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FastK Applications: MerquryFK, Smugeplot 2.0, KatFK

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Sanger K-mer Class
June 5, 2025

K-mer Counter Apps

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github@thegenemyers

FASTK
MERQURY.FK

Counter:

GenomeScope
SmudgePlot 2
Kat Tools
Merqury
Merfin

FastK
FastK
FastK
FastK
FastK

[KamilHash]

Need to
[Kamil+Gene]
[Only parts not in Merqury]
3 different counters/tables !

+ *K-mer classification*
+ *Ab initio HiFi error model*

9,773 Merqury.FK examples at
tolqc.cog.sanger.ac.uk

The FastK "Ecosystem"

More efficient algorithms wherever possible

All apps are threaded

Tables never loaded, always efficiently streamed

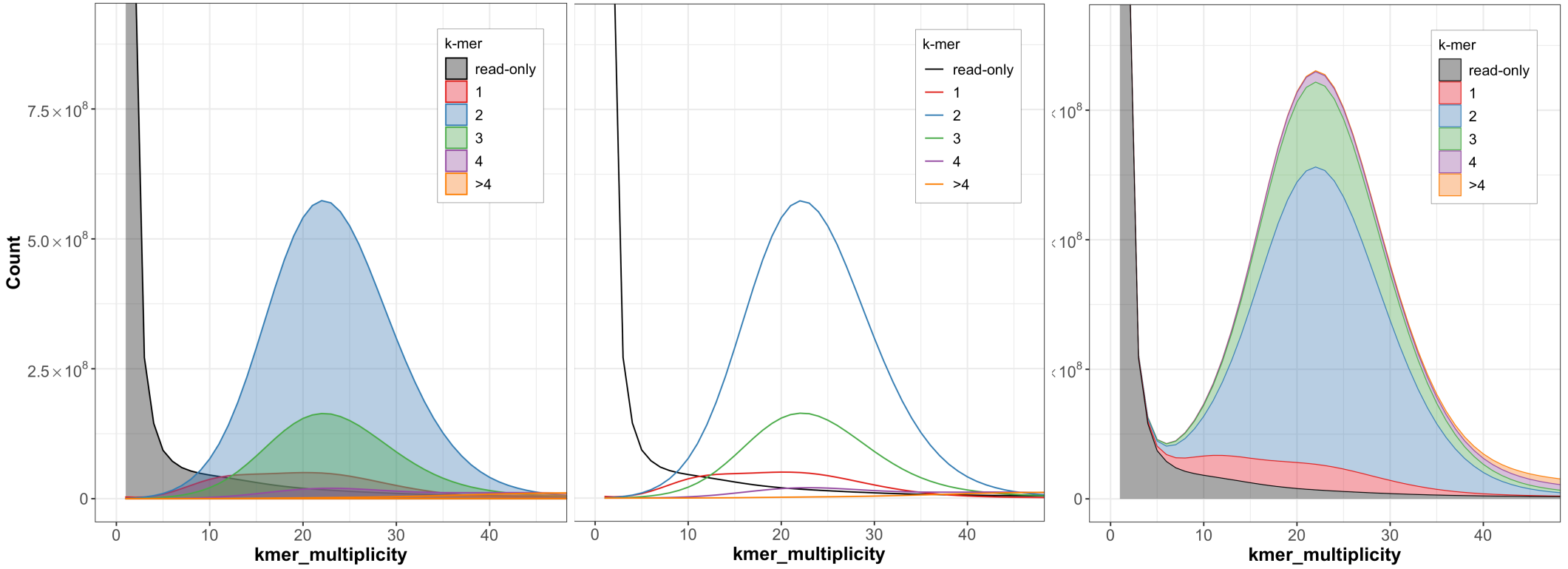
Common parameters conventions/style

Merqury Spectrum Plots

Fill, -f

Line, -l

Stack, -s



The FastK “Ecosystem”

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More efficient algorithms wherever possible

All apps are threaded

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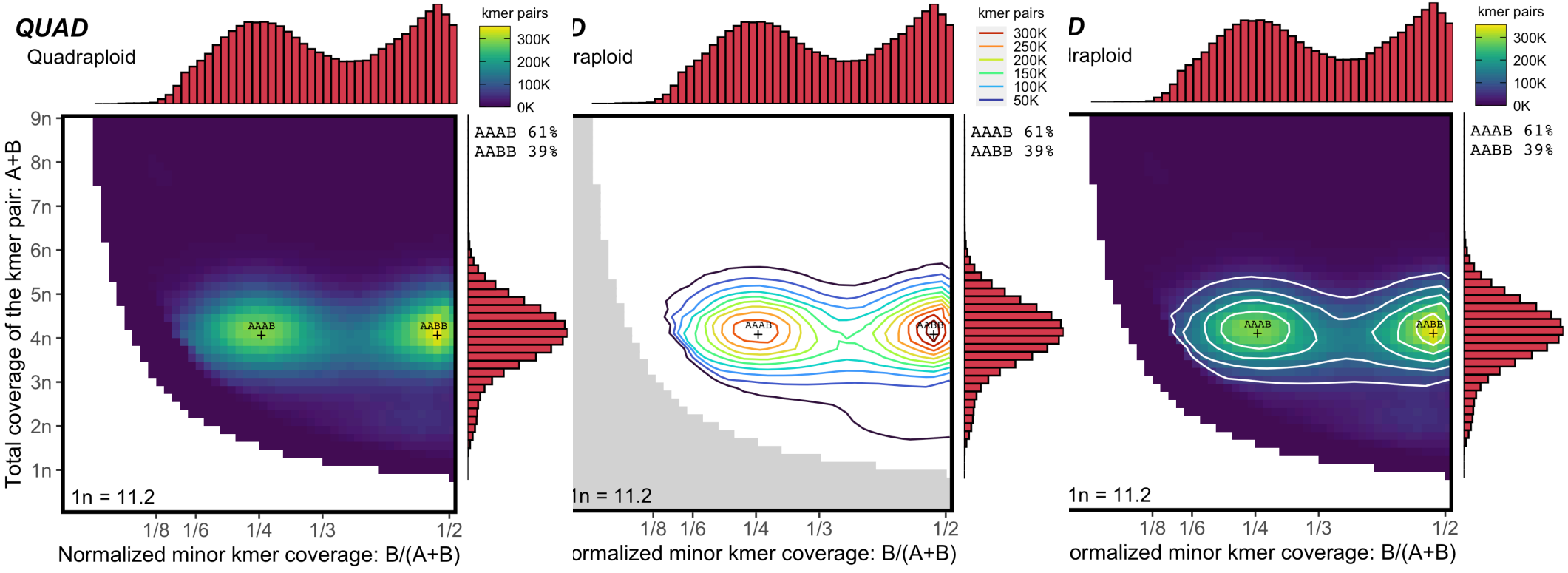
Common parameters conventions/style

PloidyPlot “smudge” Plots

Fill, -f

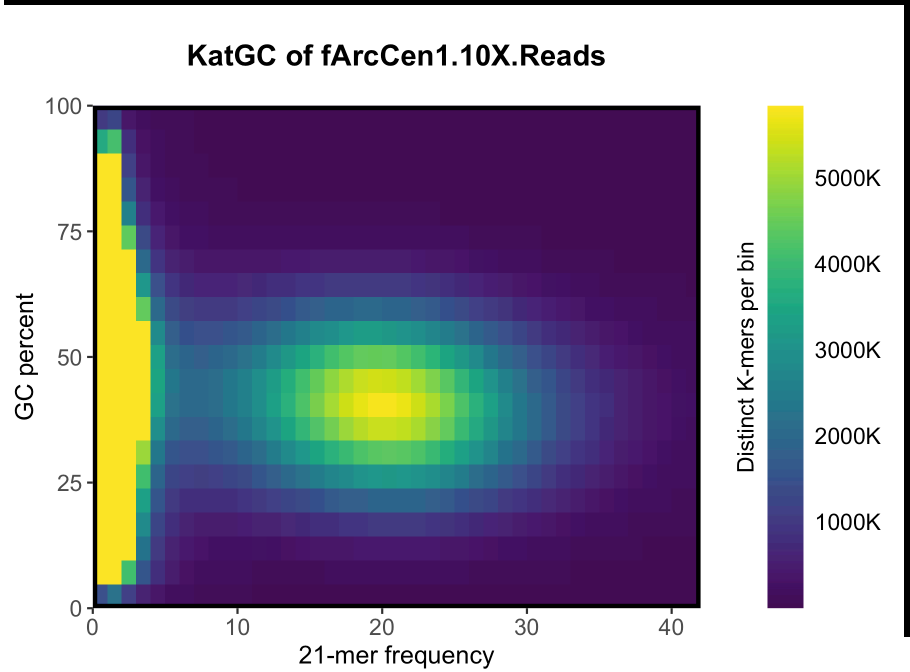
Line, -l

Stack, -s

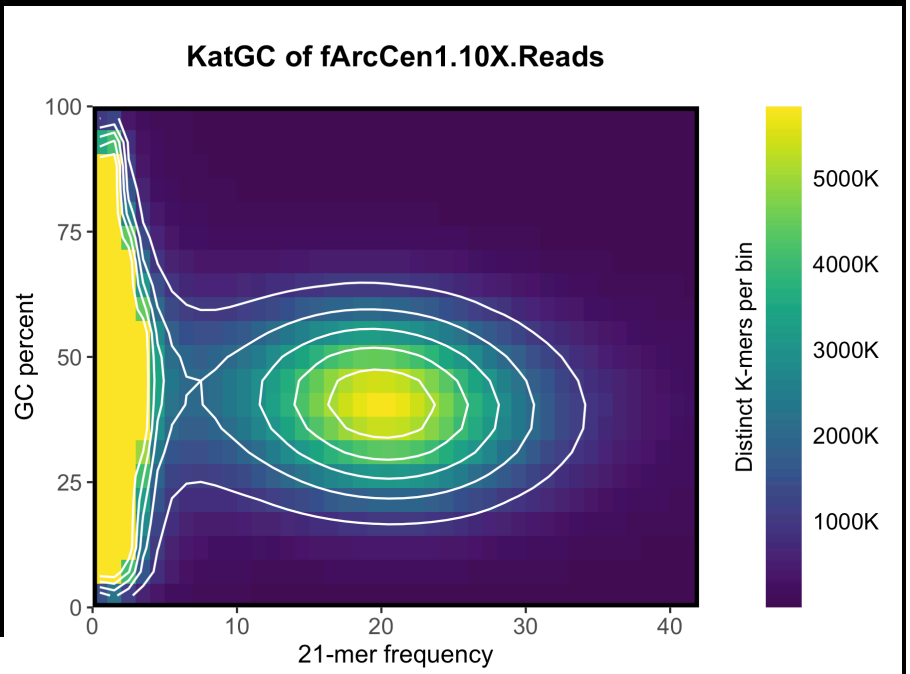


KatGC -T8 -oDemo fArcCen1.10X.Reads

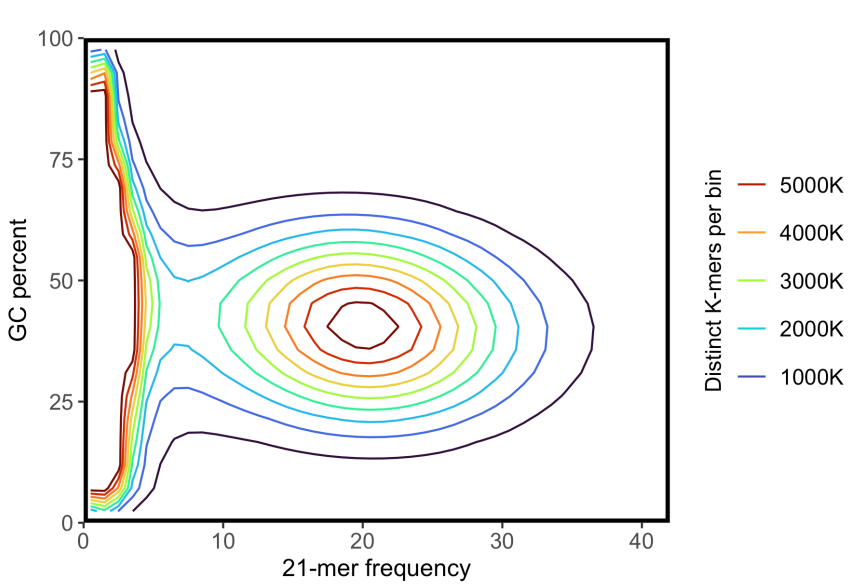
25.5 seconds later …



Demo.fi.png



Demo.st.png



Demo.ln.png

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MerquryFK

MerquryFK

Merqury is a suite of k-mer analyses for QC'ing genome assemblies/reconstructions developed by Arang Rie.

The envisioned scenarios are one unphased assembly or two haplotype phased assemblies, with or without parental trio data:

Asm1: unphased assembly or one of two phased assemblies

Asm2: a second phased assembly

Read: read data from individual sequenced,
e.g. Pacbio HiFi, Hi-C Illumina, or ONT ultra-long.

Pat: read data from individual's father

Mat: read data from individual's mother

Obviously designed for diploid genomes.

Calling MerquryFK

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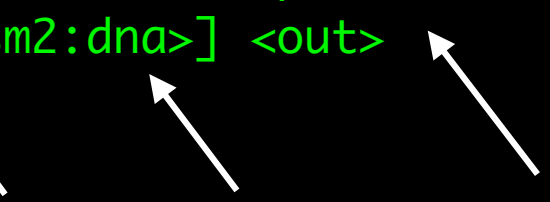
So Merqury FK has 4 possible calls:

MerquryFK Read Asm1 Out	(3 args)
MerquryFK Read Asm1 Asm2 Out	(4 args)
MerquryFK Read Pat Mat Asm1 Out	(5 args)
MerquryFK Read Pat Mat Asm1 Asm2 Out	(6 args)

```
MerquryFK [-w<double(6.0)>] [-h<double(4.5)>]
                [-[xX]<number(x2.1)>] [-[yY]<number(y1.1)>]
                [-vk] [-lfs] [-pdf] [-z] [-T<int(4)>] [-P<dir(/tmp)>]
                <read>[.ktab] [ <mat>[.hap[.ktab]] <pat>[.hap[.ktab]] ]
                <asm1:dna>[<asm2:dna>] <out>
```



Note carefully the type of each argument!

Setting up to Call MerquryFK

```
<read>[.ktab] [ <mat>[.hap[.ktab]] <pat>[.hap[.ktab]] ]
<asm1:dna> [<asm2:dna>] <out>
```

The assemblies are sequence files (sam, bam, cram, fasta, fastq) where each sequence is a scaffold and gaps between contigs are denoted by runs of n's.

FastK needs to be run on the Read and Mat & Pat datasets if present all with the same k. The Read's .ktab is the actual argument.

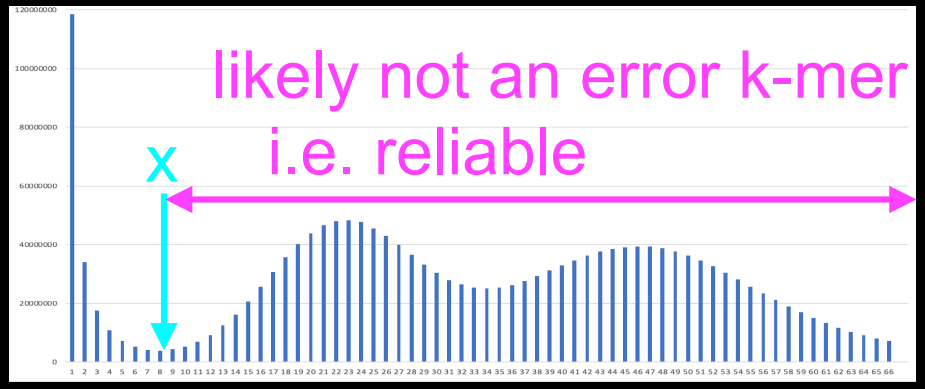
If trio data than HAPmaker needs to also be run beforehand to produce derived k-mer tables Mat.hap.ktab and Pat.hap.ktab, and these are the actual arguments.

HAPmaker

```
HAPmaker [-v] [-T<int(4)>] <mat>[.ktab] <pat>[.ktab] <read>[.ktab]
```

Pat.hap = Rel(Read&.Rel(Pat-Mat))

Rel(A) = A[x-] where x = 1st inflection in histogram of A



So, reliable k-mers in the father but not mother that are also reliable in the child.

Pat.hap = Rel(Read)&.(Rel(Pat)-Mat) *Just as good?*

Mat.hap = Rel(Read&.Rel(Mat-Pat))

MerquryFK Outputs

MerquryFK Read Pat Mat Asm1 Asm2 Out

Produces (lots of) outputs:

```
Out.Asm1.spectra-cn.fl.png
Out.Asm1.spectra-cn.ln.png
Out.Asm1.spectra-cn.st.png
Out.Asm1.qv
Out.Asm1_only.bed

Out.spectra-asm.fl.png
Out.spectra-asm.ln.png
Out.spectra-asm.st.png
Out.qv
Out.completeness.stats


Out.Asm2.spectra-cn.fl.png
Out.Asm2.spectra-cn.ln.png
Out.Asm2.spectra-cn.st.png
Out.Asm2.qv
Out.Asm2_only.bed

Out.spectra-cn.fl.png
Out.spectra-cn.ln.png
Out.spectra-cn.st.png
```

```
Out.Asm1.Mat.spectra-cn.fl.png
Out.Asm1.Mat.spectra-cn.ln.png
Out.Asm1.Mat.spectra-cn.st.png
Out.Asm1.Pat.spectra-cn.fl.png
Out.Asm1.Pat.spectra-cn.ln.png
Out.Asm1.Pat.spectra-cn.st.png
Out.Asm1.phased_block.bed
Out.Asm1.phased_block.blob.png
Out.Asm1.phased_block.stats
Out.Asm1.block.N.png
Out.Asm1.continuity.N.png

Out.hapmers.blob.png
```

```
Out.Asm2.Mat.spectra-cn.fl.png
Out.Asm2.Mat.spectra-cn.ln.png
Out.Asm2.Mat.spectra-cn.st.png
Out.Asm2.Pat.spectra-cn.fl.png
Out.Asm2.Pat.spectra-cn.ln.png
Out.Asm2.Pat.spectra-cn.st.png
Out.Asm2.phased_block.bed
Out.Asm2.phased_block.blob.png
Out.Asm2.phased_block.stats
Out.Asm2.block.N.png
Out.Asm2.continuity.N.png
```

We'll go through them one by one …

Copy Number Spectra Plots:

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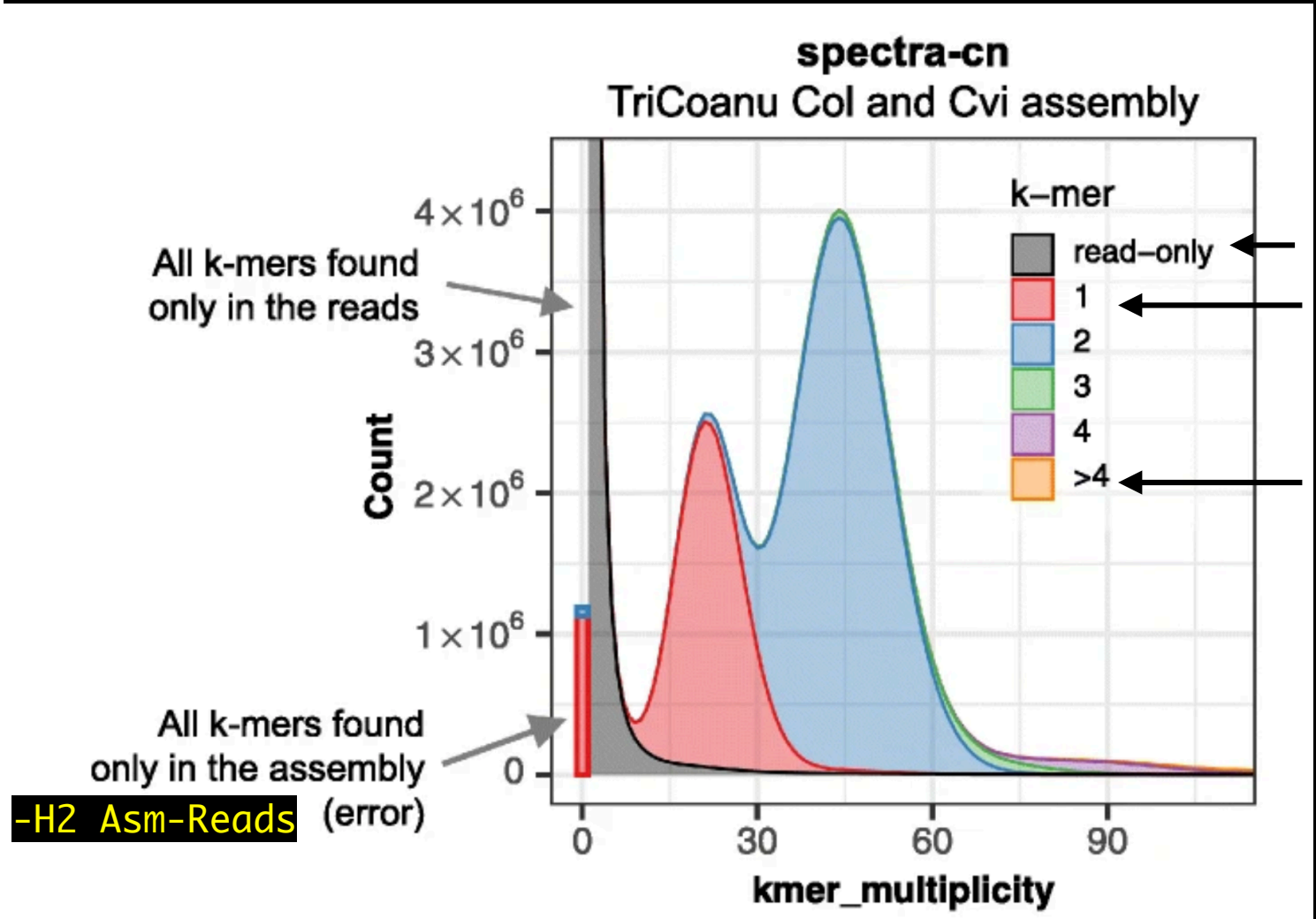
```
CNplot <reads>[.ktab] <asm:dna> <out>[.cni]
```

```
The output(s) is(are) <out>.[ln|fl|st].[png|pdf]
```




```
Reads-Asm
Reads&.Asm[1]
. . .
Reads&.Asm[5-]
```

Asm1 = *Col*
Asm2 = *Cvi*
Out = *TrioCanu*

CN-Spectra Options:

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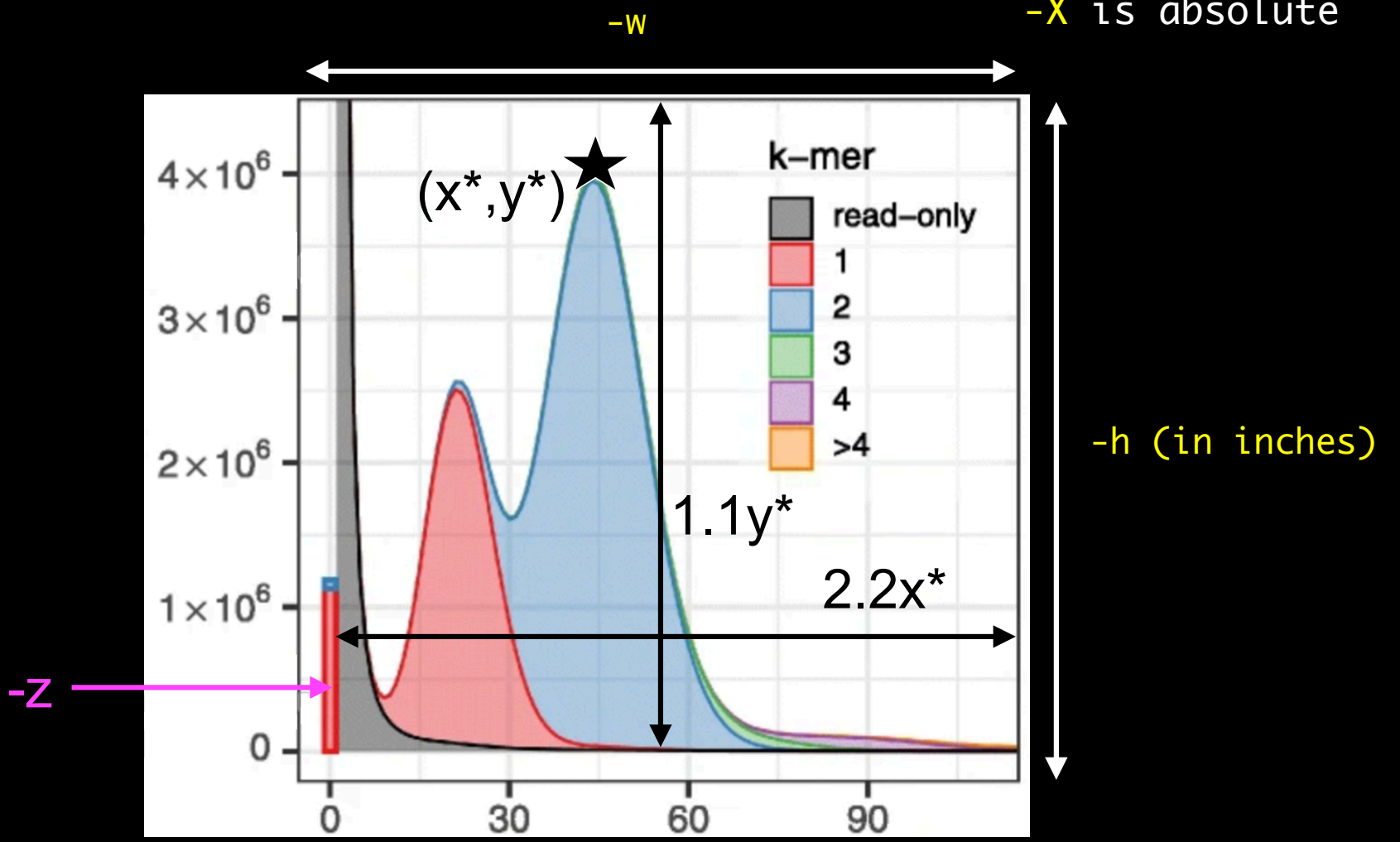
```
CNplot [-w<double(6.0)>] [-h<double(4.5)>] [-z] [-lfs]
       [-[xX]<number(x2.1)>] [-[yY]<number(y1.1)>] [-pdf]
       <reads>[.ktab] <asm:dna> <out>[.cni]
```

-x is relative to x*
-X is absolute



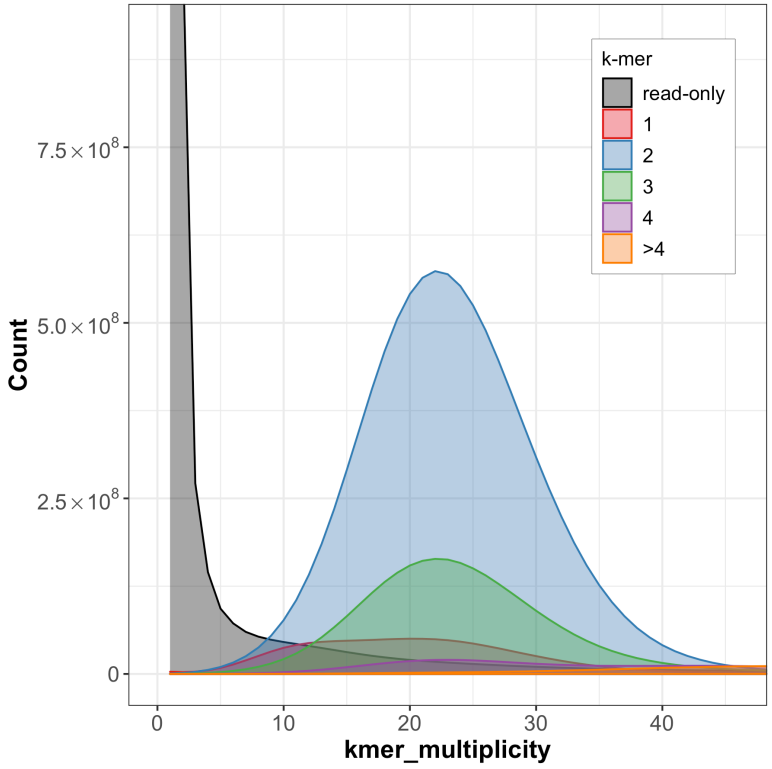
CN-Spectra Options:

default is all 3
e.g. -ls legal

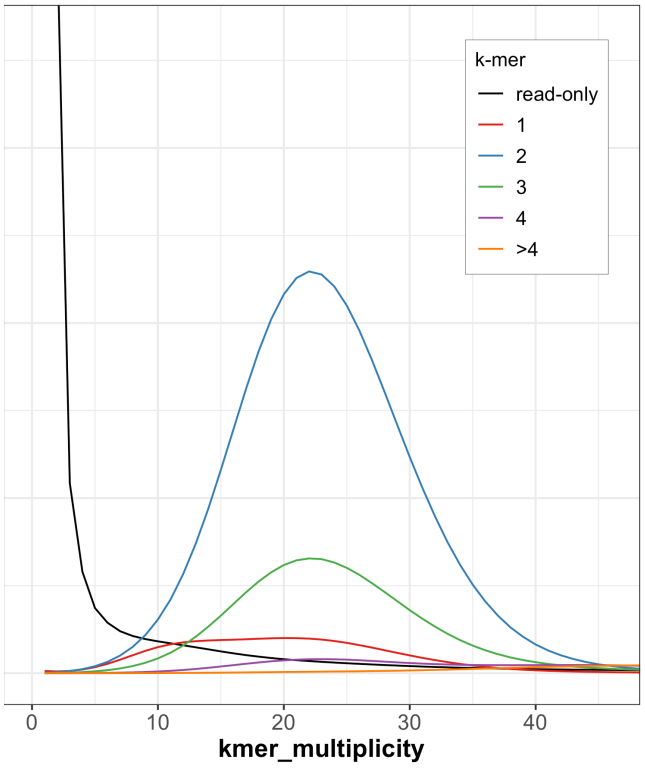
```
CNplot [-w<double(6.0)>] [-h<double(4.5)>] [-z] [-lfs]
       [-[xX]<number(x2.1)>] [-[yY]<number(y1.1)>] [-pdf]
       <reads>[.ktab] <asm:dna> <out>[.cni]
```

default is .png

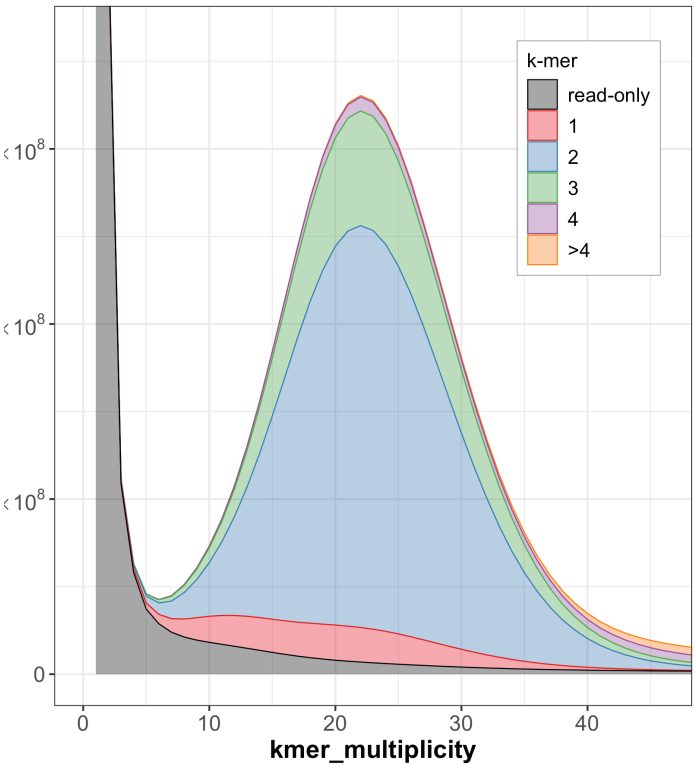
Fill, -f



Line, -l



Stack, -s



CN-Spectra Options:

```
CNplot [-w<double(6.0)>] [-h<double(4.5)>] [-z] [-lfs]
       [-[xX]<number(x2.1)>] [-[yY]<number(y1.1)>] [-pdf]
       [-vk] [-T<int(4)>] [-P<dir(/tmp)>]
       <reads>[.ktab] <asm:dna> <out>[.cni]
```

Passed through to FastK and Logex

The -k option asks CNplot to save the FastK & Logex results so that a call:

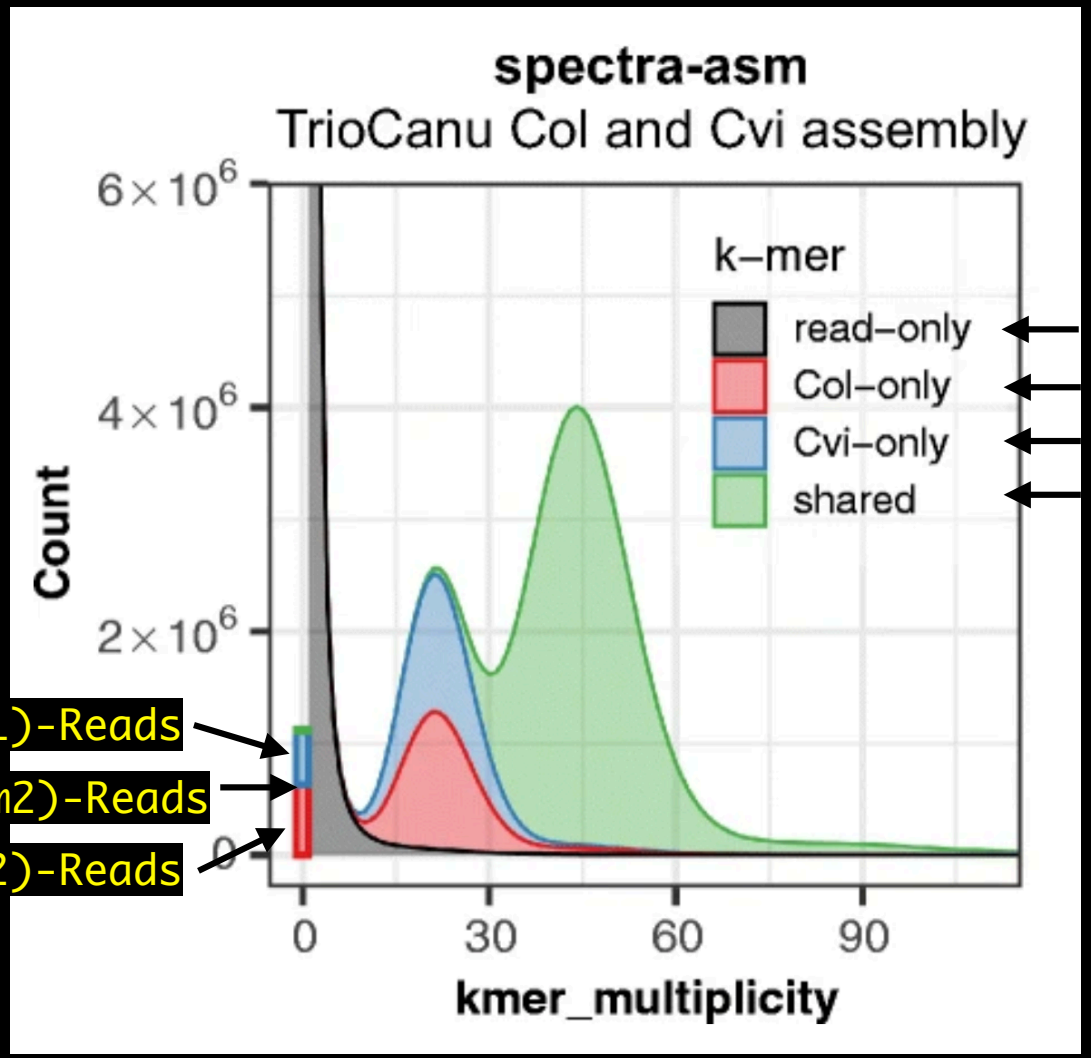
```
CNplot [-w<double(6.0)>] [-h<double(4.5)>] [-z] [-lfs]
       [-[xX]<number(x2.1)>] [-[yY]<number(y1.1)>] [-pdf]
       <out>[.cni]
```

Produces just plots and requires no significant computation. (Often a user wants to just adjust the size or aspect or style of the plot)

Assembly Spectra Plots:

-k as for CNplot save extension is different

```
ASMplot [-w<double(6.0)>] . . . [-P<dir(/tmp)>]
        <reads>[.ktab] <asm1:dna> [<asm2:dna>] <out>[.asmi]
```



NB: Expression inside # don't need count qualifier.

```
Reads - #(Asm1|Asm2)
Reads &. (Asm1-Asm2)
Reads &. (Asm2-Asm1)
Reads &. #(Asm2&Asm1)
```

```
Reads - Asm1
Reads &. Asm1
```

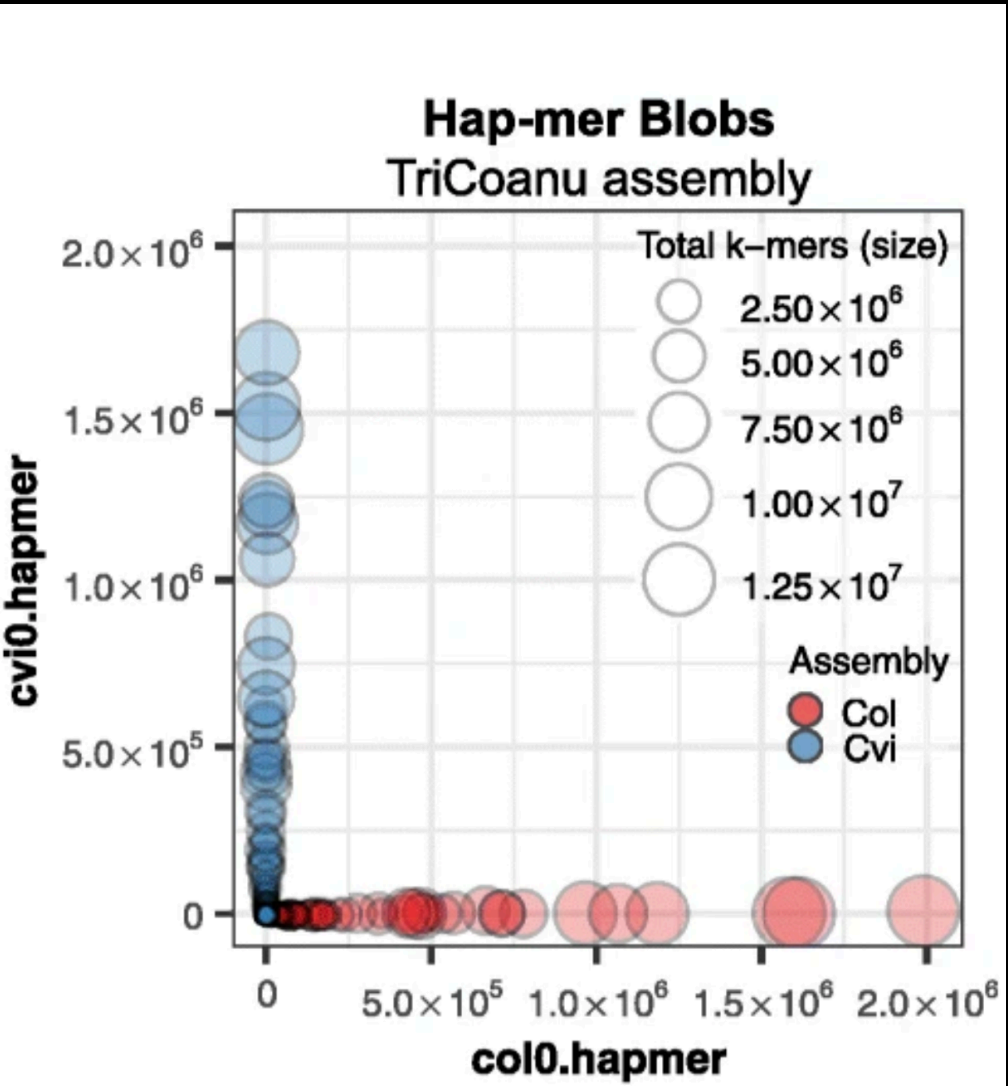
```
H1 (Asm2-Asm1)-Reads
H1 (Asm1&+Asm2)-Reads
H1 (Asm1-Asm2)-Reads
```

```
Asm1-Reads
```

Asm1 = *Col*
Asm2 = *Cvi*
Out = *TrioCanu*

Hap-mer Blob Plot: Are the contigs well phased?

```
HAPplot[-w<double(6.0)>] [-h<double(4.5)>] [-pdf]
       [-vk][-T<int(4)>] [-P<dir(/tmp)>]
       <mat>[.hap[.ktab]] <pat>[.hap[.ktab]] <asm1:dna> [<asm2:dna>] <out>[.hpi]
```



1 blob per contig whose radius is proportional to its length

M(c) = # of k-mers in mat.hap.ktab

P(c) = # of k-mers in pat.hap.ktab

position of blob center = (M(c),P(c))

opacity = (M(c)+P(c))/2|c|

color = which assembly

Asm1 = *Col*
Asm2 = *Cvi*
Out = *TrioCanu*

MerquryFK Outputs

MerquryFK Read Pat Mat Asm1 Asm2 Out

Asm1 only

```
Out.Asm1.spectra-cn.fl.png
Out.Asm1.spectra-cn.ln.png
Out.Asm1.spectra-cn.st.png
Out.Asm1.qv
Out.Asm1_only.bed

Out.spectra-asm.fl.png
Out.spectra-asm.ln.png
Out.spectra-asm.st.png
Out.qv
Out.completeness.stats
```

+ Asm2

```
Out.Asm2.spectra-cn.fl.png
Out.Asm2.spectra-cn.ln.png
Out.Asm2.spectra-cn.st.png
Out.Asm2.qv
Out.Asm2_only.bed

Out.spectra-cn.fl.png
Out.spectra-cn.ln.png
Out.spectra-cn.st.png
```

CN spectra of Asm1 U Asm2 (if both)

Asm1 + Trio

```
Out.Asm1.Mat.spectra-cn.fl.png
Out.Asm1.Mat.spectra-cn.ln.png
Out.Asm1.Mat.spectra-cn.st.png
Out.Asm1.Pat.spectra-cn.fl.png
Out.Asm1.Pat.spectra-cn.ln.png
Out.Asm1.Pat.spectra-cn.st.png
Out.Asm1.phased_block.blob.png
Out.Asm1.phased_block.bed
Out.Asm1.phased_block.stats
Out.Asm1.block.N.png
Out.Asm1.continuity.N.png

Out.hapmers.blob.png
```

All

```
Out.Asm2.Mat.spectra-cn.fl.png
Out.Asm2.Mat.spectra-cn.ln.png
Out.Asm2.Mat.spectra-cn.st.png
Out.Asm2.Pat.spectra-cn.fl.png
Out.Asm2.Pat.spectra-cn.ln.png
Out.Asm2.Pat.spectra-cn.st.png
Out.Asm2.phased_block.blob.png
Out.Asm2.phased_block.bed
Out.Asm2.phased_block.stats
Out.Asm2.block.N.png
Out.Asm2.continuity.N.png
```

CN spectra of <asm> vs <hapmer>

MerquryFK QV Metric

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T = # of k-mers in Asm

S = # of k-mers in Reads&Asm

Pr (k-mer is not an error) = $(1-\varepsilon)^k$

Pr (k-mer is not an error) = S/T (k-mers supported by reads)

$\Rightarrow \varepsilon = 1 - (S/T)^{1/k}$

$qv = 10 \log_{10} \varepsilon$

Out.Asm.qv ≡ T-S ◦ T ◦ ε ◦ qv

Out.Asm_only.bed = intervals covered by k-mers in Asm-Reads
= places where there might be an error

Out.qv = cumulative over all assemblies

MerquryFK Outputs

MerquryFK Read Pat Mat Asm1 Asm2 Out

Asm1 only

```
Out.Asm1.spectra-cn.fl.png
Out.Asm1.spectra-cn.ln.png
Out.Asm1.spectra-cn.st.png
Out.Asm1.qv
Out.Asm1_only.bed

Out.spectra-asm.fl.png
Out.spectra-asm.ln.png
Out.spectra-asm.st.png
Out.qv
Out.completeness.stats
```

+ Asm2

```
Out.Asm2.spectra-cn.fl.png
Out.Asm2.spectra-cn.ln.png
Out.Asm2.spectra-cn.st.png
Out.Asm2.qv
Out.Asm2_only.bed

Out.spectra-cn.fl.png
Out.spectra-cn.ln.png
Out.spectra-cn.st.png
```

Asm1 + Trio

```
Out.Asm1.Mat.spectra-cn.fl.png
Out.Asm1.Mat.spectra-cn.ln.png
Out.Asm1.Mat.spectra-cn.st.png
Out.Asm1.Pat.spectra-cn.fl.png
Out.Asm1.Pat.spectra-cn.ln.png
Out.Asm1.Pat.spectra-cn.st.png
Out.Asm1.phased_block.blob.png
Out.Asm1.phased_block.bed
Out.Asm1.phased_block.stats
Out.Asm1.block.N.png
Out.Asm1.continuity.N.png

Out.hapmers.blob.png
```

All

```
Out.Asm2.Mat.spectra-cn.fl.png
Out.Asm2.Mat.spectra-cn.ln.png
Out.Asm2.Mat.spectra-cn.st.png
Out.Asm2.Pat.spectra-cn.fl.png
Out.Asm2.Pat.spectra-cn.ln.png
Out.Asm2.Pat.spectra-cn.st.png
Out.Asm2.phased_block.blob.png
Out.Asm2.phased_block.bed
Out.Asm2.phased_block.stats
Out.Asm2.block.N.png
Out.Asm2.continuity.N.png
```

MerquryFK Completeness

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T = # of k-mers in Rel(Reads)

S = # of k-mers in Rel(Reads)&Asm

S/T is fraction of Asm covered by reliable read k-mers

Out.completeness.stats ≡ target ◦ source ◦ S ◦ T ◦ 100(S/T)

target = Asm1, Asm2, ‘both’

source = ‘all’, Mat, Pat

MerquryFK Phasing

With trio data, can find runs of pat or mat hap-mers in
the scaffolds of an assembly = hap-mer blocks (of the same phase)

Small switches of phase are allowed between reliable blocks
(= 20Kbp and/or 5 hap-mers in a row).

A well phased assembly has large blocks all of the same kind.

Out.Asm1.phased_block.stats = #blocks ◦ sum ◦ min ◦ avg ◦ N50 ◦ max

Out.Asm1.phased_block.bed = block intervals

= scaffold ◦ beg ◦ end ◦ phase ◦ purity ◦ switches ◦ #hap-mers

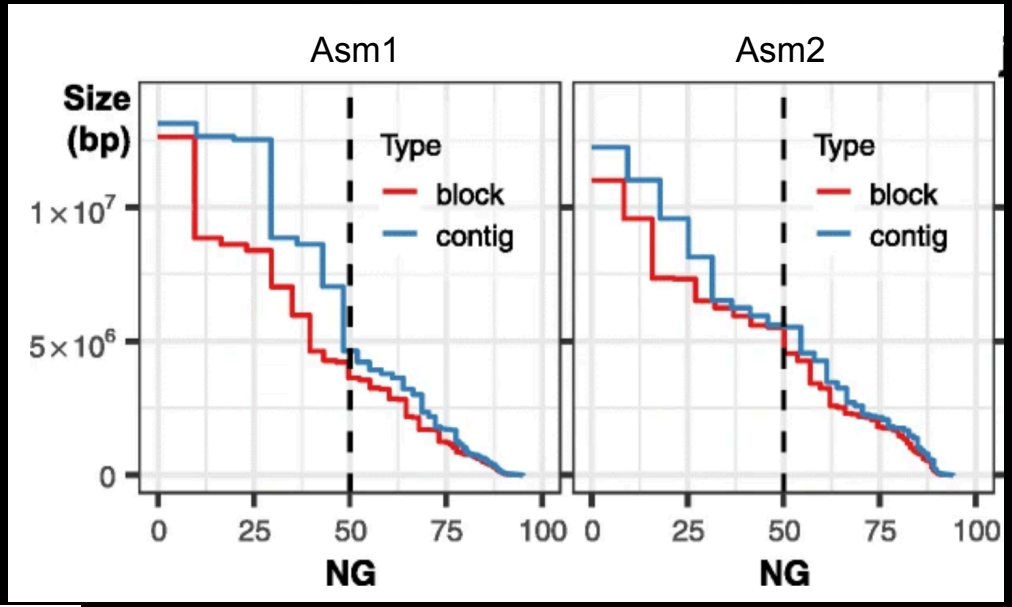
Out.Asm.phased_block.bed

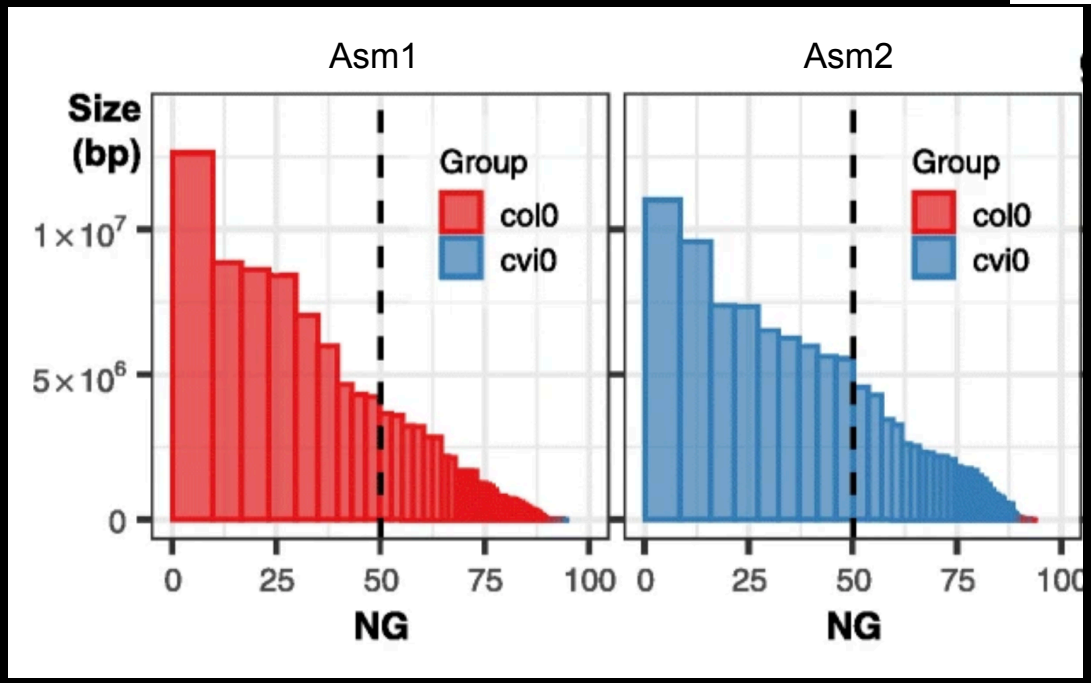
Scaffold	Start	End	Phase	Purity	Switches	Markers
1	0	24973	Gut4	0.463	43	9289
1	26394	30482	Gut3	11.364	25	220
1	30970	846998	Gut4	0.134	449	336080
1	847235	847415	Gut3	0.000	0	112
1	847708	1404608	Gut4	0.080	162	202012
2	1404621	1456858	Gut4	0.125	21	16795
2	1457275	1457620	Gut3	0.000	0	21
3	1458249	1617417	Gut4	0.000	0	53070
4	1618096	2072629	Gut4	0.136	213	156733
4	2075958	2081903	Gut3	0.000	0	47
5	2082003	3500257	Gut4	0.089	400	450404
6	3500357	5864720	Gut4	0.046	367	86355
7	5864820	6131671	Gut4	0.054	49	79745
8	6131771	10025579	Gut4	0.023	262	1133157
9	10026565	15600117	Gut4	0.038	452	1179938
10	15600217	15601057	Gut3	0.000	0	21
10	15601994	15813295	Gut4	0.076	21	27604
10	15813690	15813726	Gut3	0.000	0	22

. . . .

MerquryFK: NG plots

The size distribution of every contig, scaffold and phase blocked is captured in a “NG” continuity plot for each assembly





Similarly phase colored hapmer blocks are plotted in order of size.

MerquryFK: Options

```
MerquryFK [-w<double(6.0)>] [-h<double(4.5)>] [-z] [-lfs]
          [-[xX]<number(x2.1)>] [-[yY]<number(y1.1)>] [-pdf]
          [-vk][-T<int(4)>] [-P<dir(/tmp)>]
          <read>[.ktab] [ <mat>[.hap[.ktab]] <pat>[.hap[.ktab]] ]
          <asm1:dna> [<asm2:dna>] <out>
```

Same as CNplot! Passed through to FastK, Logex, and internal calls to CNplot, ASMplot, and HAPplot.

As before the -k option causes all .cni, .asmi, and .hpi intermediates to be saved for subsequent replotting.

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Merqury Exercises

```
Run on trio.small, trio.5.40x, trio.05.40x
```

```
Examine results
```

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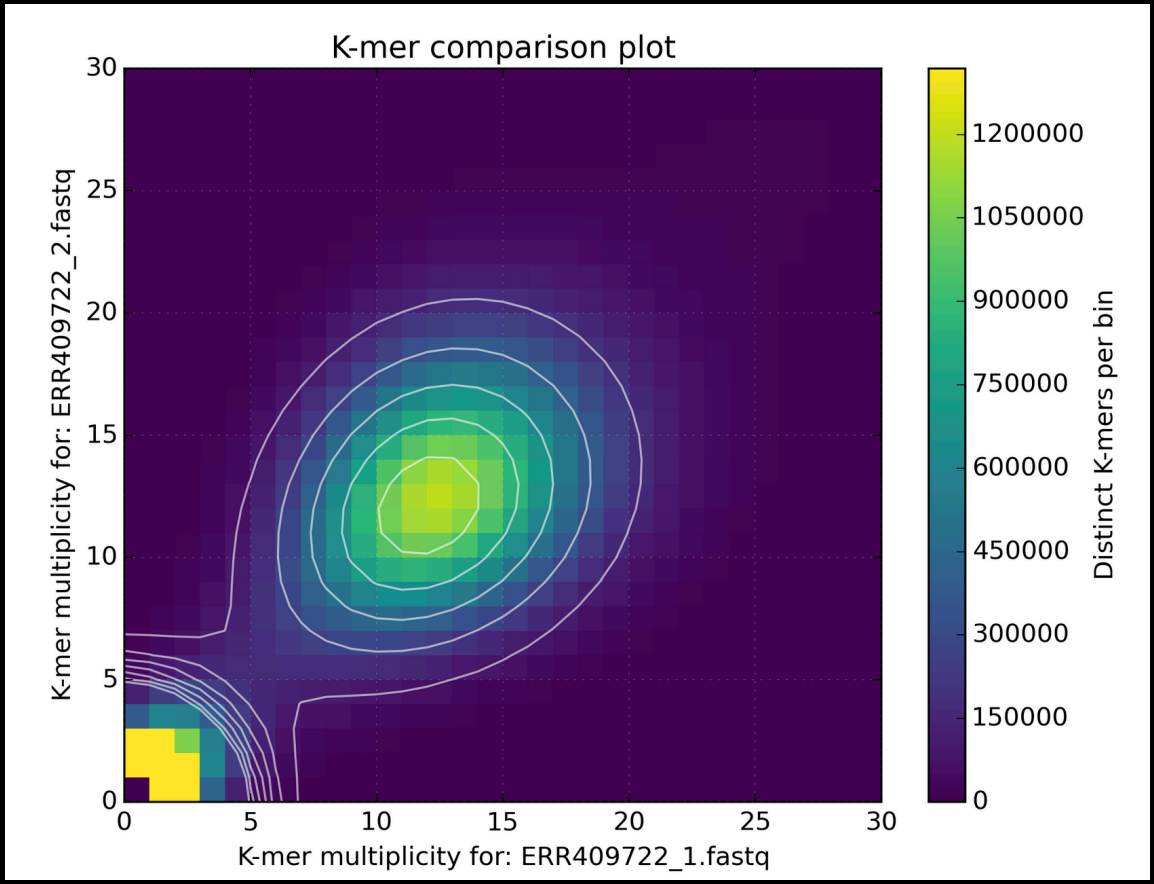
KatGC & KatComp

KatComp:

```
KatComp [-w<double(6.0)>] [-h<double(4.5)>] [lfs]
        [-[xX]<number(x2.1)>] [-[yY]<number(y2.1)>] [-pdf] [-T<int(4)>]
        <source1>[.ktab] <source2>[.ktab] <out>
```



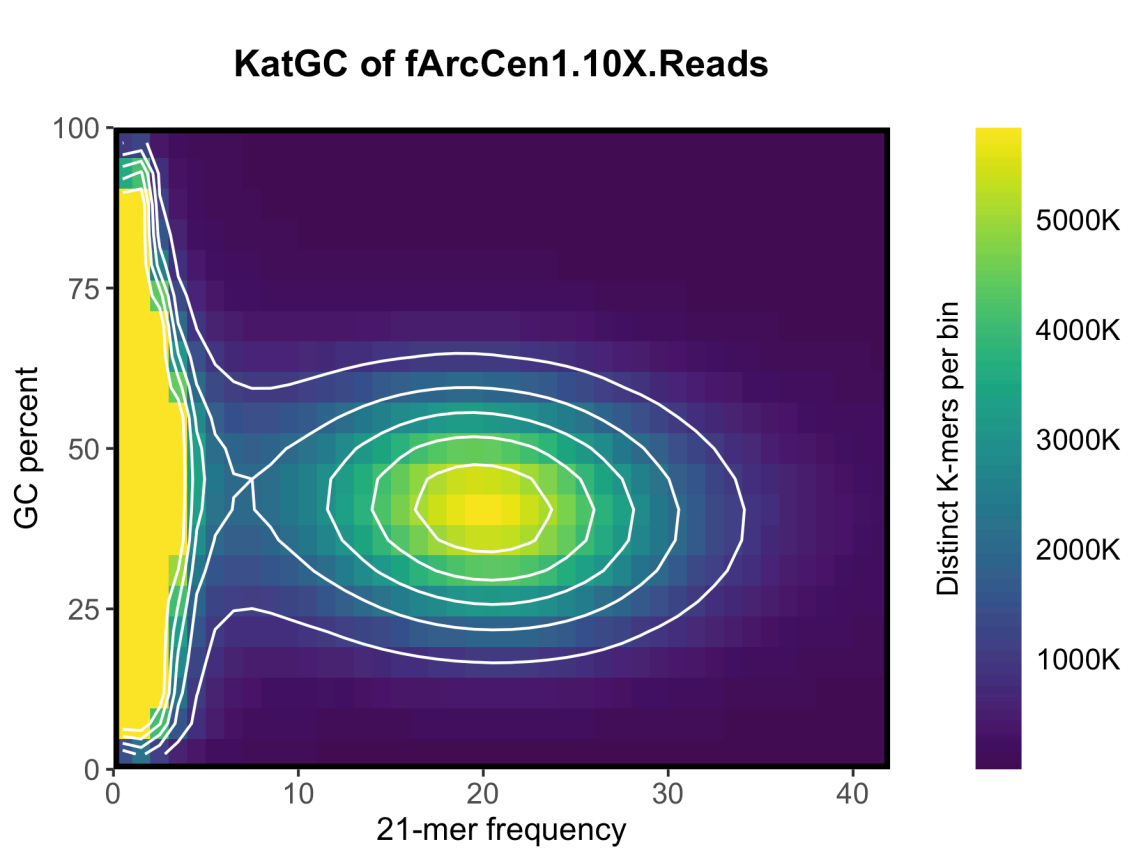
KatComp(x,y)

$= | \alpha : \alpha \in A\&B \;\&\; freq_A(\alpha) = x \;\&\; freq_B(\alpha) = y |$

where A = source1
and B = source2

KatComp:

```
KatGC [-w<double(6.0)>] [-h<double(4.5)>] [lfs]
      [-[xX]<number(x2.1)>] [-pdf] [-T<int(4)>]
      <source>[.ktab] <out>
```



KatGC(x,y)

$= | \alpha : \alpha \in A \ \& \ freq_A(\alpha) = x \ \& \ \%gc(\alpha) = y |$

where A = source1 & $y \in [0,100]$

wellcome connecting science

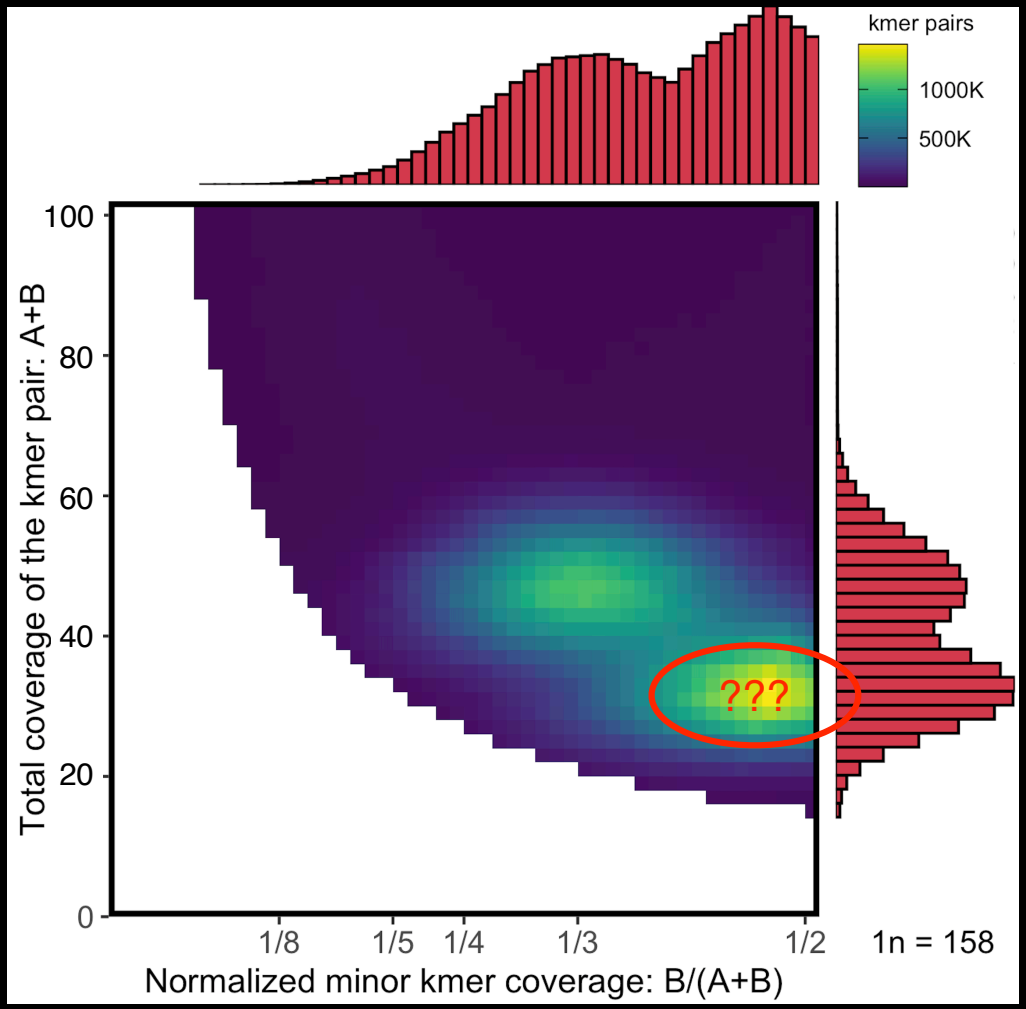
SmudgePlot 2.0

`If time permits, optional.`

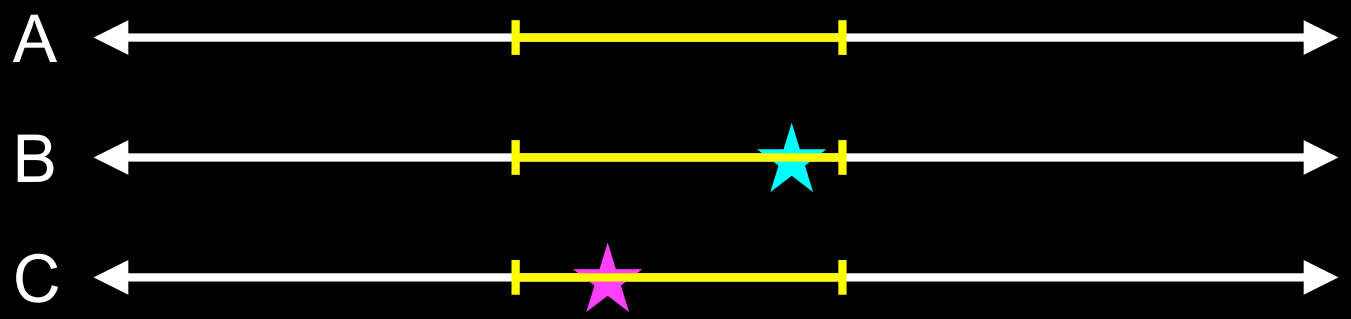
SmudgePlot 'C-Backend'

Simulated 50X triploid data set
with 1% heterogeneity

Original SmudgePlot



The explanation:



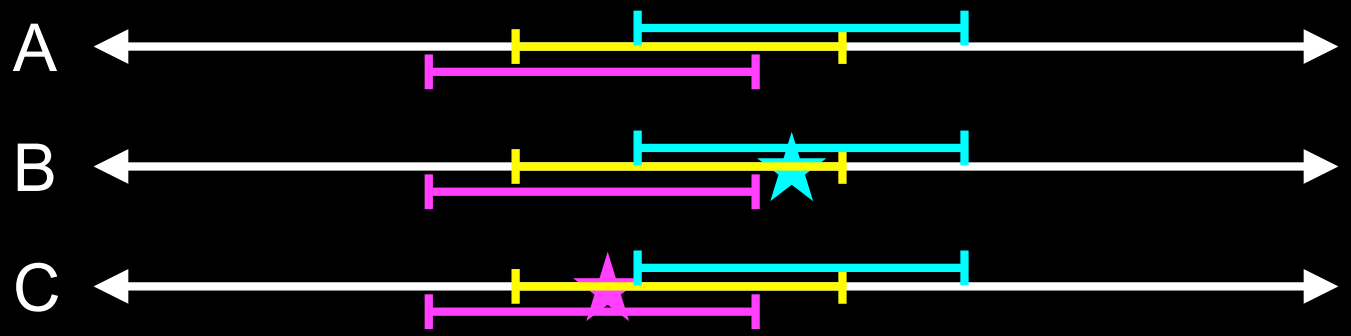
cnt(A) = cnt(B) = cnt(C) ~ 1/3c

A/(A+B) ~ A/(A+C) ~ B/(B+C) ~ 1/2

A,B / A,C / B,C contribute to the diploid smudge!

The greater k or the heterogeneity the greater the effect

The fix:



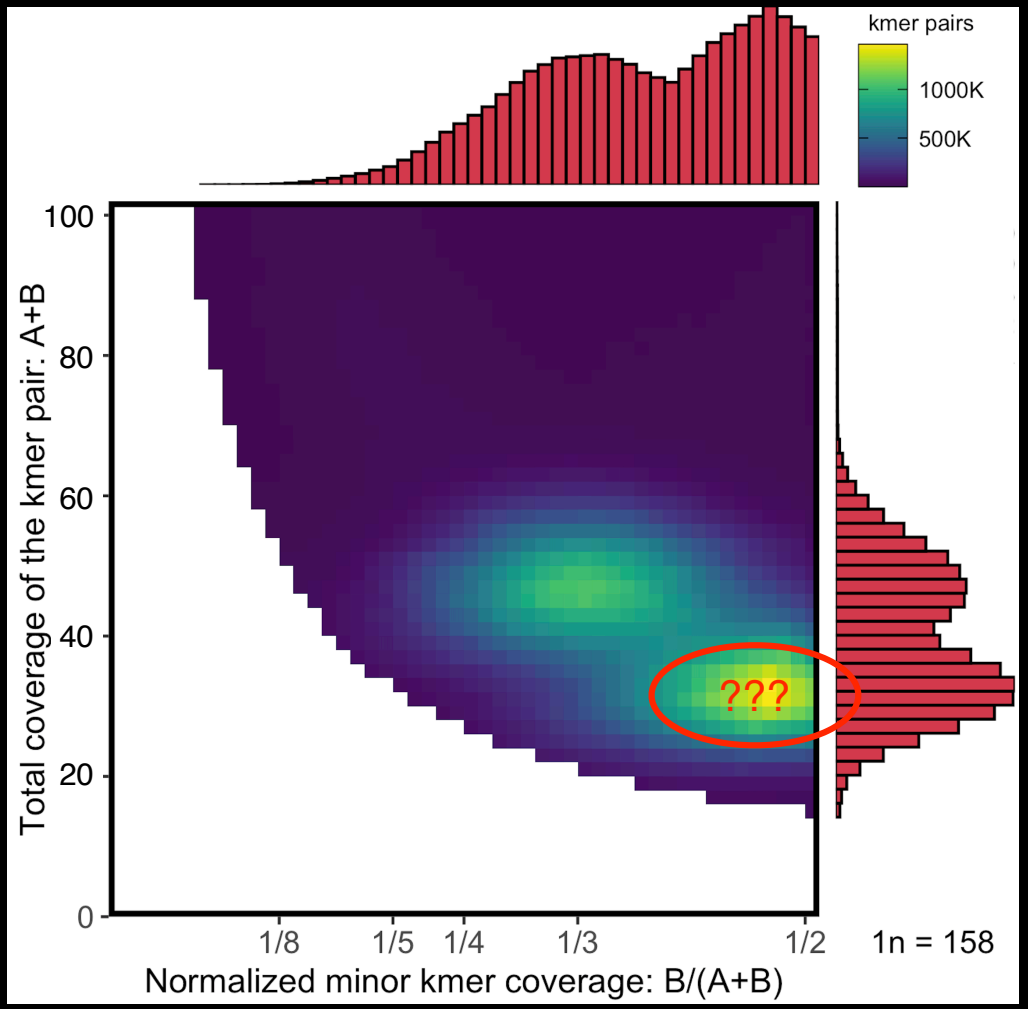
If a k-mer occurs in more than 1 non-erroneous het-pair then don’t count it.

SmudgePlot 2.0

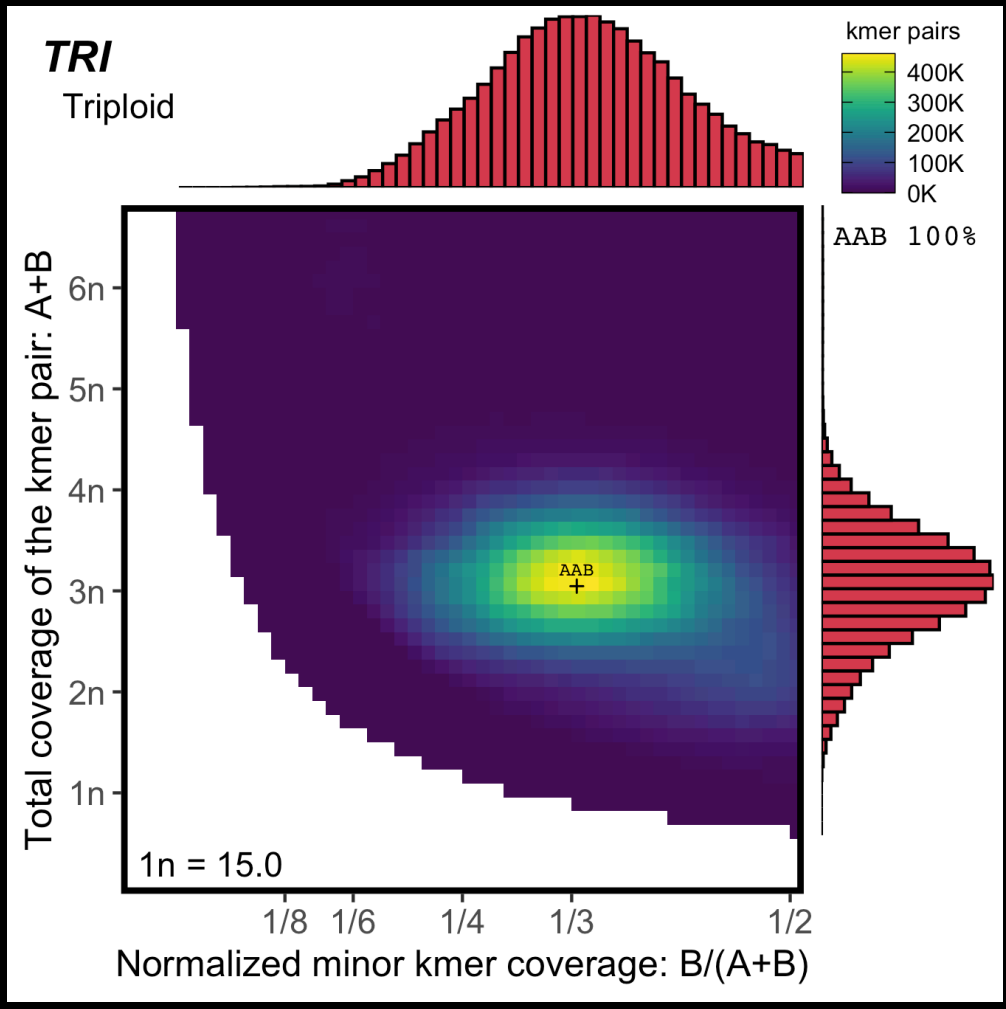
wellcome connecting science

Simulated 50X triploid data set
with 1% heterogeneity

Original SmudgePlot



SmudgePlot 2.0



`smudgeplot.py hetmers -L # <source:.ktab>`

All k-mers with count less than **-L** are considered error

Table needs to be trimmed (to **-L**) and symmetric before finding pairs

User can do it, or smudgeplot will — determined automatically

Assuming foo is an untrimmed, canonical table:

```
smudgeplot.py -t 8 -L 4 foo.ktab    331s
```

```
Logex -T8 'trim=A[4-]' foo          97s
smudgeplot.py -t 8 -L 4 trim.ktab   231s     328s
```

```
Logex -T8 'trim=A[4-]' foo          97s
Symmex -T8 trim symx                56s      335s
smudgeplot.py -t 8 -L 4 symx.ktab   182s
```

Always trim first!

```
Symmex -T8 foo symx                 640s
smudgeplot.py -t 8 -L 4 symx.ktab   482s
```

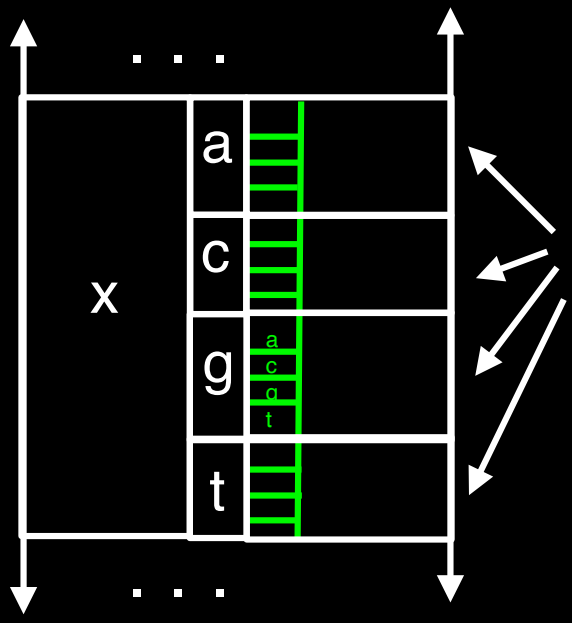
works but slow

Smudgeplot Algorithm:

Find all k-mer pairs xay,xby (w. counts A,B): plot min(A,B)/A+B

In a *symmetric* table of k-mers:

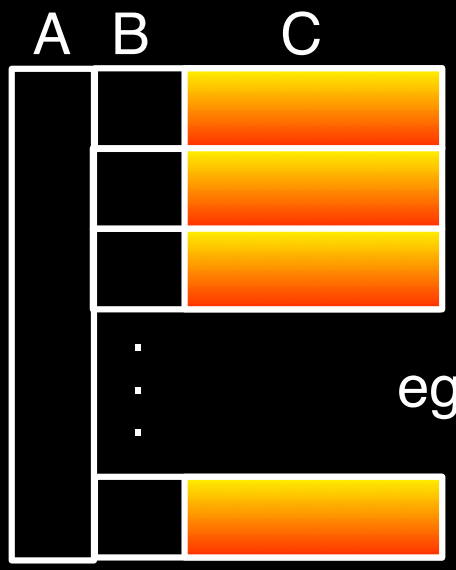
For all x, |x|<k:



Merge 4 lists looking for = suffixes

O(kN) time

Also find subdivisions for next lower level



eg: A 1-3
B 4-5
C 6-..

Threading:

A. If |x| small: divide lists into N-parts

B. If |x| not small: one thread, recurse

C. If |x| large: table range is in core

O(kN/T) time