FASTQ
95\% population
5\% wild-type

## split FASTQ (FASTX

 Barcode Splitter)population FASTQ

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trimmed population FASTQ

population BAM map reads (BWA)
wild-type FASTQ
trim adapters
(Cutadapt)

trimmed wild-type FASTQ
$\downarrow \begin{gathered}\text { mark PCR duplicates } \\ \text { (modified Picard } \\ \text { MarkDuplicates) }\end{gathered}$
deduped population BAM

(GATK)
deduped, recalibrated
population BAM

mark overlapping bases in paired-end reads (custom Java program)
deduped, recalibrated, overlap-added population
recalibrated, overlap-added wild-type BAM

BAM

per-site population allele counts
per-site wild-type allele counts
per-site population allele counts
per-site wild-type allele counts

merged per-site wild-type

| compare |
| :---: |
| population and |
| wild-type counts | allele counts

sites with non-reference allele proportion greater than wild-type

significant sites

multiple hypothesis
testing correction
significant sites, FDR-
corrected

heuristic filters
final call set

