Immobilized template assay for Mot1

1. Anneal oligos in 100 µl of 1X TE+ 0.1M NaCl by heating at 100°C for 5 mins and then slow cooling overnight. Add 10µg of biotinylated top strand and 20µg of the bottom strand so that it is always forced into the hybridized state.
2. Final concentration of the DNA is 100 ng/ul in 1xTE +0.1M NaCl. This could be ethanol precipitated to get a higher concentration.

Coupling DNA to beads

1. Wash 40 µl of Dynabeads M-280 Streptavidin (catalog no: 112.05D) for each reaction with 1X B&W buffer (manufacturer recommended) thrice.

2. Pull down the beads on a magnet and remove the washes. Resuspend the beads in 300 µl of 1X B&W buffer

3. Add DNA (600 ng) to the beads and incubate by rotating at 4°C for 4 hours

4. Then pull down the beads, remove the supernatant and wash the beads with 1X B&W buffer twice to get rid of the unbound DNA.

5. Then, wash the beads once with the final reaction buffer (1X Reaction buffer -DTT).

For catalytic reaction

1. Pull down and remove the wash. Resuspend the beads in 300 µl of Reaction mix+water (containing DTT).

2. Add 6 µl of 1 µM TBP (20nM final concentration). Incubate the tubes on a rotator at room temperature for 20 minutes.

3. Pull down the beads on a magnet and remove the flow through.

4. Resuspend the beads in 285 µl of Reaction mix+water. Add 15 µl (of 145nM) Mot1 and incubate at room temperature on the rotator for 30 minutes. *Note: if the reactions will be split in the end, you want to make sure there will be enough Mot1 to see on the gel. So although there might enough in terms of molarity for a binding reaction, each reaction should also have enough to see on a gel.*

5. Then pull down the beads and remove the supernatant.

6. Wash the beads with 1 ml of 1X Reaction buffer (-DTT) once (If reactions need to be split, this is a good point to split it).

7. Pull down on the magnet and remove the wash completely.

8. Resuspend the beads (might have been split into different tubes in reaction mix +water (+DTT). Adjust the mix+water according reactions containing ATP or competitor DNA.

9. Add ATP or competitor DNA to respective tubes.

10. Let the reaction go for 5 mins at room temperature. Then pull down the beads on the magnet and remove the supernatant carefully with a pipette. Try pulling it out by the same time to keep the time constant for each reaction.

11. Store the supernatant and beads separately on ice.

12. Mix sample buffer to a final concentration of 2X and run on a SDS-PAGE gel (percentage varies whether you are blotting for TBP or Mot1). Blot using anti-TBP antibody (Sigma, 58C9 clone) or Mot1 (anti-Py).