BISHOP LAB
DSB DETECTION BY PULSED-FIELD GEL ANALYSIS
Combination of method from Borde *et al*, 1999 and Bishop Lab protocols

Note: This protocol is optimized for meiotic yeast in liquid sporulation cultures

DAY 1

1. Harvest 5mLs of cells from meiotic liquid culture (OD_{600} 0.7-1.0)
2. Spin cells at room temperature for 5 minutes in clinical centrifuge on setting 5
3. Wash 3 x with 50mM EDTA, pH 7.5, repeating spin as above
4. Resuspend cells in 100µL 50mM EDTA, pH 7.5
5. Add 113.6µL Zymolyase Solution A per sample (work with one sample at a time)
6. Then add the following per sample:
    a. Add 1.7µL β-Me
    b. 1.7µL 20mg/mL Zymolyase
    c. 83µL 2% agarose to each sample
7. Mix well and pipet mixture into plug molds (approx 100µL per mold)
8. Allow plugs to solidify at 4°C for 10 minutes
9. Push plugs into 2mL Eppendorf tubes containing 500µL RNase Solution A
10. If processing samples at different timepoints, keep plugs in RNase Solution A at 4°C until all samples are collected
11. When all plugs are made, add 500µL RNase Solution B
12. Mix gently by inverting tube and incubate plugs at 37°C for 2 hours
13. After incubation, decant RNase solution (using pipetman or aspirator)
14. Add 750µL Proteinase K Solution to each plug
15. Incubate overnight at 50ºC

DAY 2

1. Wash plugs 2 x 30 minutes in 1mL 50mM EDTA, pH 7.5 at room temperature
2. Store plugs at –20ºC in 1mL Plug Storage Solution

*** CAN STOP HERE ***

RUNNING GEL

1. Equilibrate plugs in 1mL of 0.5X TBE for 1 hour on ice
2. Load plugs in a 1% pulsed-field certified agarose gel in 0.5X TBE
3. Run gel under following conditions in 0.5X TBE (pre-chilled in gel apparatus)
    a. Voltage: 6V/cm
    b. Switch Angle: 120º
    c. Initial Switch Time: 90 seconds
    d. Final Switch Time: 90 seconds
    e. Run Time; 23 hours
    f. Temperature: 14ºC

Plug Storage Solution
50mM EDTA
50% glycerol
Adjust pH to 7.5

General Zymolyase Solution
170mM Sorbitol
10mM EDTA
17mM Na Citrate
0.85% β-Me
0.17 mg/mL Zymolyase
0.83% Low Melt Temp. Agarose (Nu Sieve or similar)

Zymolyase Solution A (All reagents except for β-Me, Zymo, and Agarose)
170mM Sorbitol
10mM EDTA
17mM Na Citrate

* For each plug sample, add 113.6μL of Solution A, then add:
    1.7μL β-Me
    1.7μL 20mg/mL Zymolyase
    83μL 2% agarose to each sample

RNase Solution A
450mM EDTA
10mM Tris-HCl (pH 7.5)

RNase Solution B
450mM EDTA
10mM Tris-HCl (pH 7.5)
15%β-Me
0.2μL/mL RNaseA

Proteinase K Solution
450mM EDTA
10mM Tris-HCl (pH 7.5)
1% SDS
1mg/mL Proteinase K

* Add Proteinase K fresh before using