GCSA2: A scalable approach to indexing population variation graphs

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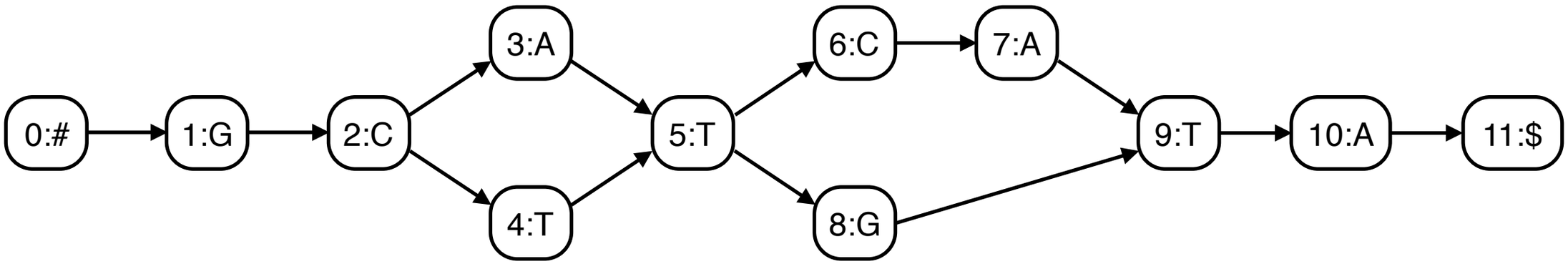


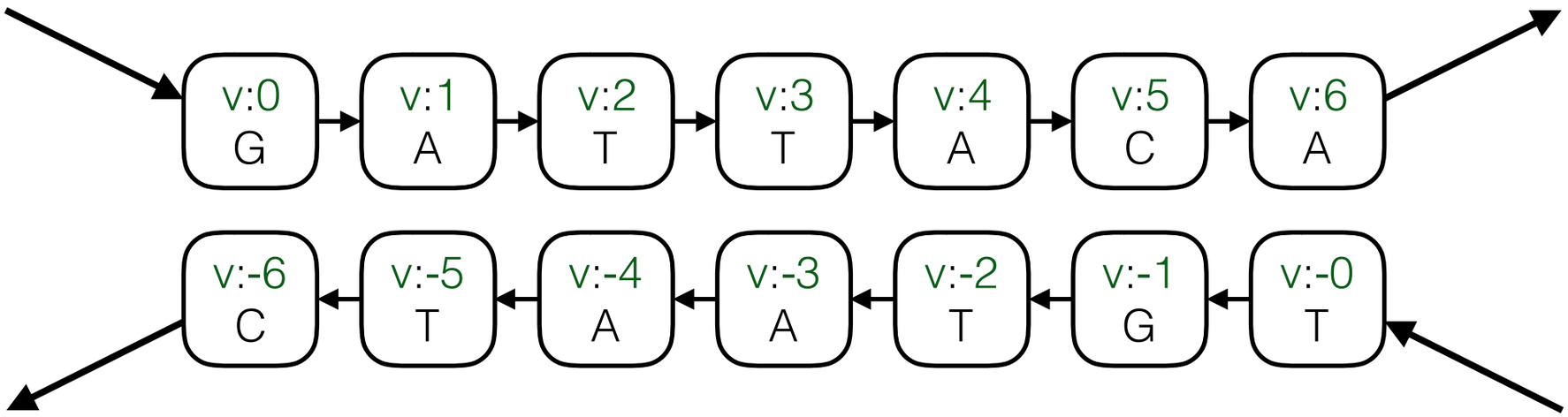
- Graphs with paths labeled by sequences are a natural way of representing genetic variation.

- Reference genomes could eventually become such graphs.

- The variation graph toolkit vg (Erik Garrison et al, https://github.com/vgteam/vg) is a community effort to develop tools for working with such graphs.

- This talk is about GCSA2, the path index used in vg.

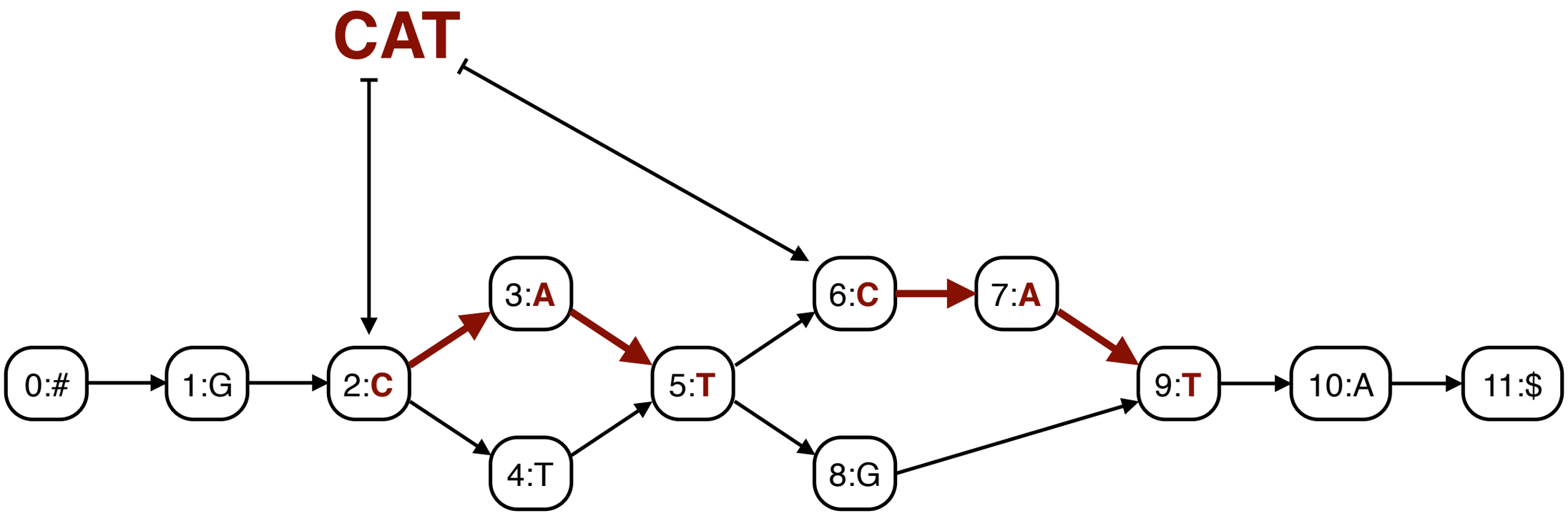
Variation graphs



Simple directed graphs are easier to handle. The transformation is also useful for other purposes.

Path Indexes

Path indexes



Path indexes are a central tool for working with variation graphs. They are text indexes for the path labels in a graph. The index finds (the start nodes of) the paths labeled by the query string.

Path indexes

- The number of kmers in a graph increases exponentially with k.

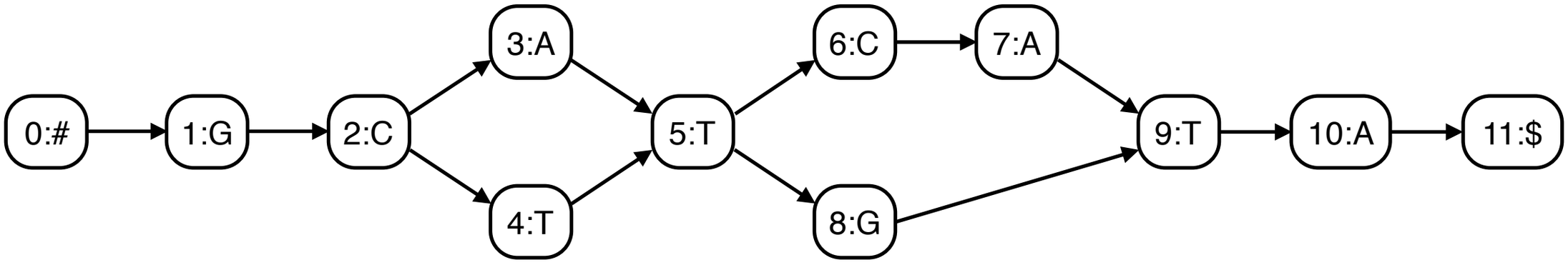
- k should be large enough to map perfectly matching short reads in one piece.

- In one human variation graph, the number of kmers is $1.031^k \cdot 2.348$ billion, or 116 billion for $k = 128$.

- The design of a path index is a trade-off between index size, query performance, maximum query length, and ignoring complex regions of the graph.

- The kmer index is a simple path index. It consists of a set of key-value pairs.
- A hash table supports fast kmer queries.
- Binary search in a sorted array is slower but supports queries shorter than k.
- Index size: terabytes.

Key	Value	Key	Value
$$$	11	GTA	8
A$$	10	TA$	9
ATA	7	TCA	5
ATC	3	TGT	5
ATG	3	TTC	4
CAT	2, 6	TTG	4
CTT	2	#GC	0
GCA	1	##G	0:1
GCT	1	###	0:2



- We can represent the kmer index as a de Bruijn graph.
- We label each node with the first character of the key.
- The de Bruijn graph approximates the variation graph. There are no false negatives, and no false positives shorter than k+1.

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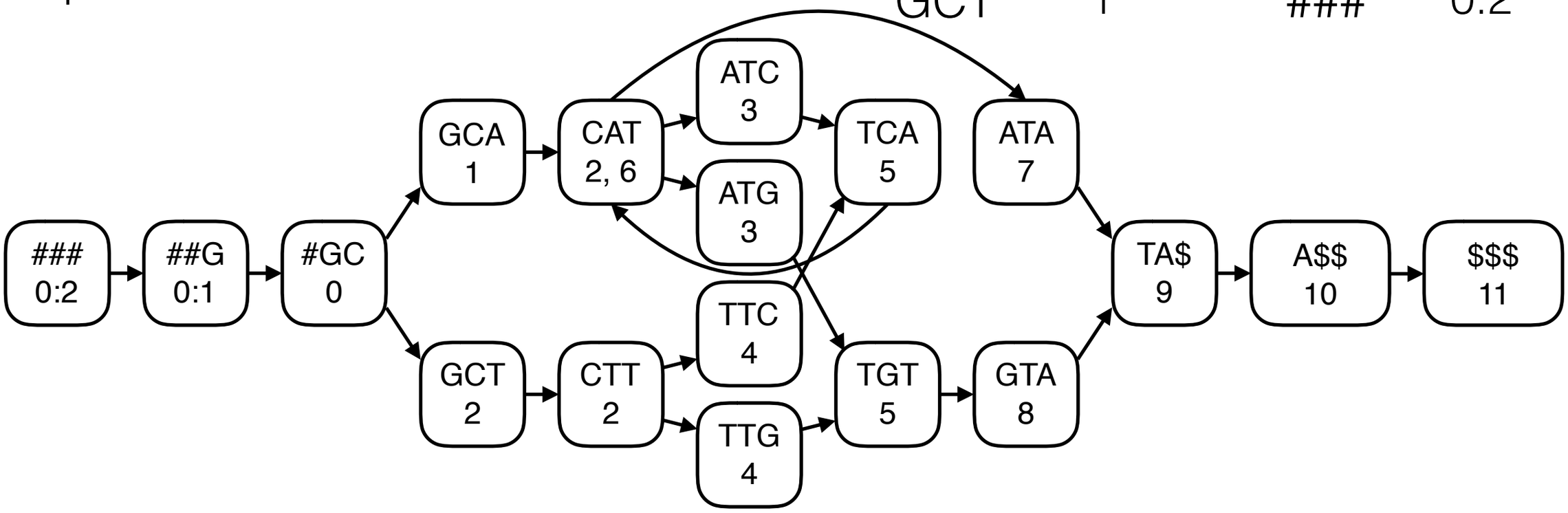
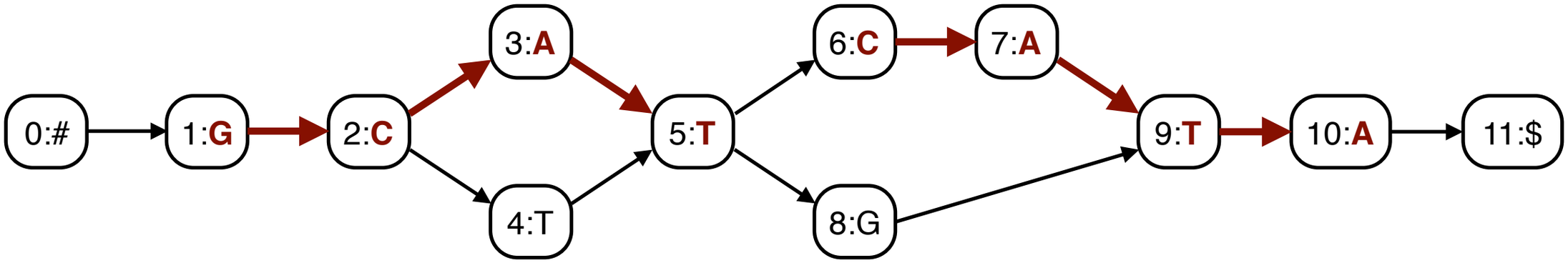
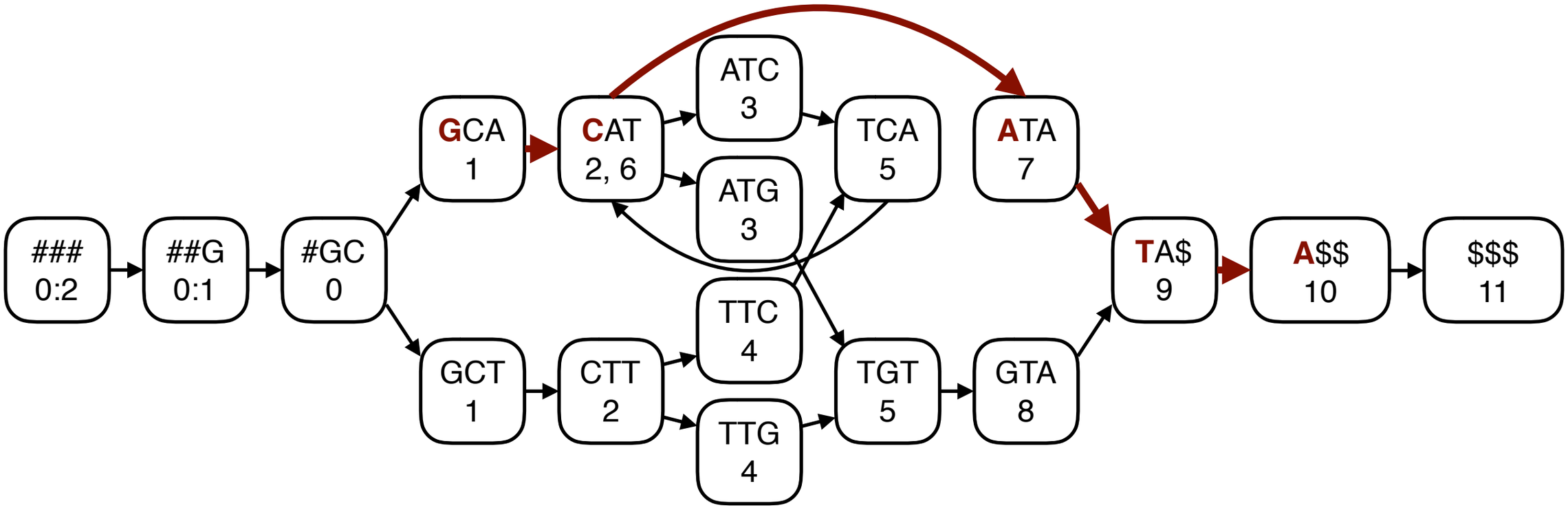




Paths longer than k+1 may be false positives, but we can verify them in the input graph.



Succinct de Bruijn graphs

Node	BWT	IN	OUT
$$$	A	1	1
A$$	T	1	1
ATA	C	1	1
ATC	C	1	1
ATG	C	1	1
CAT	GT	01	001
CTT	G	1	01
GCA	#	1	1
GCT	#	1	1
GTA	T	1	1
TA$	AG	01	1
TCA	AT	01	1
TGT	AT	01	1
TTC	C	1	1
TTG	C	1	1
#GC	#	1	01
##G	#	1	1
###	$	1	1

- Sort the nodes, write the predecessor labels to BWT, and encode the indegrees and the outdegrees in unary to bitvectors IN and OUT.
- The result is an FM-index for de Bruijn graphs.
- Bowe et al: Succinct de Bruijn graphs. WABI 2012.
- Index size: hundreds of gigabytes.

GCSA2

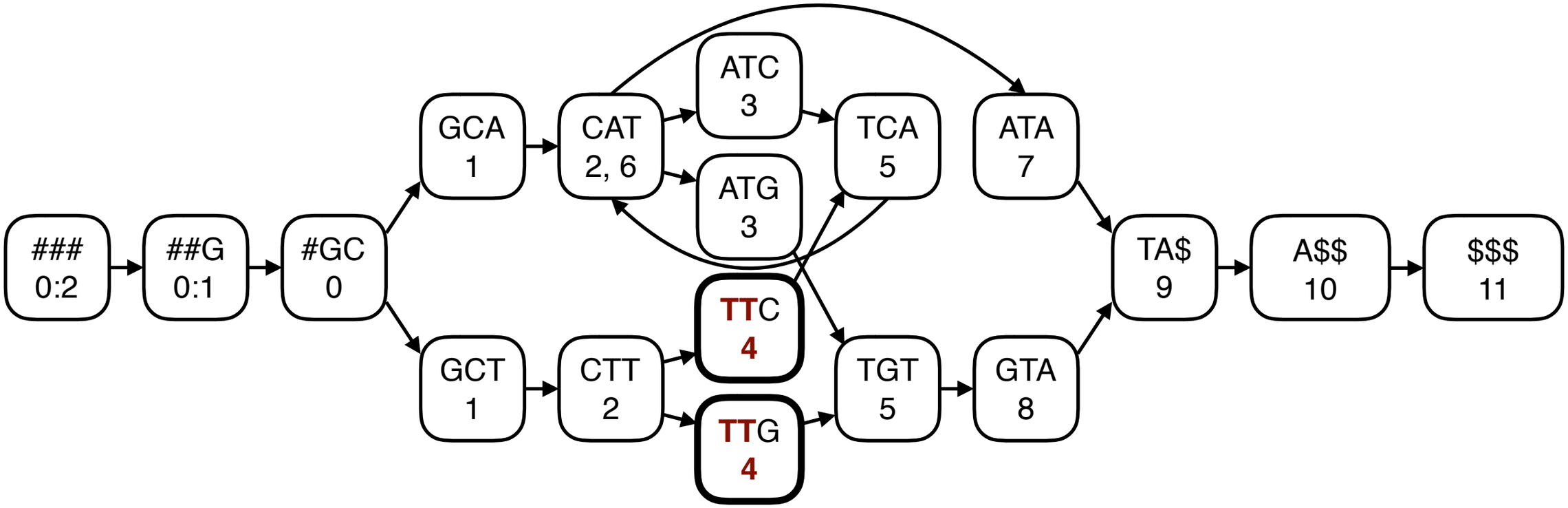
Path graphs

- High-order de Bruijn graphs of a graph have redundant subgraphs, if shorter keys already specify the position uniquely.

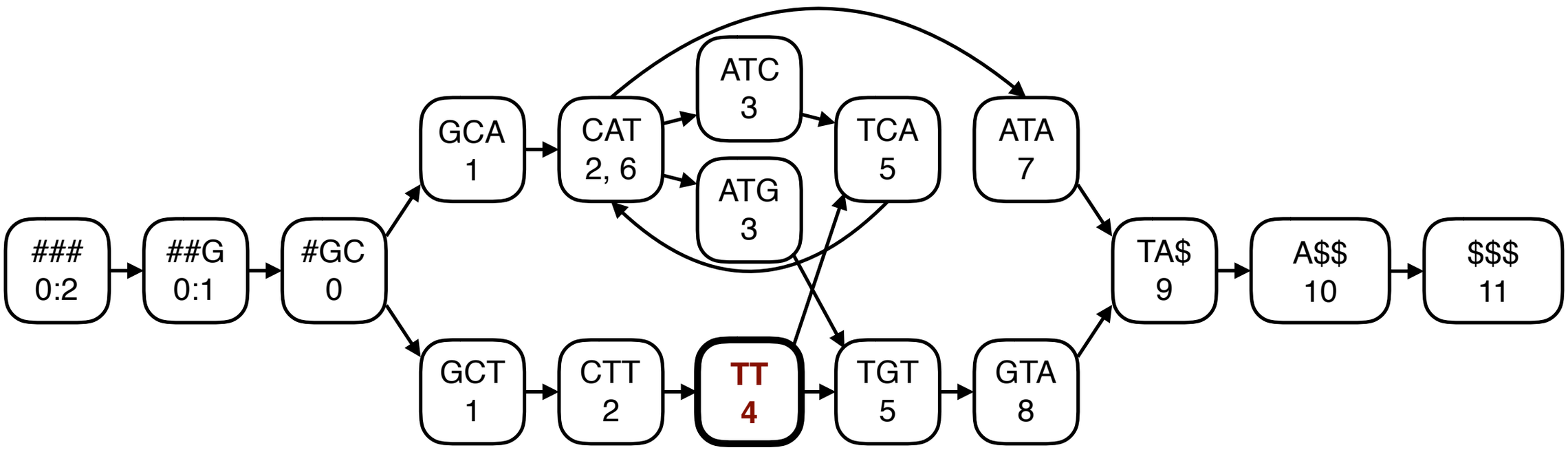
- We can compress the de Bruijn graph by merging such subgraphs.

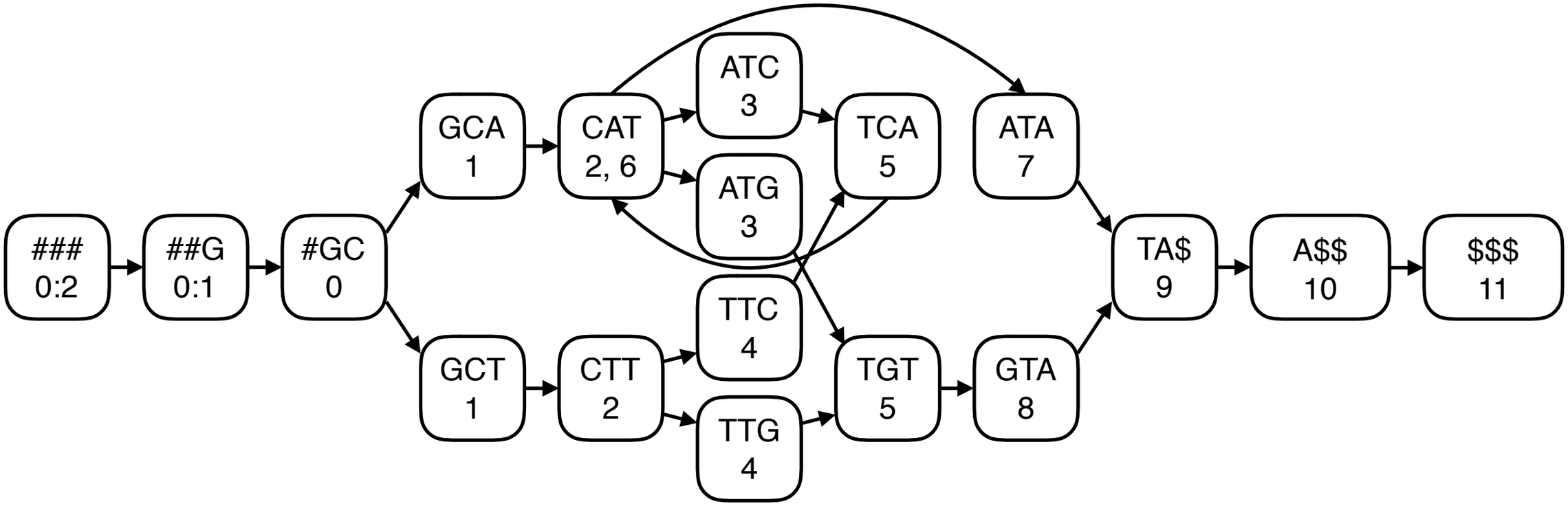
- Path graphs generalize de Bruijn graphs by using any prefix-free set of strings as keys.

- Inspired by: Sirén et al: Indexing Graphs for Path Queries with Applications in Genome Research. TCBB, 2014.

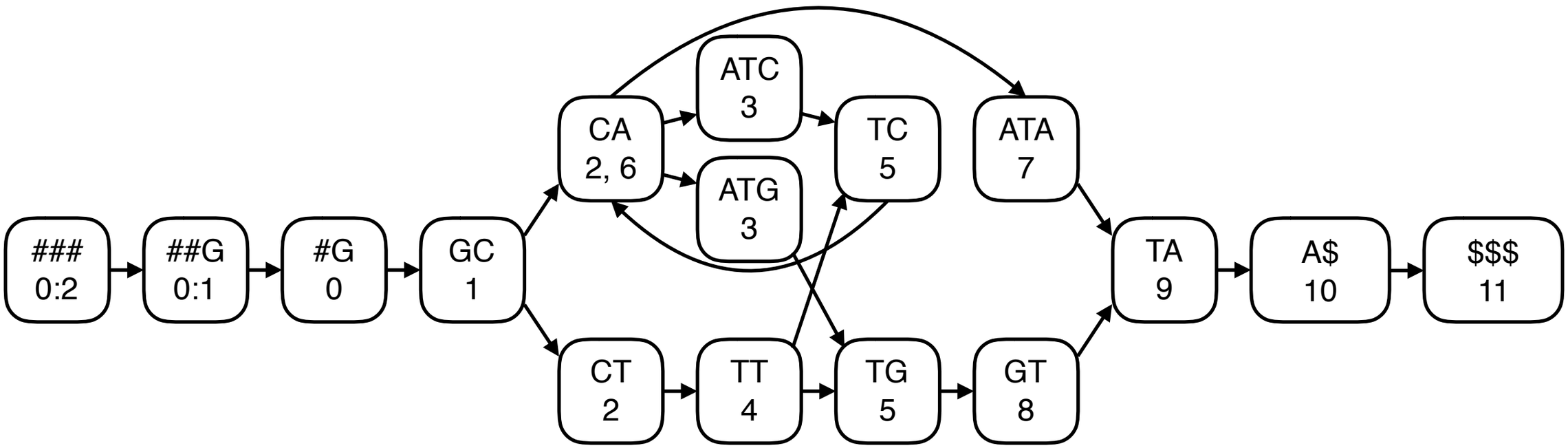


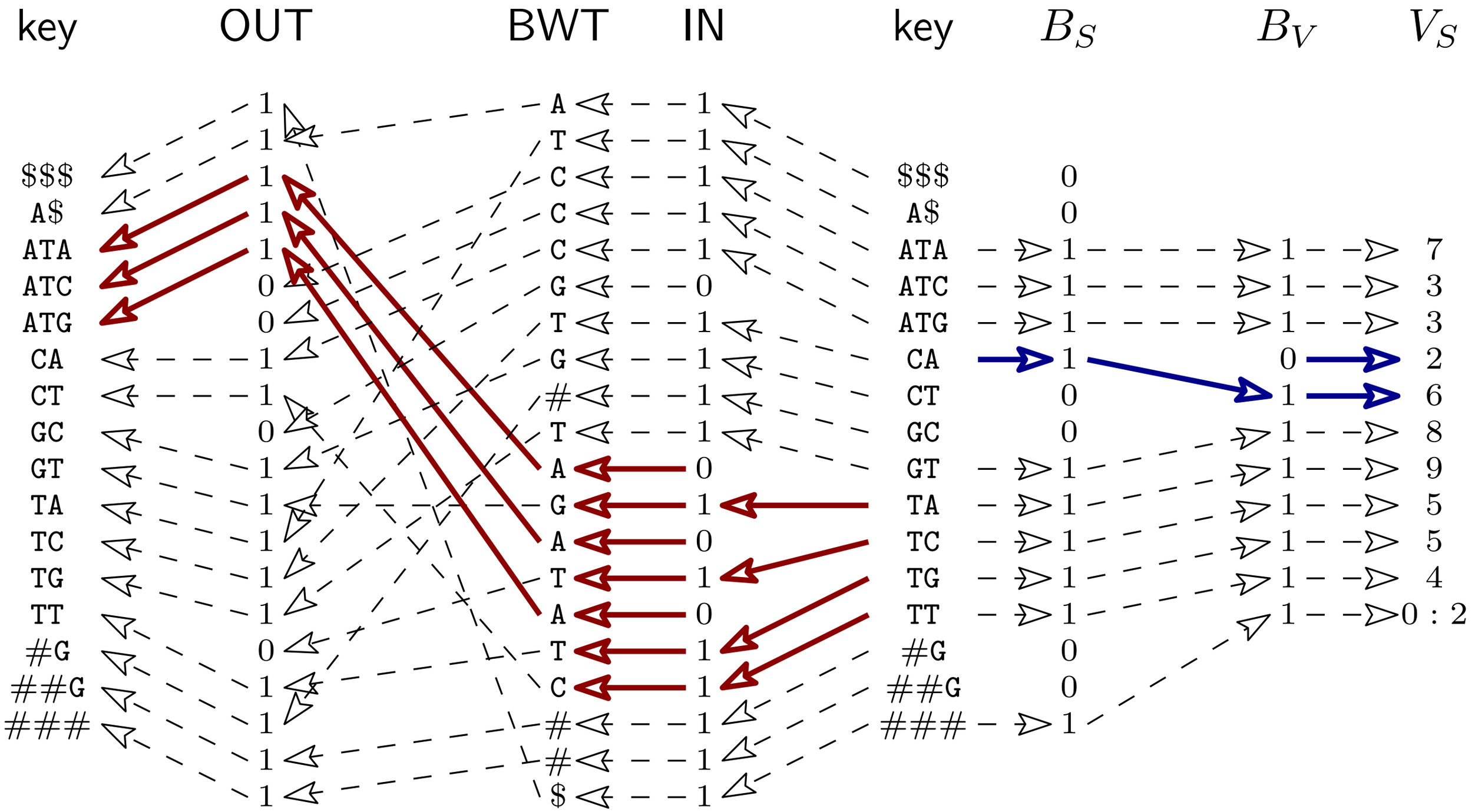
We can merge nodes sharing a prefix without affecting queries, if the value sets are identical.



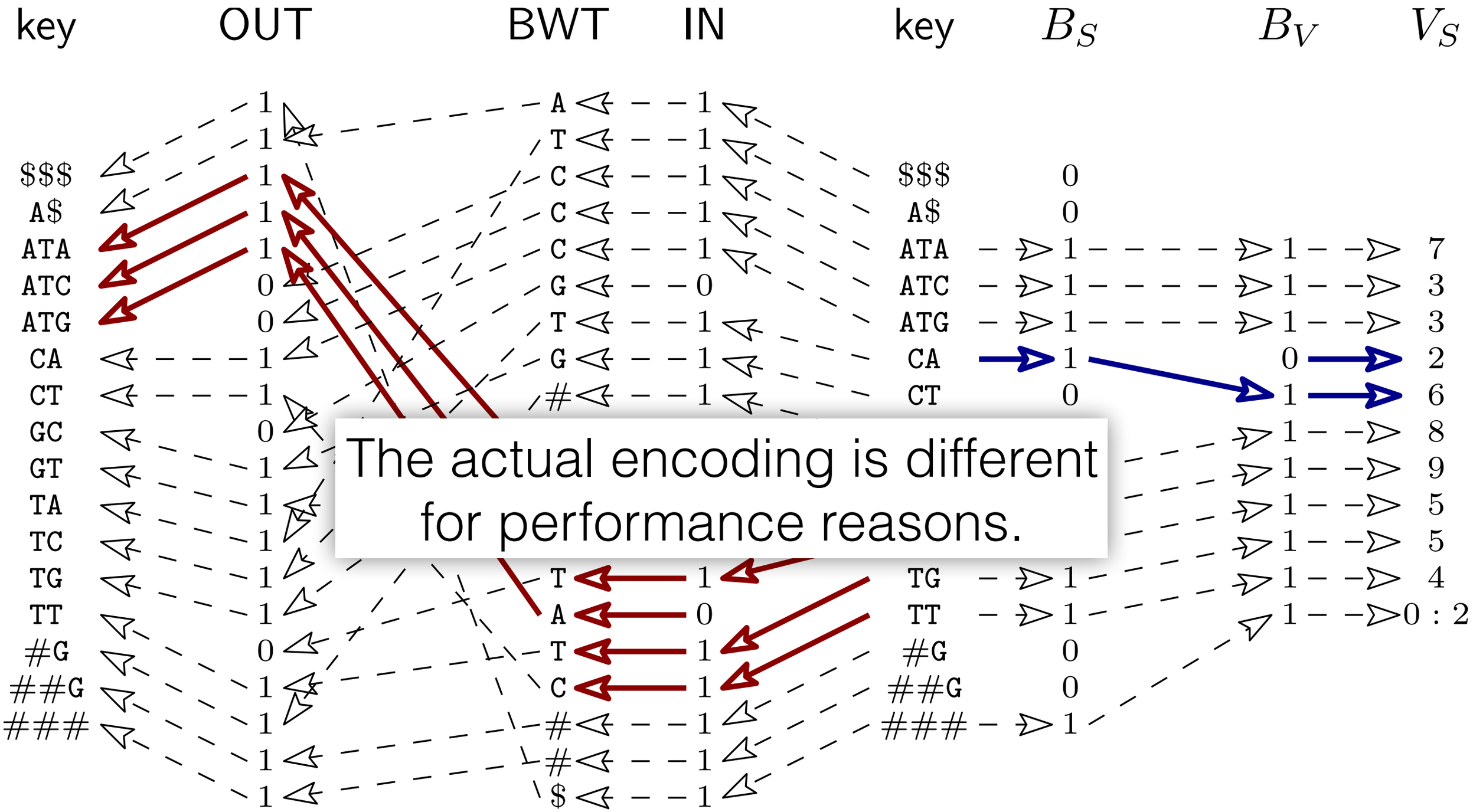


If we keep merging the nodes, we get a (maximally) pruned de Bruijn graph, which behaves intuitively.





We can encode the result in the same way as in the succinct de Bruijn graph / GCSA.



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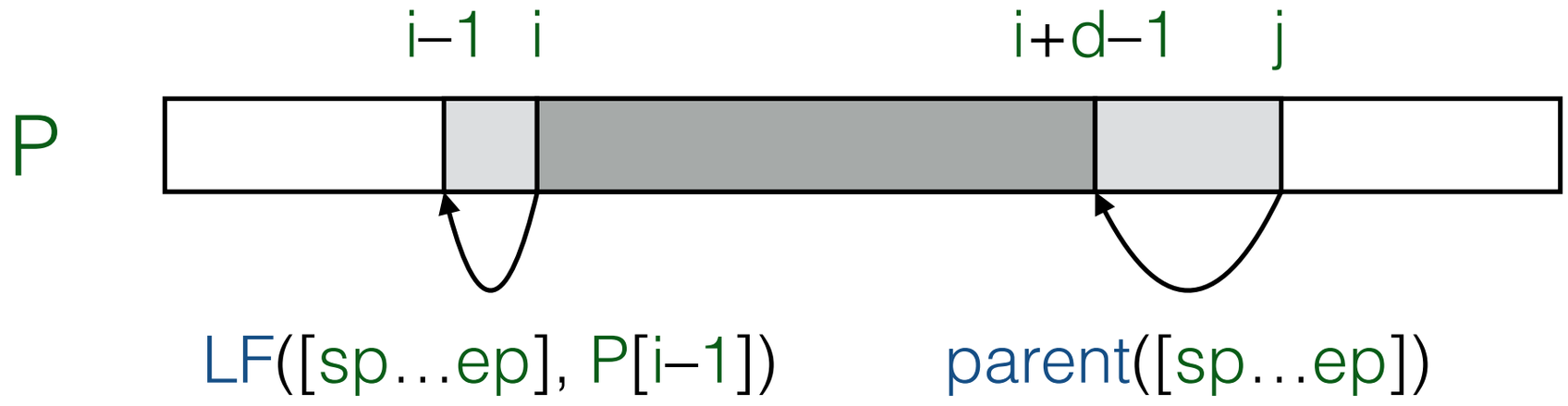

If lexicographic range [sp…ep] **matches** substring P[i…j] of the **pattern**, we can

- **extend** the match to the **left** with LF(); and
- **remove** characters from the **right** with parent().

This allows us to find **maximal exact matches**.

Ohlebusch et al: **Computing Matching Statistics and Maximal Exact Matches on Compressed Full-Text Indexes**. SPIRE 2010.

Path length	16→32	16→64	16→128
Kmers	6.20G	16.7G	**116G**
Nodes	4.37G	5.24G	**5.73G**
Index size	13.2 GB 18.2 bits / kmer	13.5 GB 6.99 bits / kmer	**14.6 GB** **1.08 bits / kmer**
Construction:			
Time	7.40 h	10.6 h	**14.0 h**
Memory	59.8 GB	51.9 GB	**52.3 GB**
Disk	387 GB	415 GB	**478 GB**
I/O:			
Read	1.37 TB	2.03 TB	**2.78 TB**
Write	0.88 TB	1.51 TB	**2.25 TB**

1000GP human variation (forward strand only)

`vg mod -p -l 16 -e 4 | vg mod -S -l 100`

32 cores, 256 GB memory, distributed Lustre file system

Index	k	kmers	Matched	find()	locate()
GCSA	16	351584	347453	4.77 µs	6.75 µs
GCSA	32	351555	333258	10.9 µs	5.66 µs
GCSA	64	351567	326101	22.6 µs	2.93 µs
GCSA	128	351596	316500	45.3 µs	3.13 µs
BWA	16	351584	320764	4.72 µs	4.84 µs
BWA	32	351555	156080	6.63 µs	3.20 µs
BWA	64	351567	88786	10.2 µs	2.34 µs
BWA	128	351596	35741	13.9 µs	3.46 µs

GCSA2 for the graph vs. the FM-index in BWA for the reference.
Query kmers extracted from the non-pruned variation graph.
Time per find query / distinct occurrence.

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BWA	**16**	351584	**320764**	**4.72 *µs***	4.84 µs
BWA	**32**	351555	156080	6.63 µs	3.20 µs
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find() stops early if it cannot match the pattern.
When the number of matched patterns is similar,
GCSA2 is as fast as BWA.

GCSA2 for the graph vs. the FM-index in BWA for the reference.
Query kmers extracted from the non-pruned variation graph.
Time per find query / distinct occurrence.

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locate() benchmarks may produce biased results when the queries are not evenly distributed. On the average, GCSA2 is a bit slower than BWA.

GCSA2 for the graph vs. the FM-index in BWA for the reference.
Query kmers extracted from the non-pruned variation graph.
Time per find query / distinct occurrence.

Index	k	kmers	Matched	find()	locate()
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BWA	**128**	351596	35741	13.9 µs	3.46 µs

The graph takes 5.7 bits/node and supports exact membership queries in k / 3 microseconds and neighbor queries in 1 / 3 microseconds. It could be useful as a de Bruijn graph representation.

GCSA2 for the graph vs. the FM-index in BWA for the reference.
Query kmers extracted from the non-pruned variation graph.
Time per find query / distinct occurrence.

Pruning the Variation Graph

Complex regions

- A whole-genome human variation graph based on 1000GP variation contains trillions (quadrillions?) of distinct 128-mers.

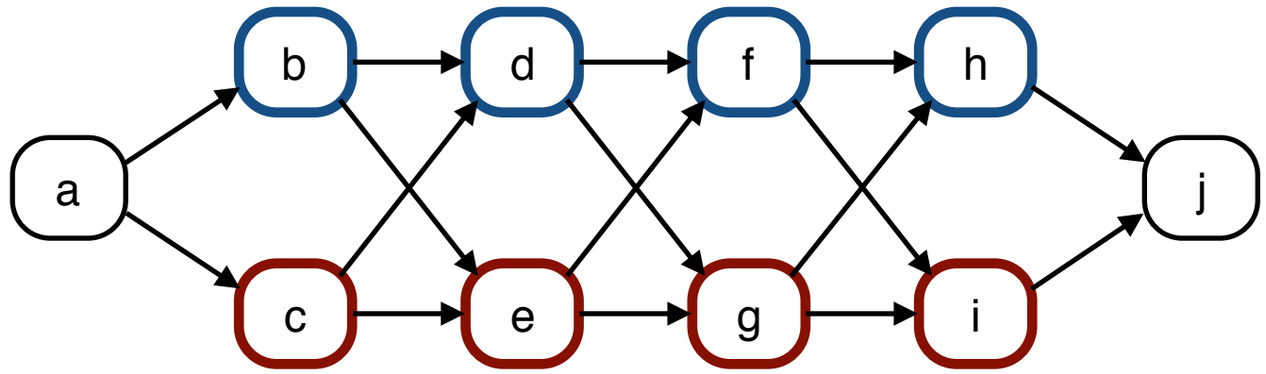
- Almost all of them are from a few complex regions.

- We cannot index all potential recombinations in such regions. Even if we could, the resulting index would probably be too biased.

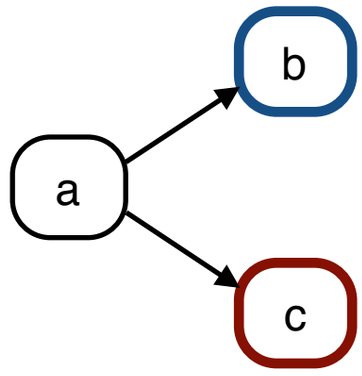
- vg and GCSA2 have several ways for dealing with the complex regions.

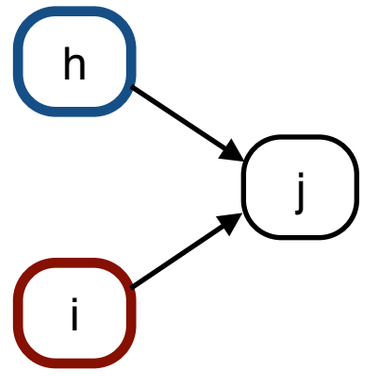
Pruning

`vg mod -p -l 16 -e 4`
Remove paths of length 16 crossing more than 4 nontrivial edges.



`vg mod -S -l 100`
Remove subgraphs shorter than 100 bases.



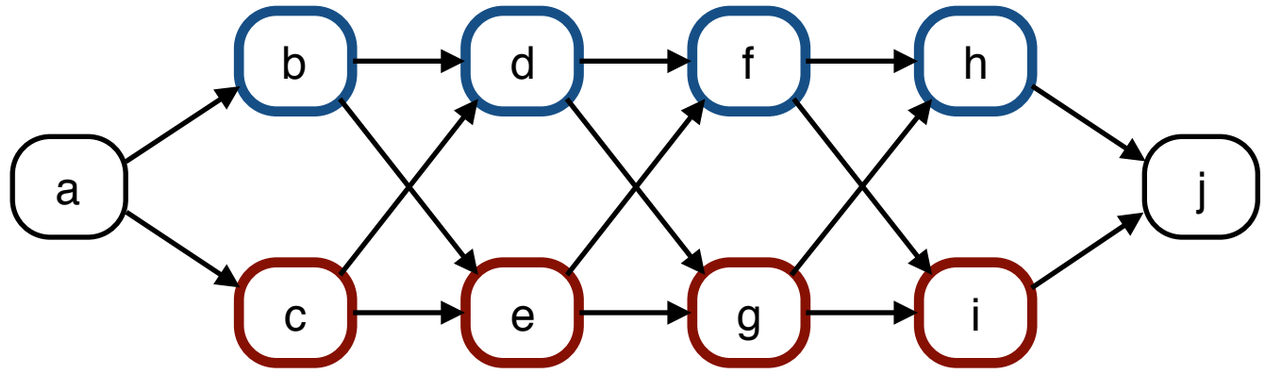


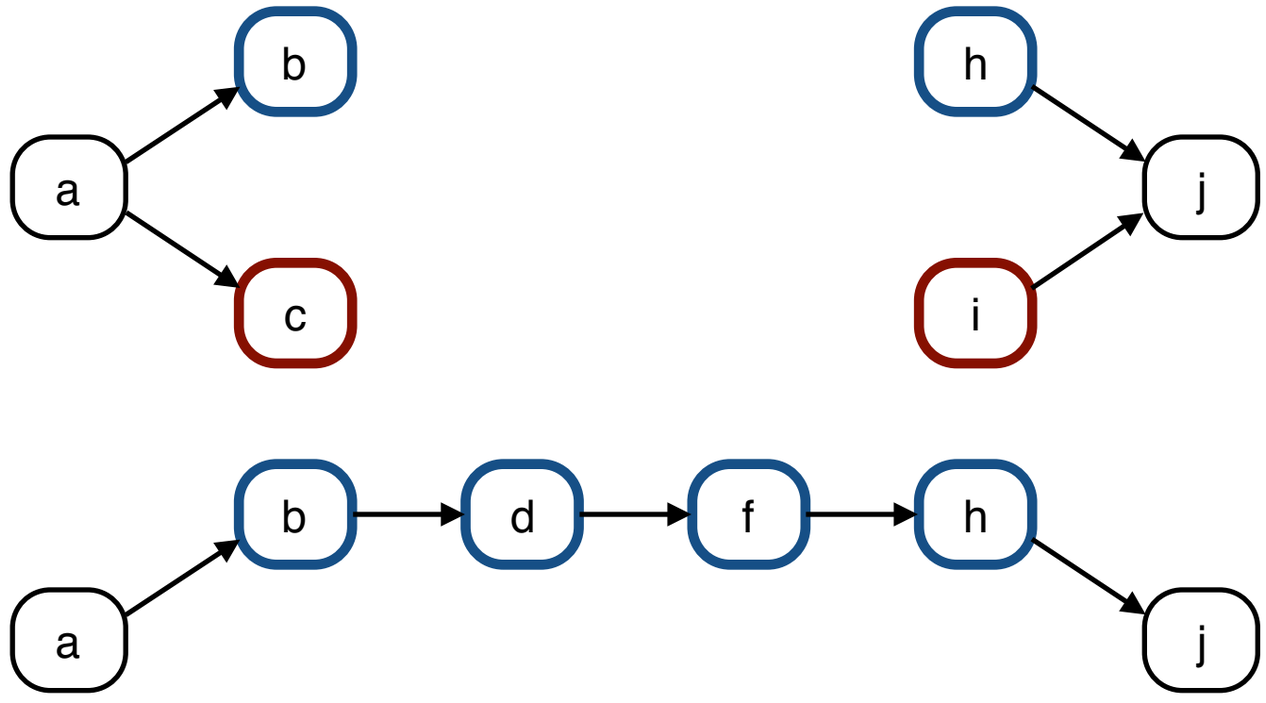
- Easy and efficient.
- Complex regions may be removed completely.

Indexing subgraphs

We can index overlapping subgraphs (e.g. a pruned variation graph and the reference path) and merge the results into a single index.

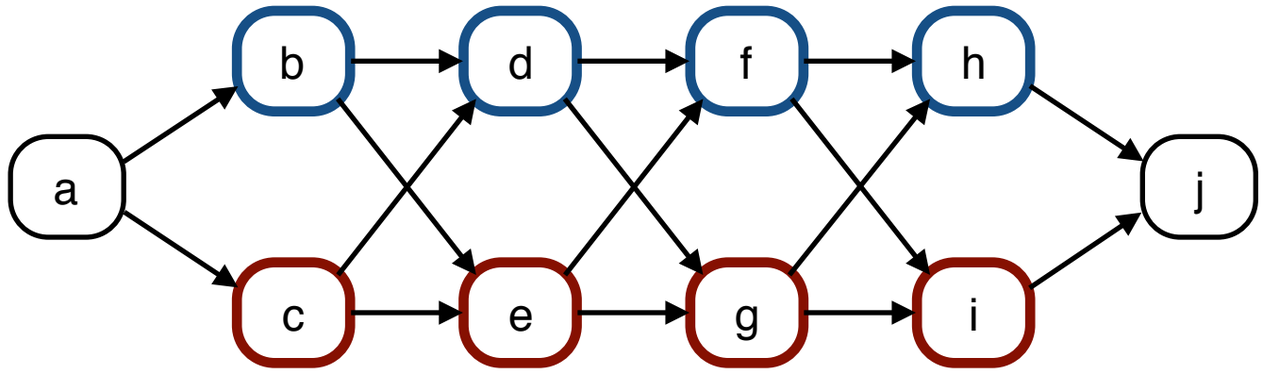
- Guarantees that the entire genome is indexed.
- Redundant paths can make index construction more expensive.





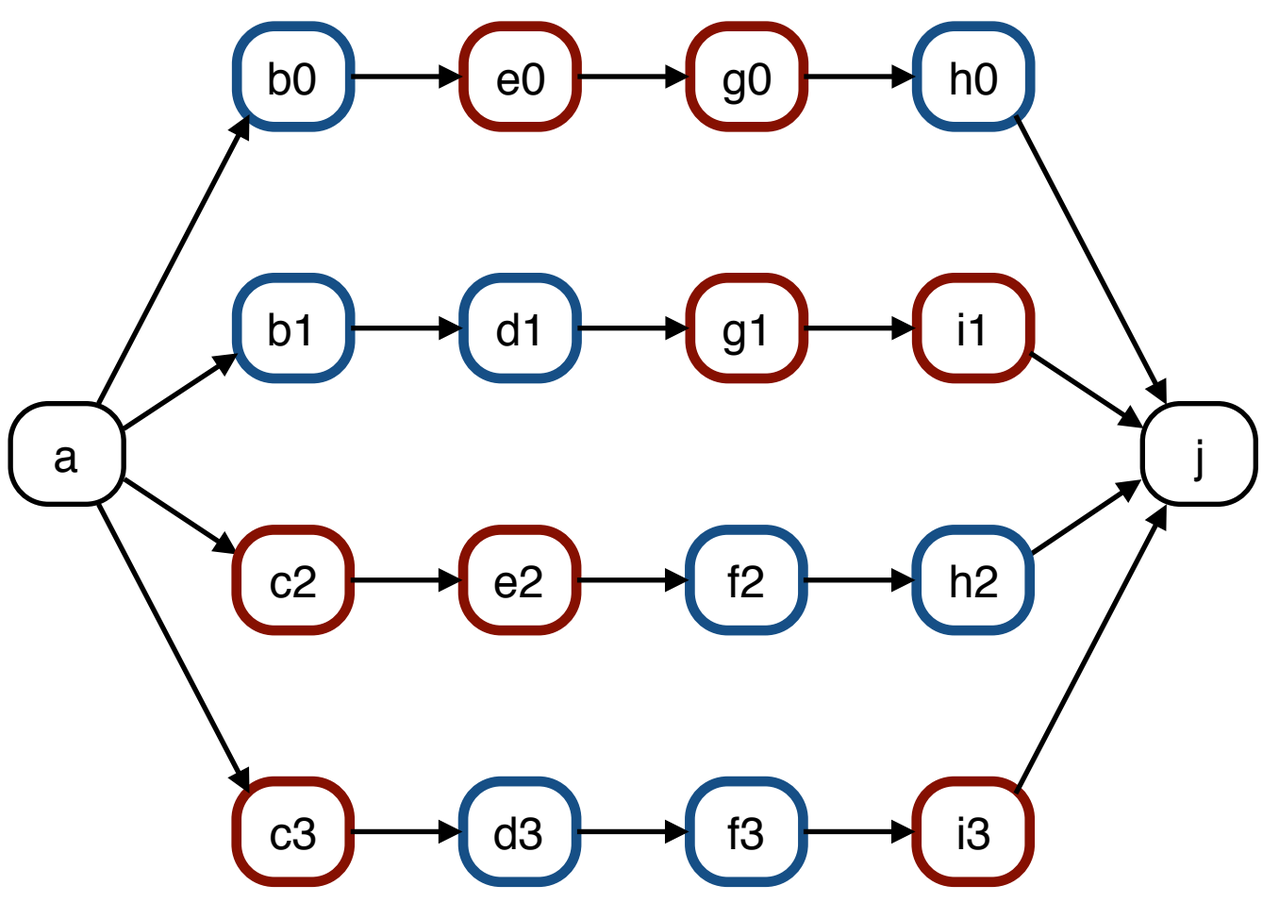
Indexing haplotypes

Index only paths corresponding to known haplotypes in complex regions.



Multiple nodes of the input graph map to the same node in the variation graph.

- Guarantees that the entire genome and all observed variation is indexed.
- Not implemented yet in vg.



Conclusions

- The design of a path index is a trade-off between index size, query performance, maximum query length, and ignoring complex regions of the graph.

- GCSA2 prioritizes performance and size, while supporting queries long enough to map short reads in one piece.

- It uses a de Bruijn graph as a kmer index, compresses it by merging redundant subgraphs, and encodes the result as a compressed suffix tree.

- Sirén: Indexing Variation Graphs. arXiv:1604.06605, 2016. https://github.com/jltsiren/gcsa2