Restriction Digest and Electrophoresis without DNA Recovery

Background:

Restriction enzymes such as *EcoRI* recognize specific sequences of DNA and cleave the phosphodiester bond on each strand at that sequence. After digestion with a restriction enzyme, the resulting DNA fragments can be separated by agarose gel electrophoresis, and their corresponding sizes can be estimated.

In this experiment, we will perform a restriction digest using the restriction enzyme *EcoRI* on λ-phage DNA that has already been digested by the enzyme *HindIII*. (Please see the attached sequence site sheet for exact fragment lengths)

The recognition site of *EcoRI* is:
5'…G'AATTC…3'
3'…C'TTAAG…5'

Materials:

λ-DNA/*HindIII* solution (5 µl = 2.5 µg)
EcoRI enzyme (0.5 µl = 10 units)
10X *EcoRI* buffer (5 µl)
DD-H_2O (double-distilled)
37ºC incubator
65ºC bath
Micropipettes and tips
Gel dish
6X loading dye
1X TAE Buffer
1X TAE-agarose gel
100 bp dyed DNA ladder

Procedure:

In a one ml centrifuge tube add the following components in this order:
5 µl λ-phase DNA/*HindIII*
5 µl 10X *EcoRI* buffer
39.5 µl DD-H_2O
0.5 µl *EcoRI* enzyme

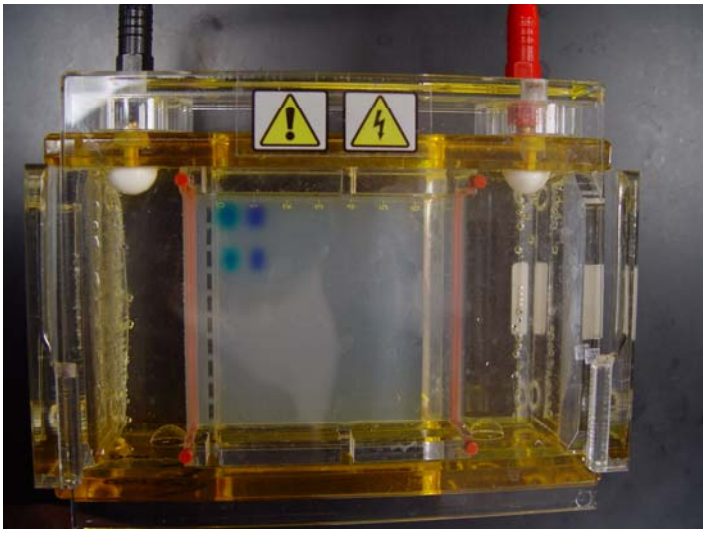
Mix the final solution with the pipette tip. *Do not vortex as this will rip the DNA.* Spin down the tube in the micro-centrifuge to ensure all liquid is at the bottom.

Place the tube in the 37º C bath for about 2 hours to facilitate digestion.

Meanwhile, clean the gel dish with ethanol and DI water. Heat the agarose gel to about 65 ºC to liquefy, and pour about 35ml (1/3 full) into the sealed gel tray ensuring there are *no bubbles* in the liquid (ask for help here). Make sure the lane comb is properly in place before pouring the gel. Allow about 25 minutes for the gel to cool and solidify. Once fully solidified, remove the lane comb, and rotate the gel tray 90 degrees so that the gel wells are exposed to the negative terminal reservoir. Fill each of the reservoirs with 1X TAE buffer until the gel is submerged with about 1mm of buffer. Set the power supply to 60VDC and 120 minutes, but do not start it yet.

About now your digestion should be complete. Place the centrifuge tube into the 65ºC bath for about 5 minutes. This effectively destroys the enzymes and stops digestion. Remove the centrifuge tube from the bath and let it cool for a few minutes. Place 10 µl of 6X loading dye in the centrifuge tube and stir gently with the pipette tip. You are now ready to start the gel.

Pick someone in your group with a steady hand to place about 15 µl of your digest in the first lane of the gel, 15 µl of the dyed 100bp ladder solution in the third lane, and 15 µl of the dyed λ-phage DNA/*HindIII* solution in the fifth lane. Secure the electrophoretic lid, and start the power supply. Be careful not to shake or jostle the gel. Within a few minutes you should be able to see the loading dye moving in the gel, as shown below.



When the 120 minutes is up, the power supply will automatically turn off. Carefully remove the gel tray and gel from the TAE buffer solution and place it in the Ethidium Bromide staining bath for approximately 8 minutes. PLEASE BE CAREFUL: ETHIDIUM BROMIDE IS A DANGEROUS MUTAGEN AND CARCINOGEN, DO NOT SPLASH OR SPILL IT!! YOU MUST WEAR NITRILE GLOVES, SAFETY GLASSES AND A LAB COAT DURING THIS STEP. Make sure to thoroughly rinse the gel tray and spatula after contact with the Ethidium Bromide. Next rinse the gel in the water bath for 20 minutes. Finally, carefully place the gel on the UV light stage. Place the UV blocking cover over the stage and turn the UV lamp on. Quickly position the camera over the gel and take the best possible image, preferably one with a longer exposure. Admire your gel, you are now done.

Using a computer, you should be able to compare the calibration for the 100 bp ladder (found below) with your digested DNA, and verify the combined *HindIII* and *EcoRI* cut lengths. Does a linear relationship exist between the fragment length and the distance it goes in the gel?

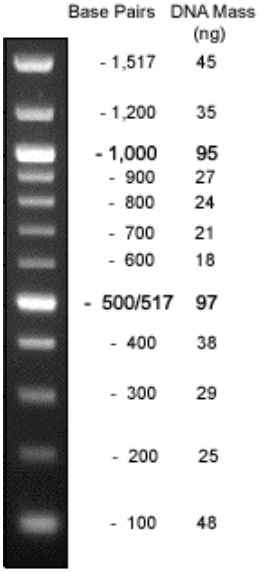


Fig: 0.5 µg of 100 bp DNA Ladder visualized by ethidium bromide staining on a 1.3% TAE agarose gel (courtesy NEB)

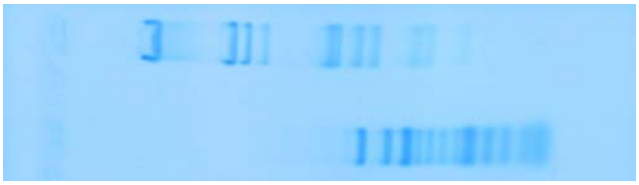


Fig: Top lane-λ-phase DNA digested by *HindIII* & *EcoRI*, bottom lane: 100bp ladder. This is a decent experimental result from this protocol.

Lambda DNA: Location of Sites

Enzyme	#	Locations				
Apa I	1	10086				
Bmt I	1	34679				
BsiW I	1	19323				
Kas I	1	45679				
Nae I	1	20040				
Nar I	1	45679				
NgoM IV	1	20040				
Nhe I	1	34679				
PaeR7 I	1	33498				
PspOM I	1	10086				
SanD I (x)	1	28797				
Sfo I	1	45679				
SnaB I	1	12188				
Tli I	1	33498				
Xba I	1	24508				
Xho I	1	33498				
Acc65 I	2	17053	18556			
Afe I	2	20995	37057			
Asc I	2	3521	16648			
AvrI I	2	24322	24396			
Bsa I	2	11424	42715			
Bsu36 I	2	26717	34318			
Eag I	2	19944	36654			
FspA I (x)	2	21804	21825			
Kpn I	2	17053	18556			
Pci I	2	628	39395			
PflF I	2	11202	36120			
Pme I	2	8459	16293			
Sac I	2	24772	25877			
Sal I	2	32745	33244			
Tth111 I	2	11202	36120			
Afl II	3	6540	12618	42630		
BstZ17 I	3	15260	18834	19473		
Drd I	3	5116	9104	11090		
EcoO109 I	3	2815	28797	48473		
Pml I	3	26529	41482	42362		
PpuM I	3	2815	28797	48473		
Pvu I	3	11933	26254	35787		
Sma I	3	19397	31617	39888		
Xma I	3	19397	31617	39888		
ApaL I	4	5619	21798	27173	40216	
Bmr I	4	7054	11608	25691	30332	
Nco I	4	19329	23901	27868	44248	
SacI I	4	20320	20530	21606	40386	
BamH I	5	5505	22346	27972	34499	41732
BsrG I	5	5220	6142	15855	29392	32496
EcoR I	5	21226	26104	31747	39168	44972
Nru I	5	4590	28050	31703	32407	41808
RsrI I	5	3800	6041	13983	19288	22242
Sbf I	5	2555	2819	11834	19832	37000
Sca I	5	16421	18684	25685	27263	32802
SexA I	5	22264	31009	32838	40497	44408
Bgl II	6	415 38814	22425	35711	38103	38754
Blp I	6	10297 39450	10682	11661	16518	20744
BssH II	6	3522 28008	4126	5627	14815	16649
SgrA I	6	7064 31824	8680	12878	15653	16974
Sph I	6	2212 39418	12002	23942	24371	27374
Stu I	6	12434 40614	31478	32997	39992	40596
AcI I	7	13529 43392	16290 43488	22580	22595	24642
Ban II	7	581 25877	10086 39453	19763	21570	24772
BbvC I	7	8012 31836	18147 35813	18465	30916	31222
BstB I	7	18048 34331	25884 42637	27980	29150	30396
Hind III	7	23130 37584*	25157 44141	27479 (**cI*857 *ind*1 mutants only)	36895	37459
Mlu I	7	458 20952	5548 22220	15372	17791	19996
Nde I	7	27630 38357	29883 40131	33679	36112	36668
PshA I	7	8920 37889	9394 48152	13512	15412	36925
Ava I	8	4720 33498	19397 38214	20999 39888	27887	31617
Bcl I	8	8844 43682	9361 46366	13820 47942	32729	37352
BsoB I	8	4720 33498	19397 38214	20999 39888	27887	31617
BspH I	8	889 28860	4650 31608	4989 40642	10249	18275
BssS I	8	20356 35219	25572 42416	27956 42737	29425	34430
Mfe I	8	22687 37186	22715 38332	23054 47880	25863	35764
Acc I	9	2190 32745	15260 33244	18834 40201	19473 42921	31301
Ahd I	9	6398 18544	11238 23460	12477 30467	12915 44669	16588
EcoN I	9	13509 35521	21292 38268	22377 41842	25174 47213	25223
Aat II	10	5105 40806	9394 41113	11243 42247	14974 45563	29036 45592
Bae I	10	694 18732	7665 19109	13267 21779	15775 43976	16271 48385
Bme1580 I	10	5619 14897	5664 21798	10086 27173	11414 32330	13039 40216
Dra III	10	2954 30365	5613 31909	6635 41479	8999 47312	14477 48434
Sap I	10	2397 24769	6489 27234	8702 34327	10370 34800	13286 47712
Sty I	10	19329 27868	21211 28793	23901 35016	24322 36505	24396 44248
Zra I	10	5105 40806	9394 41113	11243 42247	14974 45563	29036 45592
Aar I (x)	12	554 16194 37003	10835 16233 38481	13941 21242	14383 29897	16164 36293
Psi I	12	2285 24667 33236	9011 25472 37756	18941 27746	19573 29498	22014 29656
Xcm I	12	958 9460 32530	4770 9490 36165	5065 14891	5874 19329	9193 23685

There are no restriction sites for the following enzymes:
AsiS I, Fse I, I-Ceu I, I-Sce I, Not I, PI-Psp I, PI-Sce I, Pac I, Sfi I, Spe I, Srf I (x), Swa I

(x) = enzyme not available from NEB