

# Lambda HindIII Ladder

## Reagents

1. To make ~120 50  $\mu$ l aliquots of ladder, mix the following reagents in a 2.0 ml tube on ice:

|                                |              |
|--------------------------------|--------------|
| dH <sub>2</sub> O              | 1068.36      |
| NEB buffer 2                   | 150          |
| $\lambda$ DNA (500 $\mu$ g/ml) | 251.64       |
| HindIII (20 U/ $\mu$ l)        | 30           |
|                                | <hr/>        |
|                                | 1500 $\mu$ l |

## Procedure

2. Split reagent cocktail into three 1.7 ml tubes (500  $\mu$ l/tube);
3. Incubate at 37°C for 1 hour;
4. Stop digestion at 65°C for 15 minutes;
5. Pool reactions together in a 50 ml centrifuge tube (*keep track of the total volume of the pooled digestion reactions*);
6. Add 600  $\mu$ l 10X loading dye for each 1500  $\mu$ l digest;
7. Add 3.9 ml dH<sub>2</sub>O for each 1500  $\mu$ l digest;
8. Run 5  $\mu$ l and 10  $\mu$ l of ladder in several lanes of a 1.5% agarose gel to check the quality of the digestion;
9. Use repeater pipette to aliquot 50  $\mu$ l ladder to series of 0.6 ml tubes and store at -20°C.

## REAGENTS NEEDED:

NEB buffer 2 (provided with HindIII enzyme below)  
Lambda DNA, 250 µg, 500 µg/ml – www.neb.com, N3011S  
HindIII, 10,000 units, 20,000 U/ml – www.neb.com, R0104S  
10X loading dye

## NOTES:

- λ DNA = 48,502 bp in length.
- HindIII restriction produces band lengths given below:

| λ HindIII fragments | ng/band in 10 µl |
|---------------------|------------------|
| 23,130              | 100              |
| 9,416               | 41               |
| 6,557               | 28               |
| 4,361               | 19               |
| 2,322               | 10               |
| 2,027               | 9                |
| 564                 | 2                |
| 125                 | 0.5              |

- Want 100 ng of largest (23,130 bp) band in a 10 µl load of ladder (on basis of this can calculate ng for smaller bands - rounded above).
- Thus, want 209.69 ng per 10 µl ladder (sum ng of all bands).
- So, in 6 ml (result of procedure above) want 125.8158 µg λ DNA.
- λ DNA is at 500 µg/ml (= 0.5 µg/µl), so need  $125.8158 / 0.5 = 251.64$  µl λ DNA per reaction.