

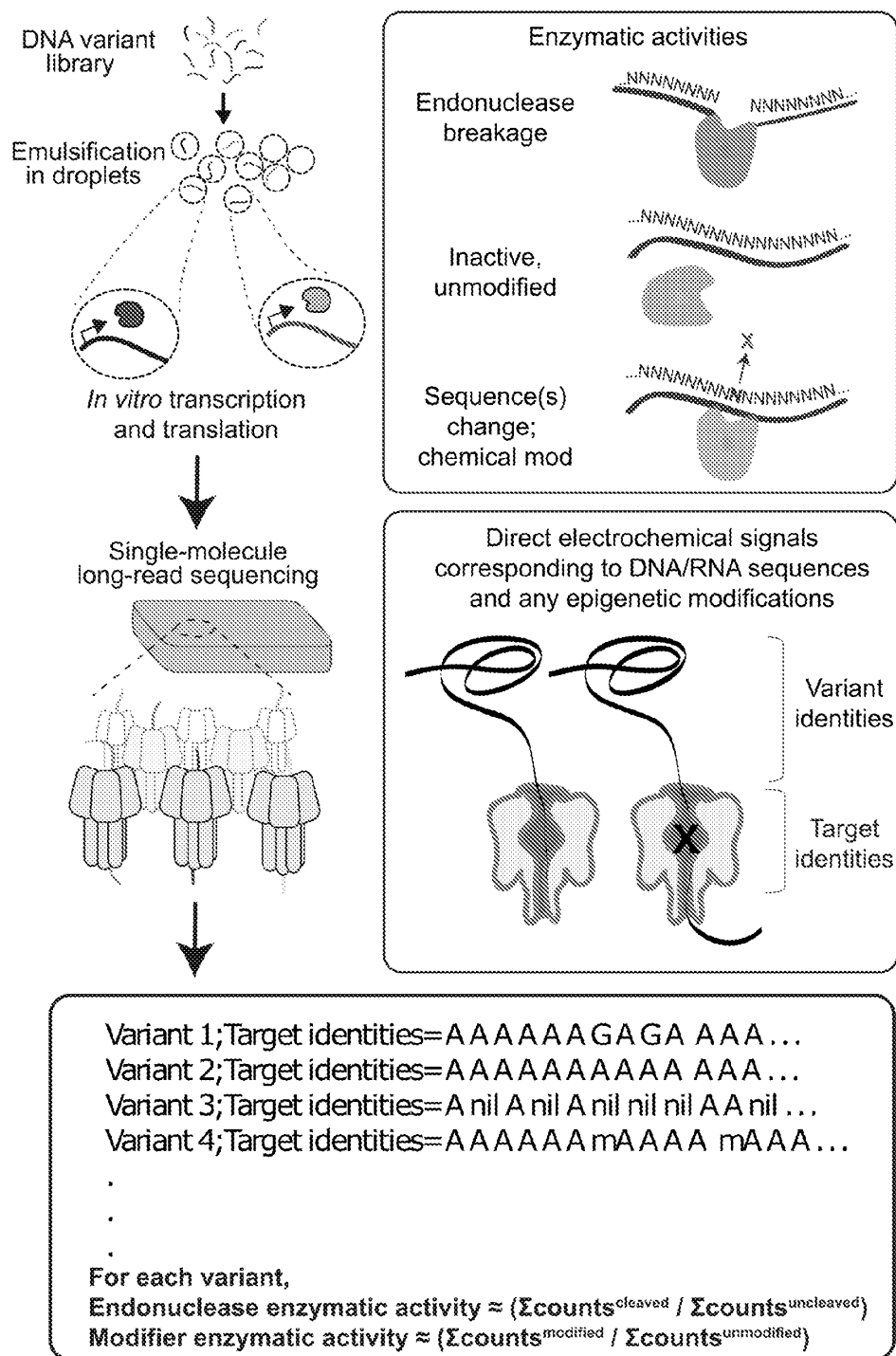
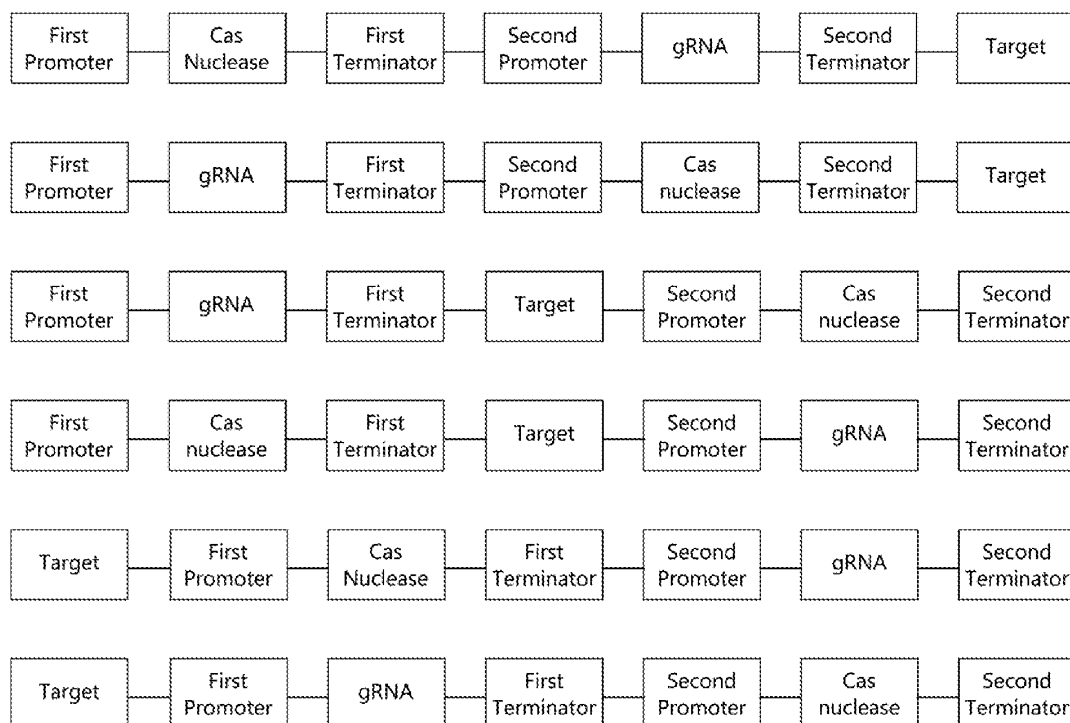


Figure 1

Single IVTT DNA constructs for DNA-targeting Cas:



Single IVTT DNA constructs for RNA-targeting Cas:

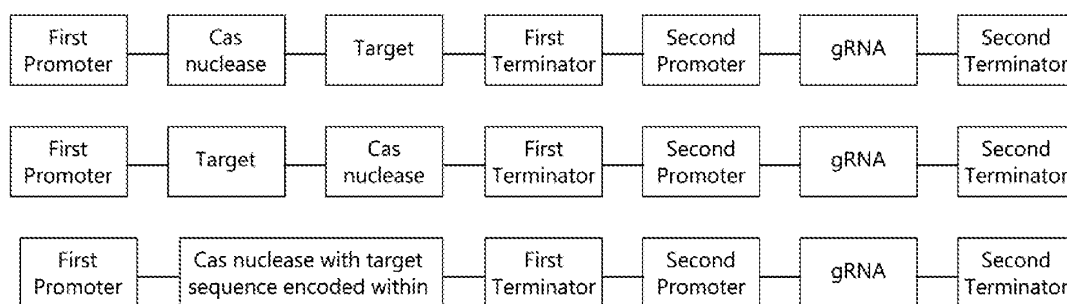


Figure 2

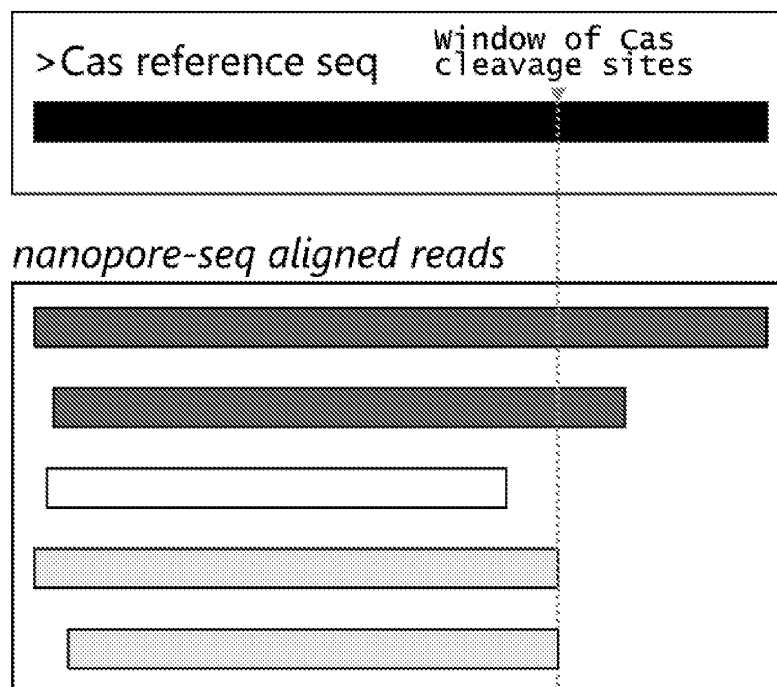


Figure 3

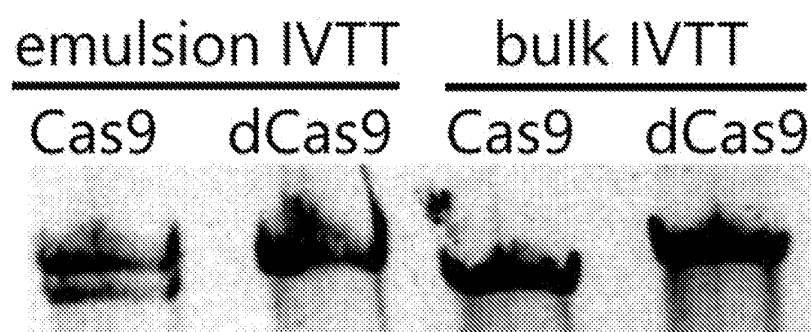


Figure 4

Emulsion IVTT reaction with high Sp Cas9 DNA input: reads mapped

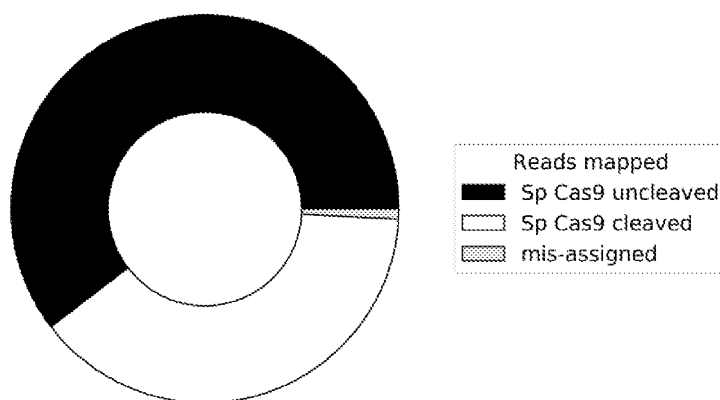


Figure 5

Emulsion IVTT reaction with high Sp dCas9 DNA input: reads mapped

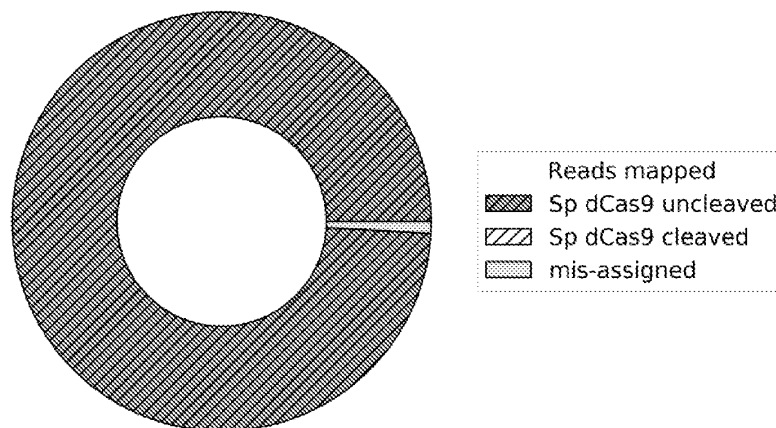


Figure 6

Bulk IVTT reactions:

1. For Cas variants SpCas9, SaCas9, AsCpf1, LbCpf1, setup individual 100 μ l PURExpress reactions + 1 μ g template DNA.

2. Start 37°C incubation for the tubes and remove the corresponding tubes per timepoint to quench with 20 mM EDTA + RNase cocktail + Proteinase K, quench at 37°C for 30 min.

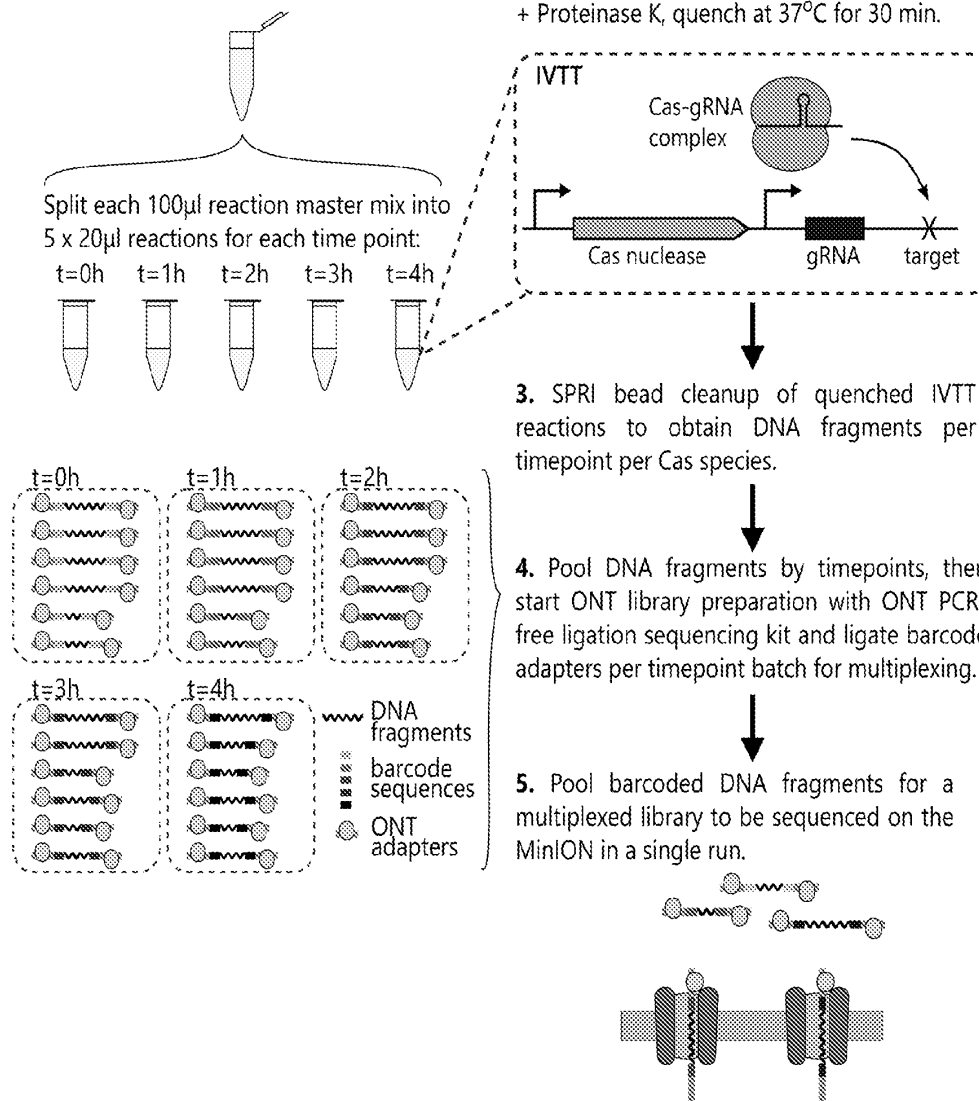


Figure 7

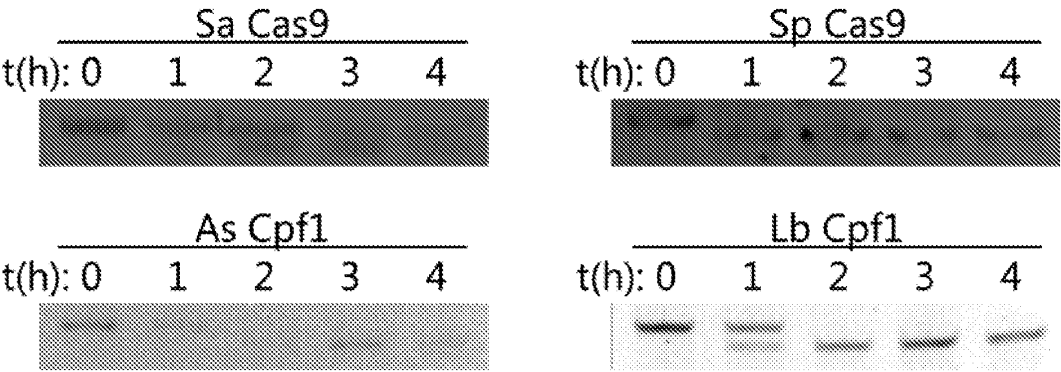


Figure 8

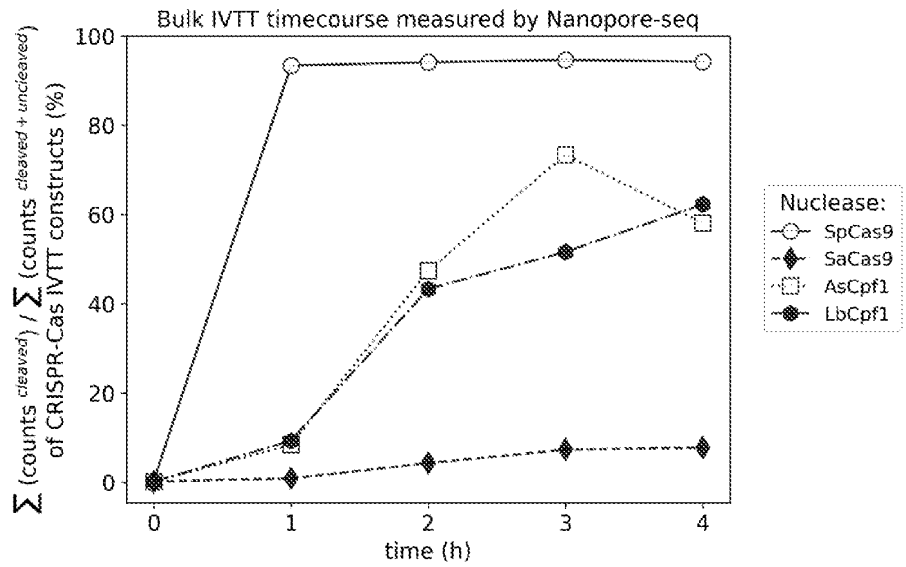


Figure 9

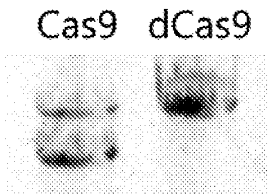


Figure 10

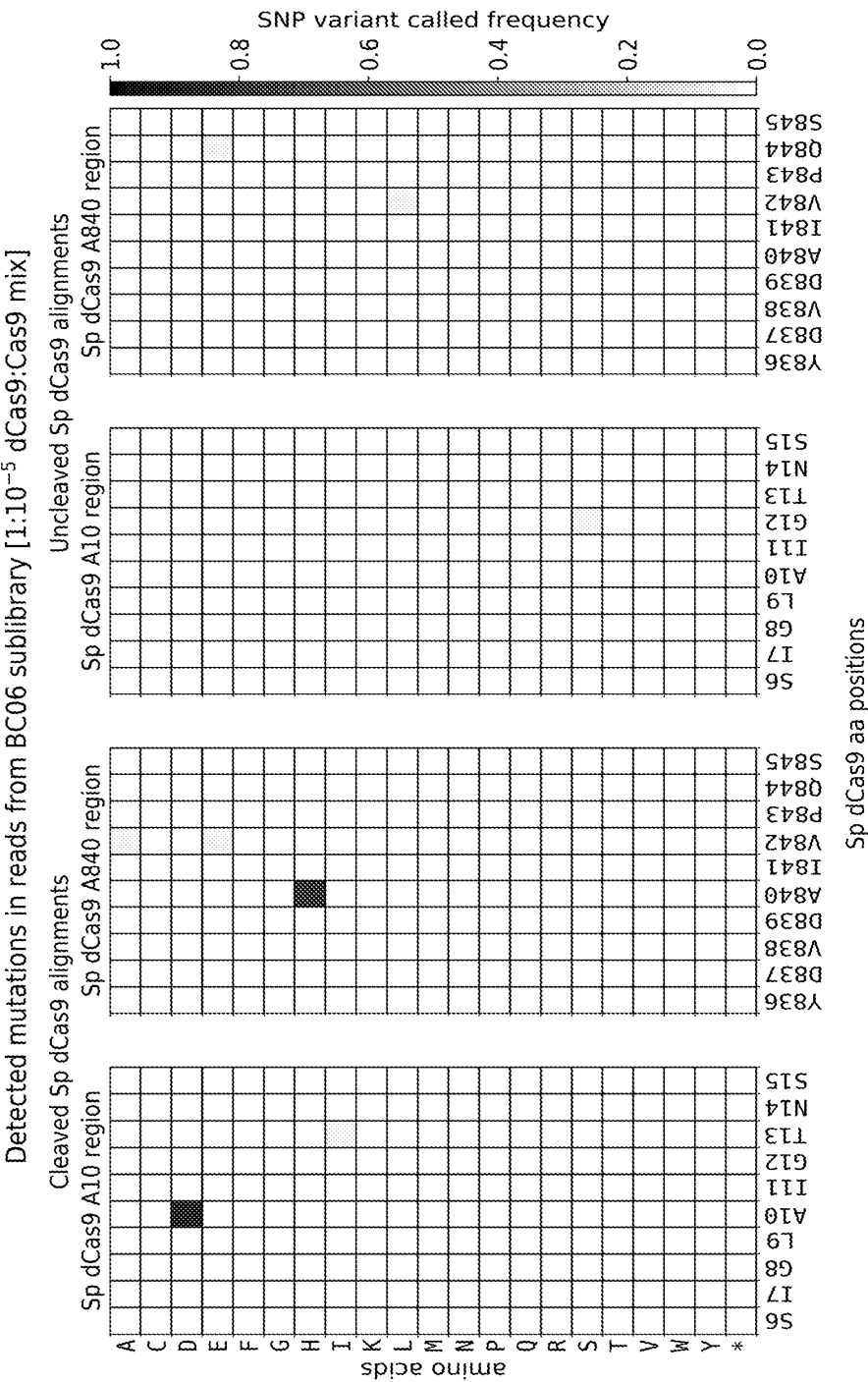


Figure 11

Emulsion IVTT reaction with limiting Sp Cas9 DNA input: reads mapped

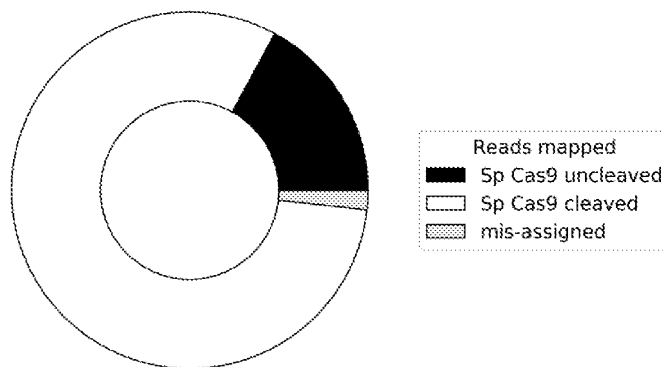


Figure 12

Emulsion IVTT reaction with limiting Sp dCas9 DNA input: reads mapped

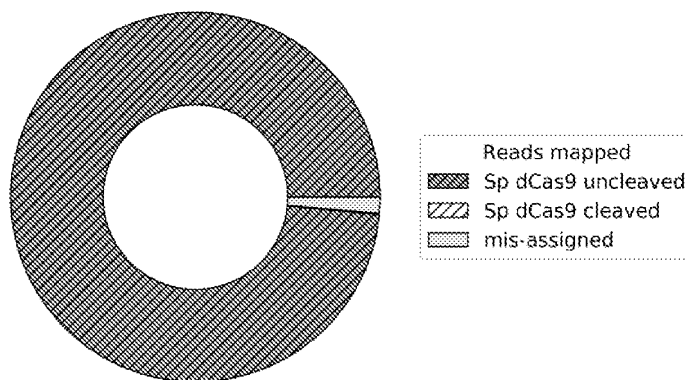


Figure 13

Emulsion IVTT reaction with limiting Sp Cas9 and dCas9 (1:1) DNA input: reads mapped

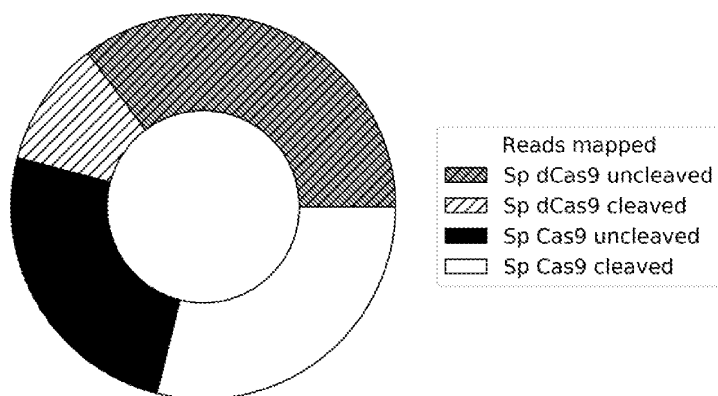


Figure 14