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Analysis Software for Overlapping Linkers on Adjacent Fragments Sequencing (OLAF-Seq) Strategy

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Need

Users:
- Dr. Xiao
- Any lab using the OLAF strategy
- No other current software analysis tool for data produced by the OLAF strategy

Design Inputs

Constraint	Description
Computing Power	Data processing with University Research Computing Facility (URCF)
Data	Large files in FASTA and FASTQ to be compatible with modern sequencing technologies

Requirement	**Description**
Node Identification	**Only select nodes with probe combination hits ≥ 50 hits**
Linkage Formation	**Find node linkages with 100% accuracy to the design**
Sequence Accuracy	**Accurately reconstruct sequence to ≥99% accuracy**

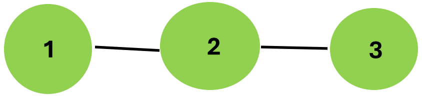
Future

- *Future revisions should focus on improving functionality on lower end computers*
- *Potential for faster results in targeted sequencing applications that use OLAF strategy*

Verification Testing

Test 1: Node Formation
- >50 reads to a combination indicates probes form a node

gRNA Combination	Number of Hits
NBN2 vs NBN3	84
NBN4 vs NBN5	66
NBN6 vs NBN7	156



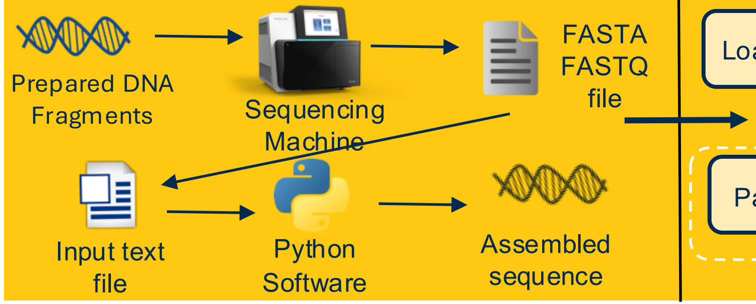
Test 2: Node Linkage (Kmer analysis)
- >100 matched kmers indicate a linkage between fragments by comparing sections

Edge	Number of matched Kmers
NBN3 to NBN4	162
NBN5 to NBN6	163

Test 3: Reference comparison
- >99% percent identity indicates successful reassembly of sequence when compared to original sequence

99.9% Accuracy to reference achieved

Solution – Design



Solution – Build