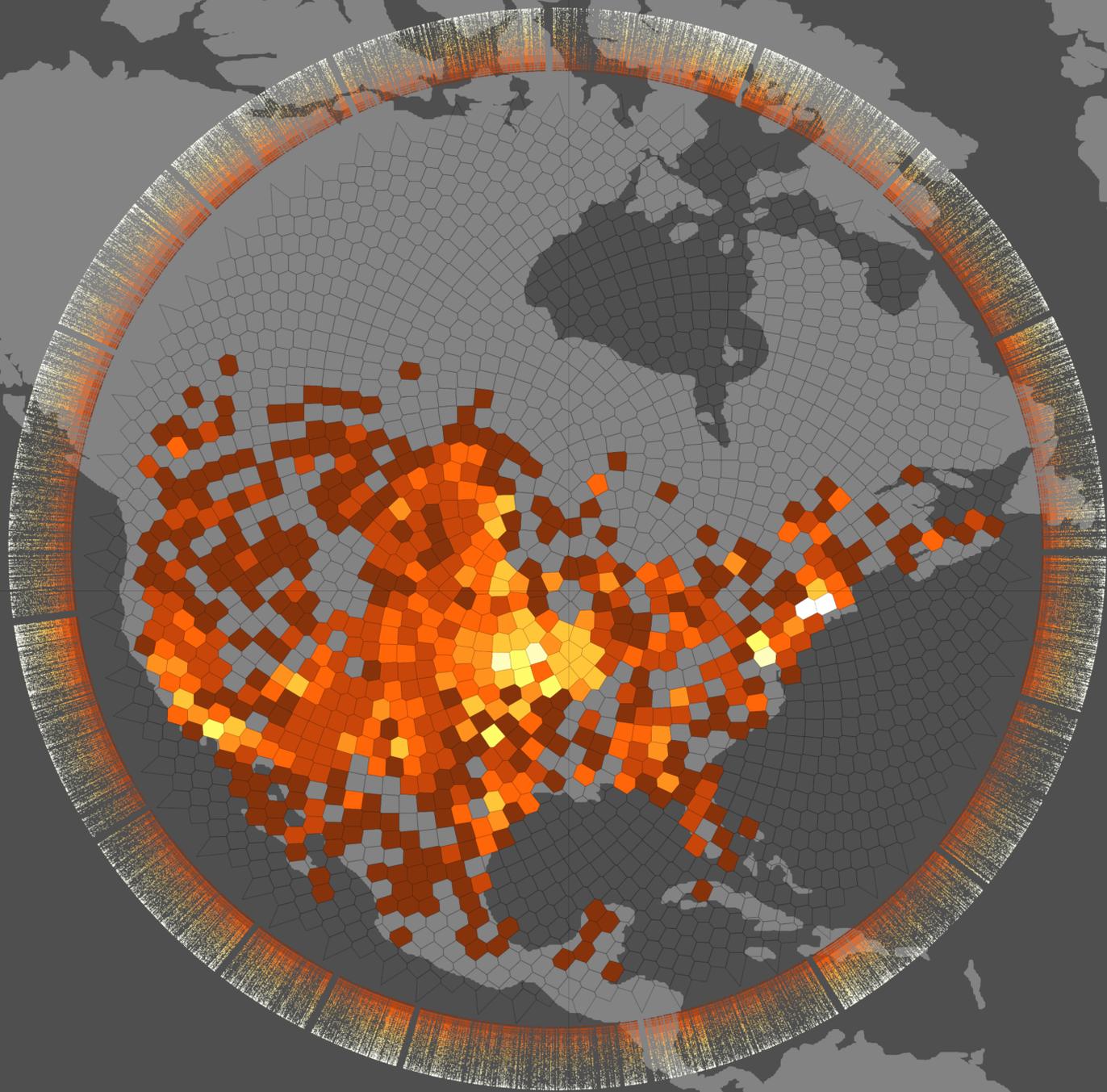


# Sunflower Genome

2015-01-14

Chris Grassa





Closest relative of  
domesticated sunflower

- Woody

- Downy-mildew resistance

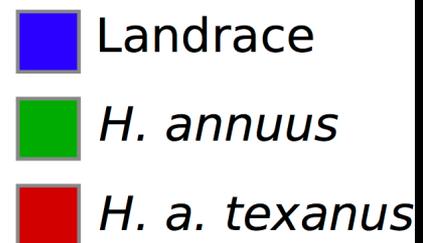
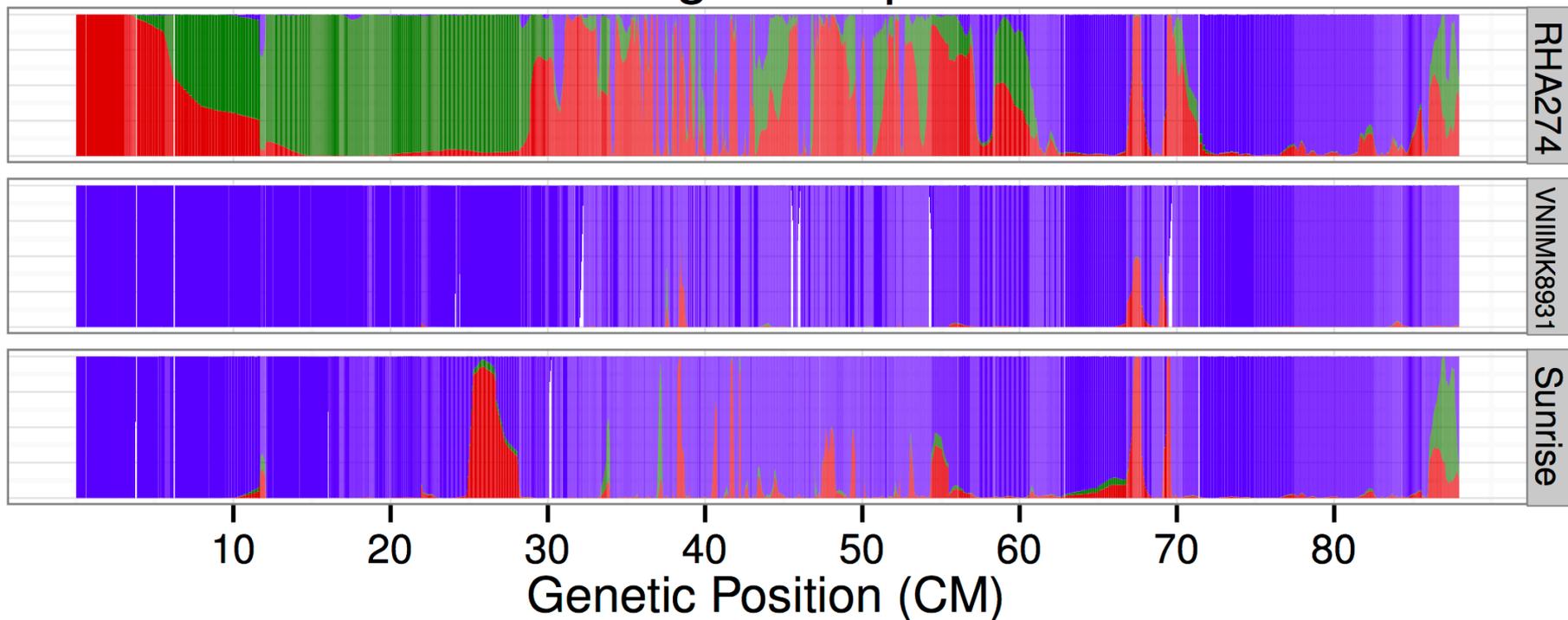
- Bi-modal flowering time

*Helianthus argophyllus* (coastal)  
Brook Moyers

# Domestication & Improvement



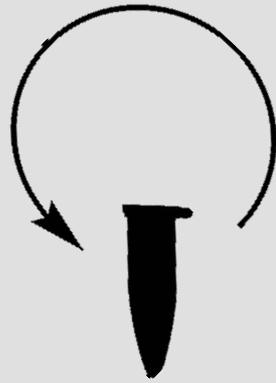
# Linkage Group 10



# Mitochondrial Genome Assembly



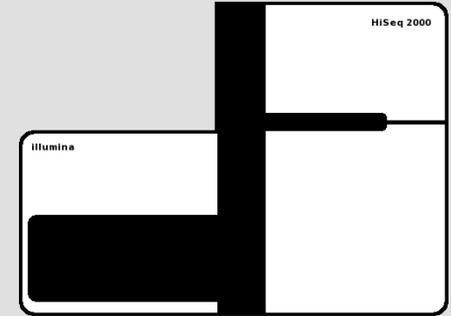
9-day-old seedlings



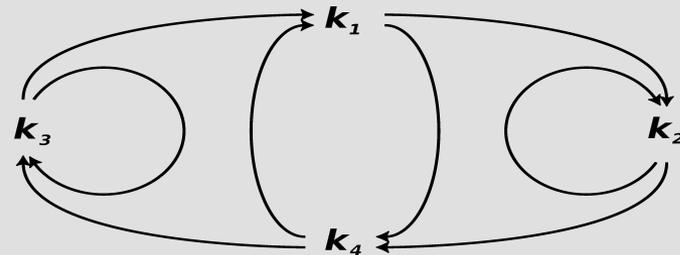
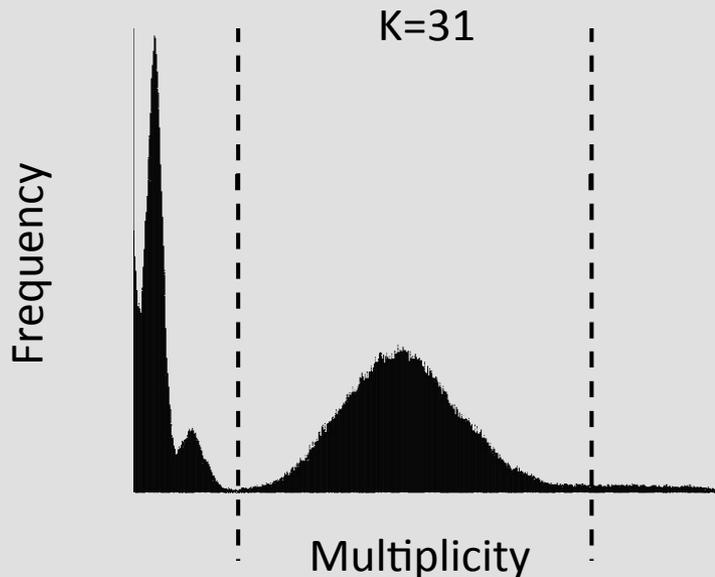
mitochondria enriched



Whole-Genome-Shotgun DNA library

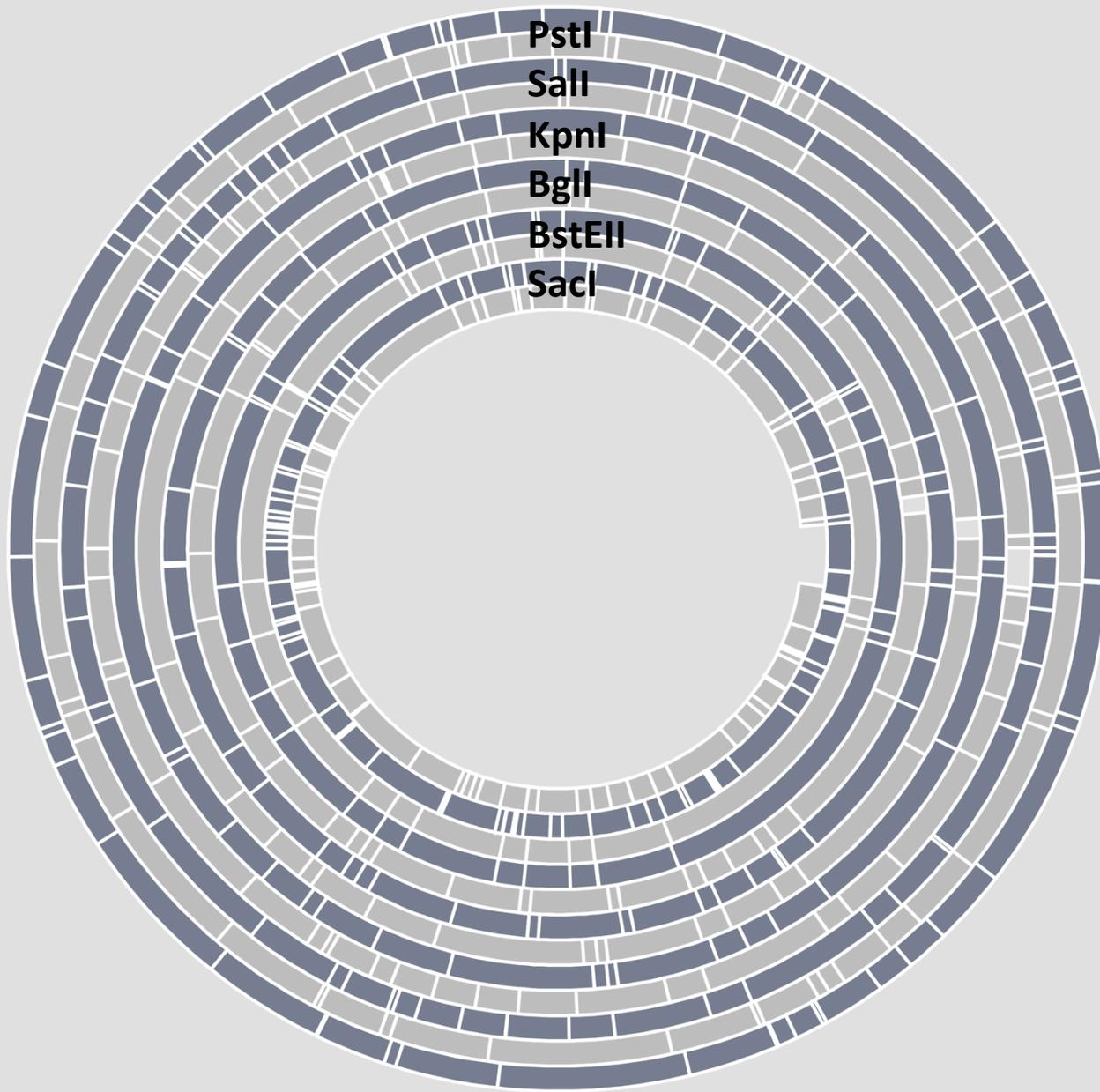


1/48<sup>th</sup> lane



De Bruijn graph assembly

# Restriction site maps



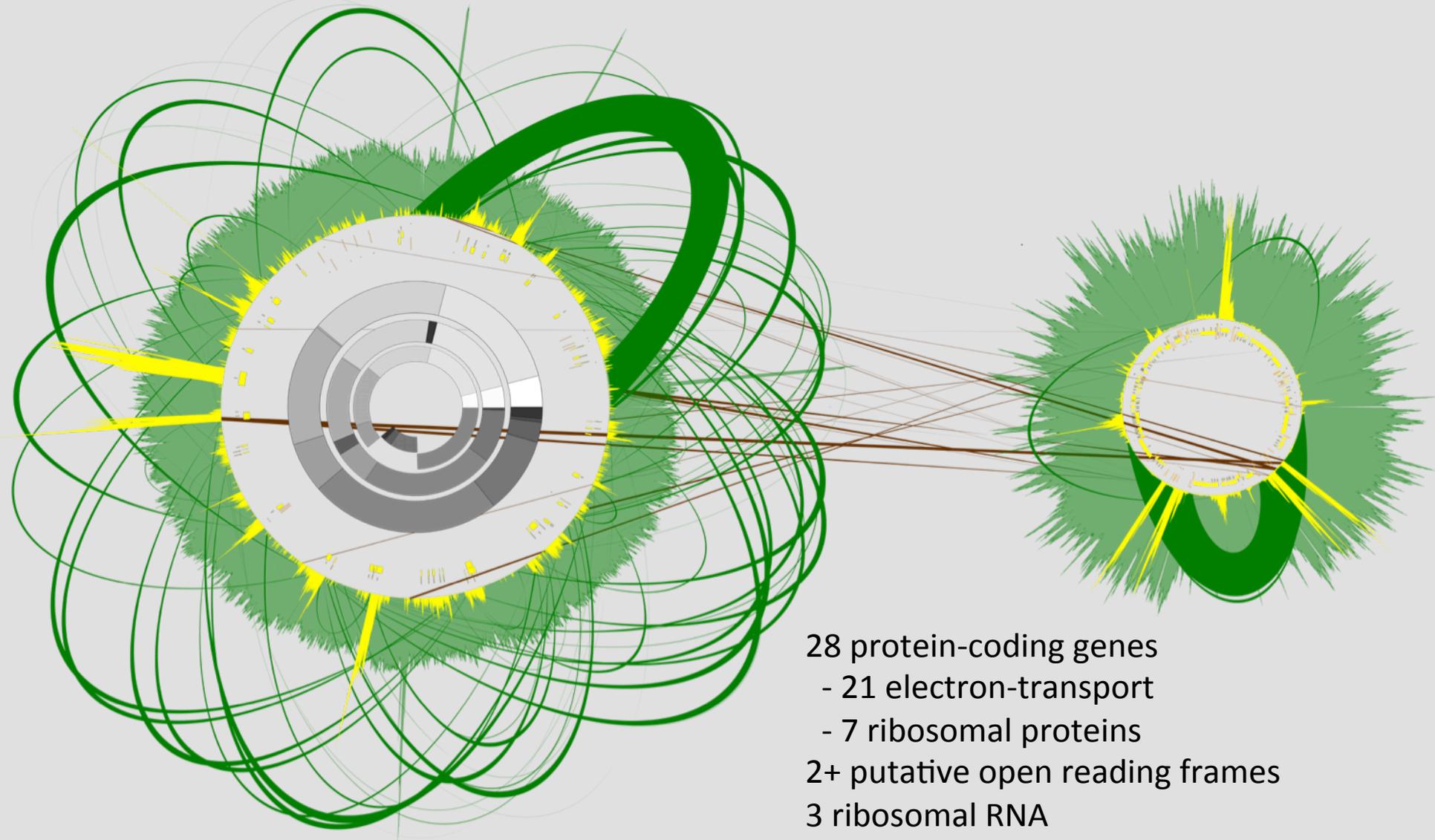
Fragments:



Siculelal & Palmer, 1988

## Mitochondrial Genome

## Plastid Genome



28 protein-coding genes

- 21 electron-transport

- 7 ribosomal proteins

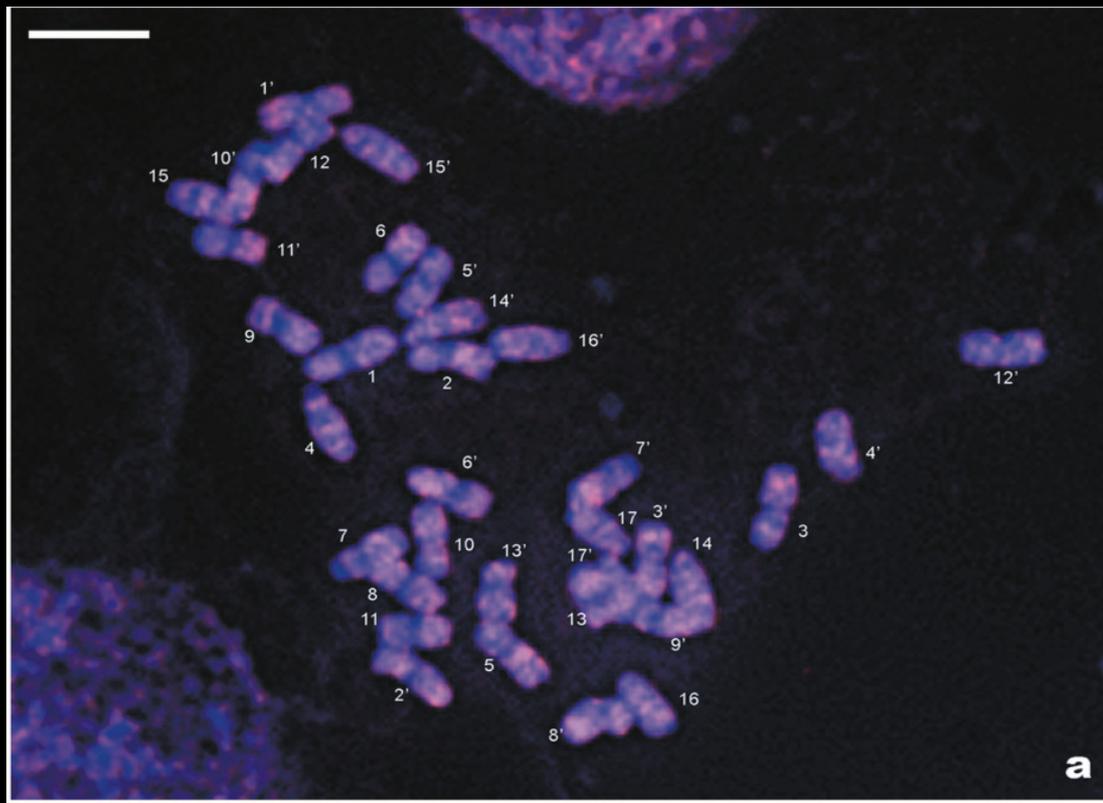
2+ putative open reading frames

3 ribosomal RNA

14 tRNA

11.87% of genome

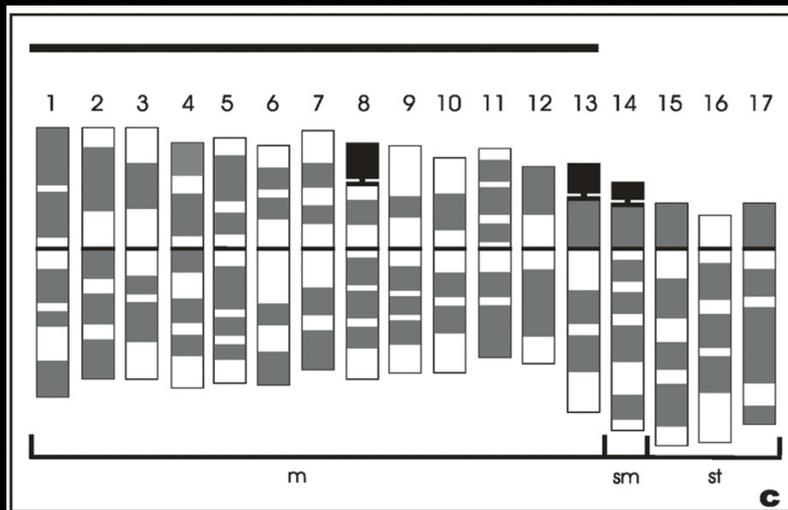
Bars represent 10  $\mu$ m.



Nuclear Genome:

~3.6 Gbp

17 chromosomes

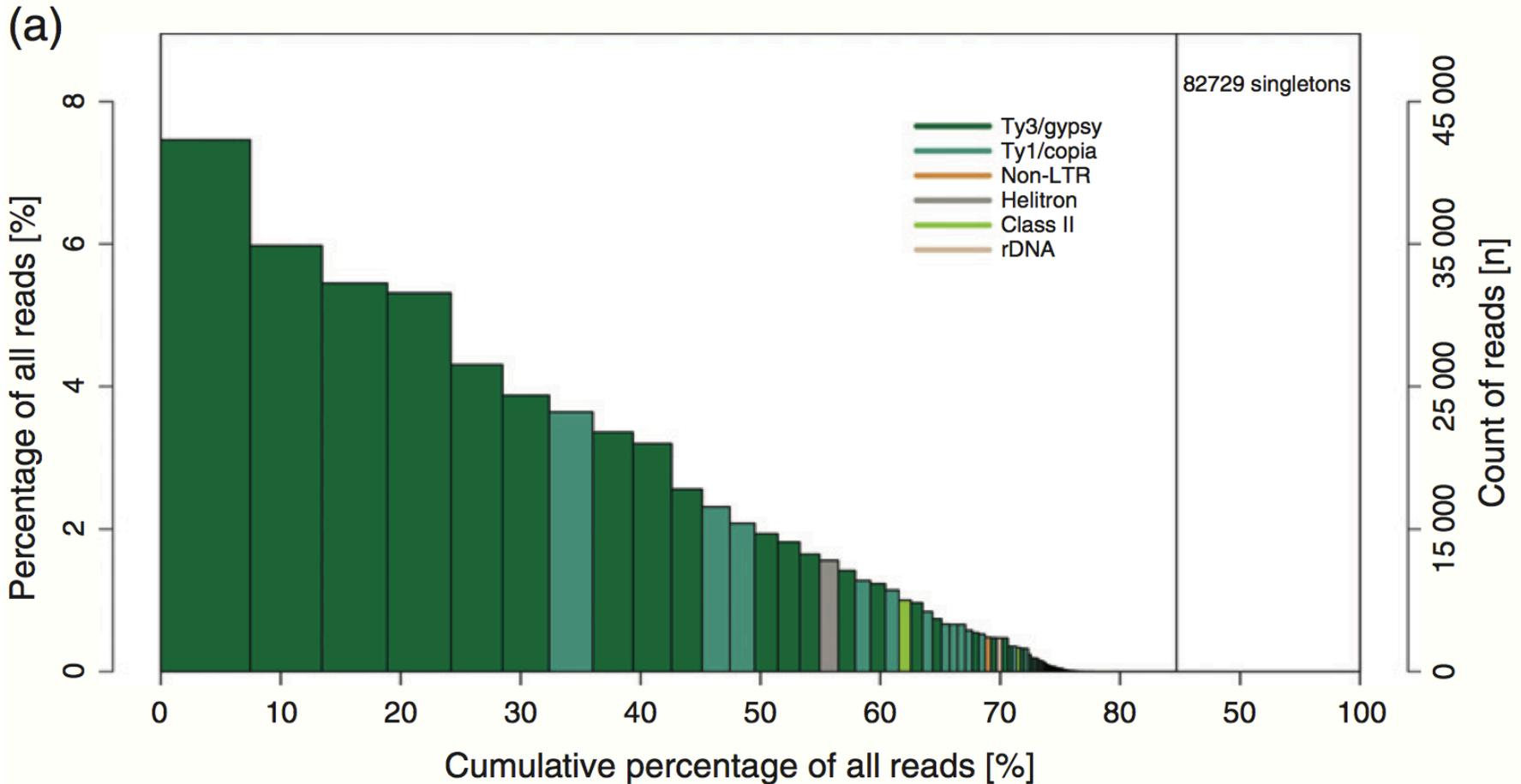


Cytological characterization of sunflower by

**Cytological characterization of sunflower by in situ hybridization using homologous rDNA sequences and a BAC clone containing highly represented repetitive retrotransposon-like sequences**

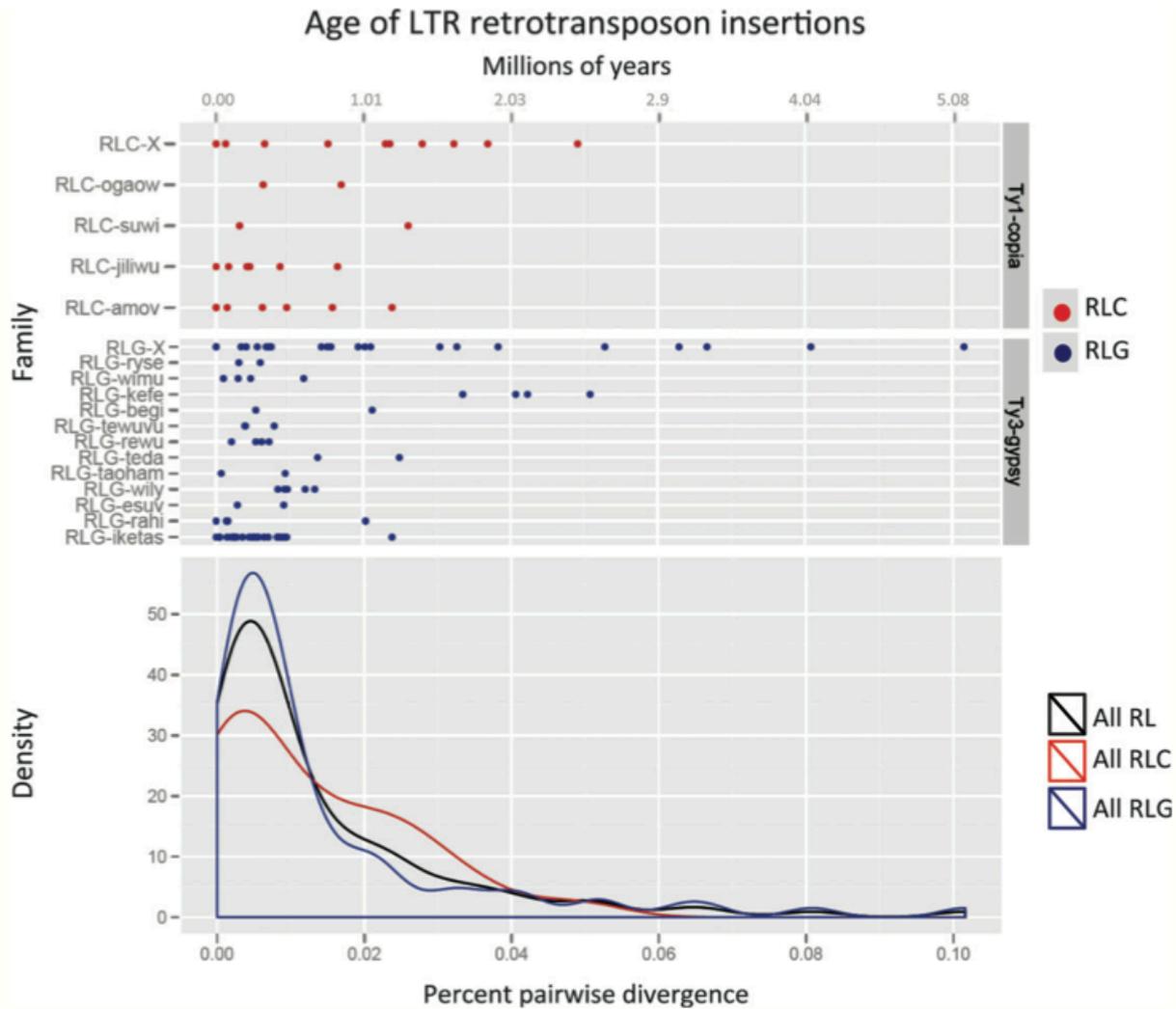
P. Talia, E. Greizerstein, C. Díaz Quijano, L. Peluffo, L. Fernández, P. Fernández, H.E. Hopp, N. Paniego, R.A. Heinz, and L. Poggio

# Highly Repetitive Genome

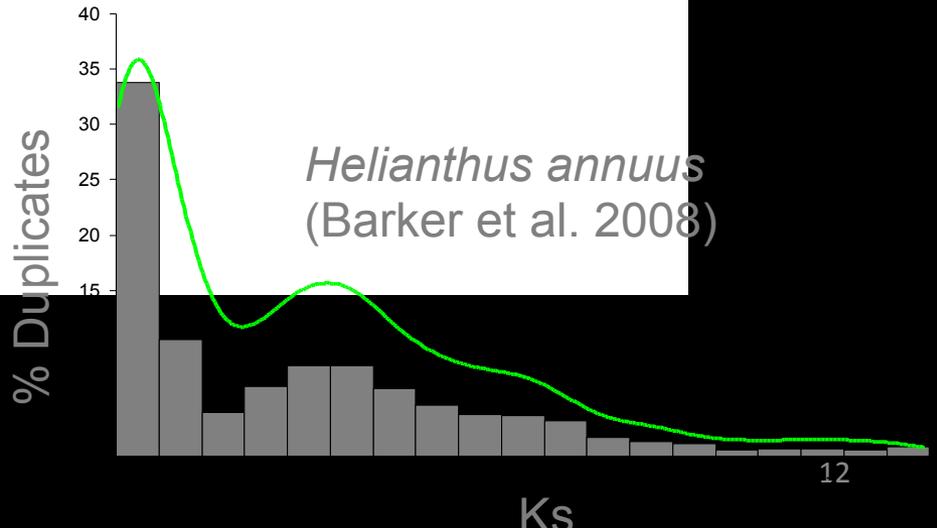
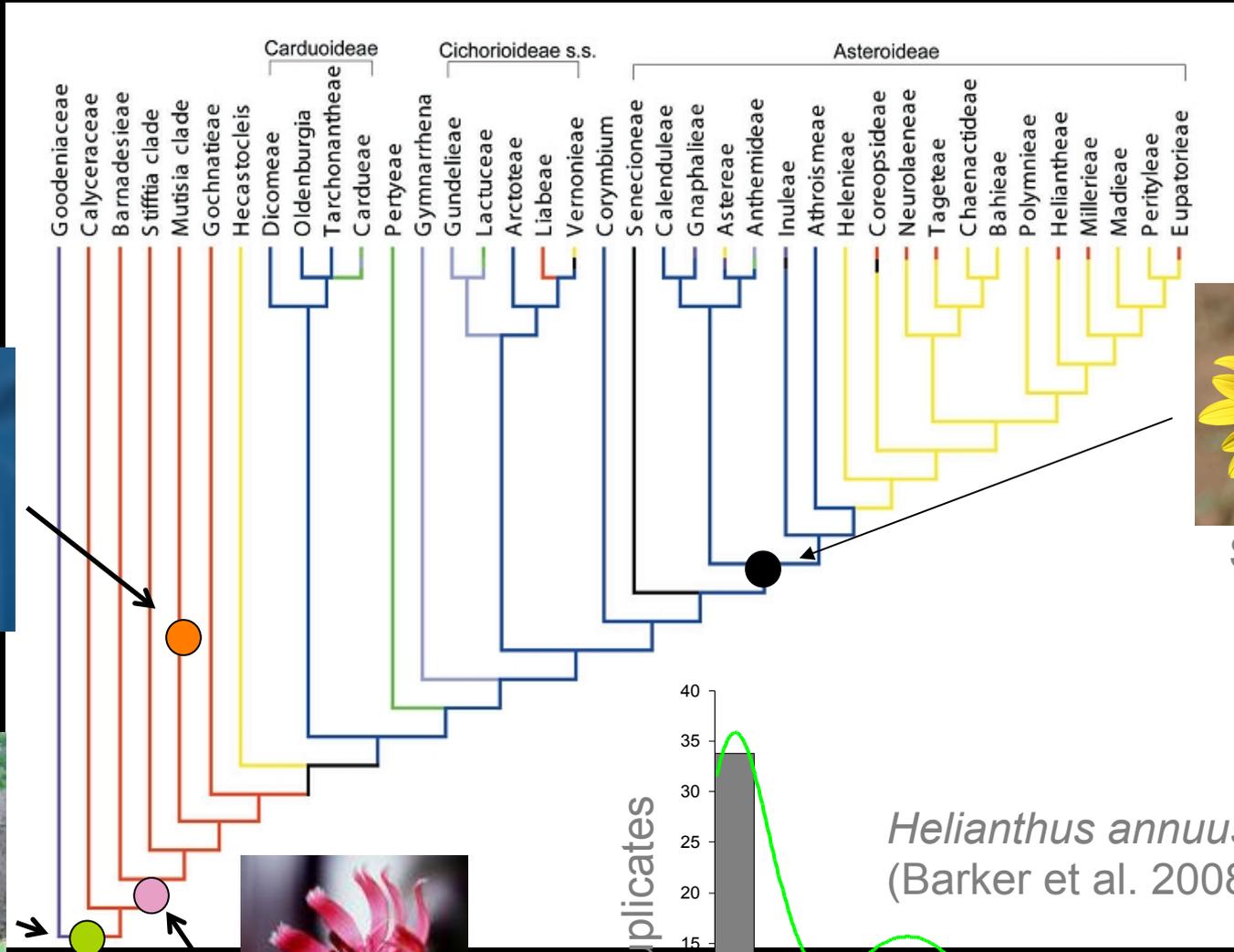


The sunflower (*Helianthus annuus* L.) genome reflects a recent history of biased accumulation of transposable elements

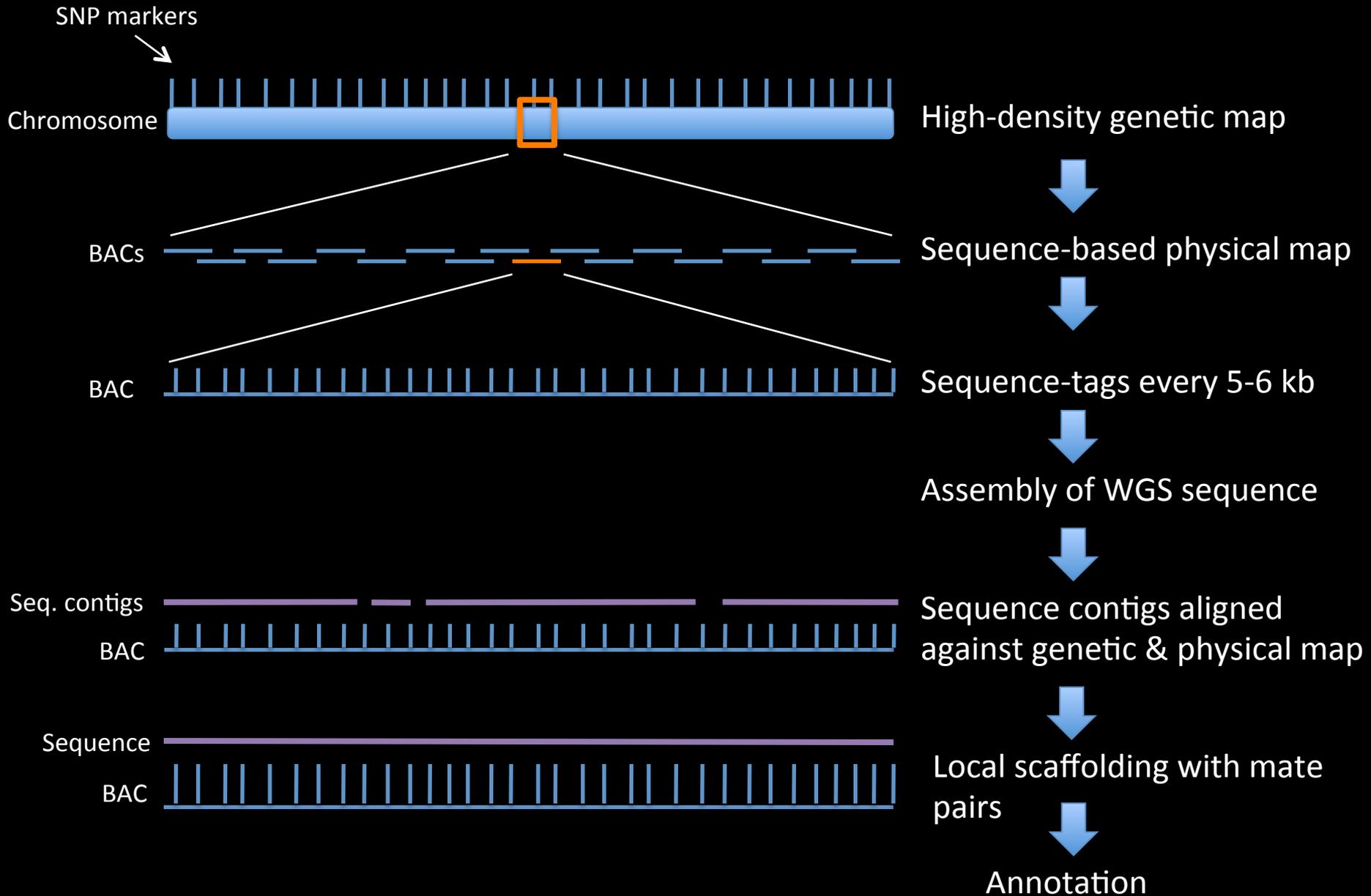
# Young Repeats, Low Divergence



# Sunflower is ancient octaploid



# Strategy



# 43 Read Libraries

	Illumina ~60x			454 ~24x		
	LIBRARY_NAME	MEAN	STDDEV	LIBRARY_NAME	MEAN	STDDEV
Short Fragment	A1	136	23	01V17GRL2	368	133
	A2	139	28	MPS004761454RL	373	124
	A5	160	34	MPS006655454RL	383	120
	200bp_HA0001	192	21	01V17G454RL	384	136
	500bp_HA0002	408	46	MPS004762454RL	394	125
				HA412Long	592	169
				MAYha412long	648	205
Mate Pair	2kbp_HA0003_61YEJAAXX_1	1510	321			
	MP1	2062	1744	01V17G454PE1	2890	485
	MP2.BD0TEHACXX_3	2451	295	01V17G454PE2	2929	515
	MP3.AC0C9VACXX_4	2500	272	MPS008920454PE55kb	3259	1148
	LBM11326_GFI-529_3kb_LJD	2550	760	MPS008921454PE6kb	3517	1412
	MP4.BD0TEHACXX_5	3320	443	MPS006655454PE20Kb	4491	3390
	HA412_GGCTAC_40kb_LJD	3458	1910	MPS004761454PE38kb	7272	1060
	MP5.AC0C9VACXX_6	3848	339	MPS008922454PE8kb	7584	1360
	5kbp_HA0004_626E6AAXX_5	4418	846	MPS004761454PE210kb	7897	1146
	MP6.BD0TEHACXX_7	4653	468	MPS008923454PE10kb	10041	1587
	INX517*	4394	321	MPS004761454PE10kb	10441	1932
	LBM11326_GFI-546_40kb_LJD	5084	3642	MPS004761454PE15kb	11157	5060
	INX518*	5286	2016	MPS009917454PE20kb	12507	4667
	LBM11325_GFI-530_8kb_LJD	7114	1090	MPS008924454PE20kb	12955	4944
	LBM_CAGATC_8kb.LJD	7132	1057	MPS009918454PE20kb	13463	5190
	LBM_GATCAG_20kb.LJD	13887	5153			
LBM1481_GFI-531_20kb_LJD	16863	4578				

# The Eulerian path/de Bruijn graph approach

1. kmer hash table
2. de Bruijn graph
3. simplification of the graph and Eulerian path search

Examples: Euler, Velvet,  
Allpath, Abyss, SOAPdenovo...

10bp read: **ATTCGACTCC**

ATTCG

TTCGA

TCGAC

for k=5,  
6 kmers:

CGACT

GACTC

ACTCC

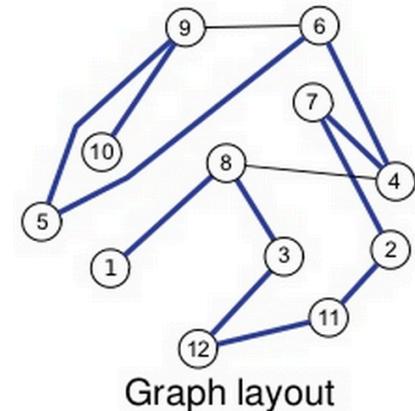
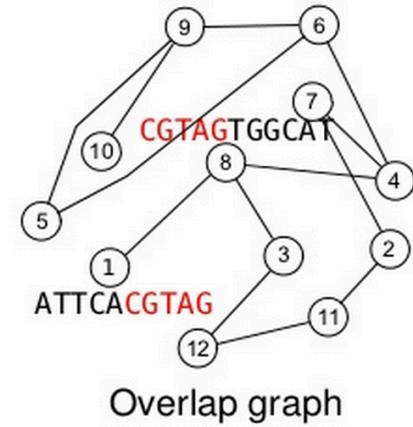


de Bruijn Graph

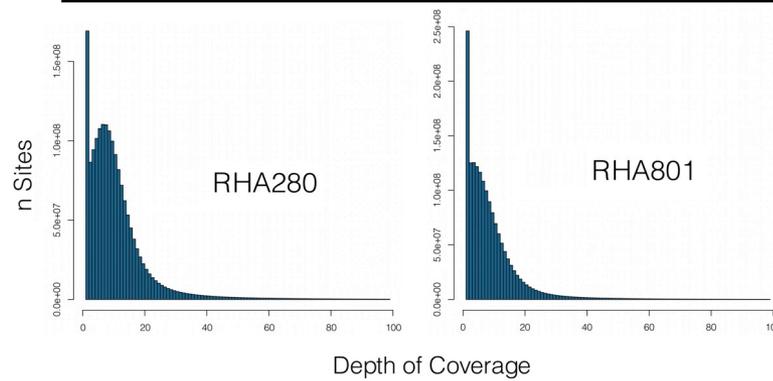
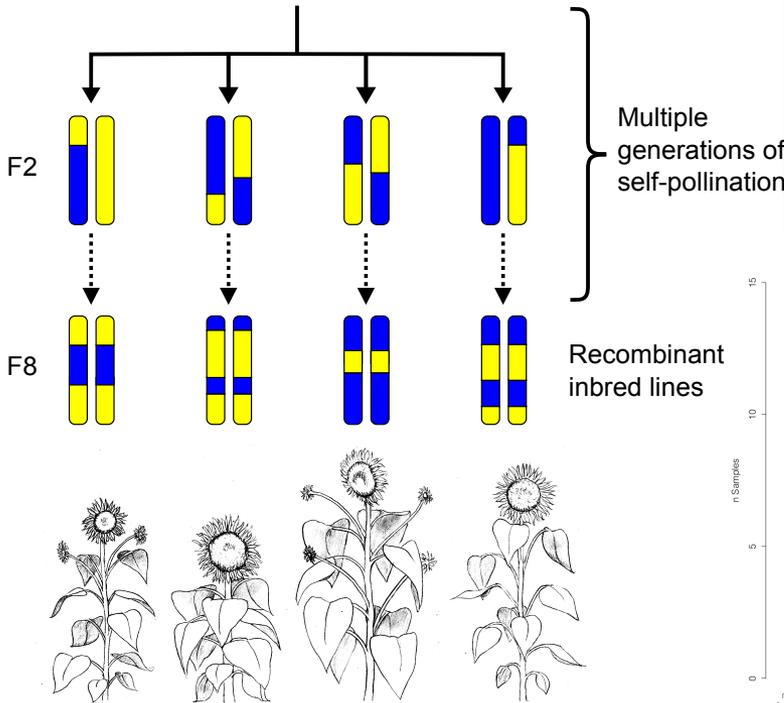
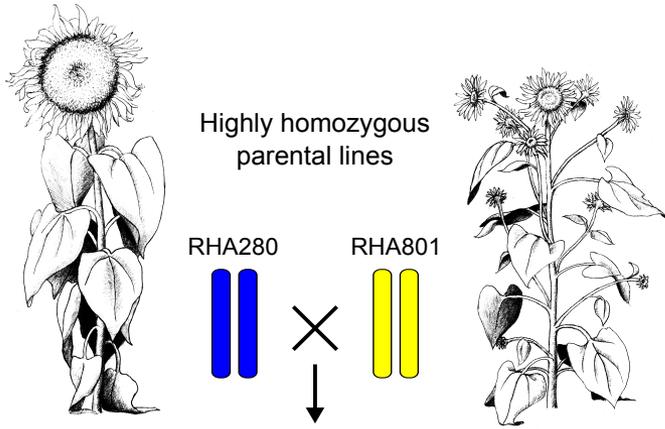
# The Overlap-layout-consensus (OLC) approach

1. Pairwise alignments and overlap graph
2. Graph Layout: search of a single path in the graph (i.e. the Hamiltonian path)
3. Multiple sequence alignments and consensus

Examples: Newbler, Celera,  
Arachne...

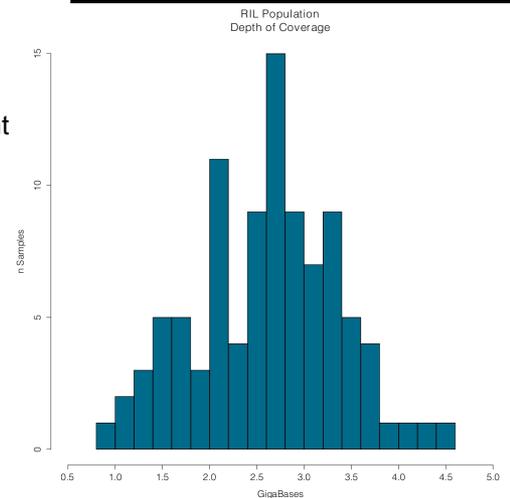


# Sequence Based Genetic Map

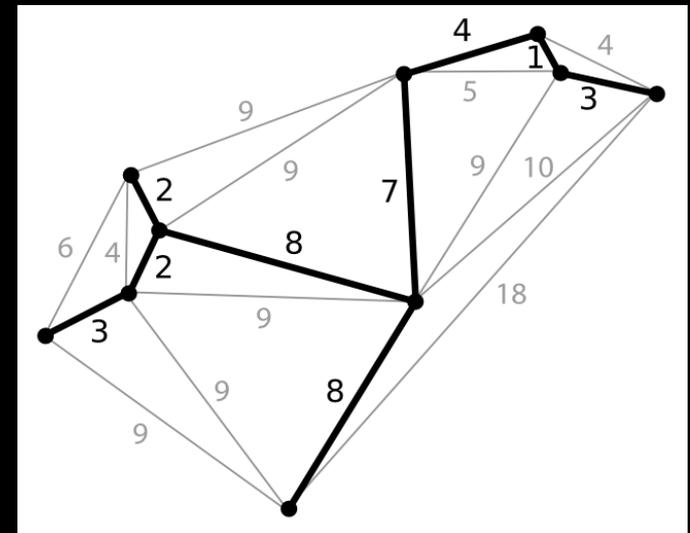
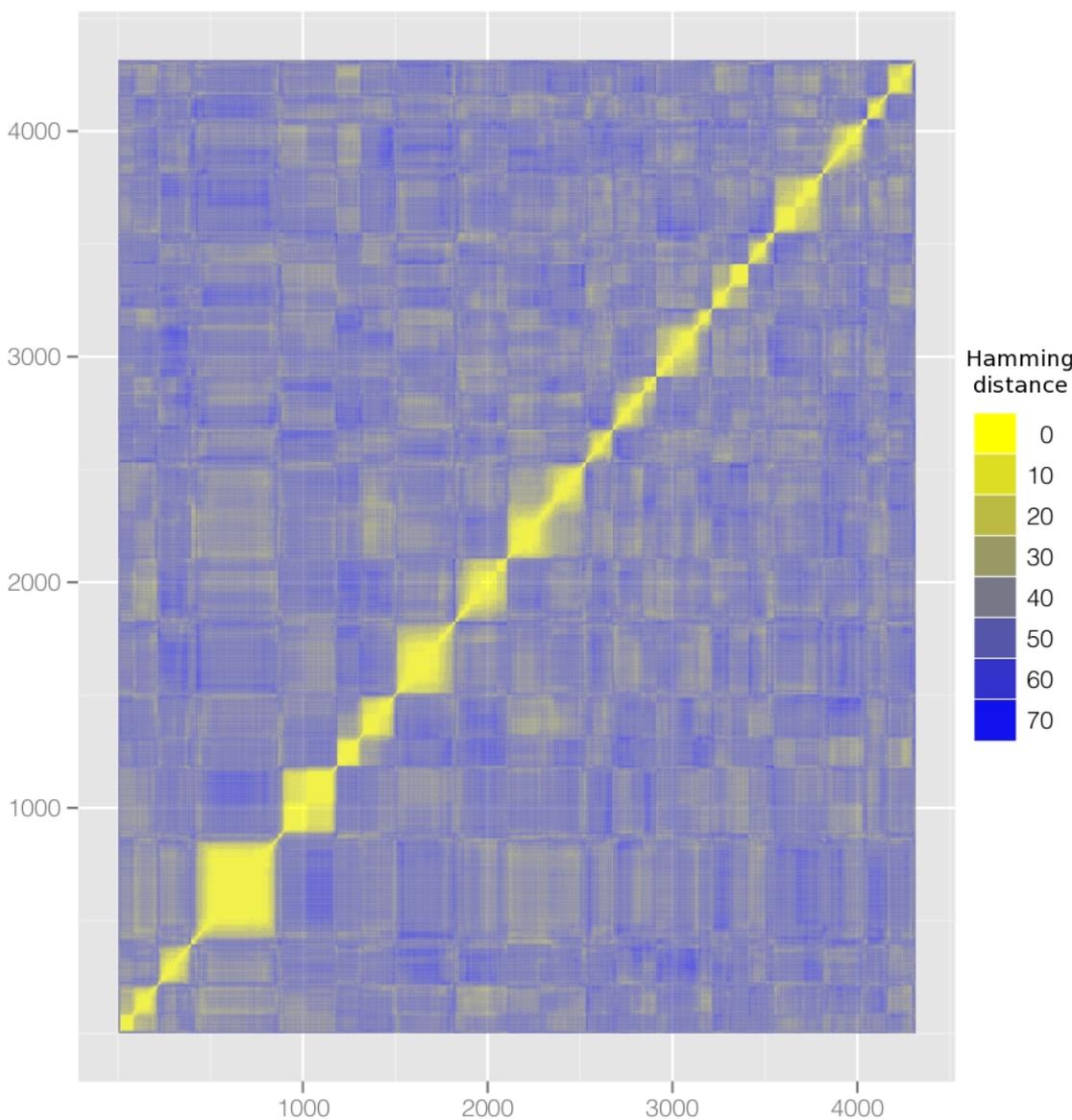


10x WGS Parental Lines

~.8x WGS of 93 RILs



# Template Map



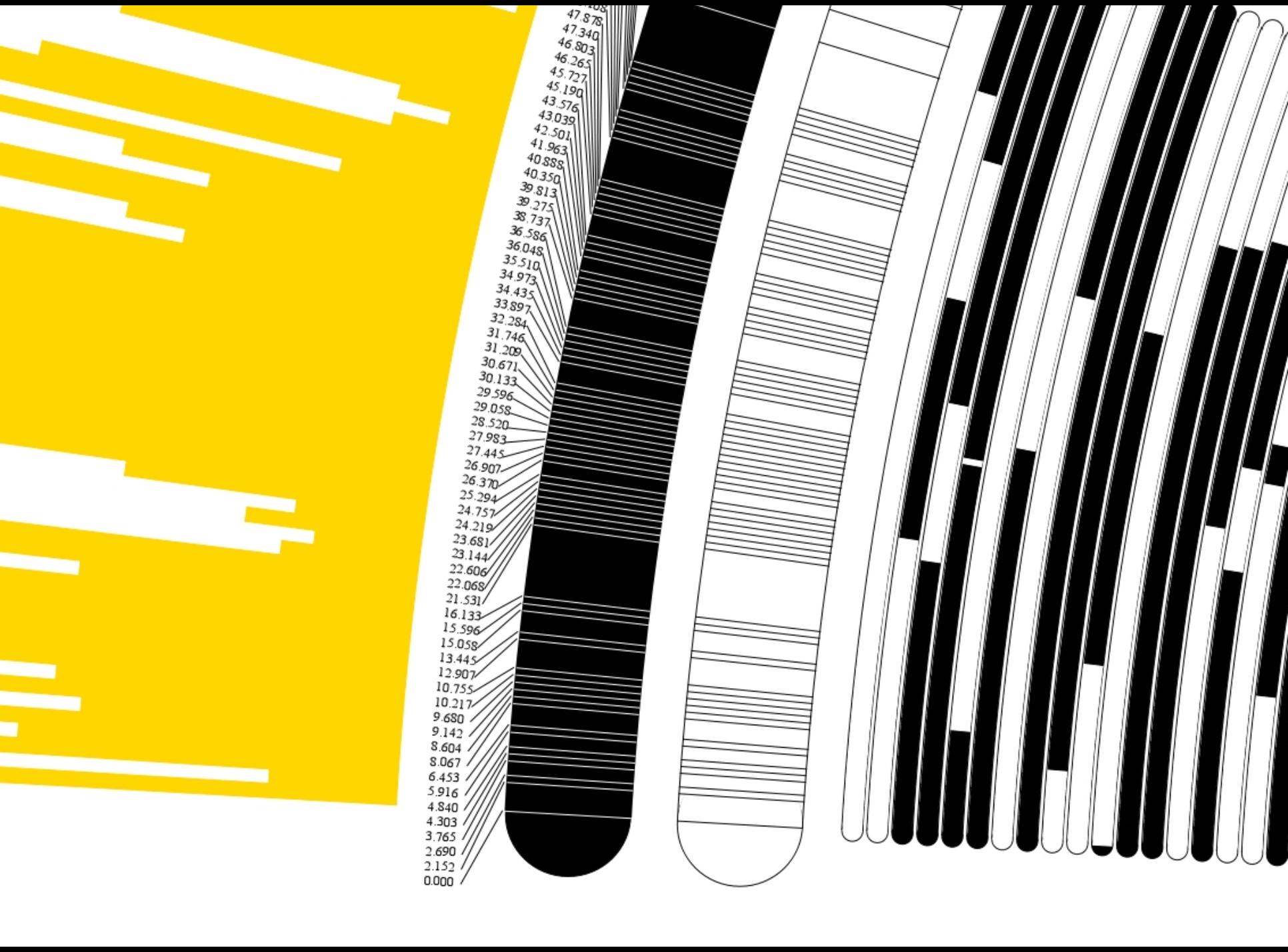
OPEN ACCESS Freely available online

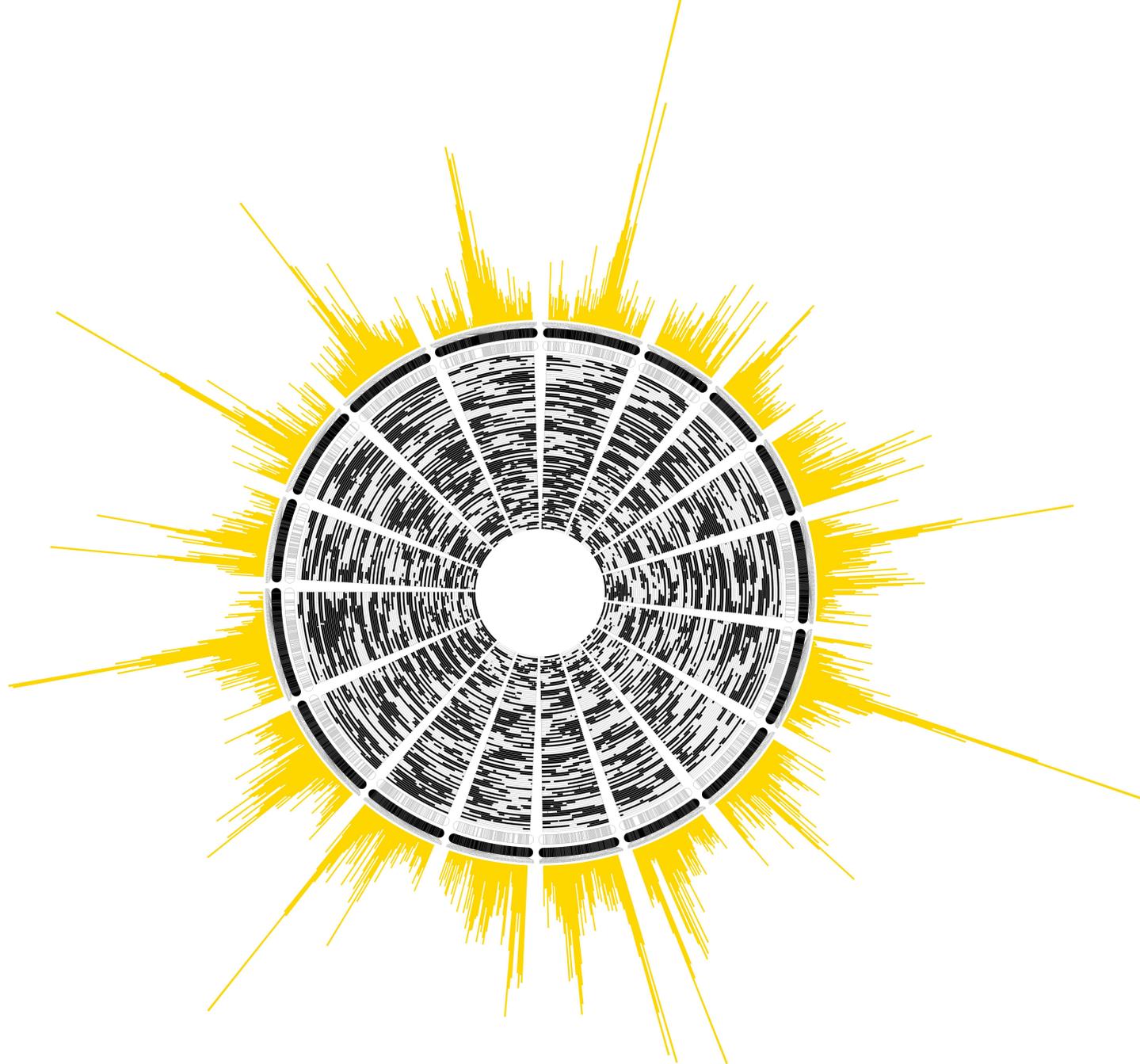
PLoS GENETICS

## Efficient and Accurate Construction of Genetic Linkage Maps from the Minimum Spanning Tree of a Graph

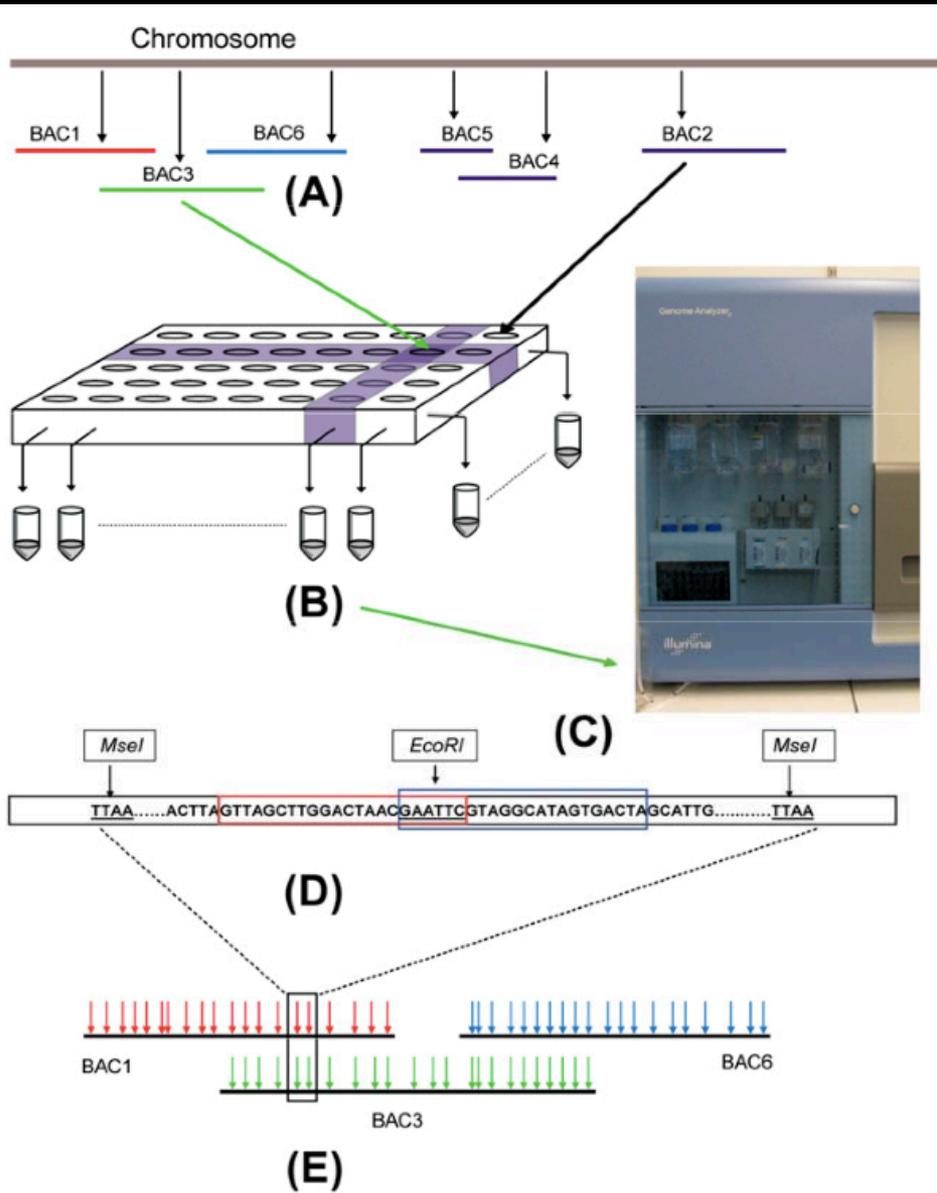
Yonghui Wu<sup>1</sup>, Prasanna R. Bhat<sup>2</sup>, Timothy J. Close<sup>2</sup>, Stefano Lonardi<sup>1\*</sup>

<sup>1</sup> Department of Computer Science and Engineering, University of California Riverside, Riverside, California, United States of America, <sup>2</sup> Department of Botany and Plant Sciences, University of California Riverside, Riverside, California, United States of America





# Whole Genome Profiling Physical Map



- A) 12x BAC library
- B) Barcoded BAC Pools
- C) Restriction Digest and Sequencing
- D) Deconvolution of BAC tags
- E) BAC-contig assembly

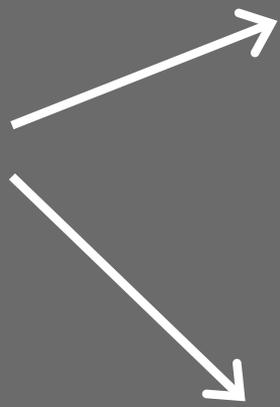
# *in silico* Chromosome Sorting

Whole Genome Shotgun  
- 280 x 801 RILs

Bowers 10k  
- HA412HO x RHA415  
- HA412HO x ANN1238  
- NMS373 x Hopi  
- RHA280 x RHA801

INRA INEDI  
- XRQ x PSC8

Talukder NSA  
- HA89 x RHA464  
- B-line x RHA464  
- CR 29 x RHA468



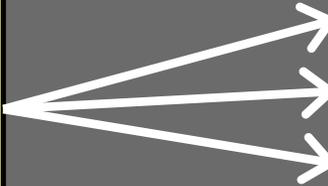
Allpaths scaffolds

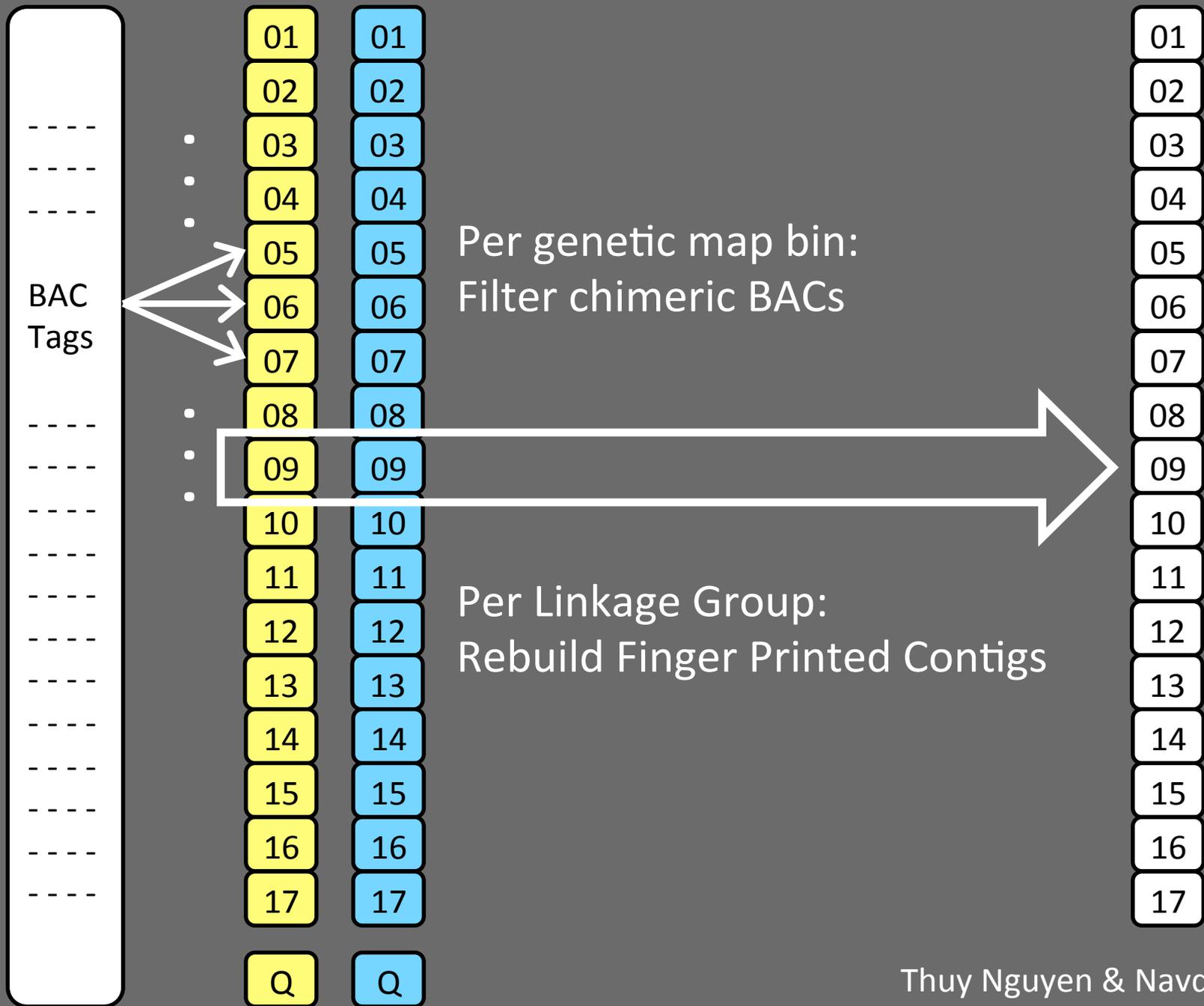
Celera scaffolds

⋮

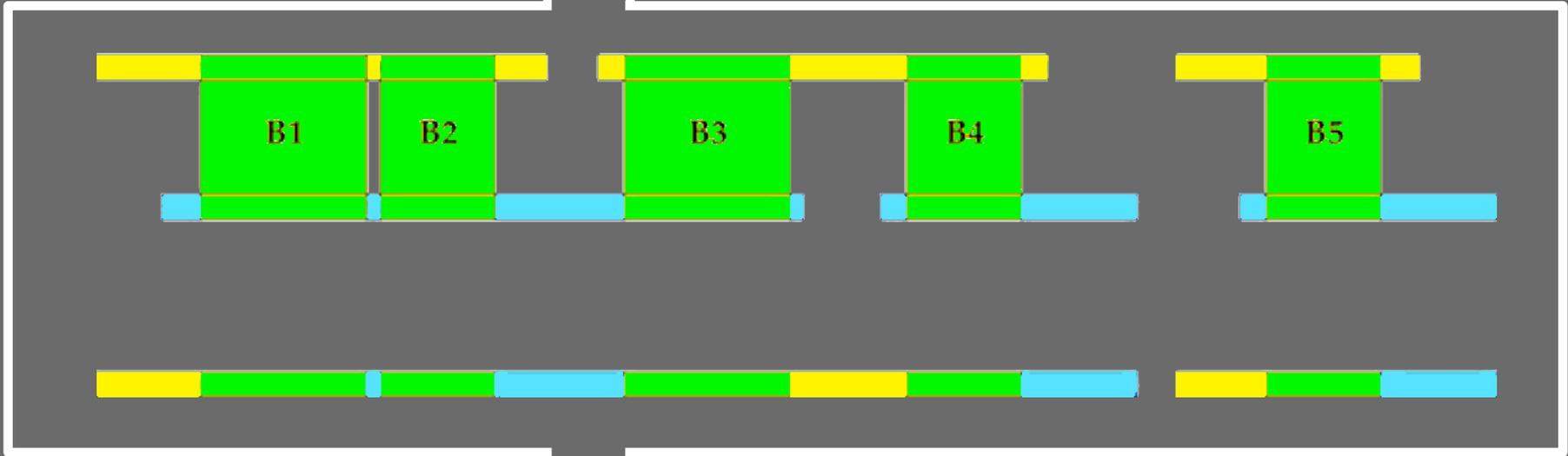
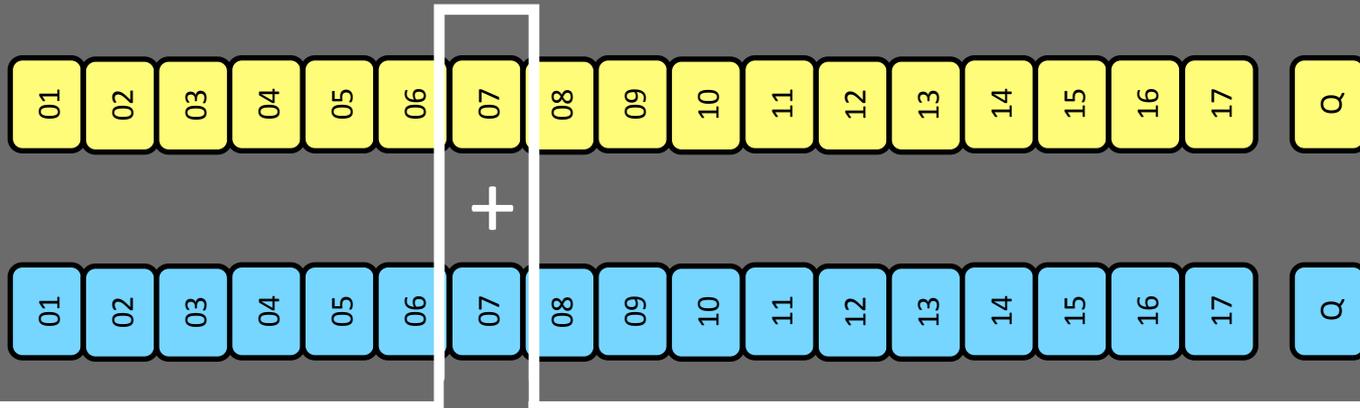
- 01
- 02
- 03
- 04
- 05
- 06
- 07
- 08
- 09
- 10
- 11
- 12
- 13
- 14
- 15
- 16
- 17
- Q

- 01
- 02
- 03
- 04
- 05
- 06
- 07
- 08
- 09
- 10
- 11
- 12
- 13
- 14
- 15
- 16
- 17
- Q





# per LG: Assembly merge & SSPACE scaffolding



Genome wide: error correction



```

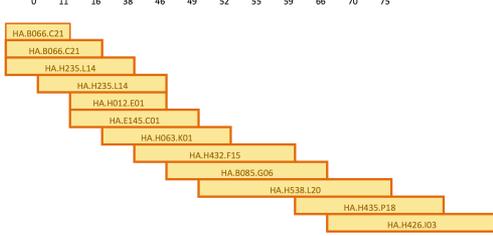
#### vvv LG08|scaffold403|size86505 TO ctg66      vvv ####
#           LG:08 cM: 66.93
#
#pMap_TAG          scaffold_bp pMap_CTG_fpc
#                   100 102 115
GAATTCCGAACACACTGATGTGATTA      17724      ..X
GAATTCGTTGTAAAACAGAGATATGATTTC  17746      ..X
GAATTCTAGAATATCCTTGAATACAACCAT  18170      ..X
GAATTCAAGGAAACACGAAATGAGTGGTTT  18974      ..X
GAATTCATTTTCATCAACATGCATCATCTT  20734      ..X
GAATTCAAGGTTGATTTTGAAGAAGAAGT   20758      ..X
GAATTCGAGCTAGCTCGGCTTGGCTCGATC  25585      .X.
GAATTCTAATCAAGCCGAGCTCGAGCCTCA  25609      X..
#### ^^ LG08|scaffold403|size86505 forward: 2 ^^ ####
GAATTCTAATCAAGCCGAGCTCGAGCCTCA  25609      X..
GAATTCGAGCTAGCTCGGCTTGGCTCGATC  25585      .X.
GAATTCAAGGTTGATTTTGAAGAAGAAGT   20758      ..X
GAATTCATTTTCATCAACATGCATCATCTT  20734      ..X
GAATTCAAGGAAACACGAAATGAGTGGTTT  18974      ..X
GAATTCTAGAATATCCTTGAATACAACCAT  18170      ..X
GAATTCGTTGTAAAACAGAGATATGATTTC  17746      ..X
GAATTCCGAACACACTGATGTGATTA      17724      ..X
#### ^^ LG08|scaffold403|size86505 reverse: 10 ^^ ####

```

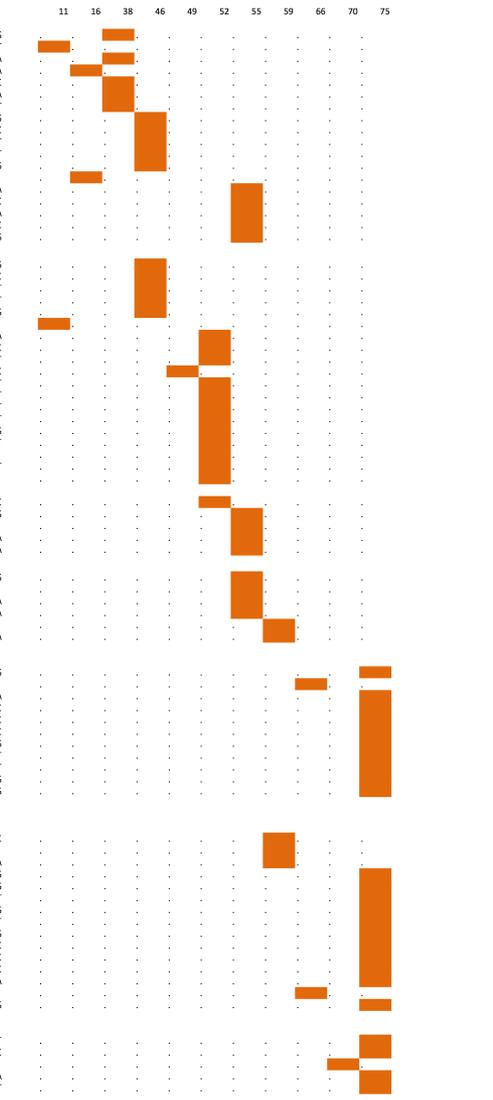
```

## alignment_scoring_scheme:
## no change in index = +1
## move forward one   = +2
## move forward >1   = 0
## move reverse       = -1

```

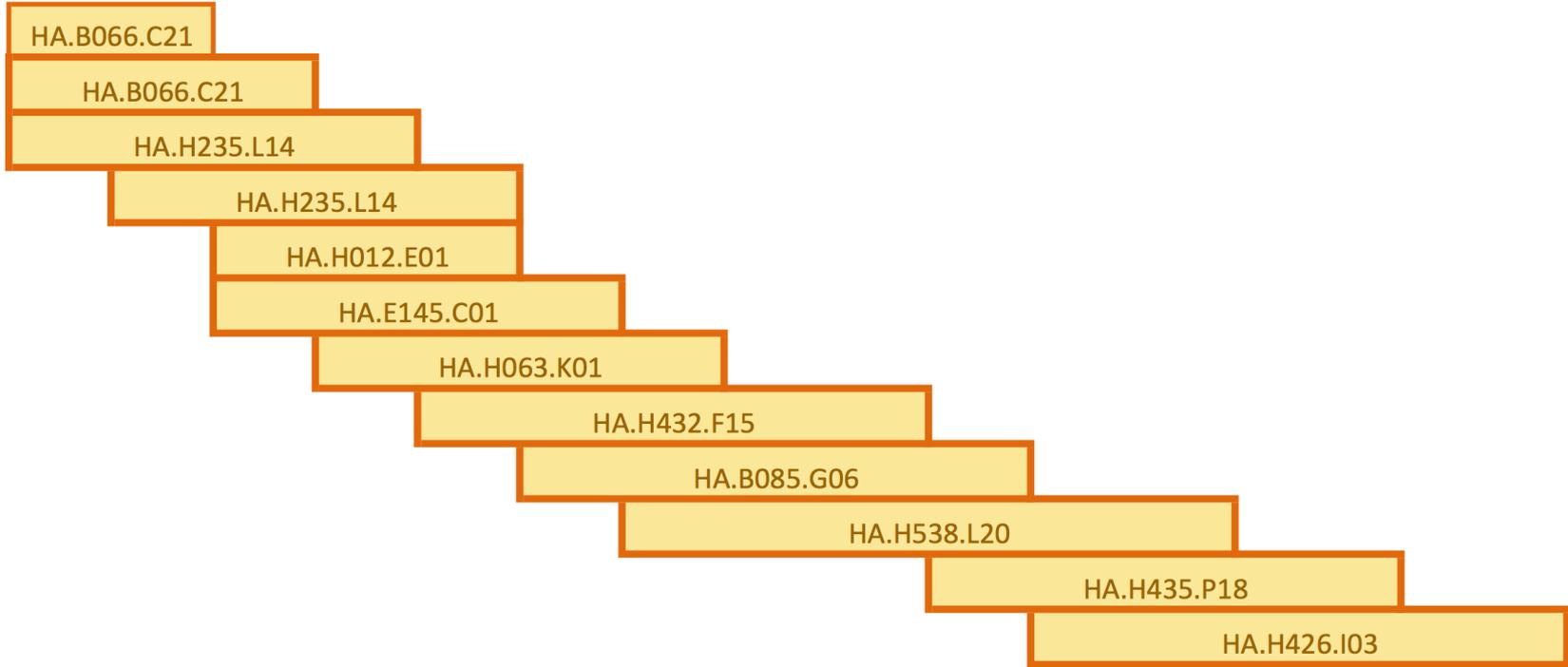


length n N50 N90  
 3131940255 14940 476491 89759



LG04-CTG80 ~632,000 bp

0 11 16 38 46 49 52 55 59 66 70 75

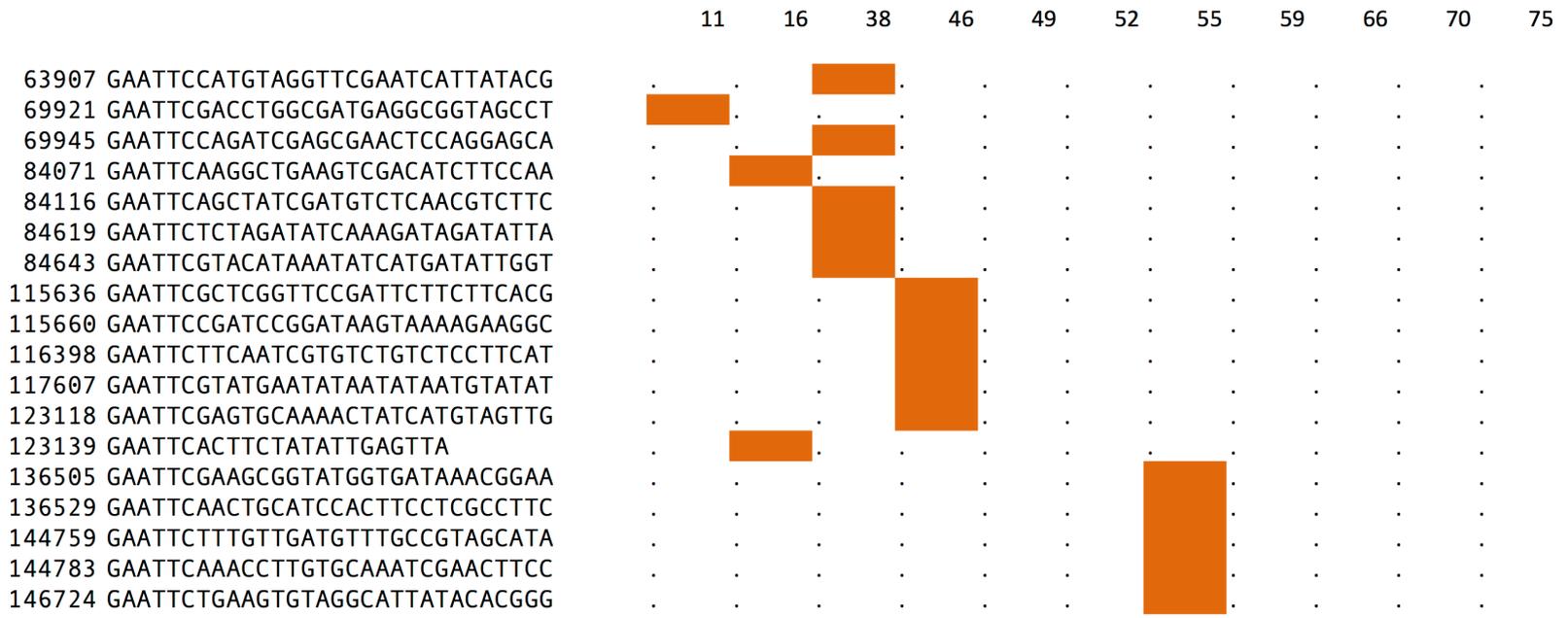


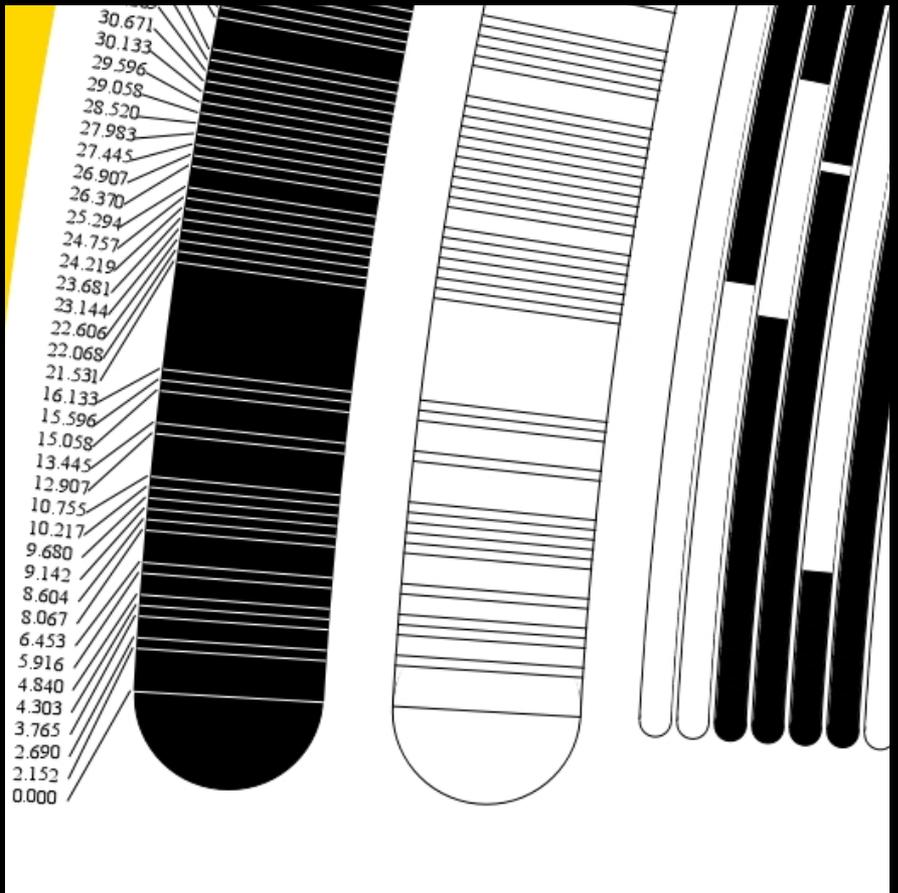
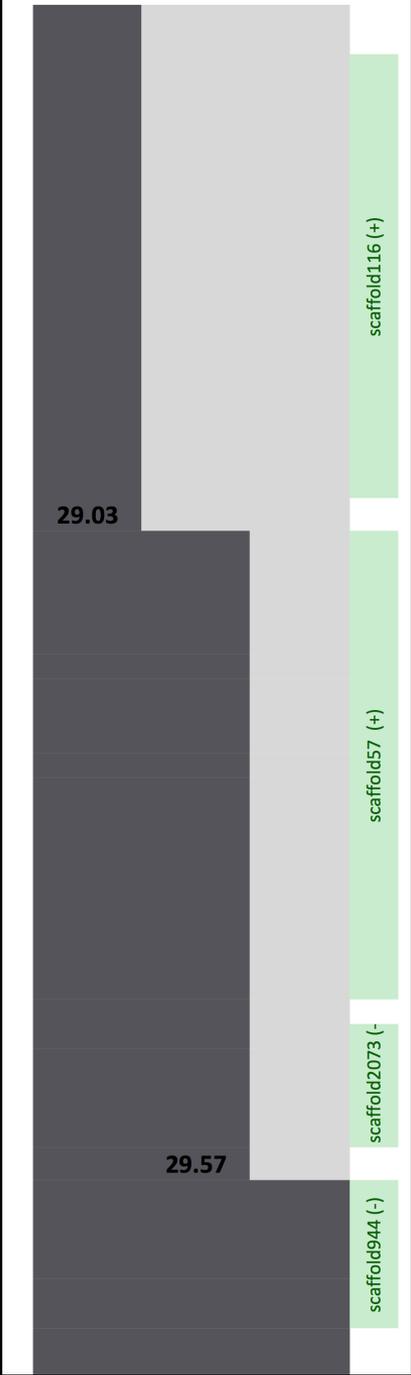
11 16 38 46 49 52 55 59 66 70 75

ACG



scaffold116 (+)



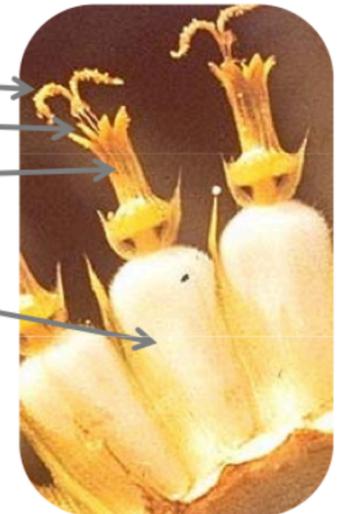


# Stats

assembly	nATCG	length	n	N50	N90
gold-superscaffolds	1658168430	3131940255	14940	476491	89759
bronze-pseudomolecules	1989332786	3641596391	17		
bronze-superscaffolds	2037102379	4168038957	31392	210251	54824
AllPathsLG	993696150	1156891308	100027	20687	6018
Nov22k22	2163238910	3082784204	453539	24876	1541
454AllContigs	1946374353	1946374353	1603248	2009	509

# mRNA discovery strategy

- Genotype XRQ (B)
  - Illumina 100bp
- 3 vegetative tissues
  - Leaves, roots and stem
- 8 floral tissues
  - Bracts
  - Ray florets: Ligule (tube and lamina)
  - *Ray florets: Sterile ovary*
  - Disc florets: Style (stigmata + style)
  - Disc florets: Stamens (anthers + filaments)
  - Disc florets: Corolla( Disc floret petals)
  - Disc florets: Seeds (Disc floret seeds)
  - Pollen
- collected at 3-5 different developmental stages





https://www.heliogene.org/HA412.v1.1.bronze.20141015/



Consortium File View Help

0 20,000,000 40,000,000 60,000,000 80,000,000 100,000,000 120,000,000 140,000,000 160,000,000 180,000,000 200,000,000

Navigation controls: back, forward, zoom in, zoom out, search. Search box: Ha4 Ha4:17163981..17186820 (22.84 Kb) Go

17,165,000 17,170,000 17,175,000 17,180,000 17,185,000

Helianthus annuus XRQ RNASeq transcripts  
HaT13I045675  
HaT13I045675

Helianthus annuus 412-HO RNASeq transcripts

EugeneAnnotation V1.1

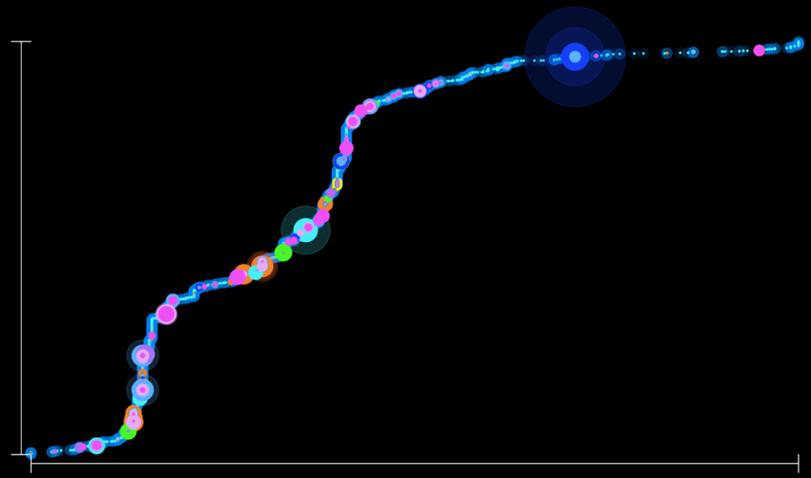
airpin glycosidase-lik...

Ha412v1r1\_04g005740  
Ha412v1r1\_04g005740

AutoIPR: IPR000922:D-galactoside/L-rhamnose binding SUEL lectin domain; IPR001944:Glycoside hydrolase, family 35; IPR017853:Glycoside ...

LG01

125.8 MBP



79.5 CM

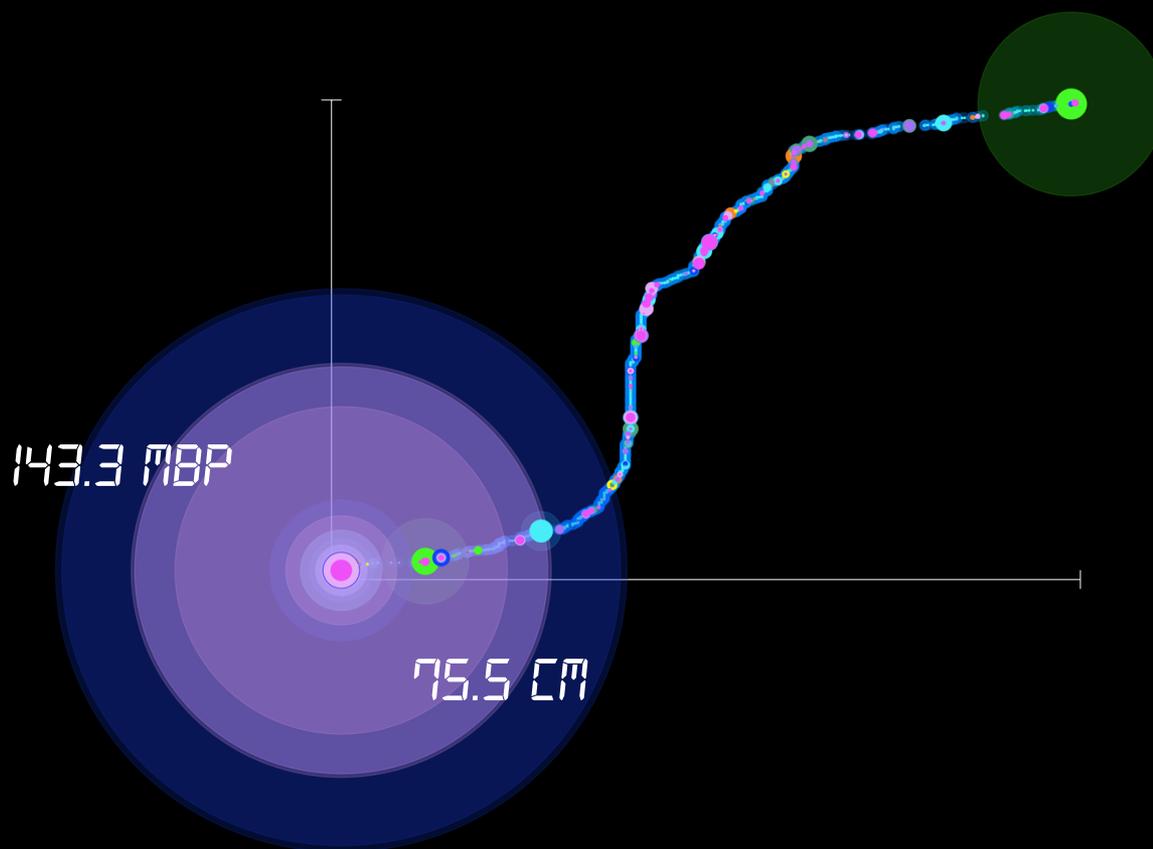
LG02

153.9 MBP



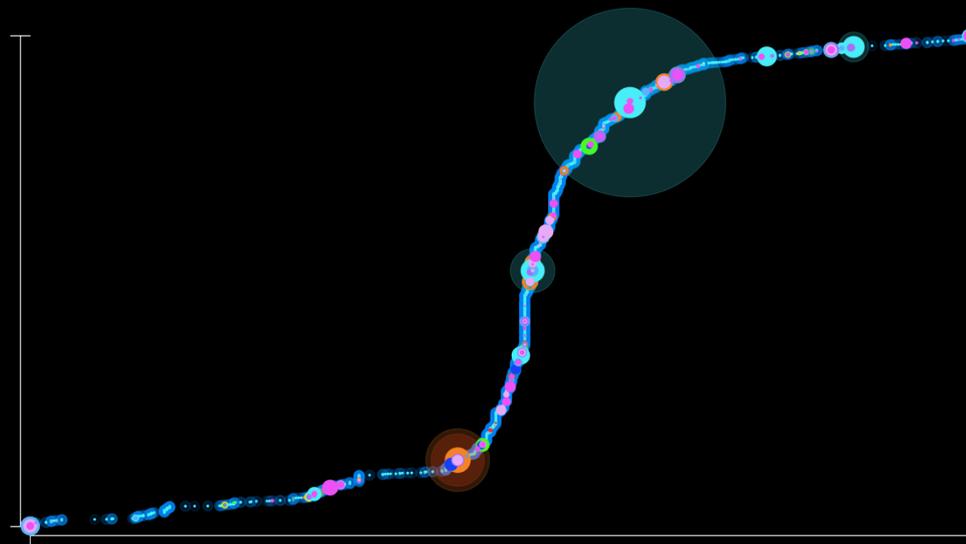
83.6 CM

LG03



LG04

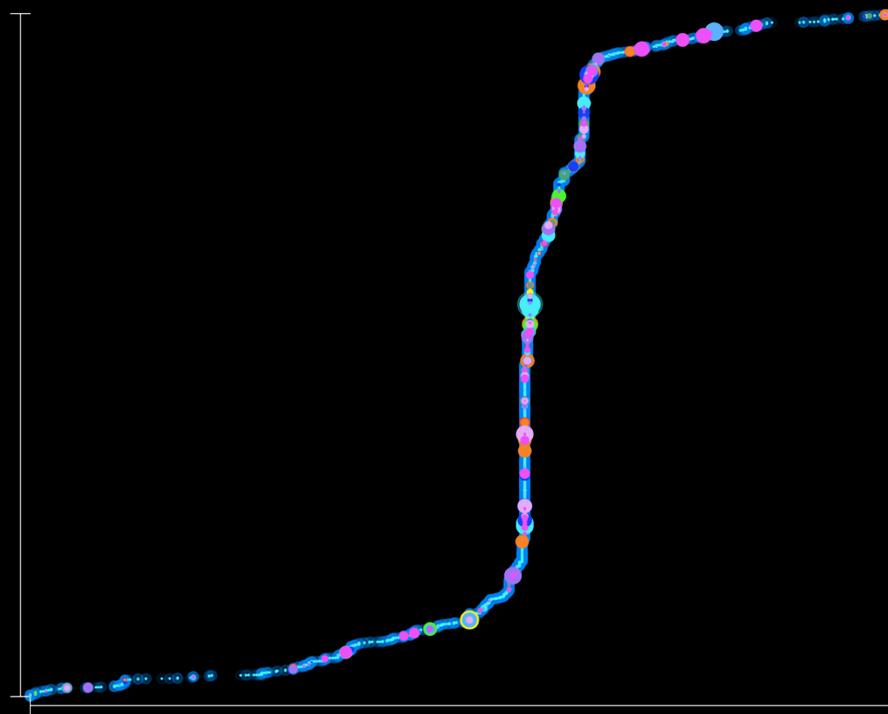
149.5 MBP



95.7 CM

LG05

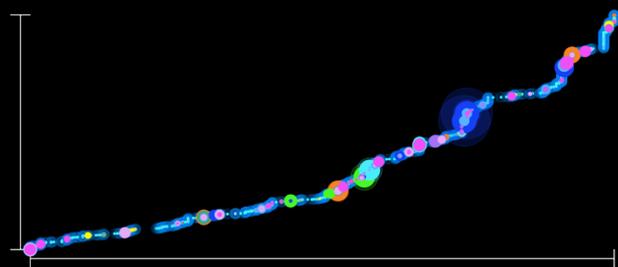
208.0 MBP



87.9 CM

LG06

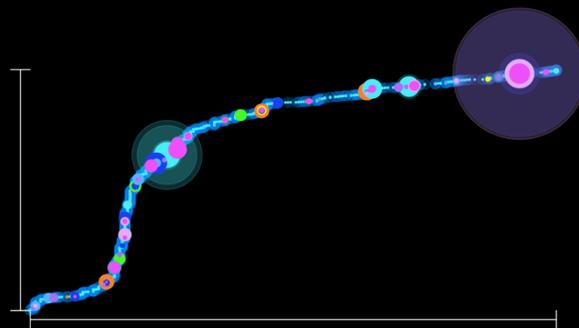
71.5 MBP



59.6 CM

L607

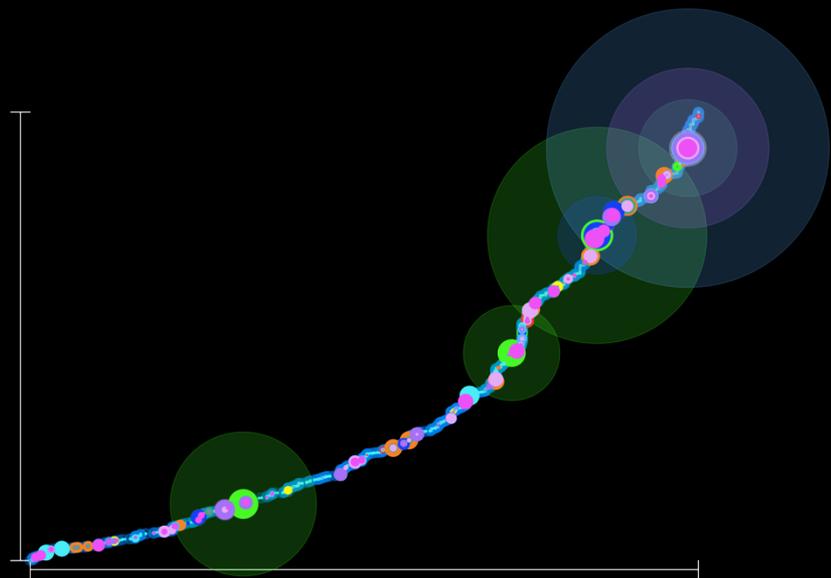
73.4 MBP



53.8 CM

1608

136.6 MBP



68.3 CM

1609

187.3 MBP

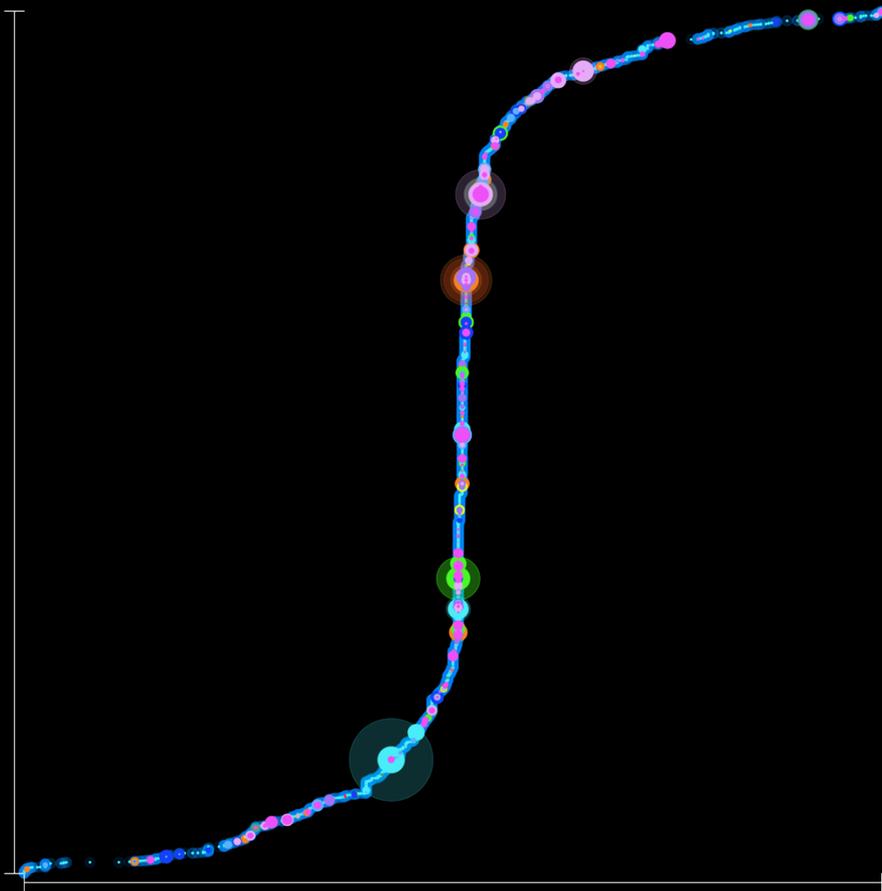


91.9 CM

262.7 MBP

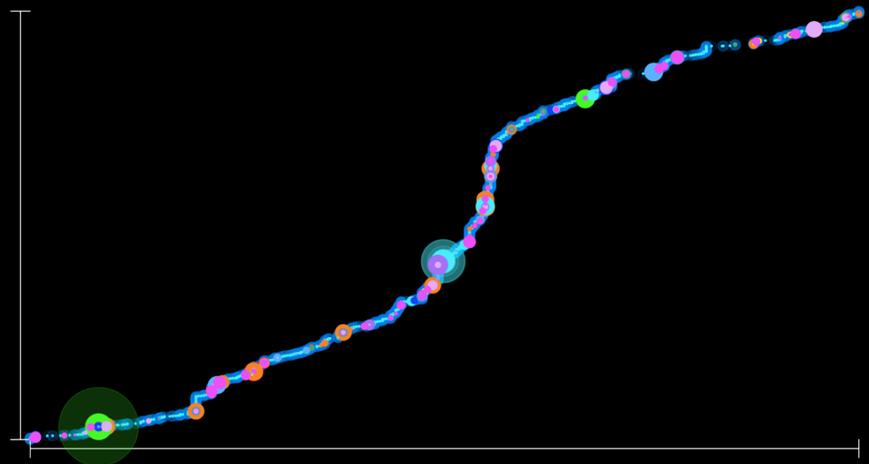
87.6 CM

LG10



LG11

130.5 MBP

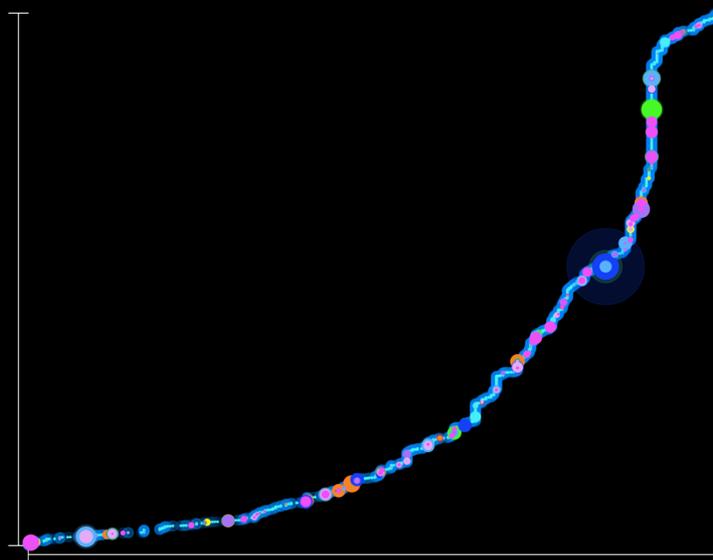


84.7 CM

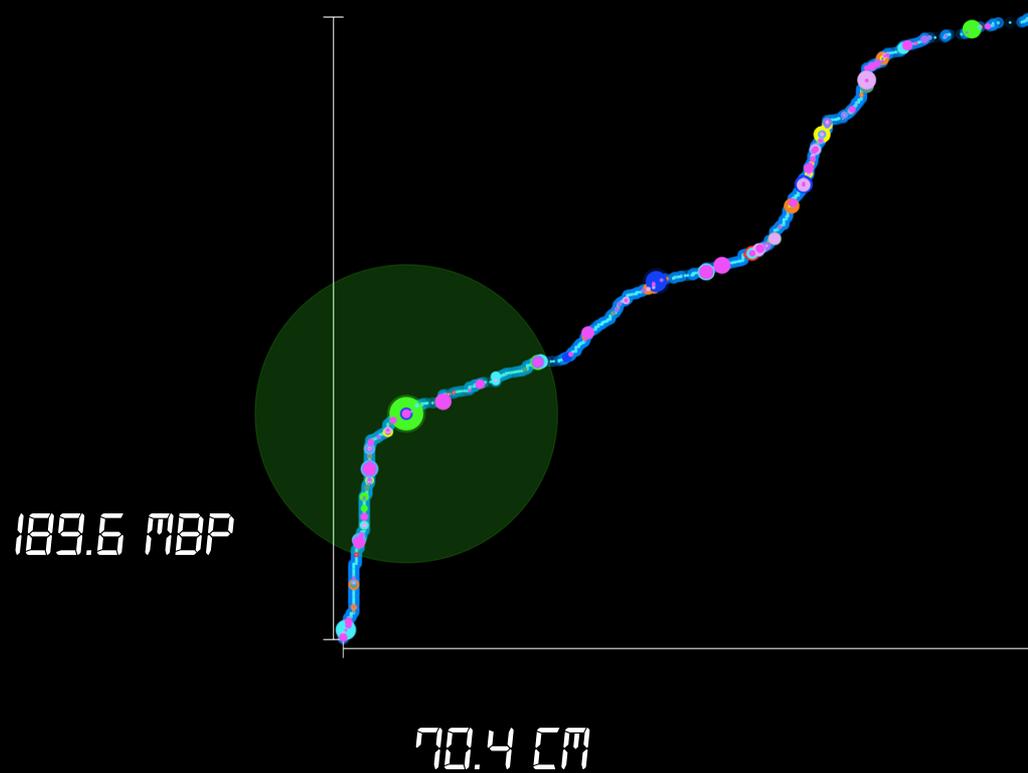
LG12

162.2 MBP

70.2 CM



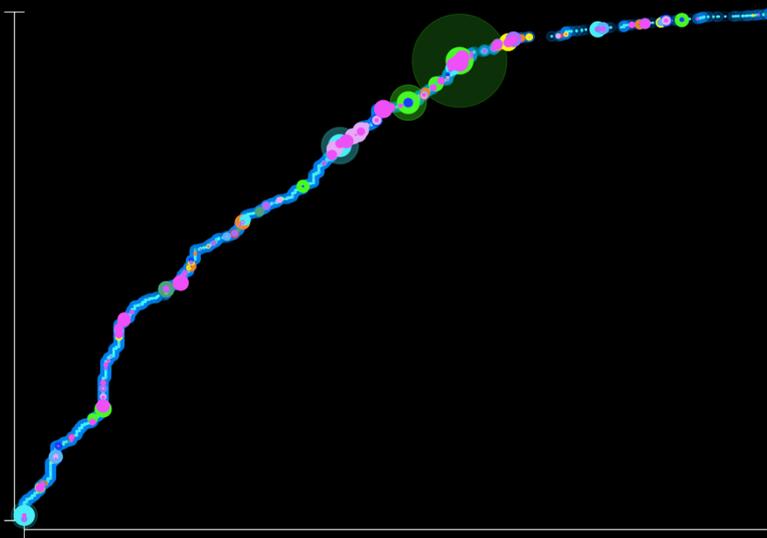
LG13



LG14

154.9 MBP

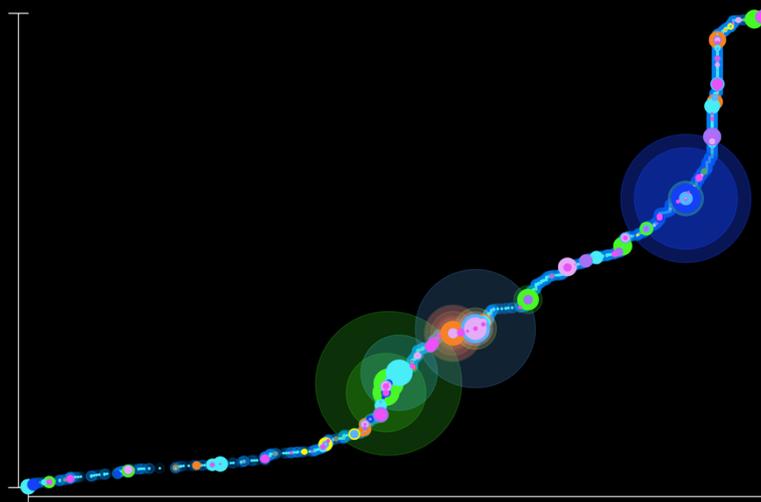
76.1 CM



LG15

144.4 MBP

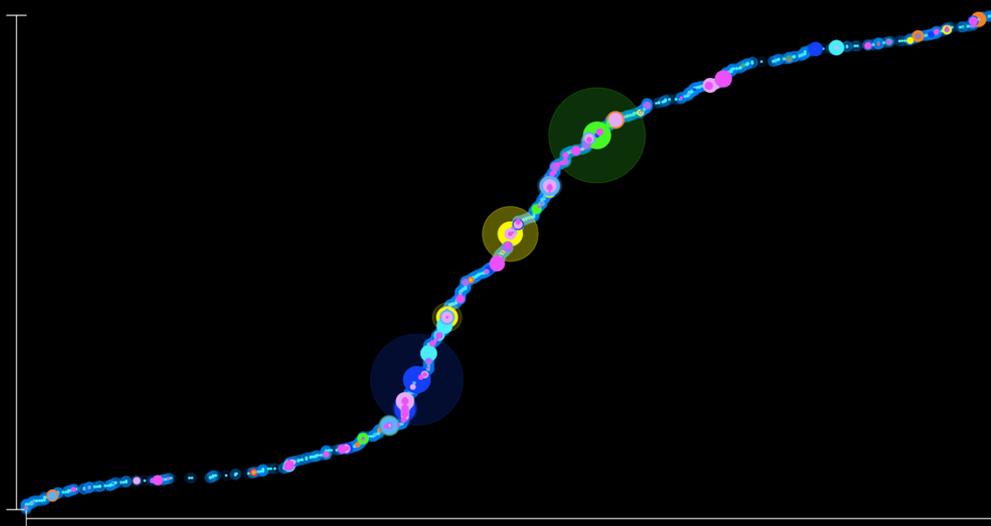
75.3 CM



LG16

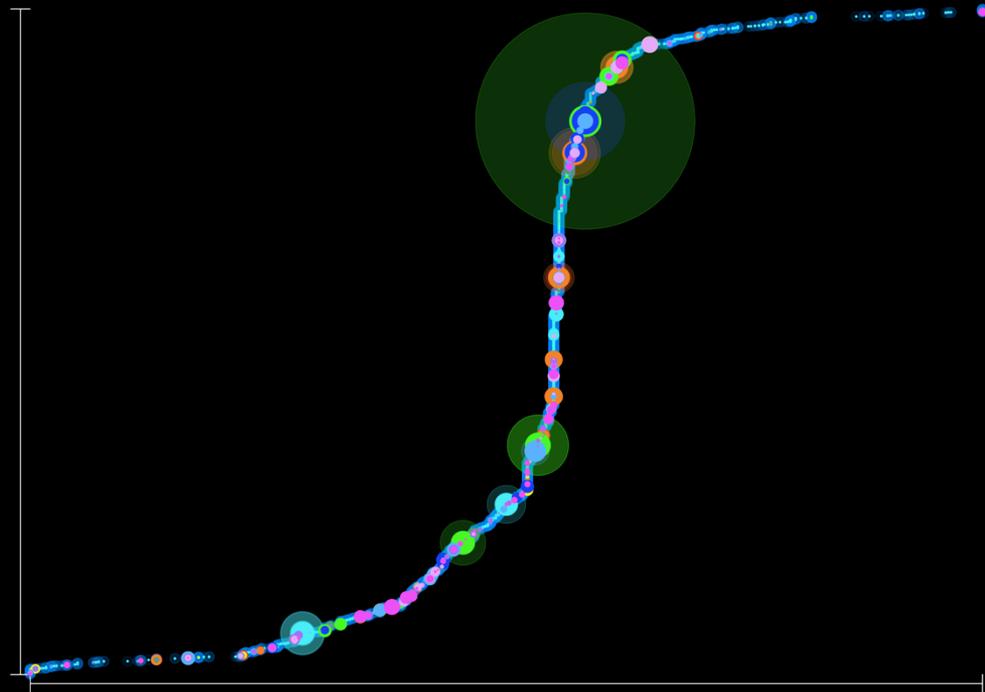
150.5 MBP

98.9 CM



LG17

202.7 MBP



97.3 CM