Informatics Interest Group Meeting

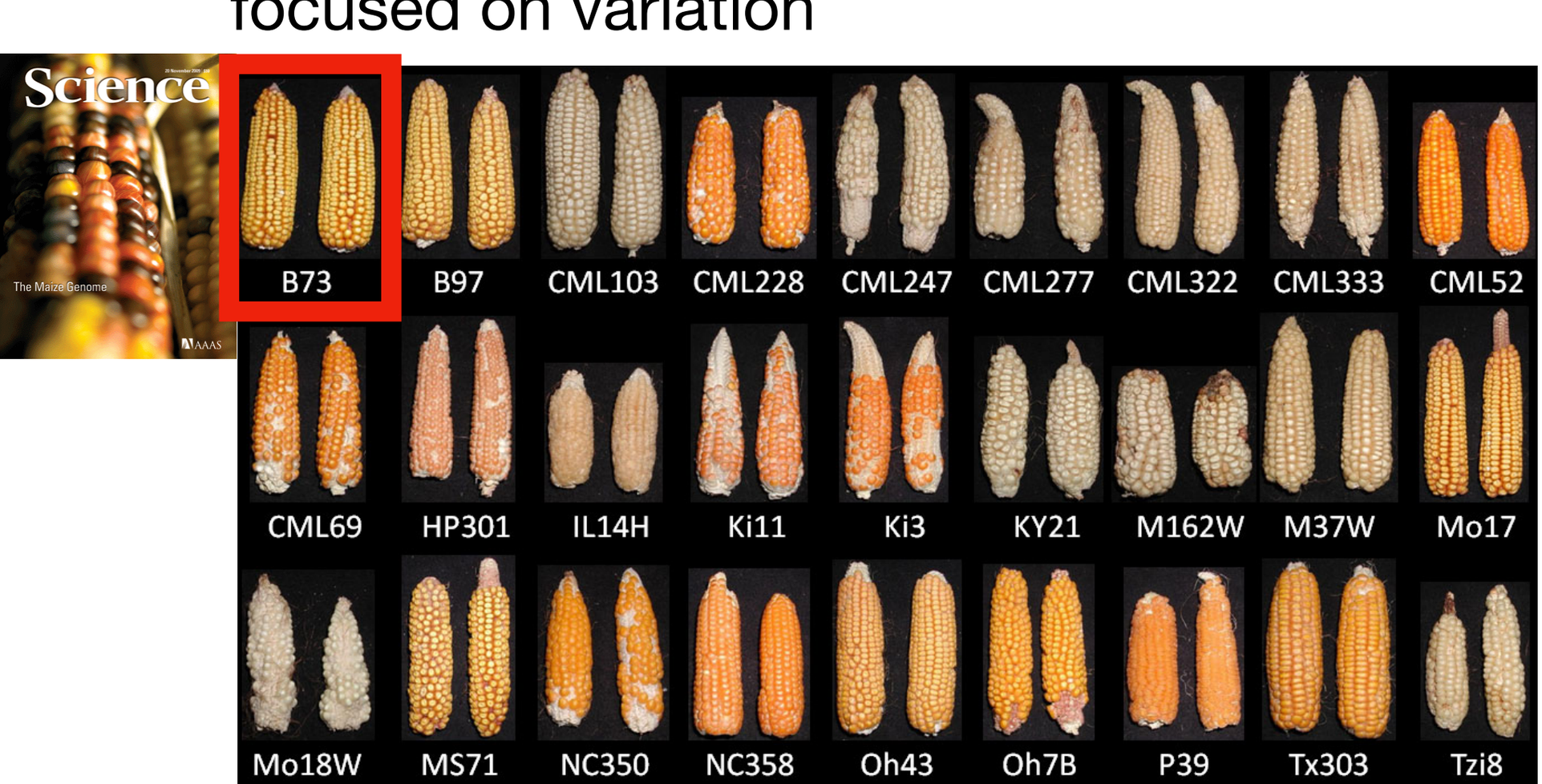
2018-05-02

Tom Kono

A Reference Genome

- String of nucleotides from a "representative" individual from a species or population

- Once a reference is assembled, questions become focused on variation



http://web.missouri.edu/~flint-garcias/pics.html

Limitations of A Single Reference

- Variation is widespread and most of it precludes discovery by comparison to a single genome

ARTICLE

OPEN

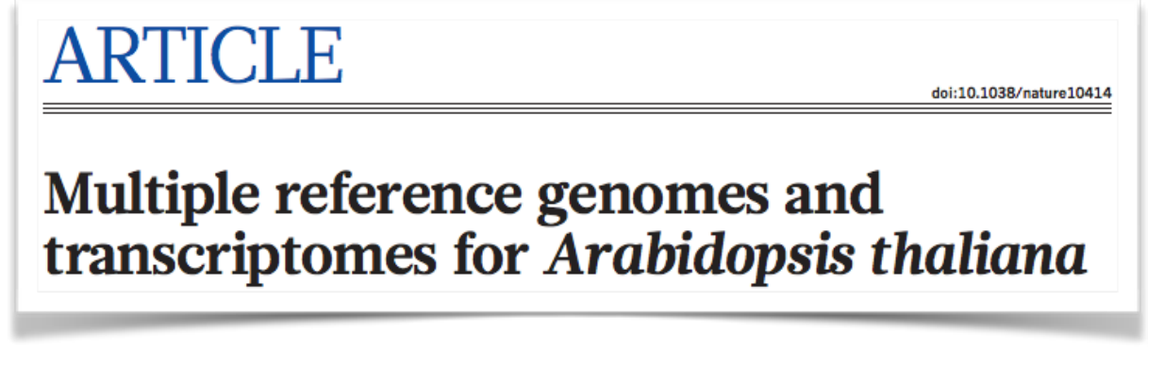
doi:10.1038/nature15394

An integrated map of structural variation in 2,504 human genomes

The Plant Cell, Vol. 26: 121–135, January 2014, www.plantcell.org © 2014 American Society of Plant Biologists. All rights reserved.

LARGE-SCALE BIOLOGY ARTICLE

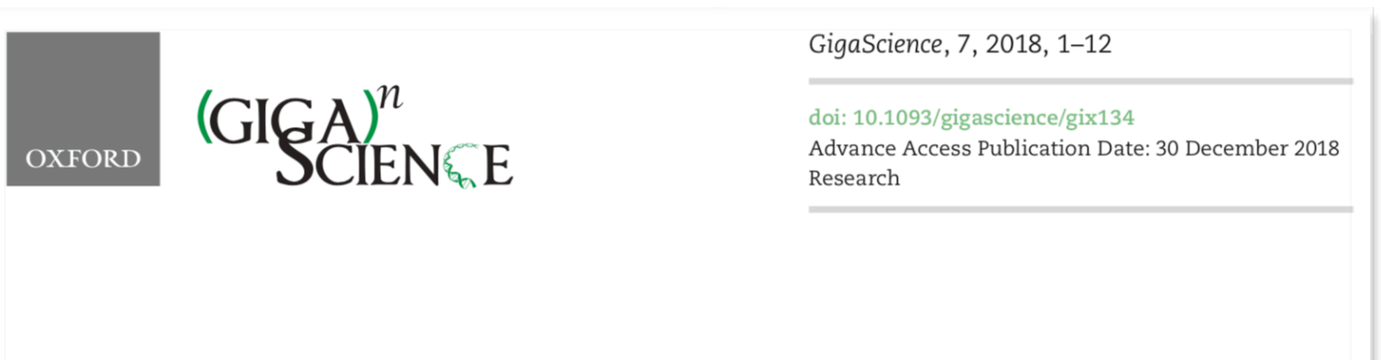
Insights into the Maize Pan-Genome and Pan-Transcriptome[W][OPEN]



ARTICLE

doi:10.1038/nature10414

Multiple reference genomes and transcriptomes for *Arabidopsis thaliana*



OXFORD

(GIGA)n SCIENCE

GigaScience, 7, 2018, 1–12

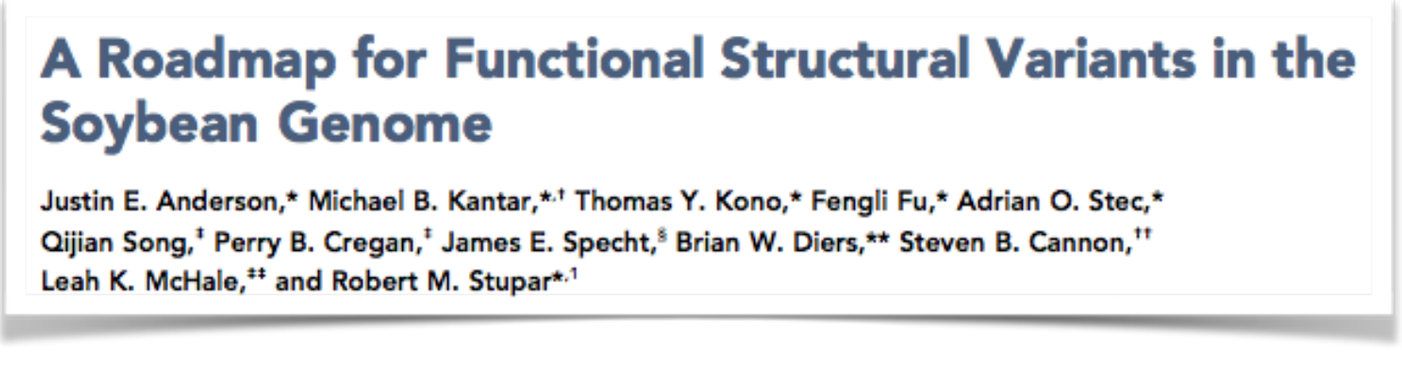
doi: 10.1093/gigascience/gix134

Advance Access Publication Date: 30 December 2018

Research

RESEARCH

Construction of the third-generation *Zea mays* haplotype map

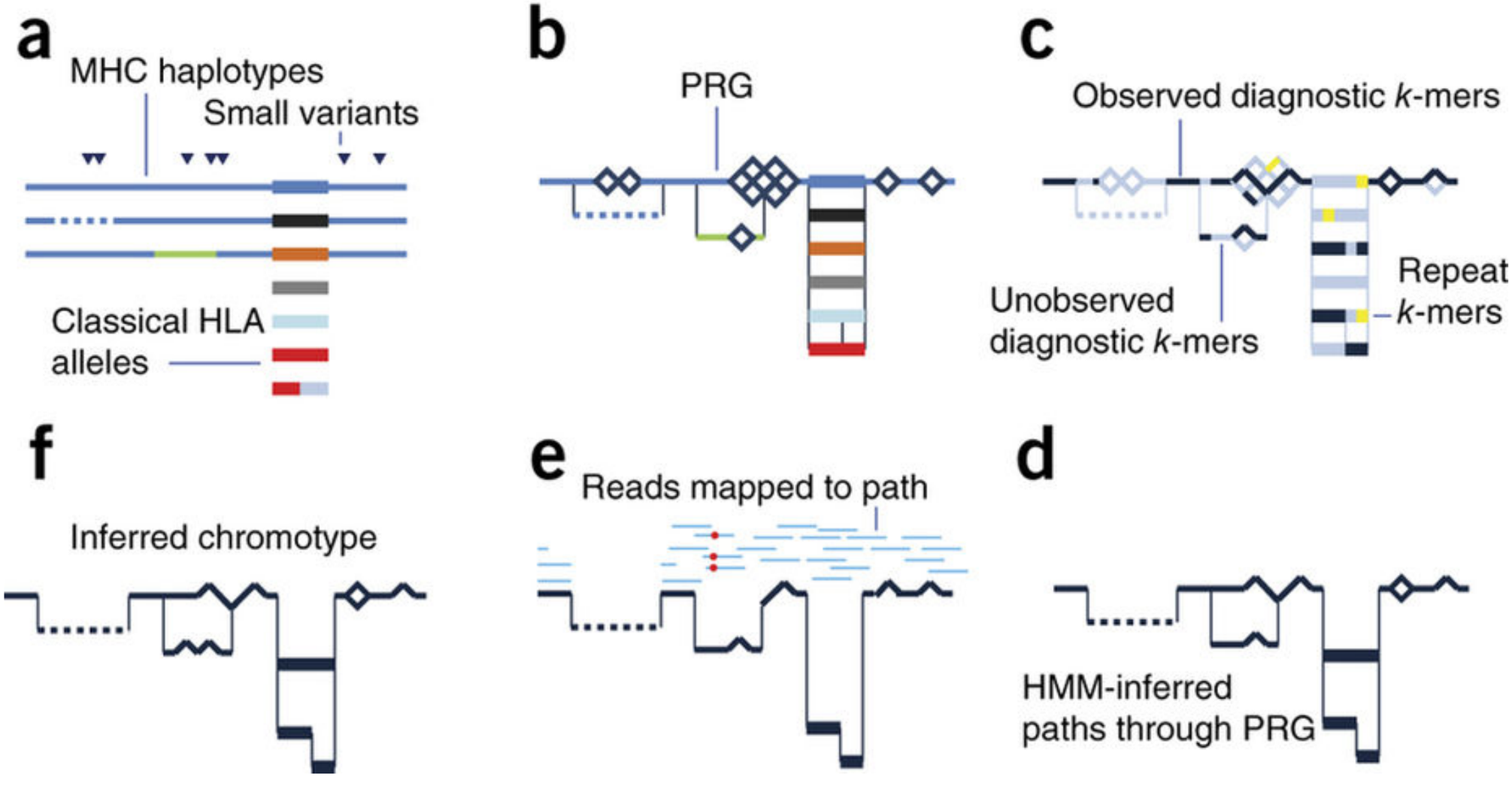


A Roadmap for Functional Structural Variants in the Soybean Genome

Justin E. Anderson,* Michael B. Kantar,*,† Thomas Y. Kono,* Fengli Fu,* Adrian O. Stec,* Qijian Song,‡ Perry B. Cregan,‡ James E. Specht,§ Brian W. Diers,** Steven B. Cannon,†† Leah K. McHale,‡‡ and Robert M. Stupar*,1

Potential Solution: de Bruijn Graphs

- Borrow from sequence assembly techniques to represent diversity



Dilthey et al. 2014, Nat Genet
doi:10.1038/ng.3257

Enter: Variation Graphs

- Represent genomes as a graph, rather than a string or series of strings

    - Invariant sites are single connections through graph

    - Variants are "bubbles" - alternate paths through graph

    - Individuals (or chromosomes) are then represented as a path through the graph

Natural Limits to the Problem

- Potential problem: the number of possible haplotypes

  - ~80m common SNPs in humans => ~$2^{80000000}$ haplotypes possible. Lower bound, because there are other types of polymorphisms, with many alleles...

- But! The number of observed haplotypes is *far fewer* than the number that are possible. Linkage disequilibrium*.

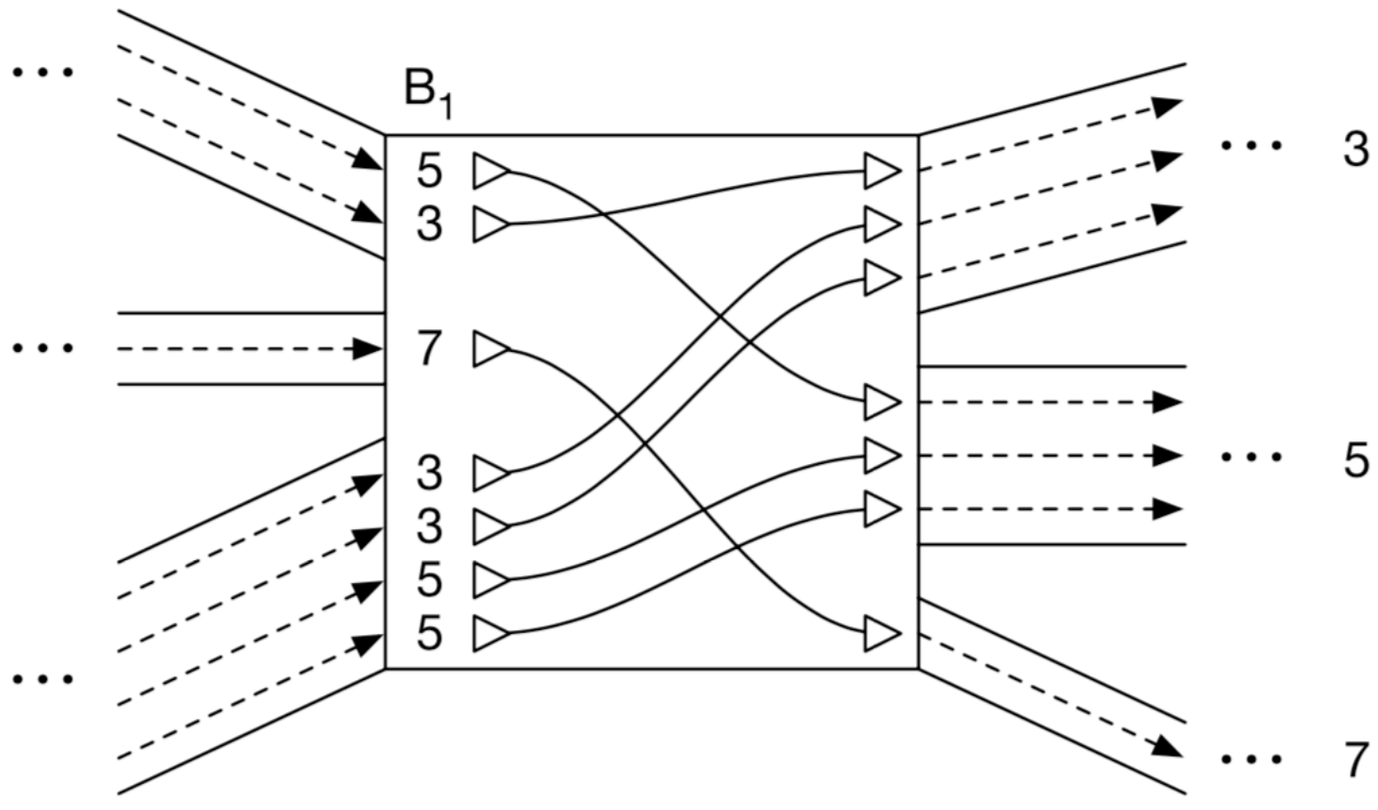
- Haplotypes in a genome graph: restricted paths through multiple variants

*Lewontin and Kojima 1960, *Evolution*

BWT Variant: gPBWT

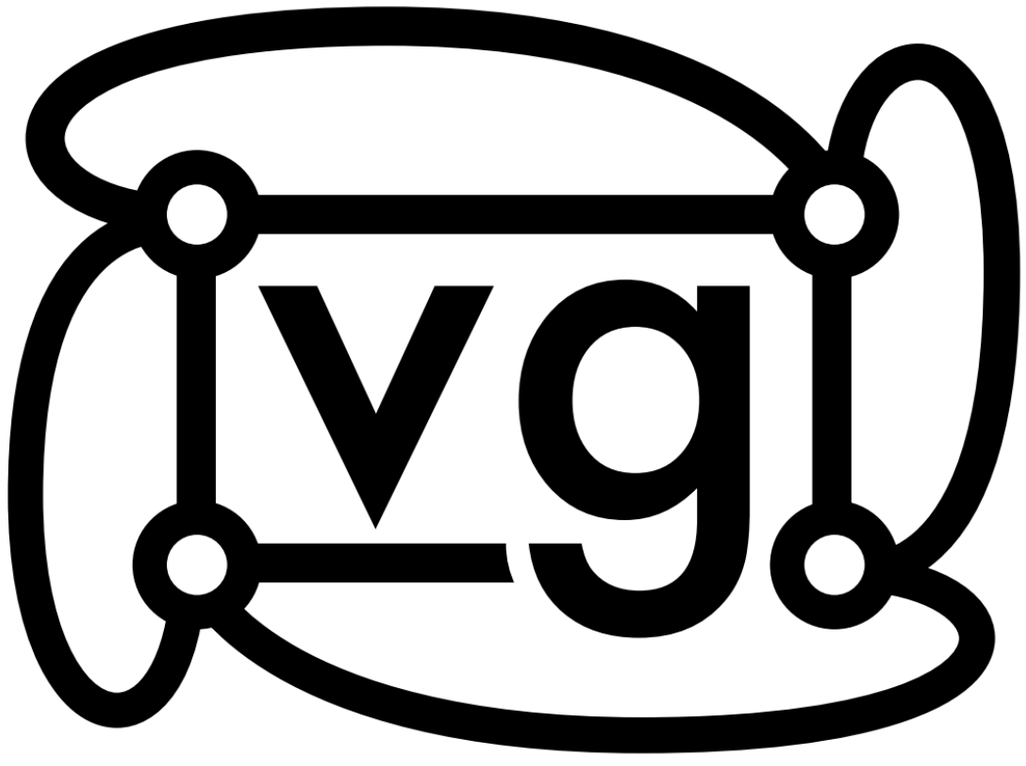
- Generalization of the Burrows-Wheeler Transform to contain positional information

  - Embeds the "threads" (linkages among variant sites) into the graph



Genome Graph Tools

- Erik Garrison (of vcflib and FreeBayes fame) is involved with the 'vg' project (variation graphs)
    - https://github.com/vgteam/vg



vg Capabilities

- vg is pretty complete now:
  - Build graph (from FASTA and VCF)
  - View/visualize graph (via graphviz)
  - Align reads to a graph (or map lots of reads)
  - Call variants

Running vg

- Building takes ~2.7 Gb on MacOS, including dependencies

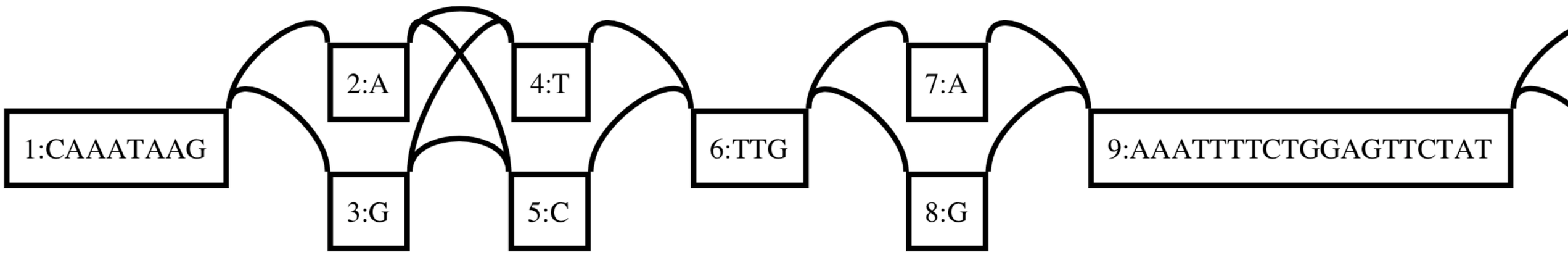
- Build graphs with `vg construct`

```
vg construct \
    -r test/small/x.fa \
    -v test/small/x.vcf.gz \
    > x.vg
```

Running vg

- Convert between formats with `vg view`. The `.dot` file is suitable for input into graphviz visualization software

```
vg view x.vg > x.gfa #default fmt is GFA1
vg view –d x.vg > x.dot
dot –Tpdf x.dot –o x.pdf
```



Running vg

- Can align and map reads to a graph: GAM (Graph Alignment/Map), looks like compressed JSON

```
vg index –x x.xg –g x.gcsa –k 16 x.vg
vg sim –n 1000 –l 150 –x x.xg > reads
vg map –x x.xg –g x.gcsa –T reads > aln.gam
vg view –a aln.gam | less
```


```
{"sequence":"GAGTCTCAAGGACAGCTCTCCCTTGTGTCCAGAGTGTATACGATTAACTCTGTTCTGGGCACTGGTGAAAGAAAACAGAGGAAATGCCTGGCTTTTTATCAGAACATGTTTCCAAGCTTATCCCTTT
TCCCTGCTCTCCTTGTCCCTCCT","path":{"mapping":[{"position":{"node_id":"120","offset":"59"},"edit":[{"from_length":3,"to_length":3}],"rank":"1"}
,{"position":{"node_id":"121"},"edit":[{"from_length":1,"to_length":1}],"rank":"2"},{"position":{"node_id":"123"},"edit":[{"from_length":17,
"to_length":17}],"rank":"3"},{"position":{"node_id":"124"},"edit":[{"from_length":1,"to_length":1}],"rank":"4"},{"position":{"node_id":"126"
},"edit":[{"from_length":6,"to_length":6}],"rank":"5"},{"position":{"node_id":"128"},"edit":[{"from_length":1,"to_length":1}],"rank":"6"},{"
position":{"node_id":"129"},"edit":[{"from_length":14,"to_length":14}],"rank":"7"},{"position":{"node_id":"131"},"edit":[{"from_length":1,"t
o_length":1}],"rank":"8"},{"position":{"node_id":"133"},"edit":[{"from_length":11,"to_length":11}],"rank":"9"},{"position":{"node_id":"134"}
,"edit":[{"from_length":1,"to_length":1}],"rank":"10"},{"position":{"node_id":"135"},"edit":[{"from_length":6,"to_length":6}],"rank":"11"},{
"position":{"node_id":"137"},"edit":[{"from_length":1,"to_length":1}],"rank":"12"},{"position":{"node_id":"138"},"edit":[{"from_length":9,"t
o_length":9}],"rank":"13"},{"position":{"node_id":"139"},"edit":[{"from_length":1,"to_length":1}],"rank":"14"},{"position":{"node_id":"141"}
,"edit":[{"from_length":15,"to_length":15}],"rank":"15"},{"position":{"node_id":"143"},"edit":[{"from_length":1,"to_length":1}],"rank":"16"}
,{"position":{"node_id":"144"},"edit":[{"from_length":42,"to_length":42}],"rank":"17"},{"position":{"node_id":"145"},"edit":[{"from_length":
1,"to_length":1}],"rank":"18"},{"position":{"node_id":"147"},"edit":[{"from_length":17,"to_length":17}],"rank":"19"},{"position":{"node_id":
"148"},"edit":[{"from_length":1,"to_length":1}],"rank":"20"}]},"mapping_quality":60,"score":160,"identity":1,"refpos":[{"offset":"625","name
":"x"}],"time_used":6113}
{"sequence":"CTGGGCACTGGTGAAAGATAACAGAGGAAATGCCTGGCTTTTTATCAGAACATGTTTCCAAGCTTATCCCTTTTCCCTGCTCTCCTTGTCCCTCCTACGATCTCTTCACTGGCCTTTTATCTTTACT
GTTACCAAATCTTTCCGGAACCT","path":{"mapping":[{"position":{"node_id":"133","offset":"10"},"edit":[{"from_length":1,"to_length":1}],"rank":"1"}
,{"position":{"node_id":"134"},"edit":[{"from_length":1,"to_length":1}],"rank":"2"},{"position":{"node_id":"135"},"edit":[{"from_length":6,"
```

Running vg

- Put it back into the linear world using `vg surject`

```
vg surject -x x.xg -b aln.gam | less -S
```

```
*       16      x       424     60      43M1D33M1I53M1I19M      *       0       0       ACCTTCCTTGACTTC
*       0       x       584     60      85M1I1M1D63M    *       0       0       ATTGCATCTCAAATCTAAGACCC
*       16      x       688     60      150M    *       0       0       TGGTGAAAGATAACAGAGGAAATGCCTGGCT
*       0       x       115     60      58M1I91M        *       0       0       TTGAAGTAACGTTTGACAATCTA
*       0       x       34      60      150M    *       0       0       AATATTCCAACTCTCTGGGTCCTGGTGCTAT
*       0       x       793     60      68M1I81M        *       0       0       CTTTTATCTTTACTGTTACCAAA
*       0       x       555     60      114M1I1M1D34M   *       0       0       TCTGCTCACCGCGATCTTCAAGT
*       0       x       212     60      150M    *       0       0       TTTGCCACAGATTAGCCATGTGACTTTGAAC
*       16      x       347     60      150M    *       0       0       GAAGCTCAGGGAATAGTGCCTGGCATCGAGG
*       0       x       76      60      150M    *       0       0       AATGGTAATGGATATGTTGGGCTTTTTTCTT
*       16      x       335     60      150M    *       0       0       GGTGATGCTTGTGAAGCTGAGGGAATAGTGC
```

Running vg

- Augment the source graph with variation identified in the mapped reads

```
vg filter aln.gam \
    -r 0.9 -fu -s 2 -o 0 -D 999 -x x.xg \
    > flt.gam
vg augment x.vg flt.gam \
    -q 10 -S aug_graph.support \
    -Z aug_graph.trans -A aug_alignment.gam \
    > aug_graph.vg
```

Running vg

- Call variants based on support for various paths through the graph

```
vg paths -v aug_graph.vg -L # Gives path names
vg call \
-b x.vg -s aug_graph.support \
-x aug_graph.trans -r x > calls.vcf
```

```
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=XDP,Number=2,Type=Integer,Description="Expected Local and Global Depth">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=AD,Number=.,Type=Integer,Description="Allelic depths for the ref and alt alleles in the order listed">
##FORMAT=<ID=XADL,Number=1,Type=Float,Description="Likelihood of allelic depths for called alleles">
##FORMAT=<ID=SB,Number=4,Type=Integer,Description="Forward and reverse support for ref and alt alleles.">
##FORMAT=<ID=XAAD,Number=1,Type=Integer,Description="Alt allele read count.">
##FORMAT=<ID=AL,Number=.,Type=Float,Description="Allelic likelihoods for the ref and alt alleles in the order listed">
##contig=<ID=x,length=1001>
#CHROM  POS     ID      REF     ALT     QUAL    FILTER  INFO    FORMAT  SAMPLE
x       533     223_315_226-223_322_226 T       A       2370    PASS    DP=161;SVLEN=0;XSEE=112,113,114,115;XREF        GT:DP:XDP:AD:XADL:SB
x       813     338_376_286-338_388_286 A       G       2550    PASS    DP=170;SVLEN=0;XSEE=158,159,160,161;XREF        GT:DP:XDP:AD:XADL:SB
x       553     226_272_325-226_325     T       TG      2250    PASS    DP=155;SVLEN=1;XSEE=115,116,117;XREF    GT:DP:XDP:AD:XADL:SB:XAAD
x       272     320_211_289_214-320_342_263_214 TA      CG      1470    PASS    DP=103;SVLEN=0;XSEE=55,56,57,58,59,60;XREF      GT:DP:XDP:AD
x       823     286_393_220-286_353_220 A       G       2340    PASS    DP=169;SVLEN=0;XSEE=161,162,163,164;XREF        GT:DP:XDP:AD:XADL:SB
x       10      303_410_319_216-303_410_229_216 C       T       150     PASS    DP=11;SVLEN=0;XSEE=1,2,3,4,5,6;XREF     GT:DP:XDP:AD:XADL:SB
x       566     325_253_294-325_352_294 T       C       2220    PASS    DP=155;SVLEN=0;XSEE=117,118,119,120;XREF        GT:DP:XDP:AD:XADL:SB
x       277     214_384_332-214_336_332 A       C       2940    PASS    DP=195;SVLEN=0;XSEE=60,61,62,63;XREF    GT:DP:XDP:AD:XADL:SB:XAAD
```

Running vg

- Or, call them with FreeBayes-like algorithm

```
vg genotype -G aug_alignment.gam \
    -E -v aug_graph.vg -r x \
    > calls.vcf
```

```
##fileformat=VCFv4.2
##ALT=<ID=NON_REF,Description="Represents any possible alternative allele at this location">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=XSBB,Number=1,Type=Integer,Description="Ultrabubble Bases">
##INFO=<ID=XSBN,Number=1,Type=Integer,Description="Ultrabubble Nodes">
##FORMAT=<ID=DP,Number=1,Type=Float,Description="Read Depth">
##FORMAT=<ID=GQ,Number=1,Type=Float,Description="Genotype Quality">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=AD,Number=.,Type=Float,Description="Allelic depths for the ref and alt alleles in the order listed">
##FORMAT=<ID=SB,Number=4,Type=Float,Description="Forward and reverse support for ref and alt alleles.">
##FORMAT=<ID=PL,Number=G,Type=String,Description="Log Likelihood">
##FORMAT=<ID=XAAD,Number=1,Type=Float,Description="Alt allele read count.">
#CHROM  POS     ID      REF     ALT     QUAL    FILTER  INFO    FORMAT  SAMPLE
x       14      .       G       A       0       .       DP=22.000000;XSBB=24;XSBN=4     GT:DP:GQ:AD:SB:PL       1/0:22.000000:118.765437:10.
x       34      .       T       A       0       .       DP=46.000000;XSBB=25;XSBN=4     GT:DP:GQ:AD:SB:PL       1/0:46.000000:302.831892:22.
x       533     .       T       A       0       .       DP=165.000000;XSBB=31;XSBN=4    GT:DP:GQ:AD:SB:PL       1/0:165.000000:1164.848097:8
x       39      .       T       A       0       .       DP=52.000000;XSBB=18;XSBN=4     GT:DP:GQ:AD:SB:PL       1/0:52.000000:331.942302:28.
x       813     .       A       G       0       .       DP=173.000000;XSBB=28;XSBN=4    GT:DP:GQ:AD:SB:PL       1/0:173.000000:1252.677669:8
x       52      .       T       G       0       .       DP=60.000000;XSBB=20;XSBN=4     GT:DP:GQ:AD:SB:PL       1/0:60.000000:407.019473:29.
x       272     .       TA      CA,CG,TG        0       .       DP=198.000000;XSBB=57;XSBN=6    GT:DP:GQ:AD:SB:PL       3/2:198.000000:46.74
x       823     .       A       G       0       .       DP=172.000000;XSBB=14;XSBN=4    GT:DP:GQ:AD:SB:PL       1/0:172.000000:1165.534775:9
x       277     .       A       C       0       .       DP=199.000000;XSBB=14;XSBN=4    GT:DP:GQ:AD:SB:PL       1/0:199.000000:1451.961774:9
x       827     .       G       C       0       .       DP=171.000000;XSBB=35;XSBN=4    GT:DP:GQ:AD:SB:PL       1/0:171.000000:1181.072965:8
x       858     .       TC      CA,CC,TA        0       .       DP=176.000000;XSBB=35;XSBN=6    GT:DP:GQ:AD:SB:PL       3/1:176.000000:10.11
```

Additional vg Links

- gPBWT: https://github.com/vgteam/vg/wiki/Building-a-Graph-Positional-Burrows-Wheeler-Transform-(gPBWT)

- vg on CentOS6 and 7: https://github.com/vgteam/vg/wiki/Building-VG-on-CentOS-6.6-or-7--and-using-it

- vg with long reads: https://github.com/vgteam/vg/wiki/Long-read-assemblies-using-vg-msga

- file format ref: https://github.com/vgteam/vg/wiki/File-Formats

Graphtyper

- Tool to call variants from reads mapped to a variation graph

- Not compatible with vg, but looks like it does similar things

TECHNICAL REPORTS

nature genetics

Graphtyper enables population-scale genotyping using pangenome graphs

Hannes P Eggertsson[1,2], Hakon Jonsson[1], Snaedis Kristmundsdottir[1,3], Eirikur Hjartarson[1], Birte Kehr[1,4], Gisli Masson[1], Florian Zink[1], Kristjan E Hjorleifsson[1], Aslaug Jonasdottir[1], Adalbjorg Jonasdottir[1], Ingileif Jonsdottir[1,5], Daniel F Gudbjartsson[1,2], Pall Melsted[1,2], Kari Stefansson[1,5] & Bjarni V Halldorsson[1,3]

Seven Bridges Genomics

- Proprietary data formats and variant caller ($$$)
- Human specific?
- https://www.sevenbridges.com/graph/

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Fast and Accurate Genomic Analyses using Genome Graphs

Goran Rakocevic, Vladimir Semenyuk, James Spencer, John Browning, Ivan Johnson, Vladan Arsenijevic, Jelena Nadj, Kaushik Ghose, Maria C. Suciu, Sun-Gou Ji, Gulfem Demir, Lizao Li, Berke C. Toptas, Alexey Dolgoborodov, Bjoern Pollex, Iosif Spulber, Irina Glotova, Peter Komar, Andrew Stachyra, Yilong Li, Milos Popovic, Wan-Ping Lee, Morten Kallberg, Amit Jain, Deniz Kural

doi: https://doi.org/10.1101/194530