UNIVERSITÀ
DEGLI STUDI
DI UDINE

Estimating the rates and modes of creation of new genetic variation in plants using NGS technologies

14/06/2016

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Co-supervisor: Fabio Marroni

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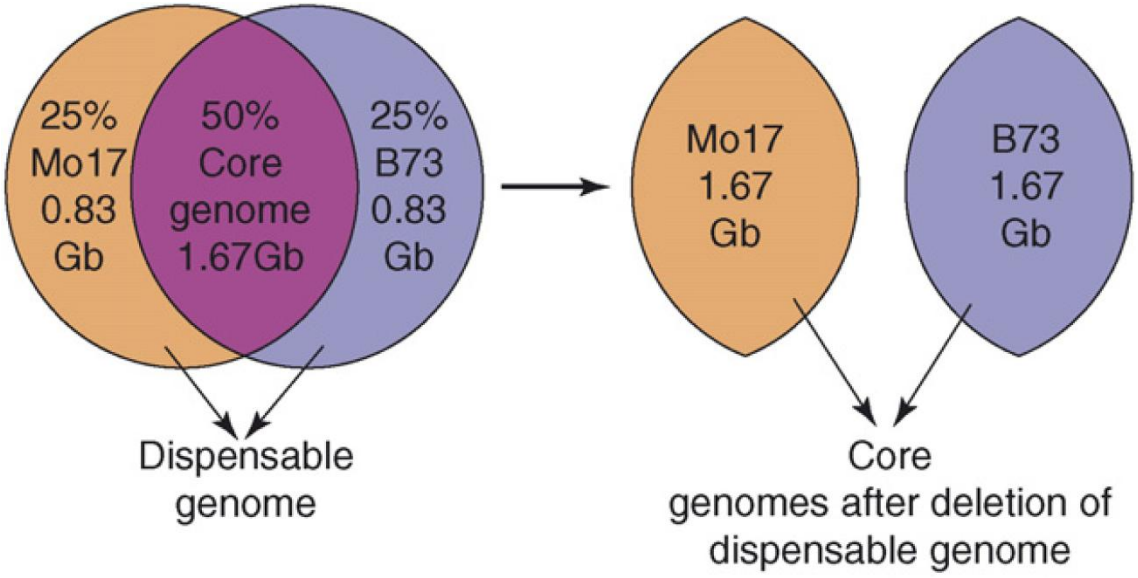
The maize pan-genome

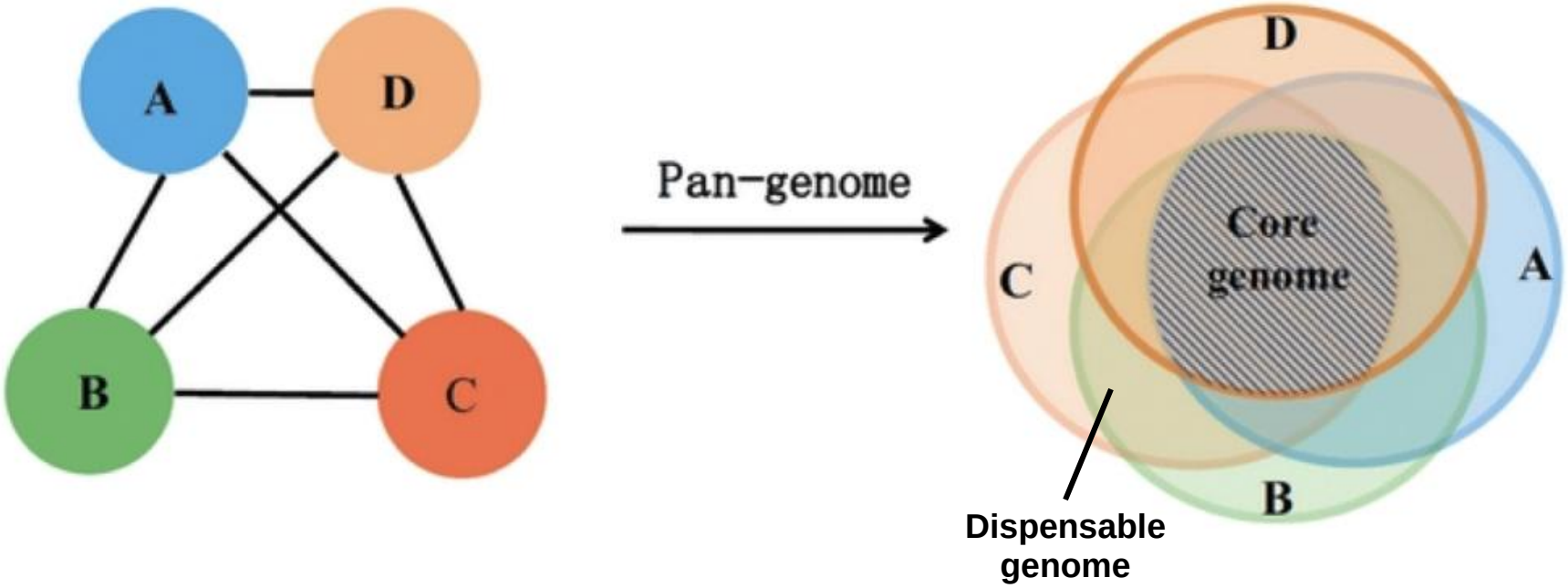
- A single individual genome is not representative of an entire species
- Pan-genome: the set of all genomic features among individuals within a species



Morgante et al., Curr Opin Plant Biol. 2007

Aim of the project

Characterizing maize dispensable genome



Modified from Novogene Corp., 2015

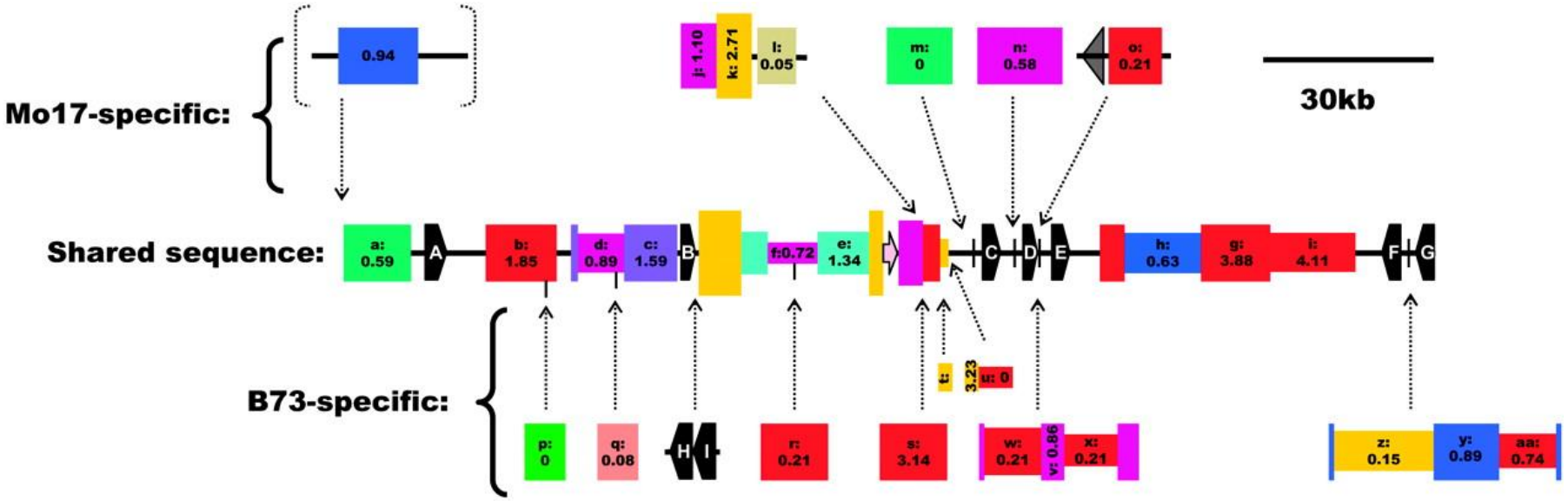
Structural variants in plants

- **Deletions** and **insertions** of TEs

- **CNV**: Copy-Number Variants

- **PAV**: Presence-Absence Variants

Insertions and deletions in maize



Genes: A) – I): geneA9008 – geneI9008

Repetitive elements:

1) Transposon:

DOPA-like

2) Retrotransposons:

- *ji*
- *opie*
- *reiver*
- *cinful*
- *flip*
- *prem*
- *huck*
- *tekay*
- *zeon*
- *shadowspawn*
- *non-LTR*

Brunner et al. Plant Cell 2005

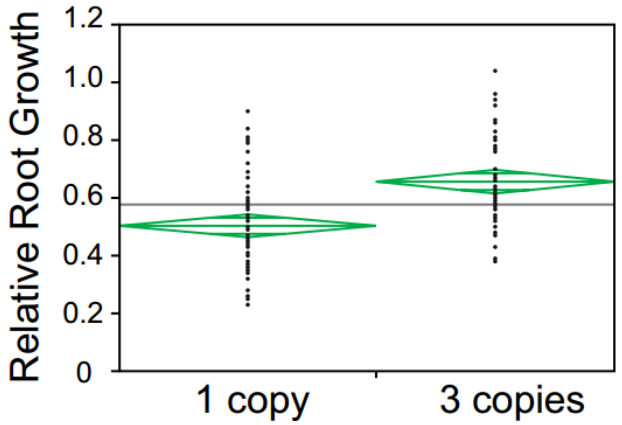
CNV in maize

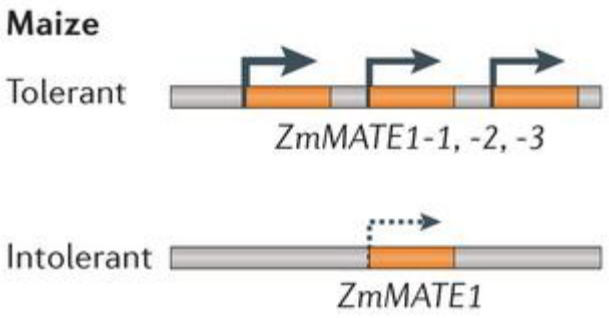
PNAS

Aluminum tolerance in maize is associated with higher *MATE1* gene copy number

Lyza G. Maron[a,1], Claudia T. Guimarães[b], Matias Kirst[c,d], Patrice S. Albert[e], James A. Birchler[e], Peter J. Bradbury[a,f], Edward S. Buckler[a,f], Alison E. Coluccio[a], Tatiana V. Danilova[e,2], David Kudrna[g], Jurandir V. Magalhaes[b], Miguel A. Piñeros[a], Michael C. Schatz[h], Rod A. Wing[g], and Leon V. Kochian[a,1]

[a]Robert W. Holley Center for Agriculture and Health, US Department of Agriculture–Agricultural Research Service, and [f]Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853; [b]Embrapa Maize and Sorghum, 35701-970, Sete Lagoas, Minas Gerais, Brazil; [c]School of Forest Resources and Conservation, University of Florida, Gainesville, FL 32611; [d]University of Florida Genetics Institute, University of Florida, Gainesville, FL 32610; [e]Division of Biological Sciences, University of Missouri, Columbia, MO 65211; [g]Arizona Genomics Institute, School of Plant Sciences, University of Arizona, Tucson, AZ 85705; and [h]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

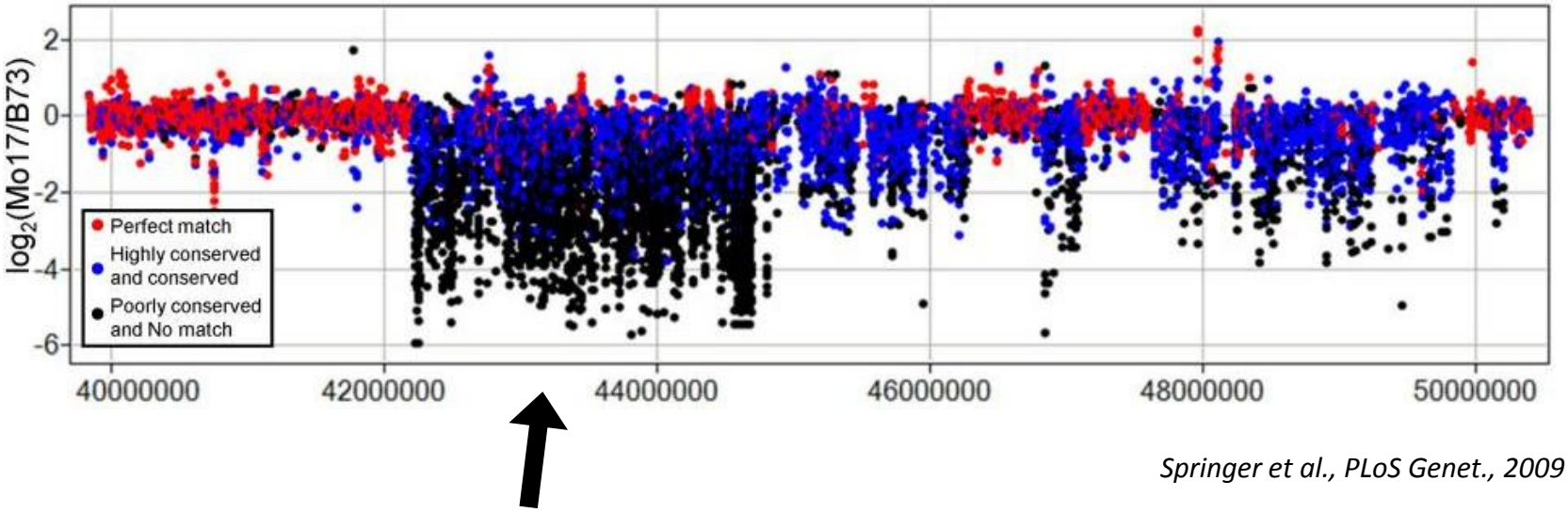




Mickelbart et al, Nature Rev Gen, 2015

PAV in maize

► ***Short arm Chr 6***

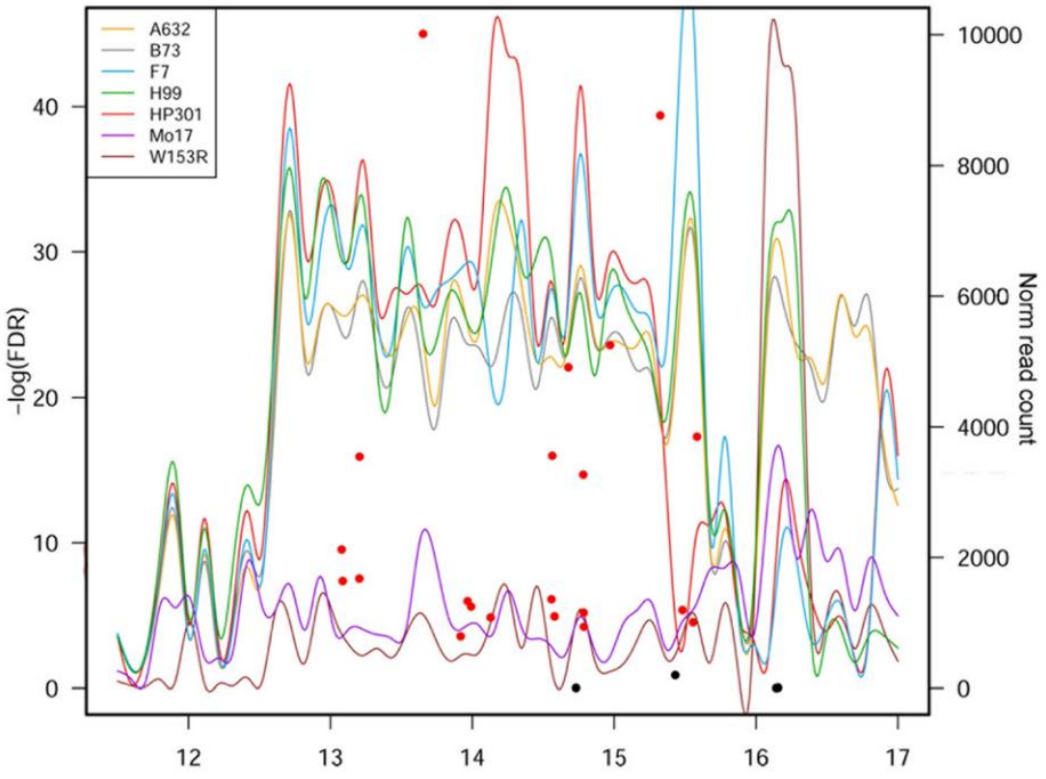


Springer et al., PLoS Genet., 2009

► ***2 Mb present in B73 and absent in Mo17***

PAV in maize

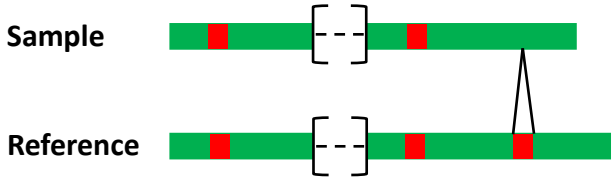
► ***Short arm Chr 6***



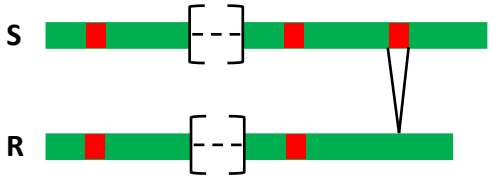
Dell'Acqua et al., Genome Biology, 2015

Structural variants discovery

1. Discovering **deletions**



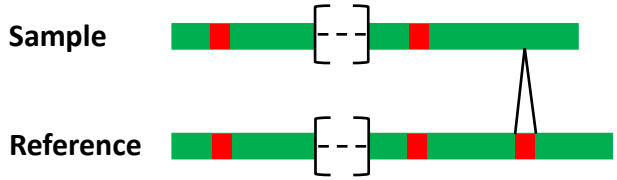
2. Discovering **insertions**



3. Discovering **CNV**s

Structural variants discovery

1. Discovering **deletions** (< 50 kbp)

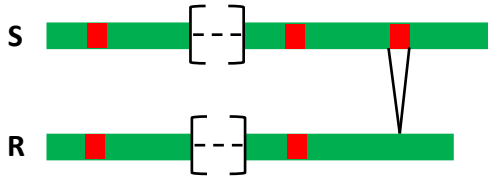


➔ Applying available methods

(paired-end mapping)

Structural variants discovery

2. Discovering **insertions** (< 50 kbp)



- Applying available methods

  (paired-end mapping)

- Developing new methods

  (split-reads mapping)

Structural variants discovery

3. Discovering **CNV**s (> 50 Kbp)

➔ Applying available methods

(depth of coverage approach)

Validation of detected deletions

- Integrated pipeline *(Magris, 2016)* calling:
  - → Delly *(Rausch et al., Bioinformatics 2012)*
  - → GASV *(Sindi et al., Bioinformatics 2009)*
- Validation in maize:
  - → BAC sequences (Mo17; >1 Mb analyzed, 33 events)
  - → genome assembly drafts (H99, HP301; 53 715 events)

Precision	Sensitivity	F1 score
0.92	**0.66**	**0.77**

Validation of detected insertions

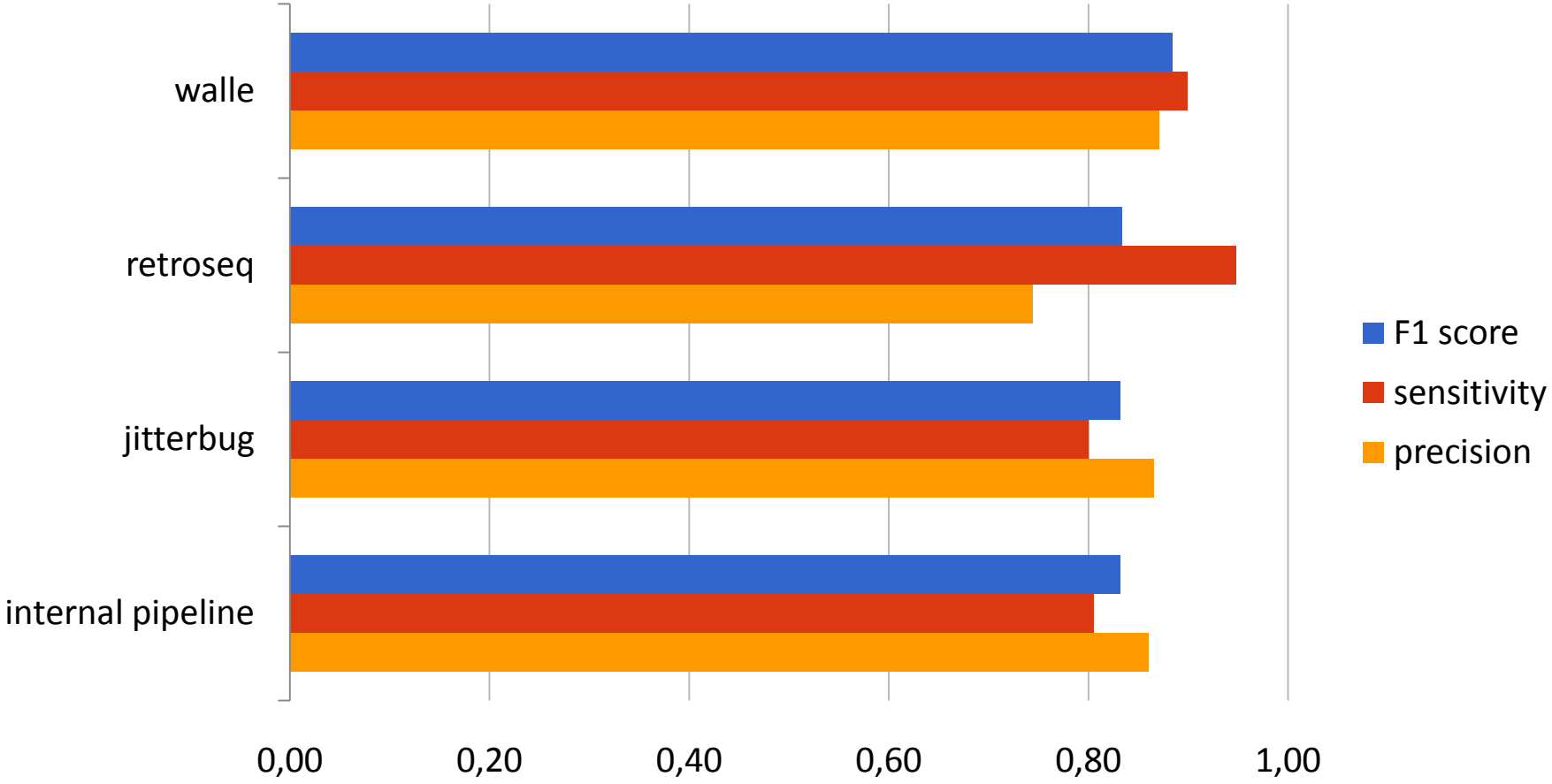
- TE DB-dependent methods
  - → IGA internal pipeline *(Pinosio, 2012)* - PEM, De Novo assembly
  - → Jitterbug *(Hénaff et al., BMC Genomics 2015)*
  - → RetroSeq *(Keane et al., Bioinformatics 2012)*
- Validation in maize:
  - → BAC sequences (Mo17; >1 Mb analyzed, 21 events)
  - → genome assembly drafts (in progress)
- Preliminary analysis on BACs reveals an overall good power (F1>0.8)

Developing Walle

- Developing a TE DB independent method: Walle
  - → Split-reads mapping approach
  - → Able to find insertions of not annotated elements

- Validation in vitis (now running in maize):
  - → Simulation of 1000 insertions
  - → Outperformed direct competitors

Validation of Walle

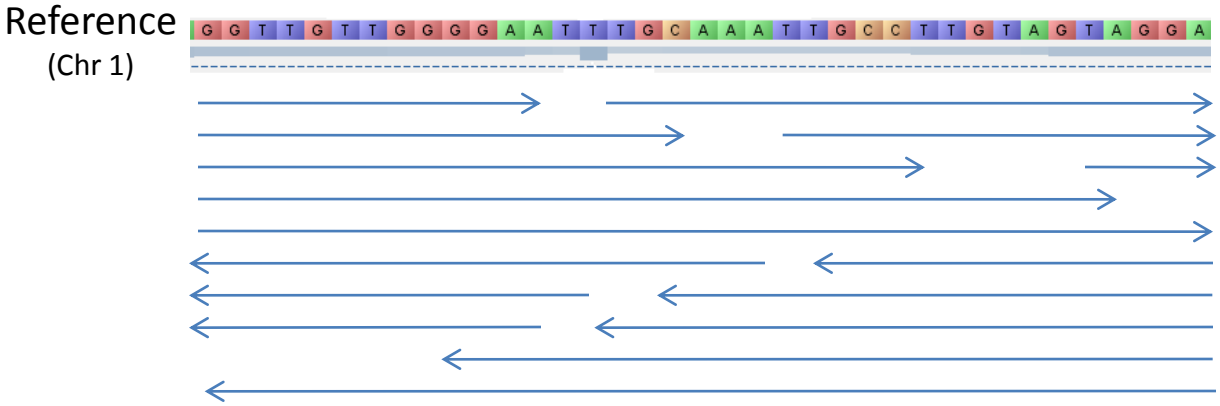


Walle methods

Split-reads mapping approach

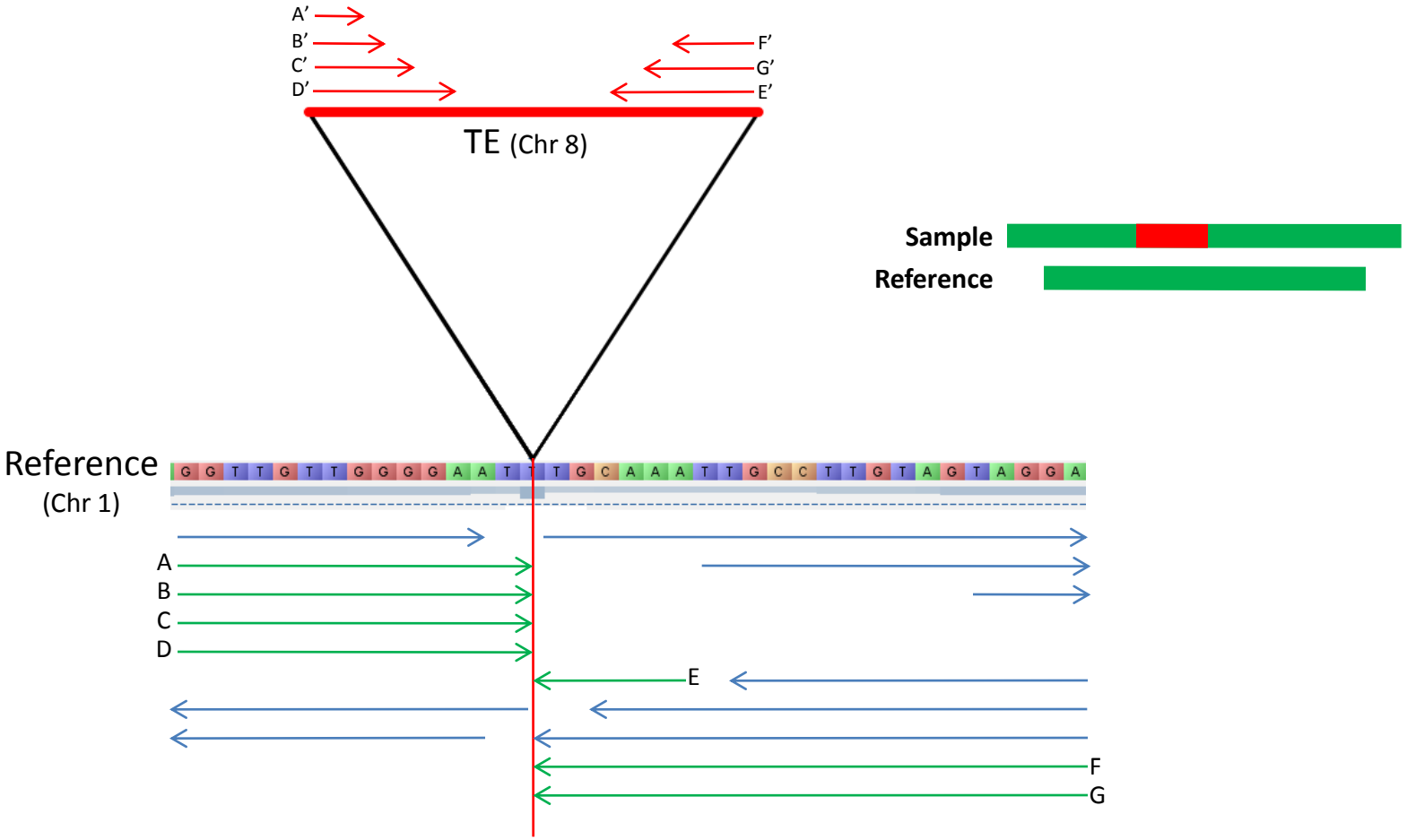
Normal alignment

(no insertion events)

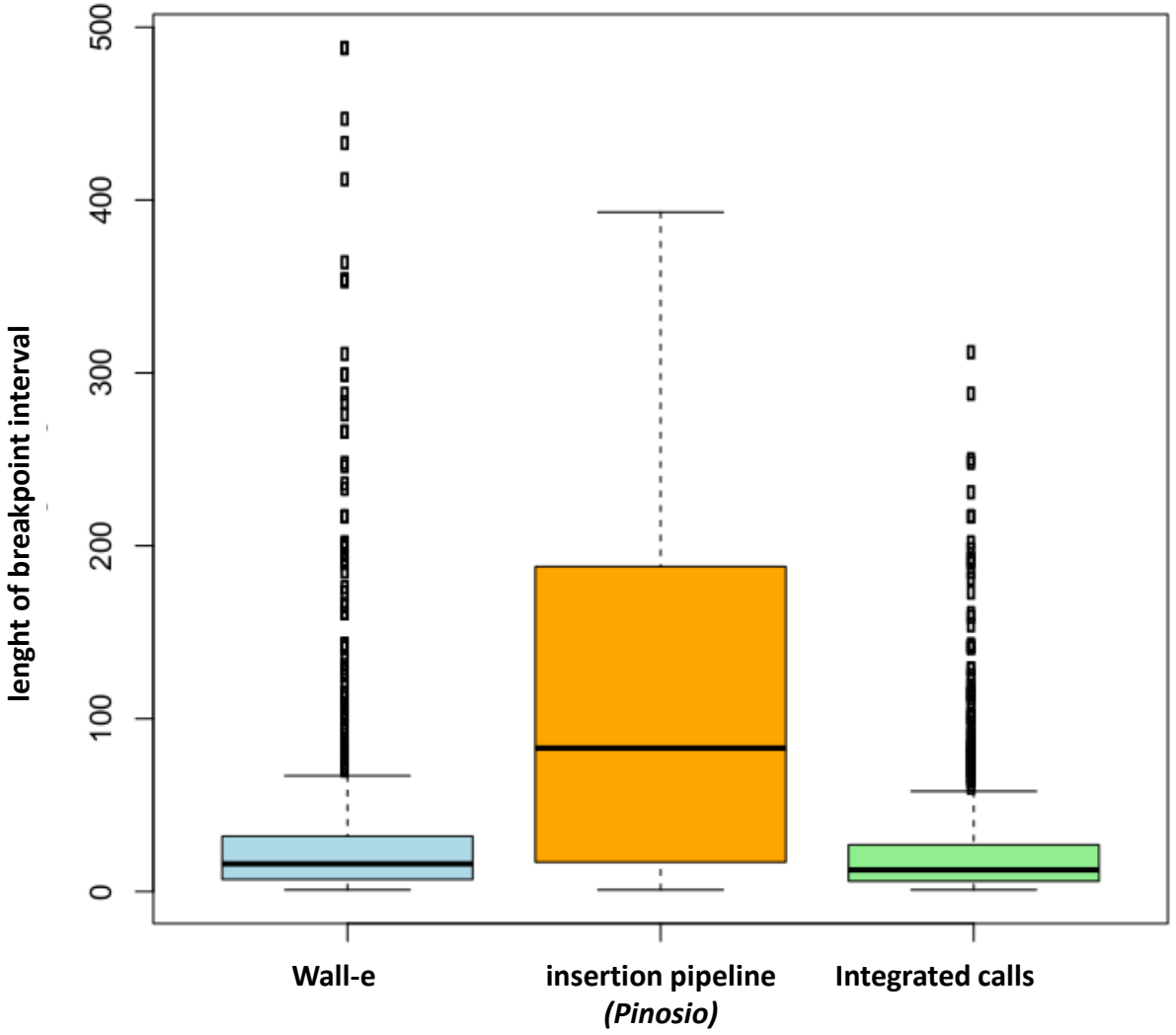


Walle methods

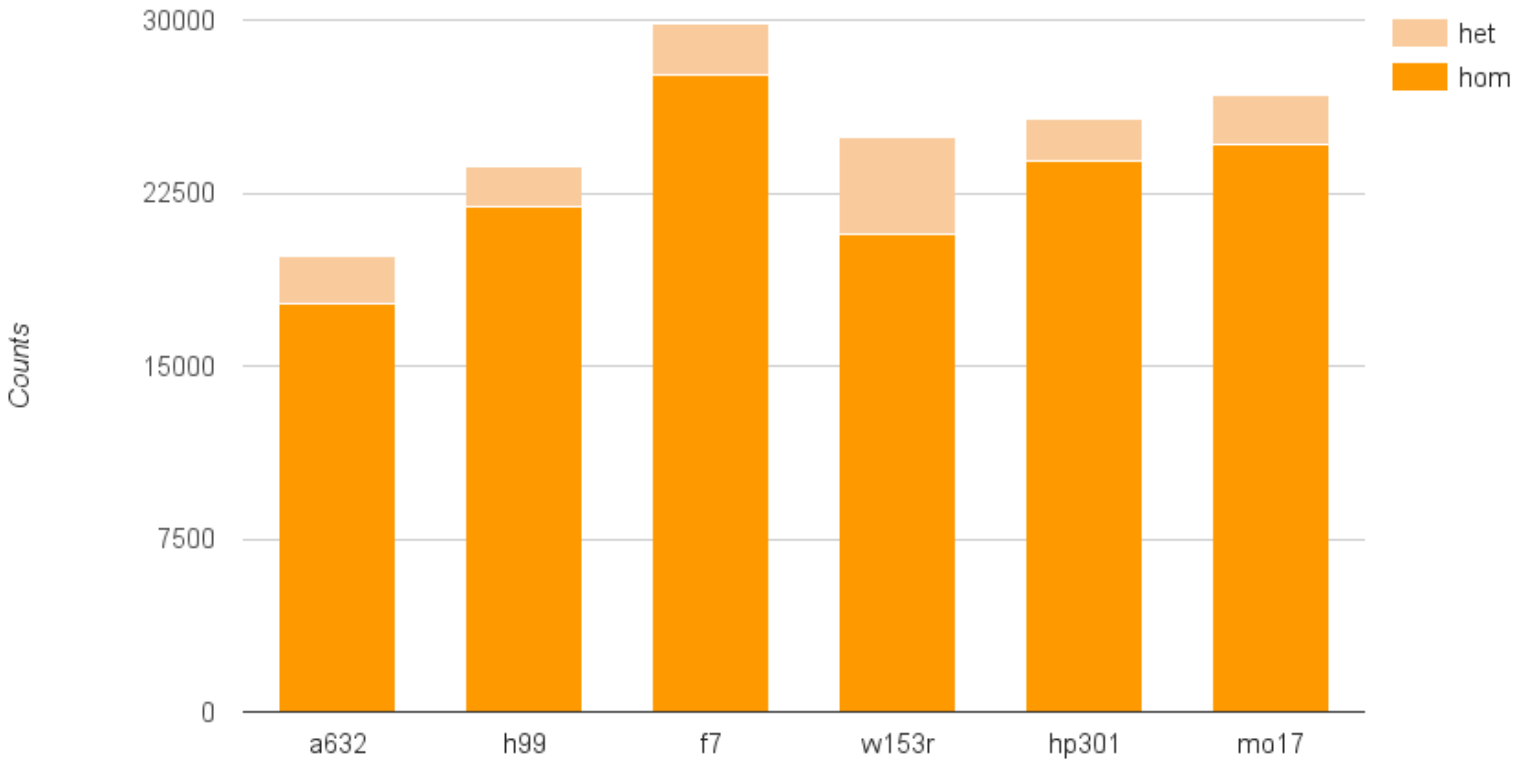
Split-reads mapping approach



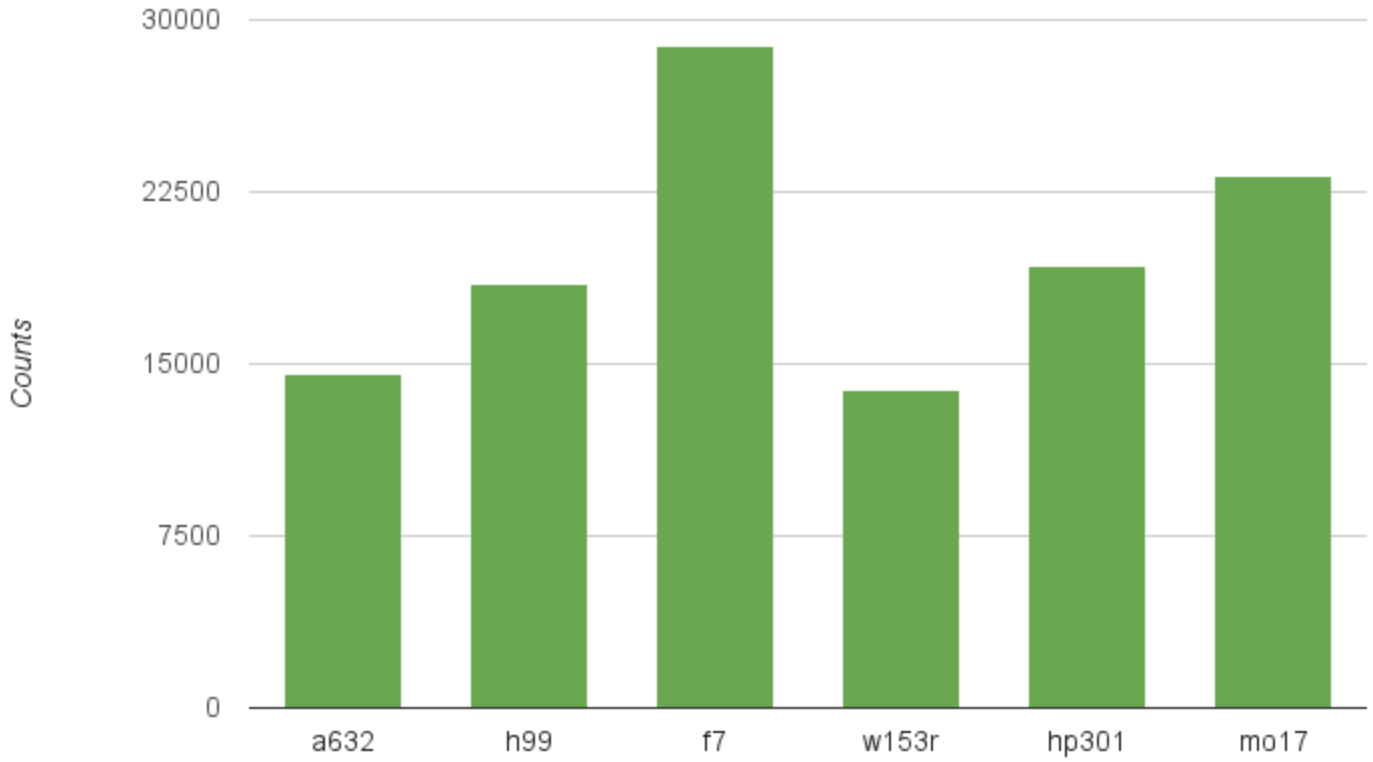
Refinement of IGA internal tool calls



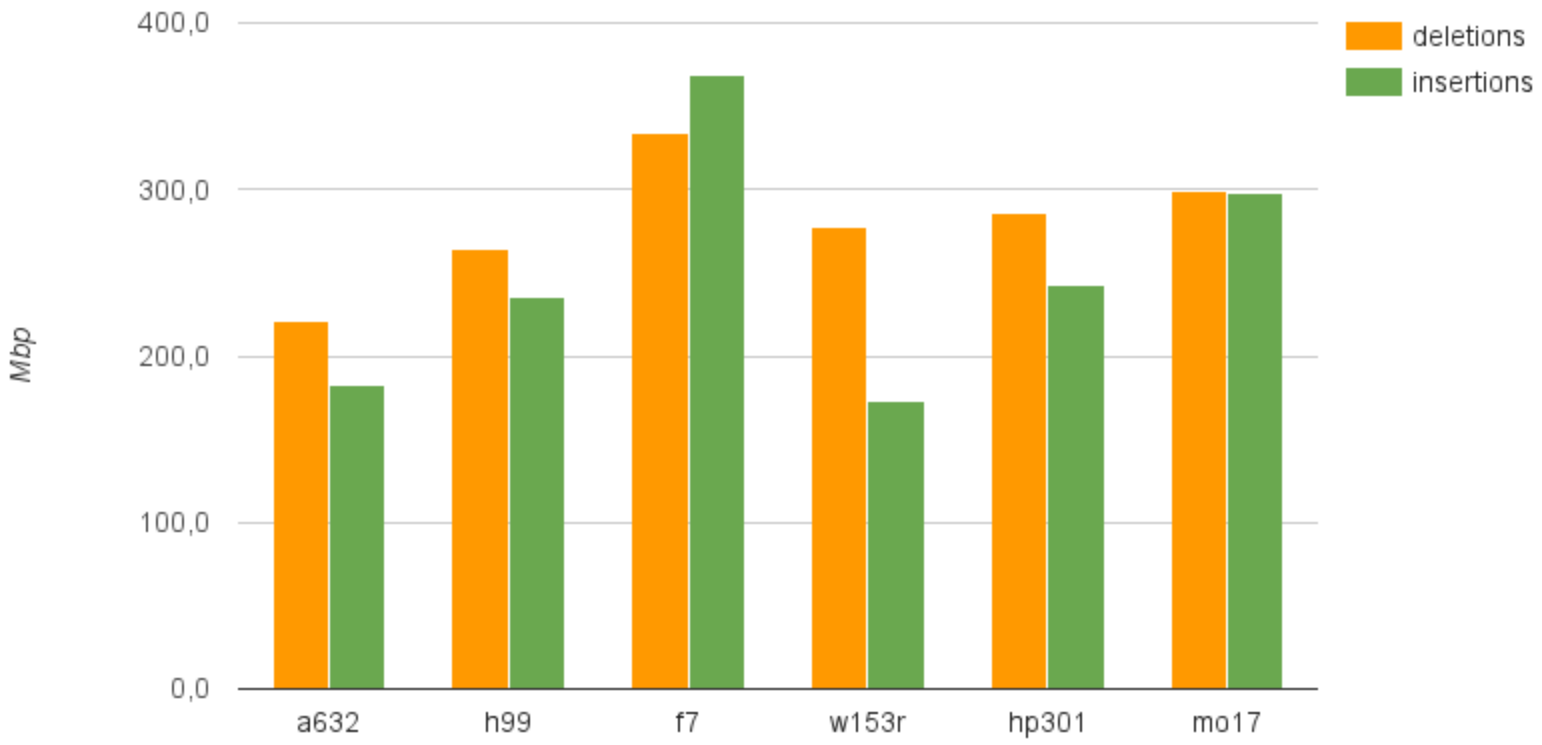
Deletions in maize



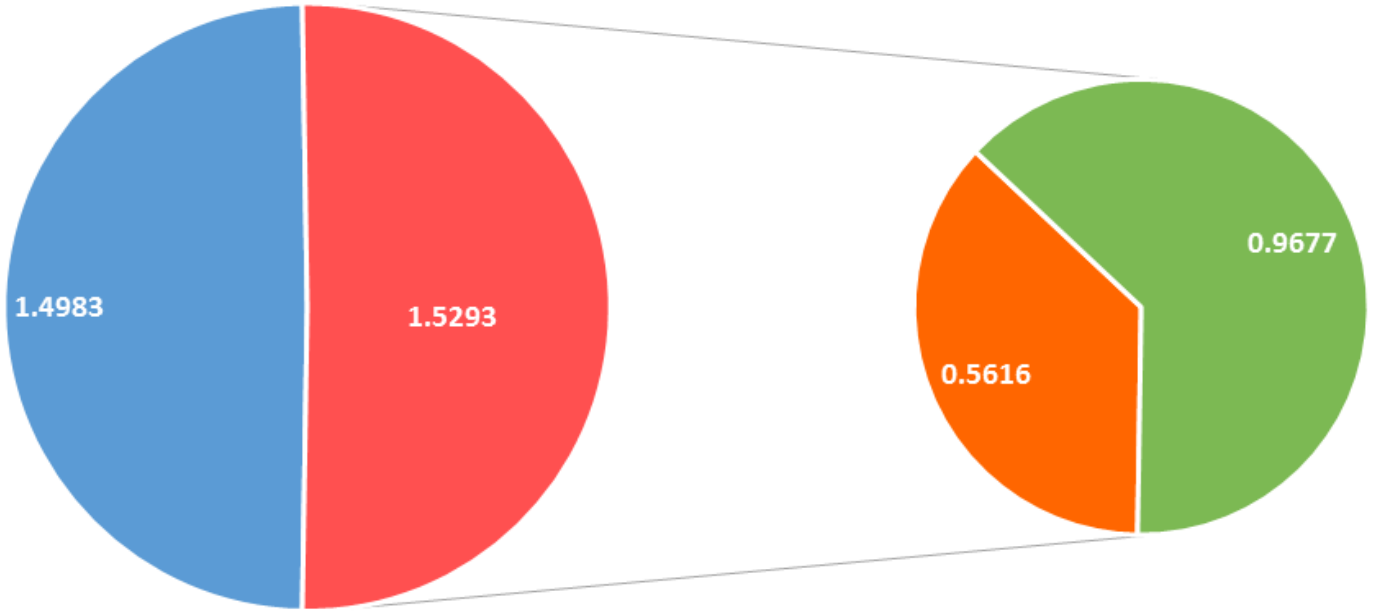
Insertions in maize



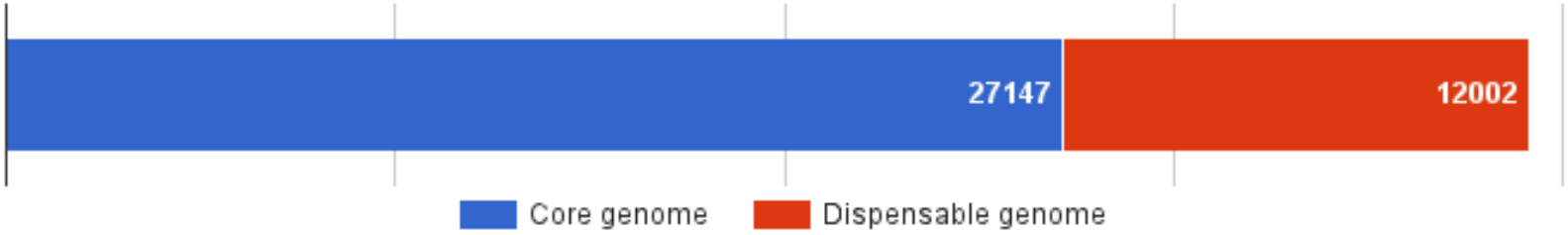
Size of deletions and insertions



Maize pan-genome



Shared genes analysis



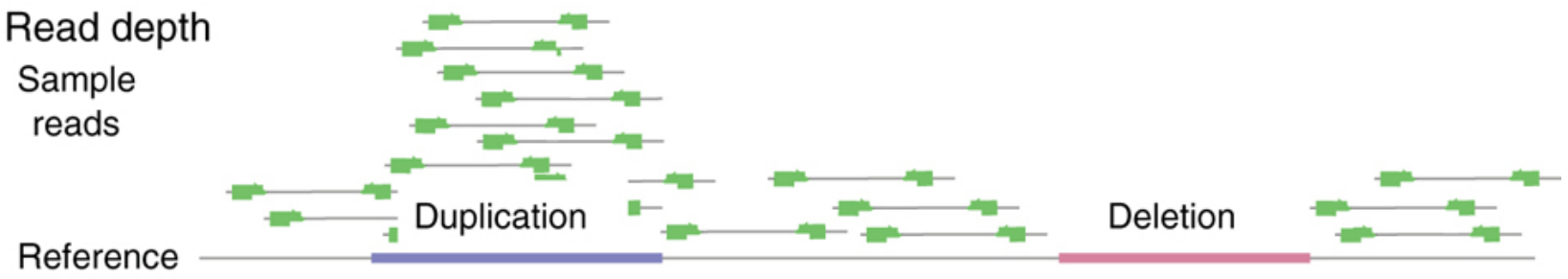
- Analysis performed on 7 varieties (included B73)

- **PanViz** *D3 based visualization for comparative genomics*

  (https://github.com/thomasp85/PanViz - *Pedersen TL, 2016*)

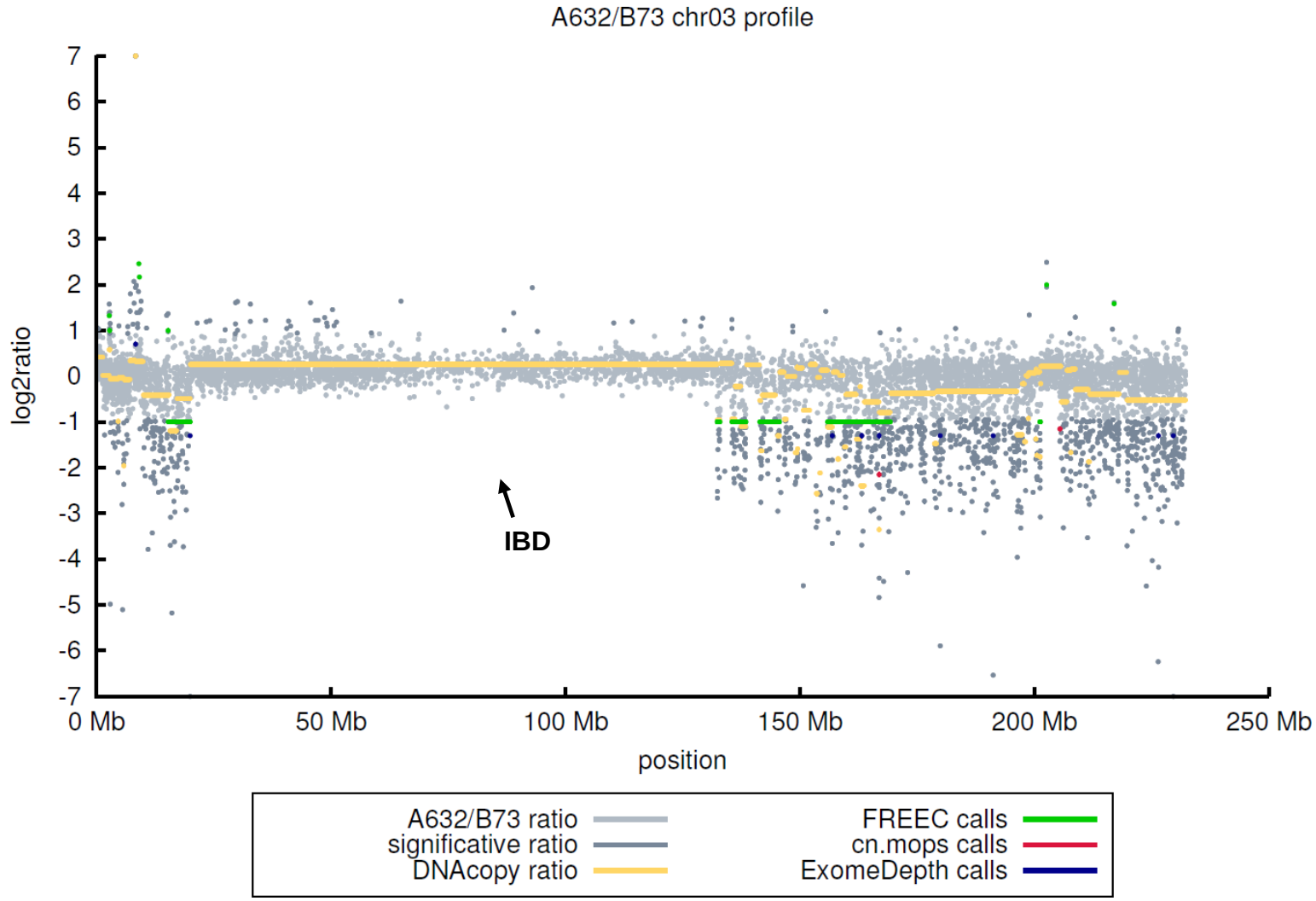
Discovering CNV

- Computing the ratio between the read counts of a sample genome and a reference genome in a region

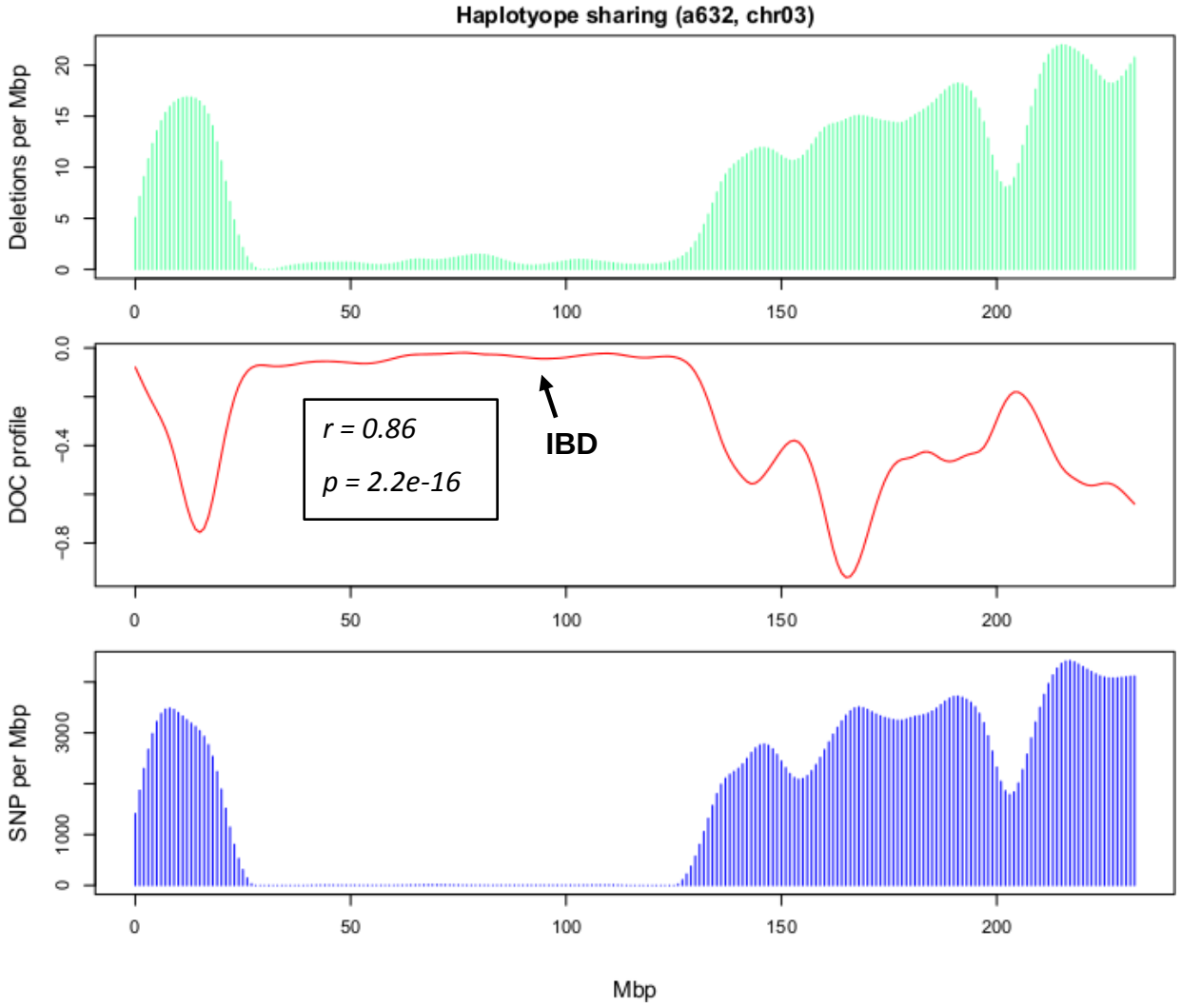


(Baker 2012)

DOC profiles



Correlating deletions and DOC



Future perspectives

- Insertions validation and genotyping
  - » Finalize Walle and validate it in maize
- Obtaining a list of CNVs in maize
- Complete the characterization of maize dispensable genome
  - » Obtaining a list of involved genes
  - » Identifying TE classes more often involved in SV
  - » Dating of amplification events of LTR sequences

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Partial Bibliography

Marroni et al.

Structural variation and genome complexity: is dispensable really dispensable?

Curr Opin Plant Biol, 2014

Morgante et al.

Transposable elements and the plant pan-genomes

Curr Opin Plant Biol, 2007

Brunner et al.

Evolution of DNA sequence nonhomologies among maize inbreds

Plant Cell, 2005