Partial Restriction Digests

4/18/2001
Leslie Vosshall

- Purpose: To generate DNA cut at a subset of possible restriction sites for a given enzyme.
- Method: A fixed amount of DNA will be cut with progressively less enzyme for a fixed period of time (1 hour).

Set up 6 tubes with the following components

2 ug DNA
5 ul 10X buffer appropriate for enzyme
5 ul 10X BSA
dH2O to a volume of 49 ul

Add enzyme according to the following schedule for digests [A] – [F]:
[A] 1.0 ul Enzyme
[B] 0.1 ul Enzyme
[C] 0.05 ul Enzyme
[D] 0.01 ul Enzyme
[E] 0.005 ul Enzyme
[F] 0.001 ul Enzyme

[A] add 1 ul of enzyme and mix well; place digest on wet ice until all tubes have received enzyme.

[B] Make a 1:10 dilution of enzyme as follows:
0.5 ul enzyme
0.5 ul 10X buffer
4 ul dH2O
mix well

add 1 ul to digest and mix well; place digest on wet ice until all tubes have received enzyme.

[C] Add 0.5 ul of 1:10 dilution and mix well; place digest on wet ice until all tubes have received enzyme.

[D] Make a 1:100 dilution of enzyme as follows:

1 ul of 1:10 dilution
1 ul of 10X buffer
8 ul of dH2O
mix well

add 1 ul to digest and mix well; place digest on wet ice until all tubes have received enzyme.

[E] Add 0.5 ul of 1:100 dilution and mix well; place digest on wet ice until all tubes have received enzyme.

[F] Make a 1:1000 dilution of enzyme as follows:

1 ul of 1:100 dilution
1 ul of 10X buffer
8 ul of dH2O
mix well

add 1 ul to digest and mix well; place digest on wet ice until all tubes have received enzyme.

INCUBATE ALL 6 DIGESTS AT 37^{o}C FOR EXACTLY ONE HOUR

At the end of the one hour digestion, place all digests on wet ice to stop enzyme reaction. Then, either gel purify immediately, or heat inactivate enzyme for 30 minutes at 65^{o}C. If enzyme cannot be heat inactivated, add 1 ul 0.5M EDTA to kill enzyme and purify by phenol extraction.