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Integrated Alignment Storage and Taxonomic Sorting for High-Throughput Sequencing

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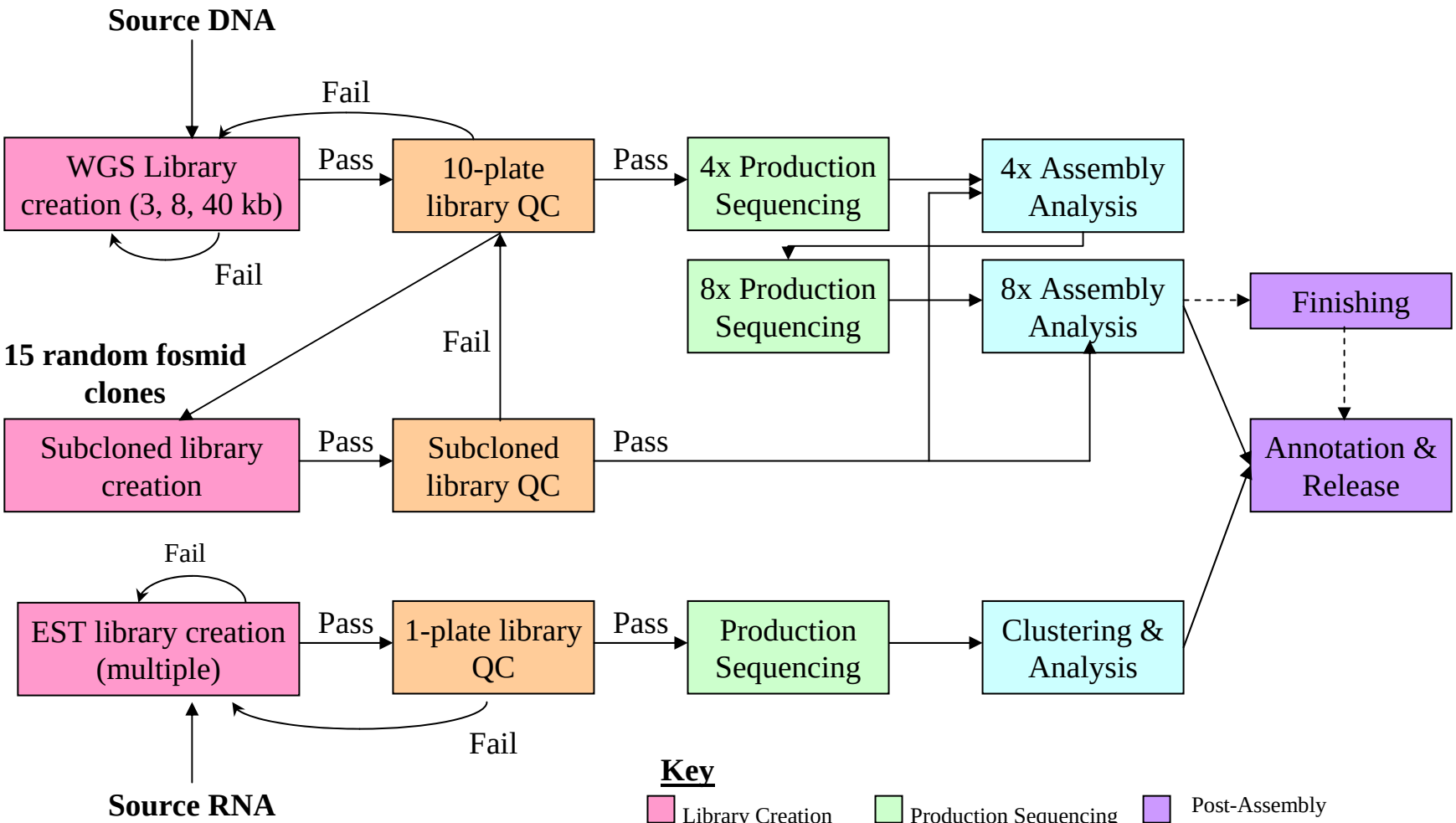
Overview

- What problem needs to be solved?
- Alignment repository & interface
- Taxonomy repository & interface
- Application examples
- Future plans

The Standard WGS Sequencing Project



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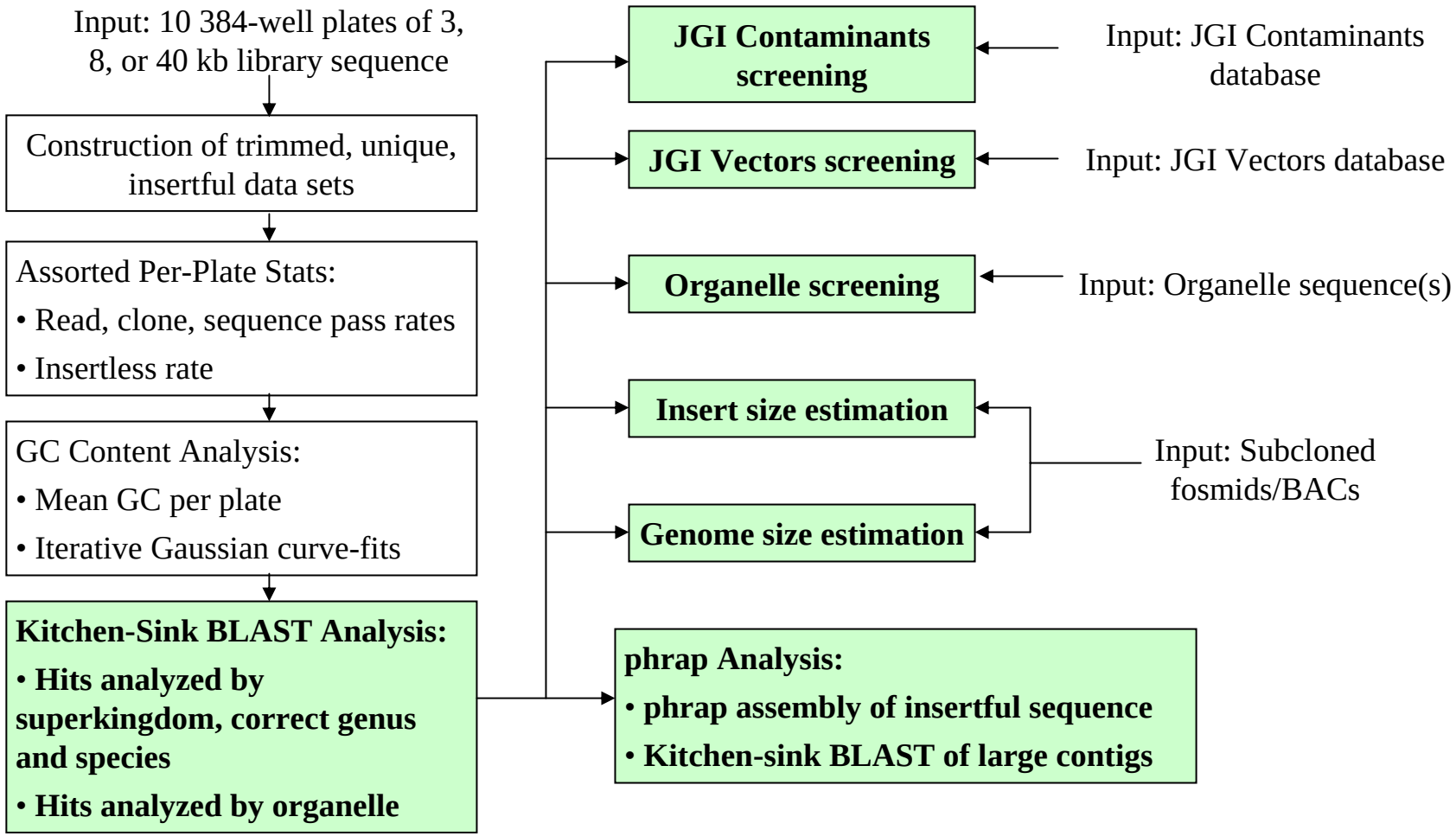
The Standard WGS Library QC Procedure



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What Problem Needs To Be Solved?

- **The goal is to fully automate the standard production analysis processes, downstream of sequence generation**
- **Alignment-related issues**
  - **Volume of alignment data**
  - **Alignment CPU time**
  - **Alignment software maintainability**
  - **Integration with taxonomic information**

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Alignment Software Maintainability

- **Different aligners often have different alignment output formats, some more efficient than others**
- **Modifying every alignment-related tool to handle every output format is a losing battle**
- **Tying tools directly to specific storage mechanisms (flatfile, database, etc.) complicates hardware management, and constrains future development**
- **Implementing an alignment repository with a standard interface decouples alignment-related tools from these details, and helps future-proof them**

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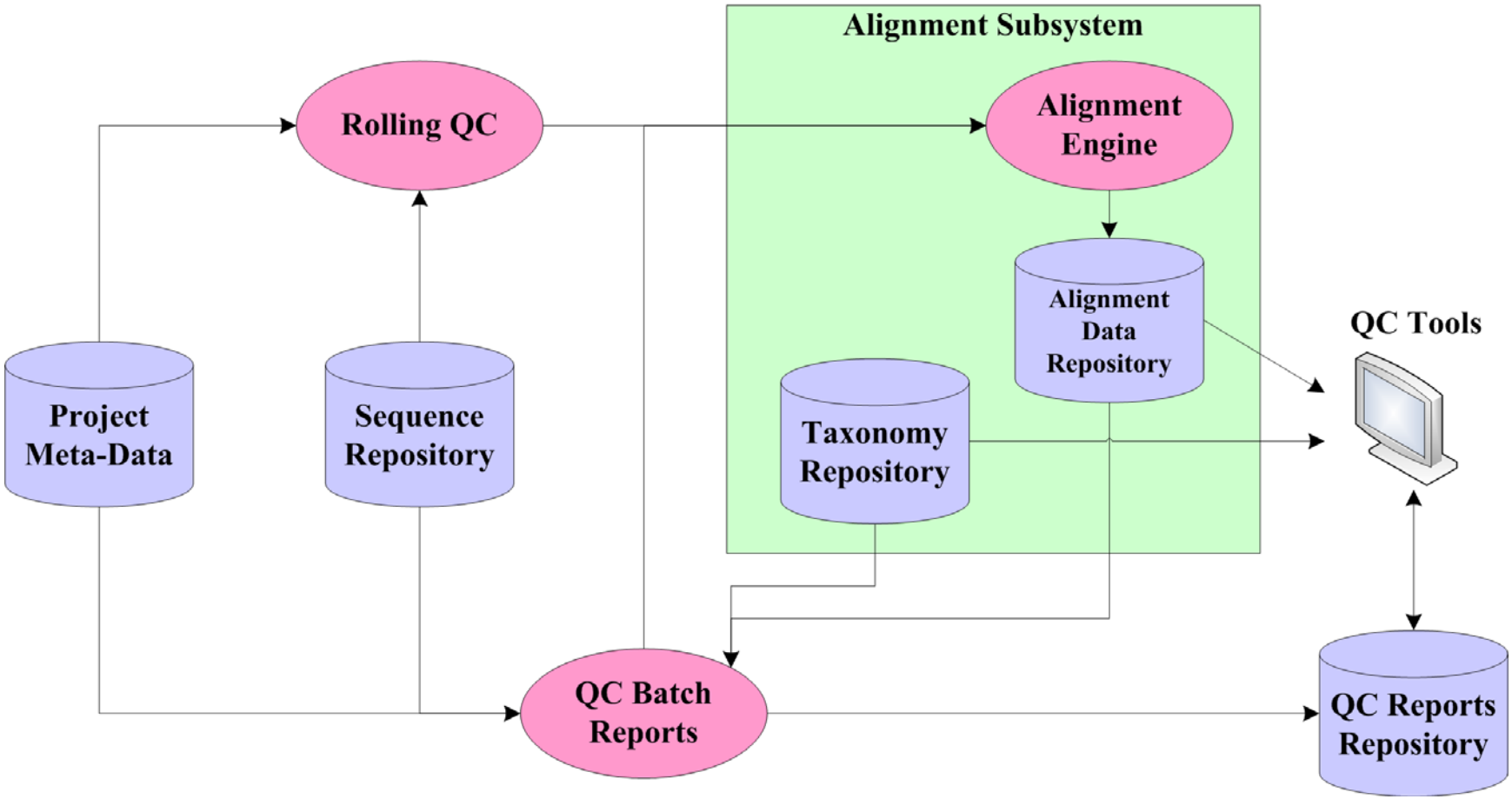
How The Parts Fit Together



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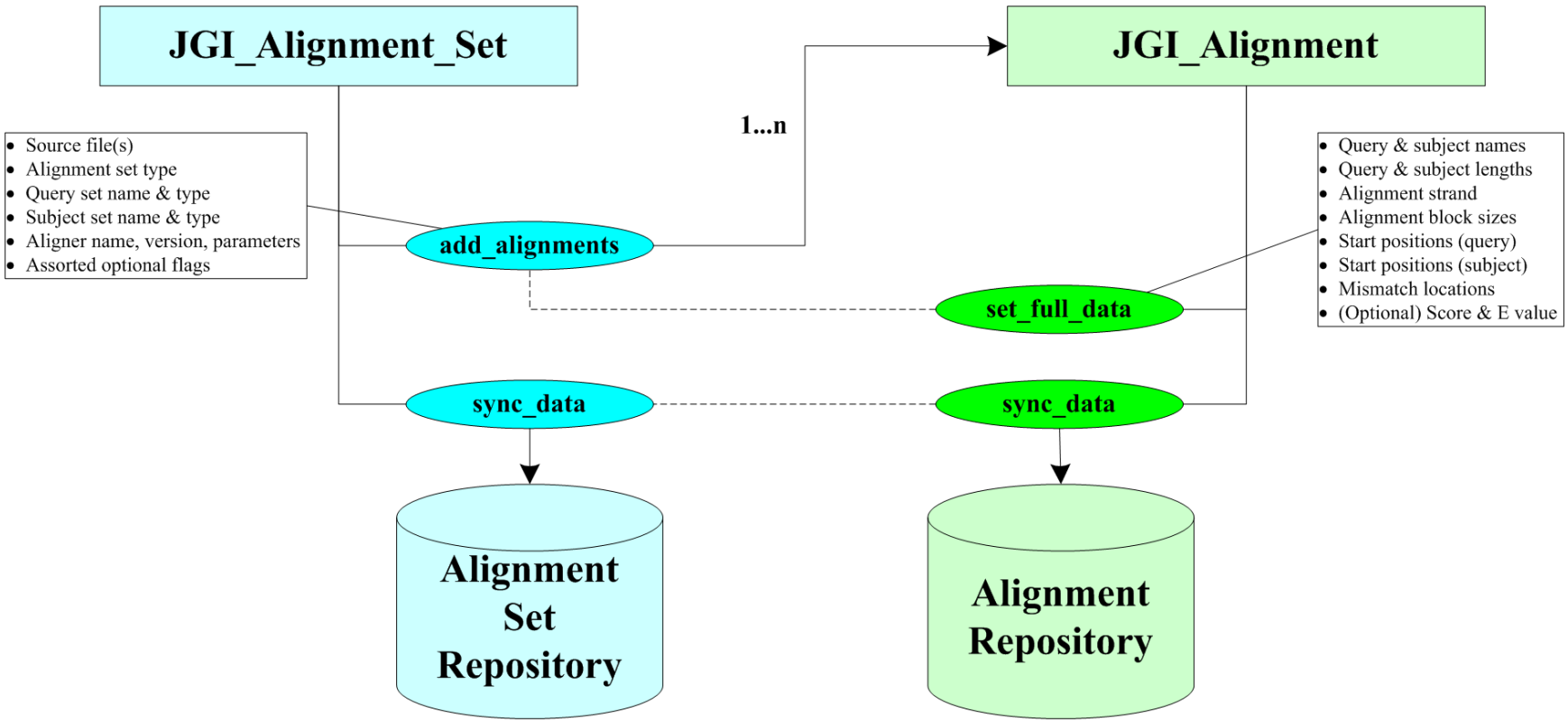
The Alignment Storage Interface



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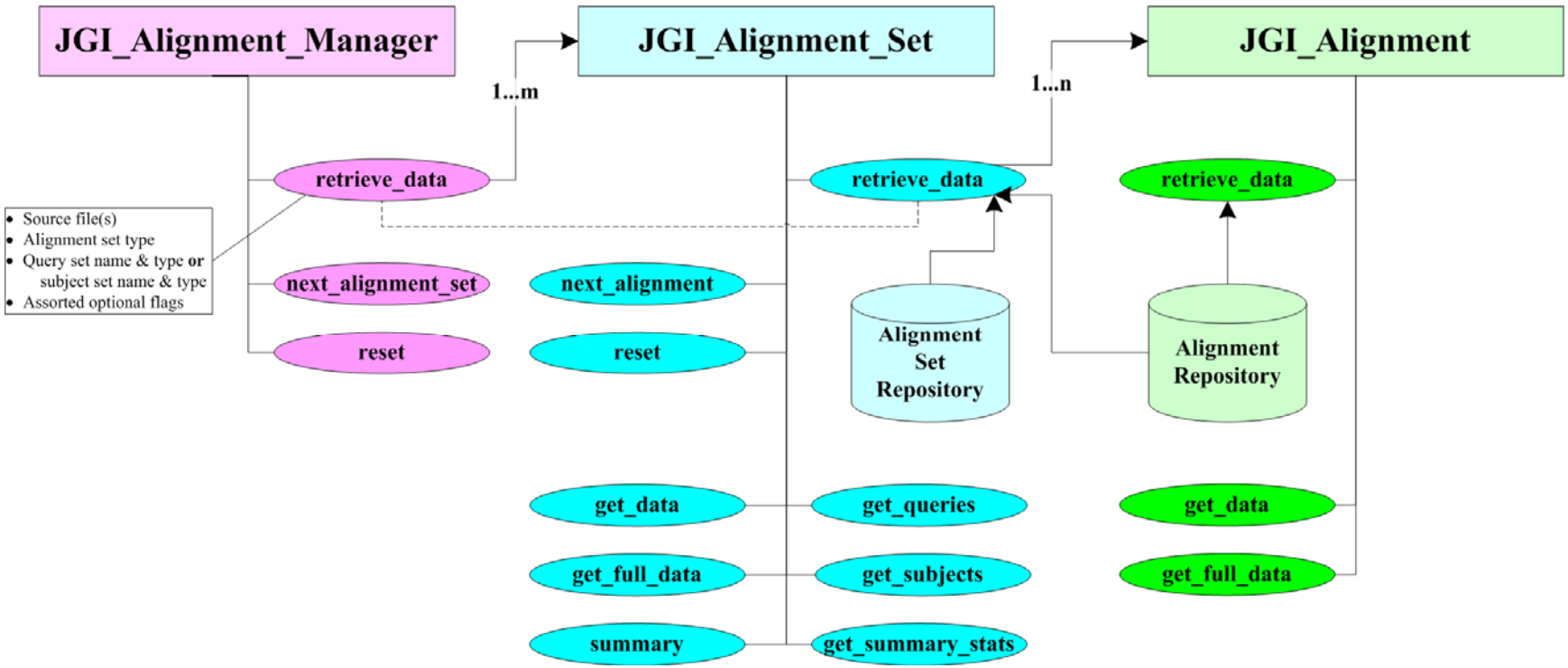
The Alignment Retrieval Interface



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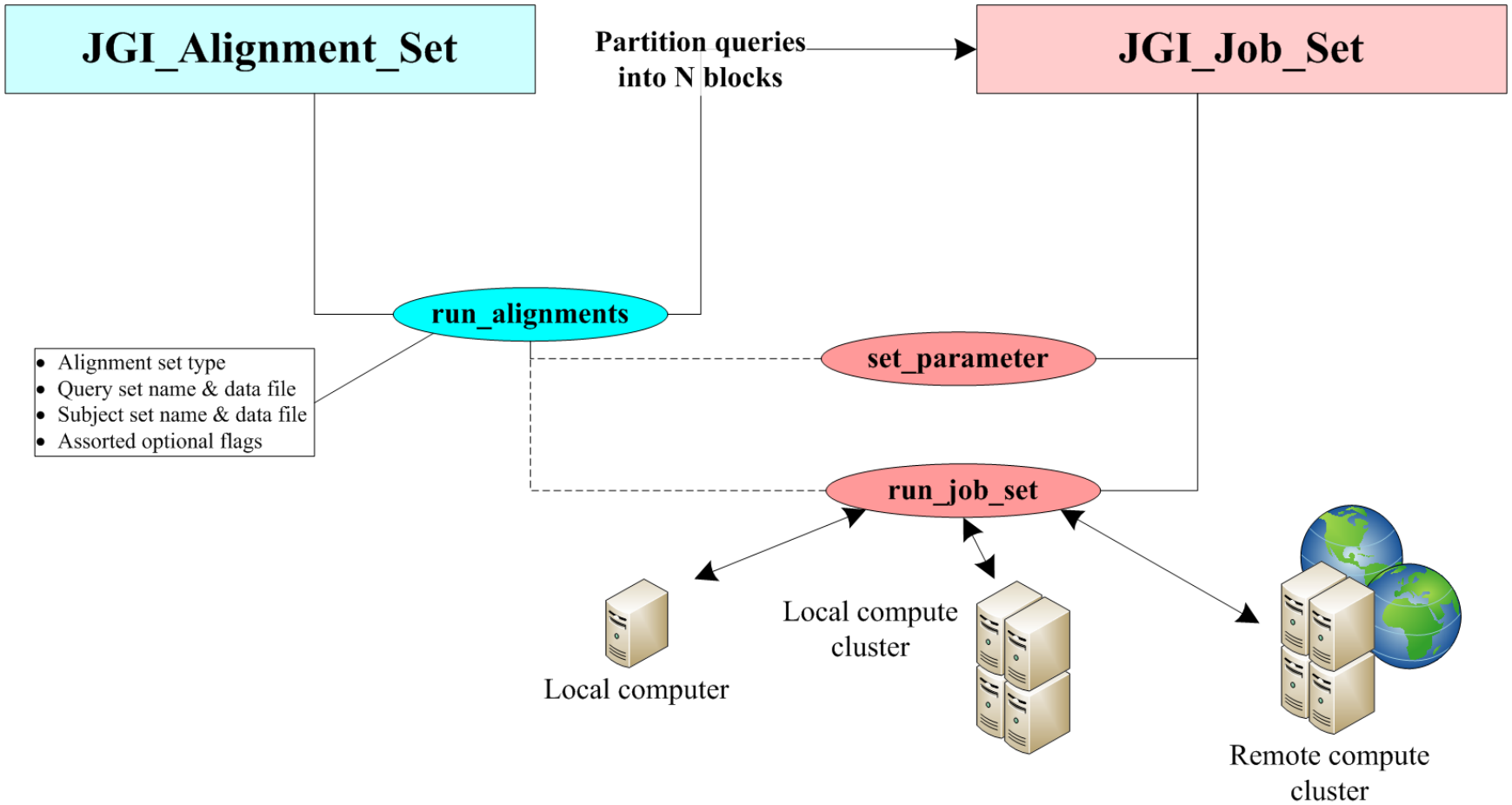
The Alignment Engine



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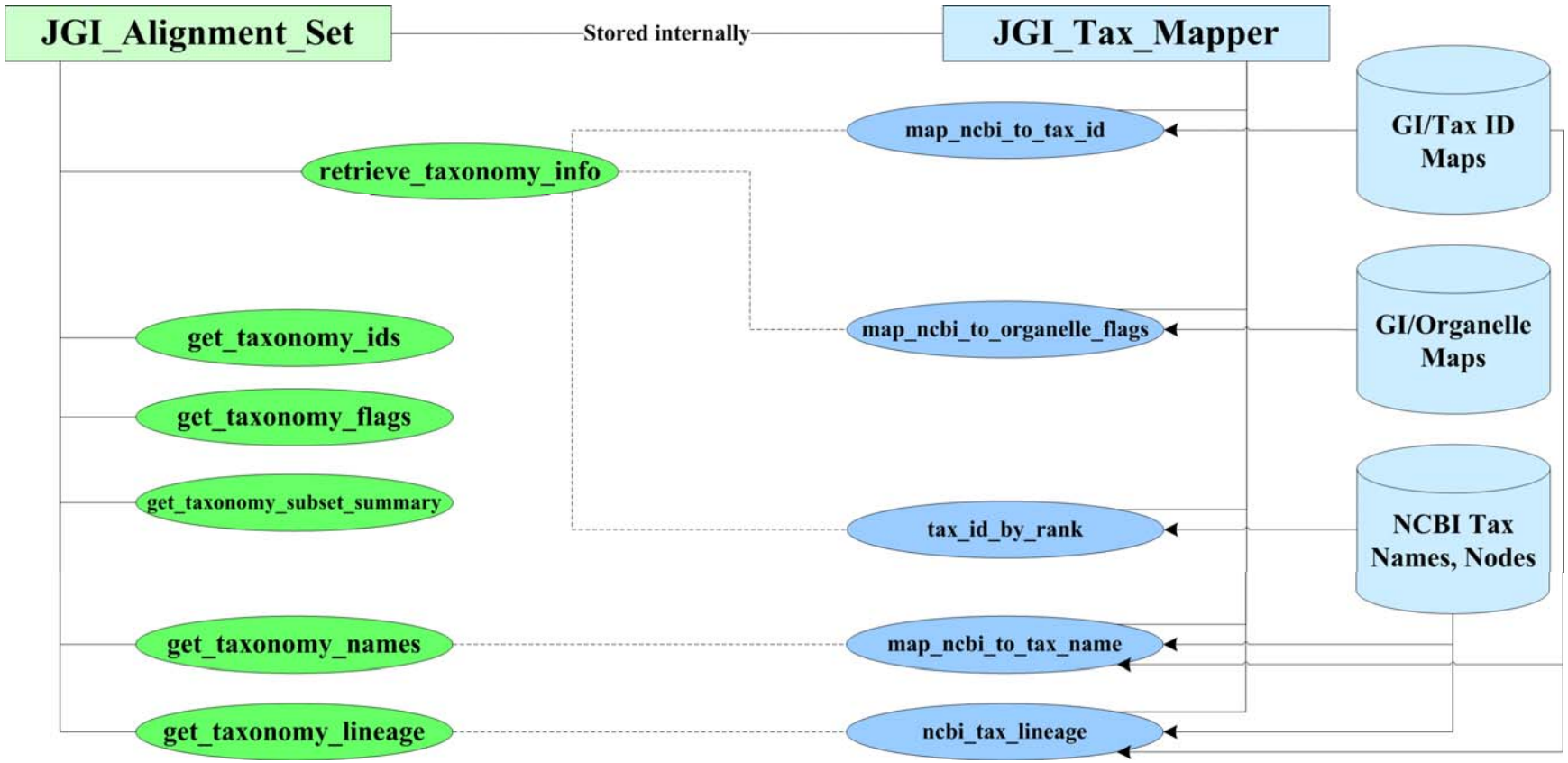
The Taxonomy Interface



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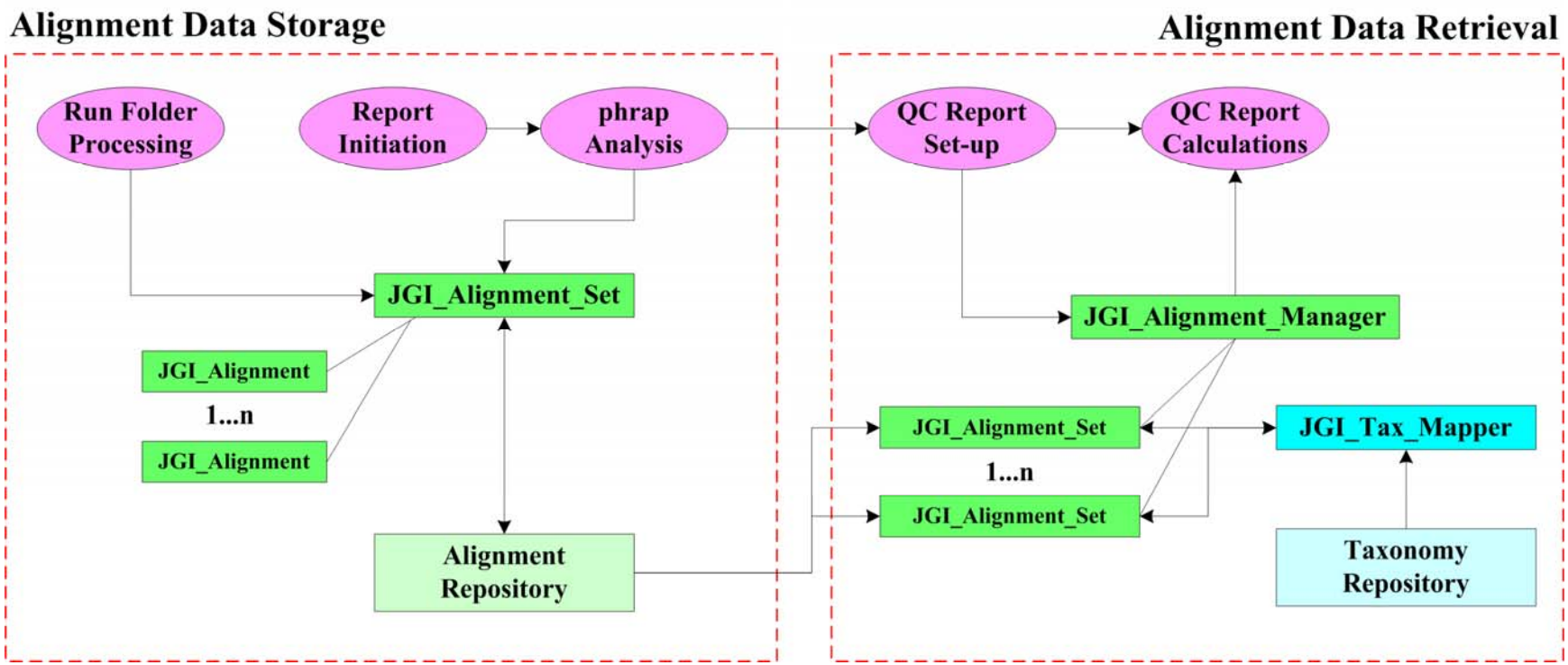
Application: The Rolling QC System



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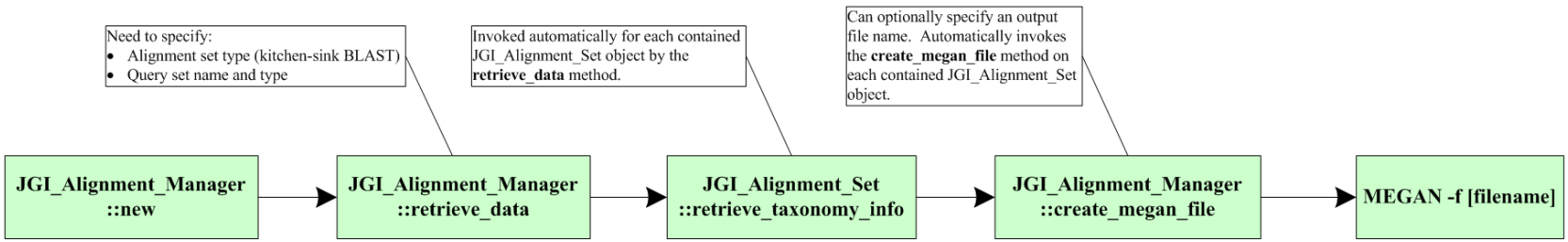
Production System Statistics (9/12/2008)

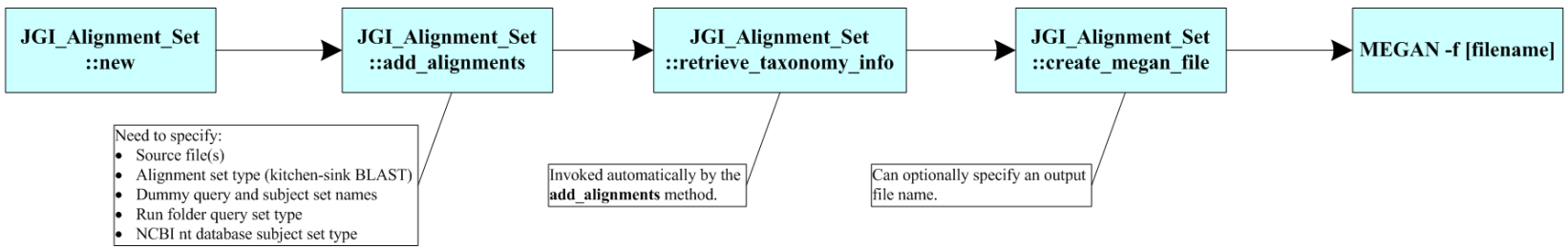
Data Type	# of Entries	Disk Space
Alignments	345,478,750	84.4 GB
Alignment sets	3,856,905	1.1 GB
Nucleotide GI/tax ID map	136,117,316	2.3 GB
Protein GI/tax ID map	21,258,420	371 MB
NCBI tax names	609,597	148.5 MB
NCBI tax nodes	435,747	37 MB
Nucleotide GI/organelle map	2,133,207	43.8 MB
Protein GI/organelle map	582,792	11.8 MB

Application: Exporting to MEGAN (Overview)

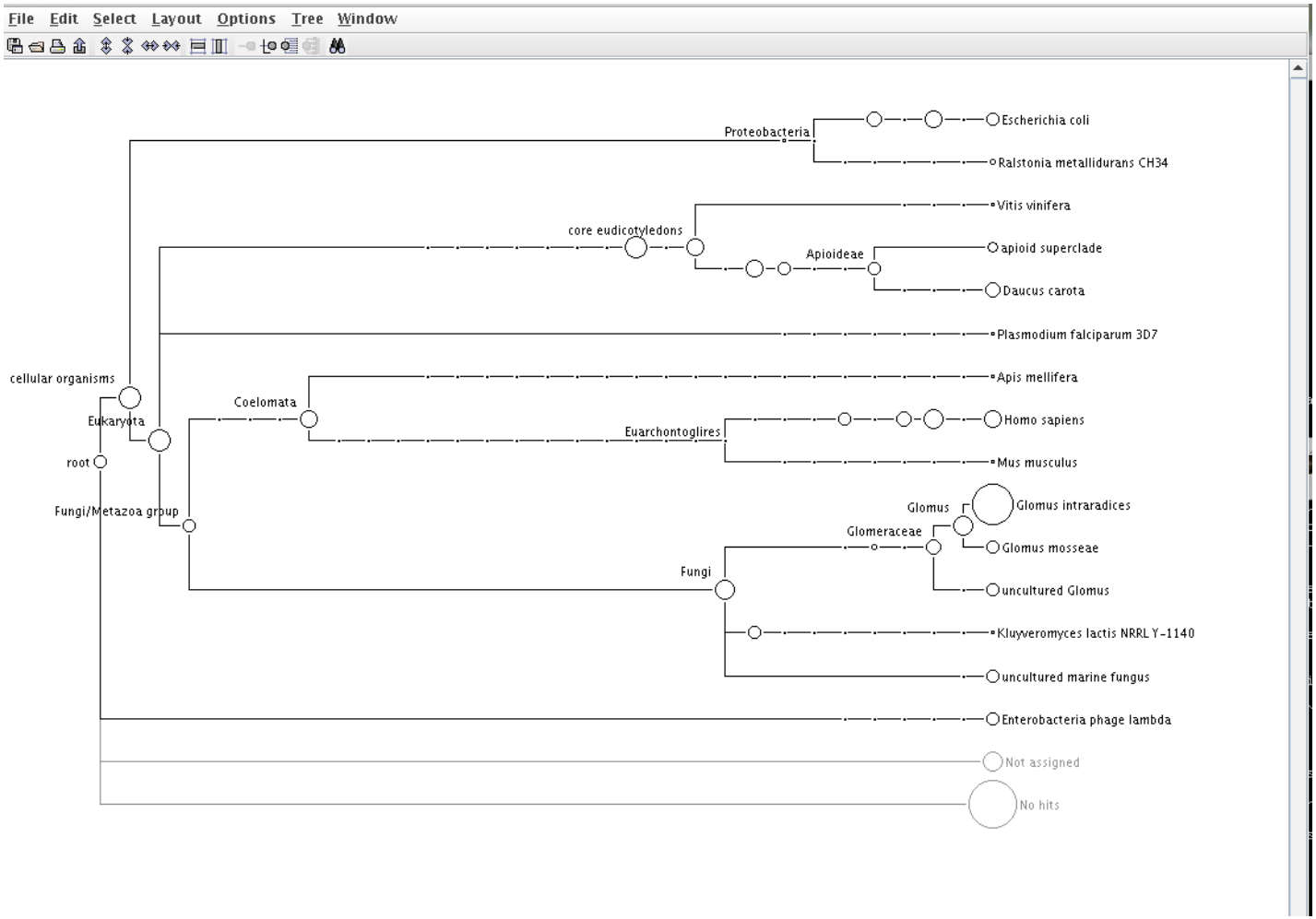


Integrated (Production) Mode

Standalone (R&D) Mode



Application: Exporting to MEGAN (Example)



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Future Plans

- **Addition of support for 454 & Illumina**
- **Full integration with the in-house sequence repository**
- **Protein alignment support**
- **Migration of assorted QC metrics**
- **Streamlined standalone R&D operation mode**
- **Implementation of an assembly repository**
- **Linkage to additional tools**
  - **Java Web Start versions of MEGAN, GAViT**
  - **AMOS bank format (Hawkeye)**

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Acknowledgements

- **Alex Copeland (Production QA/QC)**
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- **Daniel Huson (MEGAN)**

  D.H. Huson , A. Auch, Ji Qi and S.C. Schuster, *MEGAN analysis of metagenome data,* Genome Research. 17:377-386, 2007, software freely available for academic purposes from www-ab.informatik.uni-tuebingen.de/software/megan

- **Aijazuddin Syed (GAViT)**
- **Roderic Page (Schema for NCBI taxdump files)**

  http://linnaeus.zoology.gla.ac.uk/~rpage/tbmap/downloads/ncbi/

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Examples of Sequence Meta-Data

- **Sequence run meta-data**
  - **Sequencing platform**
  - **Sequencing library identifier**
- **Sequence library meta-data**
  - **Vector & insert size information**
  - **Genome size estimates**
  - **Project identifier**
- **Project meta-data**
  - **Organism name**
  - **NCBI tax ID**

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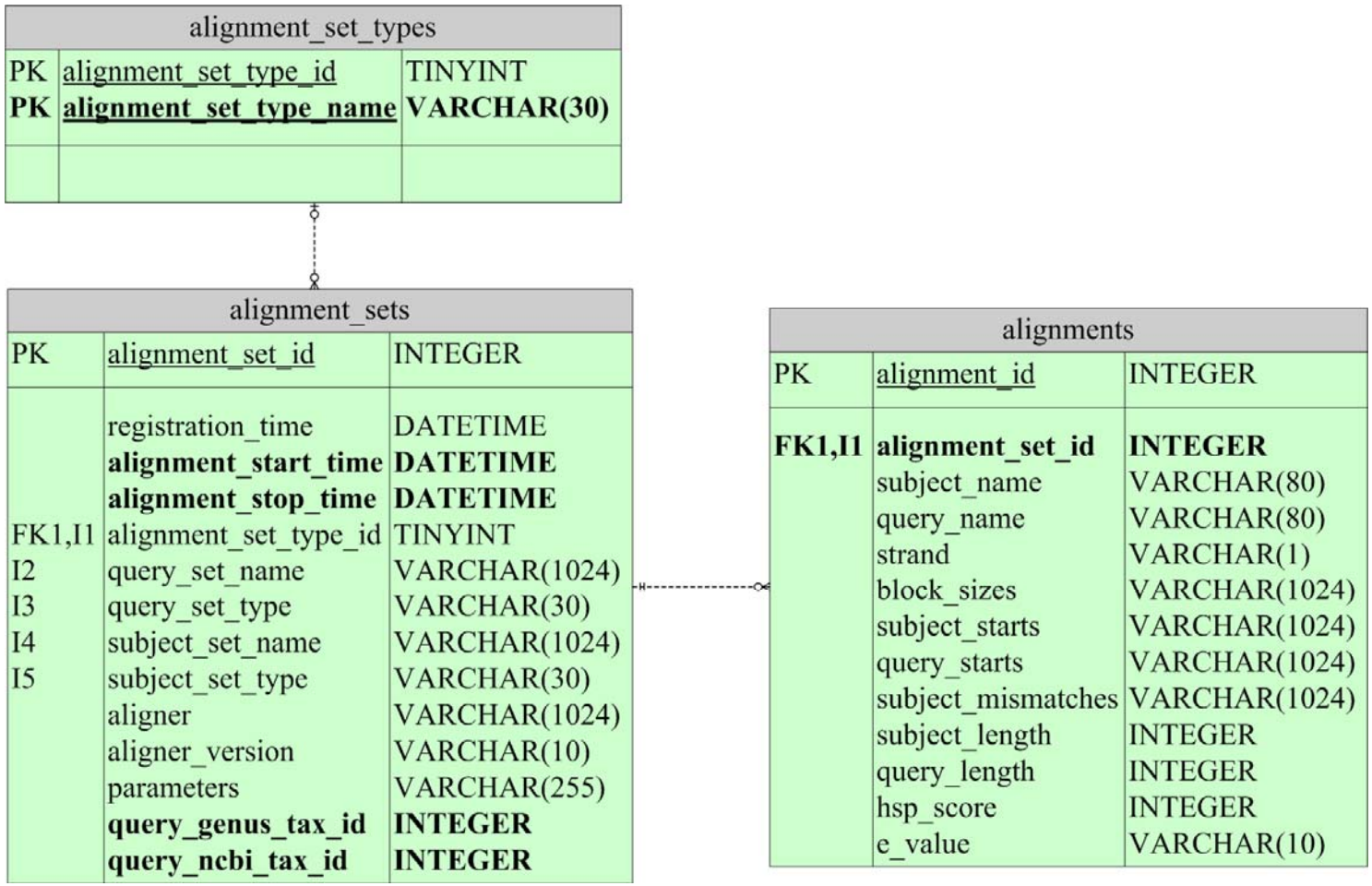
Alignment Repository Schema



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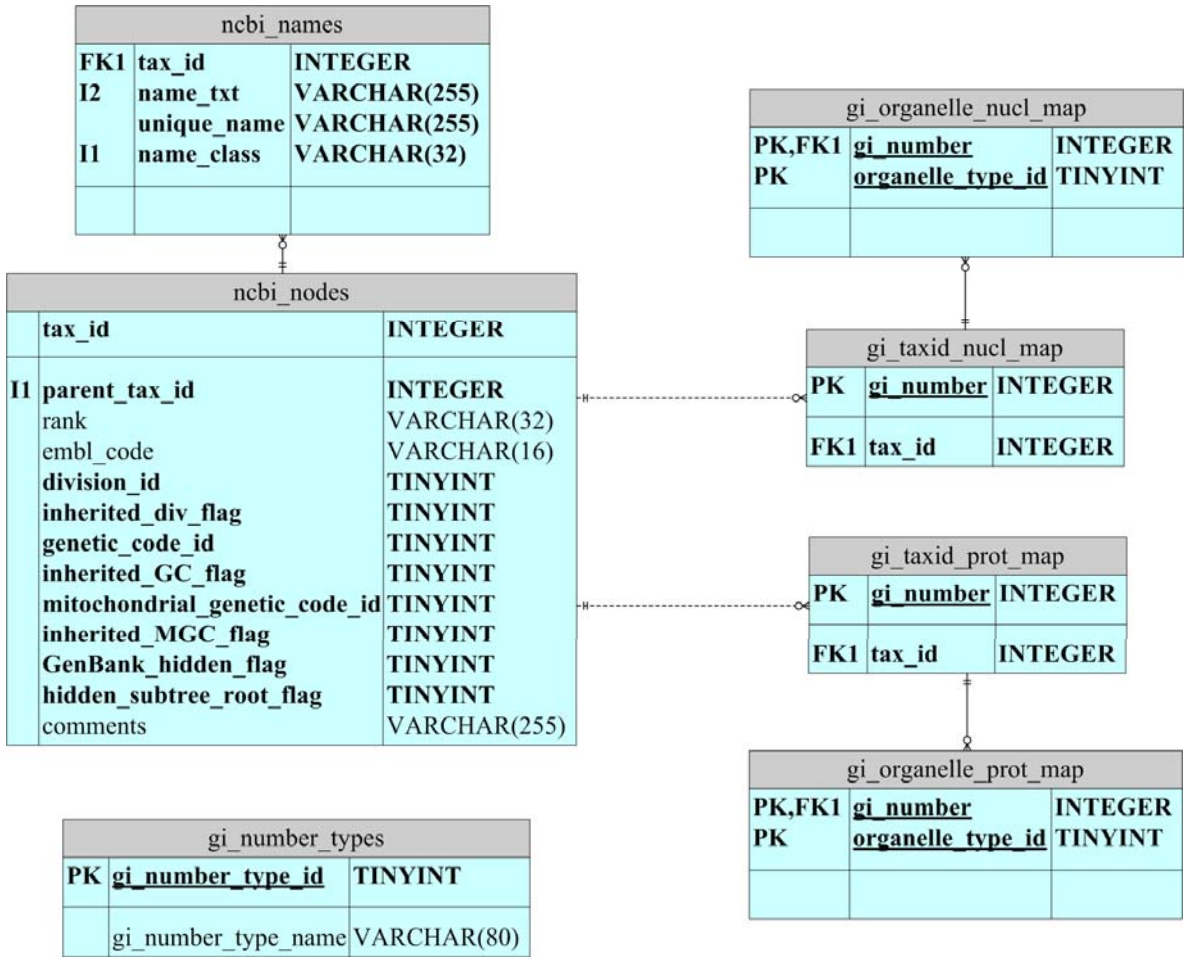
Taxonomy Repository Schema



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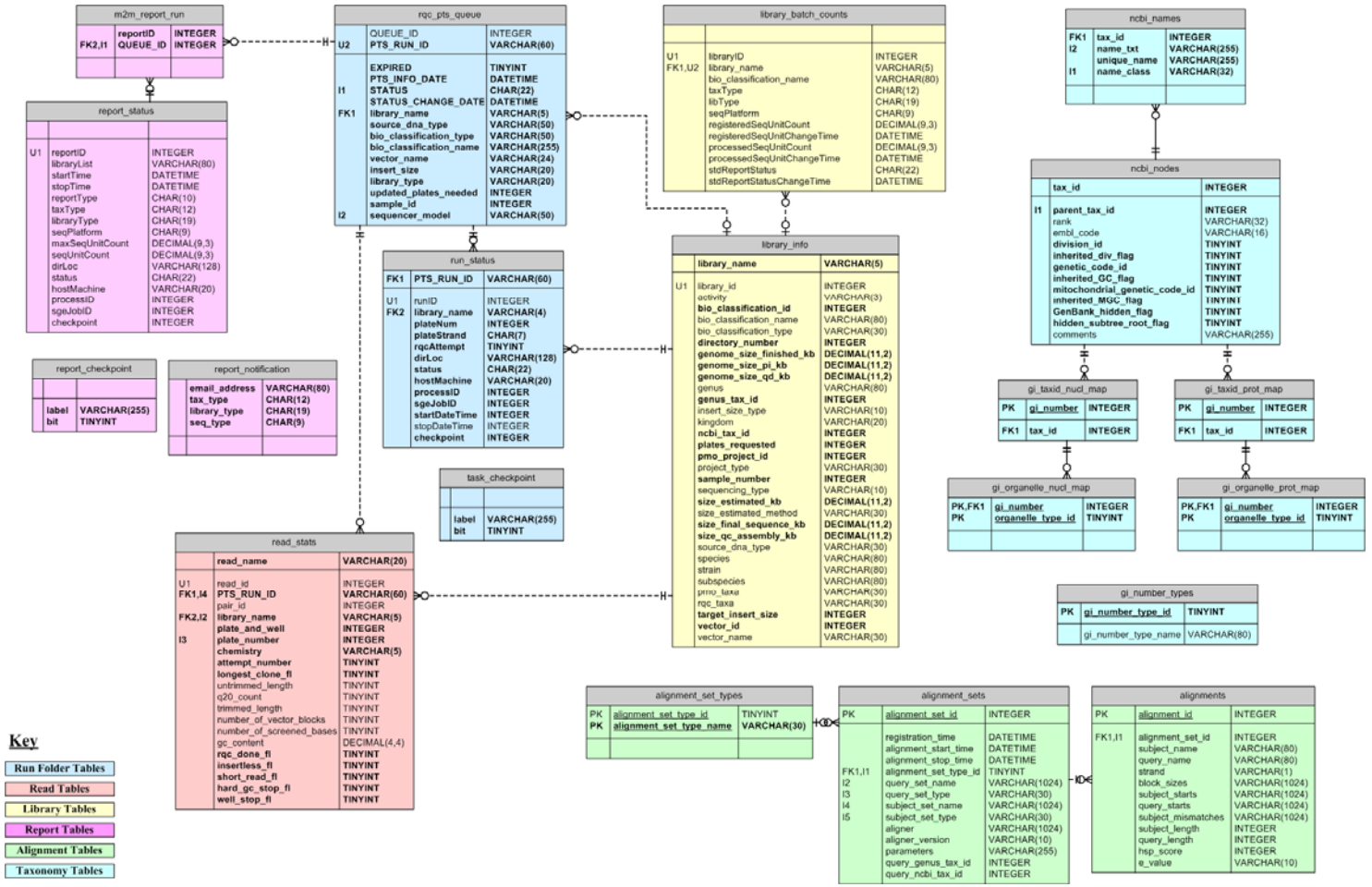
Overall Rolling QC Schema



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Nice Implementation Features of the Taxonomy Interface

- **Caching of parent tax IDs at different taxonomy tree levels**
- **Single walk-up-the-tree method, with a switch for returning application-specific sets of information**

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Nice Implementation Features of the Alignment Interface

- **Sharing of a single JGI_Tax_Mapper object across groups of JGI_Alignment_Set objects**
- **Automatic retrieval of taxonomy information, based on alignment set type**
- **Automatic retrieval of minimal alignment information, based on alignment set type**
- **Automatic mapping of different data set types (run, library, organism)**
- **Numerous internal checks & initializations, to minimize the need for users to specify parameters, and to catch inconsistencies.**

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Current Hardware Configuration

- **Production MySQL server**
  - **Dual dual-core 2.0 GHz AMD Opteron**
  - **8 GB RAM**
  - **1.1 TB local storage**
- **Two production analysis machines**
  - **Dual quad-core 1.60 GHz Intel Xeon**
  - **16 GB RAM**
  - **1.3 TB local storage**
- **Development MySQL server**
  - **Dual dual-core 2.2 GHz AMD Opteron**
  - **8 GB RAM**
  - **1.3 TB local storage**

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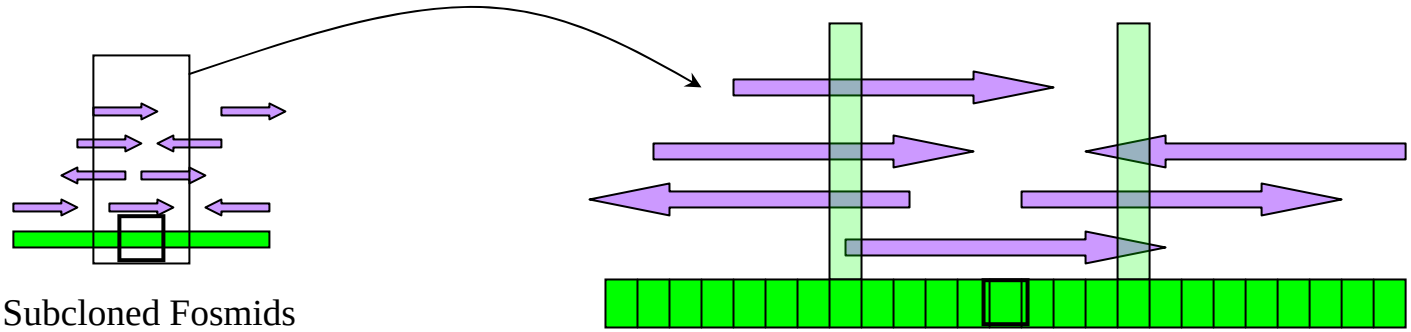
Changes Needed to Scale Up

- **Separate database servers for each sequencing platform (possibly multiple ones for each), with automatic routing**
- **Separate read/write database instances, with automatic routing**
- **Switch to automatic database table partitioning**
- **Bulk loading of alignments**

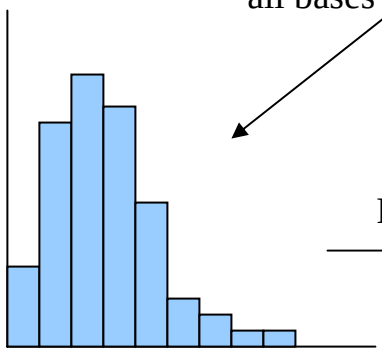
Use Case: Subcloned Fosmid Coverage Method



Subcloned Fosmids

Count the alignments covering each subcloned fosmid base

Histogram of all bases



Number of Bases

Number of Alignments

Poisson curve-fit

$$f(x) = N \cdot \exp[-d] \cdot \frac{d^x}{x!}$$

d = Estimated sequence depth

N = Number of subcloned fosmid bases

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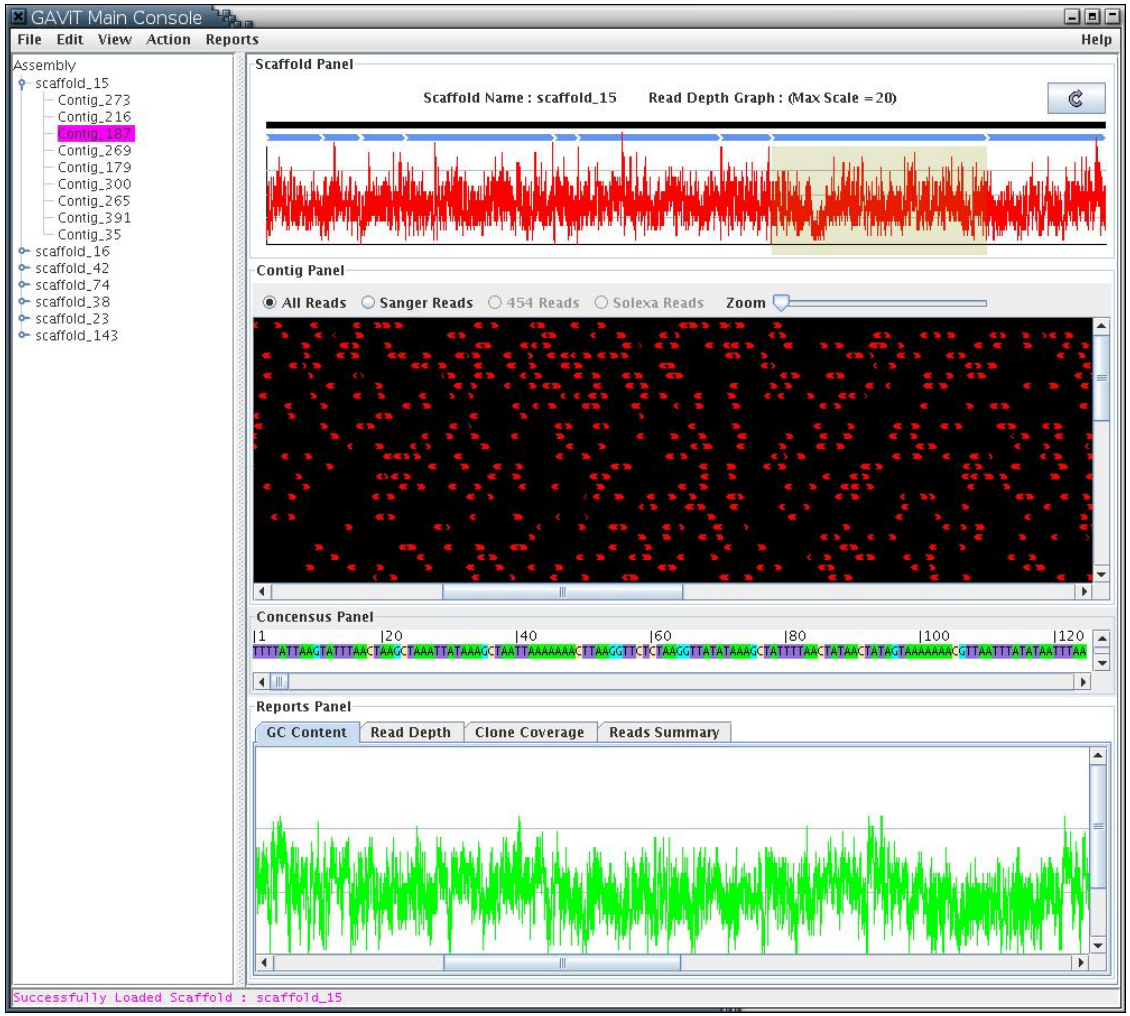
Use Case: Exporting to GAViT



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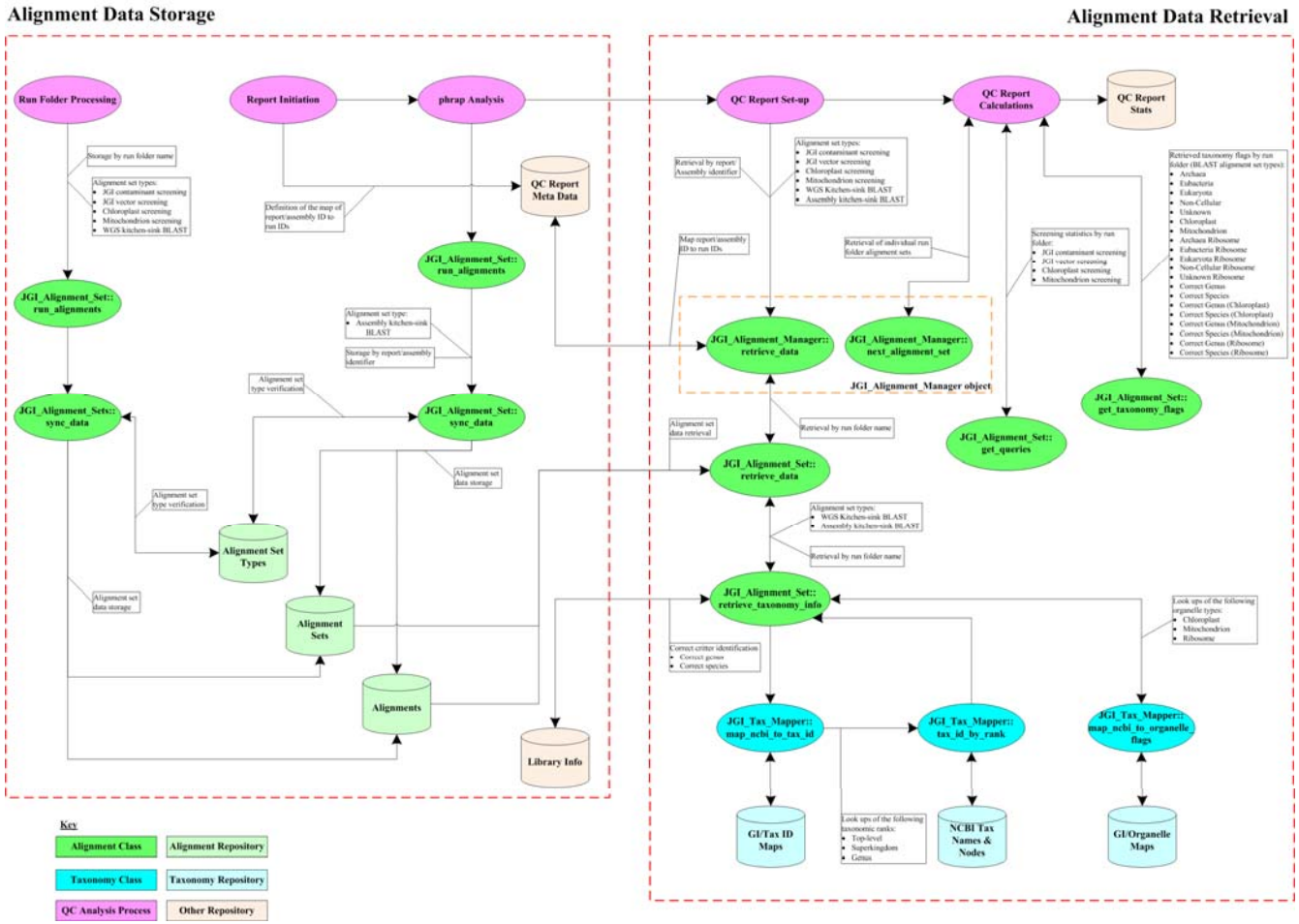
Application: The Rolling QC System (Detailed)



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Auspice Statement

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