Sequence analysis

KAT: a K-mer analysis toolkit to quality control NGS datasets and genome assemblies

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Abstract

Motivation: De novo assembly of whole genome shotgun (WGS) next-generation sequencing (NGS) data benefits from high-quality input with high coverage. However, in practice, determining the quality and quantity of useful reads quickly and in a reference-free manner is not trivial. Gaining a better understanding of the WGS data, and how that data is utilized by assemblers, provides useful insights that can inform the assembly process and result in better assemblies.

Results: We present the K-mer Analysis Toolkit (KAT): a multi-purpose software toolkit for reference-free quality control (QC) of WGS reads and de novo genome assemblies, primarily via their k-mer frequencies and GC composition. KAT enables users to assess levels of errors, bias and contamination at various stages of the assembly process. In this paper we highlight KAT’s ability to provide valuable insights into assembly composition and quality of genome assemblies through pairwise comparison of k-mers present in both input reads and the assemblies.

Availability and Implementation: KAT is available under the GPLv3 license at: https://github.com/TGAC/KAT.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

Rapid analysis of high-throughput whole genome shotgun (WGS) datasets is challenging due to their large size (Metzker, 2010), with genome size and complexity creating additional challenges (Schatz et al., 2012). Reference-free approaches for analyzing WGS data typically involve examining base calling quality, read length, GC content (Yang et al., 2013) and exploring k-mer (words of size $k$) spectra (Chor et al., 2009; Lo and Chain, 2014). A frequently used reference-free quality control tool is FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/).

K-mer spectra reveal information not only about the data quality (level of errors, sequencing biases, completeness of sequencing coverage and potential contamination) but also of genomic complexity (size, karyotype, levels of heterozygosity and repeat content; Simpson, 2014). Additional information can be extracted through pairwise comparisons of WGS datasets (Anvar et al., 2014), which can identify problematic samples by highlighting differences between spectra.

KAT, the K-mer Analysis Toolkit, is a suite of tools for rapidly counting, comparing and analysing spectra for k-mers of arbitrary length directly from sequence data (see Supplementary section 2 for a discussion on choice of $k$ and Supplementary section 3 for a comparison of k-mer tools).

2 The K-mer analysis toolkit

KAT is a C++11 application containing multiple tools, each of which exploits multi-core machines via multi-threading where possible. Core functionality is contained in a library designed to promote rapid development of new tools. Runtime and memory requirements depend on input data size, error and bias levels, and...
properties of the biological sample but as a rule of thumb, machines capable of de novo assembly of a dataset will be sufficient to run KAT on the dataset (see Supplementary section 4 for details). K-mer counting in KAT is performed by an integrated and modified version of Jellyfish2 (Marc¸ais and Kingsford, 2011), which supports large \(k\) values and is among the fastest k-mer counters available (Zhang et al., 2014).

### 2.1 Assembly validation by comparison of read spectrum and assembly copy number

The KAT comp tool generates a matrix, with a sequence set’s k-mer frequency on one axis, and another set’s frequency on the other, with cells holding distinct k-mers counts at the given frequencies. When comparing reads against an assembly, KAT highlights properties of assembly composition and quality. If represented in a stacked histogram, read k-mer spectrum is split by copy number in the assembly (see Supplementary section 5 for a primer on how to interpret KAT’s stacked histograms). In addition, KAT provides the sect tool necessary to study specific assembled sequences and track the k-mer coverage across both the read and the assembly spectra. This can help identify assembly artefacts such as collapsing or expanding events, or detect repeat regions. Figure 1 shows plots relating to two Fraxinus excelsior assemblies created from the same dataset using the comp and sect tools. The plots highlight different strategies taken by the assembler, in (a) and (c) we see some homozygous content being duplicated, and in (b) and (d) some heterozygous content eliminated.

### 2.2 Other KAT tools

KAT also includes the bist tool for computing spectrum from a single sequence set and the gcp tool to analyse gc content against k-mer frequency. The filter tool can be used to isolate sequences from a set according to their k-mer coverage or gc content from a given spectrum (see Supplementary section 1 for details on all the tools). These tools can be used for various tasks including contaminant detection and extraction both in raw reads and assemblies, analysis of the GC bias and consistency between paired end reads and other types of libraries.

### 3 Summary

KAT is a user-friendly, scalable toolkit for rapidly counting, comparing and analyzing k-mers from various data sources. The tools in KAT assist the user with a wide range of tasks including error profiling, assessing sequencing bias and identifying contaminants and de novo genome assembly QC and validation.

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### References

Anvar,S.Y. et al. (2014) Determining the quality and complexity of next-generation sequencing data without a reference genome. *Genome Biol.*, 15, 555.
Chor, R. et al. (2009) Genomic DNA k-mer spectra: models and modalities. *Genome Biol.*, 10, R108.

Lo, C.C. and Chain, P.S.G. (2014) Rapid evaluation and quality control of next generation sequencing data with faqcs. *BMC Bioinformatics*, 15, 366.

Marçais, G. and Kingsford, C. (2011) A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. *Bioinformatics*, 27, 764–770.

Metzker, M.L. (2010) Sequencing technologies - the next generation. *Nat. Rev. Genet.*, 11, 31–46.

Schatz, M.C. et al. (2012) Current challenges in de novo plant genome sequencing and assembly. *Genome Biol.*, 13, 243.

Simpson, J.T. (2014) Exploring genome characteristics and sequence quality without a reference. *Bioinformatics*, 30, 1228–1235.

Yang, X. et al. (2013) HTQC: a fast quality control toolkit for illumina sequencing data. *BMC Bioinformatics*, 14, 33.

Zhang, Q. et al. (2014) These are not the k-mers you are looking for: efficient on-line k-mer counting using a probabilistic data structure. *PLoS One*, 9, e101271.