a) PPalign Raw paired-end fastq files **PoolParty** BBMap Trim reads by quality Pool-seq Pipeline Quality-trimmed fastq files Align to reference genome Identify breakpoint reads samblaster Remove PCR duplicates samtools aligned reads Discordant and split-end .bam files Aligned .bam files Picard Tools Sort by coordinates samtools Remove unpaired reads Alignment summaries Filtered .bam files samtools bcftools Call and filter variants Mapped-read statistics .mpileup file **PPstats** Popoolation 2 Identify and mask indel regions Popoolation 2 Convert to SYNC format b) PPanalyze Population structure Filtered .sync file r structure r_anal_maf | Comparison-specific SNP filters Pairwise-FST analysis Fisher's exact test Popoolation 2 FST analysis