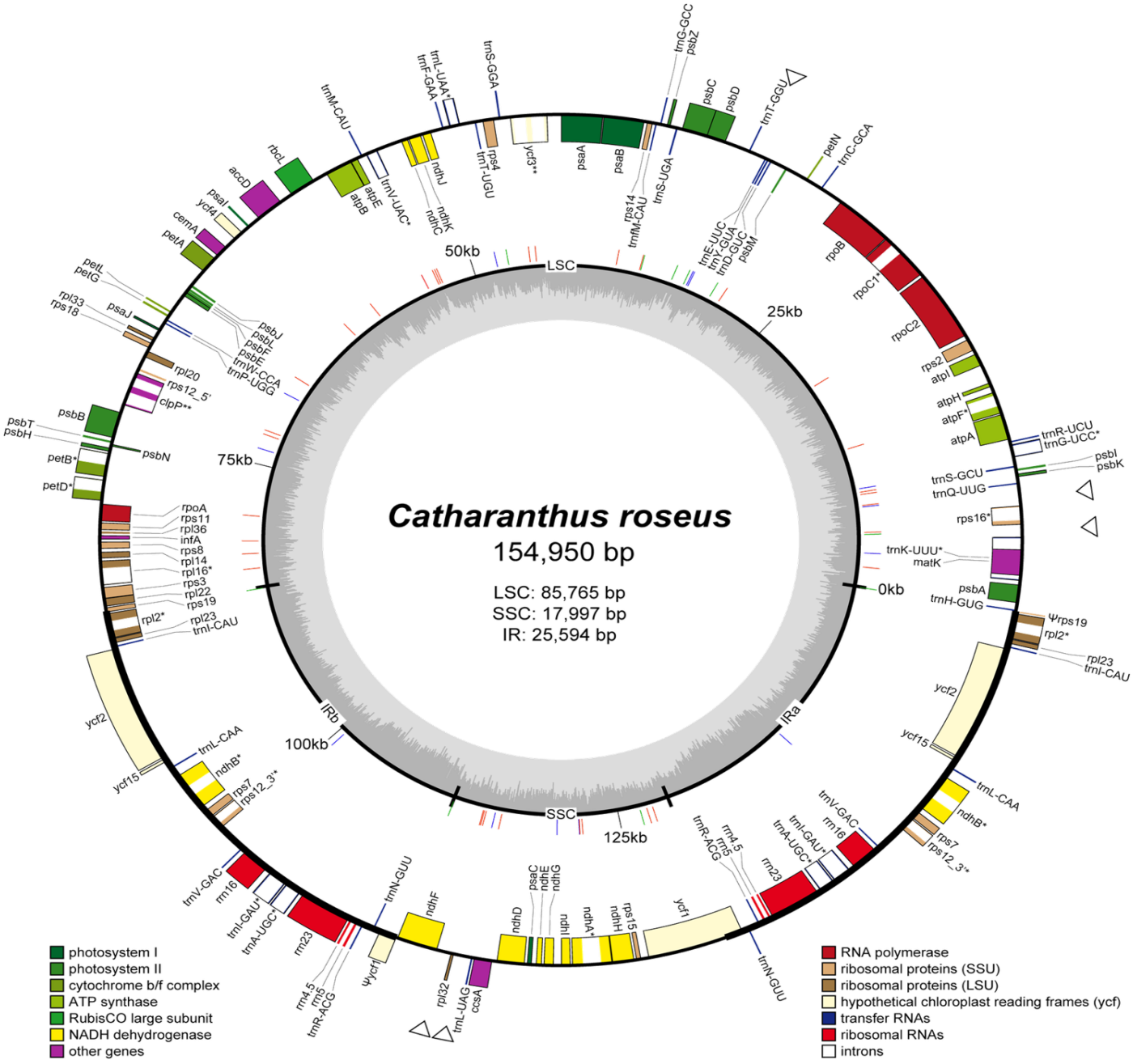
Plants have 3 genomes

1. Nuclear
2. Mitochondrial
3. **Chloroplast**

General Structure of Chloroplast Genome



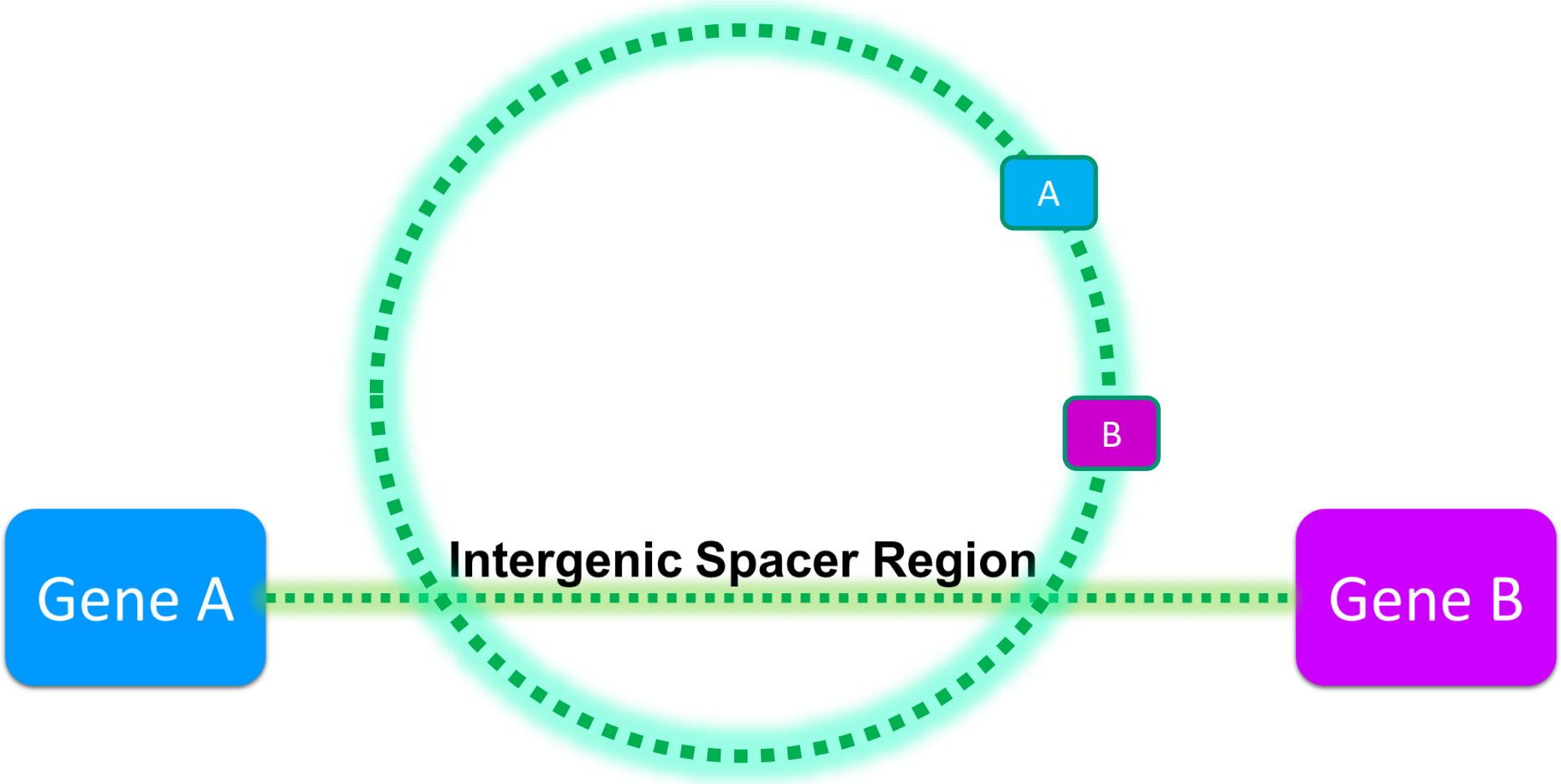
https://sk.wikipedia.org/wiki/Plastidov%C3%A1_DNA


Catharanthus roseus
154,950 bp
LSC: 85,765 bp
SSC: 17,997 bp
IR: 25,594 bp
LSC
IRb
SSC
IRa
0kb
25kb
50kb
75kb
100kb
125kb
photosystem I
photosystem II
cytochrome b/f complex
ATP synthase
RubisCO large subunit
NADH dehydrogenase
other genes
RNA polymerase
ribosomal proteins (SSU)
ribosomal proteins (LSU)
hypothetical chloroplast reading frames (ycf)
transfer RNAs
ribosomal RNAs
introns

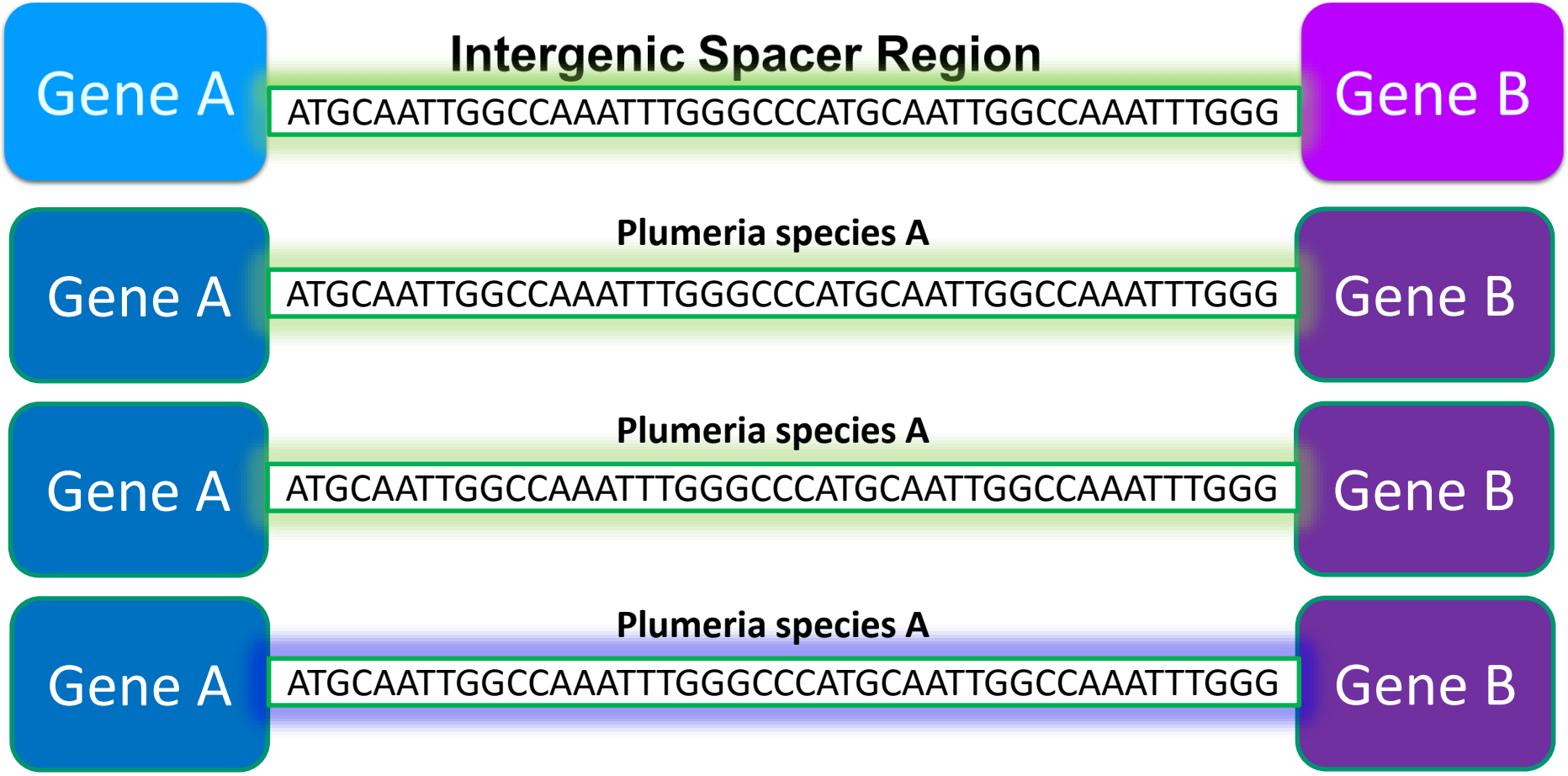
Ku et al., 2013

Intergenic Spacer Regions

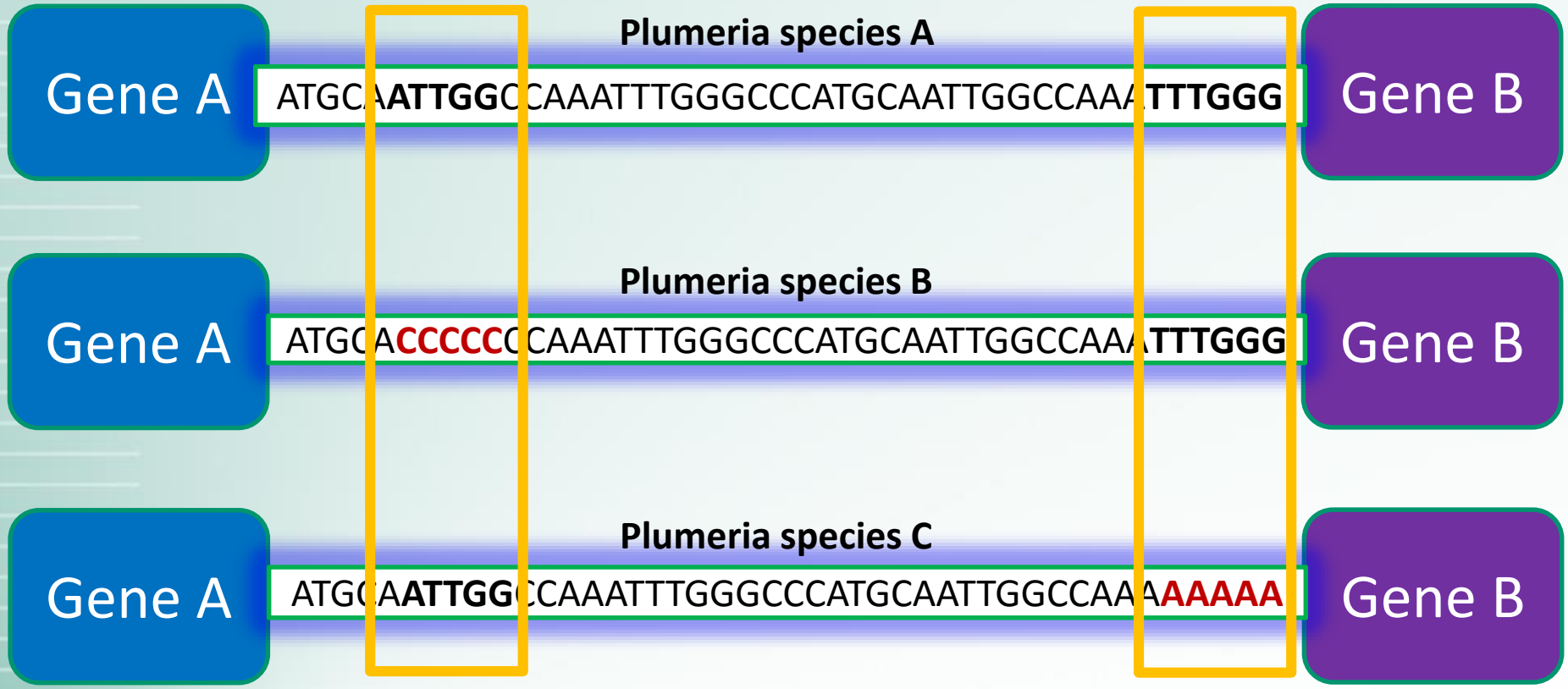
Regions that fall between genes



Comparing **same** species



Comparing **different** species

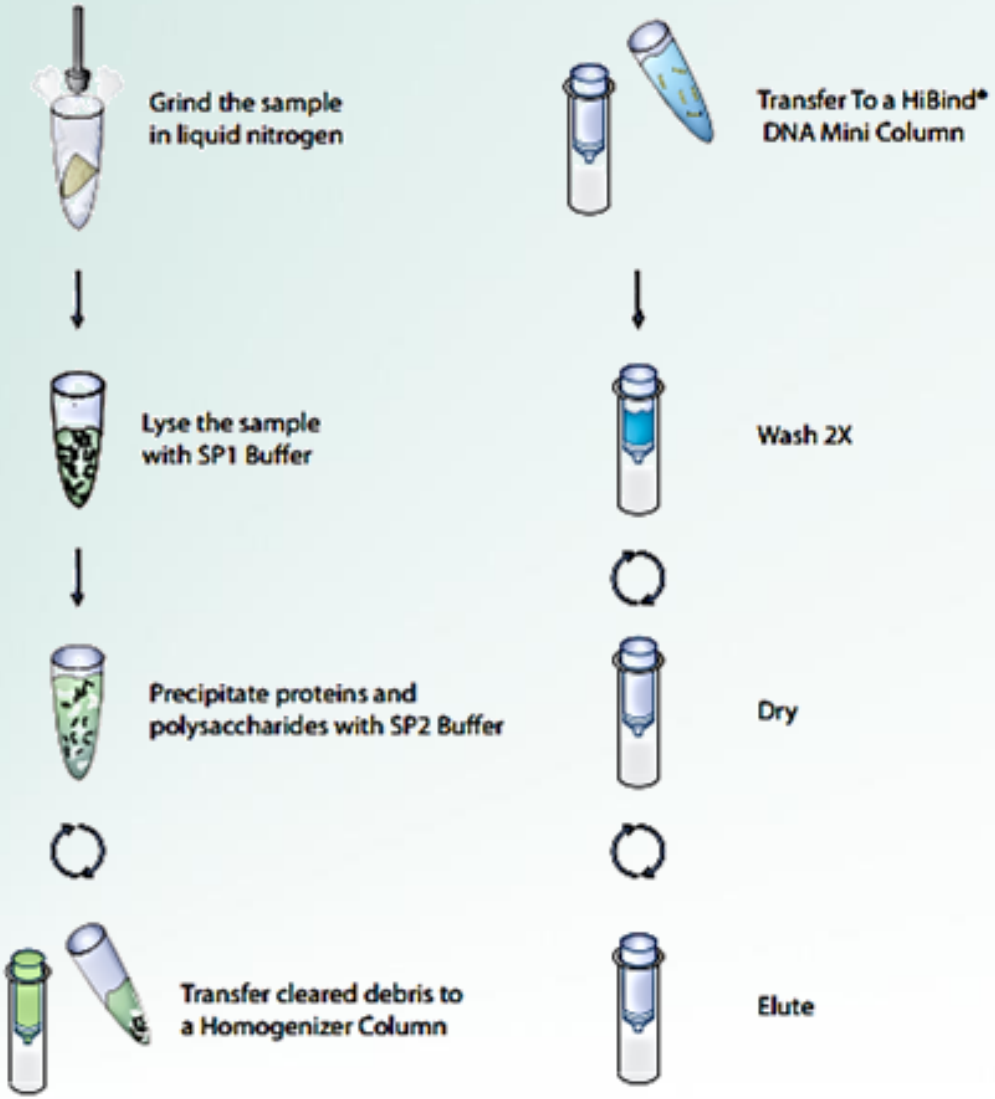


Mononucleotide repeats that distinguish a species

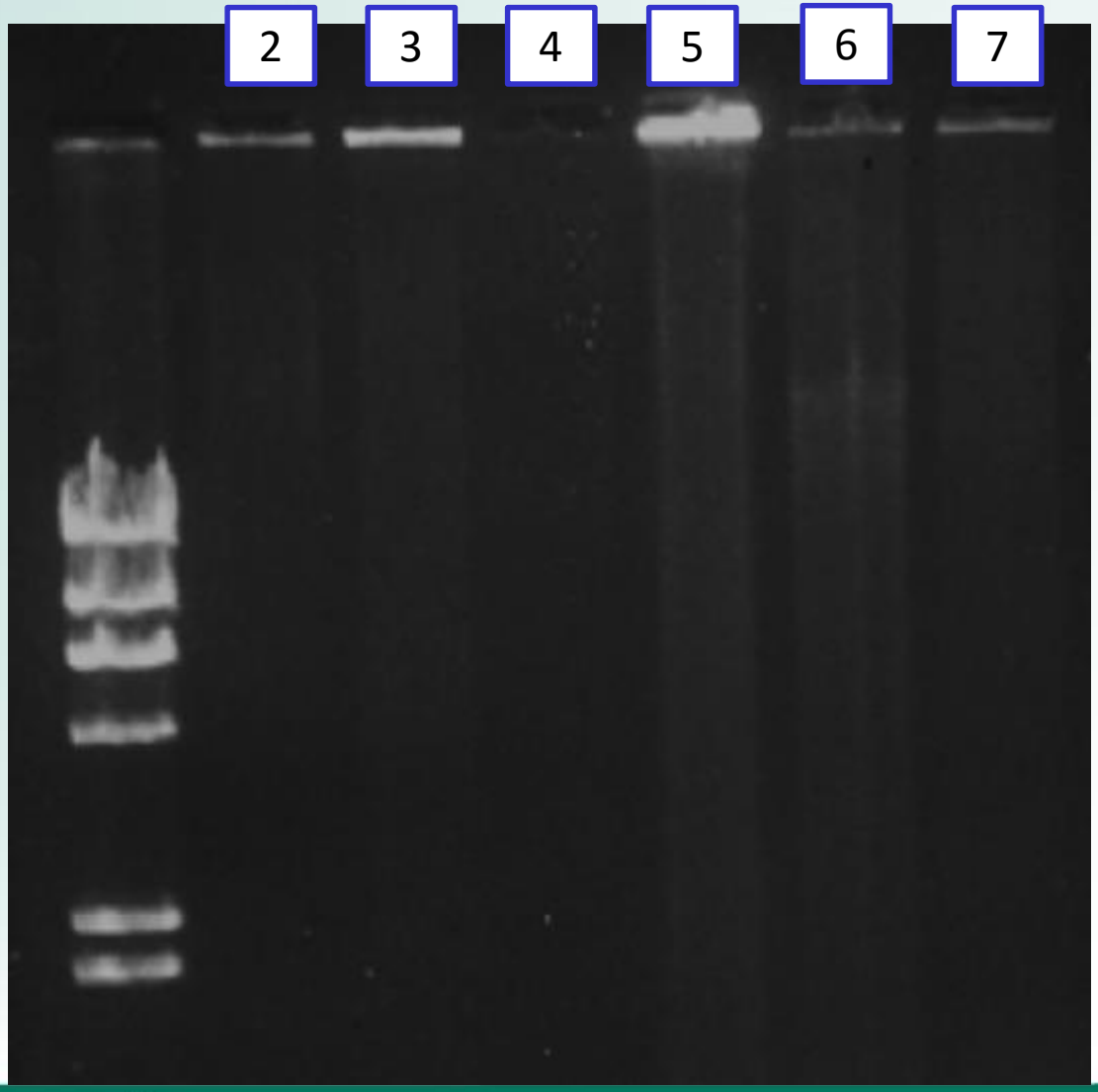
DNA Analysis in Brief

- Collect (young) leaf samples
- DNA Extraction (Qiagen, Omega Biotek, Bioline, Macherey-Nagel, CTAB)
- PCR – Multiple published protocols, multiple trials
  - Primers for intergenic spacer regions (provided by Dr. MJ Gauthier)
  - Ascertain PCR product on gel (electrophoresis)
  - Purification – Kits and ExoSAP-IT
- Sequencing
- Analyses (Free Software)
  - **Chromas Lite**—Scan for accuracy of bases
  - **MEGA** – Genetic analyses
    - Analyze DNA sequences
    - Visualize relationships (Trees)

DNA Extraction



Quality Control of Extracted DNA

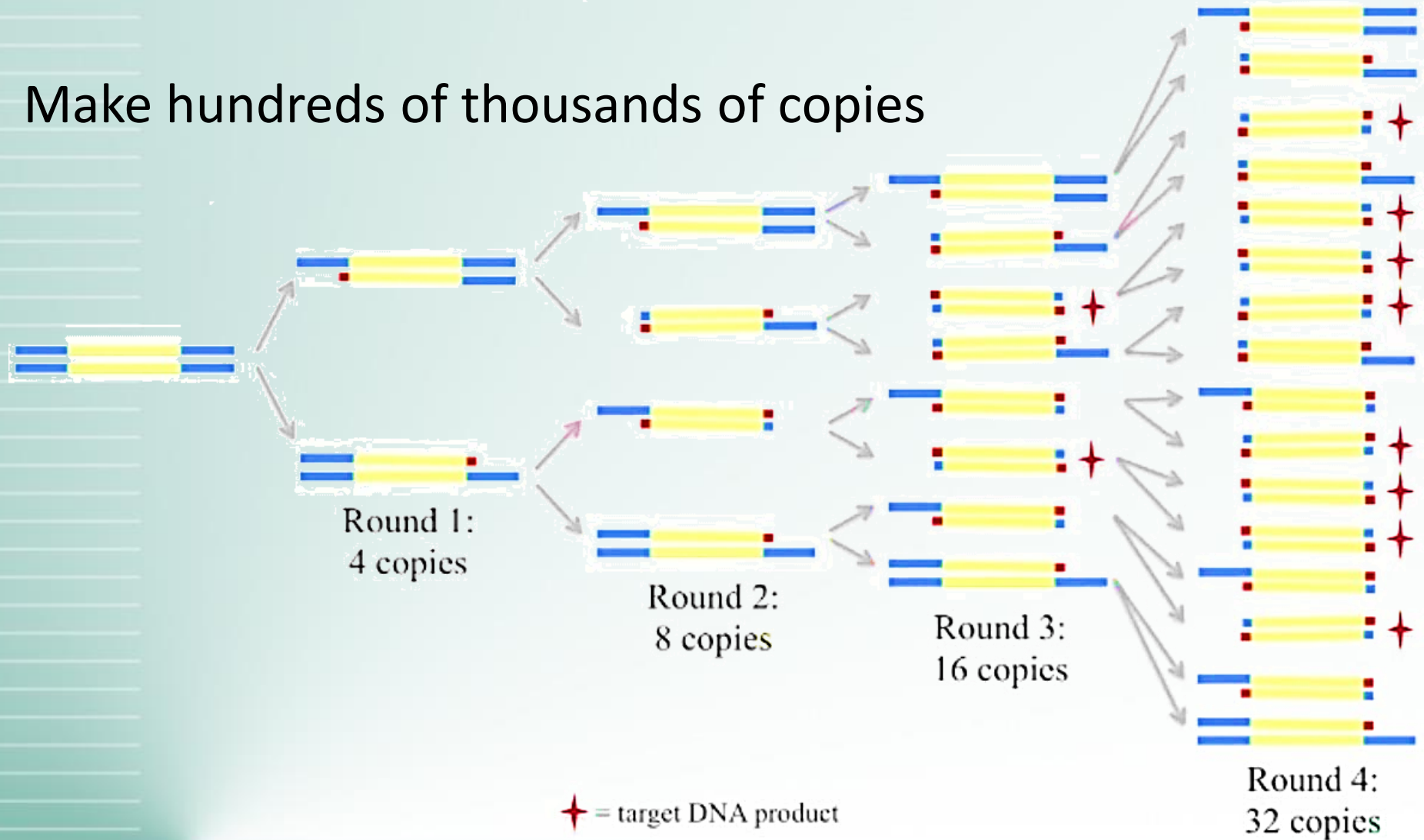


PCR

https://www.flickr.com/photos/snre/6946913449

Polymerase Chain Reaction (PCR)

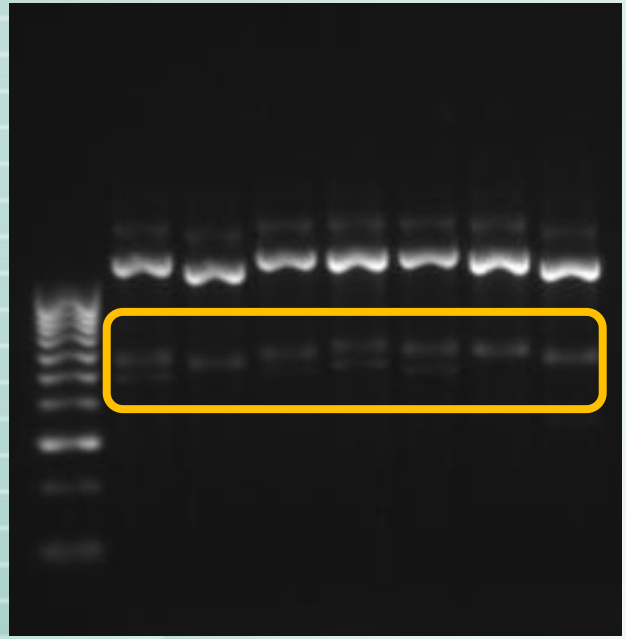
Make hundreds of thousands of copies



https://i.ytimg.com/vi/ZZ-XUG46VDI/maxresdefault.jpg

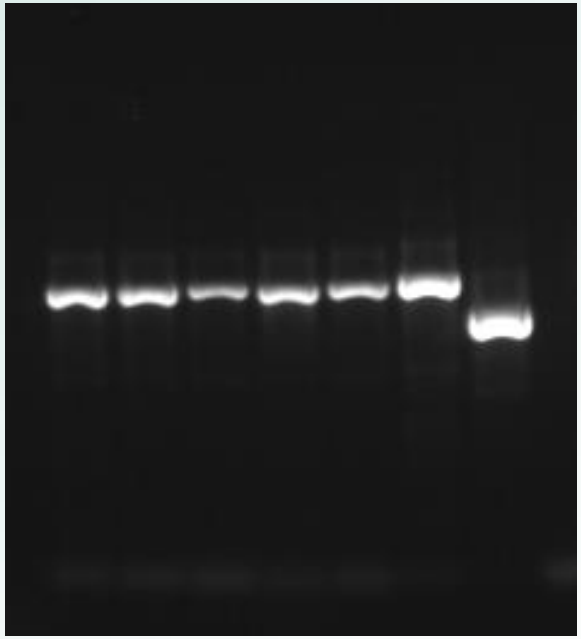
Primer Optimization

Not Good



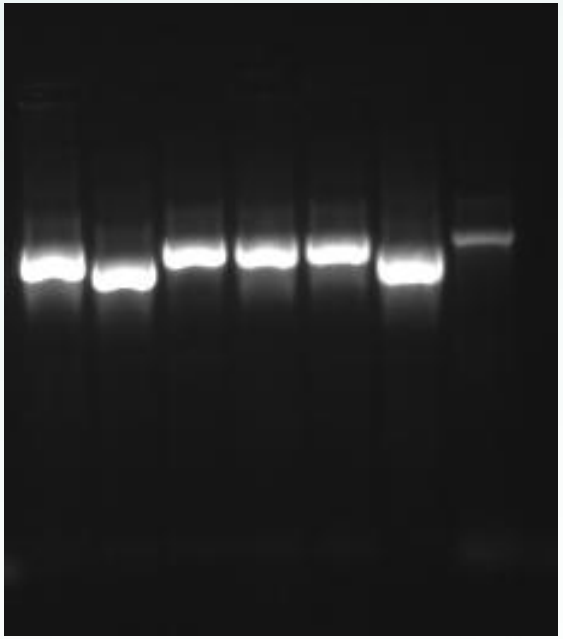
Secondary Banding

Better



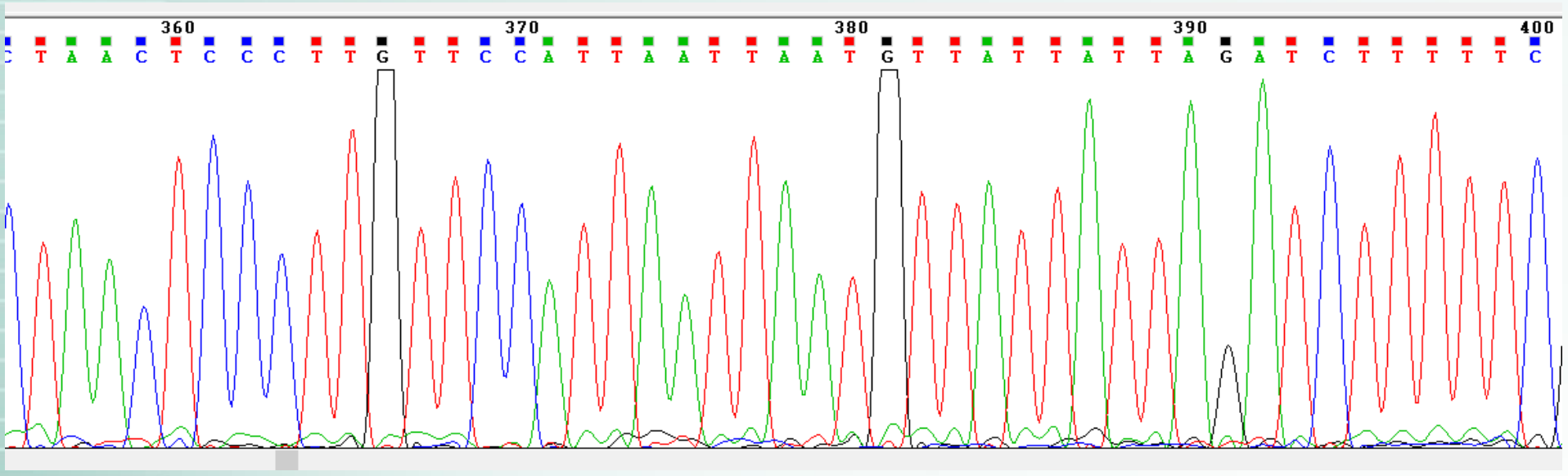
Some Secondary Banding

Good



Bright Banding

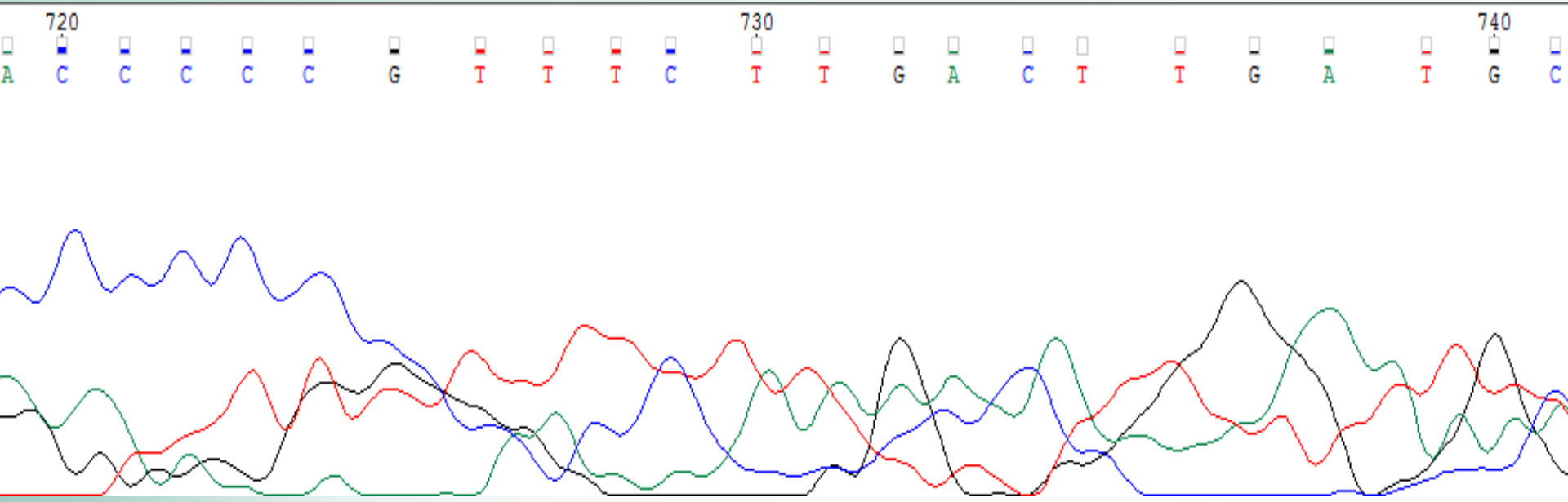
Good day in the lab…



A-Adenine, T-Thymine, C-Cytosine, G-Guanine

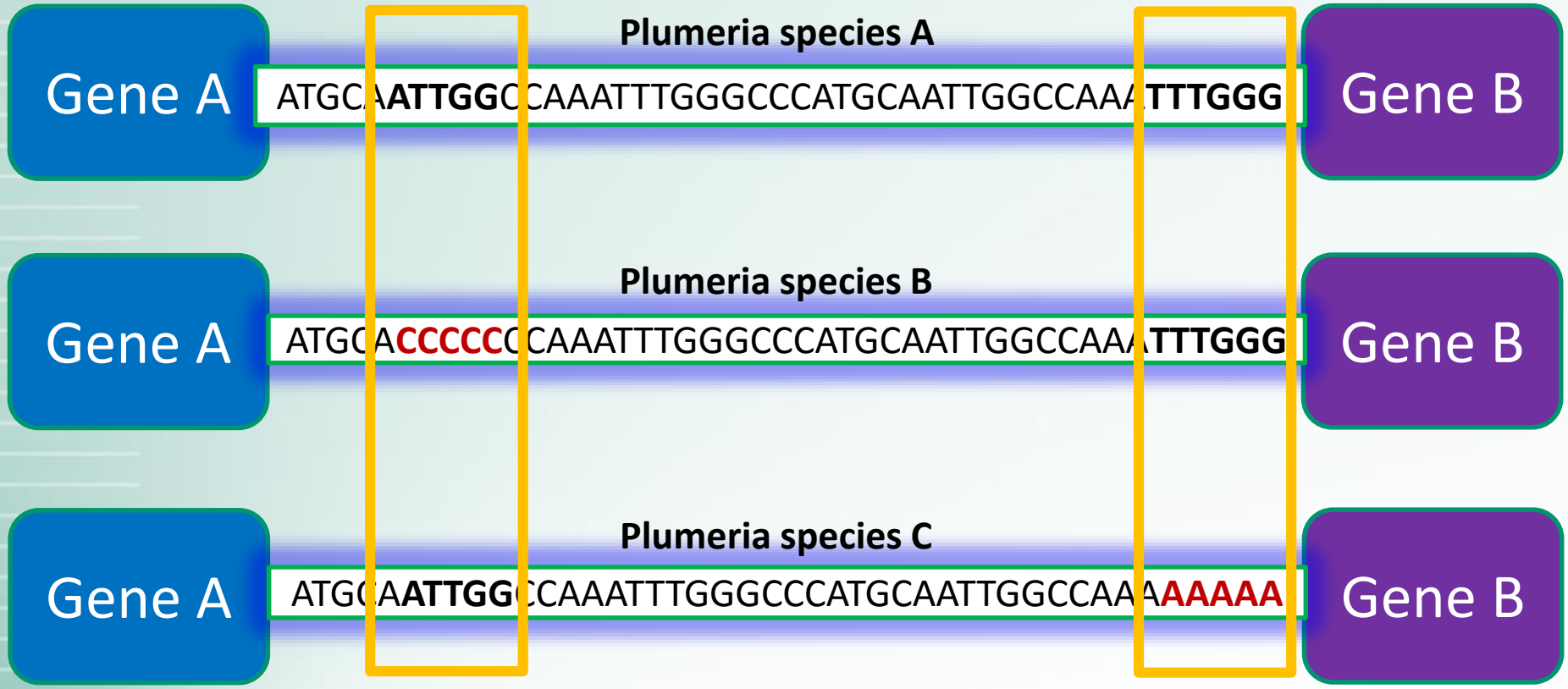
The combinations of A, T, G, and C make up a DNA sequence

Cruddy reads = BAD DAY in the lab

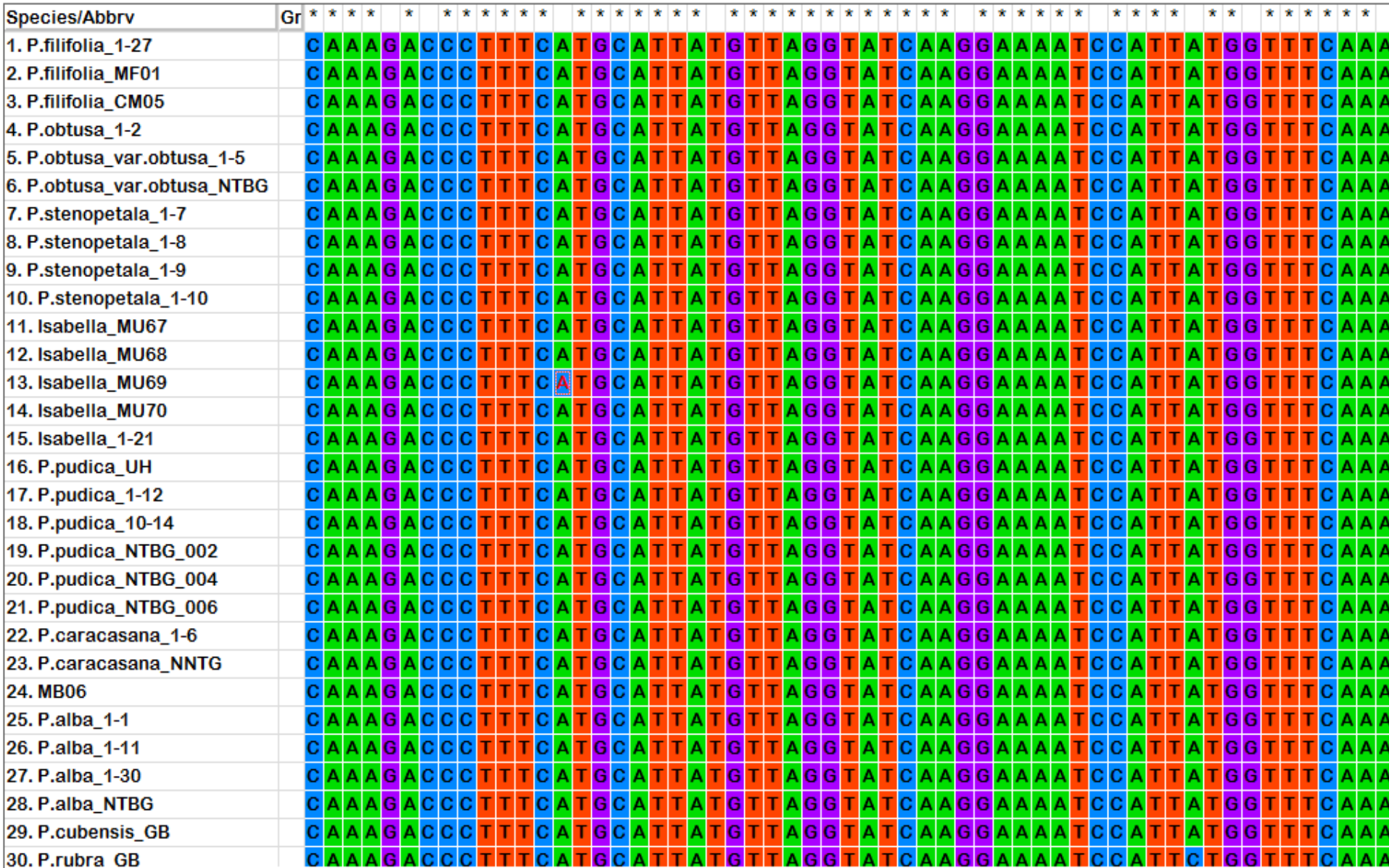


How I feel when I send samples in for sequencing…

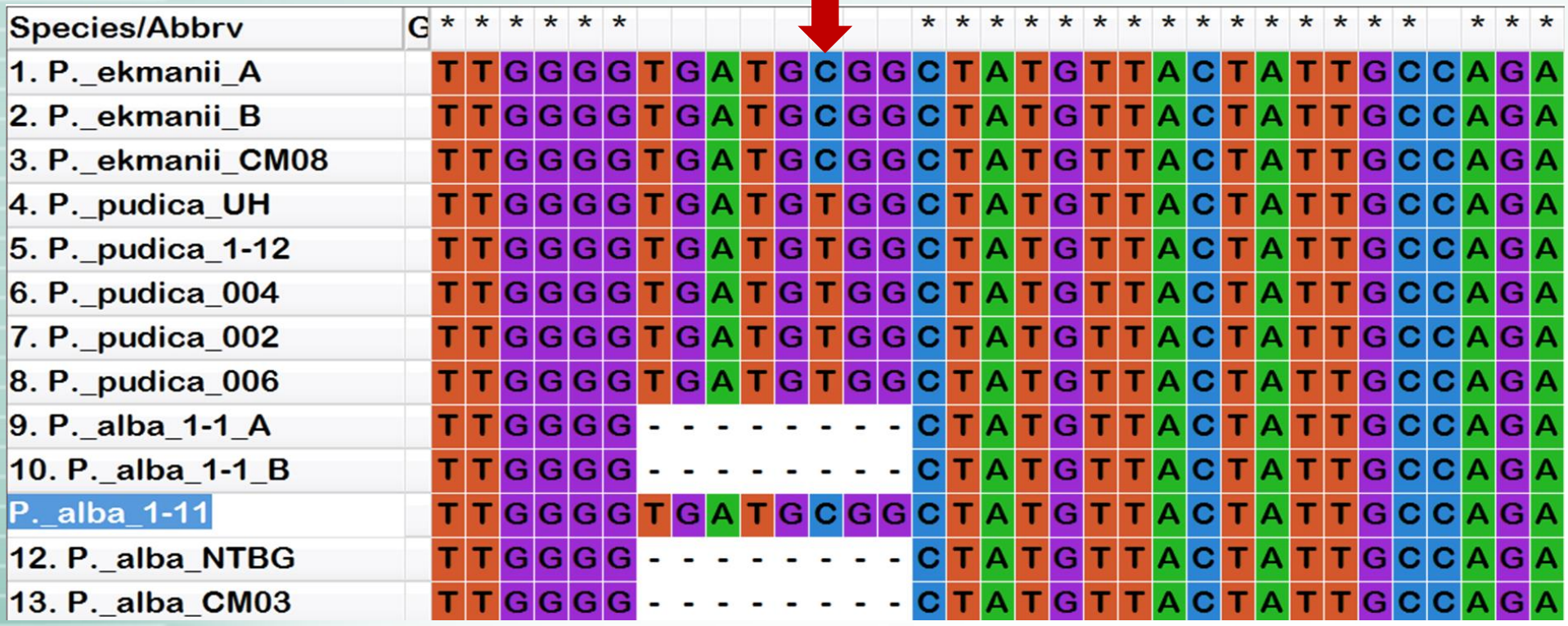
Comparing **different** species



Is this region informative?



How about this one?



A-Adenine, T-Thymine, C-Cytosine, G-Guanine

5 Morphologically Distinct Taxa

Plumeria ekmanii

Plumeria caracasana

Plumeria tuberculata

Plumeria pudica

Plumeria alba

Phase I:

6 Chloroplast Intergenic Spacer (IGS) Regions:

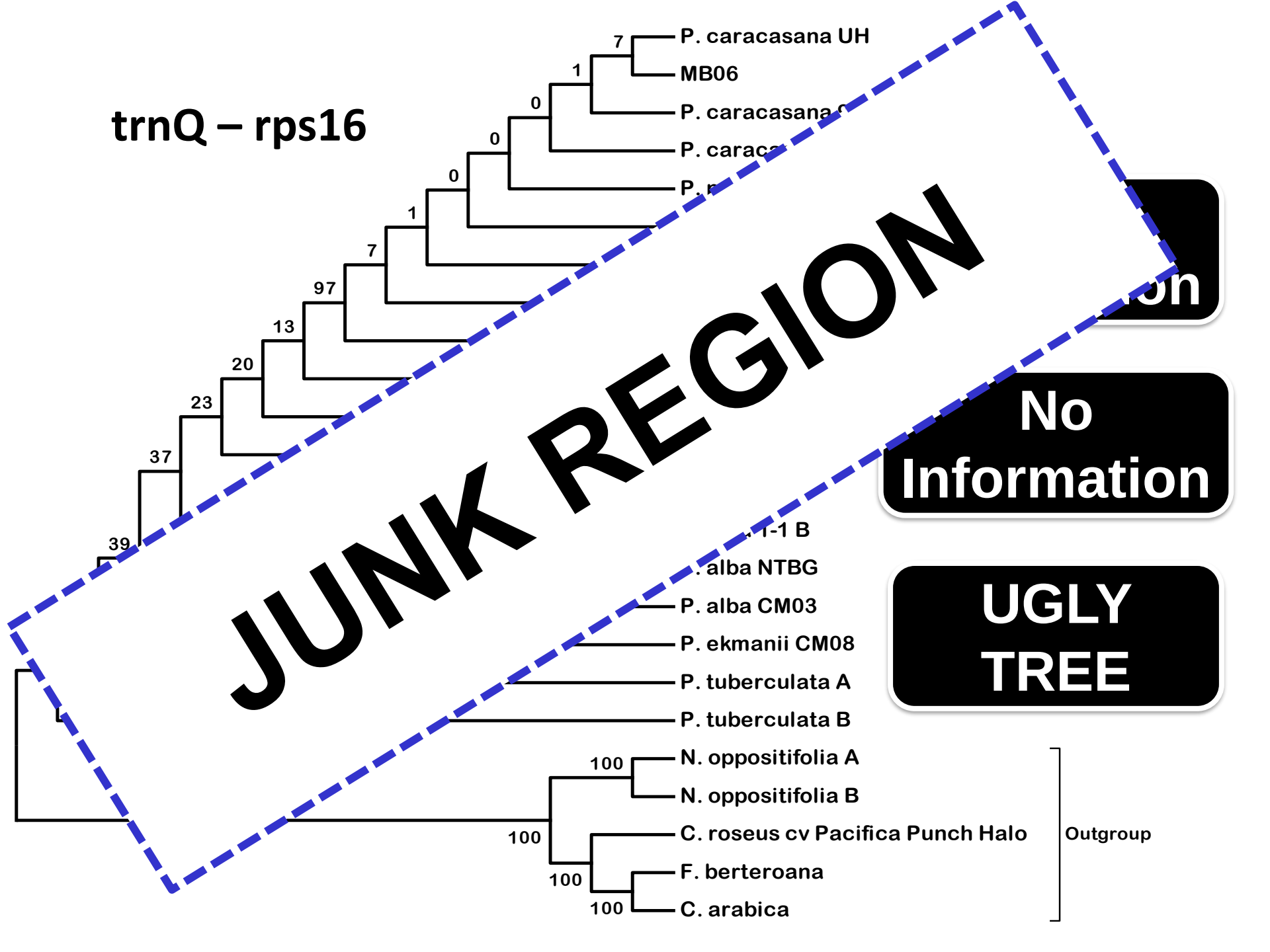
- psbJ-petA
- rpl32-trnL
- ndhF-rpl32
- psbD-trnT
- trnQ-rps16
- trnV-ndhC

QUESTION: Which region(s) can identify "distinct" species?

Lines of Evidence

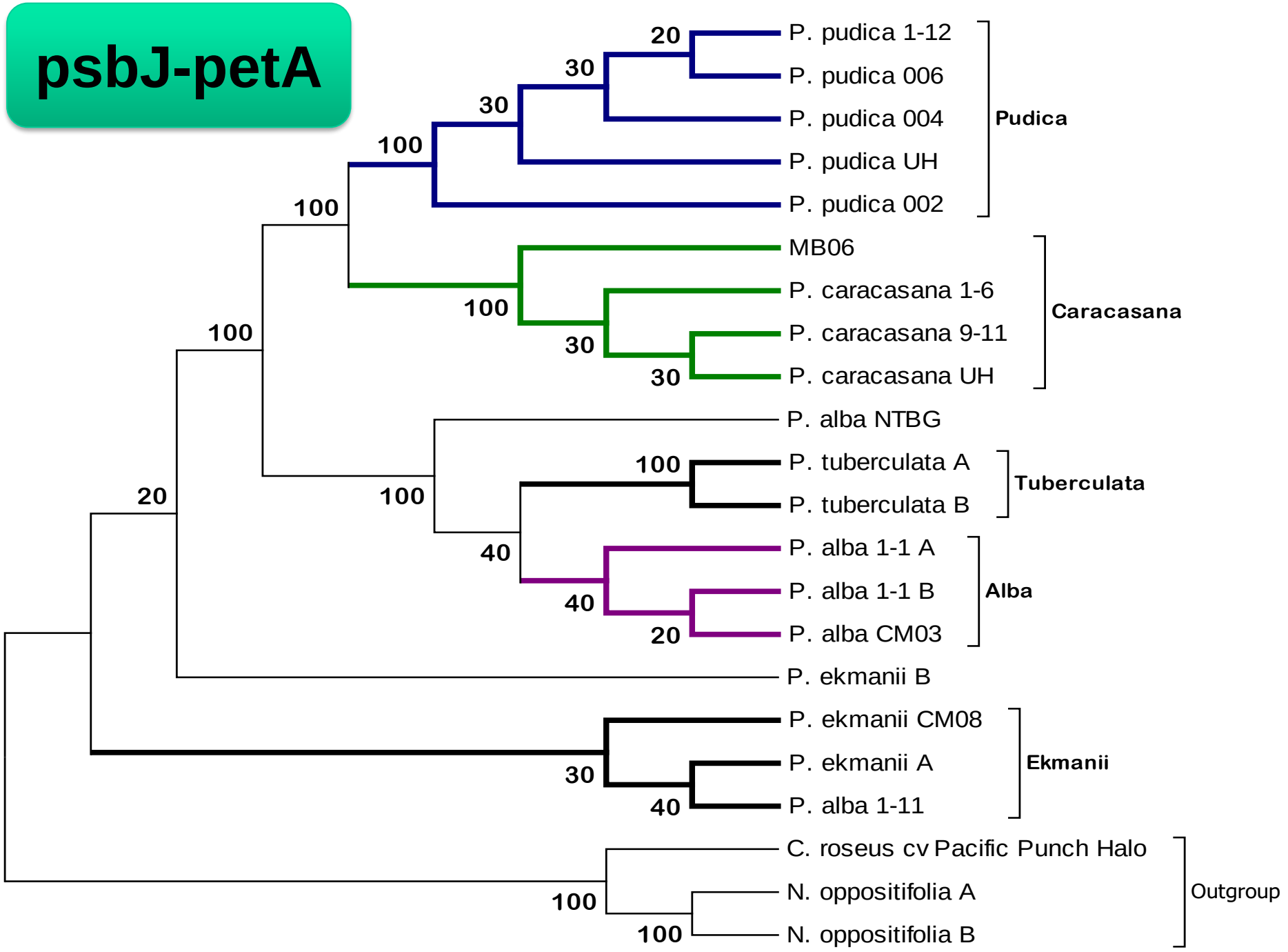
Basic Criteria for GOOD Regions:

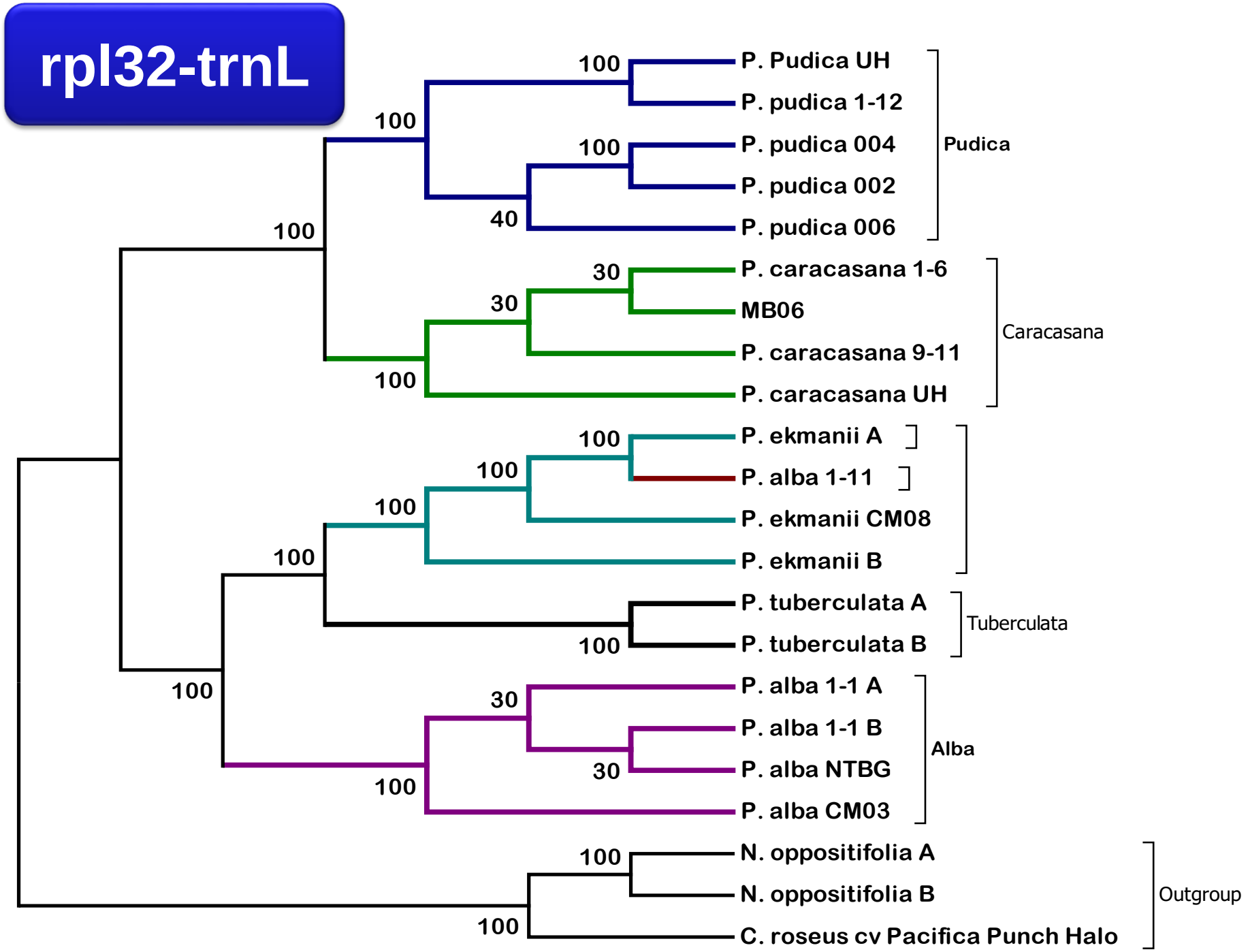
- ❑ Outgroup (Distantly related) taxa are identified
- ❑ Distinct *Plumeria* spp. cluster together
- ❑ DNA sequence variation **WITHIN** species is less than DNA sequence variation **BETWEEN** species


trnQ – rps16
JUNK REGION
No Information
UGLY TREE
P. caracasana UH
MB06
P. caracasana
P. caraca
P. n
1-1 B
. alba NTBG
P. alba CM03
P. ekmanii CM08
P. tuberculata A
P. tuberculata B
N. oppositifolia A
N. oppositifolia B
C. roseus cv Pacifica Punch Halo
F. berteroana
C. arabica
Outgroup
7
1
0
0
0
1
7
97
13
20
23
37
39
100
100
100
100

Bad day in the lab…

https://myerslifecoaching.com/


psbJ-petA
P. pudica 1-12
P. pudica 006
P. pudica 004
P. pudica UH
P. pudica 002
Pudica
MB06
P. caracasana 1-6
P. caracasana 9-11
P. caracasana UH
Caracasana
P. alba NTBG
P. tuberculata A
P. tuberculata B
Tuberculata
P. alba 1-1 A
P. alba 1-1 B
P. alba CM03
Alba
P. ekmanii B
P. ekmanii CM08
P. ekmanii A
P. alba 1-11
Ekmanii
C. roseus cv Pacific Punch Halo
N. oppositifolia A
N. oppositifolia B
Outgroup
20
30
30
100
100
100
100
30
30
100
100
40
40
20
20
30
40
100
100


rpl32-trnL
100
100
100
40
100
30
30
100
100
100
100
100
100
100
30
30
100
100
P. Pudica UH
P. pudica 1-12
P. pudica 004
P. pudica 002
P. pudica 006
Pudica
P. caracasana 1-6
MB06
P. caracasana 9-11
P. caracasana UH
Caracasana
P. ekmanii A
P. alba 1-11
P. ekmanii CM08
P. ekmanii B
P. tuberculata A
P. tuberculata B
Tuberculata
P. alba 1-1 A
P. alba 1-1 B
P. alba NTBG
P. alba CM03
Alba
N. oppositifolia A
N. oppositifolia B
C. roseus cv Pacifica Punch Halo
Outgroup

INTRAspecific Diff. Within spp. < INTERspecific Diff. Btwn. spp.

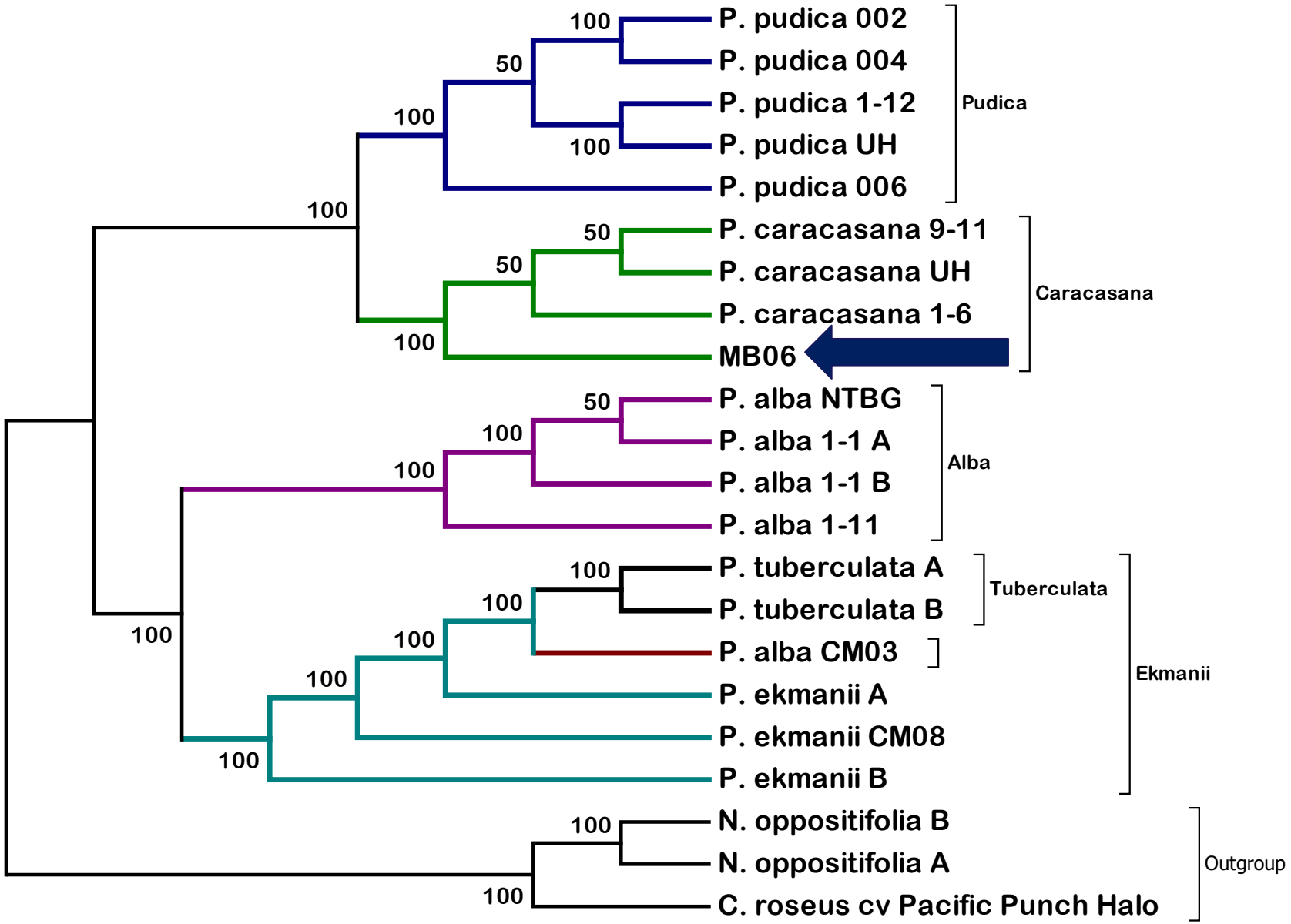
	DNA Sequence Variation			
	psbJ-petA IGS		rpl32-trnL IGS	
Plumeria spp.	WITHIN	BTWN	WITHIN	BTWN
P. ekmanii	0.000	0.002	0.005	0.014
P. pudica	0.000	0.001	0.001	0.006
P. caracasana	0.000	0.001	0.001	0.006
P. tuberculata	0.000	0.004	0.006	0.047
P. alba	0.001	0.004	0.005	0.009

Lines of Evidence

Basic Criteria for GOOD Regions:

- ✓ Outgroup taxa are identified
- ✓ Distinct *Plumeria* spp. cluster together
- ✓ DNA sequence variation **WITHIN A** species is less than DNA sequence variation **BETWEEN** species

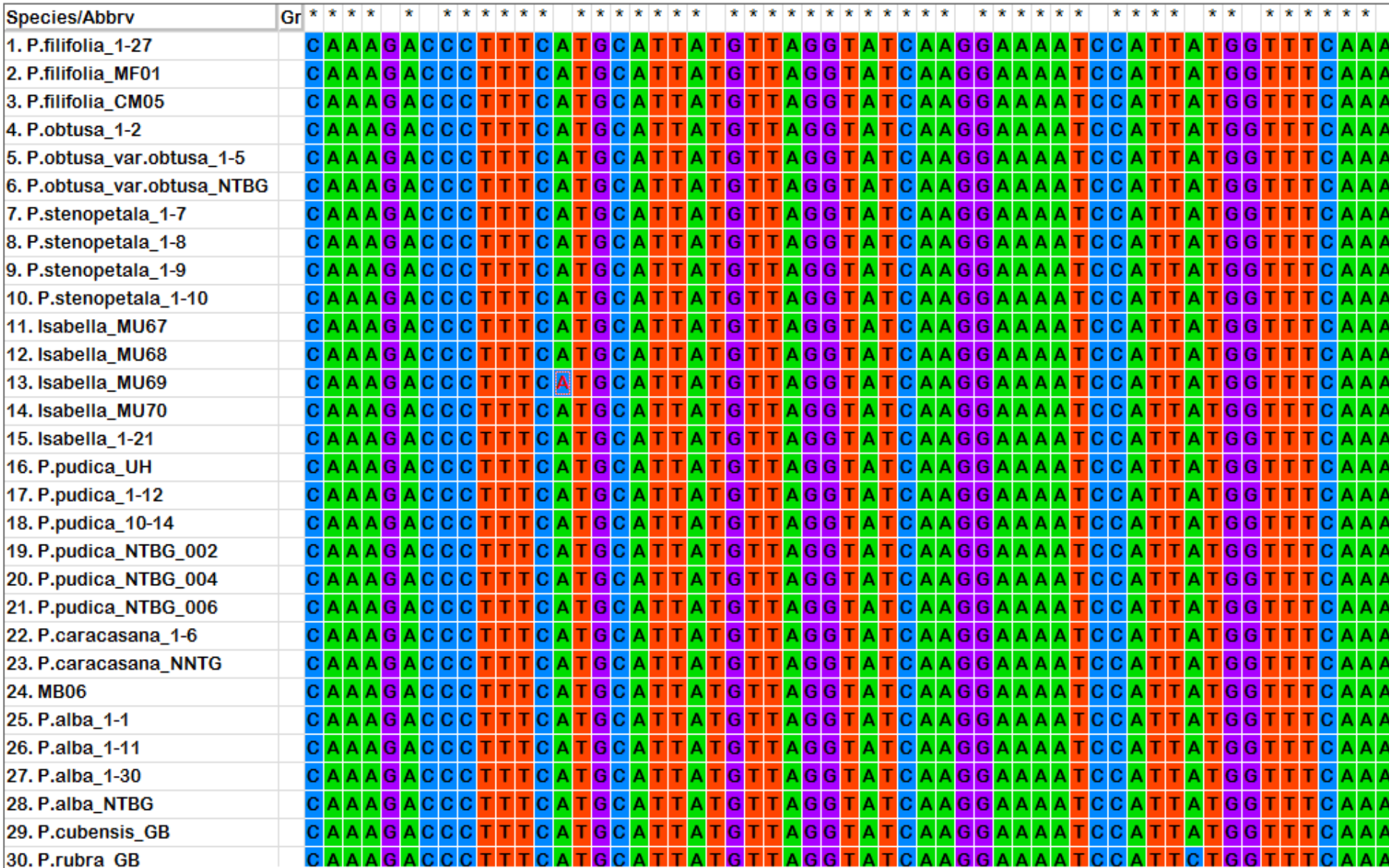
Combined psbJ-petA + rpl32-trnL



Good day in the lab…

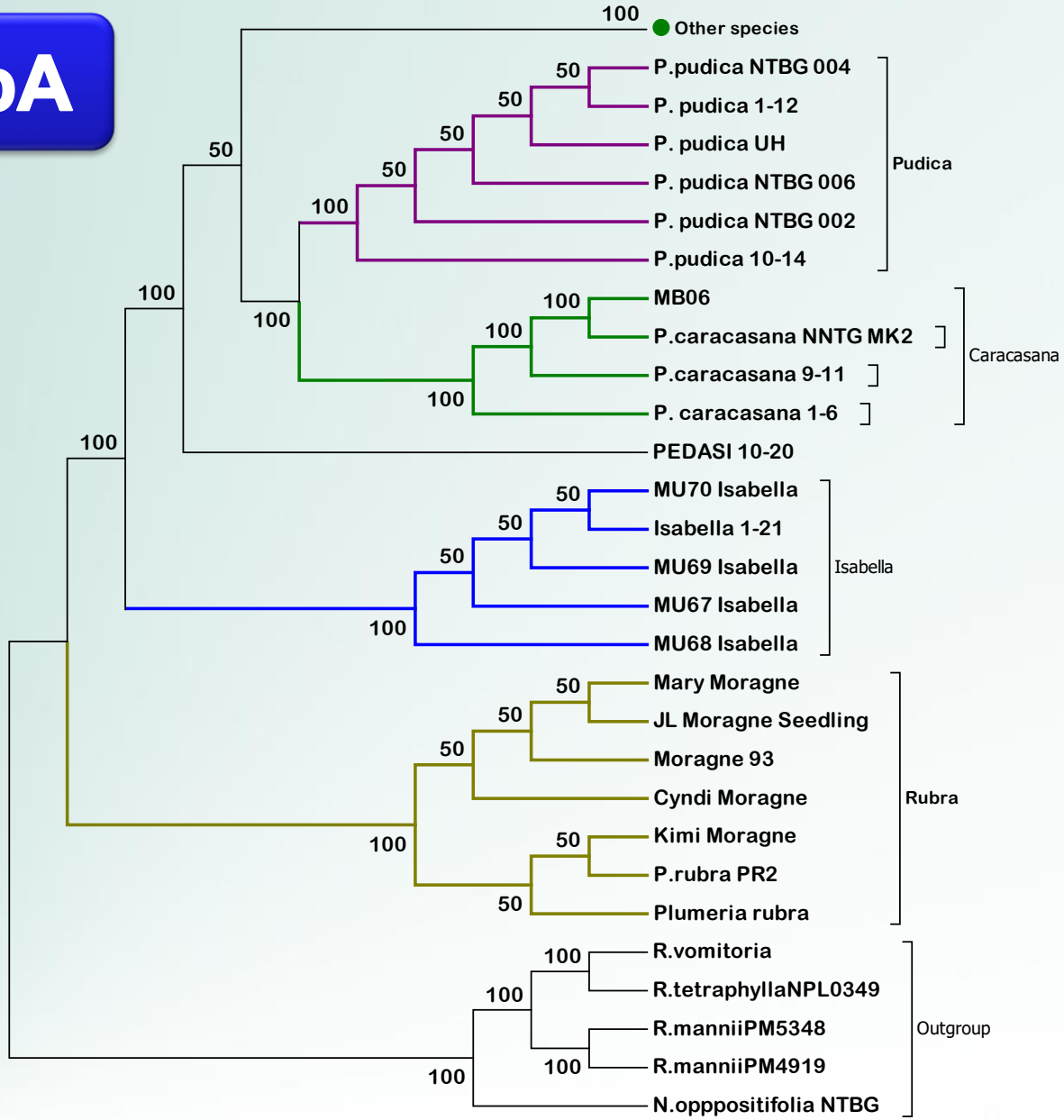
http://www.1designshop.com/photo-shop/very-excited-woman-with-glasses-rejoicing-her-success/

matK Region


Species/Abbrv
Gr
1. P.filifolia_1-27
2. P.filifolia_MF01
3. P.filifolia_CM05
4. P.obtusa_1-2
5. P.obtusa_var.obtusa_1-5
6. P.obtusa_var.obtusa_NTBG
7. P.stenopetala_1-7
8. P.stenopetala_1-8
9. P.stenopetala_1-9
10. P.stenopetala_1-10
11. Isabella_MU67
12. Isabella_MU68
13. Isabella_MU69
14. Isabella_MU70
15. Isabella_1-21
16. P.pudica_UH
17. P.pudica_1-12
18. P.pudica_10-14
19. P.pudica_NTBG_002
20. P.pudica_NTBG_004
21. P.pudica_NTBG_006
22. P.caracasana_1-6
23. P.caracasana_NNTG
24. MB06
25. P.alba_1-1
26. P.alba_1-11
27. P.alba_1-30
28. P.alba_NTBG
29. P.cubensis_GB
30. P.rubra_GB

Back to crazy lady!

trnH-psbA


Other species
P.pudica NTBG 004
P. pudica 1-12
P. pudica UH
P. pudica NTBG 006
P. pudica NTBG 002
P.pudica 10-14
Pudica
MB06
P.caracasana NNTG MK2
P.caracasana 9-11
P. caracasana 1-6
Caracasana
PEDASI 10-20
MU70 Isabella
Isabella 1-21
MU69 Isabella
MU67 Isabella
MU68 Isabella
Isabella
Mary Moragne
JL Moragne Seedling
Moragne 93
Cyndi Moragne
Kimi Moragne
P.rubra PR2
Plumeria rubra
Rubra
R.vomitoria
R.tetraphyllaNPL0349
R.manniiPM5348
R.manniiPM4919
N.opppositifolia NTBG
Outgroup

Conclusions

1. Regions **psbJ-petA** and **rpl32-trnL** can identify *Plumeria* spp. (so can **trnH-psbA**)
2. We can use these DNA regions to identify species (Who's Who)
3. We can determine genetic relationships within *Plumeria* (What's related to what)
4. We can begin to make corrections

Next Steps

- Add more samples to datasets:
  - Seedlings of *Plumeria spp.* (and seedlings)
  - Add more cultivars (breeding material)
    - 'Pops'(Red), 'Celadine'(Yellow), 'Mardi Gras', 'San Germain', 'Thornton's Lemon' (Yellow)
- Run analyses on separate datasets
  - trnH-psbA
  - rpl32-trnL
  - psbJ-petA
- Test combinations of datasets
  - trnH-psbA+rpl32-trnL
  - rpl32-trnL
  - psbJ-petA

Acknowledgements

- **Plumeria Society of America – #IPC**
- Southern California Plumeria Society – Research Funding
- Dr. Ania Wieczorek & Dr. Martha Gauthier – Primers
- Dr. Richard Manshardt – Sequencing funds
- Dr. Richard Criley – Plumeria images, slides, etc.
- Michael Ferreira – *Plumeria filifolia*
- Carlo Morici – *P. filifolia* and other *Plumeria* samples
- Hetty Ford – “Big Leaf from Naples” sample

Any questions?

References

- .
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