DIGESTION OF GENOMIC DNA FOR LIBRARY CONSTRUCTION

Test

1. Set up following digest:
   10 μg DNA
   10 μl 10 x Sau 3A buffer
   10 μl 1mgml^{-1} BSA
   10 μl 10 mM spermidine
   up to 100 μl with dH_2O

2. Divide mixture into 1 tube of 20 μl and 8 tubes of 10 μl.

3. To the tube with 20 μl mix, add 1 unit of Sau 3A enzyme.

4. Dilute as follows:

	1	2	3	4	5	6	7	8	9	10
Mix	20	10	10	10	10	10	10	10	10	10
	10									

Sau3A	1u	.125	.03125	.0078	.002					
	.25	.0625	.0156	.0039	.001					

5. 1 hour at 37°C.

6. Add 2 μl 100 mM EDTA to each tube to stop reaction.

7. Run on 0.5 % agarose gel alongside markers to check.

8. Look for sample that gives the tightest band at 15-25 kb.

Large prep.

1. Digest 100 μg DNA under conditions predetermined by test digestion (10 x more of everything).

2. Check an aliquot on 0.5% agarose gel.

3. Make a 10-30% glycerol gradient in an SW41 tube:

   Make 5 ml 10% glycerol in 10 mM Tris pH 7.5
   2 mM EDTA
   200 mM NaCl

   and 5 ml 30% glycerol in the same.

   Use a gradient mixer to pour a continuous gradient.

4. Load digest on gradient and run in SW41 rotor, 24,000 rpm 16 h 15°C.

5. Collect 500 μl fractions.

6. Check on gel and pool fractions between 10 and 20 kb.

7. Ethanol precipitate by adding 30 μl 7.5 M NH_4OAc and 500 μl isopropanol to each fraction.

8. Wash well with 70 % ethanol.

9. Resuspend in 25 μl TE.