



NIAS

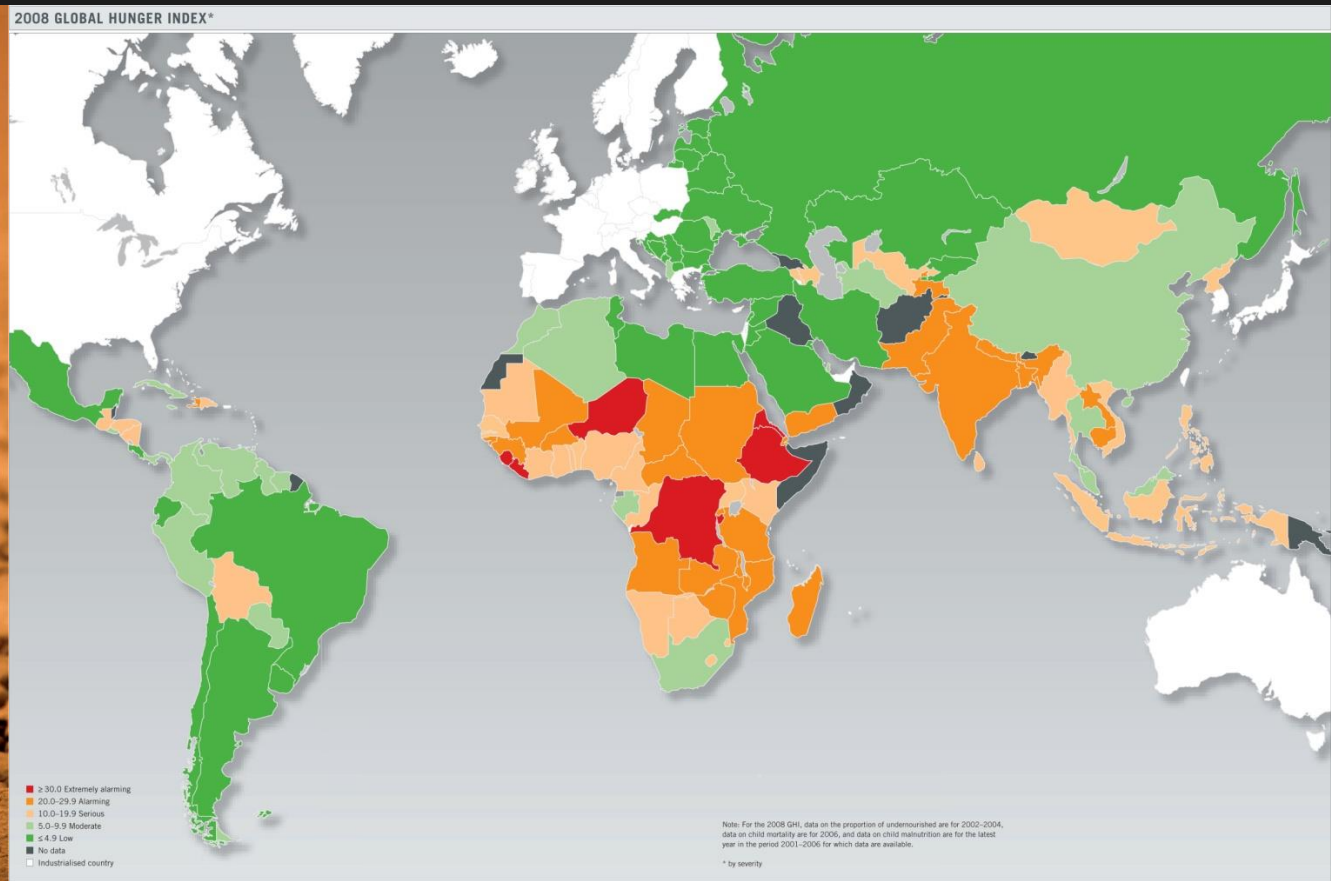
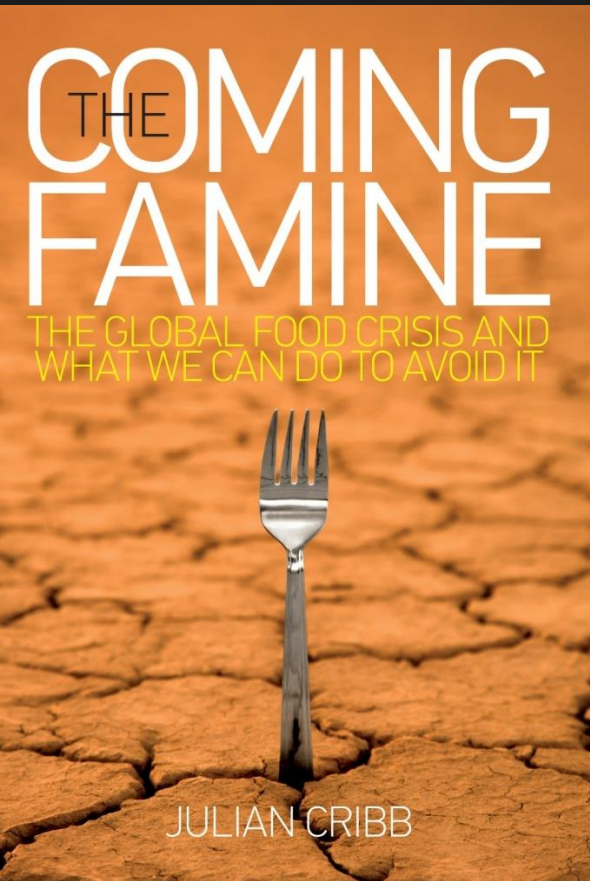
Genetic Resource Center

The Wild Vigna

JST PRESTO

Ken Naito

800M People in Malnutrition



The Problem = Stress

Salinity



800Mha

Acid Soil



4GHa

Alkaline Soil



4GHa

Drought



5Gha

Flooding



ex. Thailand, 2011

Pest & Disease



30% Product/y

Genus *Vigna*

9 domesticated

Azuki bean, Mungbean, Cowpea, etc...

82

Sexy!!

The Problem = Stress



Sal

Soil

800

Dro

la

disease

5Gha

Thailand, 2011

30% Product/y

The Problem = Stress

Salinity

Acid Soil

Alkaline Soil



pH3



pH6

5Gha

Thailand, 2011

30% Product/y

The Problem = Stress

Salinity



800Mha

Drought



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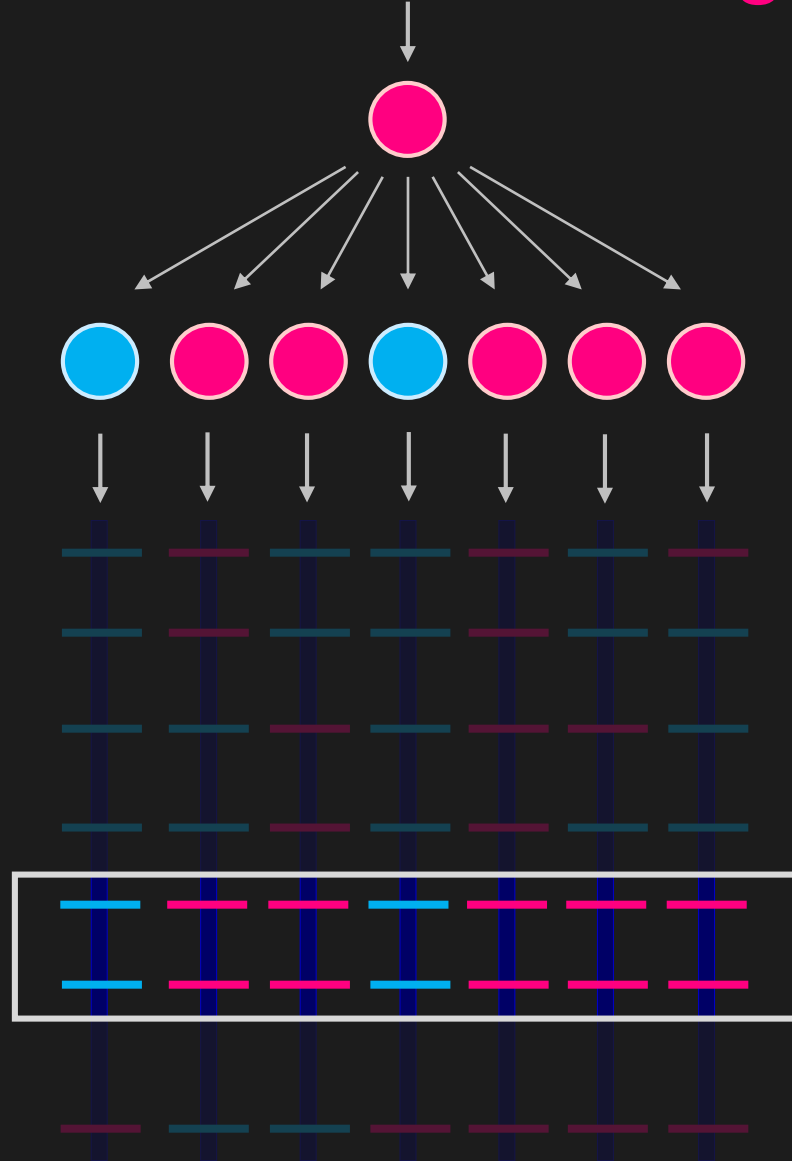


5Gha

Thailand, 2011

30% Product/y

Naïve  ×  Tough



Salinity

Acid Soil

Alkaline Soil

How can they be
so adapted??

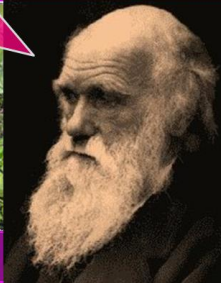
800kHa

4GHa

Drought

Flooding

Pest & Disease



5Gha

ex. Thailand, 2011

30%



The Problem = Stress

Salinity

Acid Soil

Alkaline Soil

MANIAC

5Gha

ex. Thailand, 2011

30% Product/y



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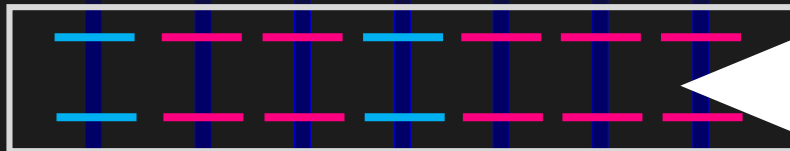
- ☐ [QTL mapping for salt tolerance and domestication-related traits in *Vigna marina* subsp. *oblonga*, a halophytic species.](#)
1. Chankaew S, Isemura T, Naito K, Ogiso-Tanaka E, Tomooka N, Somta P, Kaga A, Vaughan DA, Srinives P.
Theor Appl Genet. 2014 Mar;127(3):691-702. doi: 10.1007/s00122-013-2251-1. Epub 2013 Dec 27.
PMID: 24370961 [PubMed - in process]
[Related citations](#)
- ☐ [In-silico comparative study of inhibitory mechanism of Plant Serine Proteinase Inhibitors.](#)
2. Siva Prasad CV, Gupta S, Gaponenko A, Dhar M.
Bioinformation. 2012;8(14):673-7. doi: 10.6026/97320630008673. Epub 2012 Jul 21.
PMID: 23055608 [PubMed] **Free PMC Article**
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- ☐ [Comparison of the formation of nicotinic acid conjugates in leaves of different plant species.](#)
3. Ashihara H, Yin Y, Katahira R, Watanabe S, Mimura T, Sasamoto H.
Plant Physiol Biochem. 2012 Nov;60:190-5. doi: 10.1016/j.plaphy.2012.08.007. Epub 2012 Aug 29.
PMID: 22983143 [PubMed - indexed for MEDLINE]
[Related citations](#)
- ☐ [Community of arbuscular mycorrhizal fungi in a coastal vegetation on Okinawa island and effect of the isolated fungi on growth of sorghum under salt-treated conditions.](#)
4. Yamato M, Ikeda S, Iwase K.
Mycorrhiza. 2008 Jul;18(5):241-9. doi: 10.1007/s00572-008-0177-2. Epub 2008 May 31.
PMID: 18516629 [PubMed - indexed for MEDLINE]
[Related citations](#)

Genome Sequence Matters

Naïve  ×  Tough

*If only we had
genome sequence...*

- ~~Design markers~~
- ~~Screen for polymorphisms~~
- ~~Build up a linkage map~~
- ~~Rough mapping~~
- ~~Design & screen more markers~~
- Develop large mapping population
- Fine mapping
- Clone the gene ~~(if lucky)~~



Sequence it!



NIAS
BRAIN

THE VIGNA GENOME PROJECT

Hiroaki Sakai & Ken Naito

VigGS

BIOINFORMATICIAN
HIROAKI SAKAI



A close-up, high-resolution photograph of a large quantity of red kidney beans. The beans are a deep, vibrant red color with a glossy, reflective surface. They are densely packed, filling the entire frame. The lighting creates bright highlights and soft shadows, emphasizing their smooth, kidney-like shape. In the center of the image, the text "539 Mb" is overlaid in a large, white, sans-serif font. The text is sharp and clear, contrasting sharply with the busy, textured background of the beans.

539 Mb

De novo Assembly: Azuki Bean



Library

Yield

Single End	7.6Gb
Mate-Pair (3kb)	1.3Gb
Mate-Pair (8kb)	1.2Gb
Mate-Pair (20kb)	1.1Gb

Library

Yield

Paired-End (300bp)	37.3Gb
Mate-Pair (3kb)	31.3Gb
Mate-Pair (8kb)	24.8Gb
Mate-Pair (20kb)	25.9Gb
Mate-Pair (40kb)	11.4Gb

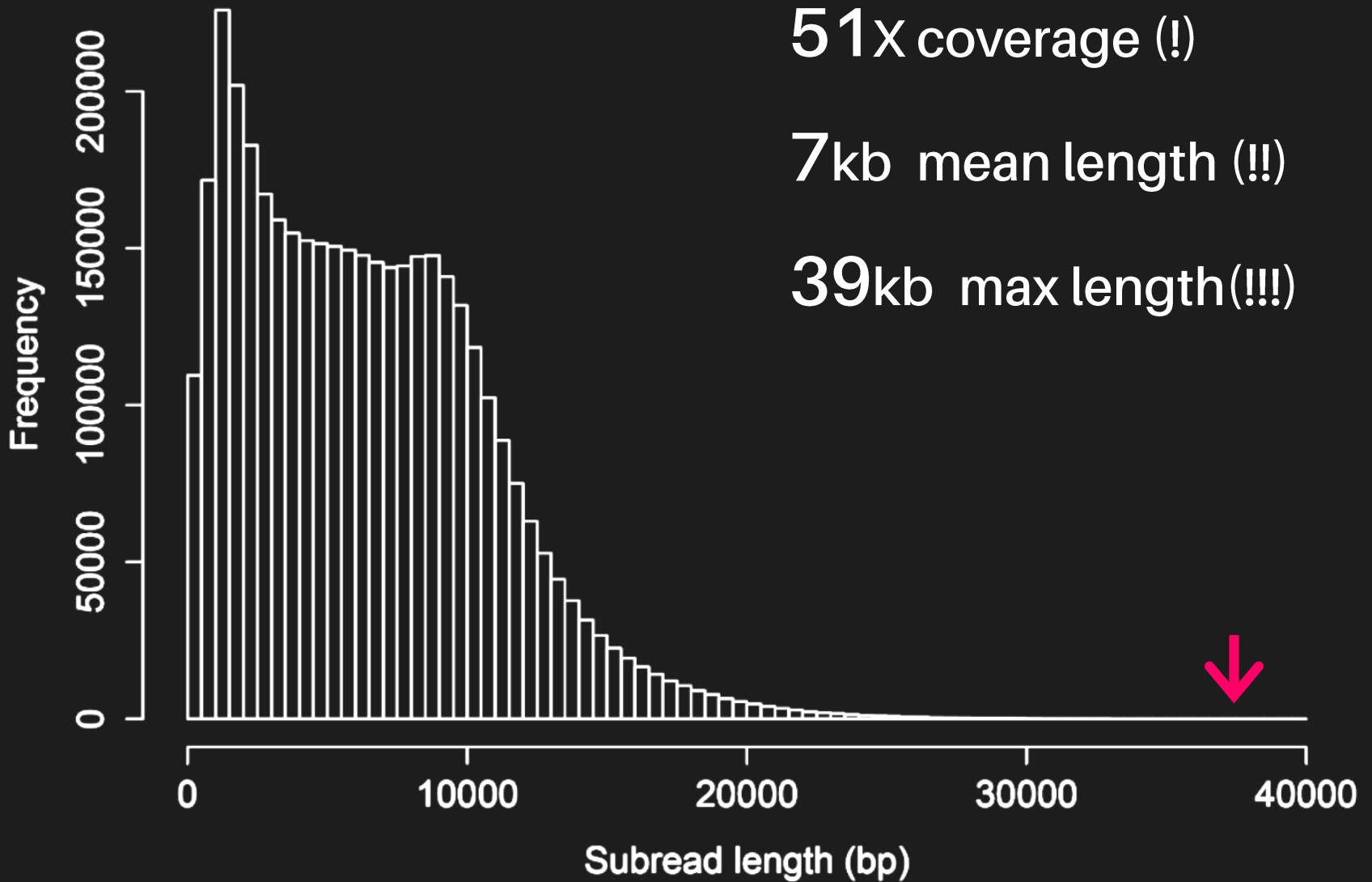


	Contig		Scaffold	
	Roche	Illumina	Roche	Illumina
#Seqs	42,291	30,487	8,910	3,739
Mean	10.7 kb	13.0 kb	56.7kb	127 kb
Max	238 kb	280 kb	2,9 Mb	14.1 Mb
N50	27.7 kb	27.7 kb	612 kb	3.0 Mb
Total (Mb)	454 Mb	397 Mb	505 Mb	473 Mb
Coverage (%)	84.2	73.7	93.7	87.8
#Anchored seqs	-	-	1,024	308
Anchored bp	-	-	462 Mb	451 Mb
Assembly errors	-	-	376	69

OIAS
Takashi Hirano



PacBio RS II



51X coverage (!)

7kb mean length (!!)

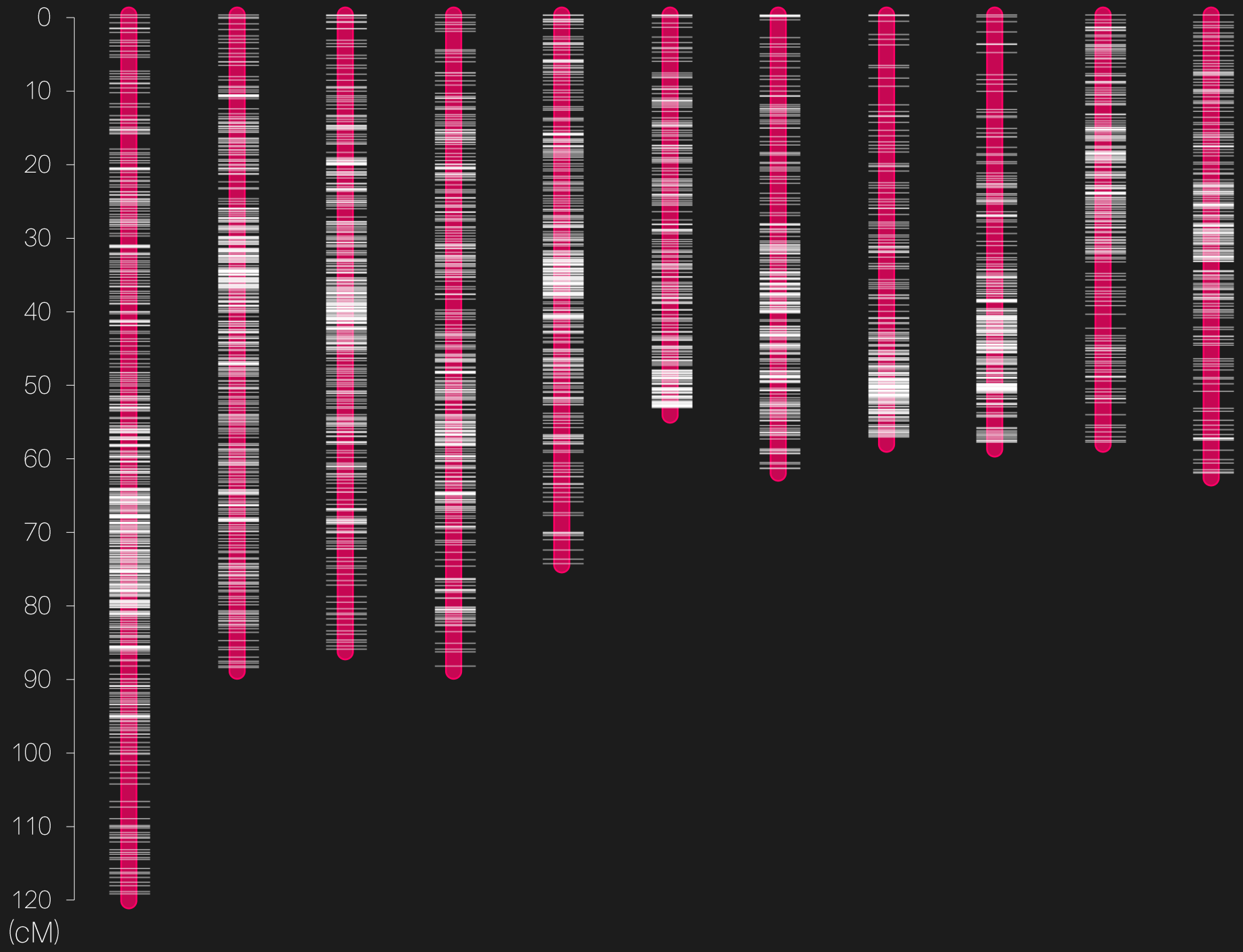
39kb max length(!!!)



S P R A Y

Roche vs. Illumina vs. Pacbio

	Contig			Scaffold	
	Roche	Illumina	Pacbio	Roche	Illumina
#Seqs	42,291	30,487	4,638	8,910	3,739
Mean	10.7 kb	13.0 kb	113 kb	56.7 kb	127 kb
Max	238 kb	280 kb	7.5 Mb	2.9 Mb	14.1 Mb
N50	27.7 kb	27.7 kb	809 kb	612 kb	3.0 Mb
Total (Mb)	454 Mb	397 Mb	504 Mb	505 Mb	473 Mb
Coverage (%)	84.2	73.7	93.5	93.7	87.8



Roche vs. Illumina vs. Pacbio

	Contig	Scaffold	
	Pacb	Roche	Illumina
#Seqs	4,638	8,910	3,739
Total (Mb)	504 Mb	505 Mb	473 Mb
#Anchored seqs	759	1,024	308
Anchored bp	449 Mb	462 Mb	451 Mb
Assembly errors	19	376	69

LG5

35.27cM

LG5

35.27cM

LG1

68.49cM

LG1

68.54cM

LG1

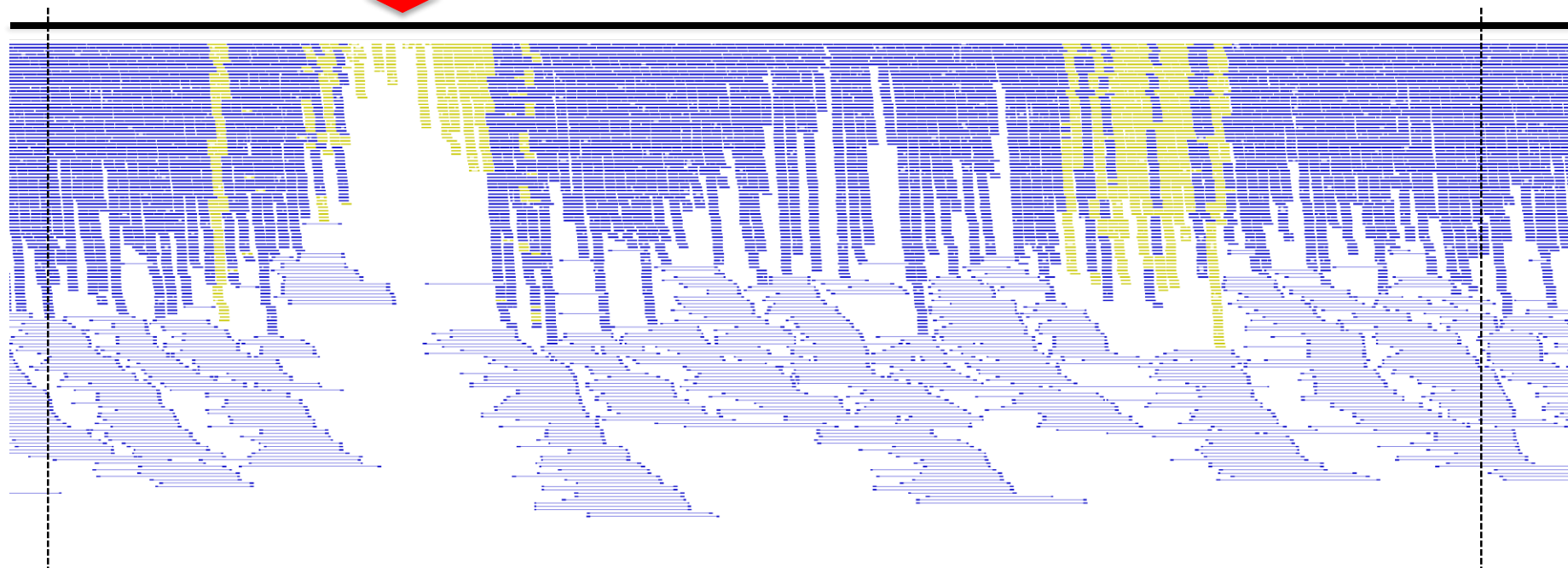
68.54cM

LG5
35.27cM

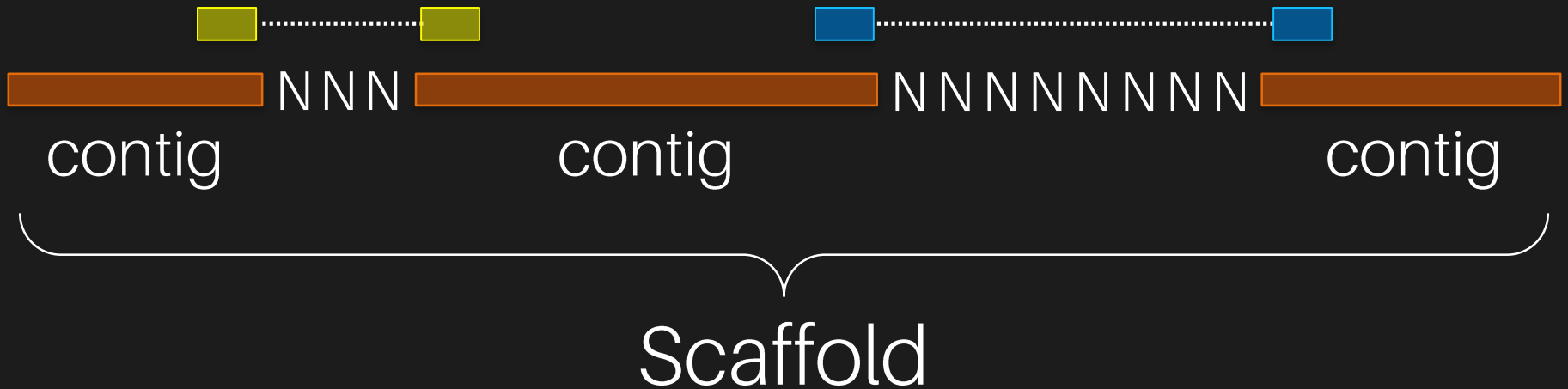
mis-assembly



LG1
68.49cM



Scaffold Pacbio Contigs?



Library	Yield
Paired-End (300bp)	37.3Gb
Mate-Pair (3kb)	31.3Gb
Mate-Pair (8kb)	24.8Gb
Mate-Pair (20kb)	25.9Gb
Mate-Pair (40kb)	11.4Gb



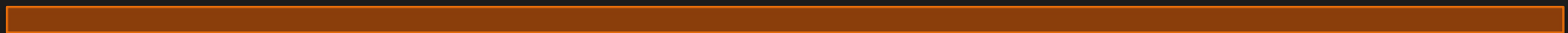
Scaffold Pacbio Contigs?



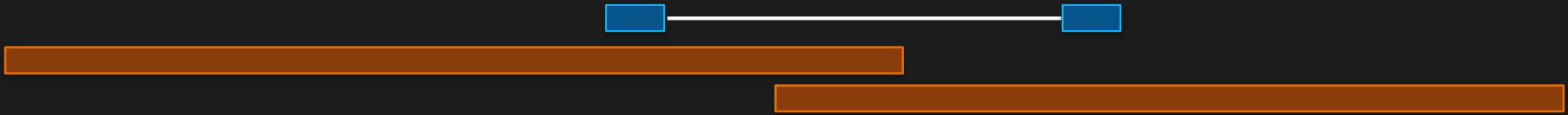
PB-Jelly2: Gap filling by Pacbio reads



Connected



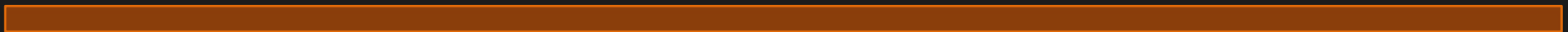
Scaffold Pacbio Contigs?



BLASTed the 2 contigs to each other



Connected



Pacbio Final Assembly

	Contig	Contig'	Scaffold
#Seqs	4,638	2,920	2,529
Mean	113	176	203
Max	7.5 _{Mb}	12.7 _{Mb}	12.7 _{Mb}
N50	809 _{kb}	1.6 _{Mb}	3.0 _{Mb}
Total (Mb)	504 _{Mb}	513 _{Mb}	514 _{Mb}
Coverage (%)	94.6	95.2	95.4
#Anchored seqs	759	-	279
Anchored bp	449 _{Mb}	-	463 _{Mb}
Assembly errors	19	-	0

	Contig			Scaffold		
	Roche	Illumina	PacBio	Roche	Illumina	PacBio
#Seqs	42,291	30,487	4,638	8,910	3,739	2,529
Mean	10.7 _{kb}	13.0 _{kb}	113 _{kb}	56.7 _{kb}	127 _{kb}	203 _{kb}
Max	238 _{kb}	280 _{kb}	7.5 _{Mb}	2.9 _{Mb}	14.1 _{Mb}	12.7 _{Mb}
N50	27.7 _{kb}	27.7 _{kb}	809 _{kb}	612 _{kb}	3.0 _{Mb}	3.0 _{Mb}
Total (Mb)	454 _{Mb}	397 _{Mb}	504 _{Mb}	505 _{Mb}	473 _{Mb}	514 _{Mb}
Coverage (%)	84.2	73.7	94.6	93.7	87.8	95.4
Gap (%)				10.1	16.1	0.1
#Anchored seqs	-	-	759	1,024	308	279
Anchored bp	-	-	449 _{Mb}	462 _{Mb}	451 _{Mb}	463 _{Mb}
Assembly errors	-	-	19	376	69	0

Genome Annotation

Gene expression

RNA-Seq, CAGE

Cotyledon, Embryo, Flower,
Leaf, Nodule, Pod, Root, Stem



Tophat – Cufflinks



ORF prediction (Trinotate)

Homology/*ab initio*

FlcDNA : Dicots

EST : Cowpea (*V. unguiculata*)

Ab initio prediction : Soybean
Protein sequences (UniProt, RefSeq)



Numa and Itoh, 2013

Genome Annotation

Gene expression

RNA-Seq, CAGE

Cotyledon, Embryo, Flower,
Leaf, Nodule, Pod, Root, Stem



Tophat – Cufflinks



ORF prediction (Trinotate)

Homology/*ab initio*

FlcDNA : Dicots

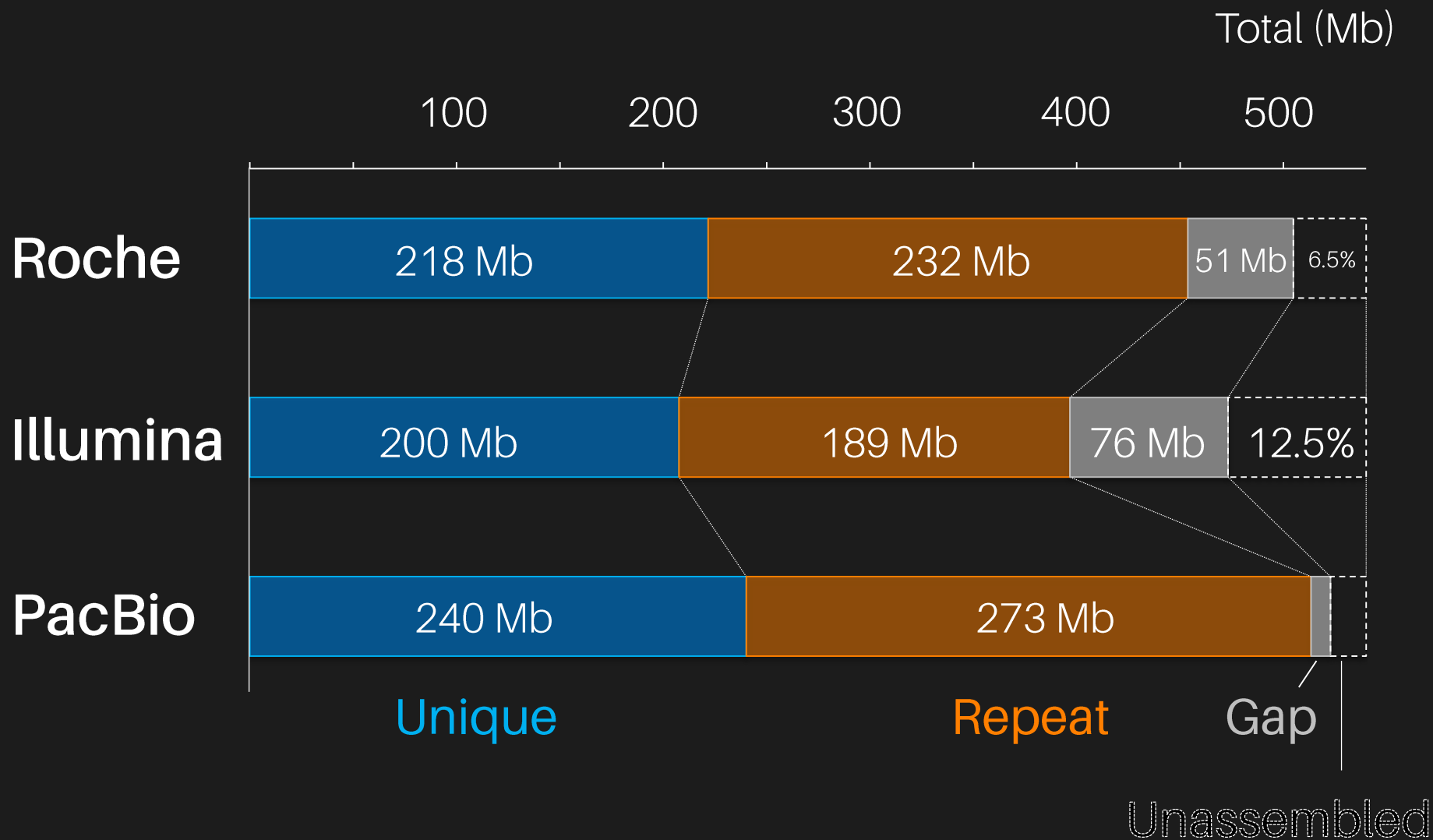
EST : Cowpea (*V. unguiculata*)

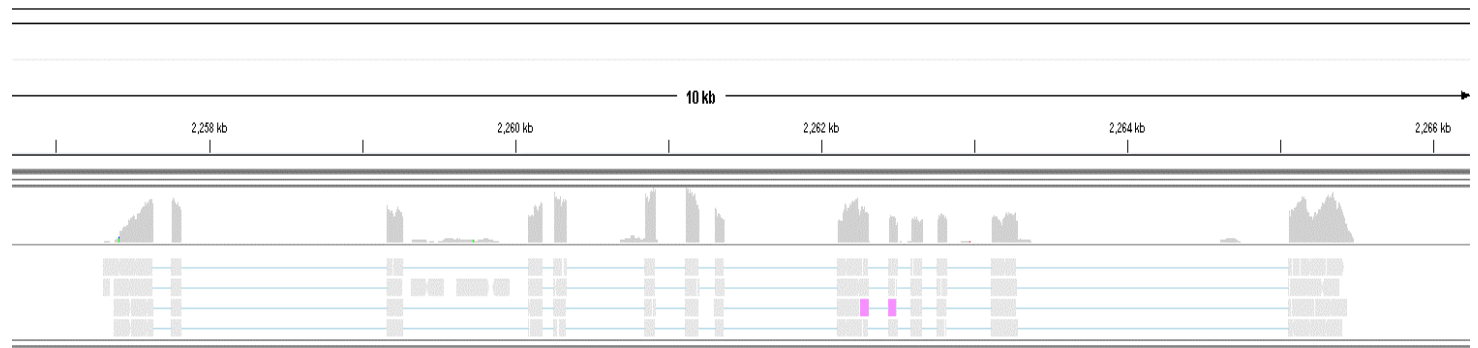
Ab initio prediction : Soybean
Protein sequences (UniProt, RefSeq)



Numa and Itoh, 2013

	Roche	Illumina	PacBio
Coding Genes	31,153	30,187	31,310
Non-coding Genes	2,658	2,482	2,493





PacBio



Illumina




 ↑
GAP



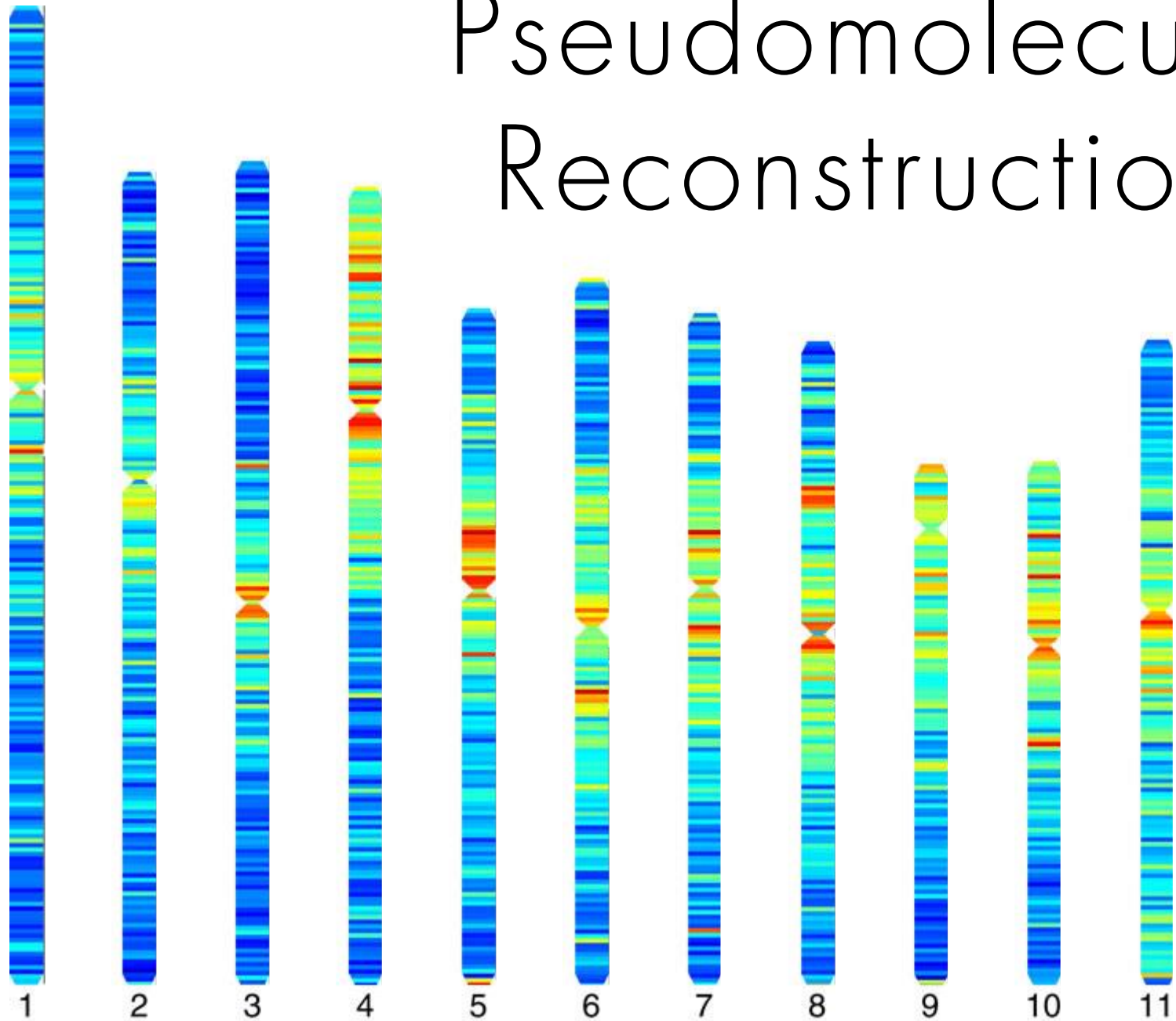
PacBio



Illumina



Pseudomolecule Reconstruction



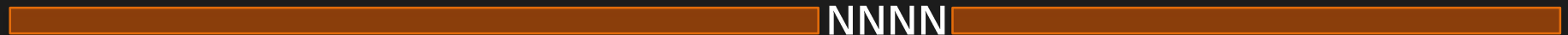
Contig/Scaffold



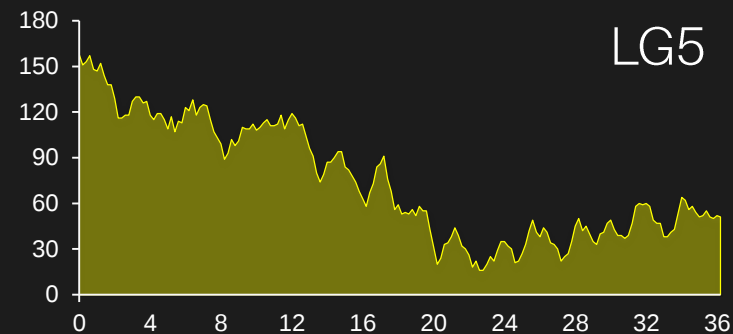
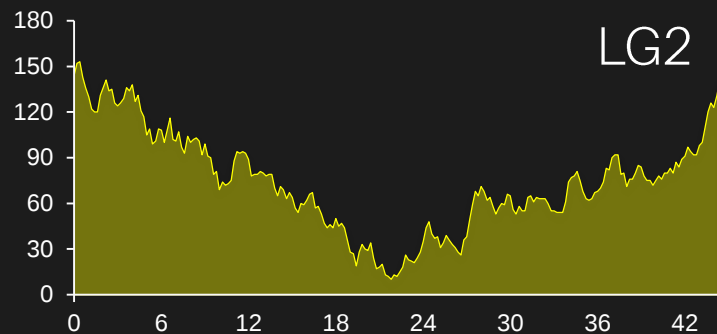
Contig/Scaffold



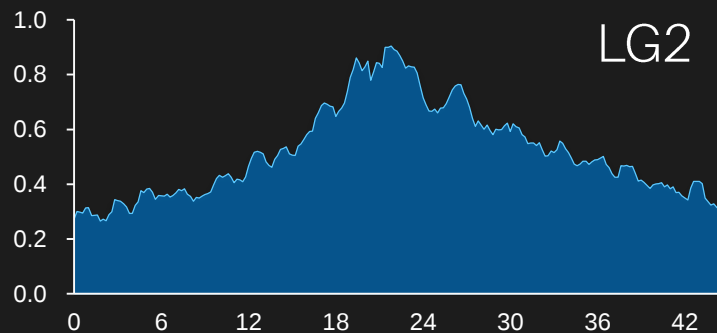
Linkage map



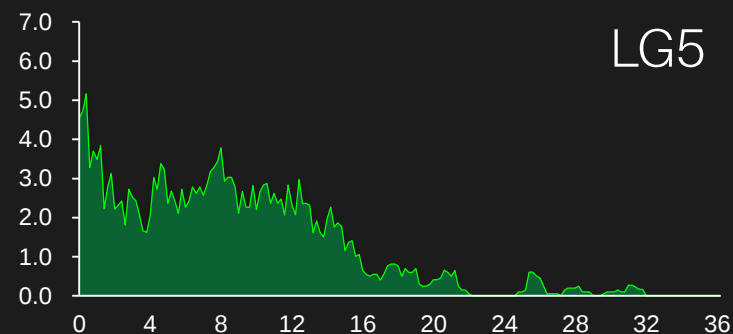
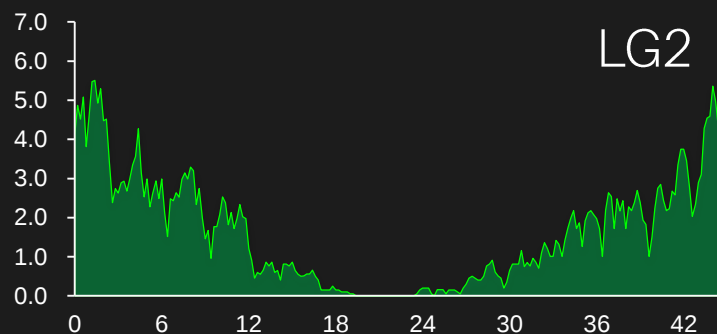
Gene Density



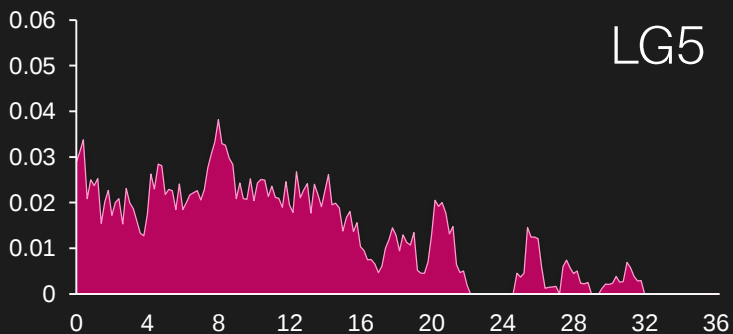
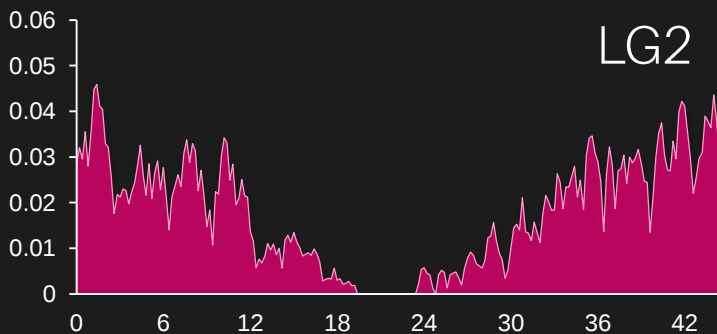
Repeat Share



Recombination
(cM/Mb)



Recombination
(cM/Gene)



Advanced

New Results

 Previous

The power of single molecule real-time sequencing technology in the de novo assembly of a eukaryotic genome

Posted June 29, 2015.

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Hiroaki Sakai, Naito Ken, Eri Ogiso-Tanaka, Yu Takahashi, Kohtaro Iseki, Chiaki Muto, Kazuhito Satou, Kuniko Teruya, Akino Shiroma, Makiko Shimoji, Takashi Hirano, Takeshi Itoh, Akito Kaga, Norihiko Tomooka

doi: <http://dx.doi.org/10.1101/021634>

 Tweet { 8 } g+1 { 0 }

Abstract

Info/History

Metrics

Data Supplements

Subject Area

Species	Salt	Acid	Alkali	Drought	Flooding	Pest/ Disease
<i>V. marina</i>	✓		✓			
<i>V. luteola</i>	✓	✓			✓	
<i>V. riukuensis</i>	✓		✓	✓		
<i>V. nakashimae</i>	✓			✓		
<i>V. trilobata</i>	✓			✓		
<i>V. aridicola</i>				✓		
<i>V. aconitifolia</i>				✓		
<i>V. mungo</i>					✓	
<i>V. stipulacea</i>					✓	✓
<i>V. vexillata</i>	✓	✓	✓	✓	✓	✓
<i>V. minima</i>		✓		✓	✓	
<i>V. exilis</i>			✓			
<i>V. indica</i>			✓			

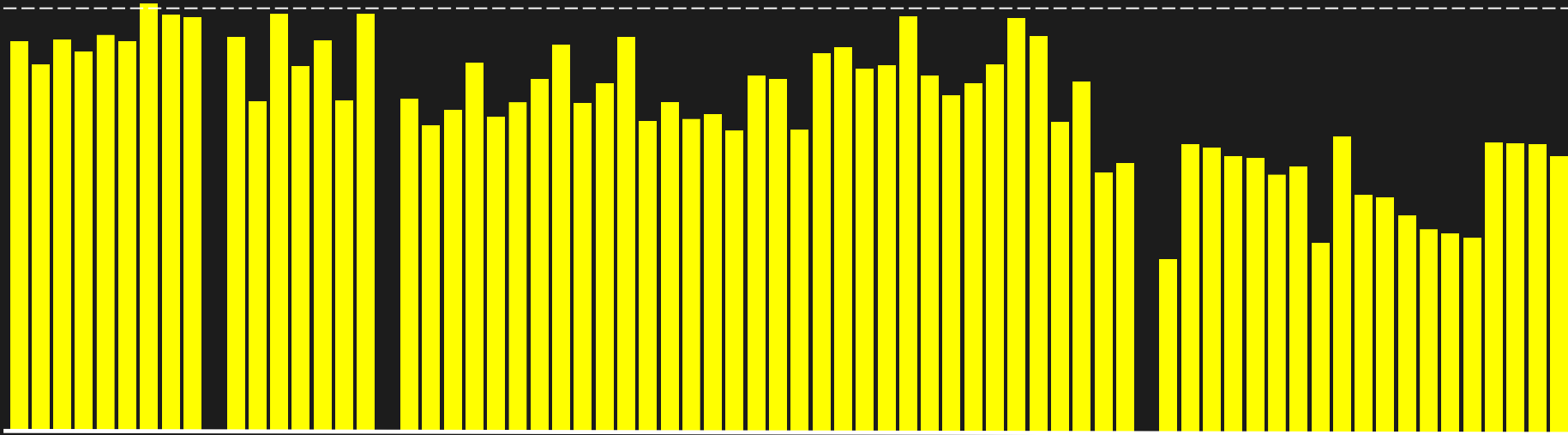
Screening for Salt tolerance



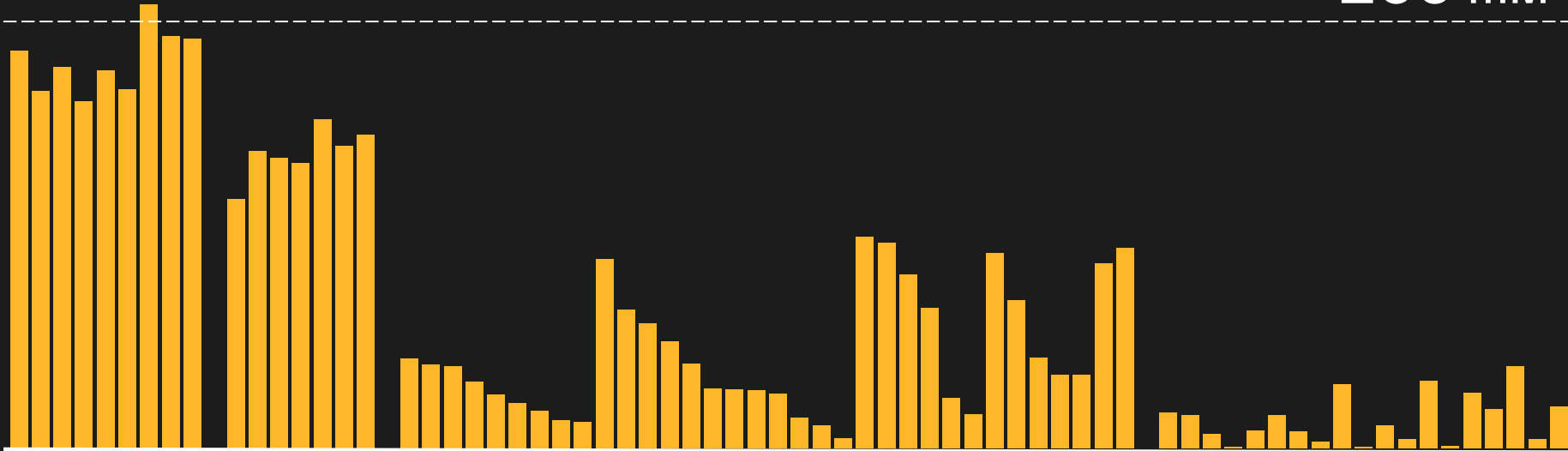
Control
50mM
200mM 2week

Photosynthetic activity

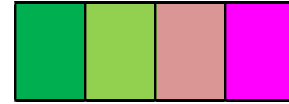
50 mM



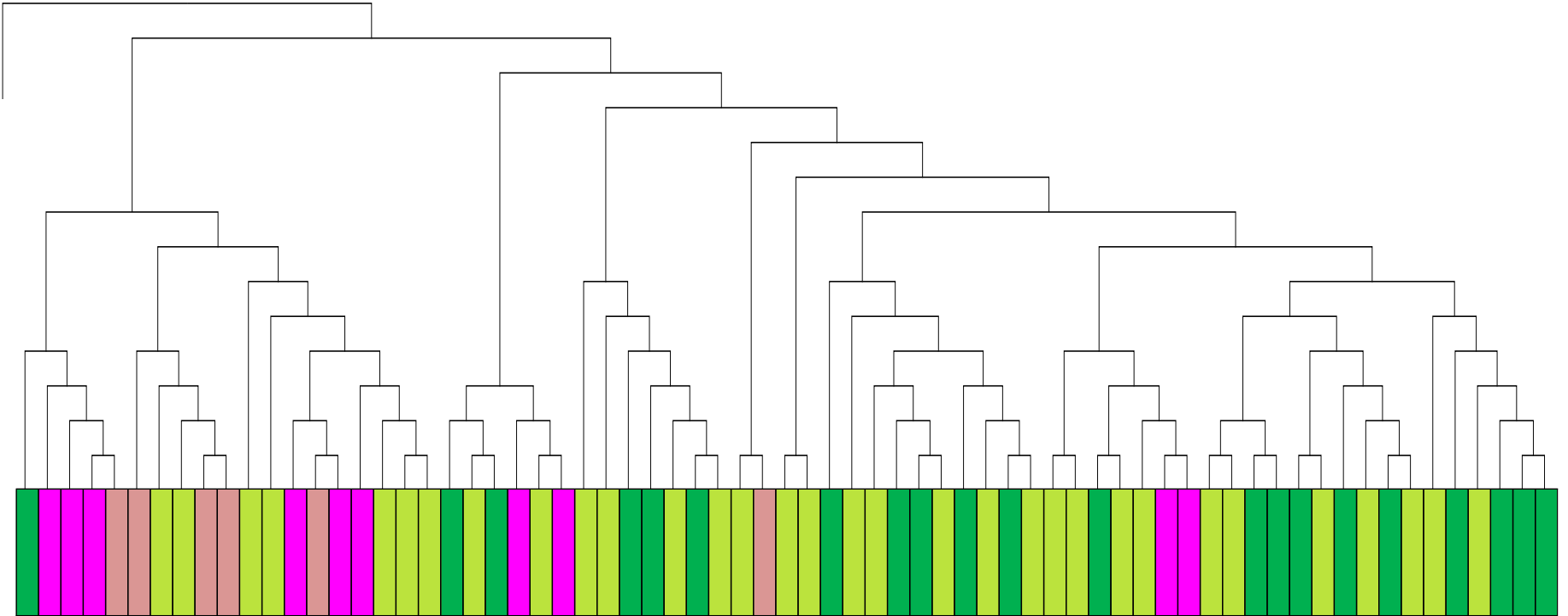
200 mM



Sensitive

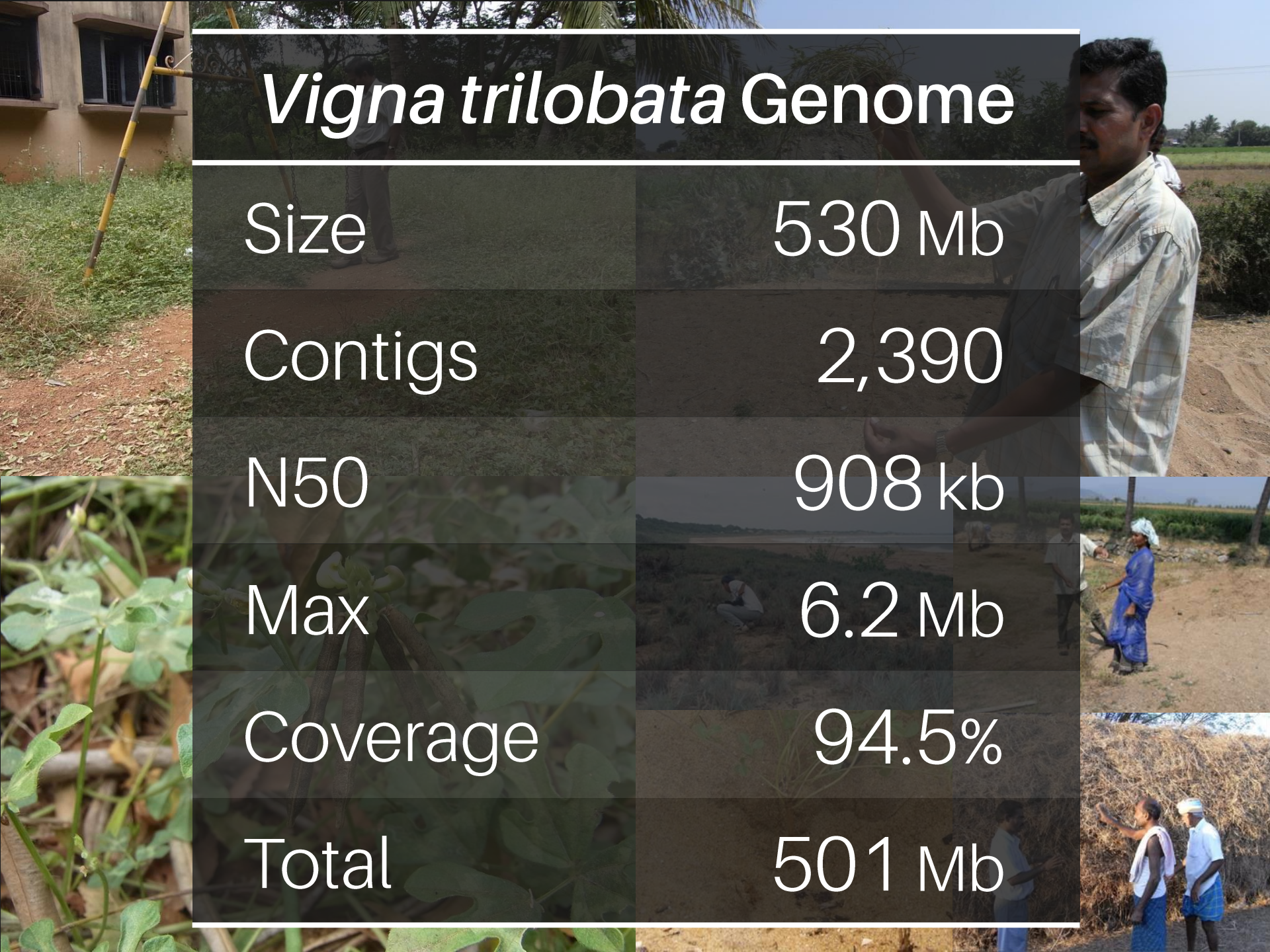


Tolerant



Vigna trilobata



The background of the slide is a collage of five photographs related to Vigna trilobata. Top left: A person standing in a field with a yellow and black striped pole. Top right: A man in a light-colored shirt holding a plant. Middle right: A woman in a blue sari walking in a field. Bottom right: Two people standing next to a large pile of harvested, dried plants. Bottom left: A close-up of green Vigna trilobata leaves and stems.

Vigna trilobata Genome

Size

530 Mb

Contigs

2,390

N50

908 kb

Max

6.2 Mb

Coverage

94.5%

Total

501 Mb

Vigna trilobata (beach)



×

Vigna trilobata (inland)



RAD-seq

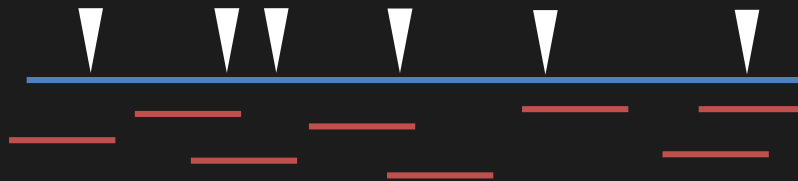


Beach

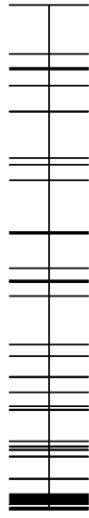
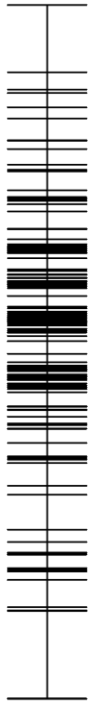


Inland

285 F_2 plants

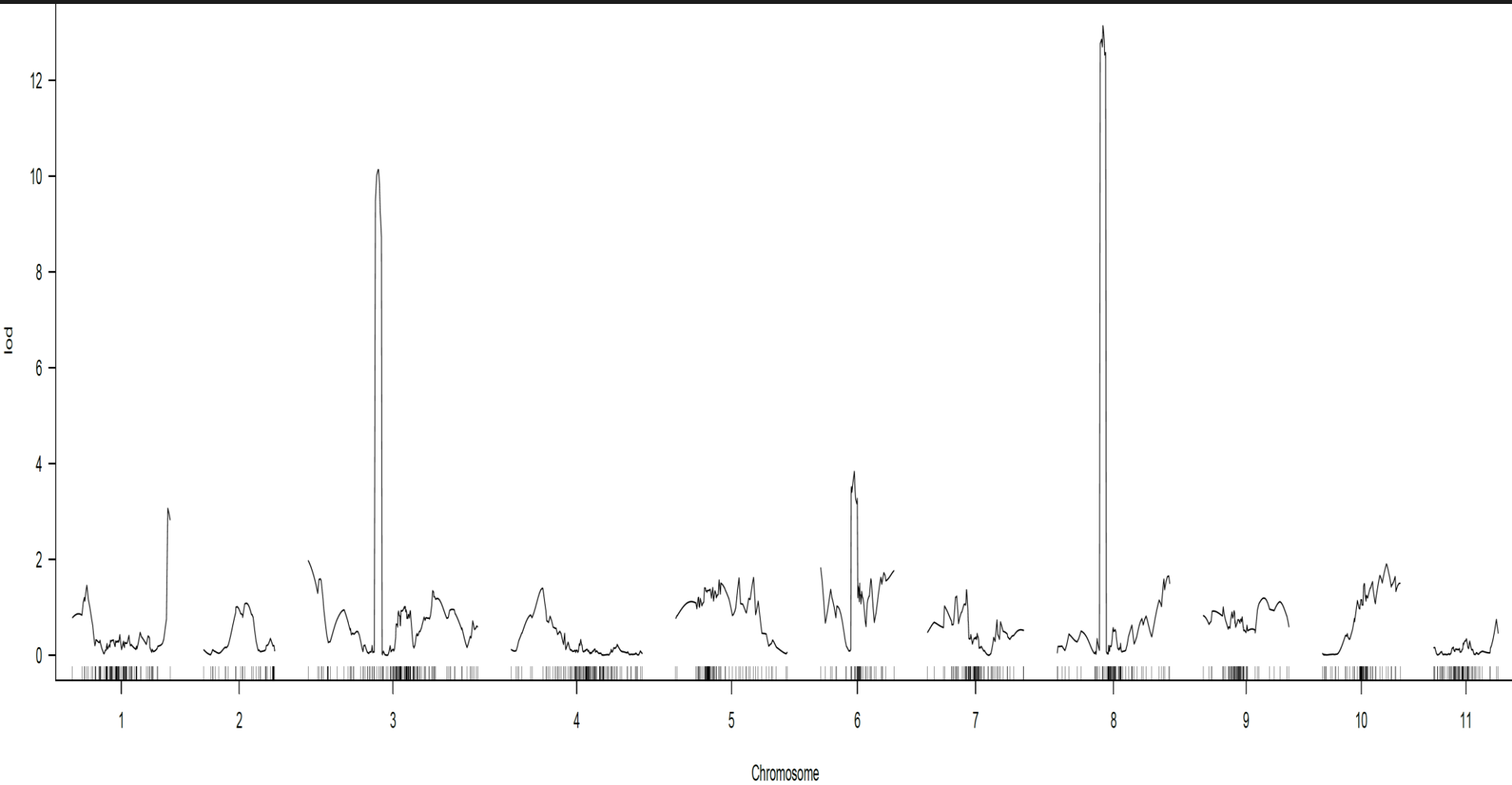


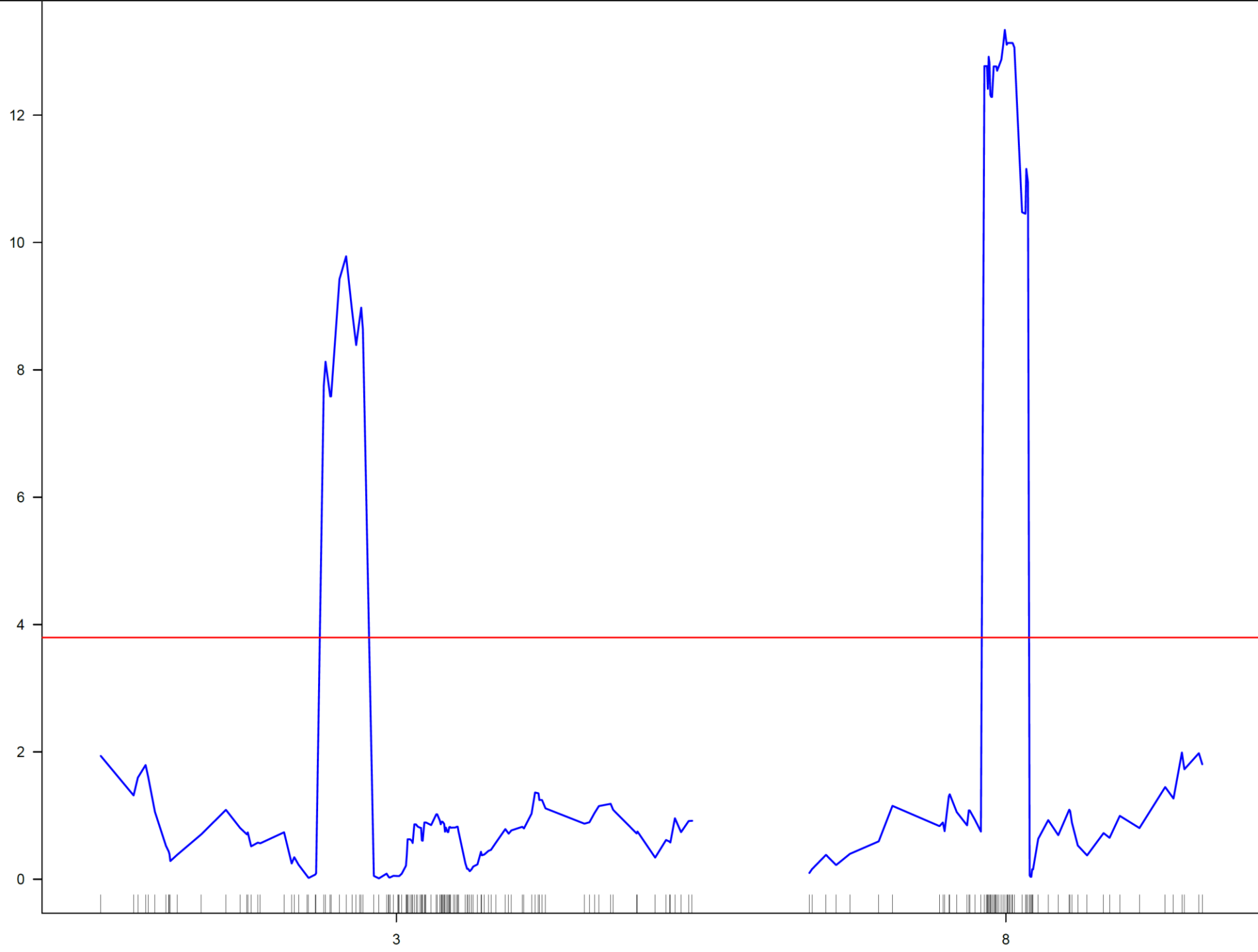
1,100 SNPs



Salt-tolerance Evaluation with F2:3





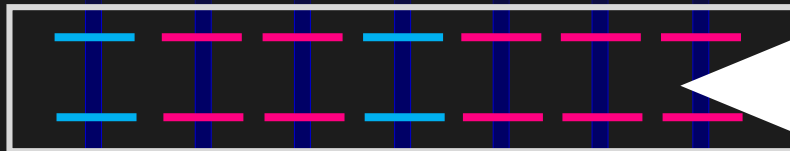


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Naïve  ×  Tough

*If only we had
genome sequence...*

- ~~Design markers~~
- ~~Screen for polymorphisms~~
- ~~Build up a linkage map~~
- ~~Rough mapping~~
- ~~Design & screen more markers~~
- Develop large mapping population
- Fine mapping
- Clone the gene ~~(if lucky)~~



Please Take Home!!

Genus *Vigna* is

Wild and Sexy

OIAS
Takashi Hirano



PacBio RS II

Kazuhito Satou
Kuniko Teruya
Akino Shiroma
Makiko Shimoji

And the Boss

NORHIKO TOMOOKA

