Construct circular consensus sequencing (CCS) reads
Demultiplex (Lima)

PacBio HiFi Reads

PacBio SMRT Link

CCS Reads

USEARCH
Cutadapt

Orient
Trim PCR primers & filter by length

Filtered Reads

USEARCH

Python

Trim single-template seqs. by UMI
Filter low read count UMI's using inflection point

UMI Bins

Cutadapt
USEARCH

Python

Trim RT Primer & UMI seqs.
Cluster reads based on 99% identity
Generate consensus seqs. from each cluster

Consensus Fasta

Consensus Seqs.

Network adjacency & knee point filtering

Final Sequences

Remove artifactual UMIs

Seqs. of interest

Remove non-Target seqs.

Consensus Fasta

Select consensus seq. from cluster with the highest abundance for each UMI bin (as Fasta)

Read count
Rank of UMI bin (by read count)

B

Read count
Rank of UMI bin (by read count)

C