dCas9 end labeling

1. synthesize RNA guides (pg 55-57, ntbk 3)
   1. we are buying these at the moment due to poor yield of synthesis reaction
2. gel purify guide RNA (pg 56-59, ntbk 3)
   1. very difficult to separate out the DNA template even with the addition of DNase
   2. DNase treatment was problematic as we had issues with contamination and degradation of our lambda DNA either due to the DNase or from an unknown source.
3. anneal tracer and guide RNAs

5x RNA folding, annealing buffer

100 mM Tris-pH 7.5

500 mM KCl

25 mM MgCl2

annealing tracer and guide RNAs (or fold composite RNA)

2 uL 5x annealing buffer

x uL RNA – 5uM final

H2O to 10 uL

95deg, 5 min

slow cool to room temp

aliquot, flash freeze immediately, and store at -20deg

1. dCas9 loading with RNA

5x dCas9-RNA loading buffer

100 mM Tris-HCl

500 mM KCl

25% glycerol

25 mM MgCl2

5 mM DTT

loading reaction

2 uL 5x dCas9-RNA loading buffer

2 uL 5 uM RNA – 1uM final

2.5 uM 400 nM dCas9-Flag – 100 nM dCas9 final

3.5 uL H2O

37 deg, 10 min, then on ice until incubation with lambda DNA

Volumes of RNA and dCas9 may vary due to stock concentrations, but maintinaing the 10:1 ratio of RNA to protein is important. Increasing the amount of RNA in the reaction may also be helpful. These complexes can also be flash frozen and stored at -80, according to Leeanne (Panning Lab).

1. end labeling lambda DNA with dCas9

5x DNA binding buffer

200 mM Tris-HCl

125 mM KCl

5 mg/mL BSA

5 mM MgCl2

5 mM DTT

Lambda binding reaction

2 uL 5x binding buffer

2 uL dCas9-RNA

2 uL 0.5 nM lambda DNA

4 uL H2O

room temp, 15 minutes

Volumes of dCas9-RNA and lambda DNA may vary due to stock concentration, but maintaining the 10:1 ratio of protein to DNA is important. It may be beneficial to increase this ratio to increase binding efficiency

This pre-loading reaction is not compatible with chromatin substrates and results in compact punctate structures stuck to the surface of the flow cell and completely immobile under flow. End labeling is compatible once the curtain has been established by incubation on the microscope. Quantum dots can be pre-incubated with the dCas9 prior to addition to the flow cell or in an additional step after incubation of dCas9 with the curtain.

End labeling in the flow-cell (pg 102 ntbk 3)

10 uL dCas9-RNa loading reaction

994 uL blocking buffer

5 uL 100 mM MgCl2

1 uL anti-Flag QD (705nm)

Inject onto flow cell and incubate ~10 minutes without flow