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**CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes**

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## Abstract

25 Large-scale recovery of genomes from isolates, single cells, and metagenomic data has been made possible by advances in computational methods and substantial reductions in sequencing costs. While this increasing breadth of draft genomes is providing key information regarding the evolutionary and functional diversity of microbial life, it has become impractical to finish all available reference genomes. Making robust biological inferences from draft genomes requires accurate estimates of their completeness and contamination. Current methods for assessing genome quality are *ad hoc* and generally make use of a limited number of ‘marker’ genes conserved across all bacterial or archaeal genomes. Here we introduce CheckM, an automated method for assessing the quality of a genome using a broader set of marker genes specific to the position of a genome within a reference genome tree and information about the collocation of these genes. We demonstrate the effectiveness of CheckM using synthetic data and a wide range of isolate, single cell and metagenome derived genomes. CheckM is shown to provide accurate estimates of genome completeness and contamination, and to outperform existing approaches. Using CheckM, we identify a diverse range of errors currently impacting publicly available isolate genomes and demonstrate that genomes obtained from single cells and metagenomic data vary substantially in quality. In order to facilitate the use of draft genomes, we propose an objective measure of genome quality that can be used to select genomes suitable for specific gene- and genome-centric analyses of microbial communities.

## Introduction

Recent advances in high-throughput sequencing combined with improving computational methods are enabling the rapid, cost effective recovery of genomes from cultivated and uncultivated microorganisms across a wide range of host-associated and environmental samples. Large-scale initiatives such as the Genomic Encyclopedia of Bacteria and Archaea (GEBA; Wu et al. 2009) aim to provide reference genomes from isolated species across the Tree of Life, while targeted efforts such as the Human Microbiome Project (HMP; Turnbaugh et al. 2007) and the GEBA-Root Nodulating Bacteria (GEBA-RNB; <http://jgi.doe.gov/>) initiatives are providing reference genomes necessary for understanding the role of microorganisms in specific habitats. These efforts are complemented by

50 initiatives such as the GEBA-Microbial Dark Matter (GEBA-MDM) project which used single-cell  
genomics to obtain genomes from uncultivated bacterial and archaeal lineages (Rinke et al. 2013).  
Several studies have also demonstrated the successful recovery of high-quality population genomes  
directly from metagenomic data (Tyson et al. 2004; Wrighton et al. 2012; Albertsen et al. 2013;  
Sharon et al. 2013). Together these initiatives have produced thousands of draft genomes, and stand to  
55 provide tens of thousands more as sequencing technology and computational methodologies continue  
to improve. While this rapid recovery of genomes stands to greatly improve our understanding of the  
microbial world, it is outpacing our ability to manually assess the quality of individual genomes.

In order to make robust inferences from the increasing availability of draft genomes, it is critical to  
distinguish between genomes of varying quality (Mardis et al. 2002; Chain et al. 2009). In particular,  
60 genomes recovered from single cells or metagenomic data require careful scrutiny due to the  
additional complications inherent in obtaining genomes using these approaches (Dick et al. 2010;  
Albertsen et al. 2013). The quality of isolate genomes has traditionally been evaluated using assembly  
statistics such as N50 (Salzberg et al. 2012; Gurevich et al. 2013), while single cell and metagenomic  
studies have relied on the presence and absence of universal single-copy ‘marker’ genes for  
65 estimating genome completeness (Wrighton et al. 2012; Haroon et al. 2013; Rinke et al. 2013; Sharon  
et al. 2013). However, the accuracy of this completeness estimate has not been evaluated and the  
approach is likely to be limited by both the uneven distribution of universal marker genes across a  
genome and their low number, typically accounting for <10% of all genes (Sharon and Banfield  
2013). These limitations have been partially addressed by identifying genes that are ubiquitous and  
70 single copy within a specific phylum, which increases the number of marker genes used in the  
estimate (Swan et al. 2013). Single-copy marker genes present multiple times within a recovered  
genome have also been used to estimate potential contamination (Albertsen et al. 2013; Soo et al.  
2014; Sekiguchi et al. 2015).

Here we describe CheckM, an automated method for estimating the completeness and contamination  
75 of a genome using marker genes that are specific to a genome’s inferred lineage within a reference  
genome tree. Using simulated genomes of varying degrees of quality, we demonstrate that lineage-

specific marker genes provide refined estimates of genome completeness and contamination compared to the universal or domain-level marker genes commonly used. Marker genes that are consistently collocated within a lineage do not provide independent evidence of a genome's quality, so collocated marker genes were grouped into marker sets in order to further refine estimates of genome quality. We show that lineage-specific collocated marker sets provide robust estimates across all bacterial and archaeal lineages, with completeness and contamination estimates generally having a low absolute error even when genomes are relatively incomplete (70%) with medium contamination (10%). We also propose a fixed vocabulary for defining genome quality based on estimates of completeness and contamination that is suitable for automated screening of genomes from large-scale sequencing initiatives and for annotating genomes in reference databases. We envisage that CheckM will help identify problematic genomes before they are deposited in public databases. For single-cell genomes and population genomes recovered from metagenomic data, the improved quality estimates provided by CheckM allow biological inferences to be made in the context of genome quality, and highlight genomes that would benefit from further refinement.

## Results

### *Simulation Models for Evaluating the Accuracy of Quality Estimates*

Three independent models were used to generate simulated genomes suitable for evaluating the completeness and contamination estimates provided by CheckM. Under the 'random fragment' model, 3324 high-quality draft genomes (spanning 39 classes and 20 phyla) obtained from IMG were fragmented into non-overlapping windows of 5 to 50 kbp and randomly sampled in order to generate genomes with varying degrees of completeness and contamination (see Methods for more details). This model allows a large number of genomes to be simulated at varying degrees of quality and provides a baseline for assessing the accuracy of completeness and contamination estimates. In order to simulate genomes reflecting the characteristics of assembled contigs, the 2430 high-quality draft genomes (spanning 31 classes and 18 phyla) comprised of  $\geq 20$  contigs were used to simulate incomplete genomes contaminated with foreign DNA. Under this 'random contig' model, incomplete

genomes were generated by randomly removing contigs to achieve a desired level of completeness and contamination introduced by randomly adding contigs from a randomly selected draft genome.

105 The final model simulates genomes that reflect the limitations of metagenomic binning methods which rely on the statistical properties of assembled contigs (e.g., tetranucleotide signatures, coverages) to determine their source genome. Since the variance of genome statistics increase with decreasing contig length, binning methods are more likely to incorrectly bin shorter contigs (Dick et al. 2010; Albertsen et al. 2013; Imelfort et al. 2014). The ‘inverse length’ model captures this  
110 limitation by generating incomplete and contaminated genomes in a manner similar to the random contig model, but with contigs removed or added with a probability inversely proportional to a contig’s length.

For all three models, genomes were generated at 50 to 100% completeness and 0 to 20% contamination. While genomes recovered using single-cell genomics are often <50% complete, we  
115 have focused on relatively complete genomes as these have a broader range of applications and we believe the methodology of combining reads from multiple single cells belonging to the sample population, which often results in highly complete genomes, will become common practice. Similarly, we have focused on genomes with  $\leq 20\%$  contamination as there are limited applications for genomes with excessive levels of contamination.

## 120 *Assessment of Universal- and Domain-level Marker Gene Sets*

Within CheckM, a gene identified as single copy in  $\geq 97\%$  of genomes is considered to be a marker gene. The genome quality estimates provided by the marker sets used by CheckM were compared with three universal (Mende et al. 2013; Rinke et al. 2013; Darling et al. 2014), three bacterial (Wu and Scott 2012; Dupont et al. 2012; Rinke et al. 2013), and two archaeal (Wu and Scott 2012; Rinke  
125 et al. 2013) marker sets using genomes simulated under the random fragment model. The universal marker sets had similar performance with the exception of the SpecI set (Mende et al. 2013), which provided superior contamination estimates at the expense of substantially less accurate completeness estimates (**Supplemental Table S1**). Among the CheckM, GEBA-MDM (Rinke et al.

2013) and PhyloSift (Darling et al. 2014) sets, the mean absolute error in quality estimates provided  
130 by CheckM never deviated by more than 1.4% and was typically within 1% of the best performing  
universal marker set. The four bacterial- and three archaeal-specific marker sets provided far more  
accurate estimates than the universal sets with the exception of the poorly performing Amphora 2 set  
(Wu and Scott, 2012; **Supplemental Table S1**). The mean absolute error in the completeness  
135 estimates provided by CheckM's domain-specific marker sets were always within 1% of the best  
performing marker set, while the contamination estimates were generally within 1% and always  
within 1.8%.

### ***Organizing Marker Genes into Collocated Sets***

As marker genes are required to be present in nearly all genomes within a lineage (e.g., all bacteria or  
archaea), they often encode essential functions and are frequently organized into operons  
140 (**Supplemental Fig. S1**). Marker genes that are consistently collocated do not provide independent  
information regarding the completeness or contamination of a genome. To address this, we grouped  
marker genes that were consistently collocated within a lineage into marker sets and used this  
grouping structure to refine estimates of genome completeness and contamination. Collocated marker  
genes are common across all taxonomic groups with 36% of marker genes, on average, being grouped  
145 into a set with one or more other marker genes (**Supplemental Table S2**).

We evaluated the benefit of collocated marker sets for assessing genome quality by applying  
CheckM's domain-specific markers (bacteria: 104 markers organized into 58 sets; archaea: 150  
markers organized into 108 sets) to genomes simulated under the random fragment model.  
Completeness and contamination estimates calculated with collocated marker sets were superior to  
150 estimates determined with individual marker genes regardless of the completeness or contamination  
of the simulated genomes (**Fig. 1; Supplemental Table S3**). The mean absolute error in completeness  
(contamination) estimates across all simulated genomes increased from 4.3% to 5.7% (3.8% to 4.7%)  
when using marker sets compared to 5.5% to 9.0% (4.7% to 6.8%) when using individual marker  
genes as the window size was increased from 5 to 50 kbp (**Supplemental Table S3**).

155 To further evaluate the benefits of using collocated marker sets, domain-specific markers were used to estimate the quality of genomes simulated under the random contig and inverse length models. Under the random contig model, the mean absolute error in the completeness and contamination estimates across all simulated genomes was reduced from 8.5% to 5.4% and 5.9% to 4.1%, respectively, when genome quality was estimated with collocated marker sets as opposed to individual marker genes  
160 **(Supplemental Fig. S2; Supplemental Table S4)**. Similar improvements were obtained under the inverse length model though estimates were less accurate for genomes generated under this model (completeness: 10.3% to 6.6%, contamination: 8.2% to 5.6%; **Supplemental Fig. S3; Supplemental Table S5**).

### *Inference of Reference Genome Tree*

165 Estimates of completeness and contamination can be further refined by using lineage-specific, collocated marker sets determined from the placement of a query genome within a reference genome tree (**Fig. 2**). The reference tree used by CheckM was inferred from the concatenation of 43 conserved marker genes with largely congruent phylogenetic histories (**Supplemental Tables S6 and S7**). It incorporates 2052 finished and 3604 draft genomes obtained from the Integrated Microbial Genomes  
170 (IMG; Markowitz et al. 2014) database identified as being near complete with minimal contamination (see Methods). The inferred tree (**Supplemental Fig. S4**) shares features in common with recently published genome trees, including the class *Clostridia* being highly paraphyletic (Yutin and Galperin, 2013) and the class *Epsilonproteobacteria* residing outside the *Proteobacteria* phylum (Rinke et al. 2013). These discrepancies between phylogeny and taxonomy will cause marker genes calculated  
175 from named lineages within the genome tree to deviate from those determined strictly from assigned taxonomy. More importantly, a reference tree allows lineage-specific marker genes to be established for any internal nodes and not just those representing a named taxonomic group.

### *Assessment of Lineage-specific Marker Sets*

Lineage-specific marker sets were determined for all nodes within the reference genome tree by  
180 identifying single-copy genes present in  $\geq 97\%$  of all descendant genomes. The quality of a genome

can be estimated using the marker set defined at any parental node between the genome's position in the reference tree and the root. A simulation framework was used to establish the parental lineage with the most favourable set of markers for assessing the quality of genomes placed along any branch in the reference tree (**Fig. 3**). Briefly, finished genomes were used to simulate incomplete and contaminated genomes placed along a branch, and the parental lineage whose marker genes most accurately estimated the quality of these genomes was determined.

We evaluated the effectiveness of the selected lineage-specific marker sets on genomes generated under all three simulation models. The quality of each simulated genome was estimated using collocated marker sets inferred from genomes within i) the archaeal or bacterial lineage, ii) the lineage selected by the simulation framework, and iii) the parental lineage producing the most accurate estimates. Case iii represents an idealized case where the parental lineage is selected independently for each simulated genome, which is in contrast to case ii where the single parental lineage selected by the simulation framework must be used for all simulated genomes placed along a given branch. Under all three models, the selected lineage-specific marker sets provided more accurate completeness and contamination estimates than domain-specific marker sets, and produced estimates nearly as accurate as the best performing lineage-specific marker sets (**Fig. 4; Table 1; Supplemental Figs S5 and S6; Supplemental Tables S8-S10**). The improvement in quality estimates can be substantial with the mean absolute error in completeness and contamination being reduced by 44.4% (5.4% to 3.0%) and 19.5% (4.1% to 3.3%), respectively, when using selected lineage-specific sets instead of the domain-specific sets to estimate the quality of genomes generated with the random contig model. In addition, the variances of the completeness and contamination estimates are substantially reduced. Summarizing results by the taxonomic group affiliated with each simulated genome indicated that the selected lineage-specific sets provided improved estimates across all 39 classes (20 phyla) considered in this study, with the exception of the poorly sampled *Synergistetes* lineage where the estimates were largely unchanged (**Supplemental Fig. S7; Supplemental Tables S11-S13**).



### ***Influence of Taxonomic Novelty***

210 'Natural' taxonomic novelty within the test set of 3324 draft genomes was examined in order to explore the influence of novelty on genome quality estimates. As expected, increasing taxonomic novelty results in the preferential use of more broadly defined lineage-specific marker sets (**Supplemental Table S14**). For example, 69.7% of the 33 test genomes which are sole representatives of different taxonomic families within the reference genome tree were evaluated with a lineage-specific marker set at a rank of class or higher, while only 17.9% of the 1923 genomes with multiple strains within the reference genome tree were evaluated with such broad marker sets. The use of more broadly defined marker sets had a direct impact on the genome quality estimates for simulated genomes generated under the random fragment model (**Supplemental Table S15**). While simulated genomes derived from the six draft genomes representing novel phyla or classes resulted in less accurate quality estimates than genomes from less novel lineages, the estimates were consistent with the performance expected for genomes evaluated with a domain-specific marker set (contrast **Supplemental Tables S8 and S15**). This suggests that the novelty of a genome is less critical than the specificity of the marker set used to evaluate genome quality, and that even genomes representing novel phyla can be assessed at the error rates expected for domain-specific marker sets. While genome quality estimates improved gradually as the taxonomic novelty of genomes decreases from order to species, the mean absolute error of the quality estimates across the test sets never deviated by more than 0.8%, suggesting that the accuracy of lineage-specific marker sets are relatively stable at this degree of novelty (**Supplemental Table S15**).

### ***Bias in Genome Quality Estimates***

230 Quality estimates based on individual marker genes or collocated marker sets exhibit a bias resulting in completeness being overestimated and contamination being underestimated (**Figs. 1 and 4**). This bias is the result of marker genes residing on foreign DNA which are otherwise absent in a genome being mistakenly interpreted as an indication of increased completeness as opposed to contamination. This bias approximately follows a binomial distribution suggesting a potential avenue for bias

235 correction (see Supplemental Methods). We have elected not to correct for this bias as confounding factors such as gene collocation make the correction approximate and the bias is small (<3%) when genomes are substantially complete (>70%) with medium contamination ( $\leq 10\%$ ; **Supplemental Fig. S8**).

### *Assessment of Isolate Genomes*

240 To benchmark CheckM on real world data, we assessed the quality of 2281 isolate genomes from the GEBA, GEBA-KMG, GEBA-PCC, GEBA-RNB, and HMP datasets (**Table 2**). Using lineage-specific marker sets, 2190 (96%) of these genomes were estimated to be  $\geq 95\%$  complete with  $\leq 5\%$  contamination (**Supplemental Table S16**) making them excellent reference genomes for analyses such as assigning taxonomy to anonymous genomic fragments (Brady and Salzberg, 2009; Parks et al. 2011) or characterizing metagenomic samples using marker genes (Darling et al. 2014). The remaining 91 (4%) genomes were found to be <95% complete or >5% contaminated making them 245 less reliable reference genomes for some analyses. A small number of the genomes have an estimated completeness <90% (14 genomes) or an estimated contamination >10% (5 genomes). These genomes suffer from a diverse range of problems which we illustrate using three public genomes from the HMP available at the time of preparing this manuscript:

- 250 • The *Capnocytophaga* sp. oral taxon 329 genome (HMP id: 9074; GenBank id: AFHP000000000; IMG id: 651324019) was estimated as 100% complete and 100% contaminated by CheckM. Investigation of the 157 contigs comprising this genome revealed a bimodal GC-distribution suggesting the presence of two distinct genomes (**Supplemental Fig. S9**). We separated the contigs into two clusters by applying *k*-means clustering with *k*=2 to the tetranucleotide signatures of each contig. Placing the resulting clusters into a genome 255 tree identified one cluster as a novel *Capnocytophaga* genome (99.0% complete, 0.2% contaminated) and the other cluster as closely related to *Paraprevotella clara* YIT 11840 (100% complete, 0.4% contaminated; **Supplemental Fig. S10**).

- 260
- The least complete HMP genome reported by CheckM was the gastrointestinal *Clostridiales* sp. SM4/1 genome (HMP id: 924; GenBank id: FP929060; IMG id: 2524023221) annotated as finished at IMG and GOLD, but estimated as only 56% complete. CheckM determined the coding density of this genome to be 66% suggesting substantial assembly or gene calling errors. Further investigation revealed that 667 kbp (21.5%) of this 3.1 Mbp genome is comprised of ambiguous base pairs (Ns).
  - The *Lactobacillus gasseri* MV-22 genome (HMP id: 515) available from IMG (id: 643886189) consists of 93 contigs comprising 1.89 Mbp with only 193 ambiguous bases. CheckM estimated the completeness of this genome as 90.9% when using the lineage-specific marker sets and 81.2% complete when using the bacterial marker set (**Supplemental Table S17**). While these low completeness estimates could be the result of lineage-specific gene loss, the other three *Lactobacillus gasseri* genomes from HMP are all estimated to be  $\geq 96\%$  complete with only the Leucyl-tRNA synthetase protein family (PF13603) exhibiting lineage-specific gene loss across the bacterial marker genes (**Supplemental Table S18**). This indicates that *Lactobacillus gasseri* MV-22 is incomplete with  $\geq 9\%$  of its genome estimated to be missing. The incomplete state of this genome is not transparent from its genome size, as available *Lactobacillus gasseri* genomes are between 1.78 and 2.01 Mbp.
- 265
- 270

275 The issues exemplified above are not limited to the HMP or large-scale sequencing efforts. For example, the *Paracoccus denitrificans* SD1 genome (Siddavattam et al. 2011) at IMG (id: 2511231195) was estimated to be only 59% complete by CheckM (**Supplemental Table S19**). Comparing this genome to *Paracoccus denitrificans* PD1222 suggests that this species has two chromosomes and a plasmid, and that the SD1 genome is currently missing both a chromosome and its plasmid. CheckM also identified several putative submission errors as exemplified by the type strain *Oligotropha carboxidovorans* OM5 (IMG id: 650716069) which is reported as 99.7% complete and 100.9% contaminated as a result of both draft and finished versions of its chromosome and plasmid being contained in its genome sequence file.

280

### *Assessment of Single-Cell Genomes*

285 The GEBA-MDM initiative applied single-cell genomics to novel uncultivated bacterial and archaeal  
cells (Rinke et al. 2013). While this is the largest single-cell sequencing initiative currently published,  
other large-scale initiatives are underway and have submitted initial genomes to IMG. To assess the  
quality of genomes recovered through single-cell genomics, we applied CheckM to i) 201 genomes  
recovered from individual cells in the GEBA-MDM initiative, ii) 21 genomes co-assembled from  
290 GEBA-MDM cells belonging to the same population, and iii) 410 additional genomes from  
unpublished studies annotated as *uncultured type* or *single cell* in IMG (**Table 2**).

Technical challenges in obtaining single-cell genomes such as low DNA yield and the associated need  
for genome amplification make it challenging to recover complete genomes. CheckM quality  
estimates indicate that only 3 of the 201 (1.5%) GEBA-MDM genomes and 17 of the 410 (4.4%)  
295 unpublished single-cell genomes have an estimated completeness  $\geq 90\%$ . Combining cells from the  
same population can substantially improve completeness with the 21 combined assemblies in GEBA-  
MDM having an average completeness of  $64.9\% \pm 24.3\%$  compared to  $34.9\% \pm 20.6\%$  for the 201  
single-cell genomes (**Supplemental Table S20**). Although current techniques for recovering genomes  
from single cells result in highly incomplete genomes, these are still valuable reference genomes for  
300 analyses such as assigning taxonomy to anonymous genomic fragments and resolving phylogenetic  
relationships (Rinke et al. 2013). However, these reference genomes should be free from substantial  
contamination as this will be a source of inaccuracy in such analyses. CheckM estimates identified 62  
of the 410 (15.1%) unpublished single-cell genomes have  $\geq 5\%$  contamination. All the GEBA-MDM  
genomes were found to have  $< 5\%$  contamination, except one *Omnitrophica* sp. with 5.3%  
305 contamination and two combined assemblies that were estimated to be 11.3% (*Marinimicrobia* sp.)  
and 21.5% (*Cloacimonetes* sp.) contaminated. Comparison of duplicate marker genes within these  
genomes suggests the contamination is the result of foreign DNA being amplified and not an  
assembly error.

### *Assessment of Population Genomes*

310 Unlike genomes recovered from cultured isolates or single cells, genomes obtained from  
metagenomic data typically represent a consensus across a microbial population. CheckM was applied  
to 146 population genomes recovered from four metagenomic studies (**Table 2**). As expected, the  
estimated completeness and contamination of these genomes vary substantially (**Fig. 5**). While  
population genomes are often incomplete (74 of 146 genomes are between 50% and 95% complete),  
315 they can be recovered with relatively little contamination (43 of the 74 partial genomes have  $\leq 5\%$   
contamination; **Supplemental Table S21**). In addition to this set of 74 partial genomes, an additional  
16 (11%) population genomes were estimated to be  $\geq 95\%$  complete with  $< 5\%$  contamination. These  
estimates account for several of the population genomes recoding the opal stop codon as CheckM  
automatically identifies such recodings (see **Supplemental Methods**).

320 Poor quality estimates are expected for genomic elements such as plasmids or phage as the marker  
genes used by CheckM are specific to bacterial and archaeal chromosomes. The 10 plasmids and 11  
phage identified within the acetate-amended aquifer (Wrighton et al. 2012) and infant gut (Sharon et  
al. 2013) datasets were estimated to be 0% complete and 0% contaminated, with the exception of two  
plasmids (CARSEP1P, ACD71) and one phage (ACD33) which were estimated as 4.2%, 2.7%, and  
325 0.15% complete, respectively (**Supplemental Table S21**). The completeness of reduced genomes  
without representation in the reference genome tree will also be underestimated when genome  
reduction has resulted in the loss of marker genes. This is illustrated by the four candidate phylum  
Saccharibacteria (TM7) genomes obtained from sludge bioreactor metagenomes which were  
estimated to be 60-70% complete by CheckM, though shown to be  $\geq 85\%$  complete after accounting  
330 for lineage-specific gene loss (Albertsen et al. 2013).

We compared the quality estimates obtained for the 90 putative population genomes recovered from  
the acetate-amended aquifer (Wrighton et al. 2012) community using domain-level and lineage-  
specific marker sets (**Supplemental Table S22**). While the completeness and contamination of these  
population genomes is unknown, these results demonstrate the degree to which quality estimates can

335 change under these two conditions. We have focused on the acetate-amended aquifer dataset as it  
contains population genomes spanning a wide range of qualities, while other studies have focused  
exclusively on high-quality population genomes. On average, completeness changed by 13.0% and  
contamination by 5.1% when using lineage-specific instead of domain-specific marker sets. Estimates  
varied substantially for some genomes with completeness estimates changing by  $\geq 15\%$  for 36  
340 genomes and contamination estimates changing by  $\geq 10\%$  for 12 genomes. While completeness  
estimates with domain-level and lineage-specific marker sets are highly correlated ( $R^2=0.84$ ), domain-  
level estimates tend to overestimate the completeness of genomes relative to lineage-specific  
estimates (**Supplemental Fig. S11**). The correlation between contamination estimates is weaker  
( $R^2=0.69$ ) and any global trend is less clear as the majority of population genomes exhibit  $<5\%$   
345 contamination (**Supplemental Fig. S12**).

### *Estimating Strain Heterogeneity*

CheckM can distinguish between contamination resulting from the presence of genomic fragments  
from multiple strains and contamination resulting from the inclusion of genomic fragments from more  
divergent taxa. This is particularly useful for genomes recovered from metagenomic data as  
350 separating strains into individual genomes remains a challenging problem (Imelfort et al. 2014). These  
two types of contamination are differentiated automatically by CheckM by using the amino acid  
identity (AAI) between multi-copy genes as a measure of phylogenetic relatedness (Konstantinidis  
and Tiedje, 2005). Reanalysis of the methanotrophic ANME-1 genome recovered from metagenomic  
data by Meyerdierks et al. (2010) with CheckM illustrates that this population genome is a chimera of  
355 closely related strains. Of the 229 lineage-specific marker genes used to evaluate the quality of this  
genome, 42 were identified as being multi-copy within the ANME-1 genome (38 present twice, 2  
present three times; 82.3% completeness). While this represents approximately 21% contamination,  
82.0% of the comparisons between multi-copy genes have an AAI  $\geq 90\%$  (76.0% at  $\geq 95\%$  AAI;  
**Supplemental Fig. S13**) revealing that the contamination is largely the result of incorporating  
360 genomic fragments from closely related taxa and that multiple ANME-1 strains are likely present  
within this environment.

### ***Proposed Genome Quality Classification Scheme***

365 Genomes recovered from isolates, single cells, or metagenomic data vary substantially in their quality (Fig. 5). To make full use of these genomes, their quality must be reported in reference databases along with other essential genome information (Field et al. 2008). A qualitative vocabulary for discussing genomes of varying quality was proposed by Chain et al. (2009), and here we supplement this effort by proposing a vocabulary based on quantitative threshold which augment existing schemes for describing draft genome quality (Table 3). The status of *finished* is reserved for genomes assembled into a single contiguous sequence containing no gaps or ambiguities, where extensive 370 efforts have been made to identify errors (Mardis et al. 2002; Chain et al. 2009). Genomes assembled into multiple sequences as a result of repetitive regions, but otherwise of a finished quality may be classified as *noncontiguous finished* (Chain et al. 2009). We propose that all other genomes be designated as *draft*, and the quality of genomes qualified based on both establish vocabularies for describing genome quality and estimates of genome completeness and contamination.

375 The proposed quantitative vocabulary permits automated assignments of completeness and contamination estimates, which are critical for quality control in large-scale genome sequencing initiatives, and for updating genome databases as new genomes are added or techniques for estimating genome quality improve. Of the 3059 genomes (2281 isolates, 632 single cell, 146 metagenomic) considered in this study, 2292 (74.9%) were classified as being near complete with either no 380 detectable (833 genomes; 27.2%) or low (1461 genomes; 47.8%) contamination. These genomes are strong candidates for being classified as *finished* or *noncontiguous finished*, but this designation should only be applied after extensive additional verification. The wide range of quality within the remaining 767 (25.1%) genomes illustrates the need for a verbose vocabulary when discussing draft genomes, e.g., 84 (2.7%) were classified as substantially-complete drafts with 22 (0.7%) exhibiting no 385 contamination, 55 (1.8%) having low contamination, and 5 (0.2%) having medium contamination. The presence of metagenomic and single-cell genomes was also transparent as 125 (4.1%) of the genomes were classified as moderately-complete drafts and 521 (17.0%) were classified as partial draft genomes.



## Discussion

390 Here we introduce CheckM, a new tool developed to estimate the completeness and contamination of  
genomes derived from isolates, single cells and metagenomes using lineage-specific marker genes. To  
evaluate the robustness of genome quality estimates, we simulated genomes under three distinct  
models: i) a random fragment model where genomic fragments were removed or added uniformly  
across the genome, ii) a random contig model which accounts for the characteristics of assembled  
395 contigs, and iii) an inverse length model reflecting the limitations of metagenomic binning methods.  
Our results on simulated genomes demonstrate that when lineage-specific marker genes are organized  
into collocated sets, they are sufficiently spaced throughout a genome to provide accurate estimates of  
genome quality. For substantially-complete genomes ( $\geq 70\%$  to  $90\%$ ) with medium contamination  
( $5\%$  to  $\leq 10\%$ ), our results suggest that completeness and contamination estimates generally have an  
400 absolute error of  $\leq 6\%$  and that the error in the quality estimates tends to decrease as the quality of a  
genome improves (**Fig. 4; Supplemental Figs. S5 and S6; Supplemental Tables S8-S10**).

The robust estimates of genome quality provided by CheckM allow for automated quality screening  
of bacterial and archaeal genomes. Using CheckM, we were able to identify isolate genomes  
exhibiting a wide range of problems. Incorporation of these low-quality genomes into reference  
405 datasets will diminish the accuracy of inferences made in many studies. For example, a study of  
horizontal gene transfer might incorrectly predict a large number of transfers between  
*Capnocytophaga* and *Paraprevotella* genomes due to the *Capnocytophaga* sp. oral taxon 329 genome  
erroneously containing genes from both of these genera. Similarly, a comparative genomics study  
including the *Clostridiales* sp. SM4/1 genome identified as  $56\%$  complete may incorrectly report the  
410 number of core genes among *Clostridiales* genomes or the ubiquity of key metabolic pathways.  
Comparison of the incomplete *Lactobacillus gasseri* MV-22 genome considered in this study to its  
GenBank (id: GL531761) counterpart revealed that this issue was localized to the IMG repository,  
illustrating the benefit of independently verifying the quality of genomes at different repositories.



Many of the erroneous genomes reported in this study were brought to the attention of IMG and have  
415 subsequently been removed from their database. The *Capnocytophaga* sp. oral taxon 329 has also  
been retracted from NCBI. While removal of contaminated or incomplete genomes is warranted, the  
statistics provided by CheckM can help identify the problems associated with these genomes. In the  
case of *Capnocytophaga* sp. oral taxon 329, the CheckM statistics directly suggested the presence of  
two distinct populations which allowed for the recovery of two near-complete genomes with low  
420 contamination.

Incomplete draft genomes are valuable references for many genomic analyses and their use is likely to  
increase as partial genomes of novel species are recovered from single cells and metagenomic data.  
While methodologies for handling genomes of varying qualities are currently in their infancy, it is  
clear many analyses will benefit from accurate estimates of completeness and contamination. The  
425 benefit of using partial genomes with low contamination for assigning taxonomy to anonymous  
genomic fragments and resolving phylogenetic relationships has already been demonstrated (Rinke et  
al. 2013). Other analyses such as comparing the metabolic capability of different groups of genomes  
will likely benefit from restricting the analyses to only near-complete genomes in order to ensure  
confident predictions can be made in regards to differences in their metabolic capabilities. Because  
430 the quality of a genome is essential for determining its suitability for different analyses, we  
recommend public genome repositories and new genome announcements include completeness and  
contamination estimates (**Table 3**).

The limitations of the proposed approach must be considered when interpreting CheckM quality  
estimates. Eukaryotic or phage genomes will be reported as highly incomplete as we have focused on  
435 marker sets suitable for evaluating bacterial and archaeal genomes. The quality of plasmids must also  
be assessed independently of CheckM. When recovering genomes from metagenomic data, the  
additional genome statistics reported by CheckM (e.g., coding density, coverage) can be used along  
with the quality estimates to help distinguish putative genomes representing fragments of an archaeal  
or bacterial chromosome from phage, plasmids, or eukaryotic genomes. However, incorporation of  
440 CheckM into a complete quality control and annotation pipeline where plasmids (Jørgensen et al.

2014), genomic islands (Langille et al. 2010), phage (Akhter et al 2012), and other genomic elements are automatically identified and screened will likely improve the quality and characterization of recovered genomes. Use of taxonomic assignment methods (Patil et al. 2011; Dröge et al. 2014) would also be of benefit within a complete quality control pipeline in order to identify genomes exhibiting clear chimerism and sources of contamination within single-cell and isolate genomes. The estimates for highly incomplete or highly contaminated genomes must be interpreted with regards to the observed systematic bias, which arises from marker genes from foreign genome being misinterpreted as an indicating of additional completeness (**Supplemental Fig. S8**). The novelty of a genome will also influence the accuracy of CheckM estimates. Estimates for bacterial and archaeal genomes from deep basal lineages with few reference genomes are necessarily determined using domain-level marker sets instead of lineage-specific markers which generally provide superior estimates. This limitation is most evident for novel lineages undergoing genome reduction as demonstrated by our reanalysis of the candidate phylum Saccaribacteria (TM7) genomes. While CheckM can provide refined estimates for reduced genomes in well-characterized lineages (see Supplemental Results), a manual assessment of gene loss or duplication is required to improve quality estimates for reduced genomes recovered from novel lineage (Albertsen et al. 2013). CheckM provides outputs to aid in performing this refinement.

We anticipate several improvements that will further refine the estimates produced by CheckM. The most substantial impact is likely to be the inclusion of additional reference genomes from lineages that are currently poorly represented. This will mitigate the number of genomes that are evaluated using broad, less accurate marker sets and improve refinements for lineage-specific gene loss and duplication. Incorporation of eukaryotic genomes into the reference tree would also be a substantive benefit when assessing population genomes recovered from environmental samples where fungi and other microbial Eukaryotes may be present. Further exploration of the parameter space of CheckM may also result in improved estimates. For instance, the 97% ubiquity criteria used to delineate marker genes is likely not optimal and the use of a probabilistic model for assessing the presence/absence of a gene across all genomes in a lineage may improve the inferred marker sets

(Segata et al., 2013). Ultimately, we expect to adopt a strategy that will allow optimal values for key parameters to be determined independently for each lineage.

470 CheckM is the first automated tool for estimating the completeness and contamination of isolate, single cell, and population genomes. The need for accurate estimates of genome quality will only grow in importance as we continue to fill out the microbial tree of life and are better able to utilize draft genomes to inform gene- and genome-centric analyses of microbial communities.

## Methods

### 475 *Inference of Reference Genome Tree*

A genome tree incorporating 5656 trusted reference genomes (see Supplemental Methods) was inferred from a set of 43 genes with largely congruent phylogenetic histories. An initial set of 66 universal marker genes was established by taking the intersection between bacterial and archaeal genes determined to be single copy in >90% of genomes. From this initial gene set, 18 multi-copy genes with divergent phylogenetic histories in >1% of the reference genomes were removed. A multi-copy gene within a genome was only deemed to have a congruent phylogenetic history if all copies of the gene were situated within a single conspecific clade (i.e., all copies were contained in a clade from a single named species) within its gene tree. Genes were aligned with HMMER v3.1b1 (480 <http://hmmer.janelia.org>) and gene trees inferred with FastTree v2.1.3 (Price et al. 2009) under the WAG (Whelan and Goldman, 2001) and GAMMA (Yang, 1994) models. Trees were then modified with DendroPy v3.12.0 (Sukumaran et al. 2010) in order to root the trees between archaea and bacteria unless these groups were not monophyletic in which case midpoint rooting was used. A further five genes found to be incongruent with the IMG taxonomy were also removed as these genes may be subject to lateral transfer. Testing of taxonomic congruency was performed as described in 485 Soo et al. (2014). The final set of 43 phylogenetically informative marker genes (**Supplemental Table S6**) consists primarily of ribosomal proteins and RNA polymerase domains, and is similar to the universal marker set used by PhyloSift (Darling et al. 2014; **Supplemental Table S7**). A reference genome tree was inferred from the concatenated alignment of 6988 columns with FastTree v2.1.3

under the WAG+GAMMA model and rooted between bacteria and archaea. Internal nodes were  
495 assigned taxonomic labels using tax2tree (McDonald et al. 2012).

### ***Identification of Marker Genes in Putative Genomes***

Genes are predicted using Prodigal v2.60 (Hyatt et al. 2012) and Pfam (Finn et al. 2014) and  
TIGRFAMs (Haft et al. 2003) protein families identified using HMMER v3.1b1  
(<http://hmmer.janelia.org>) with model specific cutoff values for both the Pfam (-cut\_gc) and  
500 TIGRFAMs (-cut\_nc) HMMs. Pfam annotations are assigned using the same methodology as the  
Sanger Institute and IMG, which accounts for homologous relationships between Pfam clans (see  
pfam\_scan.pl on the Sanger Institute FTP site). Gene calling errors occasionally occur due to  
ambiguous bases in a contig that can result in adjacent, erroneous genes being called which are  
assigned to the same marker gene. These errors are resolved by checking if adjacent marker genes  
505 have a best match to adjacent, non-overlapping portions of a marker gene's HMM.

### ***Determination of Lineage-specific Marker Genes***

Single-copy Pfam and TIGRFAMs genes were identified within reference genomes using the  
annotations provided by IMG. A gene was defined as a lineage-specific marker gene if it occurs only  
once in >97% of the genomes within a lineage. Lineage-specific marker genes were inferred for all  
510 internal nodes within the reference genome tree. Pfam and TIGRFAMs families were considered  
redundant if they matched the same genes in >90% of the finished IMG genomes in which case only  
the Pfam gene was used as a marker.

### ***Organization of Marker Genes into Collocated Marker Sets***

A pair of marker genes were considered to be collocated within a lineage if they occurred within 5  
515 kbp of each other in >95% of genomes within a lineage. Sets of collocated markers were formed from  
collocated gene pairs by clustering together all pairs with a shared gene (e.g., if genes A and B, and  
genes B and C are collocated, then they are clustered into the collocated set ABC).

### **Estimation of Completeness, Contamination, and Strain Heterogeneity**

520 Genome completeness is estimated as the number of marker sets present in a genome taking into account that only a portion of a marker set may be identified:

$$\frac{\sum_{s \in M} \frac{|s \cap G_M|}{|s|}}{|M|} \quad (1)$$

where  $s$  is a set of collocated marker genes,  $M$  is the set of all collocated marker sets  $s$ , and  $G_M$  is the set of marker genes identified in a genome. Genome contamination is estimated from the number of multi-copy marker genes identified in each marker set:

$$\frac{\sum_{s \in M} \frac{\sum_{g \in s} C_g}{|s|}}{|M|} \quad (2)$$

525 where  $C_g$  is  $N-1$  for a gene  $g$  identified  $N \geq 1$  times, and 0 for a missing gene. CheckM also supports estimating completeness and contamination without arranging marker genes into collocated sets. Equations 1 and 2 can be applied to this case by assigning each marker gene to its own set (i.e.,  $\forall s: |s| = 1$ ).

530 Contamination resulting from multiple strains or closely-related species being binned into a single putative genome is identified by examining the AAI between multi-copy marker genes. Specifically, a strain heterogeneity index is calculated as the fraction of multi-copy gene pairs above a specified AAI threshold:

$$\frac{\sum_{g \in G} \sum_{i=1}^{|g|} \sum_{j=i+1}^{|g|} aai(g_i, g_j, t)}{\sum_{g \in G} \sum_{i=1}^{|g|} \sum_{j=i+1}^{|g|} 1} \quad (3)$$

where  $g = \{g_1, g_2, \dots, g_N\}$  is the set of hits to a marker gene,  $G$  is the set of all marker genes, and  $aai$  is 1 if the AAI between  $g_i$  and  $g_j$  is greater than  $t$  (default = 0.9) and 0 otherwise.

### ***Placement of Genomes into the Reference Genome Tree***

535 Identification of the 43 phylogenetically informative marker genes within a putative genome are  
identified using HMMs as described in the preceding section “Identification of Marker Genes in  
Putative Genomes”. Identified genes are aligned with HMMER and the concatenated alignment used  
to place a genome into the reference genome tree using pplacer v2.6.32 (Matsen et al. 2010). Putative  
genomes consisting of an insufficient number of unique phylogenetic marker genes (default = 10) to  
540 be robustly placed within the reference genome tree are evaluated using the universal marker set.

### ***Selection of Lineage-specific Marker Genes***

Marker genes can be inferred for all internal nodes in the reference tree along the path from the  
putative genome to the root (**Fig. 3A**). The most suitable set of marker genes for assessing a genome  
depends on a number of factors including the novelty of the putative genome relative to the  
545 surrounding reference genomes and the breadth of diversity covered by these genomes. A simulation  
framework was used to establish the parent node producing the most suitable marker set for  
estimating the completeness and contamination of a genome placed on a given branch within the  
reference genome tree.

The simulation framework was restricted to the 2052 finished reference genomes in IMG, as draft  
550 genomes were used for evaluating the performance of CheckM. For each branch, the descendant  
lineage with the fewest genomes was removed from the reference tree (**Fig. 3B**). These genomes were  
used as proxies to simulate genomes placed on this branch. Each genome was fragmented into 10 kbp  
windows and used to simulate 100 independent genomes with completeness randomly selected  
between 50-100% and contamination randomly selected between 0-20% (**Fig. 3C**). Marker genes  
555 were then inferred for each parent node using the 97% single-copy criterion and used to assess the  
completeness and contamination of the simulated genomes. Marker genes were not formed into  
marker sets in order to reduce computational complexity and to allow a fair assessment of how this  
feature influences genome assessment. The parental node whose inferred marker genes minimize the

error in the estimated completeness and contamination over all simulated genomes was assigned to  
560 the branch (**Fig. 3D**):

$$\arg \min_{m \in M} = \sum_{g \in G} \sum_{i=1}^N |comp_{est}(g_i, m) - comp_t(g_i)| + |cont_{est}(g_i, m) - cont_t(g_i)| \quad (4)$$

where  $m$  is a set of marker genes,  $M$  is the set of marker gene for each parent node,  $comp_{est}(g_i, m)$  is the  
estimated completeness of simulated genome  $g_i$  using  $m$ ,  $comp_t(g_i)$  is the true completeness of  $g_i$ ,  
 $cont_{est}(g_i, m)$  and  $cont_t(g_i)$  are analogous functions for contamination, and  $N$  is the number of simulated  
genomes derived from  $g$ . Marker genes associated with each internal node were calculated *de novo*  
565 during the simulation to reflect removing the test genomes and then re-calculated afterwards using all  
available reference genomes in order to produced refined sets of marker genes. This simulation  
framework is computationally expensive, but only needs to be performed once and the results  
distributed with the CheckM software.

### ***Simulation of Incomplete and Contaminated Isolate and Population Genomes***

570 Simulated genomes were generated from an initial set of 3604 draft genomes within IMG identified as  
being of high quality (see Supplemental Methods). To help alleviate bias towards well-sampled  
lineages, 280 of the 3604 high-quality draft genomes with identical phylogenetic marker genes were  
not used during the generation of simulated genomes. Simulated genomes were generated at varying  
degrees of completeness and contamination using three distinct random sampling models. Under the  
575 random fragment model, each contig comprising a genome was fragmented into non-overlapping  
windows of a fixed size between 5 and 50 kbp. This size range was selected as it approximates the  
contig lengths of genomes recovered from metagenomic data or single-cell genomics: the mean N50  
of the GEBA-MDM single-cell genomes, Wrighton acetate-amended aquifer population genomes, and  
Sharon infant gut population genomes is ~28 kbp, ~17 kbp, and ~ 12 kbp, respectively. In order to  
580 generate genomes at a desired level of completeness and contamination fragments were sampled  
without or with replacement, respectively. Windows were sampled until a simulated genome had  
completeness and contamination equal to or just greater than the target values. Generation of

simulated genomes was limited to draft genomes as finished genomes were used to determine appropriate lineage-specific marker sets suitable for evaluating genomes (**Fig. 3**).

585 The 2430 draft reference genomes comprised of  $\geq 20$  contigs were used to simulate partial and contaminated genomes reflecting the characteristics of assembled contigs. Under this random contig model, genomes were generated by randomly removing contigs until the simulated genome reached or fell below a target completeness level. Contamination was introduced by randomly adding contigs with replacement from a single randomly selected genome until the desired level of contamination was reached or exceeded. These 2430 draft genomes were also used to generate genomes reflecting the limitations of metagenomic binning methods which rely on the statistical properties of contigs (e.g., tetranucleotide signature, coverage) to establish putative population genomes. To simulate this, partial genomes were generated by randomly removing contigs with a probability inversely proportional to their length until the simulated genome reached or fell below a target completeness level. Contamination was introduced by randomly selecting another draft reference genome and adding contigs from this genome with a probability inversely proportional to length until the desired level of contamination was reached or exceeded.

### ***Evaluation using Simulated Genomes***

600 Evaluation of CheckM was performed using simulated genomes generated at all combinations of 50, 70, 80, 90, 95 and 100% completeness with 0, 5, 10, 15, or 20% contamination. Marker genes and marker sets were inferred with the test genome removed from the set of reference genomes (i.e., leave-one-out testing) and their performance evaluated by considering the error in completeness and contamination estimates. To evaluate the performance of the lineage-specific markers selected by the simulation framework (**Fig. 3**), results were compared to the lineage-specific markers resulting in the best performance as determined by applying Equation 4 independently to each set of simulated genomes generated from a test genome at a specific level of completeness and contamination. This represents a highly idealized case, as it assumes a method capable of selecting different optimal



lineage-specific markers for the same genome under varying levels of completeness and contamination.

### 610 ***Evaluation of Universal- and Domain-level Marker Gene Sets***

Several universal- and domain-level marker gene sets were obtained either directly from the authors or from available software packages (**Supplemental Table S1**). PhyloSift (Darling et al. 2014) originally used the 40 marker genes suggested by Wu et al. (2013), but was reduced to 37 markers in version 1.0.1 of the PhyloSift software. Marker genes were identified using the pre-calculated 'gathering' and 'noise' cutoffs for the Pfam and TIGRFAMs models, respectively. Marker genes of COG or custom protein families were identified using an e-value cutoff of  $1e^{-5}$ ,  $1e^{-10}$ ,  $1e^{-15}$ , and  $1e^{-20}$  and results reported for the cutoff providing the best mean performance. Results were highly similar across this range of e-values.

### ***Evaluation of Taxonomic Novelty***

620 The influence of taxonomic novelty on genome quality estimates was evaluated by identifying 'natural' novelty within the test set of high-quality draft genomes used to generate simulated genomes. A test genome was classified as having taxonomic novelty at a given rank (e.g., genus) if 1) it was the only genome within the reference genome tree from the named group (e.g., the only genome from genus *Ruminobacter*), and 2) the parent group (e.g., the family *Succinivibrionacea*) contains at least 2 named groups at the rank of interest (e.g., *Ruminobacter* and at least one other *Succinivibrionacea* genus). The taxonomic rank of a lineage-specific marker set is defined as the most specific taxonomic rank containing the internal node from which the marker set was inferred (e.g., a marker set defined at an internal node between a named order and class was assigned to the broader rank of class).

### 630 ***Genome Datasets***

Population genomes from the Wrighton et al. (2012) and Sharon et al. (2013) studies were downloaded from ggKbase (<http://ggkbase.berkeley.edu/>) on March 31, 2014. Tyson et al. (2004) and

Meyerdierks et al. (2010) population genomes were obtained from NCBI. The population genomes from the Albertsen et al. (2013) study can be obtained from <http://ecogenomic.org/checkm/public-data>. Reference genomes at NCBI and IMG are occasionally removed or modified. For posterity, the reference genomes analyzed in this paper have been archived at <http://ecogenomic.org/checkm/public-data>. The GEBA, GEBA-KMG, GEBA-PCC, GEBA-RNB, GEBA-MDM, and HMP genomes comprise part of the data downloaded from IMG on April 4, 2014.

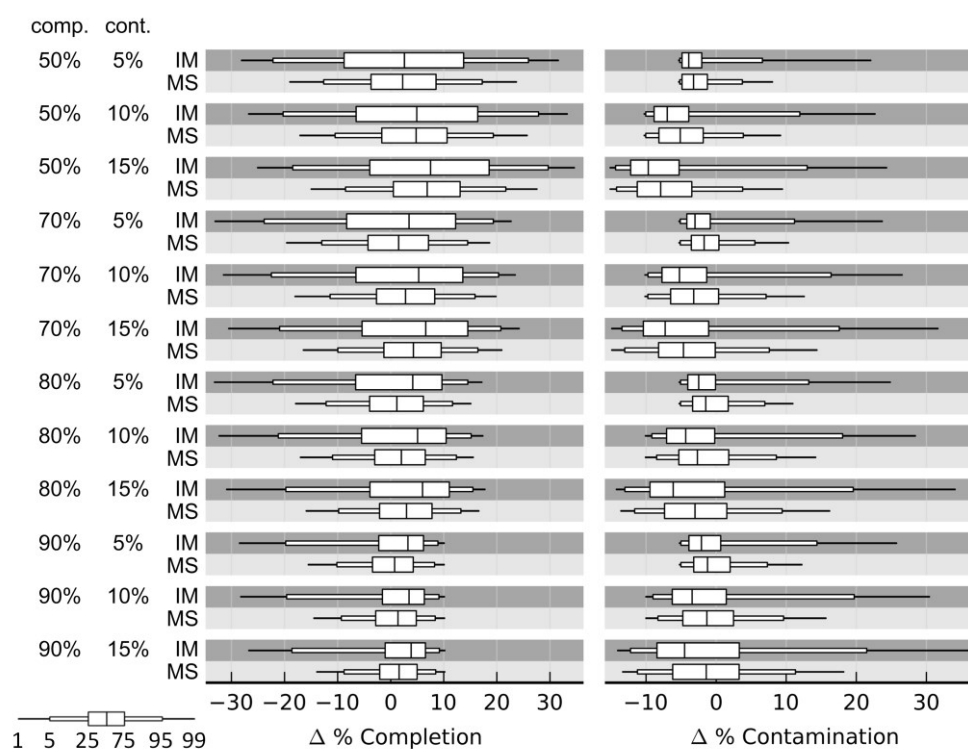
## Software Availability

640 CheckM is open source software available at <http://ecogenomics.github.io/CheckM>. CheckM v0.9.4 was used during the preparation of this manuscript and is available as Supplemental Material.

## Acknowledgements

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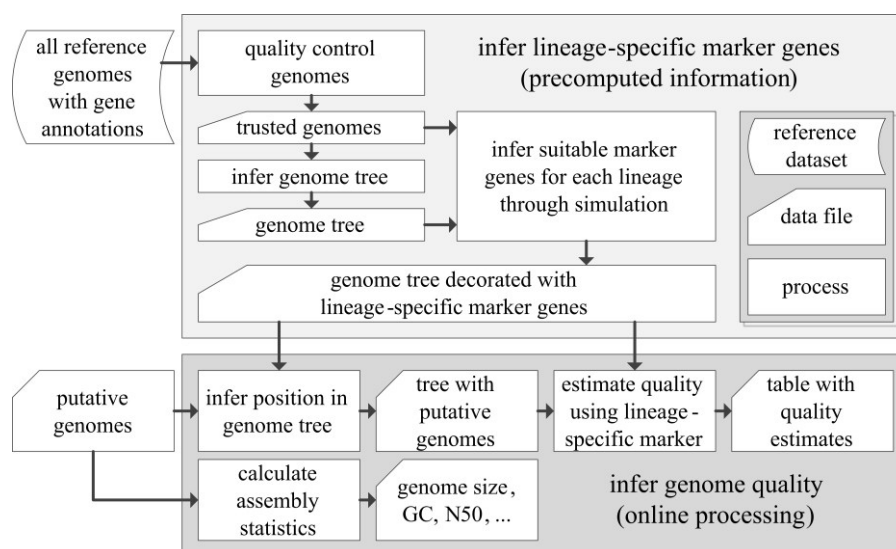
## Figure Legends



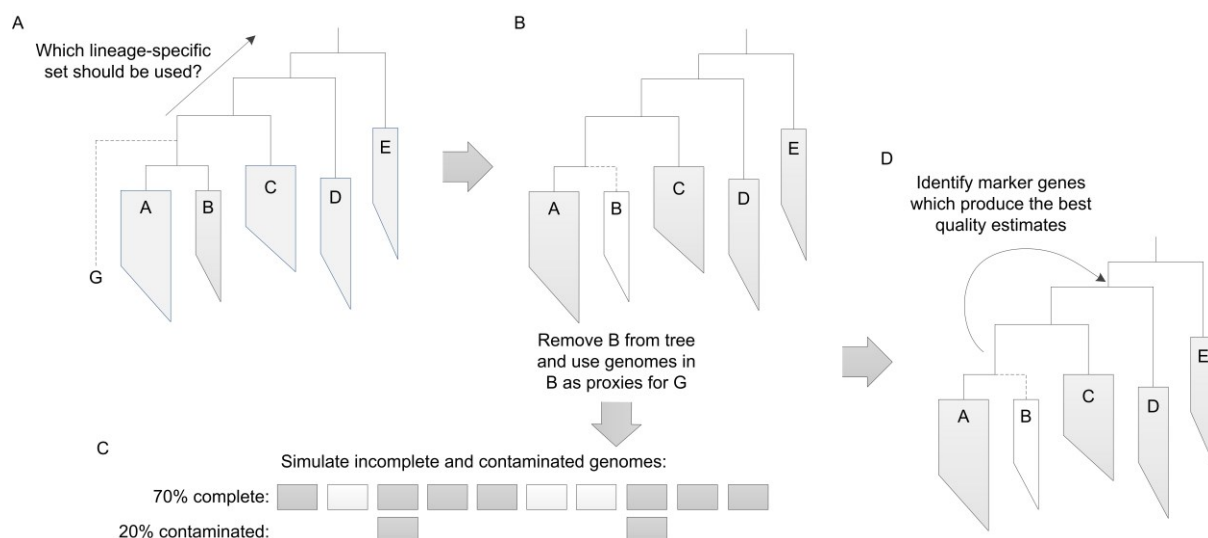
655

**Figure 1.** Error in completeness and contamination estimates on simulated genomes with 50%, 70%, 80%, or 90% completeness (comp.) and 5%, 10%, or 15% contamination (cont.). Quality estimates were determined using domain-level marker genes treated as individual markers (IM) or organized into collocated marker sets (MS). Simulated genomes were generated under the random fragment model from 3324 draft genomes spanning 39 classes (20 phyla) with each draft genome being used to generate 20 simulated genomes. A systematic bias in the estimates results in completeness being overestimated on average (median value to the right of zero) and contamination being underestimated on average (median value to the left of zero). Results are summarized using box-and-whisker plots showing the 1<sup>st</sup> (99<sup>th</sup>), 5<sup>th</sup> (95<sup>th</sup>), 25<sup>th</sup> (75<sup>th</sup>), and 50<sup>th</sup> percentiles.

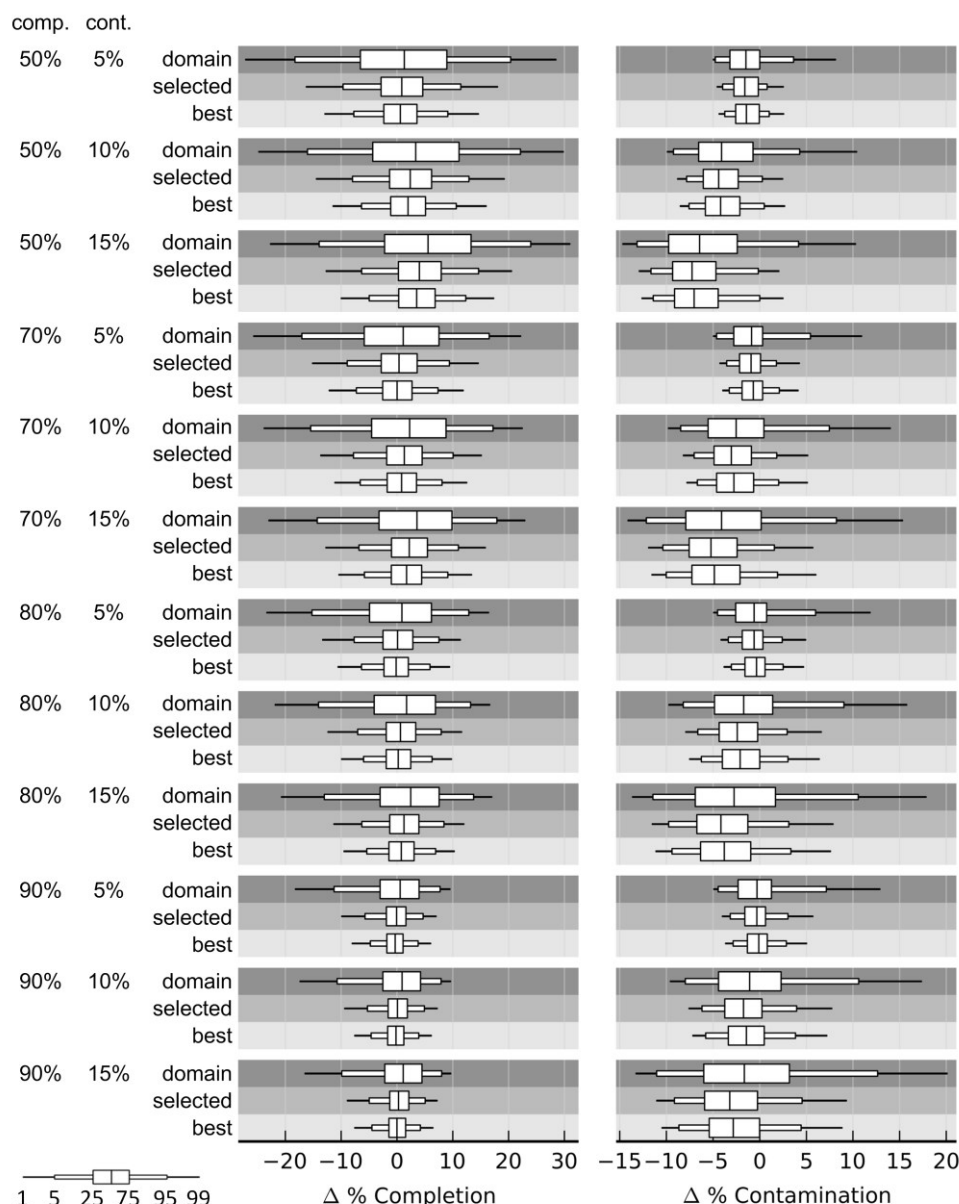
660



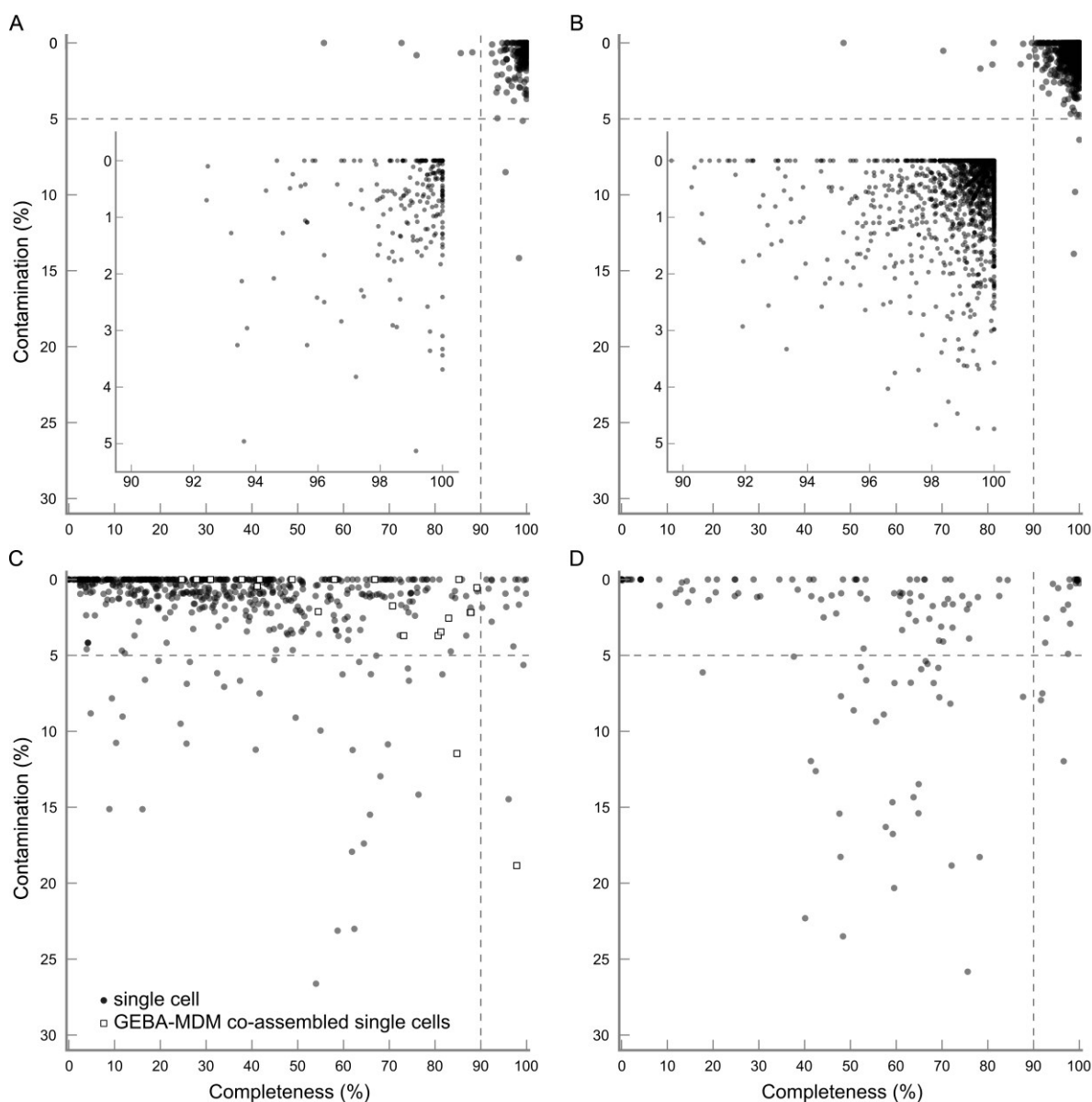
665 **Figure 2.** CheckM consists of a workflow for precomputing lineage-specific marker genes for each  
branch within a reference genome tree (top box) and an online workflow for inferring the quality of  
putative genomes (bottom box). Starting with a set of annotated reference genomes, the quality of  
these genomes is assessed in order to produce a set of near-complete genomes suitable for inferring  
marker genes. These genomes form the basis of a reference genome tree. A simulation framework is  
670 then used to associate each branch in the reference genome tree a lineage-specific marker set suitable  
for robustly estimating the quality of genomes placed along a given branch (Fig. 3). To determine the  
quality of a putative genome, its position within the reference genome tree is inferred in order to  
establish the set of marker genes suitable for assessing its quality. These marker genes are identified  
within the putative genome and the presence/absence of these genes used to estimate its completeness  
675 and contamination.



**Figure 3.** Overview of simulation framework for selecting lineage-specific marker genes. To evaluate a genome  $G$ , it is placed into a reference genome tree (A). Each parental node from the point of insertion to the root of the tree defines a lineage-specific marker set which can be used to estimate the completeness and contamination of this genome. To select a suitable set of lineage-specific marker genes for evaluating  $G$ , the genomes in the child lineage of  $G$  with the fewest genomes were used as proxies for  $G$  (B). Genomes at different levels of completeness and contamination were simulated from these proxy genomes by subsampling and duplicating fixed sized genomic fragments (C). Each parental marker set was then used to estimate the completeness and contamination of these simulated genomes, and the marker set resulting in the best average performance over all simulated genomes identified (D). This marker set is used to assess the quality of any genomes subsequently inserted along this branch.



**Figure 4.** Error in completeness and contamination estimates on simulated genomes with 50%, 70%, 80%, or 90% completeness and 5%, 10%, or 15% contamination. Quality estimates were determined using i) domain: marker sets inferred across all archaeal or bacterial genomes, ii) selected: marker sets inferred from genomes within the lineage selected by CheckM, and iii) best: marker sets inferred from genomes within the lineage producing the most accurate estimates. Marker genes were organized into collocated marker sets in all cases. Simulated genomes were generated under the random contig model from 2430 draft genomes spanning 31 classes (18 phyla) with each draft genome being used to generate 20 simulated genomes.



705

**Figure 5.** Lineage-specific completeness and contamination estimates for (A) 262 isolates annotated as finished in IMG, (B) 2019 isolates annotated as draft in IMG, (C) 632 genomes recovered using single-cell genomics, and (D) 146 population genomes recovered from metagenomic data. Dashed lines indicate the criteria required for a genome to be considered a near-complete genome with low contamination. Insets give a more detailed view of the quality of the isolate genomes. The 2281 isolate genomes were obtained from IMG and sequenced as part of the GEBA, GEBA-KMG, GEBA-PCC, GEBA-RNB, or HMP initiatives.



## Tables

710 **Table 1.** Mean absolute error ( $\pm$  std. dev.) in completeness (comp.) and contamination (cont.) estimates for i) domain: marker sets inferred across all archaeal or bacterial genomes, ii) selected: marker sets inferred from genomes within the lineage selected by CheckM, and iii) best: marker sets inferred from genomes within the parental lineage producing the most accurate estimates.

Simulation model	Domain		Selected		Best	
	Comp. (%)	Cont. (%)	Comp. (%)	Cont. (%)	Comp. (%)	Cont. (%)
random fragment, 5 kbp	4.3 $\pm$ 4.29	3.8 $\pm$ 3.73	2.6 $\pm$ 2.75	2.4 $\pm$ 2.49	2.3 $\pm$ 2.51	2.2 $\pm$ 2.37
random fragment, 20 kbp	5.0 $\pm$ 4.89	4.3 $\pm$ 4.23	3.0 $\pm$ 3.06	2.7 $\pm$ 2.73	2.6 $\pm$ 2.75	2.4 $\pm$ 2.54
random fragment, 50 kbp	5.7 $\pm$ 5.37	4.7 $\pm$ 4.65	3.4 $\pm$ 3.41	2.9 $\pm$ 3.01	2.9 $\pm$ 3.04	2.6 $\pm$ 2.77
random contig	5.4 $\pm$ 5.85	4.1 $\pm$ 4.37	3.0 $\pm$ 3.47	3.3 $\pm$ 3.43	2.5 $\pm$ 2.90	3.1 $\pm$ 3.27
inverse length	6.6 $\pm$ 6.54	5.6 $\pm$ 5.26	4.2 $\pm$ 4.38	5.3 $\pm$ 4.92	3.6 $\pm$ 3.91	4.9 $\pm$ 4.71

715 **Table 2.** Completeness and contamination of genomes from large-scale sequencing projects.

Isolates	Genomes	Completeness (% of genomes)				Contamination (% of genomes)			
		100%	$\geq 95\%$	$\geq 90\%$	$< 90\%$	0%	$\leq 5\%$	$\leq 10\%$	$> 10\%$
GEBA	244	34.0	60.7	4.5	0.8	28.3	70.5	0.4	0.8
GEBA-KMG	724	35.5	62.8	1.7	0	31.6	67.8	0.3	0.3
GEBA-PCC	55	20.0	78.2	1.8	0	20.0	78.2	1.8	0
GEBA-RNB	92	55.4	44.6	0	0	23.9	76.1	0	0
HMP	1166	26.1	71.6	1.5	0.8	36.3	63.2	0.3	0.2
<i>Single cells</i>									
GEBA-MDM	201	0	0	1.5	98.5	51.2	48.3	0.5	0.0
GEBA-MDM (combined)	21	4.8	0	4.8	90.5	28.6	52.4	9.5	9.5
IMG single cell	410	0	3.4	1.0	95.6	31.5	53.3	8.0	7.1
<i>Metagenomics</i>									
Sludge bioreactor	13	7.7	61.5	0	30.8	30.8	61.5	7.7	0
Acid mine drainage	5	0	0	20.0	80.0	0	40.0	40.0	20.0
Infant gut	16	0	43.8	0	56.2	50.0	43.8	0	6.2
Acetate-amended aquifer	90	0	1.1	2.2	96.7	15.6	44.4	13.3	26.7
Acetate-amended aquifer*	22	0	0	13.6	86.4	13.6	68.2	9.1	9.1
<i>Mixed</i>									
'Finished' IMG genomes	2360	26.0	68.4	2.6	3.0	37.4	62.0	0.5	0.1

\* re-binning of select Wrighton et al. (2012) bins by Albertsen et al. (2013)

720 References: GEBA (Wu et al. 2009), GEBA-PCC (Shih et al. 2012), HMP (Turnbaugh et al. 2007), GEBA-MDM (Rinke et al. 2013), IMG (Markowitz et al. 2014), Sludge bioreactor (Albertsen et al. 2013), Acid mine drainage (Tyson et al. 2004), Infant gut (Sharon et al. 2013), Acetate-amended aquifer (Wrighton et al. 2012), IMG (Markowitz et al. 2014). GEBA-RNB genomes were produced by the US Department of Energy Joint Genome Institute.

**Table 3.** Controlled vocabulary of draft genome quality based on estimated genome completeness and contamination.

Completeness	Classification	Contamination	Classification
$\geq 90\%$	near	$\leq 5\%$	low
$\geq 70\%$ to $90\%$	substantial	5% to $\leq 10\%$	medium
$\geq 50\%$ to $70\%$	moderate	10% to $\leq 15\%$	high
$< 50\%$	partial	$> 15\%$	very high

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