Estudios de macro- y microevolución con secuenciación masiva dirigida

SEBOT 2020

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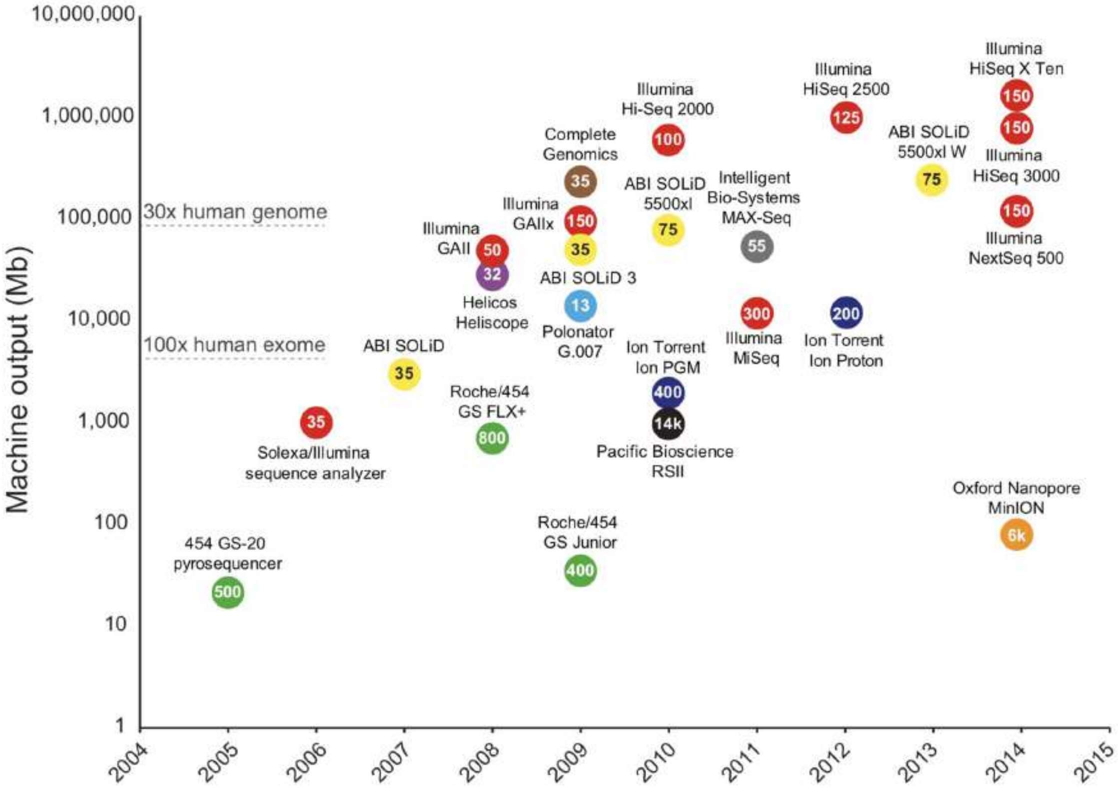
Hyb-Seq

1. Introducción: secuenciación masiva
2. Secuenciación mixta
3. Diseño de paneles de captura
4. Análisis bioinformáticos
5. Ejemplos
6. El futuro es ahora

1.

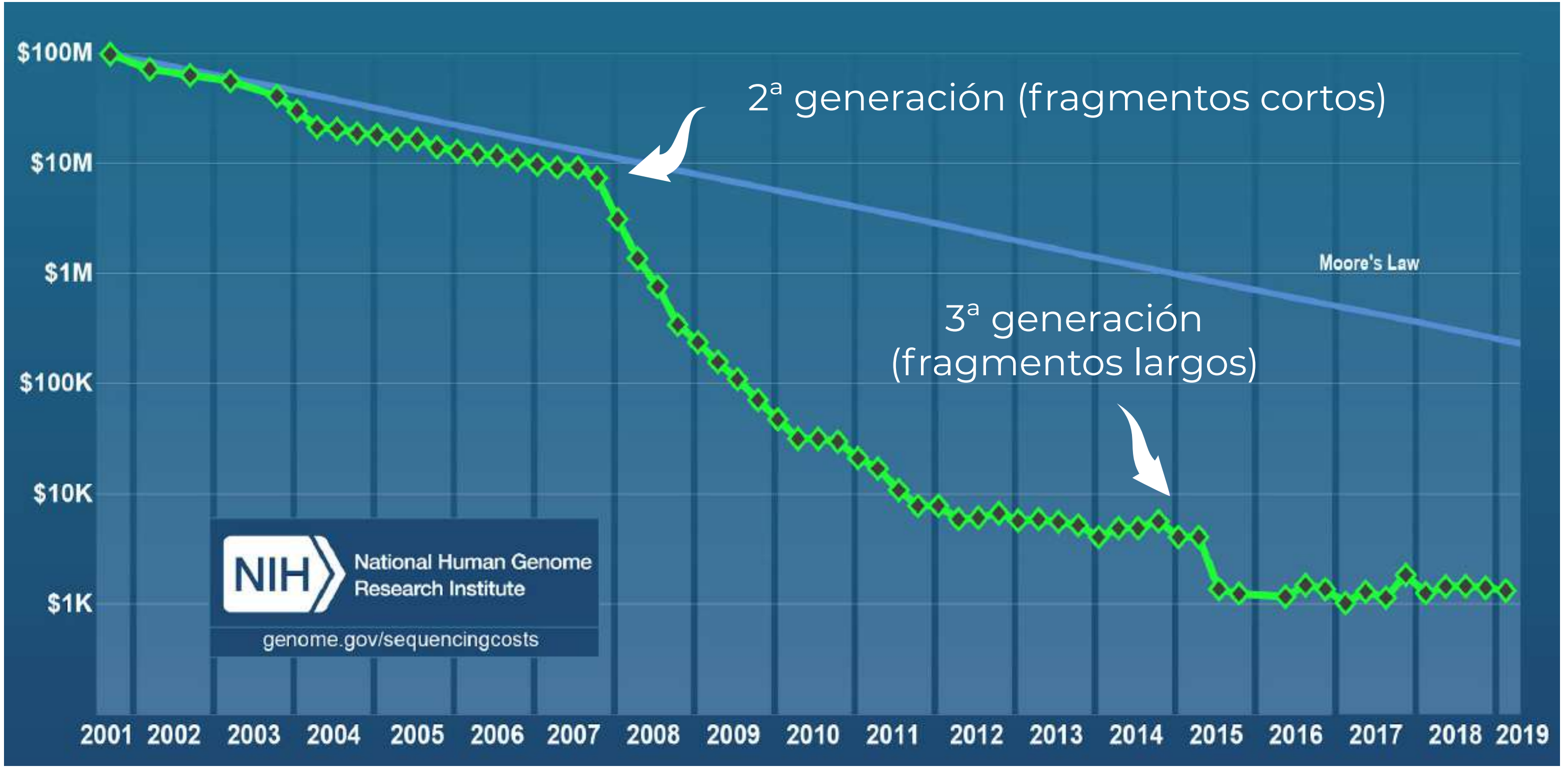
Introducción: Secuenciación Masiva

- Cronología
- Limitaciones
- Técnicas

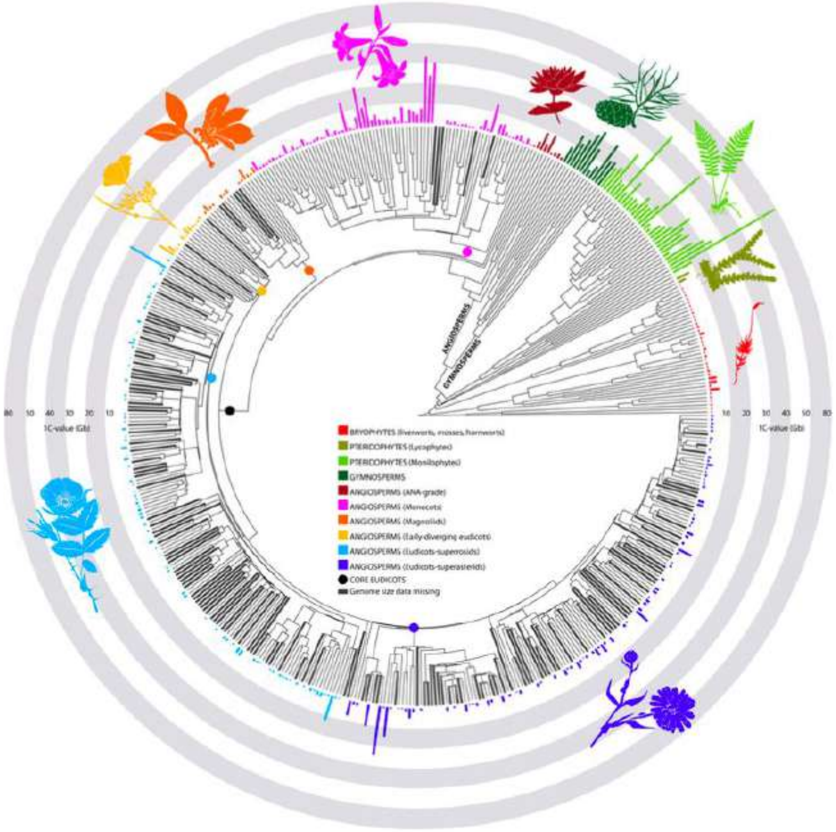


Cronología y comparación de instrumentos de secuenciación masiva

Fig. 1. Reuter et al. 2015. *Mol. Cell* 58: 586–597



Costes de secuenciación por genoma (para humanos en dólares)



Tamaños del genoma en plantas embriofitas

Fig. 1. Pellicer et al. 2018. *Genes* 9 (2), 88

Técnicas de muestreo genómico

Aleatorio

- A perdigones (**shotgun seq**)
- Genotipado por secuenciación (**GbS**, **RAD-seq**)

Estratificado

- Trascriptómica (**RNA-seq**)
- Enriquecimiento y captura (microfluídica, en fase sólida, en solución)

Mixto

- Combinando a perdigones con captura en solución (**Hyb-Seq**)

Técnicas de muestreo genómico

Aleatorio

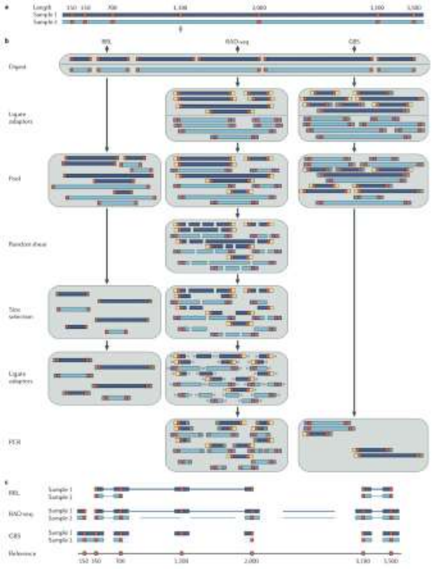


Fig. 1. Davey et al. 2011. *Nat. Rev. Genet.* 12: 499–510

Estratificado

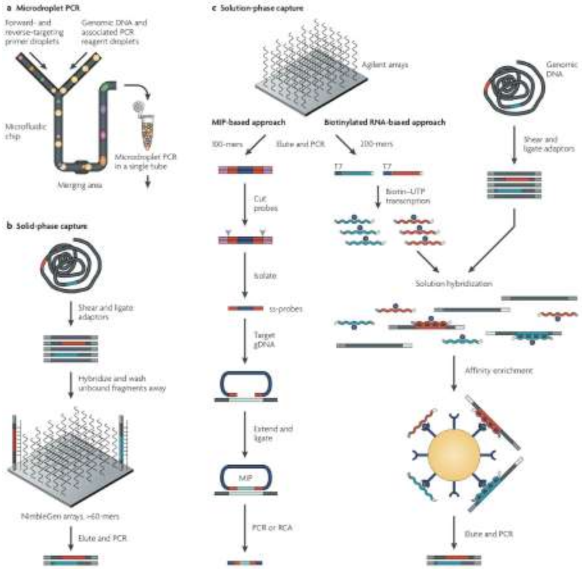
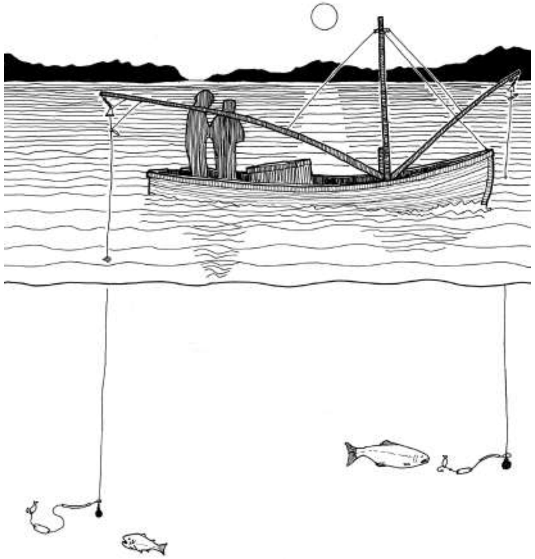


Fig. 5. Metzker. 2010. *Nat. Rev. Genet.* 11: 31–46

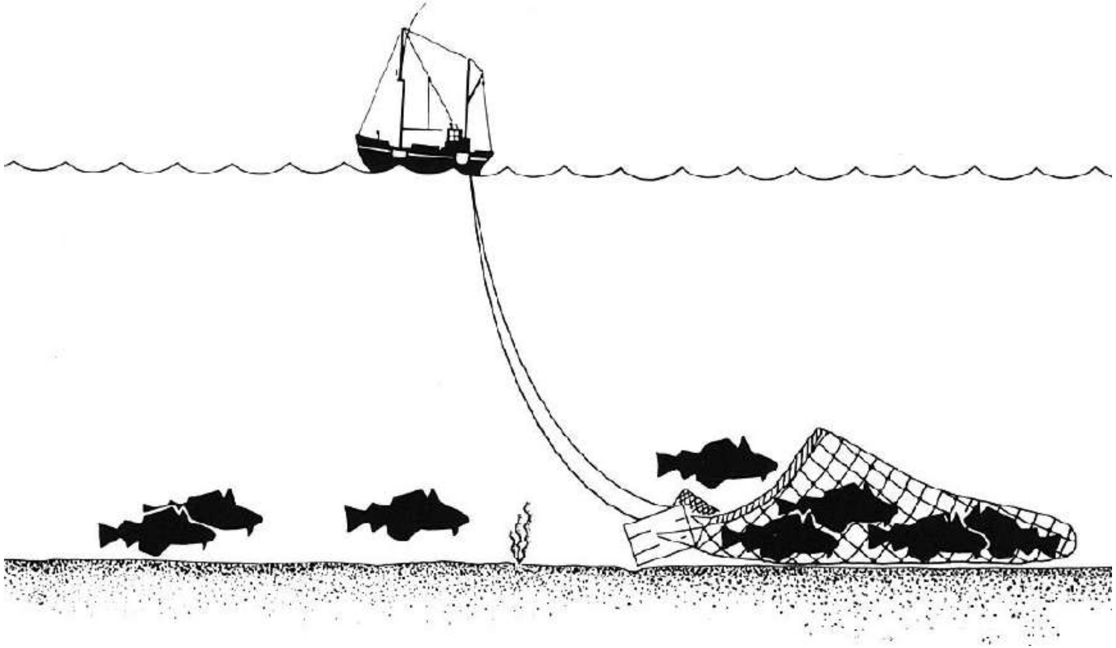
Mixto

Hyb-Seq

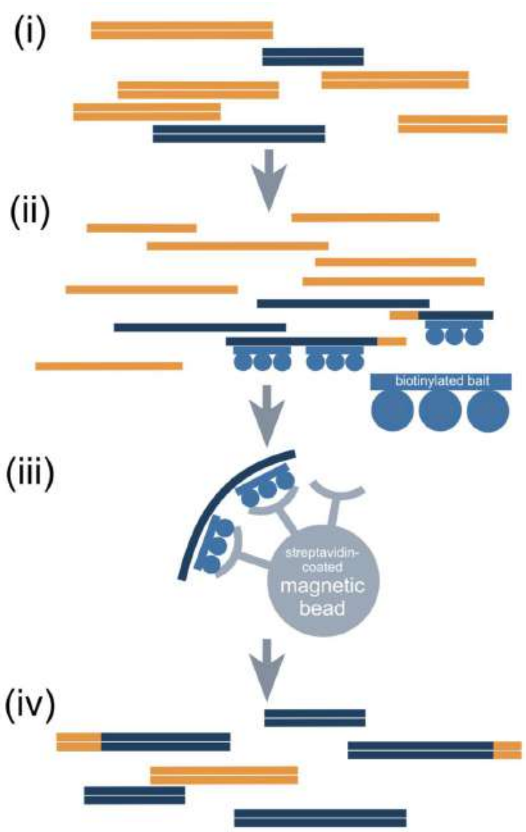


Secuenciación de terminación de cadena (Sanger sequencing)

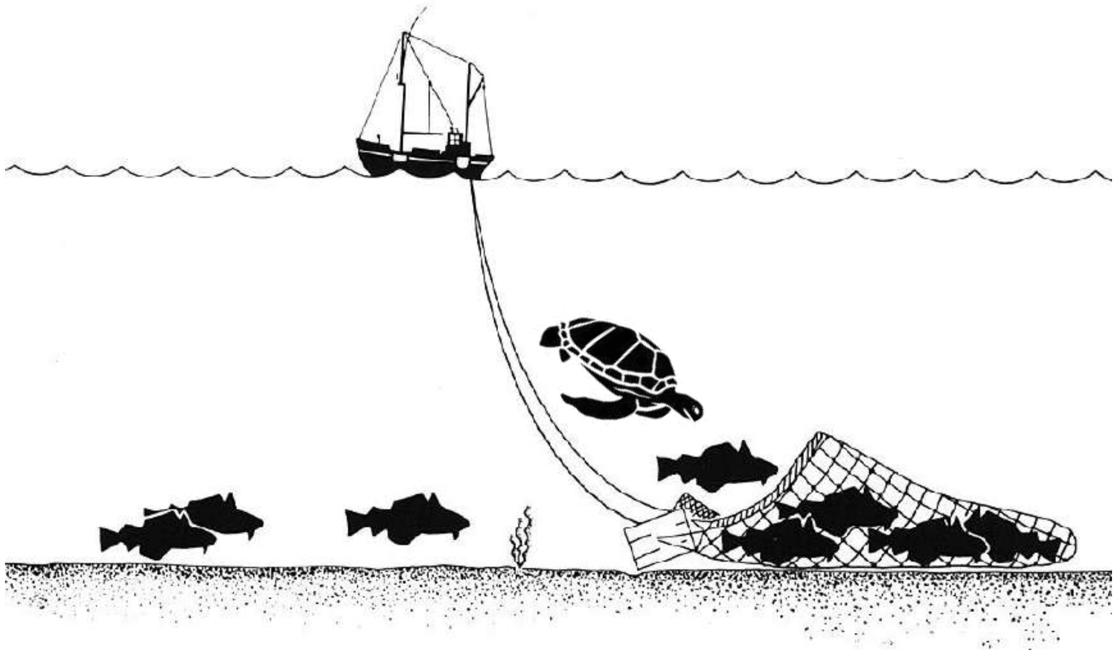
Secuenciación masiva dirigida (target capture sequencing)



Secuenciación Sanger vs. masiva dirigida



Secuenciación mixta



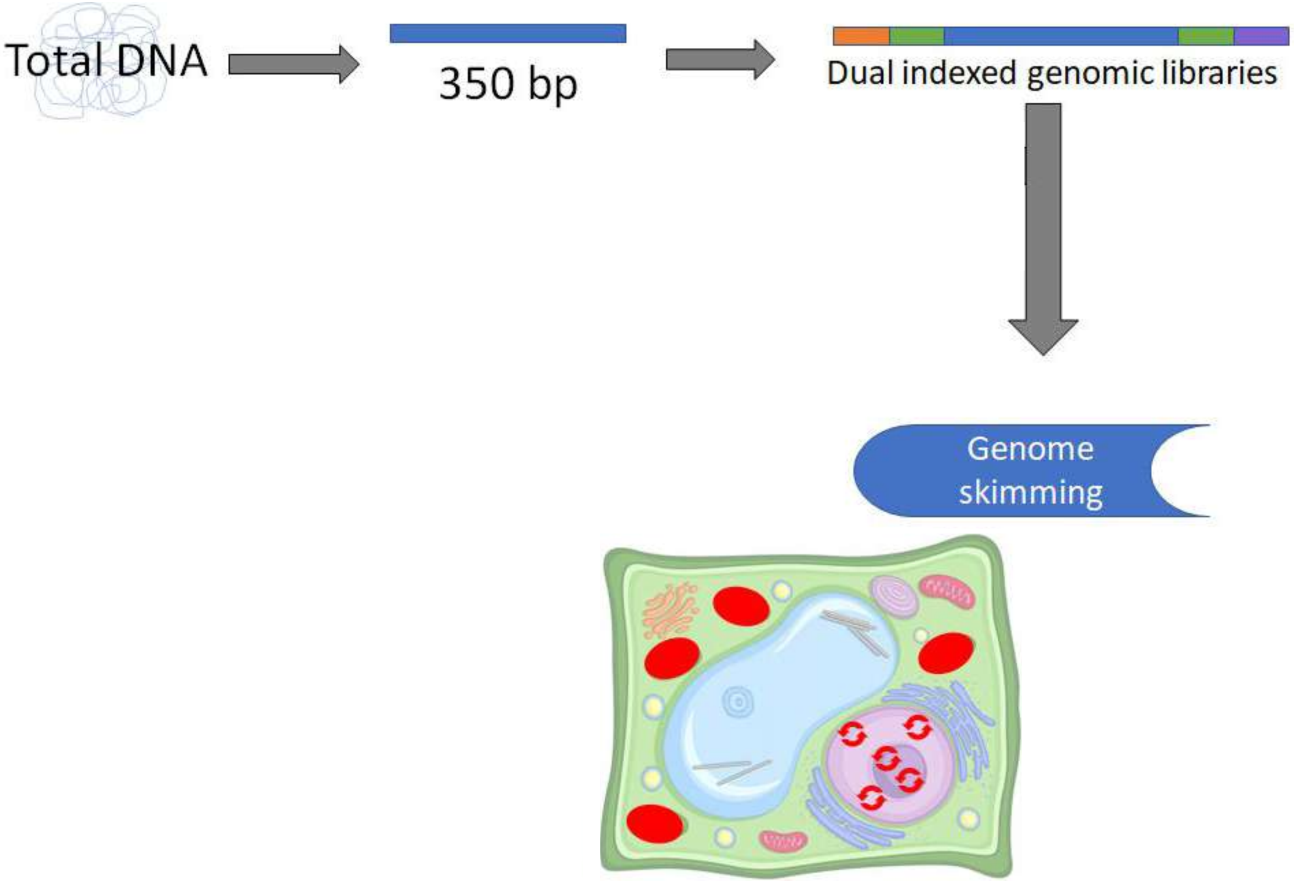
Hyb-Seq

Fig. 1. Dodsworth*, Pokorny*, Johnson* et al. 2019. *Trends Plant Sci.* 24 (10), 887–891

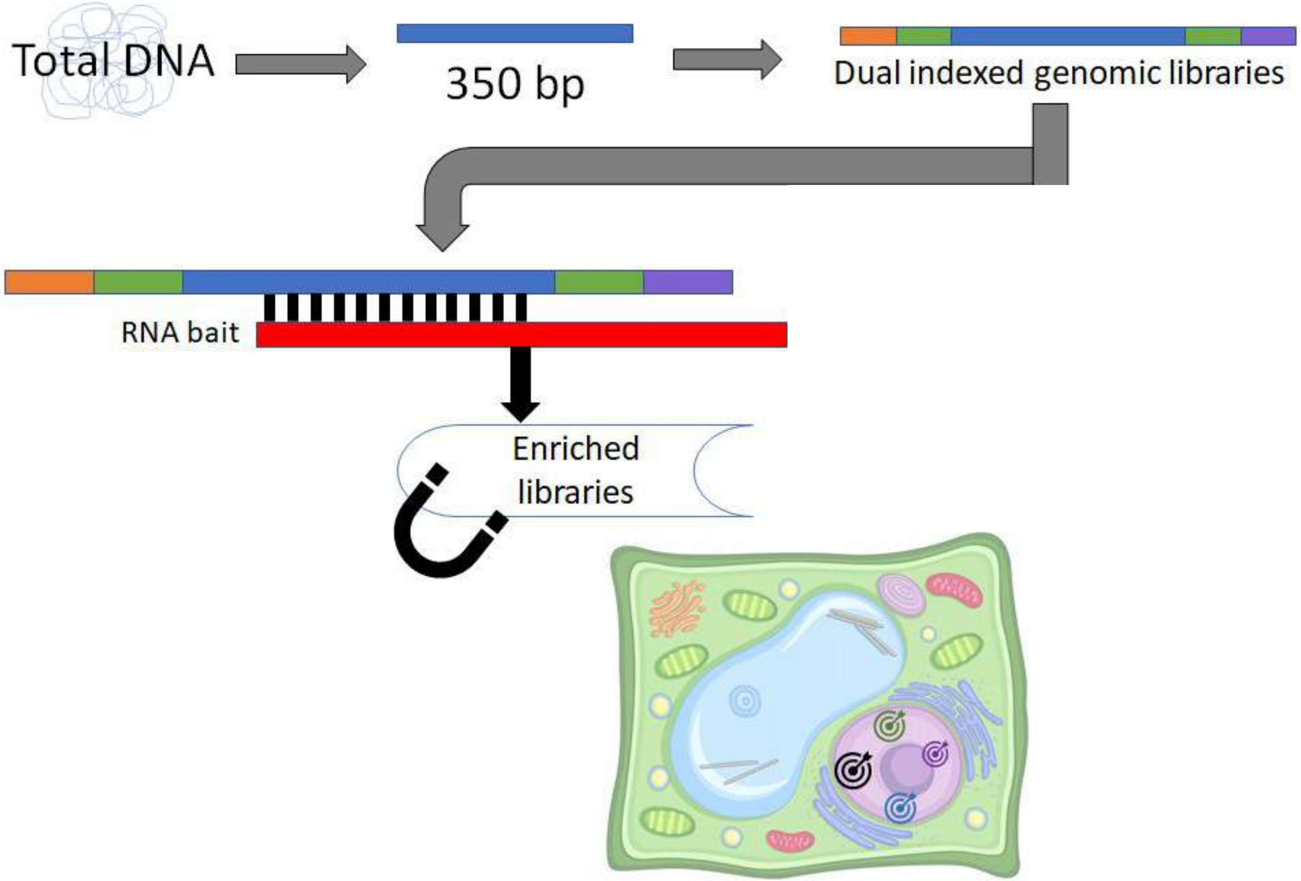
2.

Secuenciación mixta (Hyb-Seq)

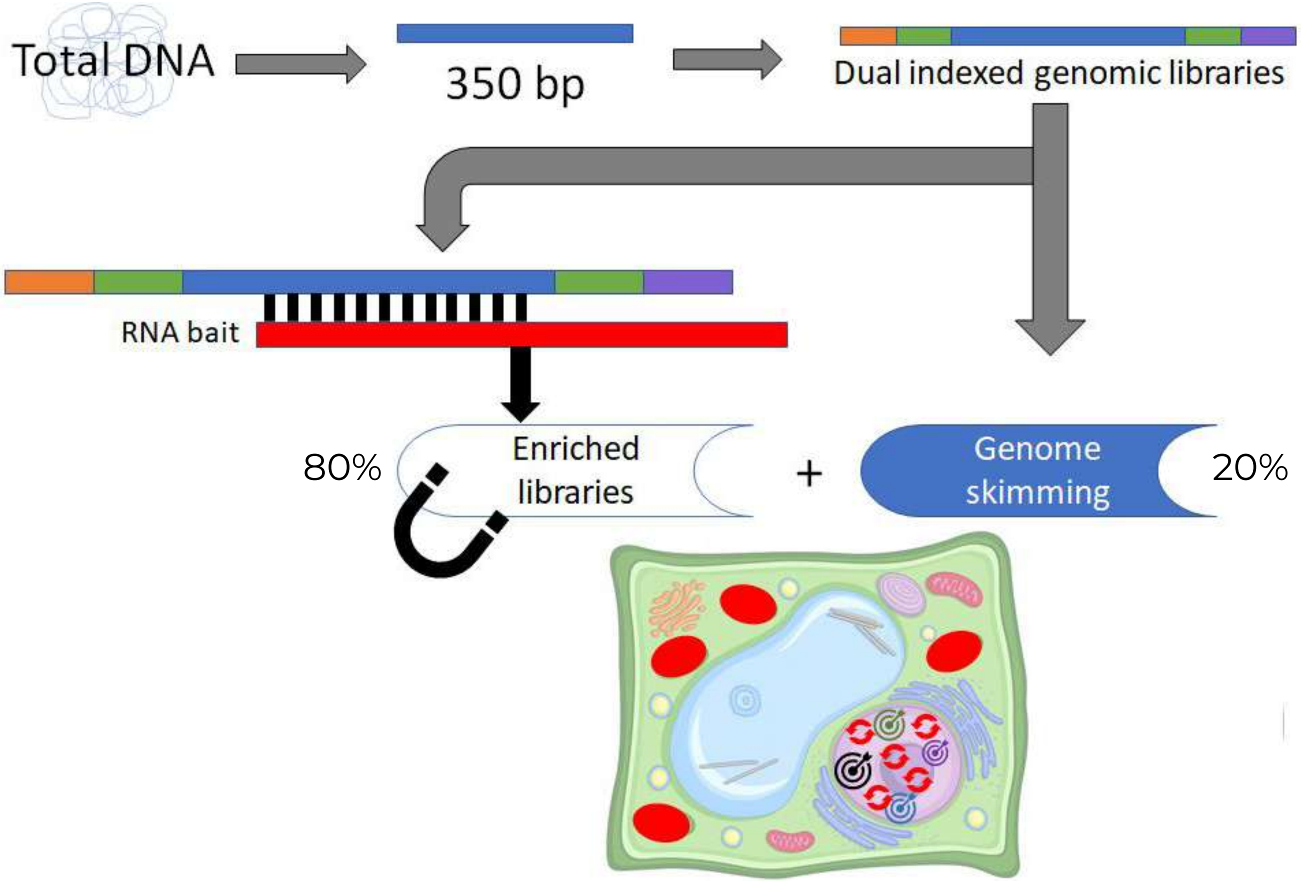
- Definición
- Materiales
- Métodos



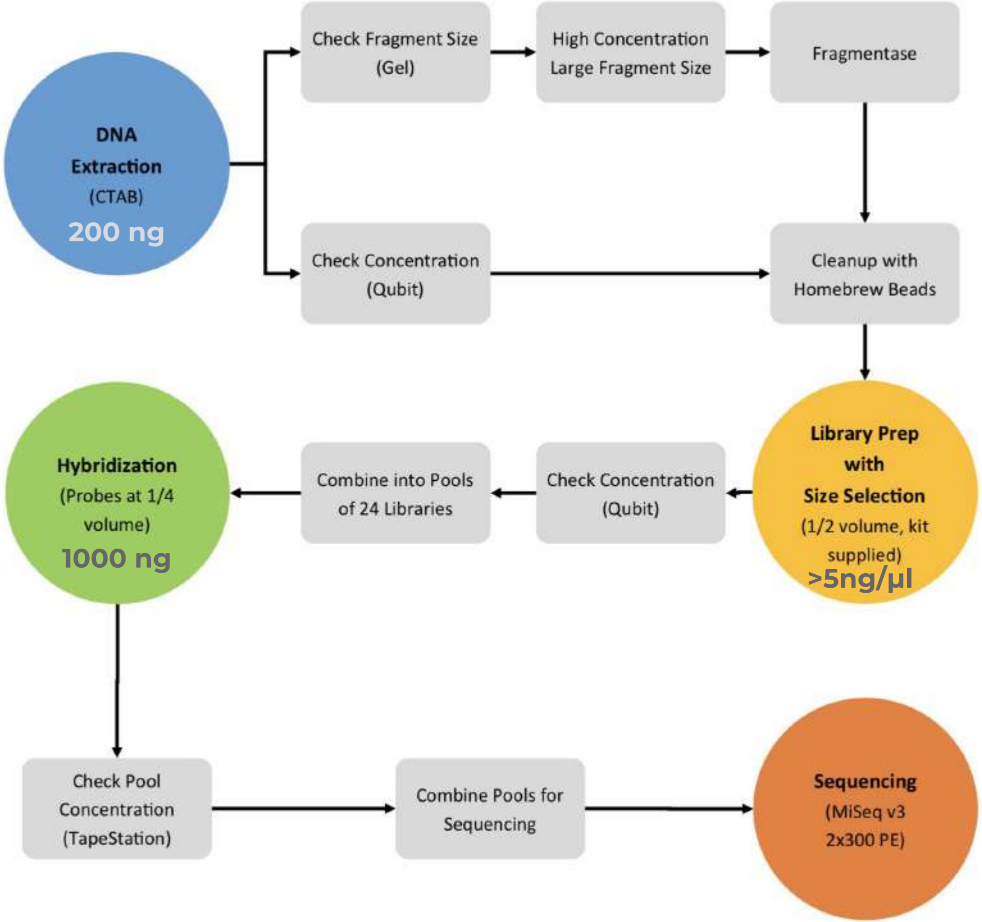
Secuenciación aleatoria a perdigones
(genome skimming)



Secuenciación masiva dirigida

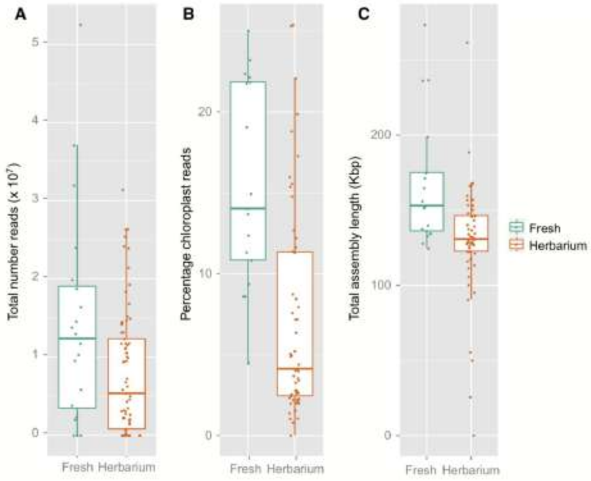


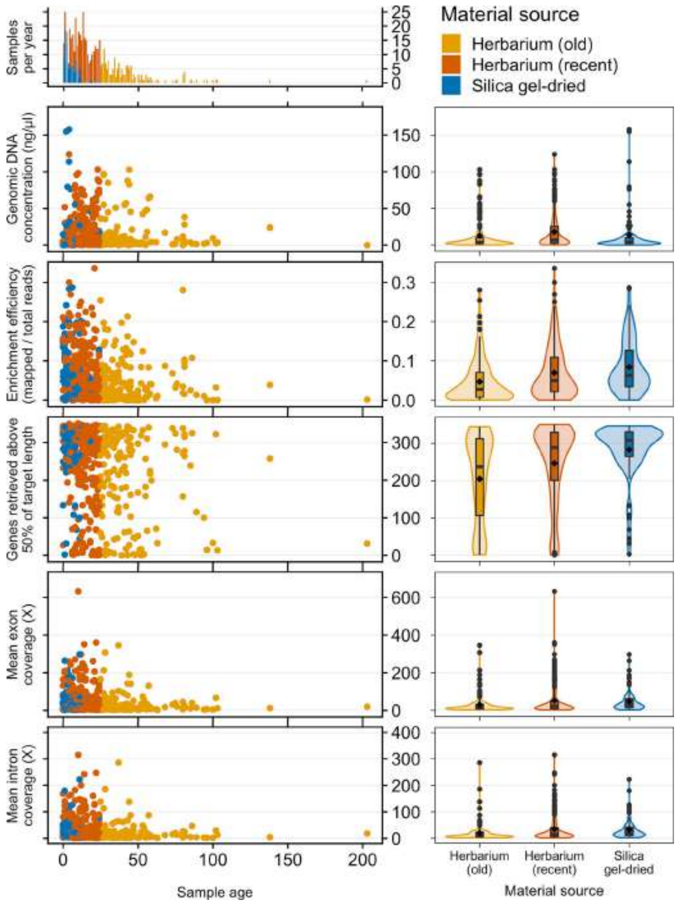
Hyb-Seq = shotgun + targeted sequencing



Hyb-Seq: flujo de trabajo molecular

Hale et al. 2020. Strategies for reducing per-sample costs in target capture sequencing for phylogenomics and population genomics in plants. *APPS*, in press.





Optimización de protocolos moleculares

Fig. 3. Bakker et al. 2015. *Biol. J. Linn. Soc.* 117: 33–43

Fig. 3. Brewer et al. 2019. *Front. Plant Sci.* 10: 1102

3.

Diseño de paneles de captura

- Específicos
- Universales
- Comparativa

Recursos genómicos

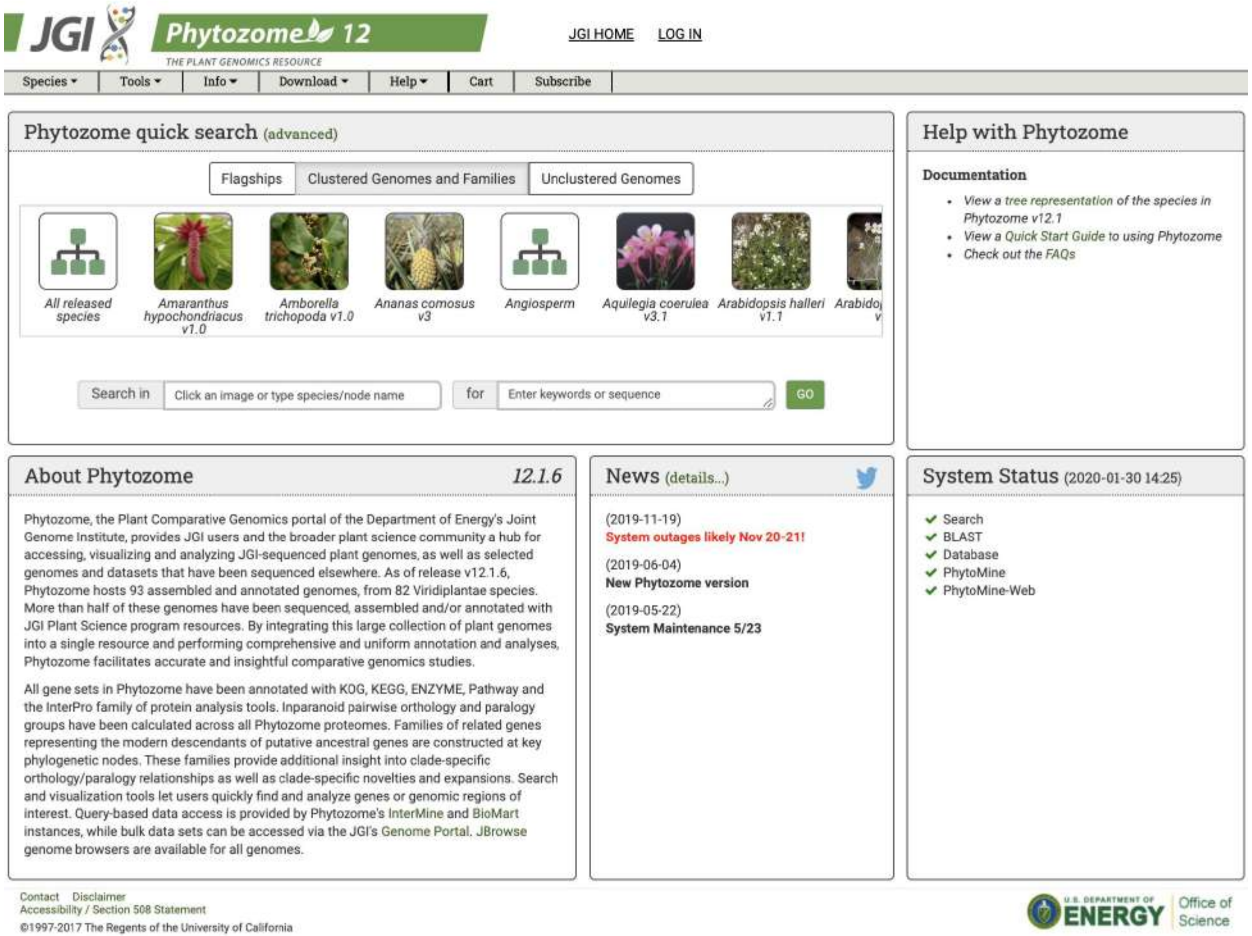
Transcriptomas, ESTs, genomas...

Recursos computacionales

Diseño con MarkerMiner, BaitFisher, DOMINO...

Recursos económicos

Producción y optimización


JGI
Phytozome 12
THE PLANT GENOMICS RESOURCE
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Flagships
Clustered Genomes and Families
Unclustered Genomes
All released species
Amaranthus hypochondriacus v1.0
Amborella trichopoda v1.0
Ananas comosus v3
Angiosperm
Aquilegia coerulea v3.1
Arabidopsis halleri v1.1
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Click an image or type species/node name
for
Enter keywords or sequence
GO
Help with Phytozome
Documentation
View a tree representation of the species in Phytozome v12.1
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Check out the FAQs
About Phytozome
12.1.6
Phytozome, the Plant Comparative Genomics portal of the Department of Energy's Joint Genome Institute, provides JGI users and the broader plant science community a hub for accessing, visualizing and analyzing JGI-sequenced plant genomes, as well as selected genomes and datasets that have been sequenced elsewhere. As of release v12.1.6, Phytozome hosts 93 assembled and annotated genomes, from 82 Viridiplantae species. More than half of these genomes have been sequenced, assembled and/or annotated with JGI Plant Science program resources. By integrating this large collection of plant genomes into a single resource and performing comprehensive and uniform annotation and analyses, Phytozome facilitates accurate and insightful comparative genomics studies.
All gene sets in Phytozome have been annotated with KOG, KEGG, ENZYME, Pathway and the InterPro family of protein analysis tools. Inparanoid pairwise orthology and paralogy groups have been calculated across all Phytozome proteomes. Families of related genes representing the modern descendants of putative ancestral genes are constructed at key phylogenetic nodes. These families provide additional insight into clade-specific orthology/paralogy relationships as well as clade-specific novelties and expansions. Search and visualization tools let users quickly find and analyze genes or genomic regions of interest. Query-based data access is provided by Phytozome's InterMine and BioMart instances, while bulk data sets can be accessed via the JGI's Genome Portal. JBrowse genome browsers are available for all genomes.
News (details...)
(2019-11-19)
System outages likely Nov 20-21!
(2019-06-04)
New Phytozome version
(2019-05-22)
System Maintenance 5/23
System Status (2020-01-30 14:25)
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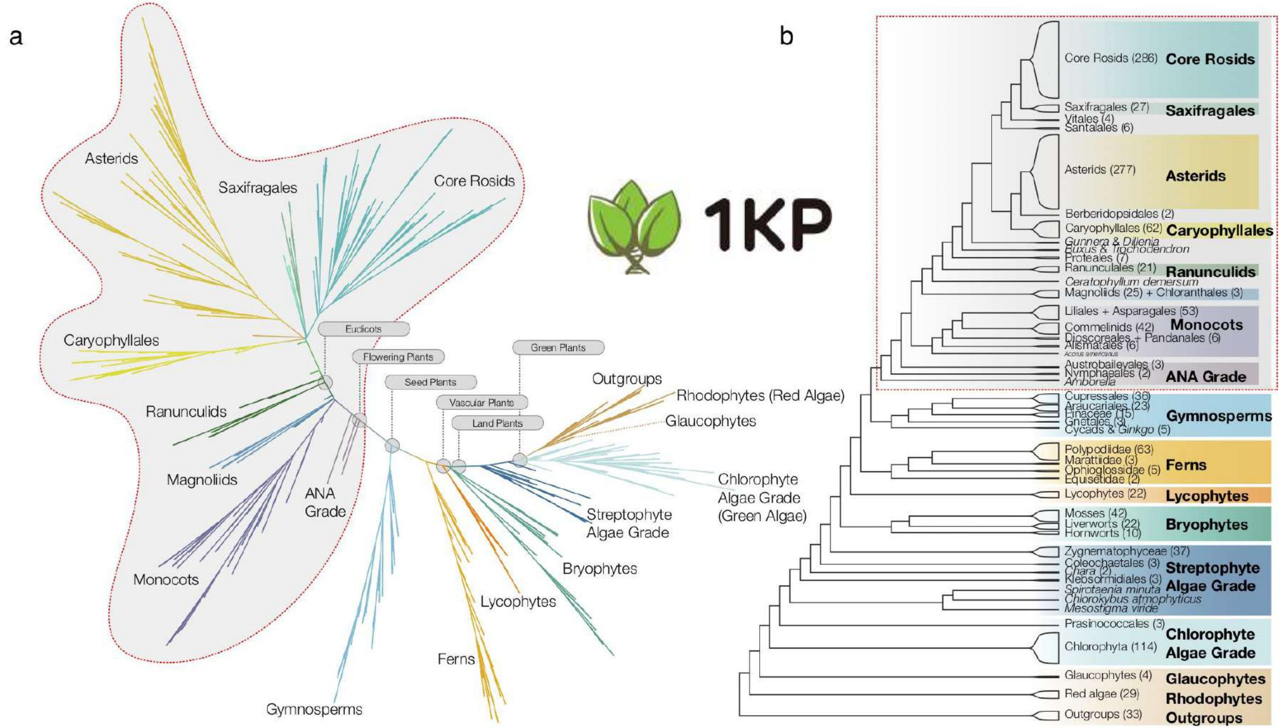
Article

One thousand plant transcriptomes and the phylogenomics of green plants

https://doi.org/10.1038/s41586-019-1693-2

One Thousand Plant Transcriptomes Initiative



OXFORD (GIGA)n SCIENCE

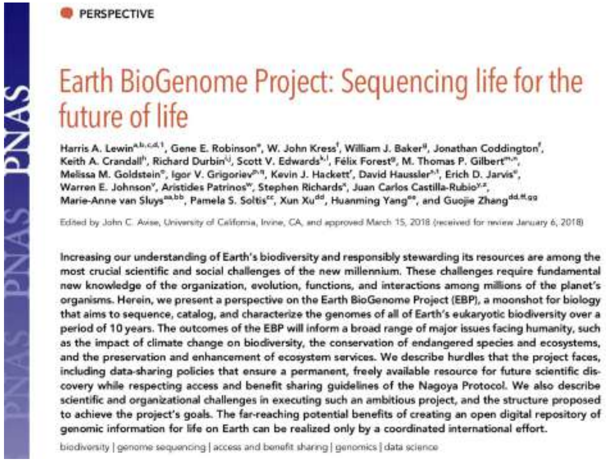
GigaScience, 7, 2018, 1–9

doi: 10.1093/gigascience/giy013
Advance Access Publication Date: 20 February 2018
Commentary

COMMENTARY

10KP: A phylodiverse genome sequencing plan

Shifeng Cheng[1,2,†], Michael Melkonian[3], Stephen A. Smith[4], Samuel Brockington[5], John M. Archibald[6], Pierre-Marc Delaux[7], Fay-Wei Li[8], Barbara Melkonian[3], Evgeny V. Mavrodiev[9], Wenjing Sun[1,2], Yuan Fu[1,2], Huanming Yang[1,10], Douglas E. Soltis[9,11], Sean W. Graham[12], Pamela S. Soltis[9,11], Xin Liu[1,2,†], Xun Xu[1,2,*] and Gane Ka-Shu Wong[1,13,14,*]



PNAS

PERSPECTIVE

Earth BioGenome Project: Sequencing life for the future of life

Harris A. Lewin[a,b,c,d,1], Gene E. Robinson[e], W. John Kress[f], William J. Baker[g], Jonathan Coddington[f], Keith A. Crandall[h], Richard Durbin[i,j], Scott V. Edwards[k,l], Félix Forest[g], M. Thomas P. Gilbert[m,n], Melissa M. Goldstein[o], Igor V. Grigoriev[p,q], Kevin J. Hackett[r], David Haussler[s,t], Erich D. Jarvis[u], Warren E. Johnson[v], Aristides Patrinos[w], Stephen Richards[x], Juan Carlos Castilla-Rubio[y,z], Marie-Anne van Sluys[aa,bb], Pamela S. Soltis[cc], Xun Xu[dd], Huanming Yang[ee], and Guojie Zhang[dd,ff,gg]

Edited by John C. Avise, University of California, Irvine, CA, and approved March 15, 2018 (received for review January 6, 2018)

Increasing our understanding of Earth's biodiversity and responsibly stewarding its resources are among the most crucial scientific and social challenges of the new millennium. These challenges require fundamental new knowledge of the organization, evolution, functions, and interactions among millions of the planet's organisms. Herein, we present a perspective on the Earth BioGenome Project (EBP), a moonshot for biology that aims to sequence, catalog, and characterize the genomes of all of Earth's eukaryotic biodiversity over a period of 10 years. The outcomes of the EBP will inform a broad range of major issues facing humanity, such as the impact of climate change on biodiversity, the conservation of endangered species and ecosystems, and the preservation and enhancement of ecosystem services. We describe hurdles that the project faces, including data-sharing policies that ensure a permanent, freely available resource for future scientific discovery while respecting access and benefit sharing guidelines of the Nagoya Protocol. We also describe scientific and organizational challenges in executing such an ambitious project, and the structure proposed to achieve the project's goals. The far-reaching potential benefits of creating an open digital repository of genomic information for life on Earth can be realized only by a coordinated international effort.

biodiversity | genome sequencing | access and benefit sharing | genomics | data science

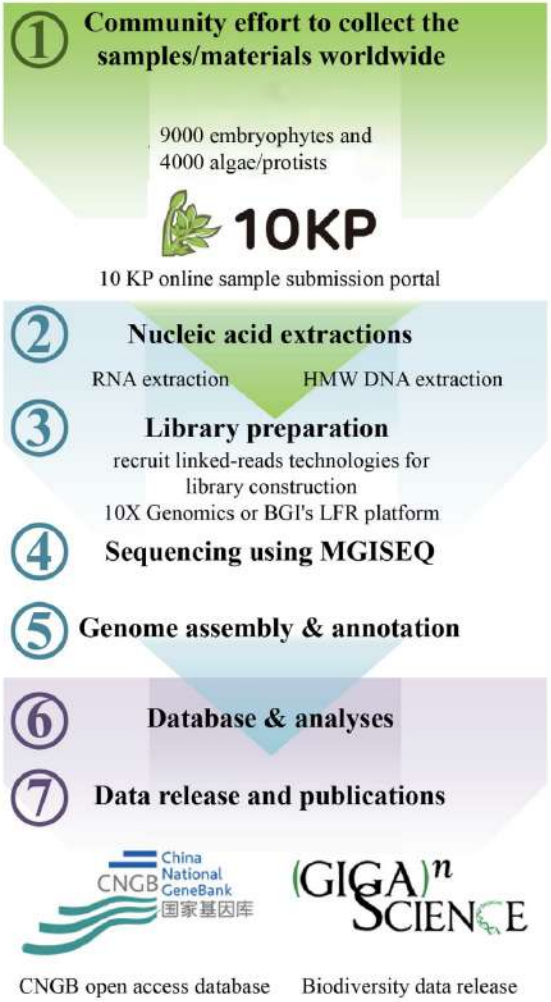


Fig. 9. Cheng et al. 2018. *GigaScience* 7: 1–9.

Applications in Plant Sciences
Official publication of the Botanical Society of America

Software Note | Open Access

MarkerMiner 1.0: A new application for phylogenetic marker development using angiosperm transcriptomes†

Srikar Chamala, Nicolás García, Grant T. Godden, Vivek Krishnakumar, Ingrid E. Jordon-Thaden, Riet De Smet, W. Brad Barbazuk, Douglas E. Soltis, Pamela S. Soltis

First published: 06 April 2015 | https://doi.org/10.3732/apps.1400115 | Citations: 32

† The authors thank all oneKP contributors, especially Gane Ka-Shu Wong, and BGI. Funding for S.C. was provided by the National Science Foundation (NSF; grant IOS-0922742 [P.S.S., D.E.S., W.B.B.]). Research funding for N.G. and G.T.G. was provided in part by NSF Doctoral Dissertation Improvement grants DEB-1310839 (P.S.S. and N.G.) and DEB-1210671 (P.S.S. and G.T.G.), respectively. The salary of I.J.T. was provided by the David Burpee Endowment and Chris Martine (Bucknell University).
‡ Authors are listed alphabetically by surname and contributed equally.

BaitFisher: A Software Package for Multispecies Target DNA Enrichment Probe Design

Christoph Mayer, Manuela Sann, Alexander Donath, Martin Meixner, Lars Podsiadlowski, Ralph S. Peters, Malte Petersen, Karen Meusemann, Karsten Liere, Johann-Wolfgang Wägele ... Show more
Author Notes

Molecular Biology and Evolution, Volume 33, Issue 7, July 2016, Pages 1875–1886,
https://doi.org/10.1093/molbev/msw056
Published: 23 March 2016

DOMINO: development of informative molecular markers for phylogenetic and genome-wide population genetic studies in non-model organisms

Cristina Frías-López, José F. Sánchez-Herrero, Sara Guirao-Rico, Elisa Mora, Miquel A. Arnedo, Alejandro Sánchez-Gracia, Julio Rozas Author Notes

Bioinformatics, Volume 32, Issue 24, 15 December 2016, Pages 3753–3759,
https://doi.org/10.1093/bioinformatics/btw534
Published: 24 August 2016 **Article history**

MOLECULAR ECOLOGY RESOURCES

Resource Article

DISCOMARK: nuclear marker discovery from orthologous sequences using draft genome data

Sereina Rutschmann, Harald Detering, Sabrina Simon, Jakob Fredslund, Michael T. Monaghan

First published: 25 July 2016 | https://doi.org/10.1111/1755-0998.12576 | Citations: 4

Applications in Plant Sciences
Official publication of the Botanical Society of America

Software Note | Open Access

MarkerMiner 1.0: A new application for phylogenetic marker development using angiosperm transcriptomes[†]

Srikar Chamala, Nicolás García, Grant T. Godden, Vivek Krishnakumar, Ingrid E. Jordon-Thaden, Riet De Smet, W. Brad Barbazuk, Douglas E. Soltis, Pamela S. Soltis

First published: 06 April 2015 | https://doi.org/10.3732/apps.1400115 | Citations: 32

[†] The authors thank all oneKP contributors, especially Gane Ka-Shu Wong, and BGI. Funding for S.C. was provided by the National Science Foundation (NSF; grant IOS-0922742 [P.S.S., D.E.S., W.B.B.]). Research funding for N.G. and G.T.G. was provided in part by NSF Doctoral Dissertation Improvement grants DEB-1310839 (P.S.S. and N.G.) and DEB-1210671 (P.S.S. and G.T.G.), respectively. The salary of I.J.T. was provided by the David Burpee Endowment and Chris Martine (Bucknell University).
[‡] Authors are listed alphabetically by surname and contributed equally.

2,840 genes de angiospermas en copia baja o única (2,663 baja + 177 única)

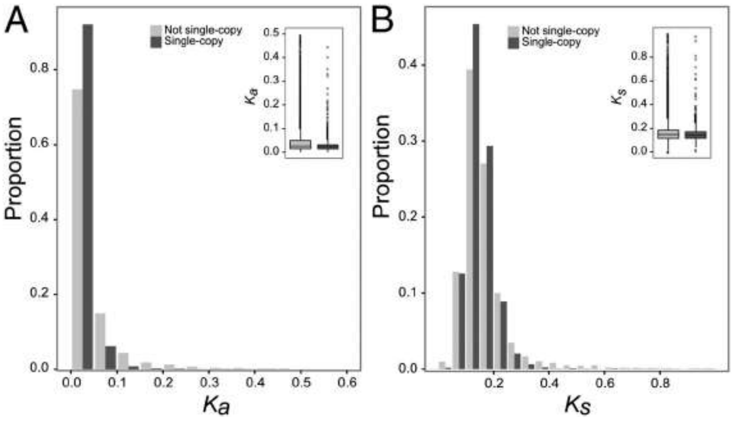


Fig. 3. De Smet et al. 2013. *PNAS* 110: 2898–2903

Diseño de panel específico

Transcriptomas

- Preexistentes o material vivo
- Ensamblaje de novo

Genoma(s) de referencia

- 16 disponibles en
- Posible añadir más

Panel de enriquecimiento y captura comercial

- Co-diseño del panel

MarkerMiner
Locus development made easy

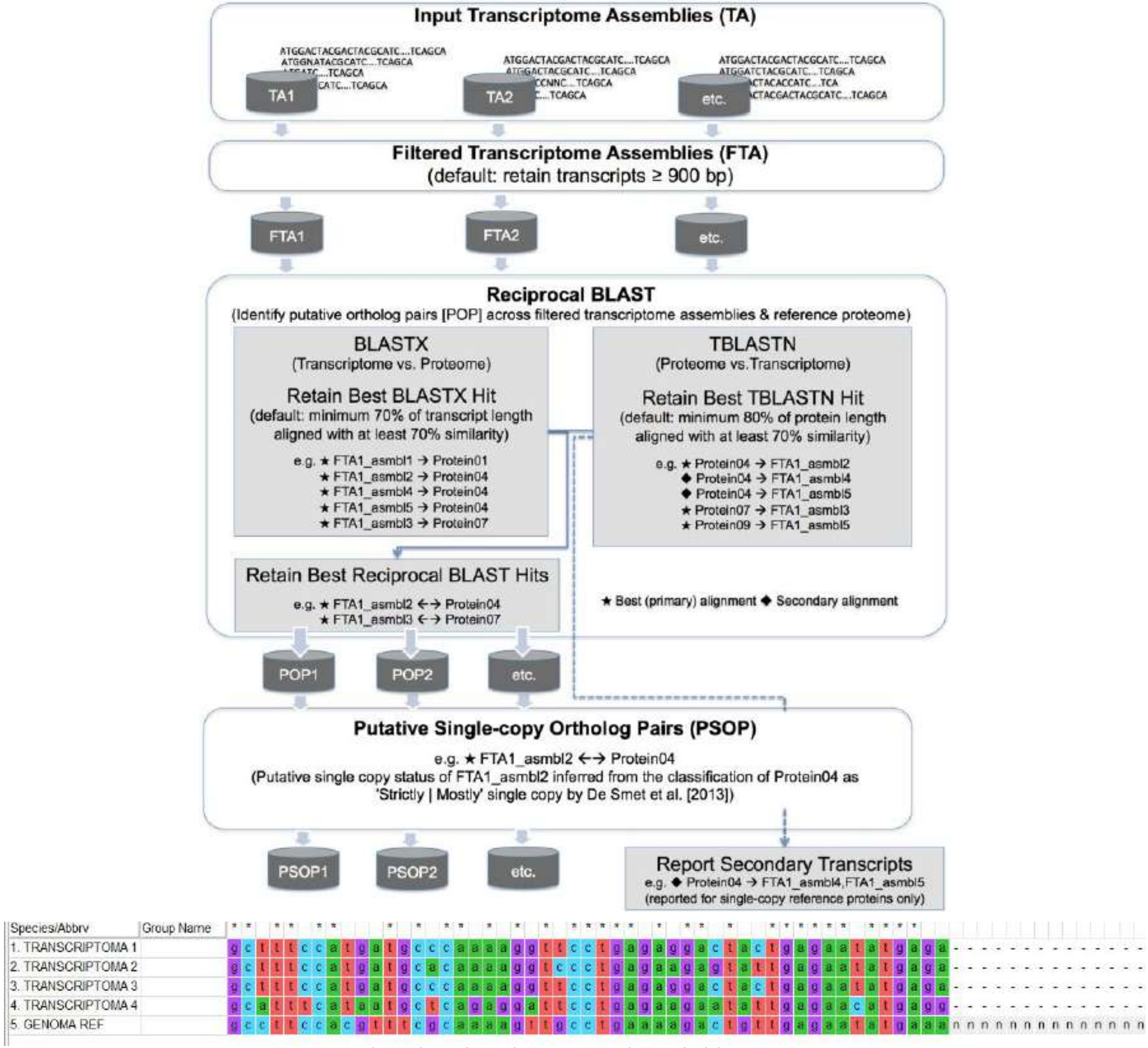


Fig. 1. Chamala et al. 2015. *Apps. Plant Sci.* 3(4): 1400115

A customized nuclear target enrichment approach for developing a phylogenomic baseline for *Dioscorea* yams (Dioscoreaceae)

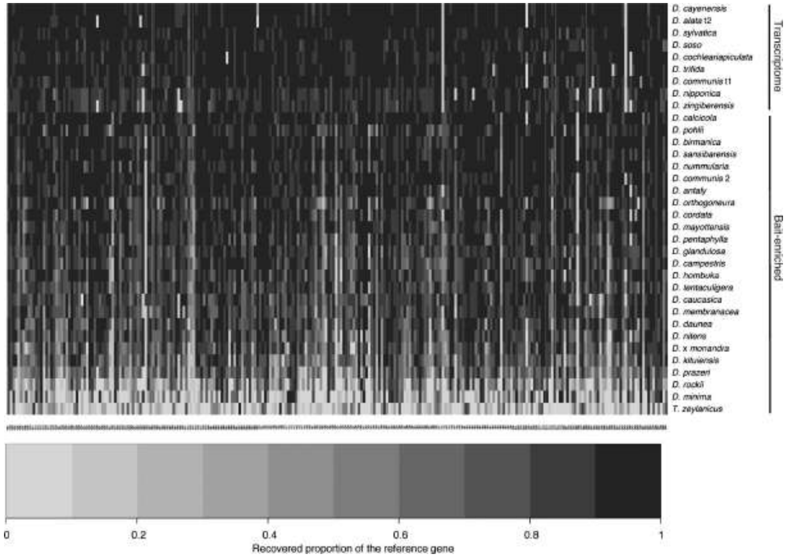
Marybel Soto Gomez[1,2,8], Lisa Pokorny[3], Michael B. Kantar[4], Félix Forest[3], Ilia J. Leitch[3], Barbara Gravendeel[5,6,7], Paul Wilkin[3], Sean W. Graham[1,2], and Juan Viruel[3]

A partir de

- Cuatro transcriptomas
  - 3x grupo de estudio (***Dioscorea***)
  - 1x género hermano (***Tacca***).
- Dos genomas de referencia
  - *Oryza sativa* (Poales)
  - *Xerophyta viscosa* (Pandanales)

Resultados

- 260 genes de copia baja o única
  - (+43 genes de interés)
  - >60% enriquecimiento
- Excelente resolución filogenética



Syst. Biol. 68(4):594–606, 2019

DOI:10.1093/sysbio/syy086
Advance Access publication December 10, 2018

A Universal Probe Set for Targeted Sequencing of 353 Nuclear Genes from Any Flowering Plant Designed Using k-Medoids Clustering

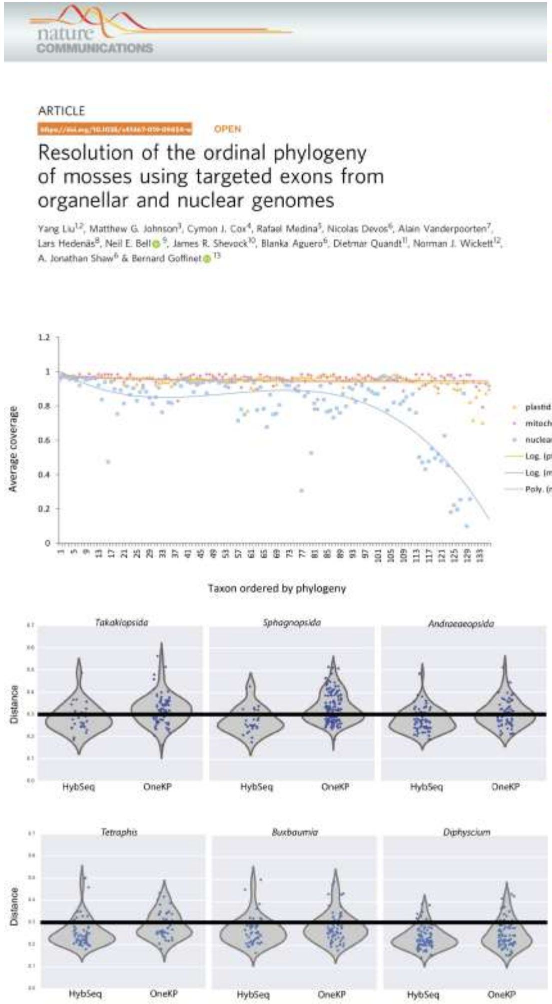
MATTHEW G. JOHNSON[1,2,*], LISA POKORNY[3], STEVEN DODSWORTH[3,4], LAURA R. BOTIGUÉ[3,5], ROBYN S. COWAN[3], ALISON DEVAULT[6], WOLF L. EISERHARDT[3,7], NIROSHINI EPITAWALAGE[3], FÉLIX FOREST[3], JAN T. KIM[3], JAMES H. LEEBENS-MACK[8], ILIA J. LEITCH[3], OLIVIER MAURIN[3], DOUGLAS E. SOLTIS[9,10], PAMELA S. SOLTIS[9,10], GANE KA-SHU WONG[11,12,13], WILLIAM J. BAKER[3], AND NORMAN J. WICKETT[2,14]

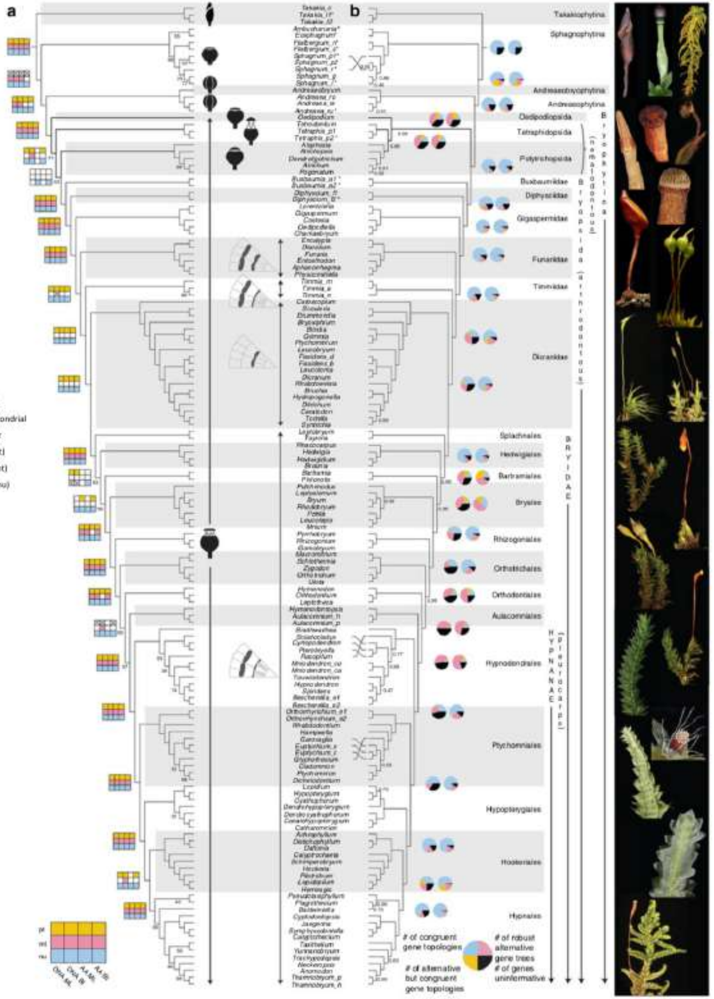
[1]*Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409, USA;* [2]*Plant Science and Conservation, Chicago Botanic Garden, 1000 Lake Cook Road, Glencoe, IL 60022, USA;* [3]*Department of Comparative Plant and Fungal Biology, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AE, UK;* [4]*School of Life Sciences, University of Bedfordshire, University Square, Luton LU1 3JU, UK;* [5]*Centre for Research in Agricultural Genomics, Campus UAB, Edifici CRAG, Bellaterra Cerdanyola del Vallès, 08193 Barcelona, Spain;* [6]*Arbor Biosciences, 5840 Interface Dr, Suite 101, Ann Arbor, MI 48103, USA;* [7]*Department of Bioscience, Aarhus University, 8000 Aarhus C, Denmark;* [8]*Department of Plant Biology, University of Georgia, 2502 Miller Plant Sciences, Athens, GA 30602, USA;* [9]*Department of Biology, University of Florida, 220 Bartram Hall, Gainesville, FL 32611-8525, USA;* [10]*Florida Museum of Natural History, University of Florida, 3215 Hull Road, Gainesville, FL 32611-2710, USA;* [11]*BGI-Shenzhen, Beishan Industrial Zone, Yantian District, Shenzhen 518083, China;* [12]*Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada;* [13]*Department of Medicine, University of Alberta, Edmonton, AB T6G 2E1, Canada; and* [14]*Program in Plant Biology and Conservation, Northwestern University, 2205 Tech Drive, Evanston, IL 60208, USA*

**Correspondence to be sent to: Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409, USA; E-mail: matt.johnson@ttu.edu.*

Matthew G. Johnson, Lisa Pokorny, Steven Dodsworth contributed equally to this article.

Received 2 July 2018; reviews returned 29 November 2018; accepted 3 December 2018
Associate Editor: Susanne Renner





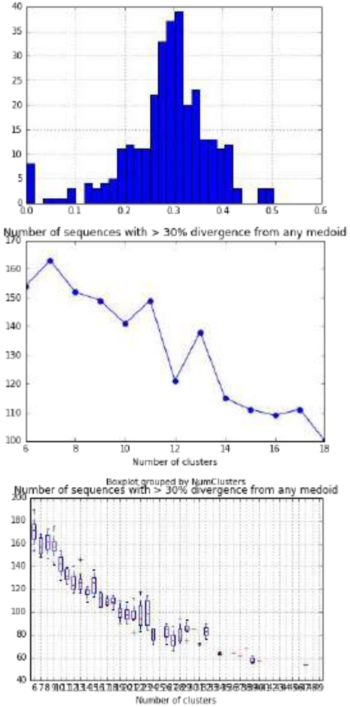
Figs. 1, S5 & S6. Liu et al. 2019. *Nat. Commun.* 10: 1485

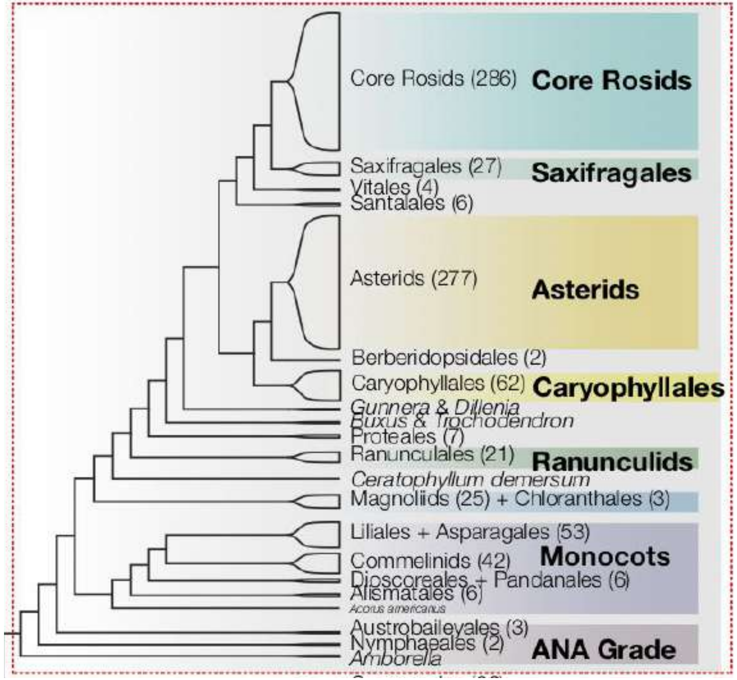
Syst. Biol. 68(4):594–606, 2019

DOI:10.1093/sysbio/syy086
Advance Access publication December 10, 2018

A Universal Probe Set for Targeted Sequencing of 353 Nuclear Genes from Any Flowering Plant Designed Using k-Medoids Clustering

Matthew G. Johnson[1,2,*], Lisa Pokorny[3], Steven Dodsworth[3,4], Laura R. Botigué[3,5], Robyn S. Cowan[3], Alison Devault[6], Wolf L. Eiserhardt[3,7], Niroshini Epitawalage[3], Félix Forest[3], Jan T. Kim[3], James H. Leebens-Mack[8], Ilia J. Leitch[3], Olivier Maurin[3], Douglas E. Soltis[9,10], Pamela S. Soltis[9,10], Gane Ka-Shu Wong[11,12,13], William J. Baker[3], and Norman J. Wickett[2,14]





http://mossmatters.net/blog/SequenceClusters.html

Syst. Biol. 68(4):594–606, 2019

DOI:10.1093/sysbio/syy086
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A Universal Probe Set for Targeted Sequencing of 353 Nuclear Genes from Any Flowering Plant Designed Using k-Medoids Clustering

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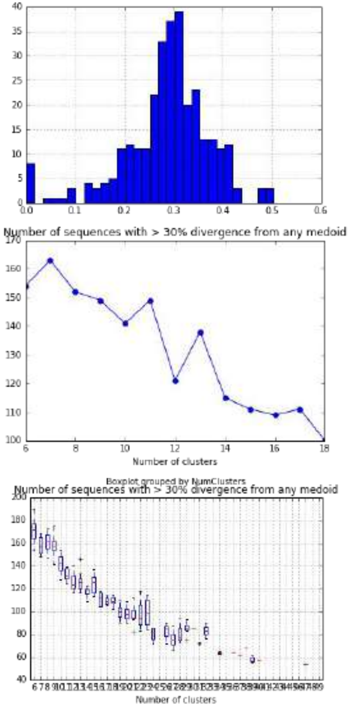
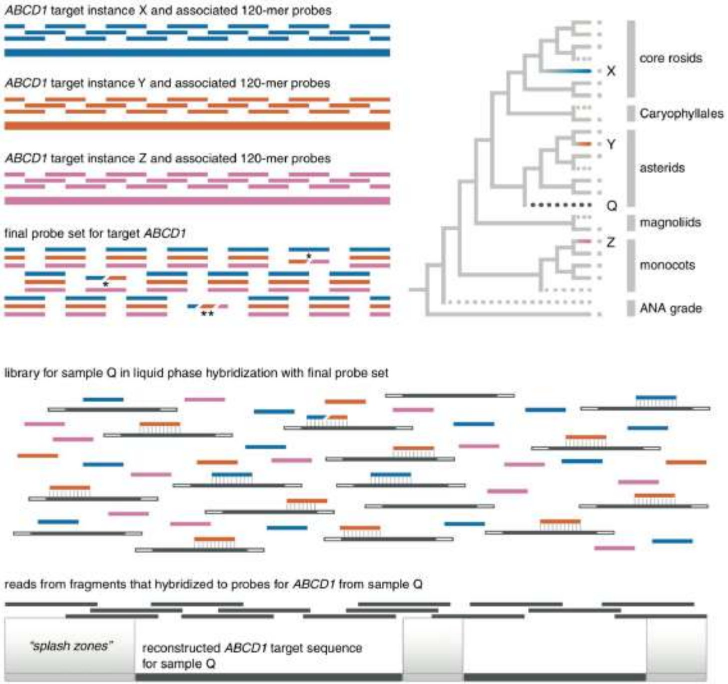




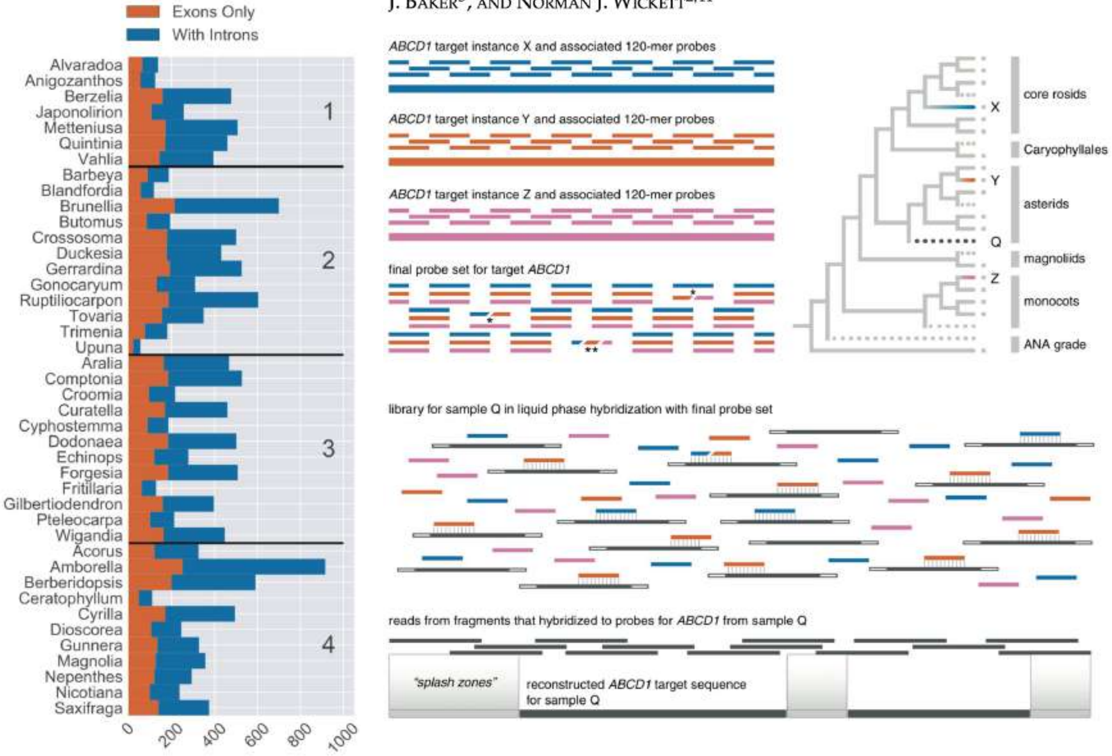
http://mossmatters.net/blog/SequenceClusters.html

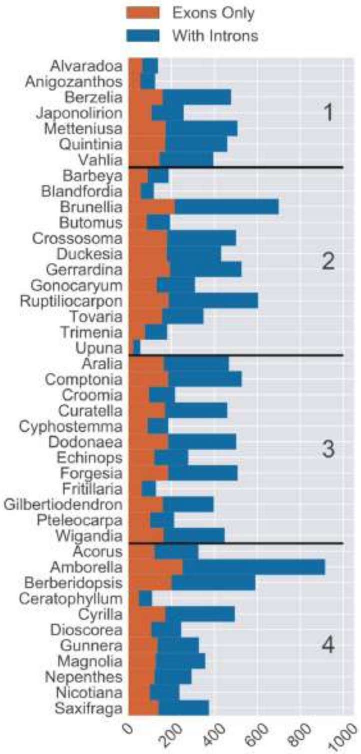
Syst. Biol. 68(4):594–606, 2019

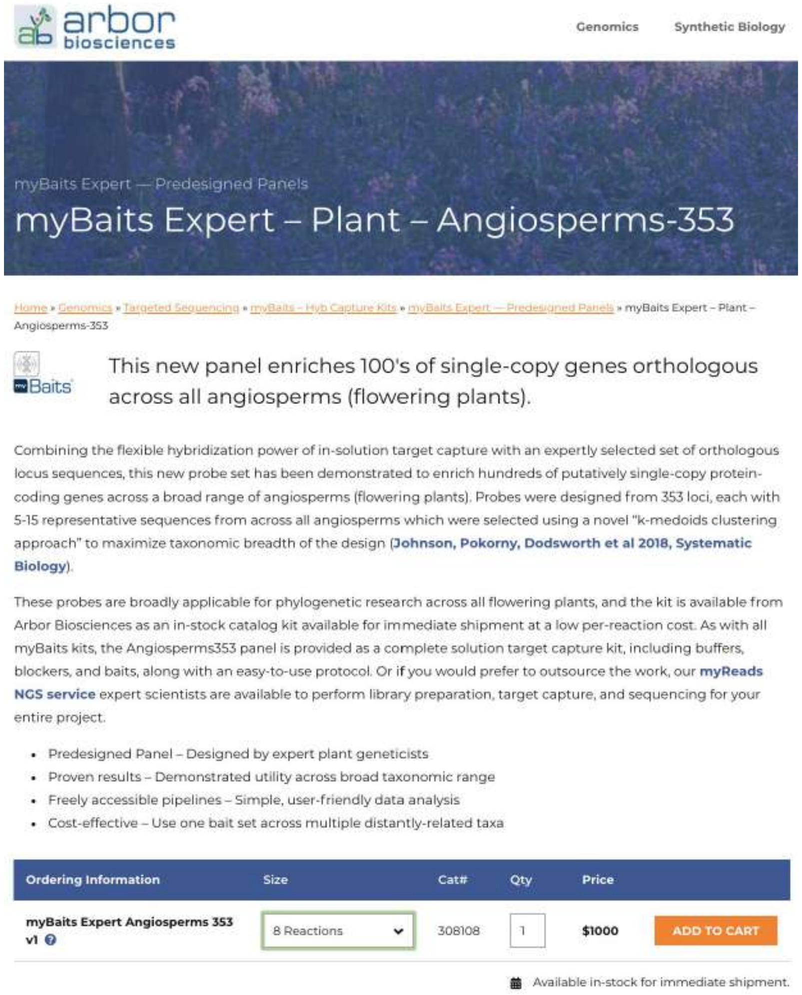
DOI:10.1093/sysbio/syy086
Advance Access publication December 10, 2018

A Universal Probe Set for Targeted Sequencing of 353 Nuclear Genes from Any Flowering Plant Designed Using k-Medoids Clustering

MATTHEW G. JOHNSON[1,2,*], LISA POKORNY[3], STEVEN DODSWORTH[3,4], LAURA R. BOTIGUÉ[3,5], ROBYN S. COWAN[3], ALISON DEVAULT[6], WOLF L. EISERHARDT[3,7], NIROSHINI EPITAWALAGE[3], FÉLIX FOREST[3], JAN T. KIM[3], JAMES H. LEEBENS-MACK[8], ILIA J. LEITCH[3], OLIVIER MAURIN[3], DOUGLAS E. SOLTIS[9,10], PAMELA S. SOLTIS[9,10], GANE KA-SHU WONG[11,12,13], WILLIAM J. BAKER[3], AND NORMAN J. WICKETT[2,14]







arbor biosciences

Genomics Synthetic Biology

myBaits Expert — Predesigned Panels

myBaits Expert – Plant – Angiosperms-353

Home » Genomics » Targeted Sequencing » myBaits – Hyb Capture Kits » myBaits Expert — Predesigned Panels » myBaits Expert – Plant – Angiosperms-353

myBaits

This new panel enriches 100's of single-copy genes orthologous across all angiosperms (flowering plants).

Combining the flexible hybridization power of in-solution target capture with an expertly selected set of orthologous locus sequences, this new probe set has been demonstrated to enrich hundreds of putatively single-copy protein-coding genes across a broad range of angiosperms (flowering plants). Probes were designed from 353 loci, each with 5-15 representative sequences from across all angiosperms which were selected using a novel "k-medoids clustering approach" to maximize taxonomic breadth of the design (**Johnson, Pokorny, Dodsworth et al 2018, Systematic Biology**).

These probes are broadly applicable for phylogenetic research across all flowering plants, and the kit is available from Arbor Biosciences as an in-stock catalog kit available for immediate shipment at a low per-reaction cost. As with all myBaits kits, the Angiosperms353 panel is provided as a complete solution target capture kit, including buffers, blockers, and baits, along with an easy-to-use protocol. Or if you would prefer to outsource the work, our **myReads NGS service** expert scientists are available to perform library preparation, target capture, and sequencing for your entire project.

- Predesigned Panel – Designed by expert plant geneticists
- Proven results – Demonstrated utility across broad taxonomic range
- Freely accessible pipelines – Simple, user-friendly data analysis
- Cost-effective – Use one bait set across multiple distantly-related taxa

Ordering Information	Size	Cat#	Qty	Price	
myBaits Expert Angiosperms 353 v1	8 Reactions	308108	1	**$1000**	ADD TO CART

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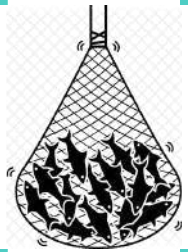
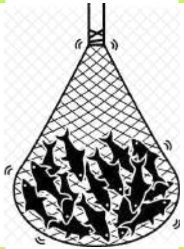
¿Panel universal o específico?

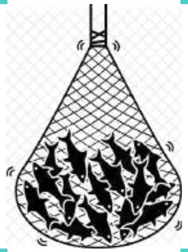


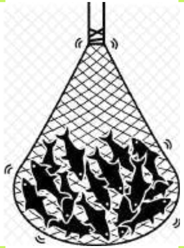


¿Panel universal o específico?

- ¿Cuál es tu hipótesis?
- ¿Qué recursos genómicos, computacionales y económicos tienes?
- ¿Y por qué no ambos?

¿Panel universal o específico?





¿Panel universal o específico?

Syst. Biol. 0(0):1–18, 2019

DOI:10.1093/sysbio/syz074

Hierarchical Hybrid Enrichment: Multitiered Genomic Data Collection Across Evolutionary Scales, With Application to Chorus Frogs (*Pseudacris*)

SARAH E. BANKER[1,2], ALAN R. LEMMON[3,*], ALYSSA BIGELOW HASSINGER[1,4], MYSIA DYE[1], SEAN D. HOLLAND[1], MICHELLE L. KORTYNA[1], OSCAR E. OSPINA[1], HANNAH RALICKI[1,5], AND EMILY MORIARTY LEMMON[1]

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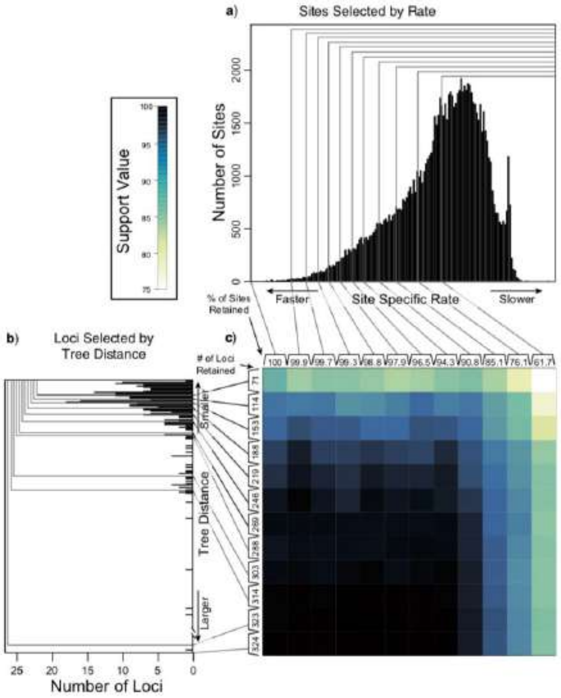
Correspondence to be sent to: Department of Scientific Computing, Florida State University 400 Dirac Science Library, Tallahassee, FL 32306 USA; E-mail: alemmon@fsu.edu.

Sarah E. Banker, Alan R. Lemmon, and Emily Moriarty Lemmon contributed equally to this article.

Received 30 June 2018; reviews returned 26 October 2019; accepted 4 November 2019
Associate Editor: Frank Burbrink

Abstract.—Determining the optimal targets of genomic subsampling for phylogenomics, phylogeography, and population genomics remains a challenge for evolutionary biologists. Of the available methods for subsampling the genome, hybrid enrichment (sequence capture) has become one of the primary means of data collection for systematics, due to the flexibility and cost efficiency of this approach. Despite the utility of this method, information is lacking as to what genomic targets are most appropriate for addressing questions at different evolutionary scales. In this study, first, we compare the benefits of target loci developed for deep- and shallow scales by comparing these loci at each of three taxonomic levels: within a genus (phylogenetics), within a species (phylogeography), and within a hybrid zone (population genomics). Specifically, we target evolutionarily conserved loci that are appropriate for deeper phylogenetic scales and more rapidly evolving loci that are informative for phylogeographic and population genomic scales. Second, we assess the efficacy of targeting multiple-locus sets for different taxonomic levels in the same hybrid enrichment reaction, an approach we term hierarchical hybrid enrichment. Third, we apply this approach to the North American chorus frog genus *Pseudacris* to answer key evolutionary questions across taxonomic and temporal scales. We demonstrate that in this system the type of genomic target that produces the most resolved gene trees differs depending on the taxonomic level, although the potential for error is substantially lower for the deep-scale loci at all levels. We successfully recover data for the two different locus sets with high efficiency. Using hierarchical data targeting deep and shallow levels: we 1) resolve the phylogeny of the genus *Pseudacris* and introduce a novel visual and hypothesis testing method that uses nodal heat maps to examine the robustness of branch support values to the removal of sites and loci; 2) estimate the phylogeographic history of *Pseudacris feriarum*, which reveals up to five independent invasions leading to sympatry with congener *Pseudacris nigrita* to form replicated reinforcement contact zones with ongoing gene flow into sympatry; and 3) quantify with high confidence the frequency of hybridization in one of these zones between *P. feriarum* and *P. nigrita*, which is lower than microsatellite-based estimates. We find that the hierarchical hybrid enrichment approach offers an efficient, multitiered data collection method for simultaneously addressing questions spanning multiple evolutionary scales. [Anchored hybrid enrichment; heat map; hybridization; phylogenetics; phylogeography; population genomics; reinforcement; reproductive character displacement.]



¿Panel universal o específico?

ORIGINAL RESEARCH ARTICLE

Front. Plant Sci., 09 January 2020 | https://doi.org/10.3389/fpls.2019.01655

Check for updates

Tackling Rapid Radiations With Targeted Sequencing

Isabel Larridon[1,2*†], Tamara Villaverde[3,4,5*†], Alexandre R. Zuntini[1], Lisa Pokorny[1,3,6], Grace E. Brewer[1], Niroshini Epitawalage[1], Isabel Fairlie[1,7], Marlene Hahn[4], Jan Kim[1], Enrique Maguilla[4,8], Olivier Maurin[1], Martin Xanthos[1], Andrew L. Hipp[4,5], Félix Forest[1] and William J. Baker[1]

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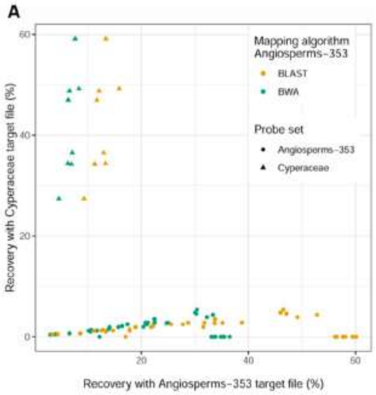
[2]Systematic and Evolutionary Botany Lab, Department of Biology, Ghent University, Ghent, Belgium
[3]Real Jardín Botánico (RJB-CSIC), Madrid, Spain
[4]The Morton Arboretum, Lisle, IL, United States
[5]The Field Museum, Chicago, IL, United States
[6]Centre for Plant Biotechnology and Genomics (CBGP, UPM-INIA), Madrid, Spain
[7]Department of Animal and Plant Sciences, University of Sheffield, Sheffield, United Kingdom
[8]Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Sevilla, Spain



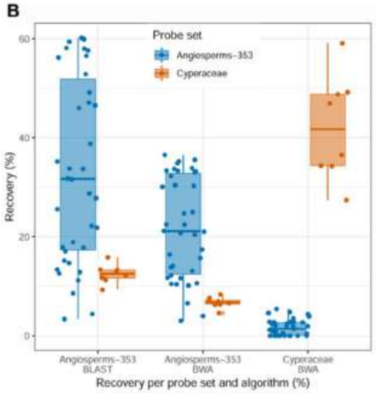


TABLE 2 | Length of the aligned contigs and number of parsimony informative sites (PIS) for data obtained after enrichment with the Angiosperms-353 and Cyperaceae-specific probes.

		Contig	PIS
Angiosperms-353	Mean	751	75
(38 accessions)	SD	438	65
Dataset 1	Min	87	0
	Max	3,103	439
	Total	**233,429**	**23,217**
Cyperaceae-specific	Mean	1,608	63
(9 accessions)	SD	830	59
Dataset 2	Min	93	0
	Max	7,527	479
	Total	**683,427**	**26,630**
Angiosperms-353	Mean	717	25
(subset of 8	SD	411	28
accessions)	Min	150	0
Dataset 3	Max	2,826	147
	Total	**221,564**	**7,613**
Cyperaceae-specific	Mean	1,471	50
(8 accessions)	SD	818	51
Dataset 4	Min	162	0
	Max	7,524	400
	Total	**667,945**	**22,767**

4.

Análisis bioinformáticos

- Calidad
- Procesado
- Filogenómica
- Ploidía

Calidad

Recorta

Pega

Colorea

Calidad

Datos en bruto

Secuencias

Calidad

Datos en bruto

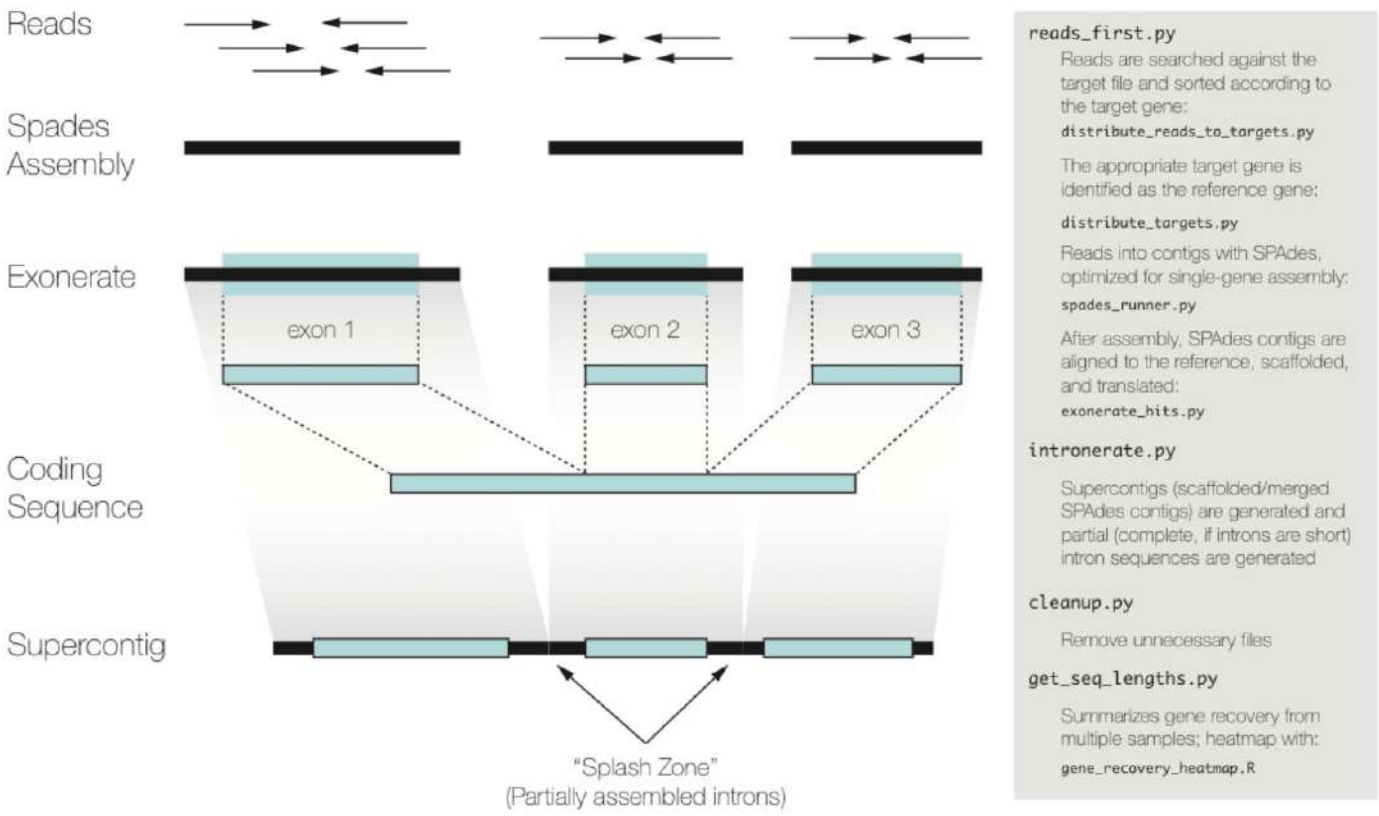
FastQC | Trimmomatic

BLASTx | BWA

SPAdes | exon(intron)erate

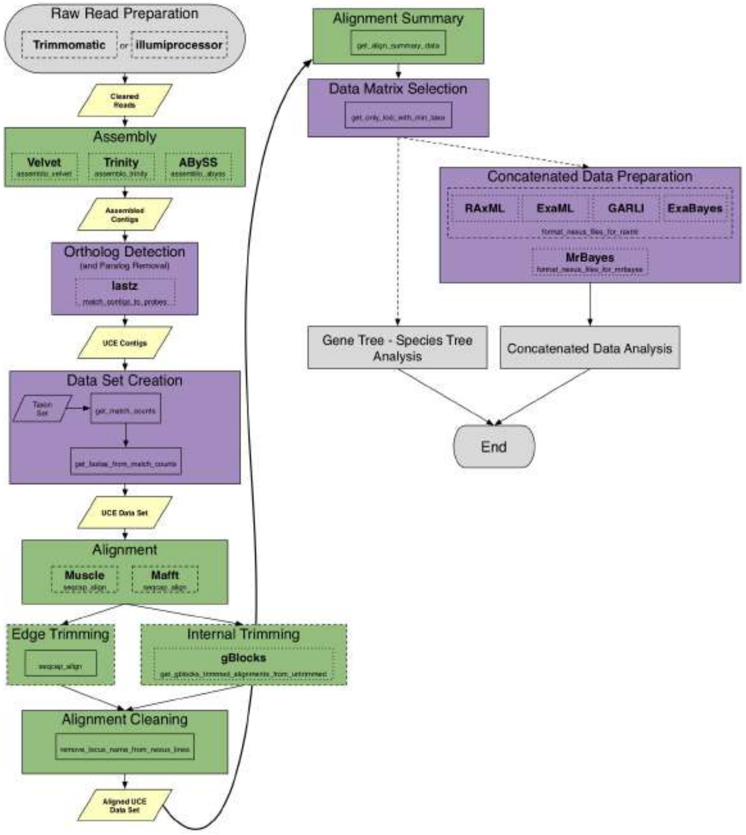
HybPiper

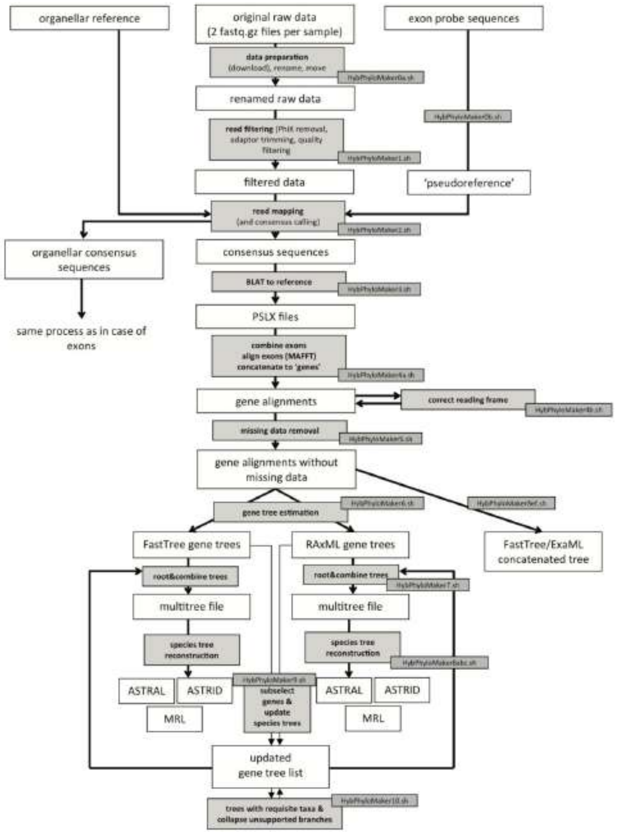
Secuencias



HybPiper

https://github.com/mossmatters/HybPiper/wiki





PHYLUCE

HybPhyloMaker

https://phyluce.readthedocs.io/en/latest/

Fig. 1. Fér & Schmickl. 2018. *Evol. Bioinfo.* 14: 1–9

Procesado

Alinea

Poda y enmascara

Refina

Procesado

Secuencias

Alinea

Poda y enmascara

Refina

Alineamientos

Procesado

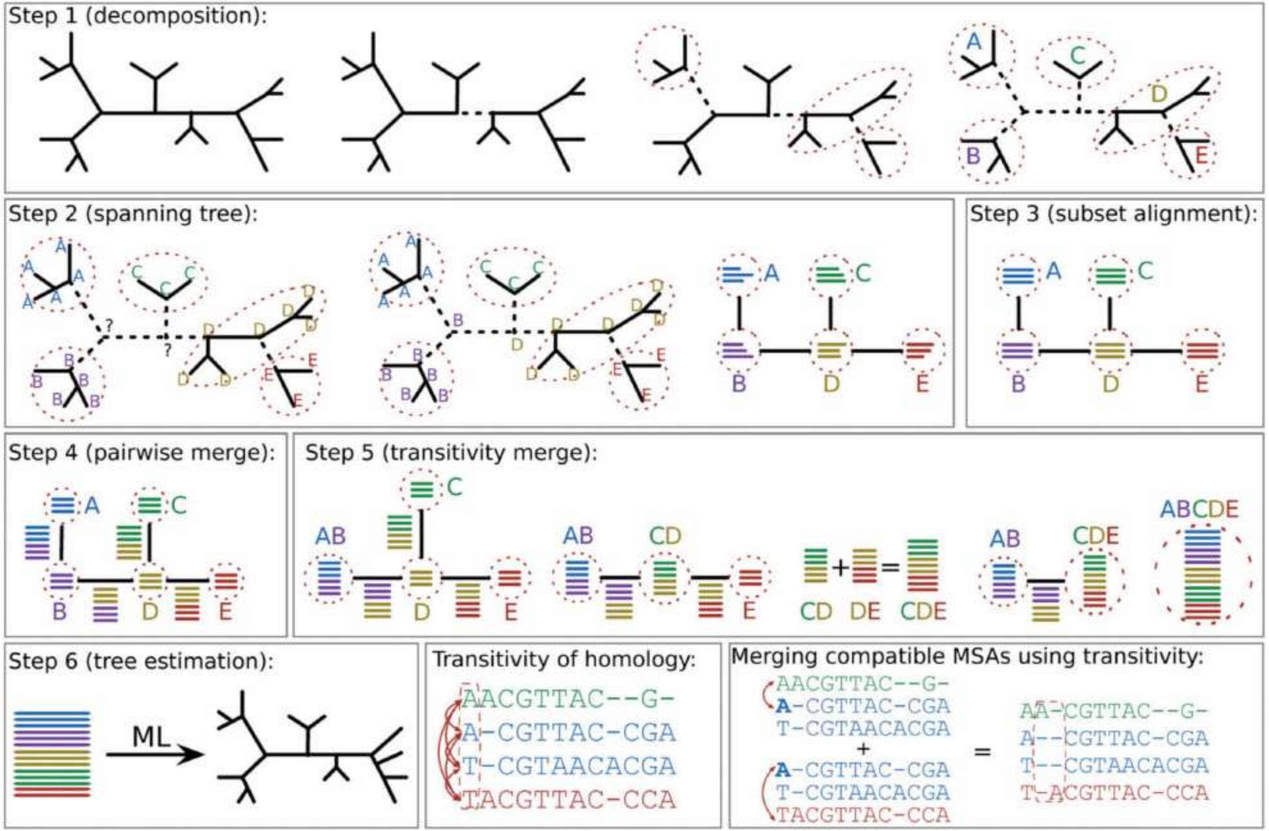
Secuencias

MAFFT | PASTA | UPP

AMAS | trimAl

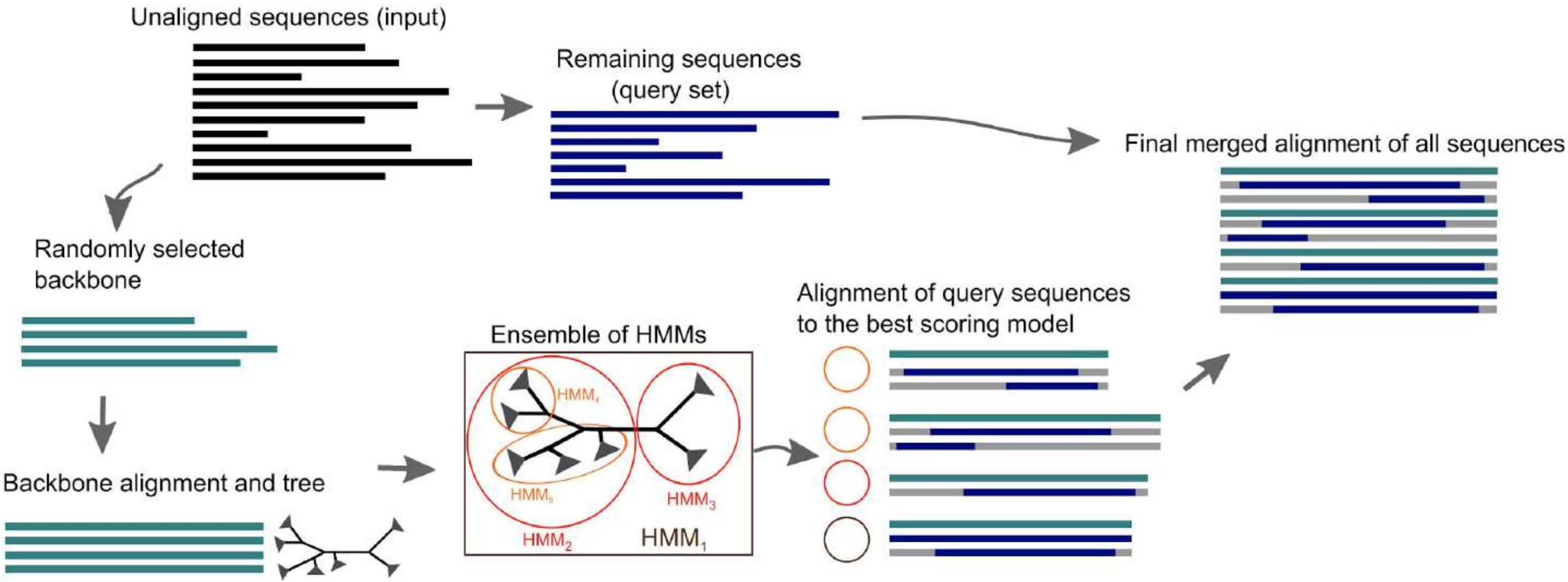
FastTree | TreeShrink

Alineamientos



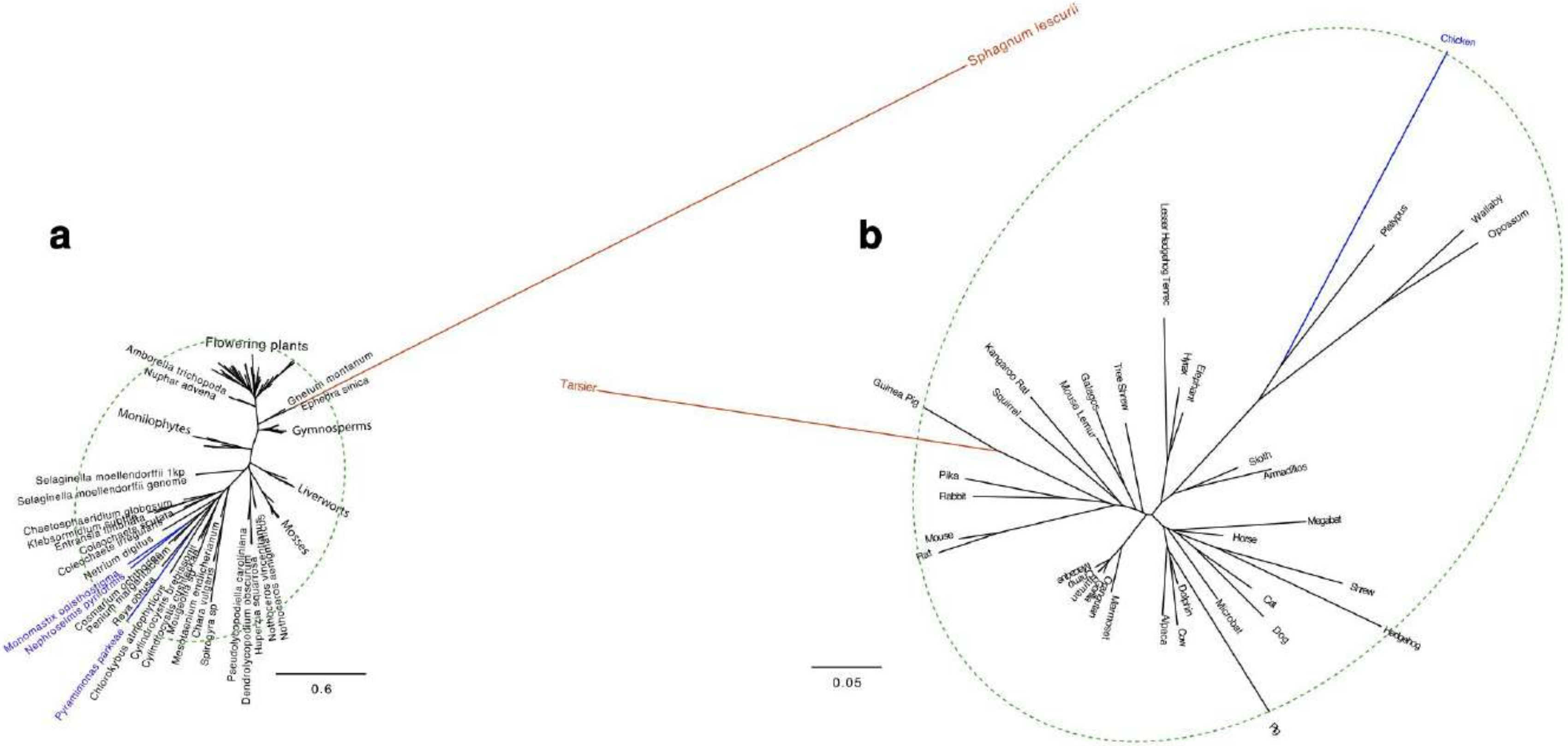
PASTA

Fig. 1. Mirarab et al. 2015. *J. Comput. Biol.* 22 (5): 377–386



UPP

Fig. 2. Nguyen et al. 2015. *Genome Biol.* 16: 123


a
b
Sphagnum lescurii
Flowering plants
Amborella trichopoda
Nuphar advena
Gnetum montanum
Ephedra sinica
Gymnosperms
Monilophytes
Selaginella moellendorffii 1kp
Selaginella moellendorffii genome
Liverworts
Mosses
Monomastix opisthostigma
Nephroselmis pyriformis
Pyramimonas parkeae
0.6
Tarsier
Chicken
Platypus
Wallaby
Opossum
Lesser Hedgehog Tenrec
Hyrax
Elephant
Tree Shrew
Galagos
Mouse Lemur
Kangaroo Rat
Squirrel
Guinea Pig
Pika
Rabbit
Mouse
Rat
Sloth
Armadillos
Megabat
Horse
Shrew
Cat
Dog
Hedgehog
Microbat
Dolphin
Cow
Alpaca
Pig
Marmoset
0.05

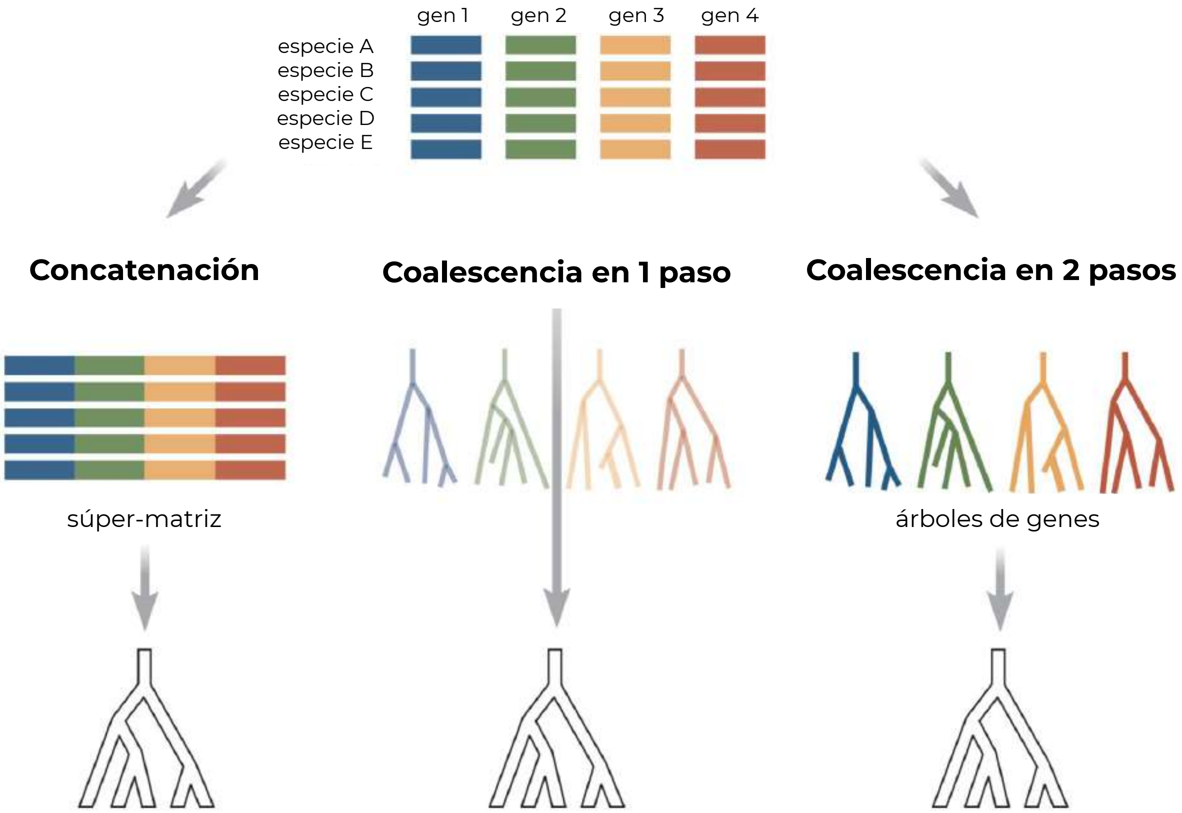
TreeShrink

Filogenómica

Filogenómica

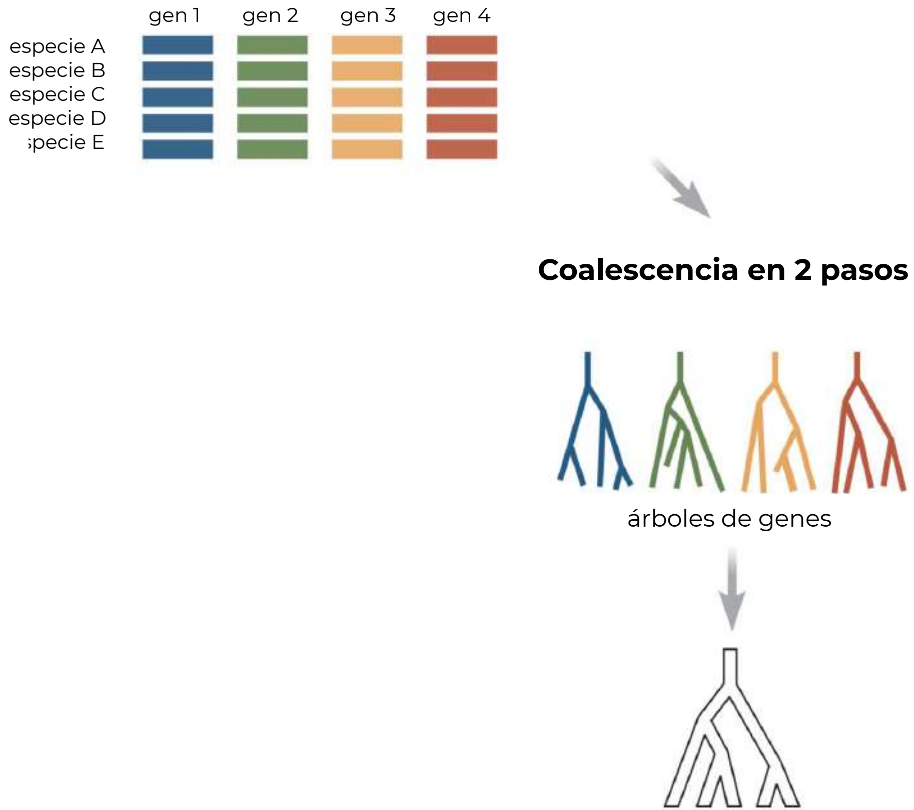
Alineamientos

Filogenias



Árboles de genes vs. árboles de especies

Fig. 1. Liu et al. 2015. *Ann. N. Y. Acad. Sci* 1360: 36–53



Árboles de genes vs. árboles de especies

Fig. 1. Liu et al. 2015. *Ann. N. Y. Acad. Sci* 1360: 36–53

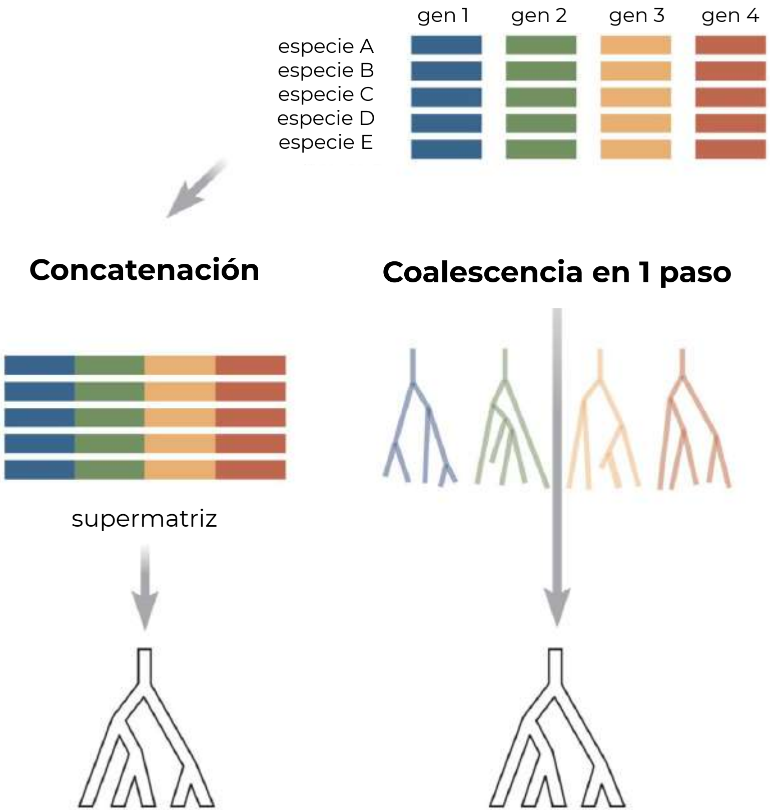
Filogenómica

Alineamientos

RAxML-NG | IQ-tree | PhyML

ASTRAL III

Filogenias



Árboles de genes vs. árboles de especies

Fig. 1. Liu et al. 2015. *Ann. N. Y. Acad. Sci* 1360: 36–53

Filogenómica

Alineamientos

ML | PoMo | SVDquartets

Filogenias

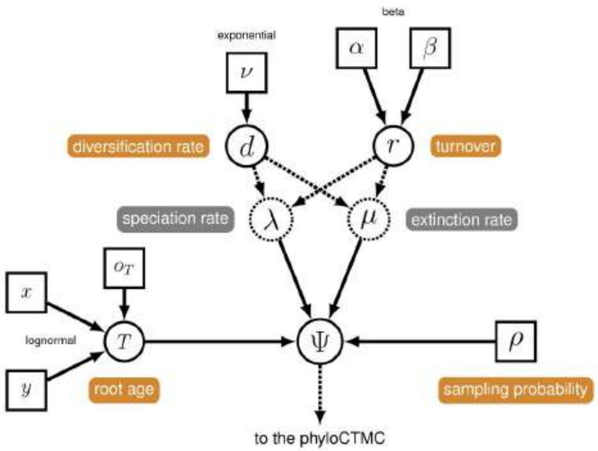
Filogenómica

Filogenómica

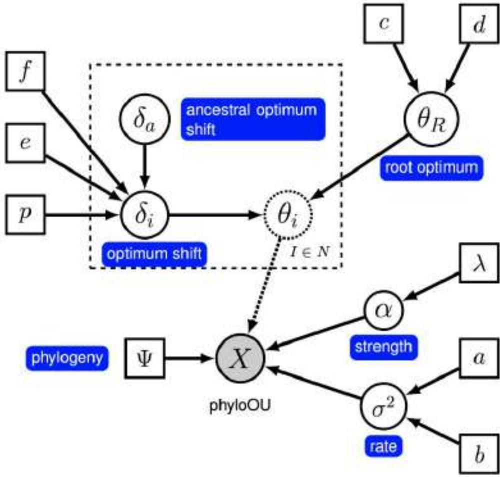
Alineamientos

 BEAST 1.x y 2.x | RevBayes 

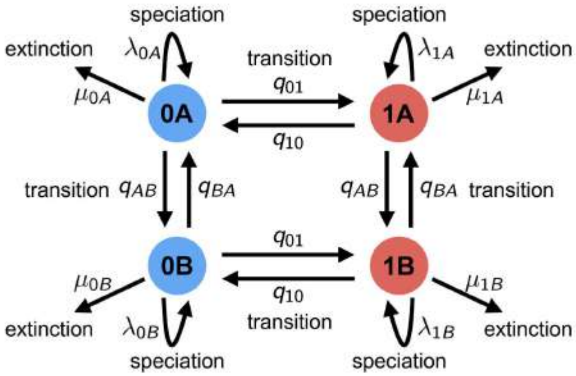
Filogenias



Tiempos de divergencia
(Birth-Death)



Métodos comparativos
(Ornstein-Uhlenbeck)



Tasas de diversificación
(HiSSE)

RevBayes

Filogenómica

Alineamientos

RAxML-NG | IQ-tree | PhyML

PAML

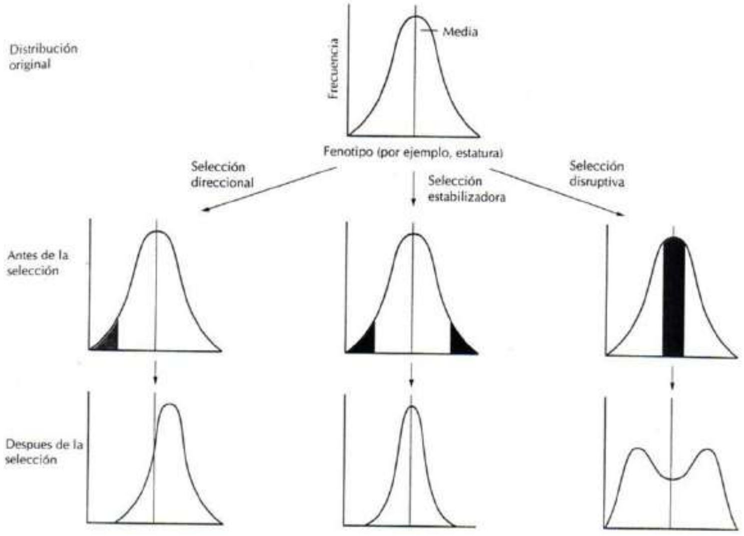
PAML | HyPhy

HyPhy

Filogenias

HyPhy

- **GARD** (**G**enetic **A**lgorithm for **R**ecombination **D**etection)
- **FEL** (**F**ixed **E**ffects **L**ikelihood)
- **SLAC** (**S**ingle-**L**ikelihood **A**ncestor **C**ounting)
- **FUBAR** (**F**ast, **U**nconstrained **B**ayesian **A**pp**R**oximation)
- **MEME** (**M**ixed **E**ffects **M**odel of **E**volution)
- **aBSREL** (**a**daptive **B**ranch-**S**ite **R**andom **E**ffects **L**ikelihood)
- **BUSTED** (**B**ranch-**S**ite **U**nrestricted **S**tatistical **T**est for **E**pisodic **D**iversification)
- **RELAX**



http://www.hyphy.org/getting-started/#characterizing-selective-pressures

ORIGINAL RESEARCH ARTICLE

Front. Plant Sci., 24 July 2019 | https://doi.org/10.3389/fpls.2019.00937

A Target Capture-Based Method to Estimate Ploidy From Herbarium Specimens

Juan Viruel[1*], María Conejero[1], Oriane Hidalgo[1,2], Lisa Pokorny[1], Robyn F. Powell[1], Félix Forest[1], Michael B. Kantar[3], Marybel Soto Gomez[4,5], Sean W. Graham[4,5], Barbara Gravendeel[6,7,8], Paul Wilkin[1] and Ilia J. Leitch[1]

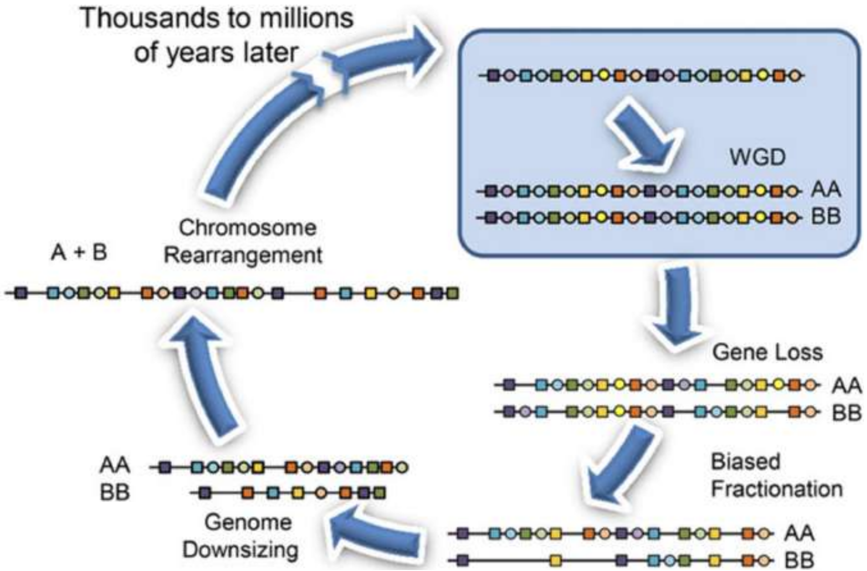
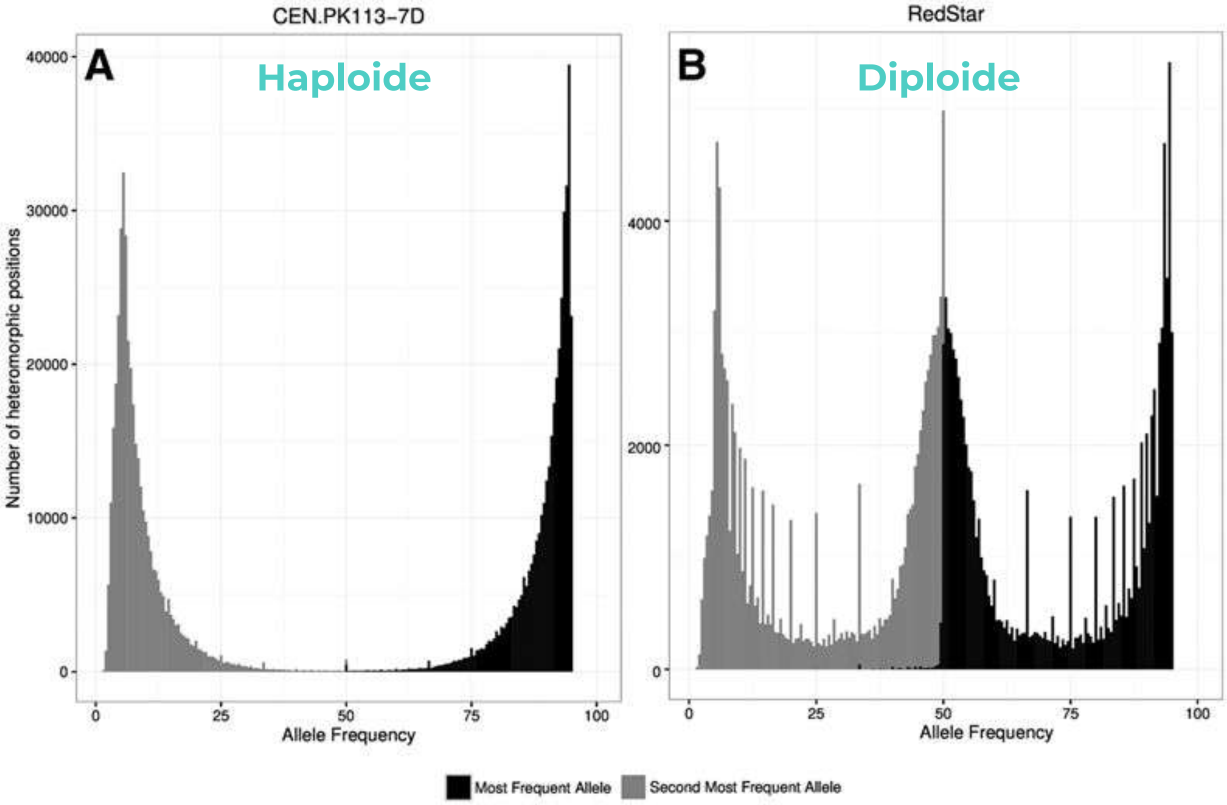
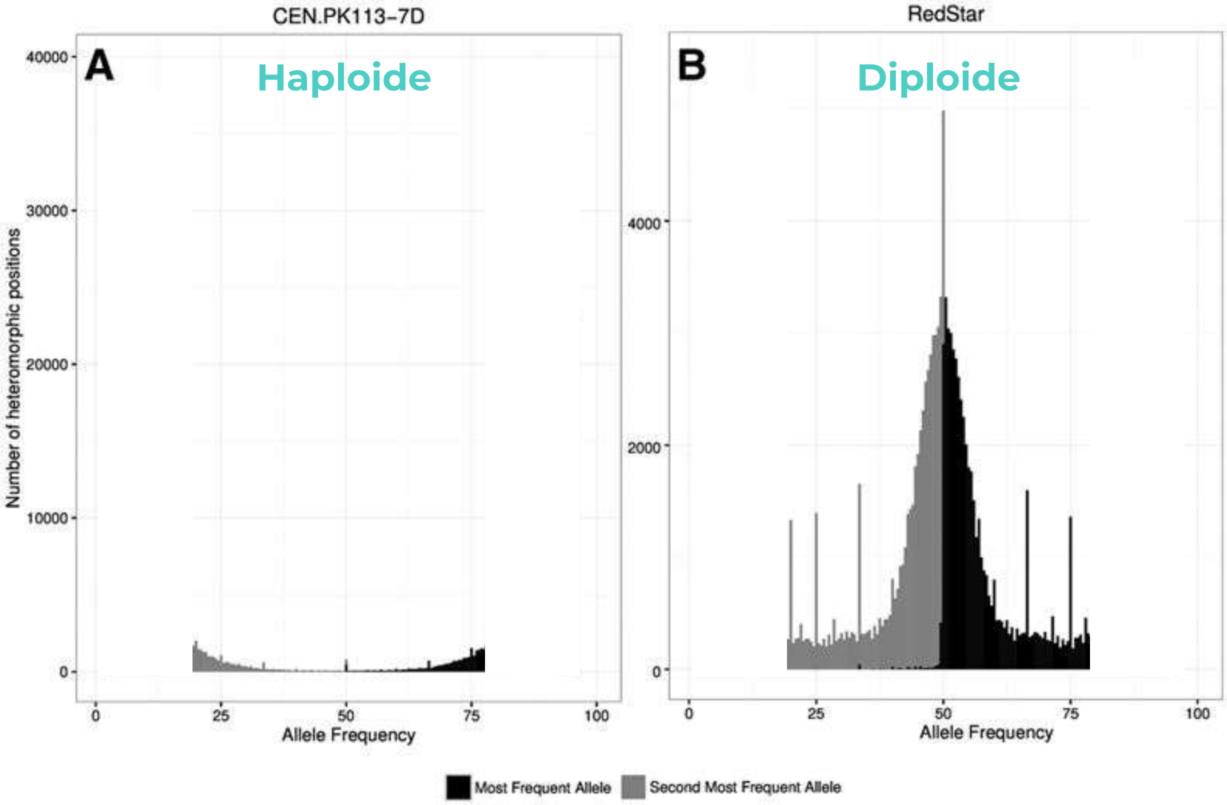


Fig. 1. Wendel. 2015. *Am. J. Bot.* 102(11): 1753–1756



Visualización del nivel de ploidía
ploidyNGS

Fig. 1. Corrêa dos Santos et al. 2017. *Bioinformatics* 33(16): 2575–2576



Visualización del nivel de ploidía
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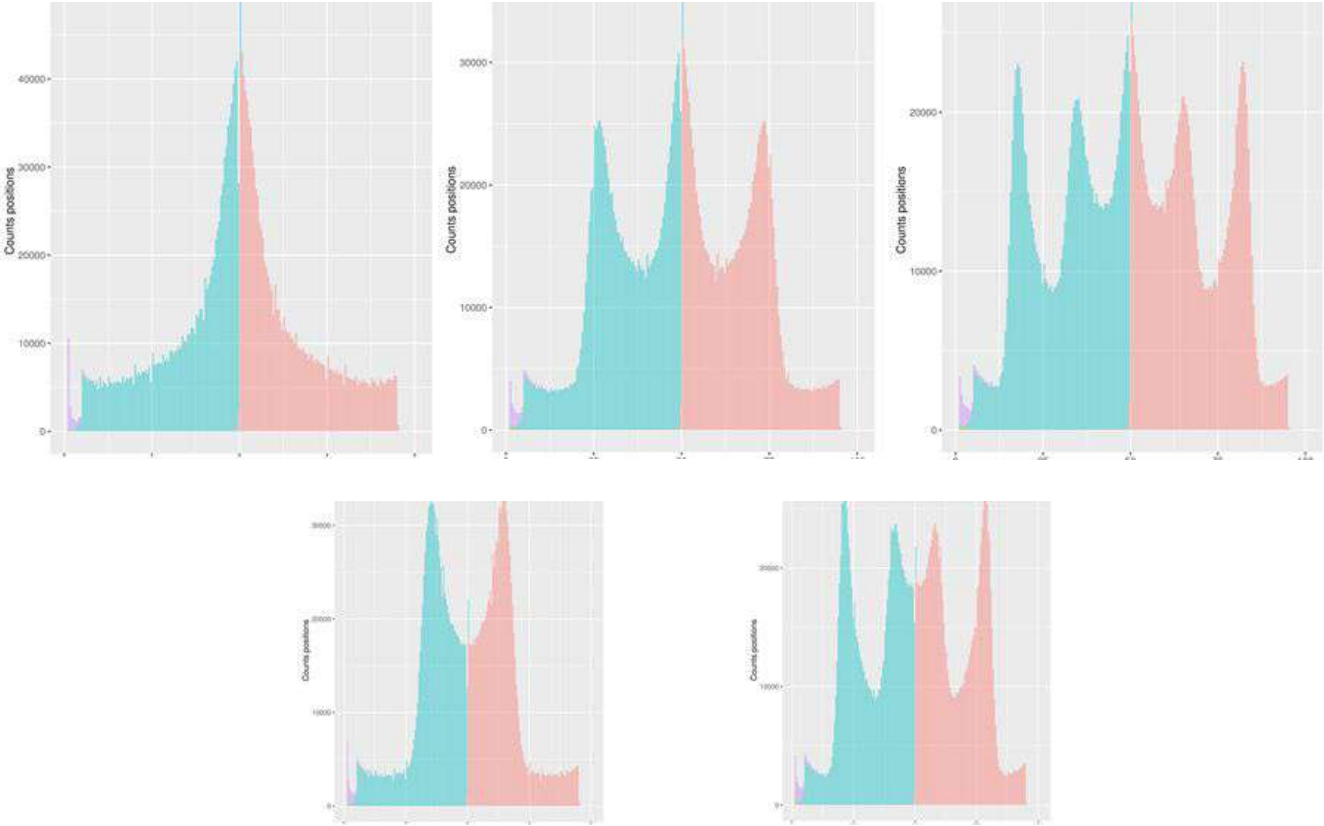
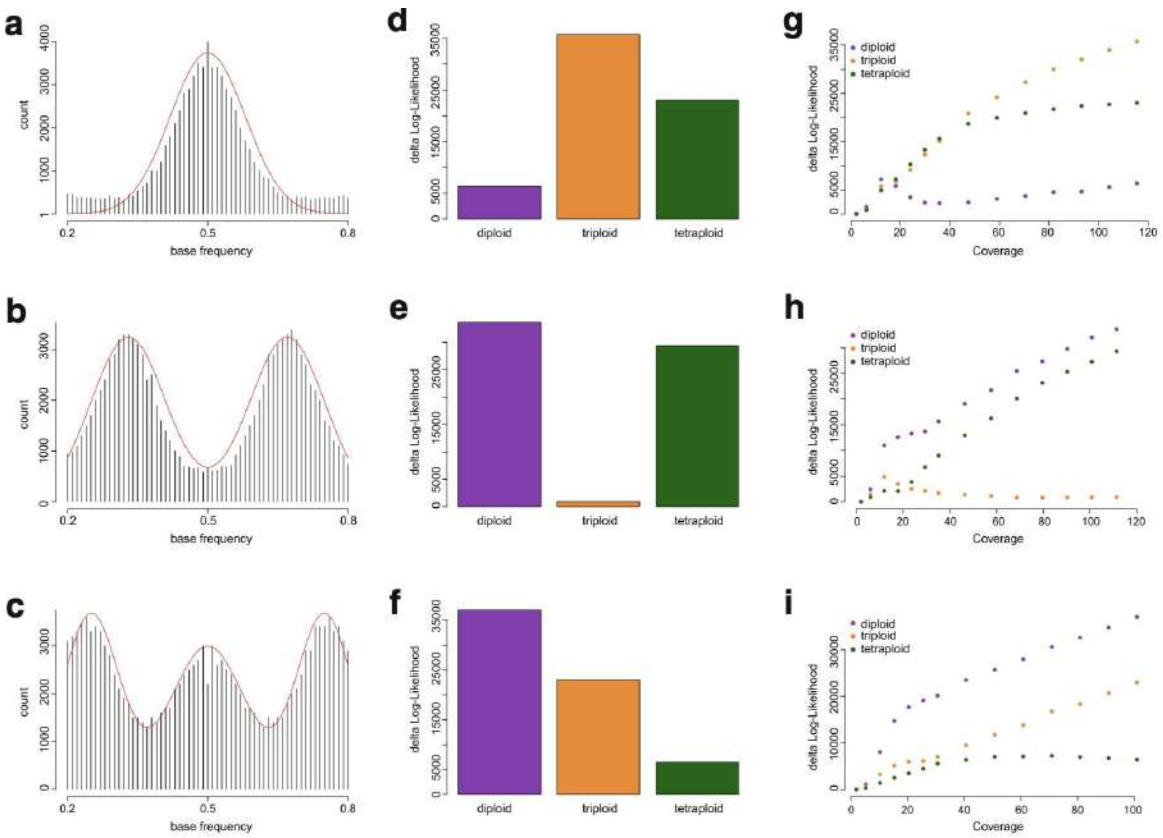


Histogramas obtenidos con simulaciones

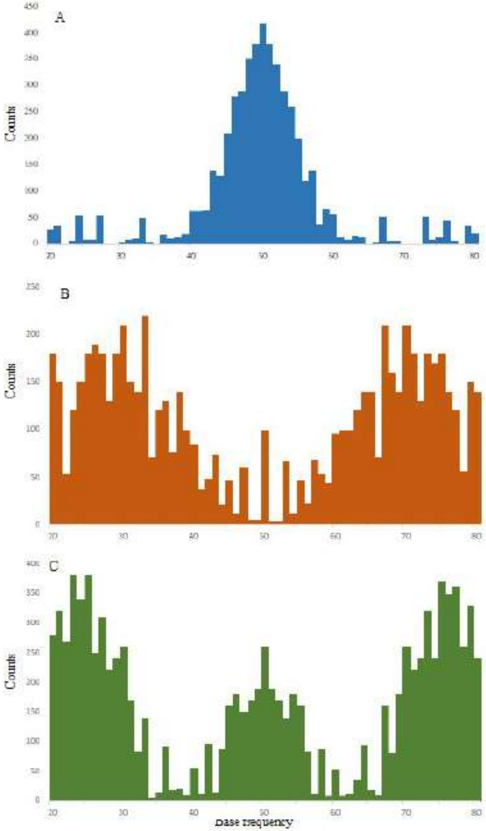
https://github.com/diriano/ploidyNGS



Estimación del nivel de ploidía

nQuire

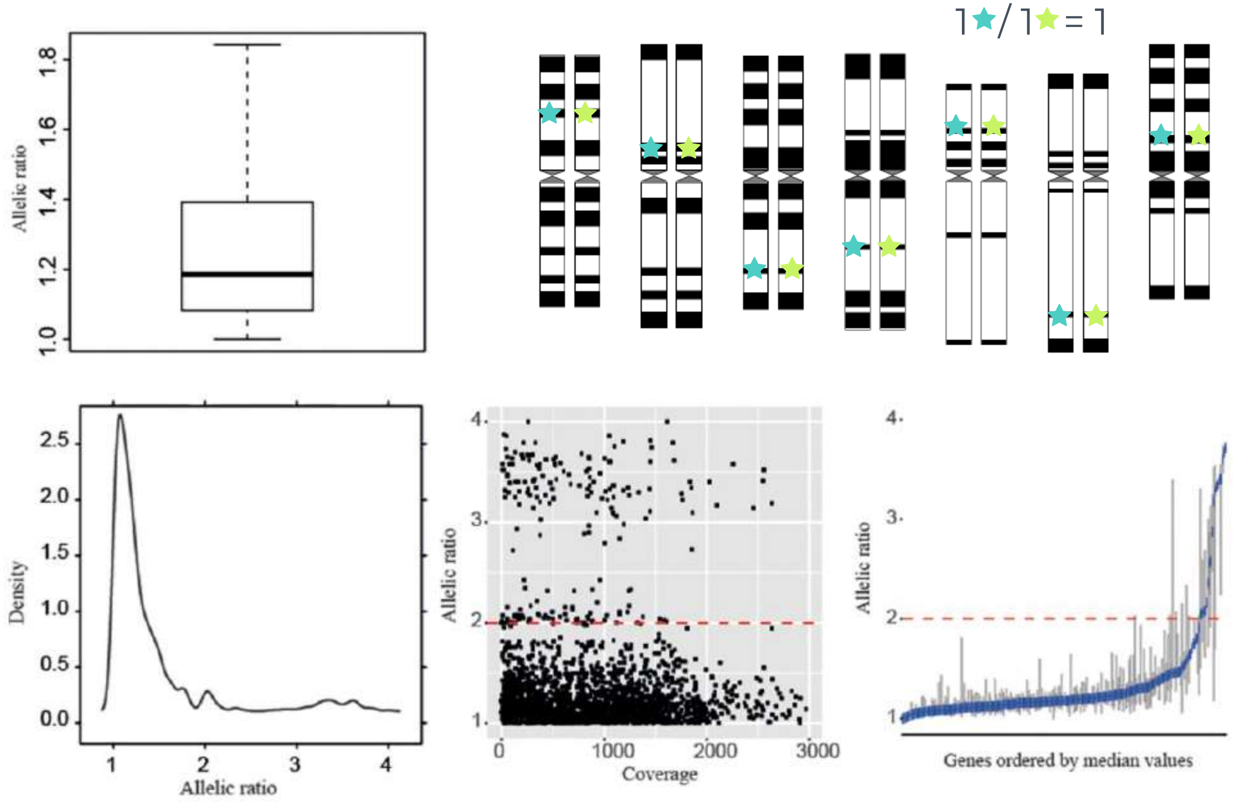
Fig. 2. Weiß et al. 2018. BMC Bioinformatics 19: 122. Disponible en https://github.com/clwgg/nQuire



M	log*L*	ΔlogL	SSR	y-y slope	std.Err	R^2	Histogram
2x	4123.4	**567.2**	**0.006**	**0.648**	**0.023**	**0.927**	
3x	1622.9	3067.6	0.056	-0.523	0.107	0.285	
4x	2774.9	1915.6	0.024	0.107	0.197	0.004	
2x	1213.0	2446.5	0.066	-0.261	0.031	**0.532**	
3x	3311.7	**347.8**	**0.014**	**0.264**	0.058	**0.257**	
4x	2800.8	858.8	**0.011**	0.072	**0.105**	0.007	
2x	1296.8	5353.6	0.052	-0.050	**0.058**	0.012	
3x	2949.3	3701.1	0.035	-0.220	0.080	0.112	
4x	6361.5	**288.9**	**0.002**	**0.883**	**0.067**	**0.764**	

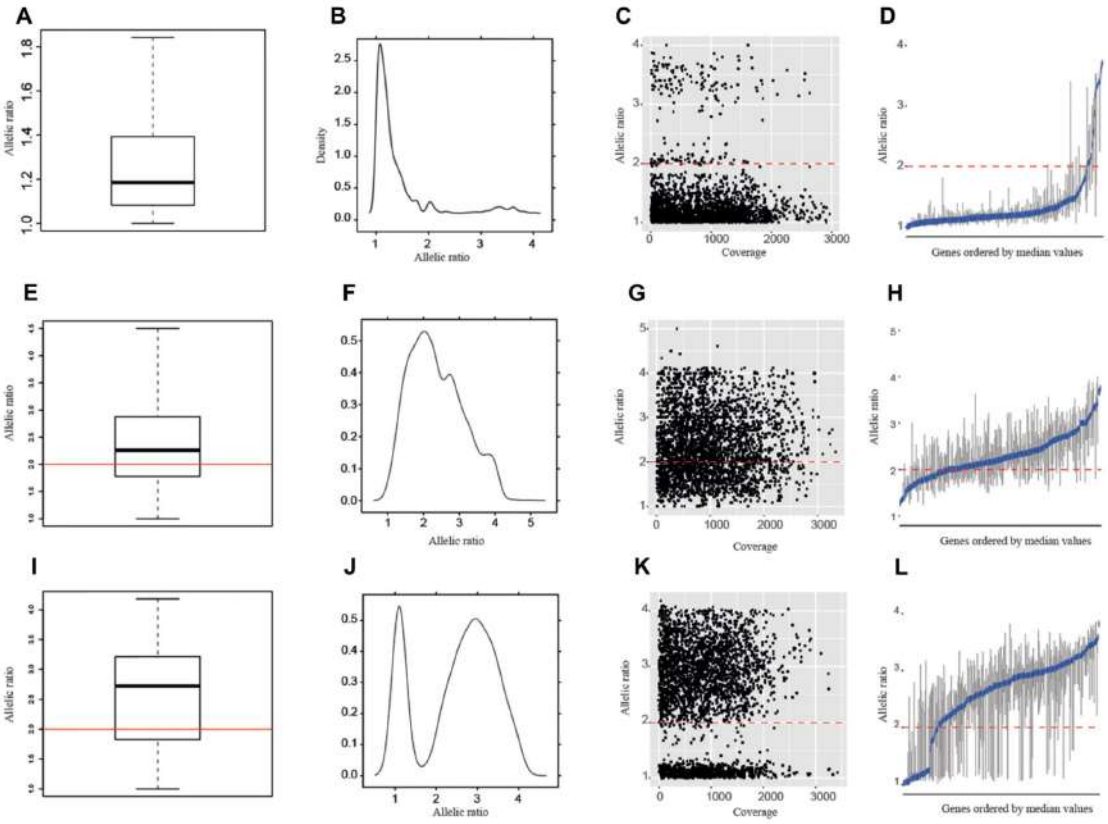
Secuencias producidas con **Hyb-Seq**

Fig. 2. Viruel et al. 2019. *Front. Plant Sci.* 10: 937



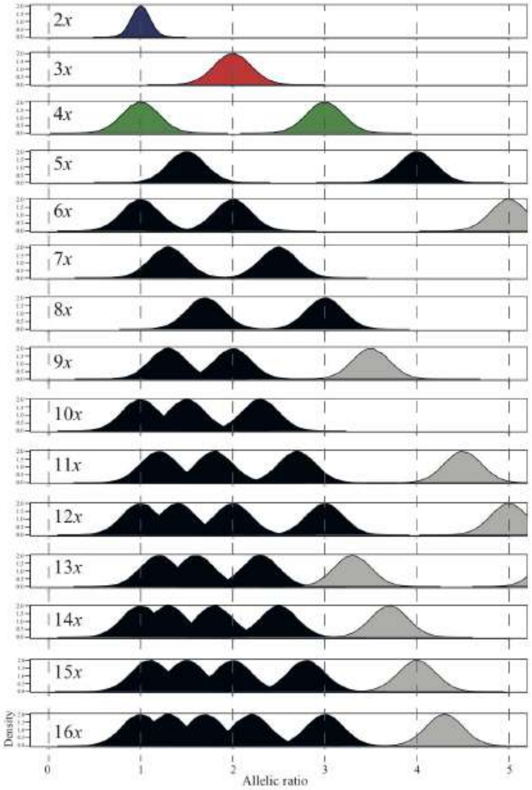
Ploidía estimada con datos de **Hyb-Seq** de material de herbario

Fig. 3. Viruel et al. 2019. *Front. Plant Sci.* 10: 937



Ploidía estimada con datos de **Hyb-Seq** de material de herbario

Fig. 3. Viruel et al. 2019. *Front. Plant Sci.* 10: 937



Datos en bruto

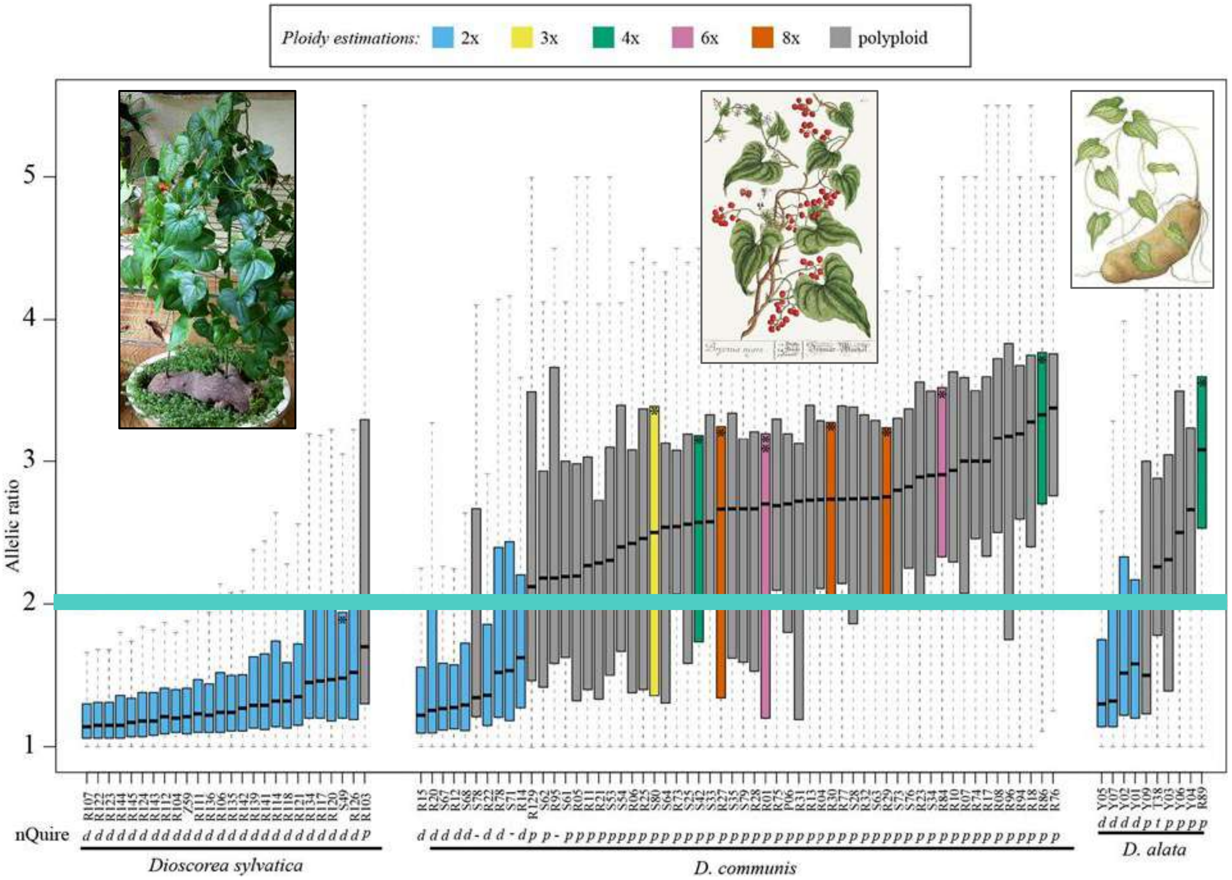
FastQC+Trimmomatic

BWA+ploidyNGS

nQuire+R

Ploidía

Fig. S5b. Viruel et al. 2019. *Front. Plant Sci.* 10: 937



Ploidía estimada con datos de **Hyb-Seq** de material de herbario

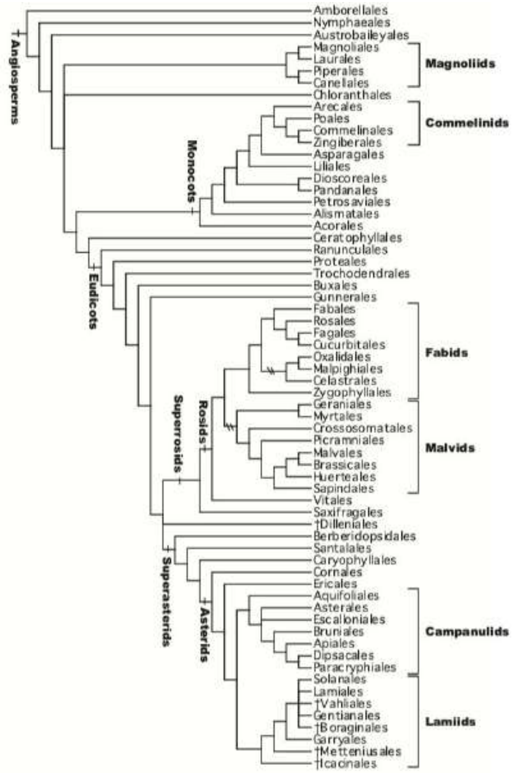
Fig. 3. Viruel et al. 2019. *Front. Plant Sci.* 10: 937

5.

Ejemplos:

- Flores 
- Tabaibas 
- Juncias 
- Chefleras 

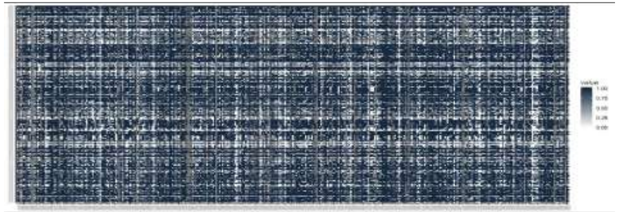
Órdenes y familias APG IV



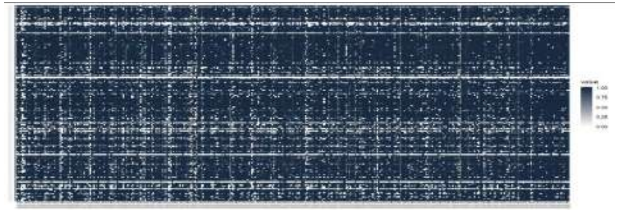
- Transcriptomas **1KP**
- Captura con **angiosperms-353**
- **SRAs** (RNA-seq) de NCBI

Órdenes y familias APG IV

- Transcriptomas **1KP**

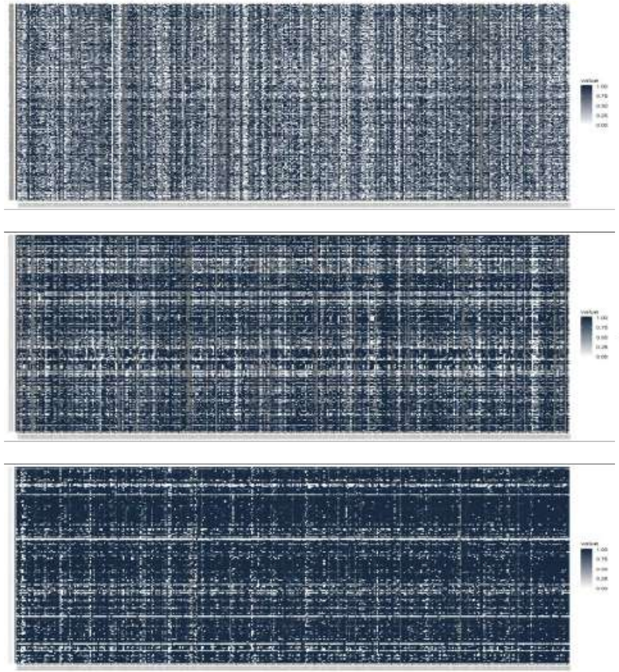


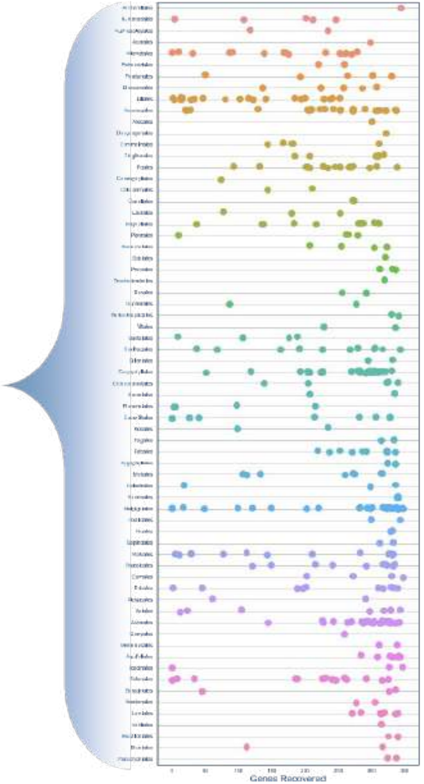
- Captura con **angiosperms-353**



- **SRAs** (RNA-seq) de NCBI

Captura y minado





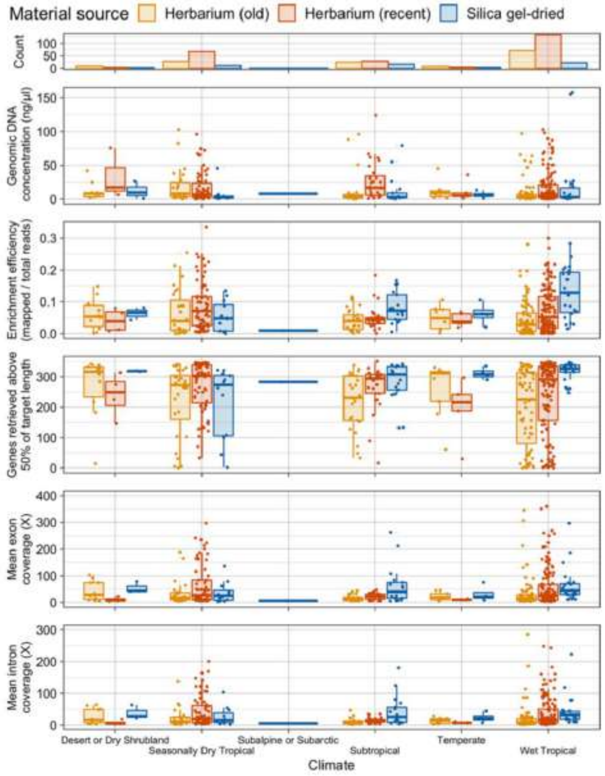
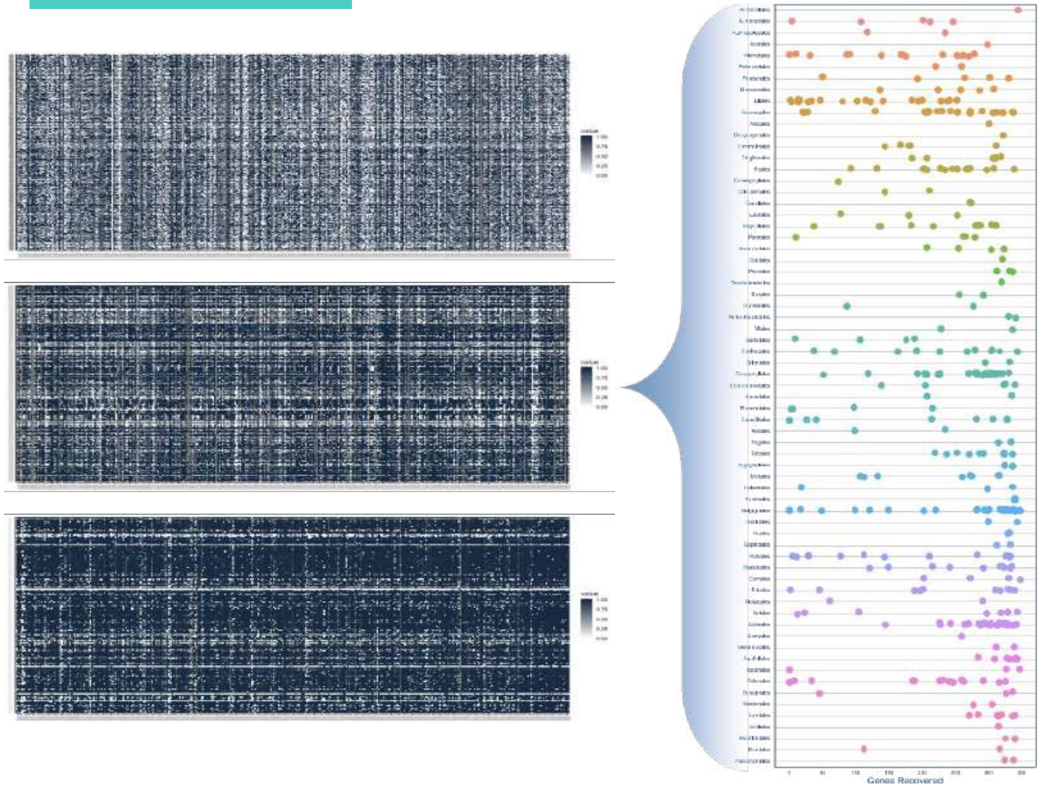


Fig. 4. Brewer et al. 2019. *Front. Plant Sci.* 10: 1102

Análisis filogenómicos



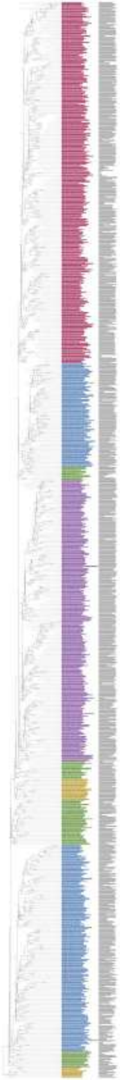
Datos en bruto

trimmomatic

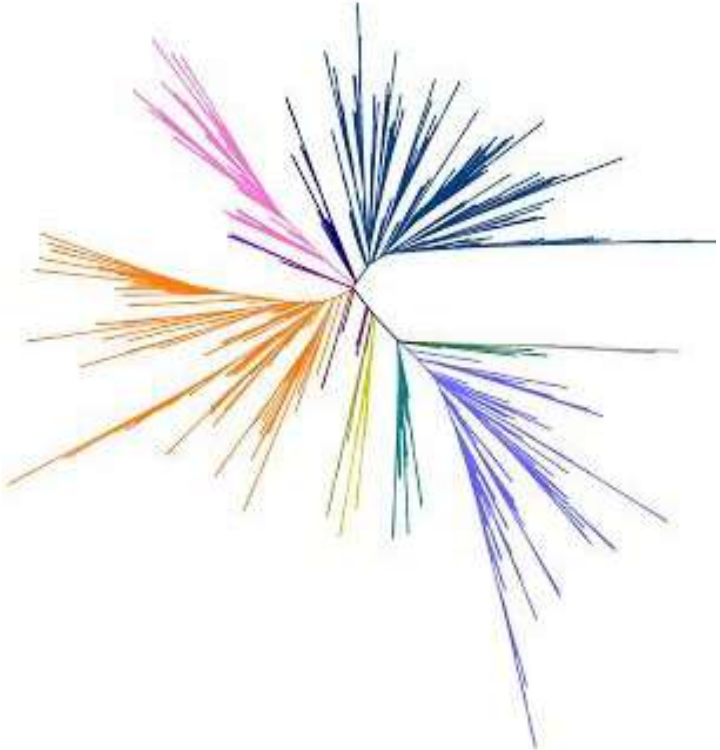
HybPiper

UPP+TS+trimAl

Alineamientos



Resultados preliminares



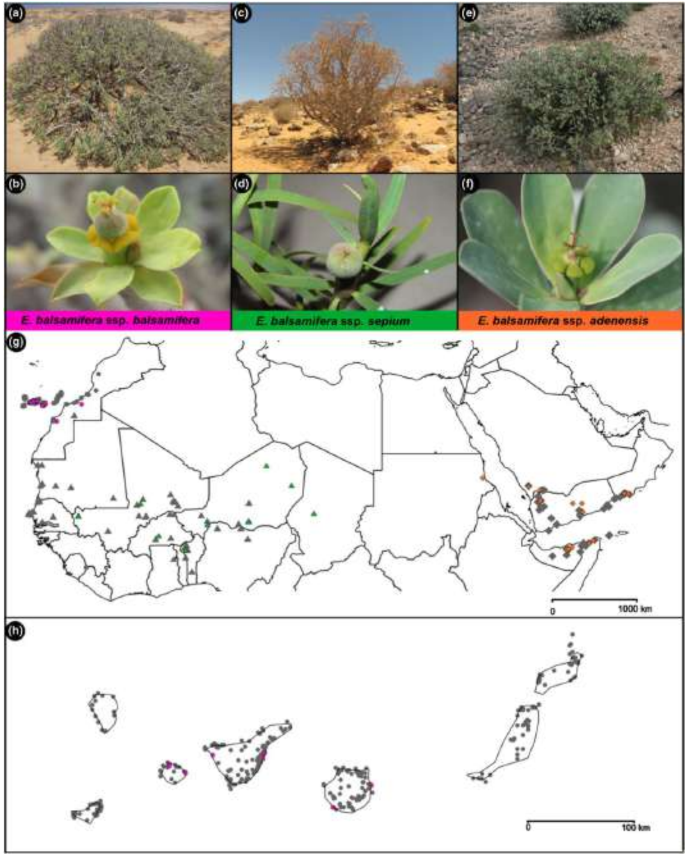
Alineamientos

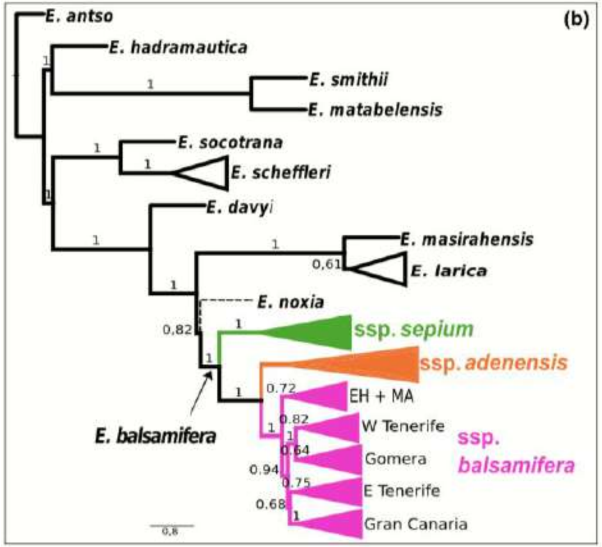
IQ-tree+UFboot

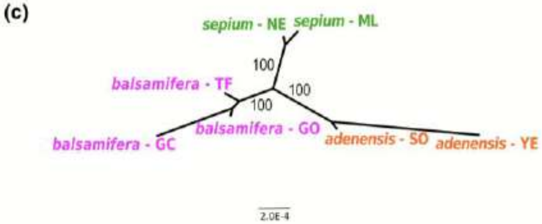
ASTRAL+LPP

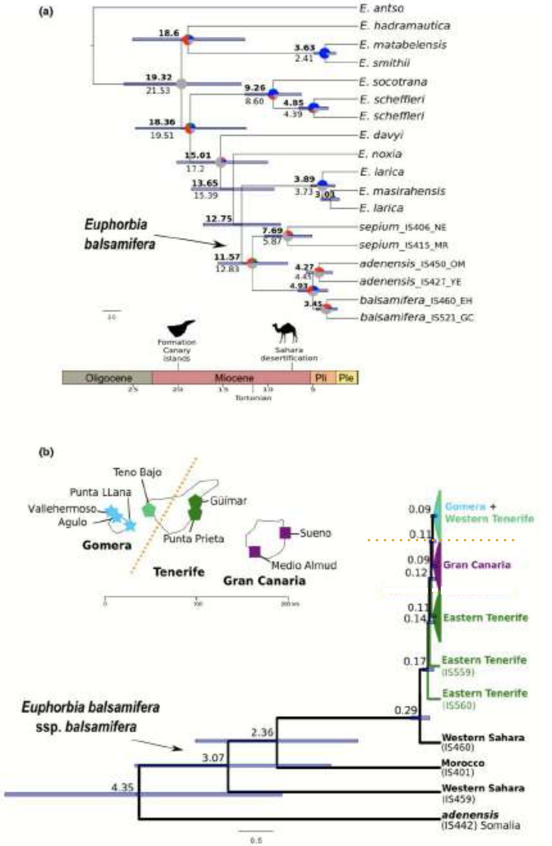
SortaDate+BEAST

Filogenias



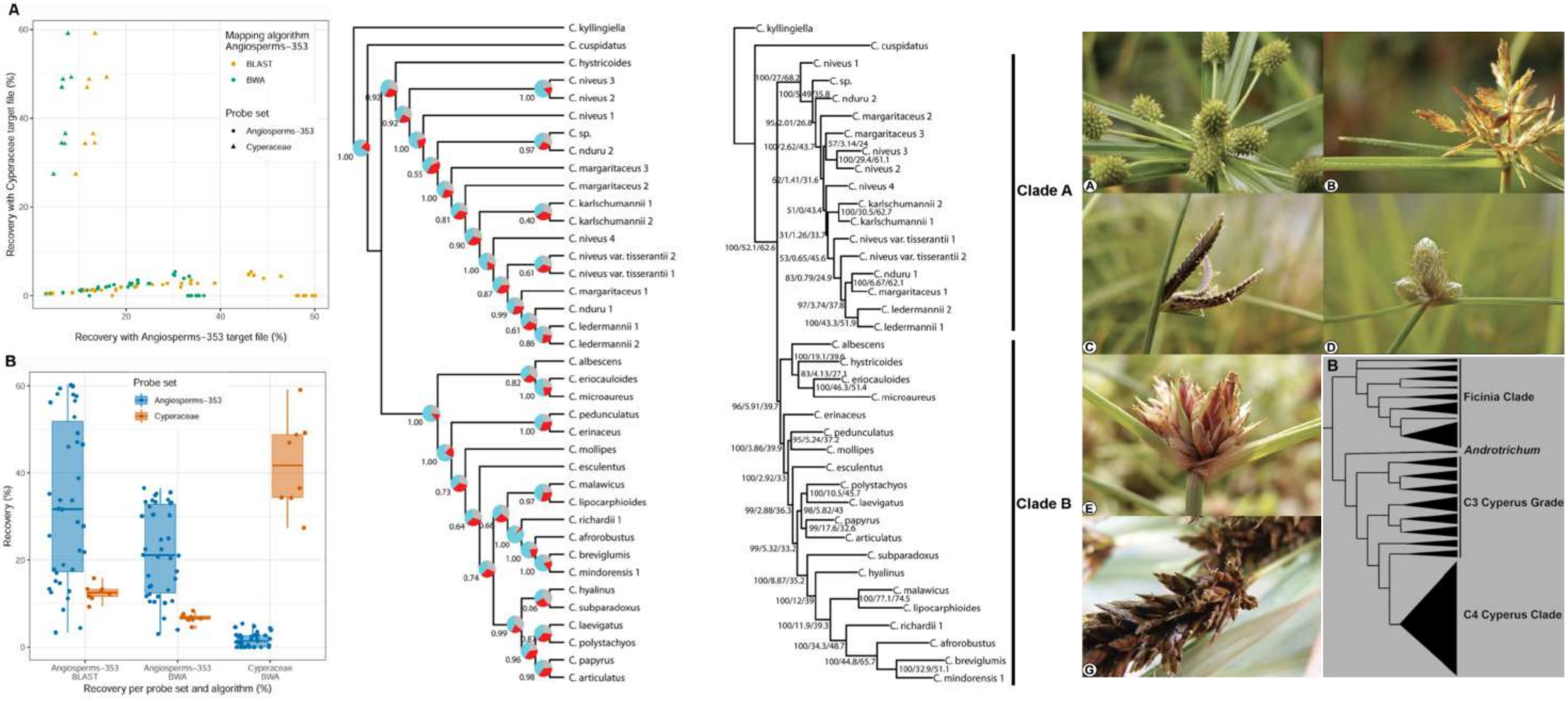






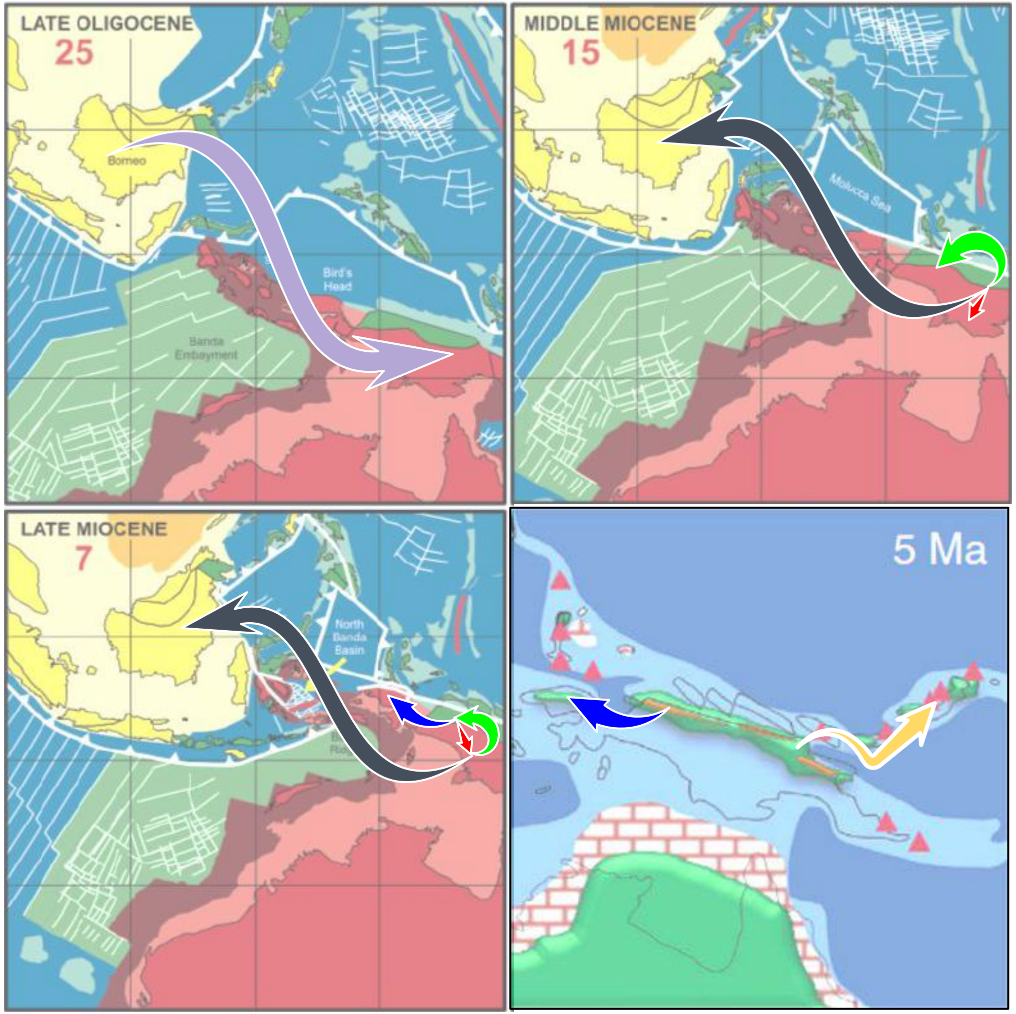
Euphorbia balsamifera s.l.

Figs. 1, 3 & 4. Villaverde*, Pokorny* et al. 2018. *New Phytol.* 220: 636–650

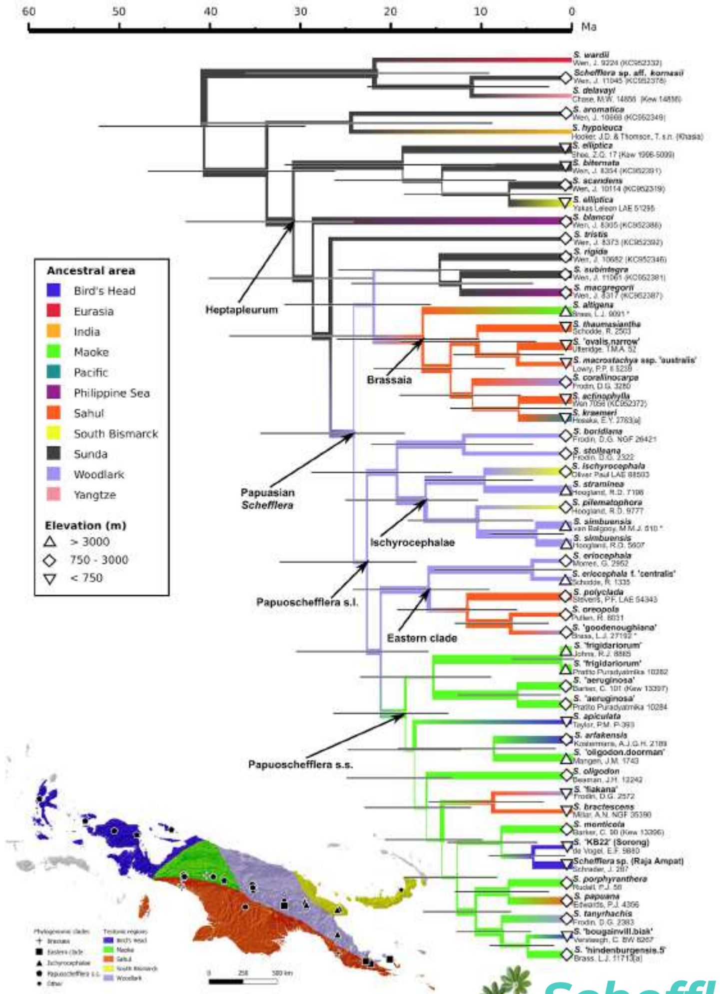


C4 *Cyperus* Clade

Figs. 1, 2 & 4. Larridon*, Villaverde* et al. 2020. *Front. Plant Sci.*



LATE OLIGOCENE
25
Borneo
Bird's Head
Banda Embayment
MIDDLE MIOCENE
15
Molucca Sea
LATE MIOCENE
7
North Banda Basin
5 Ma



60
50
40
30
20
10
0
Ma
Ancestral area
Bird's Head
Eurasia
India
Maoke
Pacific
Philippine Sea
Sahul
South Bismarck
Sunda
Woodlark
Yangtze
Elevation (m)
> 3000
750 - 3000
< 750
Heptapleurum
Brassaia
Papuasian Schefflera
Ischyrocephalae
Papuoschefflera s.l.
Eastern clade
Papuoschefflera s.s.
S. wardii
Schefflera sp. aff. kornasii
S. delavayi
S. aromatica
S. hypoleuca
S. elliptica
S. bilernata
S. scandens
S. elliptica
S. blancoi
S. tristis
S. rigida
S. subintegra
S. macgregorii
S. altigena
S. thaumasiantha
S. 'ovalis.narrow'
S. macrostachya ssp. 'australis'
S. corallinocarpa
S. actinophylla
S. kraemeri
S. boridiana
S. stolleana
S. ischyrocephala
S. straminea
S. pilematophora
S. simbuensis
S. simbuensis
S. eriocephala
S. eriocephala f. 'centralis'
S. polyclada
S. oreopola
S. 'goodenoughiana'
S. 'frigidariorum'
S. 'frigidariorum'
S. 'aeruginosa'
S. 'aeruginosa'
S. apiculata
S. arfakensis
S. 'oligodon.doorman'
S. oligodon
S. 'fiakana'
S. bractescens
S. monticola
S. 'KB22' (Sorong)
Schefflera sp. (Raja Ampat)
S. porphyranthera
S. papuana
S. tanyrhachis
S. 'bougainvill.biak'
S. 'hindenburgensis.5'

Schefflera

6.

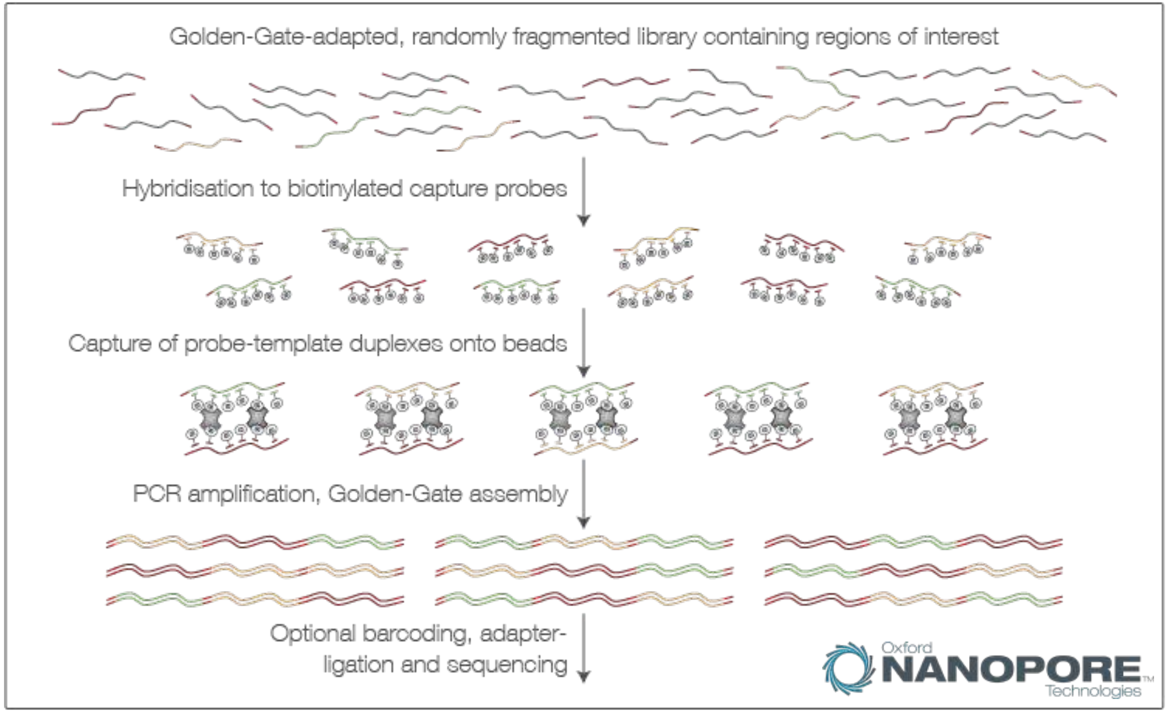
el futuro es ahora

- Fragmentos largos
- Depauperación con CRISPR

Incorporating sequence capture into library preparation for MinION, GridION and PromethION

Sequence enrichment by hybridisation of capture probes allows users to select thousands of loci of interest simultaneously prior to sequencing, making more efficient use of the sequencing run

Contact: **publications@nanoporetech.com** More information at: **www.nanoporetech.com** and **publications.nanoporetech.com**



https://nanoporetech.com/sites/default/files/s3/posters/pdf/P17013%20SeqCap%20v7.0%20A4%20_compressed.pdf

Nanopore adaptive sequencing for mixed samples, whole exome capture and targeted panels.

Alexander Payne[1], Nadine Holmes[1], Thomas Clarke[1], Rory Munro[1], Bisrat Debebe[1] and Matthew Loose[1*]

Applications in Plant Sciences
Botanical Society of America

Long-fragment targeted capture for long-read sequencing of plastomes

Kevin Bethune[1,*], Cédric Mariac[1,*], Marie Couderc[1], Nora Scarcelli[1], Sylvain Santoni[2], Morgane Ardisson[2], Jean-François Martin[3], Rommel Montúfar[4], Valentin Klein[1], François Sabot[1], Yves Vigouroux[1], and Thomas L. P. Couvreur[1,5]

Manuscript received 14 November 2018; revision accepted 21 March 2019.

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* These authors contributed equally to this work.

Citation: Bethune, K., C. Mariac, M. Couderc, N. Scarcelli, S. Santoni, M. Ardisson, J.-F. Martin, R. Montúfar, V. Klein, F. Sabot, Y. Vigouroux, and T. L. P. Couvreur. 2019. Long-fragment targeted capture for long-read sequencing of plastomes. *Applications in Plant Sciences* 7(5): e1243.
doi:10.1002/aps3.1243

PREMISE: Third-generation sequencing methods generate significantly longer reads than those produced using alternative sequencing methods. This provides increased possibilities for the study of biodiversity, phylogeography, and population genetics. We developed a protocol for in-solution enrichment hybridization capture of long DNA fragments applicable to complete plastid genomes.

METHODS AND RESULTS: The protocol uses cost-effective in-house probes developed via long-range PCR and was used in six non-model monocot species (Poaceae: African rice, pearl millet, fonio; and three palm species). DNA was extracted from fresh and silica gel–dried leaves. Our protocol successfully captured long-read plastome fragments (3151 bp median on average), with an enrichment rate ranging from 15% to 98%. DNA extracted from silica gel–dried leaves led to low-quality plastome assemblies when compared to DNA extracted from fresh tissue.

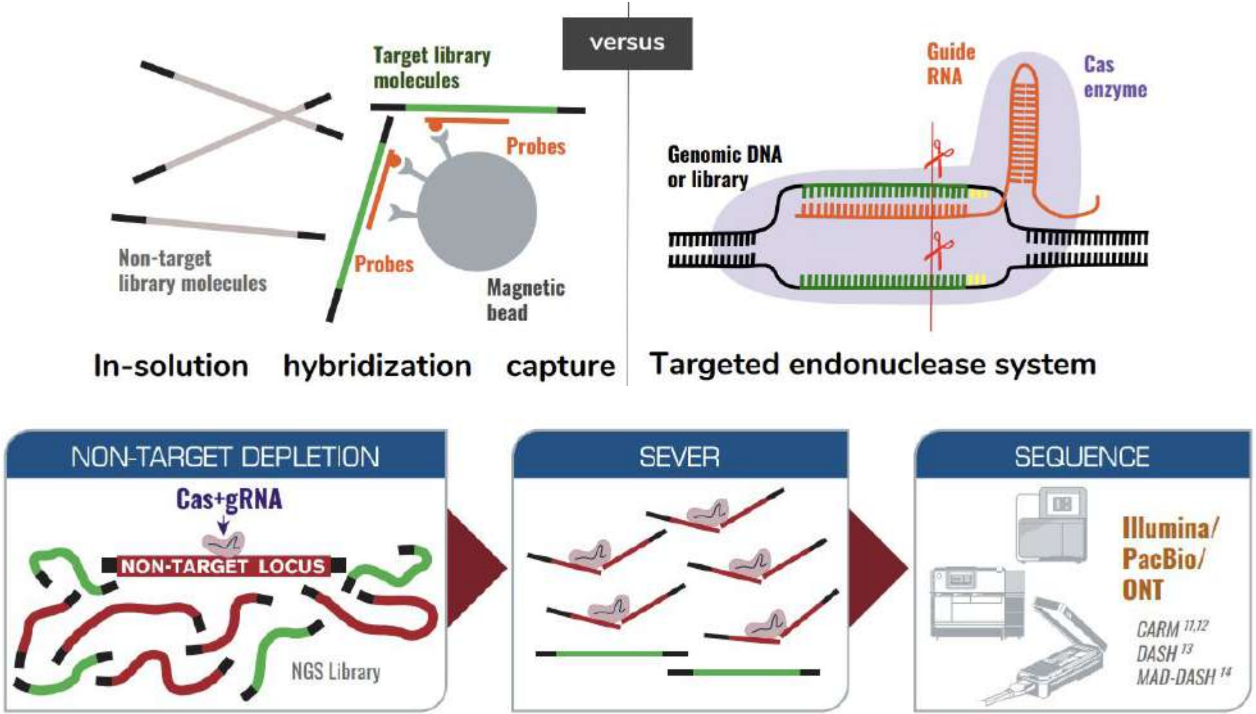
CONCLUSIONS: Our protocol could also be generalized to capture long sequences from specific nuclear fragments.

KEY WORDS de novo assembly; DNA probes; long-range PCR; MinION; whole plastome sequencing.

The CRISPR/Cas Toolkit for Targeted High-Throughput Sequencing:

Enriching complex regions and depleting unwanted elements

Jacob Enk[1], Brian Brunelle[1], Alison Devault[1], Benjamin Steil[1], Jean-Marie Rouillard[1,2]
1 Arbor Biosciences, Ann Arbor, MI, USA | 2 University of Michigan, Ann Arbor, MI, USA



https://arborbiosci.com/wp-content/uploads/2019/01/PAGXXVII-Poster-CRISPR-Cas-Targeted-Sequencing.pdf

¡Gracias!

¿Preguntas?

Lisa Pokorny [@Calyptrochaeta]

Juan Viruel [j.viruel@kew.org]

1000 PLANT TRANSCRIPTOMES INITIATIVE
GLOBAL TREE SEED BANK PROJECT
PAFTOL RESEARCH PROGRAMME

MARIE CURIE ACTIONS

Royal Botanic Gardens Kew

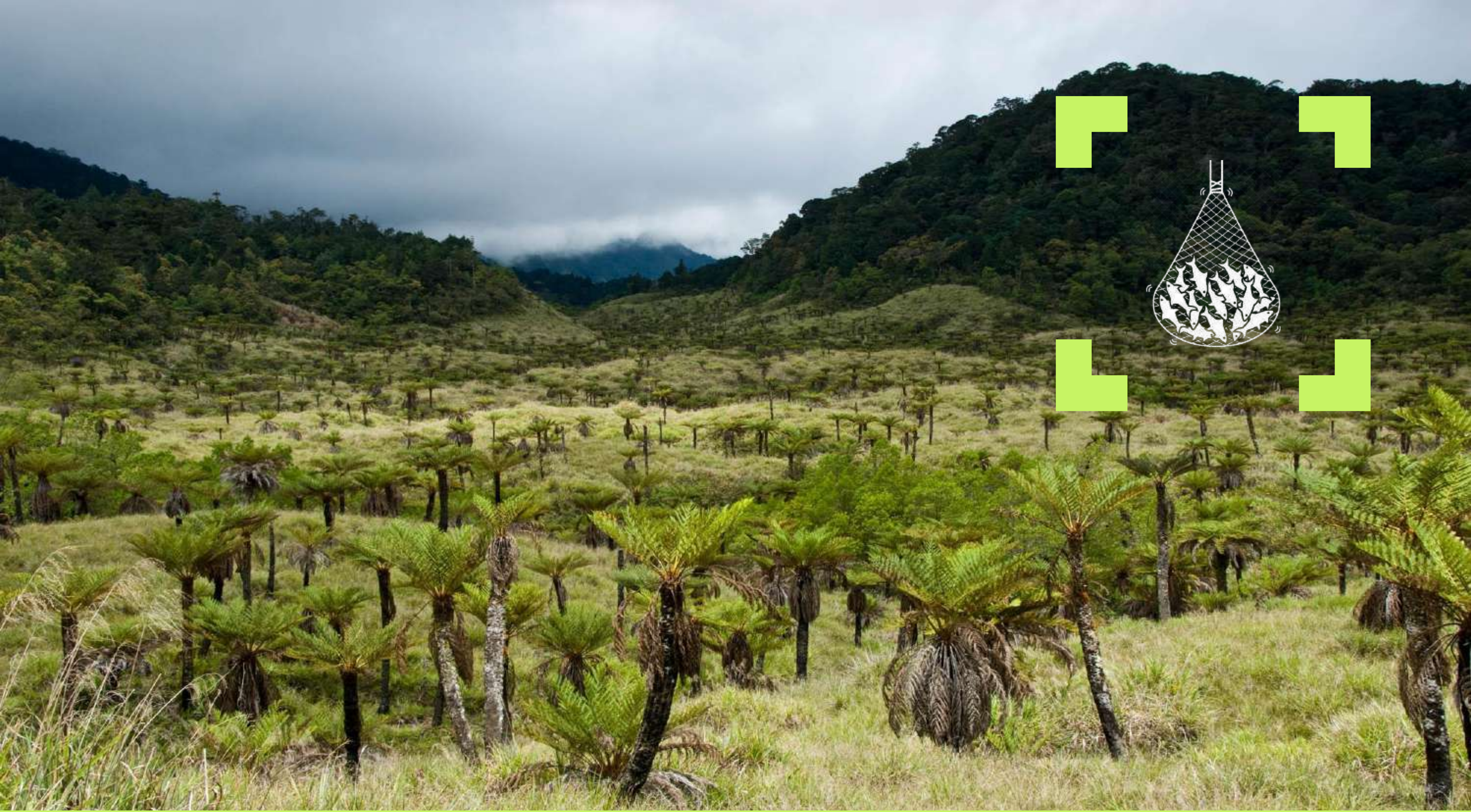


Foto: WJ Baker