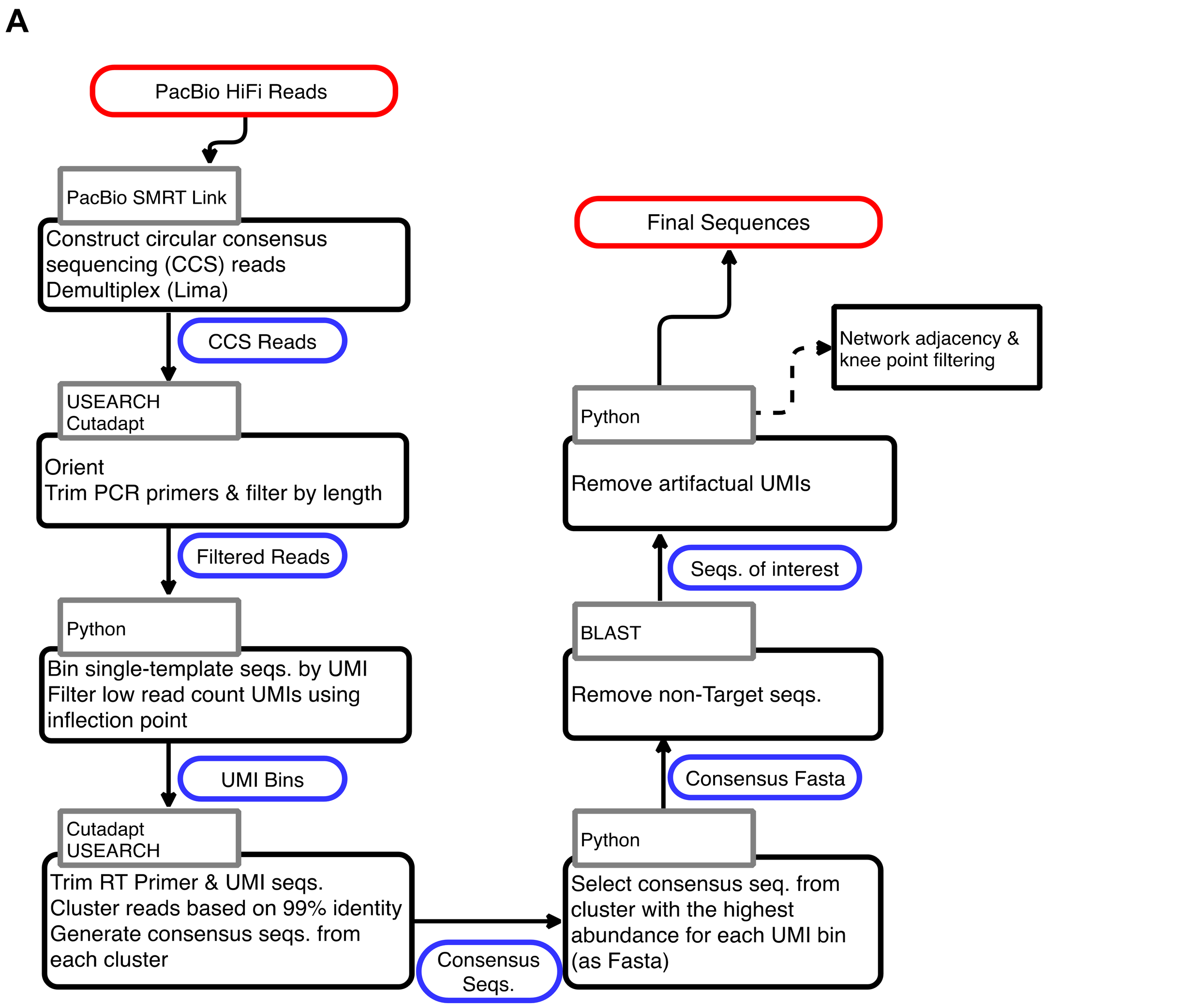
A
PacBio HiFi Reads
PacBio SMRT Link
Construct circular consensus sequencing (CCS) reads
Demultiplex (Lima)
CCS Reads
USEARCH
Cutadapt
Orient
Trim PCR primers & filter by length
Filtered Reads
Python
Bin single-template seqs. by UMI
Filter low read count UMIs using inflection point
UMI Bins
Cutadapt
USEARCH
Trim RT Primer & UMI seqs.
Cluster reads based on 99% identity
Generate consensus seqs. from each cluster
Consensus Seqs.
Python
Select consensus seq. from cluster with the highest abundance for each UMI bin (as Fasta)
Consensus Fasta
BLAST
Remove non-Target seqs.
Seqs. of interest
Python
Remove artifactual UMIs
Network adjacency & knee point filtering
Final Sequences
B
Read count
100
10
1
1
10
100
Rank of UMI bin (by read count)
C
Read count
300
100
30
1
10
100
Rank of UMI bin (by read count)