# Creating Cost-Effective DNA Size Standards for Use in Teaching and Research Laboratories

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**Abstract:** I have devised a method with which a molecular size standard can be readily manufactured using Lambda DNA and PCR. This method allows the production of specific sized DNA fragments and is easily performed in a standard molecular biology laboratory. The material required to create these markers can also be used to provide a highly robust and reliable introduction of students to the PCR technique. Protocols for demonstrating the effect of the effect of amplification cycles and DNA and Primer concentration are provided.

***Keywords:*** Size Standards, PCR, Laboratory Exercises, Cost Reduction

## INTRODUCTION

Molecular size standards consist of fragments of DNA that are a specific size and are used in electrophoresis to determine the approximate size of an unknown sample. Figure 1a shows two commercially available size standards Promega G7541 and G8291. The cost per lane for these markers is $1.23 and $2.04 respectively. A reliable technique for producing size standards was sought in order to reduce this recurring cost.

## MATERIALS AND METHODS

PCR Primers were designed (Supplemental File 1) by pasting Lambda (λ) DNA sequence (NCBI gi:215104; 48,502 bp) into the Primer3 program (Rozen & Skaletsky, 2000), then selecting a product size value equivalent to that of the target size (*i.e.* "100-100" for a 100 bp product). A 59-61° C Tm with a minimum 20% GC content were selected. The default settings of an 18-27 bp oligo length and GC% of 20-80% were used. λ DNA was ordered from Promega (Madison, WI cat # D1501) and diluted 1:100 from stock. Supplemental File 1 contains a step by step protocol for creating a specific amplification product.

Each Polymerase chain reaction (PCR) contained

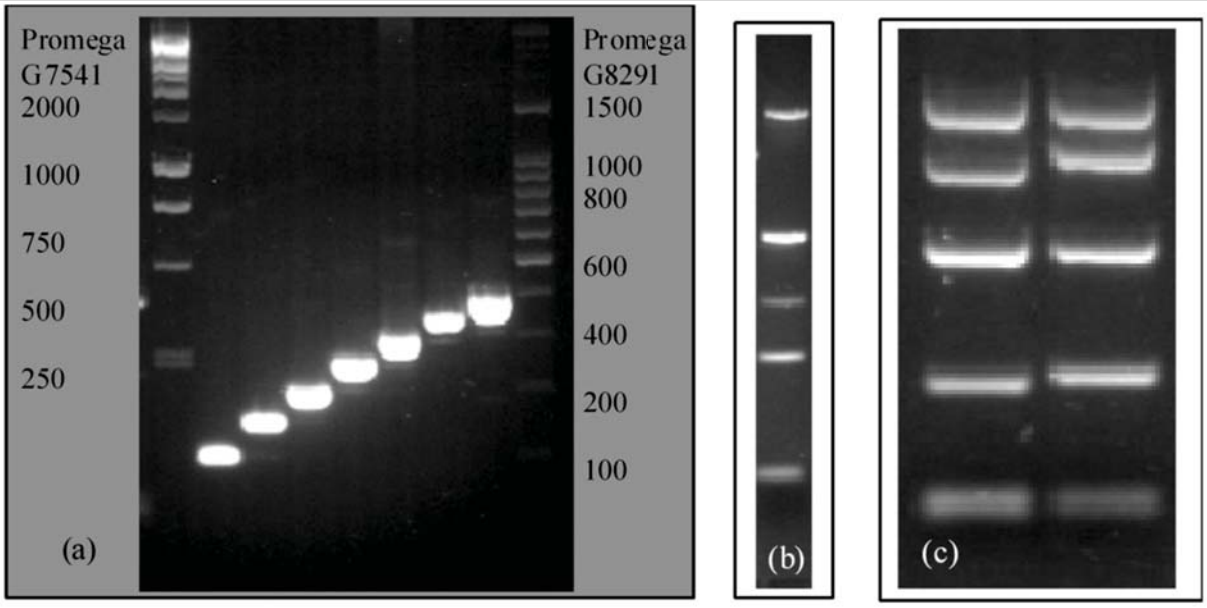


**Fig. 1**. Panel (a) Comparison of two commercially available size standards (Promega G7541 and G8291) and Lambda PCR amplification products ranging from 100-400 bp, in 50 bp increments. Lambda PCR product is highly concentrated and is typically diluted 10-20X in order to obtain clear amplification products, such as those shown in panels b and c. Panels b and c are additional examples of molecular size standards designed using this technique. Panel b shows a 100-250-350-500-1000 size standard. Panel c shows two size standards, left is 100-250-500-750-1000, right is 100-260-500-800-1000.

33 µL 2X PCR mix (cat # M7122, Promega, Madison, WI), 10 µL $H_2O$, 3 µL diluted (1:100) λ DNA and 4 µL of combined 10 mM forward and reverse primers. Amplification conditions were an initial 94° C denaturation for 3 min followed by 40 cycles of a denaturing step (94° C for 30 s), an annealing step (61° C for 60 s), primer extension (72° C for 60 s) and final extension for 5 minutes at 72° C. Each product was loaded separately into a 1.5% agarose gel (cat # BP160, Fisher Scientific, Waltham, MA) prepared with ethidium bromide (0.1 µL:20 mL gel matrix) and electrophoresed @ 4V/cm for 3-4 hours. Amplification patterns were documented with a UVP Gel documentation system (Upland, CA).

## RESULTS AND DISCUSSION

A total of 22 unique primer pairs were designed to amplify fragments of λ DNA from 100-1150 bp in 50 bp increments (Table 1). This method allows the production of specific size DNA fragments, is easily performed and offers an extensive long term cost reduction versus commercially available products. Figure 1a-c shows a comparison of two commercially available molecular size standards and a test of specific size amplification products.

### Initial Cost

Producing these fragments entails an upfront cost for the λ DNA (currently $56 for 250 µg) and for the primers. The cost for each primer pair, assuming $0.25/base, 25 nM concentration and 20 bp/primer is $10. Thus, the cost for the primers and DNA to produce a marker similar to Promegas' #8291 with 11 sizes is $166.

### Cost per 100 Lanes of Standard

Go Taq Green Polymerase contains all components except primer and template needed for PCR and costs $0.48 per 50 µL reaction. Primers are ordered at the 25 nM scale, then re-suspended into ~300 µL of 100 mM stock concentration. Once forward and reverse oligos are combined and diluted to 10 mM (see Supplemental File 2 for protocol) they are ready for PCR. At 4 µL per size, the primer cost is ~ $0.02 per reaction. The cost per size fragment is therefore $0.50 and the total cost of preparing an 11 fragment standard is $5.50. I add 200 µL of molecular biology grade water to the 550 µL of product and 50 µL of Bromophenol Blue/Glycerol loading dye, making a final volume of 800 µL. At 6-8 µL volume loaded, the cost is ~ $0.06 per lane. In essence, if the user expects to load more than one tube worth of size standard, manufacturing a 'proprietary' ladder becomes *very* cost effective.

### Educational Lab Benefits

Other than the obvious long-term savings possible, these size standards are exceptionally robust. I have used them for several quarters to introduce students to the PCR technique, with a nearly 100% PCR success rate. Supplemental File 2 contains lab exercises for the creation of a size standard and that demonstrate the influence of primer and DNA concentration and PCR extension cycle number using these primers and λ DNA.

**Table 1.** Primer sequences used to generate specific amplification product sizes with PCR of Lambda DNA.

| bp | | Sequence | bp | | Sequence |
|---|---|---|---|---|---|
| 100 | L | ATATCCGGCAGGAAACACTG | 650 | L | AAAGCCAGAACTCCCCGTAT |
| 100 | R | TAACGTTCTCCACCGACCTC | 650 | R | CTGTCACCCTTTGAGGTGGT |
| 150 | L | AGGATGACTGGTGGCGTAAC | 700 | L | GGCAGATCTCGATGATGGTT |
| 150 | R | AGTAAATTGCGGCGTGATTC | 700 | R | CACGTTACCGGACCAGAAGT |
| 200 | L | GCAGACTGGAGGAGTTTTCG | 750 | L | TGATACTGTGCCGGATGAAA |
| 200 | R | GTTTGCCGGGTCAATAAATG | 750 | R | TTAGGCAGAGACAGGCGAAT |
| 250 | L | GGGAACGGGCGTTTTATTAT | 800 | L | TAAGCGGTGAGGCTCAGATT |
| 250 | R | CACACTGTCCGTCAGCTCAT | 800 | R | AGACACCTTCACGCTGGACT |
| 300 | L | GTCATACGCCAGCAGTGAAA | 850 | L | AGCCTGTAGCTCCCTGATGA |
| 300 | R | TGTTTGTCGTTCTGGCTGAG | 850 | R | ATGCCTGGTACTTTGCCAAC |
| 350 | L | TTTTGATGAGGCGGATTTTC | 900 | L | GCAGAACGAAAAAGGTGAGC |
| 350 | R | ACCTTTCACCGGCATTGTAG | 900 | R | GCACACAGCGGAACTTATGA |
| 400 | L | CTCTGGCGGTGATAATGGTT | 950 | L | CGCAGATGAGCTTGTCCATA |
| 400 | R | TCGATTCGTAGAGCCTCGTT | 950 | R | ATCTCGCTTTCCACTCCAGA |
| 450 | L | ACCACCTCAAAGGGTGACAG | 1000 | L | TAAATTCGCACAGCAGCAAC |
| 450 | R | GGCCATGTTGTTGCTGTATG | 1000 | R | ACGTTTTCAGGTTGGCATTC |
| 500 | L | GATGCACGTAAATCCCGTCT | 1050 | L | TGCATGTCCAGAGCTCATTC |
| 500 | R | GTATGAGCCGGGTCACTGTT | 1050 | R | AAGGCATTCCTACGAGCAGA |
| 550 | L | CCAGCTGCATCAGGATCATA | 1100 | L | GACGCACTGAATACGCTGAA |
| 550 | R | CTGCTCCATCACGCTGTAAA | 1100 | R | TGCGTCACCTTCACCAATAA |
| 600 | L | AGTCGAGCTGACGGAGGATA | 1150 | L | TCCAGACATGCTCGTTGAAG |
| 600 | R | ATCTGGCGATCAAAAGGATG | 1150 | R | ACGGCAATAATCCGCATAAG |

## CONCLUSION

I have used λ DNA sequence to design PCR primers that amplify a specific fragment length of DNA. This procedure can be used on any DNA for which the sequence is known to produce specific sized DNA fragments. These amplification products are easily produced and offer an extensive long-term cost reduction versus commercially available products. In addition, these robust PCR components are useful for demonstration of basic PCR concepts.


## ACKNOWLEDGEMENTS

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## REFERENCES


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