Gene And Cell Technologies

Open Source DNA Ladder Plasmids

Gene And Cell Technologies' Open-Source DNA Ladder Plasmids can be used to manufacture your own DNA size standards easily and inexpensively.

Most people working with DNA use a size-standard containing DNA fragments of known sizes, the well known "Ladder". As shown in Figure 1, ladders are produced by restriction-digests of plasmids containing appropriately spaced restriction sites. These "source plasmids" can be grown inexpensively at large scale. But the linear fragments can no longer be replicated. These basic facts of nature allow anyone who goes through the trouble of actually creating a source plasmid to keep the plasmid itself locked away, while selling only the digested fragments that the user cannot make more of. To this day, every reagent vendor in the world capitalizes on this natural mechanism to keep their customers dependent on continued purchases, while securing a steady stream of profits.

These days have ended. Gene And Cell Technologies is proud to release the world's first open-source DNA ladder plasmids. Our plasmids have been synthesized from scratch by our private company. They are not bound by any third party restrictions, MTAs or other intellecutal property. We release them to be used for any legal purpose including commercial. We hope that these plasmids will free scientists around the world from millions of unnecessary "rents" paid to the proprietary reagent vending industry.

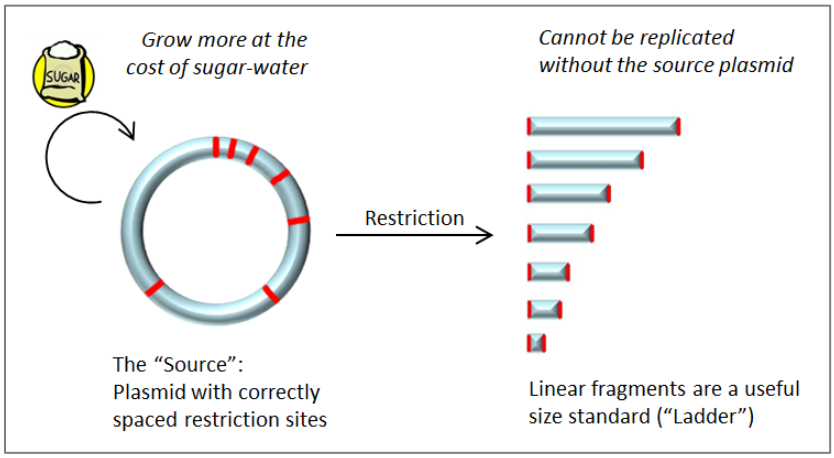

Figure 1: How to Make a DNA Size-Standard ("ladder"). A DNA size-standard is produced by restriction digestion of a special "source" plasmid containing appropriately spaced cut-sites.

Why are we the only ones in the whole wide world releasing these plasmids?

It's because at Gene And Cell Technologies, we don't' just say we're different, but we really are different. We are not primarily a reagent company. We are primarily a science company, attempting to develop futuristic regenerative medicine therapies. There's nothing wrong with earning some money along the way. But primarily, we do it because we want those medicines to exist. And you, the world's scientists, and are discovering the knowledge needed for that. We will do business with you as equals at reasonable prices, but we will not attempt to trap you in the kinds of dependencies that traditional science vending companies do. Our open source ladder plasmids are just one small example of that.

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Overview & Background Considerations

Three open source ladders currently exist, to serve a variety of needs. They can all be ordered directly through the Gene And Cell Technologies website http://www.geneandcell.com/collections/open-source.

Product Name	Description	Spacing	Range
p100bp	A single plasmid giving rise to a basic ladder for size-estimation. Some bands have highlighted intensities for ultraconvenient recognition.	100 bp	100-1000 bp
pML	A single plasmid giving rise to a mass-ladder, allowing the estimation of unknown bands' sizes and masses simultaneously.	100 bp	100-1000 bp
1kb ladder	A collection of plasmids that can configured by the user to become either a regular linear ladder, a mass ladder, or the equivalent supercoiled plasmid ladders.	1000 bp	1000-8000 bp

Reconstituting DNA from filter paper

All plasmids from Gene And Cell Technologies ship dried on a small piece of filter paper in a regular postal envelope. It may be necessary to let your administrative staff know that the plasmid will arrive in this form, and not in a package. We don't want these to end up in a filing cabinet or being discarded.

DNA on filter paper is very stable. It can be preserved for at least several years at room temperature or in the freezer in this state.

Retrieve the filter from its zip-loc bag with forceps.
Optional: If unsure, you can cut the filter in two, and preserve half of it for later as a backup.

Place the filter in a standard microcentrifuge tube and add 50 µl of Tris: 10 mM, pH 8.
The Tris buffer is user-supplied. Water will work as well. Tris just stabilizes the DNA in case it needs to be stored in the wet state for prolonged periods. Use a pipet tip to push the filter paper into the liquid and make sure it's completely submerged.

Soak for 5 minutes and spin in a table top centrifuge at top speed for 5 minutes.
This will physically squeeze the trapped plasmid out of the filter mesh. This step is generally necessary to get sufficient plasmid yield.

Transform 2 µ into competent E. coli (e.g. 5α, 10β strains) and plate ~5% of the mixture.
Most manufacturers call for recovering the E. coli in 1 ml SOC medium after transformation. Plating 50 µl of that should give you a manageable number of colonies on a single 10 cm plate. If your cells aren't quite elite, or you cut some corners during the protocol, it is possible to spin the mixture down and plate all the cells, for increased colony yield.

Incubate overnight. Pick a strong-looking colony, and grow it up in liquid culture.
Perpare plasmid for digestion. Prepare stocks in 10% glycerol for long-term storage at -80^{o}C.

Producting ladders from the plasmids by restriction digestion

All our ladder plasmids use exclustively EcoRI sites for digestion (cut-site GAATTC). This enzyme is commercially available from a variety of vendors at extremely high concentrations, and should not pose an economic limitation. We generally refer to each individual enzyme manufacturer's protocol for detailed pipetting instructions.

It is useful to refer to each manufacturer's "unit definion" for an initial estimate on how much enzyme to use. It is important to keep in mind the number of restriction sites in each ladder. For example, p100bp contains 17 individual EcoRI sites, and requires therefore 17 times the manufacturer's stated minimum enzyme / DNA ratio. When in doubt, a small pilot digestion can shed light upon how much of a given enzyme preparation is needed for how much time. It's OK to overdigest. These enzymes are very specific, and our plasmids do not contain any nonspecific * sites (TAATTC or CAATTC). At the end, the enzyme should be inactivated as specified by the manufacturer. For complete peace of mind, and extreme long-term stability, the DNA should be purified by phase separation (phenol/chloroform) or chromatographically using size-exclusion or ion-exchange.

p100bp and pML are single-plasmid ladders. They are simply quantitated and brought to their final intended concentration with distilled water (accounting for buffer and enzyme to be added by the enzyme maker's instructions). An example for what DNA concentrations to use is shown in each ladder's individual section in this manual below.

The 1kb ladder is a collection of plasmids. Each of them should be quantified separately and then united in the intended concentrations just before digestion. It will take some calculations to get this right. Again, and example is shown in the 1kb ladder section below.

Band intensity

In gel electrophoresis, the most commonly used DNA stains will produce acceptable signals in the range of 10 to 100 ng DNA per band. When multiple bands are produced by digesting a single plasmid, the fragments are released in equimolar concentration. That means longer bands will appear proportionally brighter than shorter bands.

For the DNA mass ladder (pML), this works in our favor. Each fragment in the mass ladder occurs at a copy number of 1. Therefore, the 1000 bp fragment will be 10 times brighter than the 100 bp fragment. This is the principle allowing mass quantitation (for details, see the mass ladders' individual chapter).

But for the regular size ladders, for reasons of ease of reference, we want each band to be approximately equally bright. Therefore, the size-ladder plasmid (p100bp), contains extra copies of the shorter fragments, bringing their band intensities to approximately the same as the single-copy 1000 bp fragment. The 1000 bp ladder consists of individual plasmids for most bands, which can be mixed by the user in different proportions to achieve any desired intensity pattern.

p100bp

Our basic 100 bp plasmid contains 17 EcoRI restriction sites spaced such that digestion with this single enzyme will release a ready-to-use ladder with 100 bp spacings. The resultant banding and intensity pattern is shown below.

p100bp
(EcoRI)

Length	Copy#	Mass
1000	1	100 ng
800	1	80 ng
600	1	60 ng
500	2	100 ng
400	1	40 ng
300	2	60 ng
200	3	60 ng
100	6	60 ng

The masses are given for a starting material of 560 ng / lane. For example, if the total DNA concentration in the digestion reaction is brought to 56 ng/µl, then a 10 µl / well loading volume would give the results shown above.

pML

The mass ladder plasmid pML contains 8 EcoRI restriction sites spaced so that digestion with this single enzyme will release a mass ladder suitable for the estimation of the sizes and masses of unknown bands:

pML (EcoRI)

Length	Copy#	Mass
1000	1	100 ng
800	1	80 ng
600	1	60 ng
500	1	50 ng
400	1	40 ng
300	1	30 ng
200	1	20 ng
100	1	10 ng

The masses are given for a starting material of 390 ng / lane. For example, if the total DNA concentration in the digestion reaction is brought to 39 ng/µl, then a 10 µl / well loading volume would give the results shown above.

Mass quantitation with pML

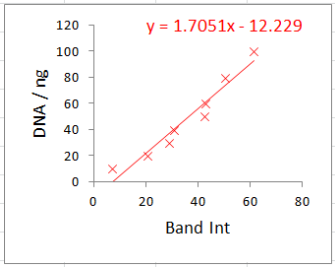
First, acquire your image such that no pixels are completely saturated (255 white). Masses from the mass ladder image can be quantified with the free NIH ImageJ or another suitable software program. This usually involves subtracting each band from a nearby background square:

Measuring a band:

Measuring its background:

Then, a standard curve can be calculated by plotting each band's intensity against its known mass.

Band	Back	Int	DNA amount
105	43	62	100 ng
87	37	51	80 ng
76	33	43	60 ng
75	32	43	50 ng
64	33	31	40 ng
60	30	29	30 ng
47	26	21	20 ng
29	22	7	10 ng



The measured intensity of an unknown band can now be used to read its mass directly off the chart. Alternatively, a standard curve equation can be calculated using linear regression with programs such as Open Office or Microsoft Excel. Then, the DNA concentration (y) can be formally calculated form the band intensity (x) using your unique line equation (red).

1 kb Ladder

The 1 kb ladder is a collection of plasmids, each containing a single EcoRI site that gives rise to its unique band. The names of these plasmids indicate their sizes – p2kb to p8kb. The only exception is the 1kb band, which is too small to accommodate the elements necessary to make a plasmid (origin + selection cassette). So there is no p1kb. Instead, the 1kb band is released from two of the other plasmids (p3+1 and p6+1). This puts stochiometric limitations on the relative ratios of the 1, 3 and 6 kb bands to each other as shown in the table below. Other than that, the user is free to use each plasmid at whatever concentration they choose.

Plan for composing a useful ladder out of the 1kb ladder plasmid collection:

Plasmid used	Plasmid mass	Band size	Band mass
p3+1, p6+1		1 kb	50 ng
p2kb	50 ng	2 kb	50 ng
p3+1	133 ng	3 kb	100 ng
p4kb	50 ng	4 kb	50 ng
p5kb	50 ng	5 kb	50 ng
p6+1	117 ng	6 kb	100 ng
p8kb	50 ng	8 kb	50 ng
p10kb	50 ng	10 kb	50 ng
Total			500 ng

The 1kb band is produced by decomposition of p3+1 and p6+1, together. This results in the follwing relationships describing band masses:

1kb band mass = (1/4 * p3+1 plasmid mass) + (1/7 * p6+1 plasmid mass).
3kb band mass = 3/4 * p3+1 plasmid mass.
6kb band mass = 6/7 * p6+1 plasmid mass.

Within these physical constraints it is possible to assemble the open source 1kb ladder plasmid collection into either a regular 1kb size ladder (as shown in the table above) or into a 1kb mass ladder, by changing the plasmid masses accordingly.

1kb ladder digestion plan

The following is just one example for how to compose a useful ladder out of these plasmids. The best way to get a handle on this fairly complicated math is to make a spreadsheet that automatically controls all the relevant numbers.

For digesting the 1kb ladder plasmid collection, it is convenient to combine the plasmids first, in a total volume that will be useful later, and then treat them as a single plasmid for digestion as suggested for the small ladders above.

For example, if you want to make 1000 lanes worth of 1kb ladder. Assume the following:

- The protocol provided by the manufacturer of your EcoRI enzyme calls for using a 10x buffer.
- Your total amount of 500 µg of DNA indicates the use of 25 µl of that enzyme preparation.
- You want to add one of the classical 6x gel loading dyes at the end.

Combine all 1kb ladder plasmids for 500 µg total plasmids.
Multiply all the amounts in the above table by 1000.

Bring the volume to 8.225 ml with distilled water.
Your concentration is now ~ 60 µg / ml total fragments. The average starting concentrations of your plasmids needs to be higher than this, in order to make this possible. If the concentrations are too low, concentrate the most dilute fragments by ethanol precipitation.

Add 833 µl of 10x buffer and 25 µl of enzyme.
Your volume is now 8.33 ml and your concentration ~55 µg / ml total fragments.

When the digestion is complete, destroy the enzyme by boiling.
Boiling alone requires the resultant ladder to be stored frozen. For increased stability, phenol/chloroform or chormatography is recommended. We're just keeping it simple in this example.

Add 1667 µl of 6x loading dye.
This brings your volume to 10 ml, and your total fragment concentration to 50 µg/ml. With this preparation, a gel loading volume of 10 µl will give you the desired 500 ng of total fragments per lane.

Supercoiled Ladder

The 1kb ladder collection can also be made to produce a supercoiled DNA ladder, simply by omitting the restriction digest. A supercoiled ladder is useful for estimating the size of supercoiled DNA plasmids, as they come out of E. coli, without the need for prior restriction digestion. Currently, no supercoiled DNA ladder with regularly sized spacings exists on the entire reagent market, presumably to prevent the user from separating the plasmids and making more on their own. So here is another very real science barrier to fall before Gene And Cell Technologies' open source policy:

Conveniently-spaced supercoiled DNA ladders are now available and accessible. To anyone. With our full permission and encouragement to grow more on your own.