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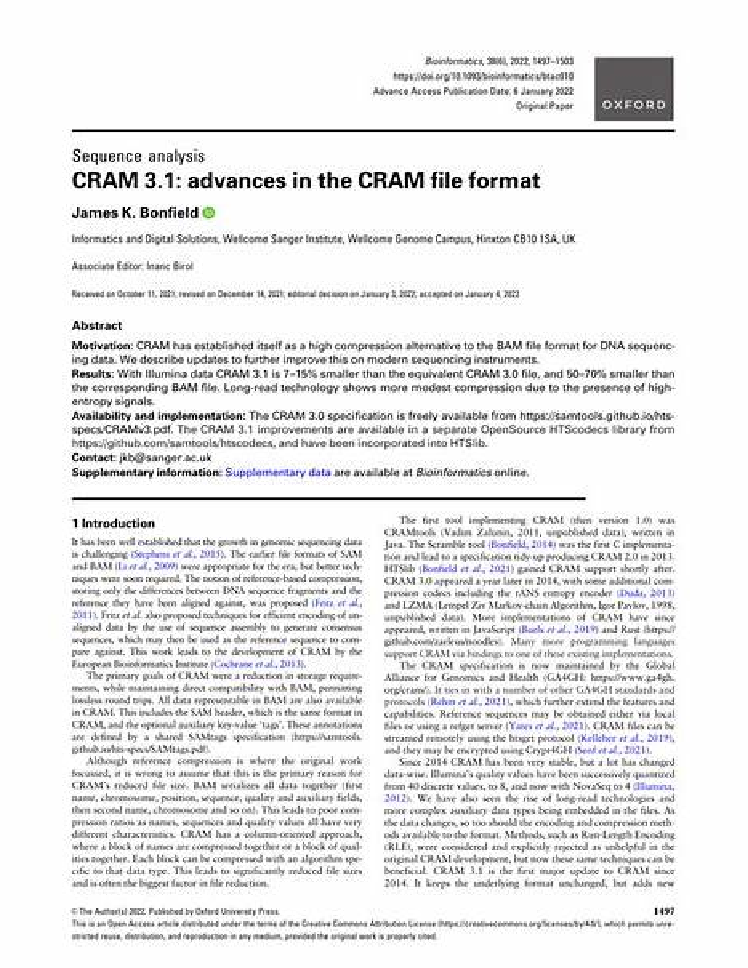

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Sequence analysis

CRAM 3.1: advances in the CRAM file format

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Abstract

Motivation: CRAM has established itself as a high compression alternative to the BAM file format for DNA sequencing data. We describe updates to further improve this on modern sequencing instruments.
Results: With Illumina data CRAM 3.1 is 7–15% smaller than the equivalent CRAM 3.0 file, and 50–70% smaller than the corresponding BAM file. Long-read technology shows more modest compression due to the presence of high-entropy signals.
Availability and implementation: The CRAM 3.0 specification is freely available from https://samtools.github.io/hts-specs/CRAMv3.pdf. The CRAM 3.1 improvements are available in a separate OpenSource HTScodecs library from https://github.com/samtools/htscodecs, and have been incorporated into HTSlib.
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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

It has been well established that the growth in genomic sequencing data is challenging (Stephens *et al.*, 2015). The earlier file formats of SAM and BAM (Li *et al.*, 2009) were appropriate for the era, but better techniques were soon required. The notion of reference-based compression, storing only the differences between DNA sequence fragments and the reference they have been aligned against, was proposed (Fritz *et al.*, 2011). Fritz *et al.* also proposed techniques for efficient encoding of unaligned data by the use of sequence assembly to generate consensus sequences, which may then be used as the reference sequence to compare against. This work leads to the development of CRAM by the European Bioinformatics Institute (Cochrane *et al.*, 2013).

The primary goals of CRAM were a reduction in storage requirements, while maintaining direct compatibility with BAM, permitting lossless round trips. All data representable in BAM are also available in CRAM. This includes the SAM header, which is the same format in CRAM, and the optional auxiliary key-value 'tags'. These annotations are defined by a shared SAMtags specification (https://samtools.github.io/hts-specs/SAMtags.pdf).

Although reference compression is where the original work focused, it is wrong to assume that this is the primary reason for CRAM's reduced file size. BAM serializes all data together (first name, chromosome, position, sequence, quality and auxiliary fields, then second name, chromosome and so on). This leads to poor compression ratios as names, sequences and quality values all have very different characteristics. CRAM has a column-oriented approach, where a block of names are compressed together or a block of qualities together. Each block can be compressed with an algorithm specific to that data type. This leads to significantly reduced file sizes and is often the biggest factor in file reduction.

The first tool implementing CRAM (then version 1.0) was CRAMtools (Vadim Zalunin, 2011, unpublished data), written in Java. The Scramble tool (Bonfield, 2014) was the first C implementation and lead to a specification tidy up producing CRAM 2.0 in 2013. HTSlib (Bonfield *et al.*, 2021) gained CRAM support shortly after. CRAM 3.0 appeared a year later in 2014, with some additional compression codecs including the rANS entropy encoder (Duda, 2013) and LZMA (Lempel Ziv Markov-chain Algorithm, Igor Pavlov, 1998, unpublished data). More implementations of CRAM have since appeared, written in JavaScript (Buels *et al.*, 2019) and Rust (https://github.com/zaeleus/noodles). Many more programming languages support CRAM via bindings to one of these existing implementations.

The CRAM specification is now maintained by the Global Alliance for Genomics and Health (GA4GH: https://www.ga4gh.org/cram/). It ties in with a number of other GA4GH standards and protocols (Rehm *et al.*, 2021), which further extend the features and capabilities. Reference sequences may be obtained either via local files or using a refget server (Yates *et al.*, 2021). CRAM files can be streamed remotely using the htsget protocol (Kelleher *et al.*, 2019), and they may be encrypted using Crypt4GH (Senf *et al.*, 2021).

Since 2014 CRAM has been very stable, but a lot has changed data-wise. Illumina's quality values have been successively quantized from 40 discrete values, to 8, and now with NovaSeq to 4 (Illumina, 2012). We have also seen the rise of long-read technologies and more complex auxiliary data types being embedded in the files. As the data changes, so too should the encoding and compression methods available to the format. Methods, such as Run-Length Encoding (RLE), were considered and explicitly rejected as unhelpful in the original CRAM development, but now these same techniques can be beneficial. CRAM 3.1 is the first major update to CRAM since 2014. It keeps the underlying format unchanged, but adds new

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F5 BIG-IP Administration Data Plane Configuration Sample Questions (Q12-Q17):

NEW QUESTION # 12
A node is a member of multiple pools and hosts different applications. If one application becomes unavailable, only that pool member should be marked down.
What should the BIG-IP Administrator deploy?

- A. HTTP monitor with custom send/receive
- B. TCP monitor
- C. ICMP + TCP monitor
- D. UDP monitor

Answer: A

Explanation:
Application-level monitors ensure that only the affected service is marked down, not the entire node.

NEW QUESTION # 13
The BIG-IP Administrator needs to load balance a pool of web servers. Load balancing should consider the number of connections that are active on that pool member.
Which load balancing method meets this requirement? (Choose one answer)

- A. Round Robin
- B. Ratio (node)
- C. Ratio (member)
- D. Least Connections (member)

Answer: D

Explanation:
The requirement states that load balancing decisions must be based on the number of active connections on each pool member. This directly maps to the Least Connections (member) load balancing method.
According to the BIG-IP Administration: Data Plane Configuration documentation:
Least Connections (member) selects the pool member with the fewest active connections at the time of the request.
This method dynamically adapts to real-time traffic patterns and ensures that more heavily loaded pool members receive fewer new connections.
It is especially effective for web servers where connection duration may vary and equal distribution of active sessions is desired.
Why the other options are incorrect:
B . Round Robin
Distributes connections sequentially without considering current load or active connections.
C . Ratio (member)
Distributes traffic based on static ratios, not real-time connection counts.
D . Ratio (node)
Uses predefined ratios at the node level and does not account for active connection counts.
Correct Resolution:
Using Least Connections (member) ensures that new connections are directed to the pool member currently handling the fewest active connections, meeting the stated requirement.

NEW QUESTION # 14
During a high-demand event, the BIG-IP Administrator needs to limit the number of new connections per second to a Virtual Server.
What should be applied?

- A. Connection Rate Limit
- B. Connection Limit
- C. OneConnect profile
- D. HTTP Compression profile

Answer: A

Explanation:
Connection rate limits restrict how many new connections are accepted per second, protecting application resources.

NEW QUESTION # 15
All pool members are online. All other virtual server settings are at default.
What might alter the load balancing behavior? (Choose one answer)

- A. Adding a persistence profile
- B. Adding a OneConnect profile
- C. Enabling a fallback host in the HTTP profile
- D. Enabling SNAT automap

Answer: A

Explanation:
By default, BIG-IP load balancing algorithms (such as Round Robin) distribute connections evenly across all available pool members. However, persistence profiles override normal load balancing decisions by forcing subsequent connections from a client to be sent to the same pool member.
According to the BIG-IP Administration: Data Plane Configuration documentation:
Persistence creates a client-to-server mapping that is honored before load balancing algorithms are applied.
When persistence is enabled, BIG-IP may repeatedly select the same pool member even if others are available.
This directly alters load balancing behavior.
Why the other options are incorrect:
A . Adding a OneConnect profile
OneConnect optimizes server-side TCP connections but does not change which pool member is selected.
B . Enabling SNAT automap
SNAT affects source address translation, not pool member selection.
C . Enabling a fallback host in the HTTP profile
A fallback host is only used when no pool members are available.
Correct Resolution:
Adding a persistence profile alters load balancing behavior by maintaining client affinity to a specific pool member.

NEW QUESTION # 16
A BIG-IP Administrator configures an SSH pool with five members.
Which health monitor should be applied?

- A. HTTP
- B. TCP
- C. HTTPS
- D. UDP

Answer: B

Explanation:
SSH is a TCP-based service. A TCP monitor validates service availability without requiring application-layer inspection.

NEW QUESTION # 17
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