Two-dimensional Analysis of Meiotic Recombination Intermediates at *HIS4LEU2*

1. Day 1. Digest 2µg DNA to completion. Typically 2 µg in 80 µL final volume with 20-fold excess of restriction enzyme; 4hours.
2. Precipitate: add 5 µL 3M NaOAc pH5.5 and 190 µL EtOH (200 proof). Mix well by inversion and let stand for 20 minutes.
3. Spin full speed in ependorf centrifuge for 10 minutes. Pour off supernatant and drain on spiky rack with paper towels.
4. Rinse pellet with 100 µL 70% EtOH. Repeat spin for 5 mins.
5. Drain tube, pulse spin and pull off traces of ethanol with yellow tip. Air dry for ~10 mins.
6. Add 15 µL TE and flick tube to resuspend. Let stand for ~10 minutes and gently mix again.
7. Add 5 µL loading buffer (regular loading buffer but 4 x NEB3 restriction buffer; extra slat prevents sample jumping out of well), mix gently.
8. ~ 5pm. Load onto 0.4% SeaKem Gold agarose gel (in 1 X TBE – NO EtBr). [Prepare 3L, 1 XTBE plus 0.3 to 0.5 µg/mL EtBr and place in cold room to chill overnight]
9. Run first dimension for 17 hrs at 1V/cM (35V in long tank) at room temperature.
10. Day 2. ~10pm Stain gel in 1 X TBE + 0.3 to 0.5 µg/mL EtBr for 30 minutes at room temp. with gentle shaking. Prepare 500 mL 0.8% agarose in 1 X TBE WITH ethidium and place to cool in 50oC water bath.
11. View gel on LONG WAVE UV box. Cut 9.5 cm slice to cover range of interest i.e. from 2.2 kb marker on lambda *Bst*EII marker upwards; typically 1-2cm down from wells. Cut off top and bottom and then excise lanes as cleanly and as quickly as possible.

12. Place excised lanes in three rows of two in 20 x 27 cm gel tray. In cold room, carefully pour 0.8% gel around slices to *just* cover them. Allow to harden 30 minutes.
13. Run in pre-cooled TBE with ethidium at 6V/cm (~150 volts in our large tanks) for 6 hrs. Can also recirculate buffer cathode-to-anode with peristaltic pump.
14. Blot gel by alkaline transfer overnight.