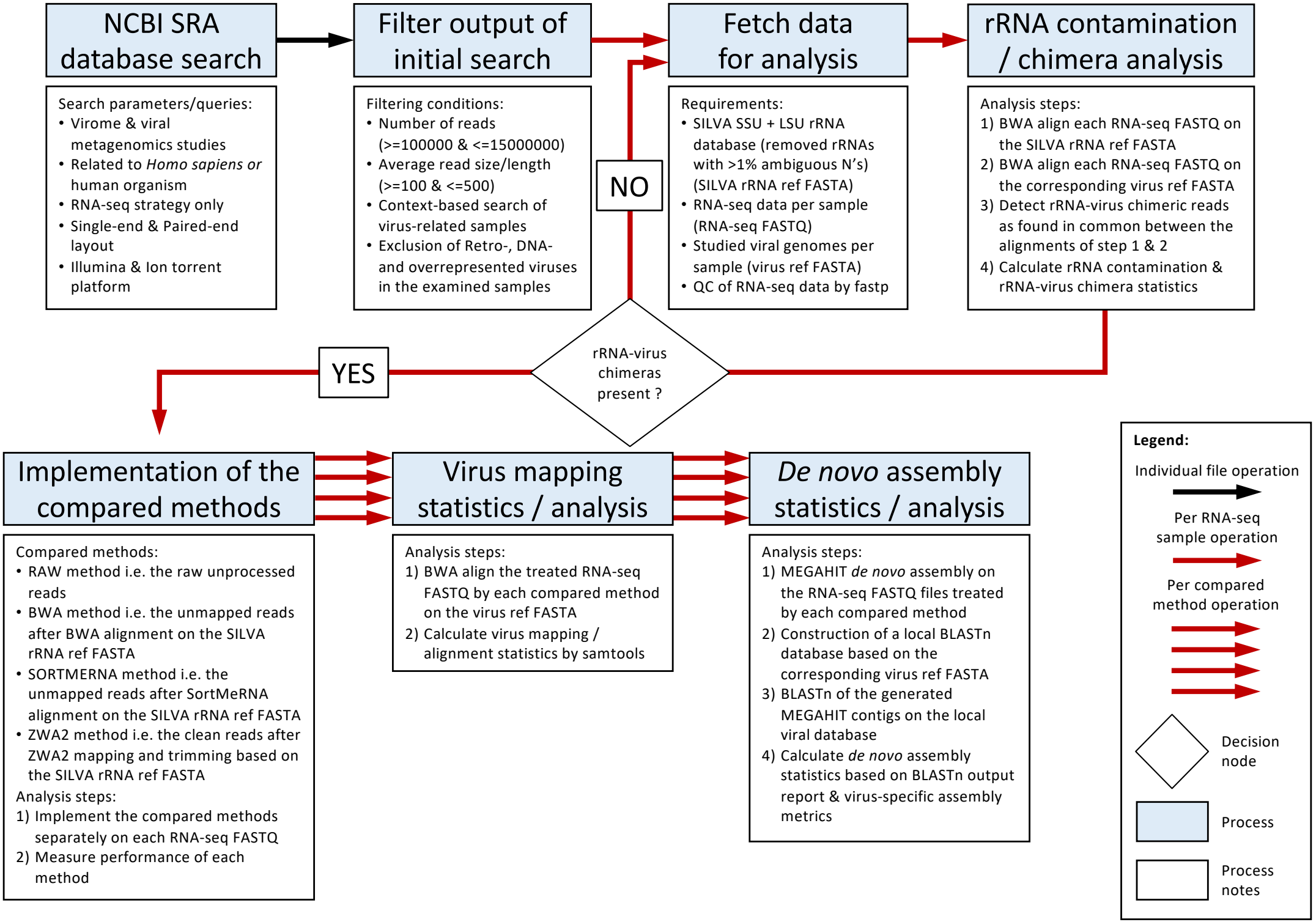
NCBI SRA database search
Search parameters/queries:
• Virome & viral metagenomics studies
• Related to Homo sapiens or human organism
• RNA-seq strategy only
• Single-end & Paired-end layout
• Illumina & Ion torrent platform
Filter output of initial search
Filtering conditions:
• Number of reads (>=100000 & <=15000000)
• Average read size/length (>=100 & <=500)
• Context-based search of virus-related samples
• Exclusion of Retro-, DNA- and overrepresented viruses in the examined samples
Fetch data for analysis
Requirements:
• SILVA SSU + LSU rRNA database (removed rRNAs with >1% ambiguous N's) (SILVA rRNA ref FASTA)
• RNA-seq data per sample (RNA-seq FASTQ)
• Studied viral genomes per sample (virus ref FASTA)
• QC of RNA-seq data by fastp
rRNA contamination / chimera analysis
Analysis steps:
1) BWA align each RNA-seq FASTQ on the SILVA rRNA ref FASTA
2) BWA align each RNA-seq FASTQ on the corresponding virus ref FASTA
3) Detect rRNA-virus chimeric reads as found in common between the alignments of step 1 & 2
4) Calculate rRNA contamination & rRNA-virus chimera statistics
rRNA-virus chimeras present ?
NO
YES
Implementation of the compared methods
Compared methods:
• RAW method i.e. the raw unprocessed reads
• BWA method i.e. the unmapped reads after BWA alignment on the SILVA rRNA ref FASTA
• SORTMERNA method i.e. the unmapped reads after SortMeRNA alignment on the SILVA rRNA ref FASTA
• ZWA2 method i.e. the clean reads after ZWA2 mapping and trimming based on the SILVA rRNA ref FASTA
Analysis steps:
1) Implement the compared methods separately on each RNA-seq FASTQ
2) Measure performance of each method
Virus mapping statistics / analysis
Analysis steps:
1) BWA align the treated RNA-seq FASTQ by each compared method on the virus ref FASTA
2) Calculate virus mapping / alignment statistics by samtools
De novo assembly statistics / analysis
Analysis steps:
1) MEGAHIT de novo assembly on the RNA-seq FASTQ files treated by each compared method
2) Construction of a local BLASTn database based on the corresponding virus ref FASTA
3) BLASTn of the generated MEGAHIT contigs on the local viral database
4) Calculate de novo assembly statistics based on BLASTn output report & virus-specific assembly metrics
Legend:
Individual file operation
Per RNA-seq sample operation
Per compared method operation
Decision node
Process
Process notes