

MG7: Configurable and scalable 16S data analysis

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ABSTRACT

As part of the Cambrian explosion of omics data, metagenomics brings to the table a specific, defining trait: its social essence. The *meta* prefix exerts its influence, with multitudes manifesting themselves everywhere; from samples to data analysis, from actors involved to (present and future) applications. Of these dimensions, data analysis is where needs lay further from what current tools provide. Key features are, among others, scalability, reproducibility, data provenance and distribution, process identity and versioning. These are the goals guiding our work in MG7, a 16S metagenomics data analysis system. The basic principle is a new approach to data analysis, where configuration, processes, or data locations are static, type-checked and subject to the standard evolution of a well-maintained software project. Cloud computing, in its Amazon Web Services incarnation, when coupled with these ideas, produces a robust, safely configurable, scalable tool. Processes, data, machine behaviors and their dependencies are expressed using a set of libraries which bring as much as possible checking and validation to the type level, without sacrificing expressiveness. Together they form a toolkit for defining scalable cloud-based workflows composed of stateless computations, with a static reproducible specification of dependencies, behavior and wiring of all steps. The modeling of taxonomy data is done using Bio4j, where the new paradigm of graph databases allows for both a simple expression of taxonomic assignment tasks and the calculation of taxa abundance values considering the hierarchic structure of the taxonomy tree. MG7 includes a new 16S reference database, *16S-DB7*, built with a flexible and sustainable update system, and the possibility of project-driven personalization.

Keywords: Metagenomics, 16S, taxonomic profiling, Bio4j, Graph databases, Cloud computing, NGS, Genomics, big data, Microbiome, Environmental, 16S Database

INTRODUCTION

During the past decade, metagenomics data analysis is growing exponentially. Some of the reasons behind this are the increasing throughput of massively parallel sequencing technologies (with the derived decrease in sequencing costs), and the wide impact of metagenomics studies (Oulas et al., 2015), especially in human health (diagnostics, treatments, drug response or prevention) (Bikel et al., 2015). We should also mention what could be called the microbiome explosion: all kind of microbiomes (gut, mouth, skin, urinary tract, airway, milk, bladder) are now routinely sequenced in different conditions of health and disease, or after different treatments. The impact of microbiome analysis is also being felt in environmental sciences (Ufarté et al., 2015), crop sciences, the agrifood sector (Coughlan et al., 2015), bioenergy (Yang et al., 2016), and biotechnology in general (Cowan et al., 2015) (Kodzius and Gojbori, 2015). These new possibilities for exploring the diversity of micro-organisms in the most varied environments are opening new research areas, and drastically changing the existing ones.

As a consequence, the challenge is thus moving (as in other fields) from data acquisition to data analysis: the amount of data is expected to be overwhelming in a very short time (Stephens et al., 2015).

Genome researchers have raised the alarm over big data in the past (Hayden, 2015), but even a more serious challenge might be faced with the metagenomics boom. If we compare metagenomics data with other genomics data used in clinical genotyping we find a differential feature: the key role of time. Thus, for example, in some longitudinal studies, serial sampling from the same patient (Faust et al., 2015) along several weeks (or years) is being used for the follow up of some intestinal pathologies,

for studying the evolution of the gut microbiome after antibiotic treatment, or for colon cancer early detection (Zeller et al., 2014) (Garrett, 2015). This need of sampling across time adds more complexity to metagenomics data storage and demands adapted algorithms to detect state variations across time as well as idiosyncratic commonalities of the microbiome of each individual (Franzosa et al., 2015). In addition to the intra-individual sampling-time dependence, metagenomic clinical test results vary depending on the specific region of extraction of the clinical specimen. This local variability adds complexity to the analysis since different localizations (different tissues, different anatomical regions, healthy or tumor tissues) are required to have a sufficiently complete landscape of the human microbiome. Moreover, re-analysis of old samples using new tools and better reference databases might be also demanded from time to time.

Other disciplines such as astronomy or particle physics have faced the big data challenge before. A key difference is the existence of standards for data processing (Stephens et al., 2015); in metagenomics global standards for converting raw sequence data into processed data are not yet well defined, and there are shortcomings derived from the fact that most bioinformatics methodologies used for metagenomics data analysis were designed for scenarios very different from the current one. These are some of the aspects that have suffered crucial changes and advances with a direct impact in metagenomics data analysis:

1. **Sequence data:** the reads are larger, the sequencing depth and the number of samples of each project are considerably bigger. The first metagenomics studies were very local projects, while nowadays the most fruitful studies are done at a global level (international, continental, national). This kind of global studies has yielded the discovery of clinical biomarkers for diseases of the importance of cancer, obesity or inflammatory bowel diseases and has allowed exploring the biodiversity of varied earth environments.
2. **The genomics explosion:** its effect being felt in this case in the reference sequences. The immense amount of sequences available in public repositories demands new strategies for curation, update and storage of metagenomics reference databases: current models will (already) have problems to face the future avalanche of metagenomic sequence data.
3. **Cloud computing:** the appearance of new models for massive computation and storage such as the cloud-based platforms, or the widespread adoption of programming methodologies like functional programming, or, more speculatively, dependently typed programming. The new possibilities that these advances offer must have a direct impact in metagenomics data analysis.
4. **Open science:** the new social manner to do science, particularly so in genomics, brings its own set of requirements. Metagenomics evolves in a social and global scenario following a science democratization trend in which many small research groups from distant countries share a common big metagenomics project; this global cooperation demands systems allowing for reproducible data analysis, data interoperability, and tools and practices for asynchronous collaboration between different groups.

RESULTS

Overview

Considering the current new metagenomics scenario and to tackle the challenges posed by metagenomics big data analysis outlined in the Introduction we have designed a new open source methodology for analyzing metagenomics data. It exploits the new possibilities that cloud computing offers to get a system robust, programmatically configurable, modular, distributed, flexible, scalable and traceable in which the biological databases of reference sequences can be easily updated and/or frequently substituted by new ones or by databases specifically designed for focused projects.

These are some of the more innovative MG7 features:

- Static reproducible specification of dependencies and behavior of the different components using *Statika* and *Datasets*
- Parallelization and distributed analysis based on AWS, with on-demand infrastructure as the basic paradigm
- Definition of complex workflows using *Loquat*, a composable system for scaling/parallelizing stateless computations especially designed for AWS

- A new approach to data analysis specification, management and specification based on working with it in exactly the same way as for a software project, together with the extensive use of compile-time structures and checks
- Modeling of the taxonomy tree using the new paradigm of graph databases (Bio4j, (Pareja-Tobes et al., 2015)). It facilitates the taxonomic assignment tasks and the calculation of the taxa abundance values considering the hierarchic structure of taxonomy tree
- Exhaustive per-read taxonomic assignment using two complementary assignment algorithms: Lowest Common Ancestor (LCA) and Best BLAST Hit
- Using a new 16S database of reference sequences (16S-DB7) with a flexible and sustainable system of updating and project-driven customization

Libraries and resources

In this section we describe the resources and libraries developed by the authors on top of which MG7 is built. All MG7 code is written in [Scala](#), a hybrid object-functional programming language. Scala was chosen based on the possibility of using certain advanced programming styles, and Java interoperability, which let us build on the vast number of existing Java libraries; we take advantage of this when using Bio4j as an API for the NCBI taxonomy. It has support for type-level programming, type-dependent types (through type members) and singleton types, which permits a restricted form of dependent types where types can depend essentially on values determined at compile time (through their corresponding singleton types). Conversely, through implicits one can retrieve the value corresponding to a singleton type.

Statika: machine configuration and behavior

[Statika](#) is a Scala library developed by AA and EPT which serves as a way of defining and composing machine behaviors statically. The main component are **bundles**. Each bundle declares a sequence of computations (its behavior) which will be executed in an **environment**. A bundle can *depend* on other bundles, and when being executed by an environment, its DAG (Directed Acyclic Graph) of dependencies is linearized and run in sequence. In our use, bundles correspond to what an EC2 instance should do and an environment to an AMI (Amazon Machine Image) which prepares the basic configuration, downloads the Scala code and runs it.

Datasets: a mini-language for data

[Datasets](#) is a Scala library developed by AA and EPT with the goal of being a Scala-embedded mini-language for datasets and their locations. **Data** is represented as type-indexed fields: keys are modeled as singleton types, and values correspond to what could be called a denotation of the key: a value of type `Location` tagged with the key type. Then a **Dataset** is essentially a collection of data, which are guaranteed statically to be different through type-level predicates, making use of the value–type correspondence which can be established through singleton types and implicits. A dataset location is then just a list of locations formed by locations of each dataset key. All this is based on what could be described as an embedding in Scala of an extensible record system with concatenation on disjoint labels, in the spirit of (Harper and Pierce, 1990) (Harper and Pierce, 1991). For that *Datasets* uses the [ohnosequences/cosas](#) library.

Data keys can further have a reference to a **data type**, which, as the name hints at, can help in providing information about the type of data we are working with. For example, when declaring Illumina reads as a data, a data type containing information about the read length, insert size or end type (single or paired) is used.

A **location** can be, for example, an S3 object or a local file; by leaving the location type used to denote particular data free we can work with different “physical” representations, while keeping track of to which logical data they are a representation of. Thus, a process can generate locally a `.fastq` file representing the merged reads, while another can put it in S3 with the fact that they all correspond to the “same” merged reads is always present, as the data that those “physical” representations denote.

Loquat: Parallel data processing with AWS

[Loquat](#) is a library developed by AA, EK and EPT designed for the execution of embarrassingly parallel tasks using S3, SQS and EC2 Amazon services.

A *loquat* executes a process with explicit input and output datasets (declared using the *Datasets* library described above). Workers (EC2 instances) read from an SQS queue the S3 locations for both input and

149 output data; then they download the input to local files, and pass these file locations to the process to be
150 executed. The output is then put in the corresponding S3 locations.

151 A manager instance is used to monitor workers, provide initial data to be put in the SQS queue and
152 optionally release resources depending on a set of configurable conditions.

153 Both worker and manager instances are *Statika* bundles. The worker can declare any dependencies
154 needed to perform its task: other tools, libraries, or data.

155 All configuration such as the number of workers or the instance types is declared statically, the
156 specification of a loquat being ultimately a Scala object. Deploy and resource management methods make
157 easy to use an existing loquat either as a library or from (for example) a Scala REPL.

158 The input and output (and their locations) being defined statically has several critical advantages.
159 First, composing different loquats is easy and safe; just use the output types and locations of the first one
160 as input for the second one. Second, data and their types help in not mixing different resources when
161 implementing a process, while serving as a safe and convenient mechanism for writing generic processing
162 tasks. For example, merging paired-end Illumina reads generically is easy as the data type includes the
163 relevant information (insert size, read length, etc) to pass to a tool such as FLASH.

164 **Type-safe eDSLs for BLAST and FLASH**

165 We developed our own Scala-based type-safe eDSLs (embedded Domain Specific Languages) for **FLASH**
166 ([Magoč and Salzberg, 2011](#)) and **BLAST** ([Camacho et al., 2009](#)) expressions and their execution.

167 In the case of BLAST we use a model where we can guarantee for each BLAST command expression
168 at compile time that

- 169 • all required arguments are provided
- 170 • only valid options are provided
- 171 • correct types for each option value
- 172 • valid output record specification

173 Generic type-safe parsers returning a heterogeneous record of BLAST output fields are also available,
174 together with output data defined using *Datasets* which have a reference to the exact BLAST command
175 options which yielded that output. This lets us provide generic parsers for BLAST output which are
176 guaranteed to be correct.

177 In the same spirit as for BLAST, we implemented a type-safe eDSL for FLASH expressions and their
178 execution, supporting features equivalent to those outlined for the BLAST eDSL.

179 **Bio4j and Graph Databases**

180 Bio4j ([Pareja-Tobes et al., 2015](#)) is a data platform integrating data from different resources such as
181 UniProt, the NCBI taxonomy, or GO, in a graph data paradigm. In the assignment phase we use a
182 subgraph containing the NCBI Taxonomy, wrapping in Scala its Java API in a tree algebraic data type.

183 **16S-DB7 Reference Database Construction**

184 The starting point for our 16S reference database is RNA Central ([Consortium et al., 2014](#)), [version 5](#).
185 RNAcentral was chosen being the most up to date, comprehensive RNA sequence repository, including
186 among others all RNA data from Silva, GreenGenes, RDP, ENA, and RefSeq. First we take those
187 sequences which

- 188 1. are annotated as being of rRNA type¹
- 189 2. their length is at least 1300²
- 190 3. have at least one taxonomic assignment to a descendant of *Bacteria* or *Archaea*
- 191 4. their lineage does not contain a set of taxa deemed uninformative³

192 After this first step, we drop redundant assignments: if sequences $S_1 \subseteq S_2$ share an assignment T ,
193 it gets dropped from S_1 ; sequences which as a result of this process end up having no assignments are
194 removed.

¹We are aware of the existence of a gene annotation corresponding to 16S in RNAcentral, that we are **not using** due to a significant amount of 16S sequences lacking it

²Note that 16S sequences are sometimes part of an entry corresponding to whole small subunits; that's why we do not set a maximum length threshold

³for example: "unclassified Bacteria (miscellaneous)" or "unclassified". Assigning a read to an "unclassified" taxon defeats the first and foremost goal of taxonomic profiling: *classification*.

195 Once we have a non-redundant set of ribosomal RNA sequences (containing all those corresponding
196 to 16S), we can apply our procedure for checking internal consistency of taxonomic assignments. We run
197 MG7 using this set of sequences as query, with reference all but the one we are trying to assign. Under
198 the (reasonable) assumption of 16S sequence similarity being correlated with the taxonomy tree topology,
199 the resulting MG7 taxonomic assignment should either

- 200 1. be a taxon *close* to the original assignment, if there are sequences similar enough in the reference
201 database; what we consider as close is: the parent of the MG7 assignment should be contained in
202 the lineage of the original assignment.
- 203 2. be empty, due to this sequence having no similar sequences in the reference database, thus making
204 impossible to do any taxonomic assignment.

205 In any other case, under our assumptions, this assignment should be discarded.

206 All these steps are automated, and integrated with every release of our reference database. The whole
207 process can be repeated and adapted to other subsets of RNACentral; we do this for an 18S reference
208 database.

209 **Workflow Description**

210 The MG7 analysis workflow is summarized in Figure 1. The input files for MG7 are the FASTQ files
211 resulting from a paired-end NGS sequencing experiment.

212 ***Joining reads of each pair using FLASH***

213 In the first step the paired-end reads, designed with an insert size that yields pairs of reads with an
214 overlapping region between them, are assembled using FLASH (Magoč and Salzberg, 2011). FLASH is
215 designed to merge pairs of reads when the original DNA fragments are shorter than twice the length of
216 reads. Thus, the sequence obtained after joining the 2 reads of each pair is larger and has better quality
217 since the sequence at the ends of the reads is refined merging both ends in the assembly. To have a
218 larger and improved sequence is crucial to do more precise the inference of the bacterial origin based on
219 similarity with reference sequences.

220 ***Parallelized BLASTN of each read against the 16S-DB7***

221 The second step is to search for similar 16S sequences in our 16S-DB7 database. The taxonomic
222 assignment for each read is based on BLASTN of each read against the 16S database. Assignment
223 based on direct similarity of each read one by one compared against a sufficiently wide database is
224 considered in different reviews of metagenomics analysis methodologies (Segata et al., 2013) (Morgan
225 and Huttenhower, 2012) as a very exhaustive method for assignment. Some methods of assignment
226 compare the sequences only against the 16S genes from available complete bacterial genomes or avoid
227 computational cost clustering or binning the sequences first, and then doing the assignments only for
228 the representative sequence of each cluster. MG7 carries out an exhaustive comparison of all the reads
229 under analysis and it does not apply any binning strategy. Every read is specifically compared with all the
230 sequences of the 16S database.

231 ***Taxonomic Assignment Algorithms***

232 All the reads are assigned under two different algorithms of assignment: i. Lowest Common Ancestor
233 based taxonomic assignment (LCA) and ii. Best BLAST Hit based taxonomic assignment (BBH). Figure
234 2 displays schematically the LCA algorithm.

235 All the assignment procedures work by default with input the set of BLAST hits which have maximum
236 bitscore among those covering the whole query sequence; we will call this the set of valid hits of a query.
237 Note that the criteria which determines this set of valid hits is fully configurable, and should be adjusted
238 to conform to its meaning in each particular dataset: those hits which we should take into account as valid
239 for assignment. This is of course experiment-dependent, and the criteria for valid hits should account for
240 read quality or technology-specific error profiles.

241 **LCA assignment** The LCA assignment simply computes the lowest common ancestor of the set of
242 valid hits. Other metagenomics analysis approaches (Huson and Weber, 2012) have adopted LCA-based
243 assignment algorithms, as it provides the most precise assignment for a set of taxa, with no further



Figure 1. MG7 analysis workflow. The paired reads in fastq format are merged resulting in only one sequence per read pair. The next step is a parallelized BLASTN of every merged sequence against the 16S reference database 16S-DB7. Then, the mapping of the detected similar sequences in the database to the taxonomy node to which they belong is carried out. This is done using Bio4j that includes a module with all the NCBI taxonomy in a graph connected with the Gene Ontology, Uniprot, and RefSeq graphs. Then the taxonomic assignment is done for each sequence following two different approaches: LCA and BBH, and finally the abundances corresponding to direct and cumulative assignments for each node in percentage and absolute counts are provided for each assignment mode.



Figure 2. Lowest Common Ancestor algorithm for taxonomic assignment. TODO UPDATE

THIS FIGURE! The Left panel displays an example of the application of LCA algorithm in a *sensu stricto* mode. A, B, C and D represent taxonomy tree nodes with assigned reads. Right panel displays the *in line* mode of assignment which is an exception for the *sensu stricto* mode of application of LCA algorithm. The *in line* mode is used when all the nodes are located in a line without bifurcations. In that case the taxon assigned is the most specific (the most distant from the root).

information available⁴; as our reference database is already heavily preprocessed and checked for internal consistency, there is no need for altering the lowest common ancestor in any way, as is customarily done in other cases. MG7 operates under the assumption that hits which fulfill the user-defined criteria for being valid hits should be treated on an equal footing, and in that case taking their LCA is the coherent assignment choice with minimum information loss.

Best BLAST hit assignment We decided to maintain the simpler method of Best BLAST Hit (BBH) for taxonomic assignment because, in some cases, it can provide information about the sequences that adds information to that obtained using the LCA algorithm. With the LCA algorithm, when some reference sequences with BLAST alignments over the required thresholds map to a not sufficiently specific taxID, the read can be assigned to an unspecific taxon near to the root of the taxonomy tree. If the BBH reference sequence maps to more specific taxa, this method, in that case, gives us useful information.

Output for LCA and BBH assignments

MG7 provides independent results for the 2 different approaches, LCA and BBH. The output files include, for each taxonomy node (with some read assigned), abundance values for direct assignment and cumulative assignment. The abundances are provided in counts (absolute values) and in percentage normalized to the number of reads of each sample. Direct assignments are calculated counting reads specifically assigned to a taxonomic node, not including the reads assigned to the descendant nodes in the taxonomy tree. Cumulative assignments are calculated including the direct assignments and also the assignments of the descendant nodes. For each sample MG7 provides 8 kinds of abundance values: LCA direct counts, LCA cumu. counts, LCA direct %, LCA cumu. %, BBH direct counts, BBH cumu. counts, BBH direct % and BBH cumu. %.

Data analysis as a software project

The MG7 16S data analysis workflow is indeed a set of tasks, all of them based in *Loquat*. For each task, a set of inputs and outputs as well as configuration parameters must be statically defined. The user is also free to leave the reasonable defaults for configuration, needing only to define the input and output of the whole workflow. The definition of this configuration is Scala code and the way of starting an MG7 analysis is compiling the project code and launching it from the Scala interactive console.

Code compilation prior to launching any analysis assures that no AWS resources are launched if the analysis is not well-defined, avoiding expenses not leading to any analysis. Besides compile-time checks, runtime checks are made before launch to ensure existence of input data and availability of resources.

⁴the lowest common ancestor of a set of nodes in a tree is just their product in the tree viewed as a poset, with the “parent of” partial order

274 An MG7 analysis is then a Scala project where the user only needs to set certain variables at the code
275 level (input, output and parameters), compile the code and run it. To facilitate the process of setting up
276 the Scala project, a template with sensible defaults is provided.

277 In order to be able to exploit AWS infrastructure for the MG7 analysis, the user needs to set up an
278 AWS account with certain IAM (Identity and Access Management) permission policies that will grant
279 access to the resources used in the workflow.

280 **Availability**

281 MG7 is open source, available at <https://github.com/ohnosequences/mg7> under an [AGPLv3](#) license.

282 **DISCUSSION**

283 We could summarize the most innovative ideas and developments in MG7:

- 284 1. Treating data analysis as a software project. This makes for radical improvements in *reproducibility*,
285 *reuse*, *versioning*, *safety*, *automation* and *expressiveness*
- 286 2. Checking at compile-time: input and output data, their locations and type are expressible and
287 checked at compile-time using *Datasets*
- 288 3. Management of dependencies and machine configurations using *Statika*
- 289 4. Automation of AWS cloud resources and processes, including distribution and parallelization
290 through the use of *Loquat*
- 291 5. Taxonomic data and related operations are treated natively as what they are: graphs, through the
292 use of *Bio4j*
- 293 6. MG7 provides a sustainable model for taxonomic assignment, appropriate to face the challenging
294 amount of data that high throughput sequencing technologies generate

295 We will expand on each item in the following sections.

296 **A new approach to data analysis: data analysis as a software project and checking at compile-time**

297 MG7 proposes to define and work with a particular data analysis task as a software project, using Scala.
298 The idea is that *everything*: data description, their location, configuration parameters and the infrastructure
299 used should be expressed as Scala code, and treated in the same way as any (well-managed) software
300 project. This includes, among other things, using version control systems (`git` in our case), writing tests,
301 making stable releases following [semantic versioning](#) or publishing artifacts to a repository.

302 What we see as key advantages of this approach (when coupled with compile-time specification and
303 checking), are

- 305 • **Reproducibility** the same analysis can be run again with exactly the same configuration in a trivial
306 way.
- 307 • **Versioning** as in any software project, there can be different versions, stable releases, etc.
- 308 • **Reuse** we can build standard configurations on top of this and reuse them for subsequent data
309 analysis. A particular data analysis *task* can be used as a *library* in further analysis.
- 310 • **Decoupling** We can start working on the analysis specification, without any need for available data
311 in a much easier way.
- 312 • **Documentation** We can take advantage of all the effort put into software documentation tools
313 and practices, such as in our case Scaladoc or literate programming. As documentation, analysis
314 processes and data specification live together in the files, it is much easier to keep coherence
315 between them.
- 316 • **Expresiveness and safety** For example in our case we can choose only from valid illumina read
317 types, and then build a default FLASH command based on that. The output locations, being declared
318 statically, are also available for use in further analysis.

319 **Input and output data declaration**

320 An important aspect of the MG7 workflow is the way it deals with data resources. All the data that is
321 going to be used in the analysis or produced as an output is described as Scala code using rich types from
322 the *Datasets* language. This allows the user to specify information about types of data, information that

323 can then be utilized by tools analyzing this data. For example, we can specify that, for the first part of the
324 MG7 workflow, running FLASH in parallel requires illumina paired end reads and produces joined reads.

325 On one hand, specification of the input data allows us to restrict its type and force users to be conscious
326 about what they pass as an input. On the other hand, specification of the output data helps to build a
327 workflow as a *composition* of several parts: we can ensure on the Scala code type level that the output
328 of one component fits as an input for the next component. This is crucial as, obviously, the way a data
329 analysis task works depends a lot on the particular structure of the data. For instance, in the MG7 workflow,
330 using BLAST eDSL, we can precisely describe which format will have the output of the BLAST step,
331 which information it will include, and then in the next step we can reuse this description to parse BLAST
332 output and retrieve the part of the information needed for the taxonomy assignment analysis. Having
333 the data structure described statically as Scala code allows us to be sure that we will not have parsing
334 problems or other issues with incompatible data passed between workflow components.

335 All this does not compromise flexibility in how the user works with data in MG7: having static data
336 declarations as a part of the configuration allows the user to reuse analysis components, or modify them
337 according to particular needs. Besides that, an important advantage of the type-level control is the added
338 protection from the execution (and deployment) of a wrongly configured analysis task, which may lead to
339 significant costs in both time and money.

340 **Tools, data, dependencies and automated deployment**

341 Bioinformatics software often has a complicated installation process and requires various dependencies
342 with unclear versions. This makes the deployment of the bioinformatics tools an involved task and
343 resolving it manually is not a solution in the context of cloud computations. To face this problem,
344 one needs an automated system of managing tools and resources, which will allow an expressive way
345 for describing dependencies between parts of a pipeline and provide a reproducible procedure of its
346 deployment. We have developed *Statika* for this purpose and successfully used it in MG7.

347 Every external tool involved in the workflow is represented as a *Statika* bundle, which is essentially a
348 Scala project describing the installation process of this tool and declaring dependencies on other bundles
349 which will be installed prior to the considered tool itself. Describing relationships between bundles on
350 the code level allows us to track the directed acyclic graph of their dependencies and linearize them to
351 automatically install them sequentially in the right order. Meanwhile, describing the installation process
352 on the code level allows the user to utilize the wide range of available Scala and Java APIs and tools,
353 making installation a well-defined sequence of steps rather than an unreliable script, dependent on a
354 certain environment. *Statika* offers an easy path towards making deployment an automated, reproducible
355 process.

356 Besides bioinformatics tools like BLAST and FLASH, *Statika* bundles are used for wrapping data
357 dependencies and all inner components of the system that require cloud deployment. In particular, all
358 components of *Loquat* are bundles; the user can then define which components are needed for the parallel
359 processing on each computation unit in an expressive way, declaring them as bundle dependencies of the
360 loquat “worker” bundle. This modularization is also important for the matter of making components of
361 the system reusable for different projects and liberating the user from most of the tasks related to their
362 deployment.

363 **Parallel computations in the cloud**

364 The MG7 workflow consists of certain steps, each of which performs some work in parallel, using
365 the cloud infrastructure managed by *Loquat*. It is important to notice the horizontal scalability of this
366 approach. Irrespectively of how much data needs to be processed, MG7 will handle it, by splitting data into
367 chunks and performing the analysis on multiple computation units. The Amazon Elastic Compute Cloud
368 (EC2) service provides a transparent way of managing computation infrastructure, called autoscaling
369 groups. The User can set MG7 configuration parameters, adjusting for each task the amount and hardware
370 characteristics of the EC2 instances they want to use for it. But it is important to note that, as each
371 workflow step is not very resource demanding, it is not needed to hire EC2 instances with some advanced
372 hardware. Instead, an average type will work and you can reduce execution time by simply scaling out the
373 number of instances.

Taxonomy and Bio4j

The hierarchic structure of the taxonomy of the living organisms is a tree, and, hence, is also a graph in which each node, with the exception of the root node, has a unique parent node. It led us to model the taxonomy tree as a graph using the graph database paradigm. Previously we developed Bio4j (Pareja-Tobes et al., 2015), a platform for the integration of semantically rich biological data using typed graph models. It integrates most publicly available data linked with sequences into a set of interdependent graphs to be used for bioinformatics analysis and especially for biological data. MG7 works based on the Bio4j taxonomy module, which contains all the NCBI taxonomy data. It opens the possibility to connect the taxonomic profiling data obtained with MG7 to all the biological knowledge associated to each taxon. Using the information available in Bio4j for all the proteins assigned to each taxon we are connected to all the functional data available in Uniprot related with it.

Future developments

Shotgun metagenomics

It is certainly possible to adapt MG7 to work with shotgun metagenomics data. Simply changing the reference database to include whole genome sequence data could yield interesting results. This could also be refined by restricting reference sequences according to all sort of criteria, like biological function or taxonomy. Bio4j would be an invaluable tool here, thanks to its ability to express complex predicates on sequences using all the information linked with them (GO annotations, UniProt data, NCBI taxonomy, etc).

Comparing groups of samples

The comparison of the taxonomic profiles between different groups of samples is a need for many metagenomics studies. Tasks related with this group-based analysis, such as the extraction of the minimal tree with all the taxa with some direct or accumulated assignment, will be part of a new MG7 module, already in development.

Interactive visualizations based on Biographika

New visualization tools for metagenomics results are undoubtedly needed. Interactivity is a especially interesting feature for metagenomics data visualization, since the expert needs to explore the results in a knowledge-driven way. The majority of the available metagenomics data visualizations are static. We are working in the *Biographika* project (Tobes et al., 2015), to provide interactive rich visualizations on the web for Bio4j data. The development of visualizations specific for MG7 is one of Biographika current goals. Biographika is based on D3.js, the de-facto standard JavaScript data visualization library, and is open source.

MATERIALS AND METHODS

Amazon Web Services

MG7 uses the following Amazon Web Services:

- **EC2** (Elastic Compute Cloud) autoscaling groups for launching and managing computation units
- **S3** (Simple Storage Service) for storing input and output data
- **SQS** (Simple Queue Service) for communication between different components of the system
- **SNS** (Simple Notification Service) for e-mail notifications

These services are used through a Scala wrapper of the official [AWS Java SDK v1.9.25](#): [ohnosequences/aws-scala-tools v0.13.2](#).

Scala

MG7 itself and all the libraries used are written in Scala v2.11.

Statika

MG7 uses [ohnosequences/statika v2.0.0](#) for specifying the configuration and behavior of EC2 instances.

Datasets

MG7 uses [ohnosequences/datasets v0.2.0](#) for specifying input and output data, their type and their location.

Loquat

MG7 uses [ohnosequences/loquat v2.0.0](#) for the specification of data processing tasks and their execution using AWS resources.

BLAST eDSL

MG7 uses [ohnosequences/blast v0.2.0](#). The BLAST version used is v2.2.31+.

FLASH eDSL

MG7 uses [ohnosequences/flash v0.1.0](#). The FLASH version used is v1.2.11.

Bio4j

MG7 uses [bio4j/bio4j v0.12.0-RC3](#) and [bio4j/bio4j-titan v0.4.0-RC2](#) as an API for the NCBI taxonomy.

DISCLOSURE/CONFLICT-OF-INTEREST STATEMENT

All authors work at the *Oh no sequences!* research group, part of Era7 Bioinformatics. Era7 offers metagenomics data analysis services based on MG7. MG7 is open source, available under the OSI-approved AGPLv3 license.

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AUTHOR CONTRIBUTIONS

- **AA** developed *MG7*, *Loquat*, *Statika*, *Datasets*, and *aws-scala-tools*; wrote the paper;
- **EK** developed *nispero* (a prototype for *Loquat* ([Kovach et al., 2014](#))) and *aws-scala-tools*.
- **MM** *MG7* workflow design; curation and design of the *16S-DB7* reference database; wrote the paper.
- **PPT** design and development of the first *MG7* prototype
- **EP** *MG7* workflow design; wrote the paper.
- **RT** *MG7* workflow design, assignment strategy; curation and design of the *16S-DB7* reference database; wrote the paper.
- **EPT** developed *MG7*, *Statika*, *Datasets*, *FLASH/BLAST eDSLs*; data analysis approach and design; reference database automated curation and filtering; wrote the paper.

All authors have read and approved the final manuscript.

REFERENCES

- Bikel, S., Valdez-Lara, A., Cornejo-Granados, F., Rico, K., Canizales-Quinteros, S., Soberón, X., Del Pozo-Yauner, L., and Ochoa-Leyva, A. (2015). Combining metagenomics, metatranscriptomics and viromics to explore novel microbial interactions: towards a systems-level understanding of human microbiome. *Computational and structural biotechnology journal*, 13:390–401.
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., and Madden, T. L. (2009). Blast+: architecture and applications. *BMC bioinformatics*, 10(1):421.
- Consortium, R. et al. (2014). Rnacentral: an international database of ncRNA sequences. *Nucleic acids research*, page gku991.
- Coughlan, L. M., Cotter, P. D., Hill, C., and Alvarez-Ordóñez, A. (2015). Biotechnological applications of functional metagenomics in the food and pharmaceutical industries. *Frontiers in microbiology*, 6.
- Cowan, D. A., Ramond, J.-B., Makhalanyane, T. P., and De Maayer, P. (2015). Metagenomics of extreme environments. *Current opinion in microbiology*, 25:97–102.
- Faust, K., Lahti, L., Gonze, D., de Vos, W. M., and Raes, J. (2015). Metagenomics meets time series analysis: unraveling microbial community dynamics. *Current opinion in microbiology*, 25:56–66.
- Franzosa, E. A., Huang, K., Meadow, J. F., Gevers, D., Lemon, K. P., Bohannan, B. J., and Huttenhower, C. (2015). Identifying personal microbiomes using metagenomic codes. *Proceedings of the National Academy of Sciences*, page 201423854.
- Garrett, W. S. (2015). Cancer and the microbiota. *Science*, 348(6230):80–86.

466 Harper, R. and Pierce, B. (1991). A record calculus based on symmetric concatenation. In *Proceedings of*
467 *the 18th ACM SIGPLAN-SIGACT symposium on Principles of programming languages*, pages 131–142.
468 ACM.

469 Harper, R. W. and Pierce, B. C. (1990). Extensible records without subsumption.

470 Hayden, E. C. (2015). Genome researchers raise alarm over big data. *Nature*.

471 Huson, D. H. and Weber, N. (2012). Microbial community analysis using megan. *Methods in enzymology*,
472 531:465–485.

473 Kodzius, R. and Gojobori, T. (2015). Marine metagenomics as a source for bioprospecting. *Marine*
474 *genomics*.

475 Kovach, E., Alekhin, A., Manrique, M., Pareja-Tobes, P., Pareja, E., Tobes, R., and Pareja-Tobes, E.
476 (2014). Nispero: a cloud-computing based scala tool specially suited for bioinformatics data processing.
477 In *IWBBIO*, pages 1414–1415.

478 Magoč, T. and Salzberg, S. L. (2011). Flash: fast length adjustment of short reads to improve genome
479 assemblies. *Bioinformatics*, 27(21):2957–2963.

480 Morgan, X. C. and Huttenhower, C. (2012). Chapter 12: human microbiome analysis. *PLoS Comput Biol*,
481 8(12):e1002808.

482 Oulas, A., Pavloudi, C., Polymenakou, P., Pavlopoulos, G. A., Papanikolaou, N., Kotoulas, G., Arvani-
483 tidis, C., and Iliopoulos, I. (2015). Metagenomics: Tools and insights for analyzing next-generation
484 sequencing data derived from biodiversity studies. *Bioinformatics and biology insights*, 9:75.

485 Pareja-Tobes, P., Tobes, R., Manrique, M., Pareja, E., and Pareja-Tobes, E. (2015). Bio4j: a high-
486 performance cloud-enabled graph-based data platform. *bioRxiv*, page 016758.

487 Segata, N., Boernigen, D., Tickle, T. L., Morgan, X. C., Garrett, W. S., and Huttenhower, C. (2013).
488 Computational meta’omics for microbial community studies. *Molecular systems biology*, 9(1):666.

489 Stephens, Z. D., Lee, S. Y., Faghri, F., Campbell, R. H., Zhai, C., Efron, M. J., Iyer, R., Schatz,
490 M. C., Sinha, S., and Robinson, G. E. (2015). Big data: Astronomical or genetical? *PLoS Biol*,
491 13(7):e1002195.

492 Tobes, P. P., Tobes, E. P., Manrique, M., Pareja, E., and Tobes, R. (2015). Biographika: rich interactive
493 data visualizations on the web for the research community. *bioRxiv*, page 021063.

494 Ufarté, L., Potocki-Véronèse, G., and Laville, E. (2015). Discovery of new protein families and functions:
495 new challenges in functional metagenomics for biotechnologies and microbial ecology. *Name: Frontiers*
496 *in Microbiology*, 6:563.

497 Yang, C., Xia, Y., Qu, H., Li, A.-D., Liu, R., Wang, Y., and Zhang, T. (2016). Discovery of new cellulases
498 from the metagenome by a metagenomics-guided strategy. *Biotechnology for Biofuels*, 9(1):1.

499 Zeller, G., Tap, J., Voigt, A. Y., Sunagawa, S., Kultima, J. R., Costea, P. I., Amiot, A., Böhm, J., Brunetti,
500 F., Habermann, N., et al. (2014). Potential of fecal microbiota for early-stage detection of colorectal
501 cancer. *Molecular systems biology*, 10(11):766.