

ENDONUCLEASE DIGESTION OF DNA

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http://biology.clc.uc.edu/fankhauser/Labs/Genetics/DNA_Digestion/DNA_Digestion.htm

Endonucleases are the highly specific enzymes which recognize unique palindromic sites on DNA at which they hydrolyze the phosphoester linkage. These enzymes have been crucial in the burgeoning fields of genetic sequencing and engineering. Because endonucleases are commonly found on the surface of the skin, as well in bacteria, latex gloves are routinely worn and good sterile technique is required when performing DNA manipulations.

We will perform three digestions on purified lambda DNA: 1) with Hind III (A[^]AGCTT), isolated from *Hemophilus influenzae*, 2) with Eco RI (G[^]AATTC), isolated from *Escherichia coli*, and 3) with both. [*BamHI* (G[^]GATCC) may also be used] The resulting fragments yield a restriction map of the lambda genome.

EQUIPMENT:

All equipment and supplies listed in the protocol on Electrophoresis of DNA Fragments

37°C incubator

SUPPLIES:

Microcentrifuge tubes
Lambda DNA: 0.5 ug/μL [50 ug in 100 uL]
Eco RI, 10 units/μL [2000 units]
Hind III, 10 units/μL [2000 units]
specific buffers for Hind III and Eco RI
Hind III lambda digest, 0.125 ug/μL
loading dye

1. Prepare the digestion tubes in an Eppendorf microcentrifuge tube:

Single digestion:

0.0 μL ddH₂O (enough to *q.s.* to 30 μL, depending on DNA conc.)
3.0 μL 10x buffer for the endonuclease you are using
17.0 μL Lambda DNA (at ~0.286 ug/μL, = ~5 ug DNA)
10.0 μL Eco RI or Hind III (10 units/μL = 100 units total enzyme)

total volume: 30.0 μL

Double digestion:

0.0 μL ddH₂O (enough to *q.s.* to 30 μL, depending on DNA conc.)
1.5 μL 10x buffer for Eco RI
1.5 μL 10x buffer for Hind III
17.0 μL lambda DNA (at ~0.286 ug/μL, = ~5 ug DNA)
5.0 μL endonuclease (10 units/μL = 100 units total enzyme)
5.0 μL endonuclease (10 units/μL = 100 units total enzyme)

total volume: 30.0 μL

2. **Incubate** at least 30 minutes at 37°C, an hour might be OK, perhaps needed for double digestion.
3. **Halt the reaction** by placing on ice, add 6 μL of loading dye, mix with same pipet, reset volume to 25 μL for next step (leave the tip in the Eppendorf tube).
4. **Load the wells:** Pipet 5 μL into one well, reset pipet to 25 μL, pipet that into second well. On the same gel, run a lane with undigested lambda DNA, and one with a Hind III standard. Run the gel, photograph or carefully diagram the resulting bands.

There are eight Hind III fragments: 23,130, 9416, 6557, 4361, 2322, 2027, 564, 125 bp long. The 564 band is in the bromphenol blue, and the 125 band appears beyond the dye band.