pBR322 Mapping by Restriction Digestion with EcoR1/HincII/PvuII and Electrophoresis

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March 15, 2019

Introduction

In order to defend the attack from virus, some bacterias can produce enzymes to digest invasive DNA molecule of virus. Among these kinds of enzymes, some of them cut DNA randomly, while others called restriction enzymes only cut at specific sequence. Thus, we can use these enzymes to digest the plasmid and get DNA fragments with different length.

As the cut sites are fixed in a plasmid, we can reconstruct the relative positions of restriction sites of a plasmid, called mapping.

Firstly, we digest the plasmid with all possible combinations of 3 restriction enzymes. Then, we can use the agarose gel and certain voltage to separate negatively charged DNA fragments and use loading dye to indicate their positions under UV. And their travel distances are proportional to the reciprocal of logarithm of the number of base pairs, namely, the size of molecule compared with the size of holes in gel, which allows us to draw a standard curve from a reference plasmid with known structure. Finally, we can get the length from travel distance based on the standard curve and use these length to rebuild a plasmid.

1 Results

1.1 Raw Result Photo

To make the photo more readable, I convert[1] the photo to black-and-white, adjust the contrast and label 9 wells.

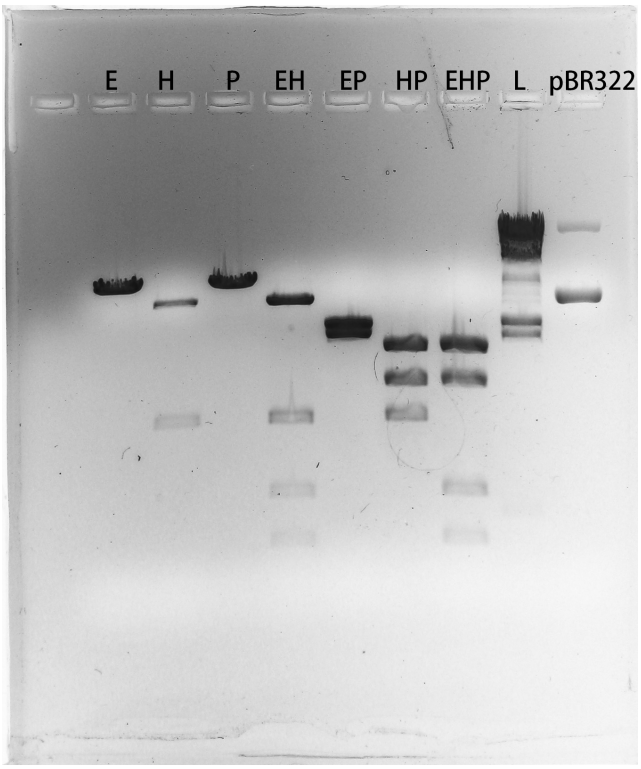

Figure 1: Raw Gel Photo

1.2 λ DNA data

Here is the known information of λ DNA's fragments bands digested by **HindIII**, which is the 8^{th} well in the photo named **L**.

[1] All the picture analysis below is based on the *Fiji ImageJ*, including color converting and distance measuring.

Table 1: λ DNA Data

	base pairs	log(bps)	distance (pixels)
1	23130	4.364175633	266
2	9461	3.97386645	292
3	6557	3.816705184	326
4	4361	3.639586087	376
5	2322	3.365862215	468
6	2027	3.306853749	502
7	564	2.751279104	870

1.3 Standard Curve

We can write distance in terms of d, and base pairs in terms of n. According to the relationship, we will have,

$$d \propto \frac{1}{log(n)}$$

The raw curve is plotted as follows,

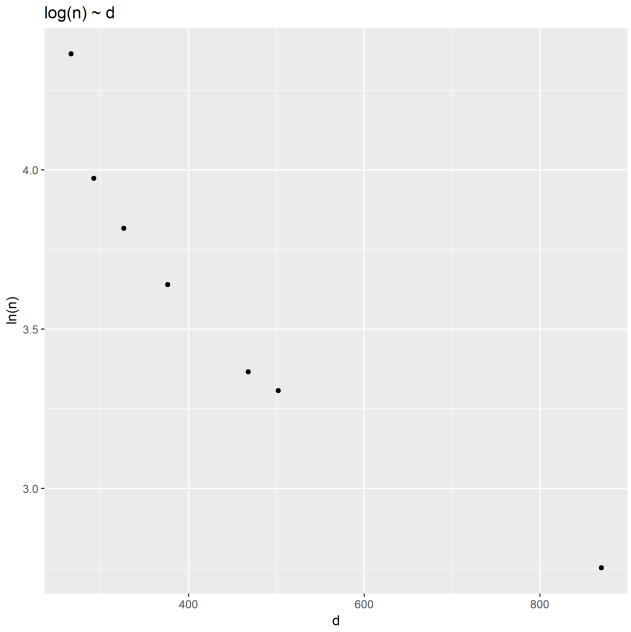

Figure 2: The realtionship between distance and logarithm of number of base pairs

After choosing some of the data points and using linear regression fitting algorithm, I derived the equation that:

$$n = exp(4.2 - 1.7 \times 10^{-3} \cdot d)$$

where n is the length (bps) of DNA fragments and d is the distance these fragments travel.

The combination of this line and data points is as follows,

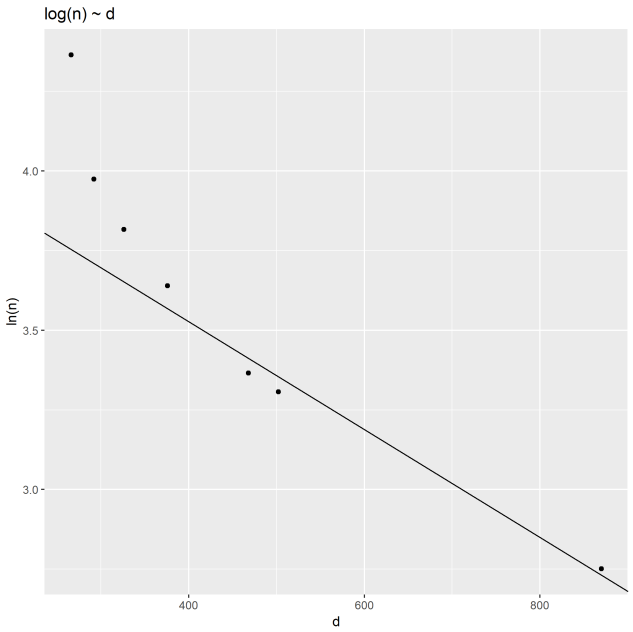

Figure 3: Standard Curve with Fitting Line

1.4 pBR322 Digestion Data

The distance of every well is shown below, using pixel unit.

Table 2: Distance

tube	E	H	P	EH	EP	HP	EHP
distance	400.125	418.12	388.082	424.118	472.004	520.004	530.015
(pixels)		682.237		676.027	486.037	596.03	602.003
				830.039		668.012	830.002
				924.078			928.002

So using the equation derived above, we can get the estimated sequence length (rounded to integer),

Table 3: Length

tube	E	H	P	EH	EP	HP	EHP
length	3342	3112	3499	3041	2523	2089	2009
(bps)		1107		1135	2388	1552	1517
				621		1169	621
				430			423

and also the logarithm of length,

Table 4: Logarithm of Length

tube	E	H	P	EH	EP	HP	EHP
log(length)	3.524	3.493	3.544	3.483	3.402	3.32	3.303
		3.044		3.055	3.378	3.191	3.181
				2.793		3.068	2.793
				2.633			2.626

1.5 pBR322 Mapping

2 Discussion

2.1 Standard Curve

First, I tried to discard the points with larger length, which is out of the gel's resolving ability. The lines are derived from linear regression, namely, least squared method (calculation not shown).

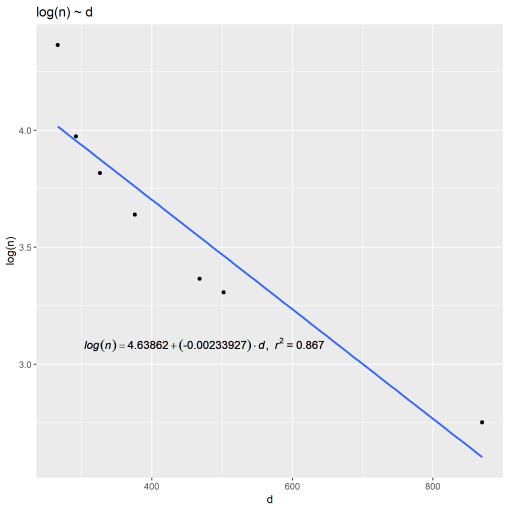

Figure 4: No Discarding

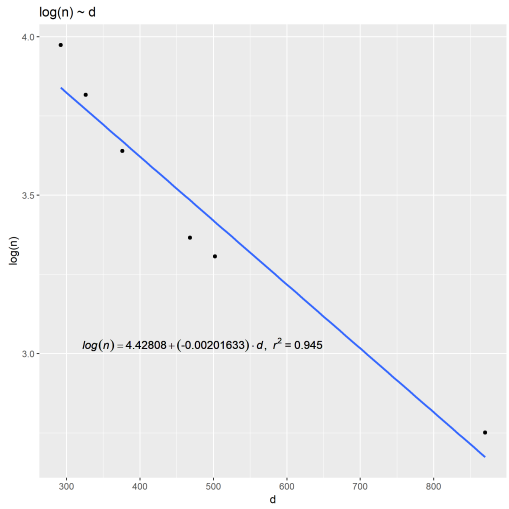

Figure 5: Discarding Point 1

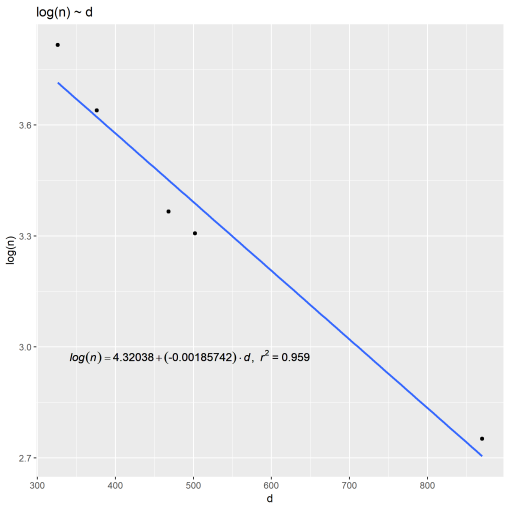

Figure 6: Discarding Point 1∼2

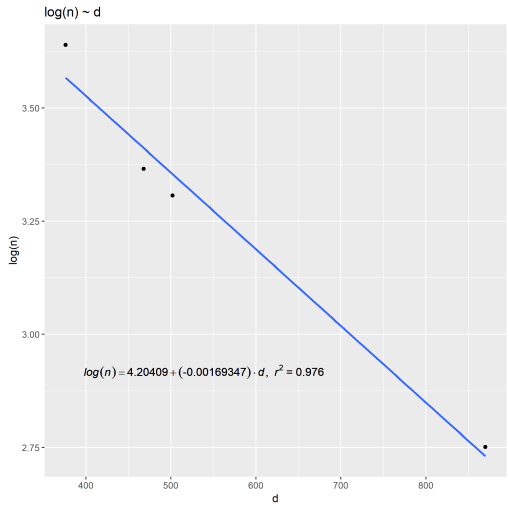

Figure 7: Discarding Point 1∼3

Then compared the R^2, we can see that discarding first 3 points leads to the highest R^2 as 0.976. In addition, these three points are also longer than 5000bp, larger than the ability of gel resolution.

If we discard more points, we would get higher R^2, but we will lose data information and increase the effect of random error.

Finally we will use Figure 7 as the fitting line, which has already been plotted in Figure 3 in **Result** part.

In conclusion,

$$\log(n) = 4.2 - 1.7 \times 10^{-3} \cdot d$$

$$n = exp(4.2 - 1.7 \times 10^{-3} \cdot d)$$

2.2 Relative Position Analysis

For convenience, I label bands from tube **E, H, P, EH, EP, HP, EHP**.

Figure 8: Labeled Bands

2.2.1 Single Enzyme Digestion

For tube **E, H, P**, we can see that **E, P** have two similar single bands, but **H** has two bands, which indicates that enzyme EcoR1 and PvuII cut the plasmid at one site, while HincII cuts at two sites. The picture below demonstrates this more clearly.

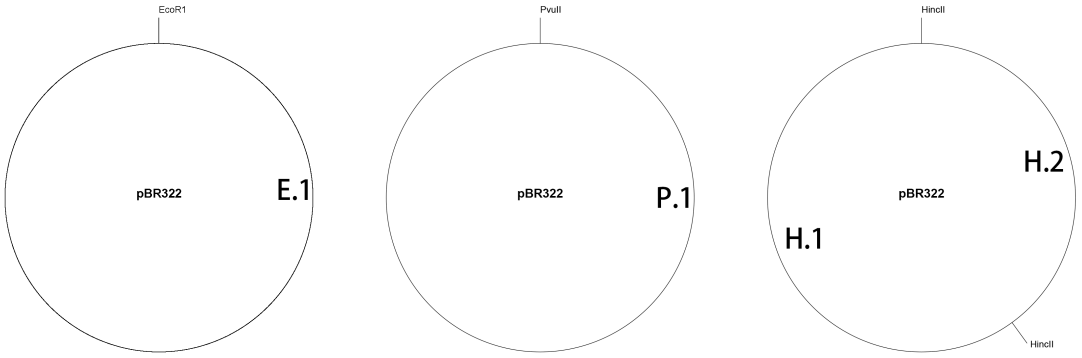

Figure 9: Number of Cutting Sites

Here, I choose different lengths for H.1 and H.2, because their bands' distances are different, causing H.1 to be longer with small distance, and H.2 to be shorter.

2.2.2 Two Enzymes Digestion

After we get the number of cutting sites of three enzymes, we can analysis their combination digestion.

EH tube There are 4 different bands, and two of them (EH.1, EH.2) are almost the same as bands of **H**'s (H.1, H.2). However, as it is digested by EcoR1 and HincII, who have 1 and 2 cutting sites. So there should be 3 sites, and 3 bands. That is, HincII cuts the plasmid into two part, and EcoR1 cuts one of these two in to another two parts. Considering two same bands as **H**'s, the reaction of EcoR1's cutting is not complete, remaining small amount of HincII's product. In addition, as the signal of EH.1 is stronger than EH.2's, it is very likely that EH.2 (also H.2) should be cut into EH.3 and EH.4 but reaction is not complete, which means EcoR1's site might be located on EH.2 (also H.2), namely, shorter part between two HincII sites.

EP tube The phenomenon is similar to **H**'s. Simply put, EcoR1's and PvuII's cutting sites lead to two fragments with different but similar lengths, since their distances are similar. So, EcoR1's cutting site should be about the opposite of PvuII's site.

HP tube This can be analyzed as **EH** tube, but with better and clearer result. The digestion process can be explained as follows[2]. HincII cuts plasmid into H.1 and H.2. Then, PvuII cuts H.1 into HP.1 and HP.2, leaving H.2 as HP.3, for their lengths are similar. So, PvuII's cutting site is located in H.1, namely, the longer part between two hincII sites.

Two Enzymes Conclusion We have got three conclusions:

1. EcoR1 is on shorter part between HincII. (Assumption with high likelihood)
2. EcoR1 is about the opposite of PvuII.
3. PvuII is on longer part between HincII.

In addition, it is mentioned above that EH.3/EH.4 are products of H.1 or H.2. And HP.1/HP.2 are products of H.1. However, both EH.3 and EH.4 is shorter than HP.1 and HP.2, for their smaller distances, which indicates that if we connect EH.3 with EH.4, the summation of length is less than HP.1 plus HP.2. That is, EH.3/EH.4 and HP.1/HP.2 come from two different reagents, EH.3/EH.4 from shorter one (H.2), and HP.1/HP.2 from longer one (H.1). So, here is the map.

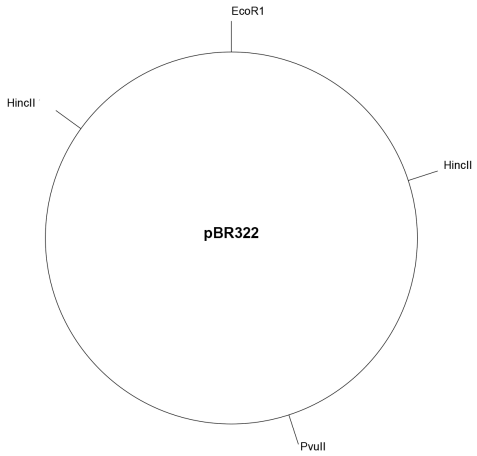

Figure 10: Possible Map

[2] There is no order of two enzyme reactions, but explaining them in order can make reaction process clear.

2.2.3 Three Enzyme Digestion

For **EHP** tube, EHP.1 and EHP.2 are similar with HP.1 and HP.2. Meanwhile, EHP.3 and EHP.4 are similar with EH.3 and EH.4.

The bands correspond with the mapping result[3]:

$$\begin{aligned} \text{pBR322} &\xrightarrow[\text{HincII}]{} \text{H.1} + \text{H.2} \\ \text{H.1} &\xrightarrow[\text{PvuII}]{} \text{EHP.1} + \text{EHP.2} \\ \text{H.2} &\xrightarrow[\text{EcoR1}]{} \text{EHP.3} + \text{EHP.4} \end{aligned}$$

2.3 Length Analysis

Before we start to calculate the length, we have to clean the raw length data (Table 3 and Table 4). As we have known that some bands have equal length, some are the summation of other bands, and some bands equal to the whole plasmid.

First, the total length sould be standardized, using the average of 7 wells' results.

Table 5: Total Length of Each Well

tube	E	H	P	EH	EP	HP	EHP
length (bps)	3342	3112 1107	3499	3041 (1135) 621 430	2523 2388	2089 1552 1169	2009 1517 621 423
total	3342	4219	3499	4092*	4911	4810	4570
average	4206						

* EH.2 (length = 1135) is remaining of incomplete reaction, not included.

Then, using the average length as the total plasmid length, we can standardize the raw data.

[3] No fixed order in real reaction.