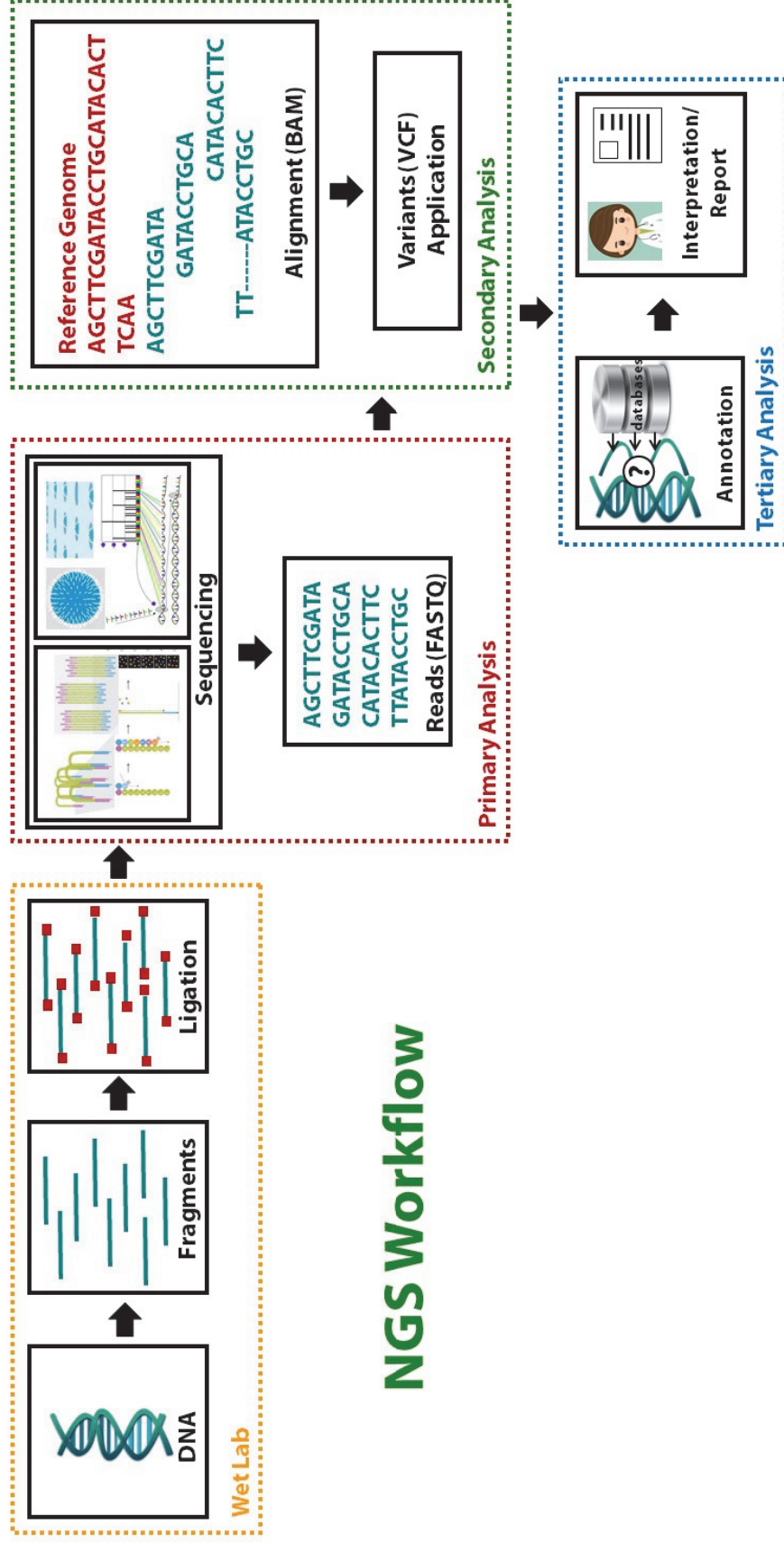


Molecular In My Pocket™ ...

Bioinformatics: Basic File Formats Part I

Analysis Schema and FASTQ (Primary Analysis)



NGS Workflow

FASTQ format: A text-based format for storing both biological sequences and their corresponding quality scores. FASTQ files contain 4 lines for each sequence.

Line1: A sequence identifier with information about the sequencing run and cluster.

Line2: The sequence of the read (the base calls; A, C, T, G and N).

Line3: A “+” and is optionally followed by sequence related information.

Line4: The ASCII encoded quality scores of the base calls in the sequence in Line 2.

Example 1: An ASCII characters and error probabilities table in ASCII_BASE 33 (the Phred quality score starts with ASCII code 33 to exclude the control characters). The Phred quality score calculation is described in http://drive5.com/usearch/manual/quality_score.html.

ASCII Character	Phred Quality Score	Probability of Incorrect Base Call	Base Call Accuracy
+	10	1 in 10	90%
5	20	1 in 100	99%
?	30	1 in 1,000	99.9%
I	40	1 in 10,000	99.99%

Example 2:

```
@NB551591.7:HJTSDBGX9:1:1101:9719:1040 1:N:0:CATCAAGT
TACTGNTAATGGTGGCAGGGTCTGTTCTTCCAAATCCTGAGGACTCCATGGGCACAAAATCTCCTG
+
AAAAA#EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE
```

@instrument name:run ID:flowcell ID:lane number:
tile number:x-coord within the tile:y-coord within
the tile[space]member of a pair:if read
filtered:control bits:index sequence

ASCII Character	Phred Quality Score	Probability of Incorrect Base Call	Base call Accuracy	Error Probability from ASCII_BASE33
#	2	1 in 2	37%	0.63096
A	32	1 in 1,587	99.94%	0.00063
E	36	1 in 4,000	99.98%	0.00025

The Sequence of the Read

References

- <https://support.illumina.com/bulletins/2016/04/fastq-files-explained.html>
- http://drive5.com/usearch/manual/quality_score.html

“Molecular in My Pocket” reference cards are educational resources created by the Association of Molecular Pathology (AMP) for laboratory and other health care professionals. The content does not constitute medical or legal advice, and is not intended for use in the diagnosis or treatment of individual conditions. See www.amp.org for the full “Limitations of Liability” statement.