A major step in most deep-sequencing data analysis is mapping of the short reads to a reference genome (Cloonan et al., 2009). In this strategy, tags are first mapped at full length, and then if unaligned, truncated then mapped again. Although reads are truncated when mapped, the data shown represents the original length and quality. Truncated reads show a steeper decline in quality than full-length reads (Fig. 1; Supplementary Fig. 1). The wobble, particularly noticeable in (a) and also evident in (b), is a quality profile characteristic of the SOLiD five primer ligation cycle. The general trend is consistent in base-space data (Supplementary Fig. 1).

Although RNA-MATE was specifically designed for (and effectively limited to) colour-space stranded RNA-Seq data, the recursive approach works equally well on all sequencing datasets. To increase the utility of this software, and provide further enhancements, we have significantly revised and updated the RNA-MATE pipeline. Here, we present X-MATE, a system for flexible and comprehensive mapping of all deep-sequencing datasets. A comparison of features between RNA-MATE and X-MATE can be found in Table 1.

1 SUPPORT FOR MULTIPLE DATATYPES

X-MATE provides full support for both unstranded and stranded RNA- and DNA-derived datasets, including CAGE and ChIP-Seq.
Table 1. Feature matrix summarizing the major improvements between RNA-MATE and X-MATE

<table>
<thead>
<tr>
<th>Feature</th>
<th>RNA-MATE</th>
<th>X-MATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mapping Engine</td>
<td>Fixed</td>
<td>Flexible</td>
</tr>
<tr>
<td>Data type</td>
<td>Stranded RNA-Seq only</td>
<td>Stranded or unstranded RNA-Seq, gDNA, CAGE, ChIP-Seq, etc</td>
</tr>
<tr>
<td>Data Encoding</td>
<td>Colour-space only</td>
<td>Colour-space or base-space</td>
</tr>
<tr>
<td>Map to junctions</td>
<td>Required</td>
<td>Optional</td>
</tr>
<tr>
<td>Output formats</td>
<td>BED, Wiggle</td>
<td>BED, Wiggle, SAM</td>
</tr>
<tr>
<td>Multi-map rescue</td>
<td>MuMRescue</td>
<td>MuMRescueLite</td>
</tr>
<tr>
<td>Configuration</td>
<td>Cumbersome</td>
<td>Improved</td>
</tr>
</tbody>
</table>

The expected strand for a dataset can be easily configured, and when mapping stranded datasets, output files summarizing mapping results are created for each strand, allowing analysis and visualization of strand mapping specificity. When mapping unstranded data these results are combined into single output files. In both cases, libraries of known exon–exon junctions can be optionally used during alignment, either simultaneously with chromosome mapping, or after chromosome mapping.

2 ALTERNATIVE MAPPING ENGINES SUPPORTING BASE- AND COLOUR-SPACE

By default mapreads is used, but if licensed, ISAS can be chosen. ISAS is an extremely fast alignment system capable of performing exhaustive searches, or using a heuristic algorithm to greatly increase search speeds (see Supplementary File 1 for notes on performance). ISAS also implements a variable-length mapping mode and can be chosen in place of the X-MATE recursive mapping. The modular redesign of X-MATE allows for further alignment programs to be incorporated into the package if required. As both mapreads and ISAS can map in base-space, we have passed this additional capability to X-MATE, and it now maps base-space data natively (fasta format for mapreads, and fastq format for ISAS).

3 SAM FORMAT OUTPUT NOW SUPPORTED

To assist with interrogating the mapped data, X-MATE provides multiple data output formats (bed, and wiggle files) consistent with commonly used visualization tools such as UCSC genome browser (Kent et al., 2002) and powerful bioinformatic middleware platforms like Galaxy (Goecks et al., 2010). In addition, to provide for backward compatibility of previously mapped data (output in ‘collated’ format), and to assist in tertiary analysis, we have included a Java utility for data transformation of colour-space mapped data into Sequence Alignment/Map (SAM) format. SAM files can be converted to Binary Alignment/Map (BAM) files using Samtools (Li et al., 2009), and are becoming the default input file format for many genome browsers (e.g. the Integrative Genomics Viewer, IGV, http://www.broadinstitute.org/igv). Using SAM and BAM files, mapped data can be viewed in single nucleotide resolution, and can also be passed directly to downstream software for tertiary analysis such as transcript assembly, SNP calling, structural re-arrangement detection and more.

4 RESOURCE USAGE AND UTILITIES

X-MATE requires modest memory (4 GB is sufficient), and although running on desktop computers is possible, the use of a distributed cluster is recommended. We have updated the multi-map rescue strategy module to use the more memory efficient MuM Rescue Lite (Hashimoto et al., 2009), and included a utility to restart a mapping run from the ‘rescue’ stage. Utilities tailored to RNA-Seq data for the creation of junction libraries and for the quantification of reads mapping to the expected strand when library protocols generate stranded data are added, the latter useful in determining the quality of a sequencing library. Finally, utilities for analysis of mapping yield and for cleaning up residual files after a mapping run have been included (see descriptions in Supplementary File 1).

5 SIMPLIFIED CONFIGURATION FILES

Setting up an X-MATE run is now straightforward using our new configuration format. Example configuration files for many possible mapping strategies and dataset types have been provided with the X-MATE distribution. The system contains improved code quality, commenting and modularization, facilitating further enhancements and ongoing maintenance.

6 FUTURE DIRECTIONS

Future enhancements planned are the addition of more mapping engines, utilities to compare mapping runs and provide information to optimize mapping parameters and upkeep with file input and output formats. All source code and documentation, test datasets with results and junction libraries are freely available from http://grimmond.imb.uq.edu.au/X-MATE/.

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