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Methods Article

pHAPE: a plasmid for production of DNA size marker ladders for gel electrophoresis

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Abstract

DNA size markers (also known as 'molecular weight markers' or 'DNA ladders') are an essential tool when using gel electrophoresis to identify and purify nucleic acids. However, the cost of these DNA ladders is not insignificant and, over time, impinges on the funds available for research and training in molecular biology. Here, we describe a method for the generation of 'pHAPE', a plasmid from which a variety of DNA ladders can be generated via simple restriction enzyme digestions. The pHAPE plasmid can be generated by mutagenesis of the commonly used pBluescript II SK+ phagemid followed by insertion of a 7141 bp sequence (comprised of three smaller, synthetic fragments). Our use of pHAPE allows us some small relief from the ever-rising costs of performing molecular biology experiments ('Don't worry, pHAPE').

Keywords: gel electrophoresis; plasmid; DNA ladder; size markers; restriction enzymes

Introduction

Gel electrophoresis for the identification and purification of nucleic acids is a fundamental technique in molecular biology. Nucleic acids (those lacking secondary structure) move through electrophoresis gels at rates inversely related to fragment length/size [1, 2]. This allows the lengths of DNA fragments to be estimated by comparison to markers of known length, collections of which are commonly referred to as 'size markers', 'molecular weight markers', or 'DNA ladders'.

The first source of molecular weight markers that found widespread use was DNA from the lambda bacteriophage digested with restriction enzymes such as EcoRI or HindIII [3]. This produces a versatile but irregular range of DNA fragment lengths. Inconveniently, the use of the bacteriophage lambda fragments to estimate the length of other DNA fragments of interest often required the measurement of electrophoresis migration distances and comparison of these using log-linear plots [1]. Consequently, the commercial provision of DNA ladders with convenient fragment length intervals and different scales (e.g. 1 kb increments up to ~10 kb, 100 bp increments up to 1 kb) proved very popular and the regular purchase of such ladders is now common practice. However, the cost of these DNA ladders is not insignificant and, over time, impinges on the funds available for research and training in molecular biology.

The drive to minimize costs in molecular biology has encouraged laboratories to find methods to generate their own size markers, although all have their caveats. The cost of lambda phage DNA has been reported to be increasing in addition to it being harder to source [4]. A number of PCR-based techniques for ladder production have been described, although these are limited to lower molecular weight bands as larger products are more difficult to synthesize [5, 6]. Additionally, PCR-based methods are more costly than using restriction enzymes and rely on the availability of commercially produced primers. Progress in DNA synthesis technology has allowed the design and construction of plasmids specifically for the generation of DNA ladders after cleavage with restriction enzymes, for example, the pPSU plasmids produced by Henrici *et al.* [4]. These are attractive sources of DNA size markers as plasmids are easy to propagate at low cost, and molecular biology laboratories hold restriction enzymes as standard tools. Methods of DNA size marker production in which both PCR products and fragments from restriction enzyme digestion of phage and plasmid DNA are combined can provide an excellent compromise solution to some of these problems [7, 8]. However, we desired to develop a method of DNA size marker production that was as simple and time-effective as possible.

To achieve this while bypassing intellectual property issues, we embarked on a project to design, assemble, and test a single plasmid for size marker production. Our plasmid is based on the pBluescript II SK+ phagemid that is widely available and in common use [9]. The plasmid can easily be reconstructed by synthesis of the described insert sequences and their ligation into a modified form of pBluescript II SK+. We named the resulting plasmid 'pHAPE' after the initials of the restriction enzymes required to produce its largest ladder output: **H**indIII, **A**paI, **P**stI, and **E**coRI.

A variety of size markers can be produced by restriction enzyme digestion of pHAPE including a ladder featuring 1 kb increments (with fragments ranging from 100 bp to 10 kb) and a ladder with 50 and 100 bp increments (spanning 50 bp to 1.2 kb). The resulting digests can be used immediately in gel electrophoresis by the addition of loading dye or the DNA be precipitated and resuspended in 1× TE buffer to allow stable storage of the ladders

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at room temperature. Our use of pHAPE allows us to further reduce molecular biology consumables costs ('Don't worry, pHAPE').

Materials and methods

Reagents

All enzymes and buffers were purchased from New England Biolabs Inc. (Ipswich, MA, USA) (see the table below).

Restriction enzymes	Cat. No.
ApaI	R0114
DpnI	R0176
EcoRI-HF	R3101
HindIII-HF	R0104
KpnI	R0142
NgoMIV	R0564
PstI	R0140
SacI-HF	R3156
XhoI	R0146
XmaI	R0180
Other enzymes	
T4 DNA ligase	M0202
Phusion HF DNA polymerase	M0530
Gibson assembly enzyme master mix	E2611
Buffers and other	
100× BSA	B9001
10× CutSmart buffer	B7204
10× NEBuffer 3.1	B7203
10× NEBuffer 2	B7002
10× NEBuffer 1	B7001
5× Phusion HF reaction buffer	B0518

Sequence design

A random DNA sequence ultimately intended for insertion into a modified pBluescript II SK+ phagemid was generated by the FaBox online toolbox [10] constrained to contain equal quantities of A, C, G, and T nucleotide residues. The resulting sequence was then modified to possess the desired restriction enzyme recognition sequences only at appropriate positions.

Synthetic insert fragments for construction of pHAPE, the 305 bp primer used to modify insert fragment 1a, and the synthetic sequence used for comparisons of DNA fragment migration in polyacrylamide gel electrophoresis (PAGE) were synthesized as IDT gBlocks™ (Integrated DNA Technologies, Inc., Coralville, IA, USA).

Molecular cloning

Protocols for molecular cloning were adapted from Sambrook and Russell [11]. *Escherichia coli* strains DH5α (#18265017) and Stbl3™ (#C737303) were used for cloning (derived from chemically competent cells supplied by Thermo Fisher Scientific, Inc., Waltham, MA, USA). Preparation of electrocompetent cells and electroporation were performed at room temperature [12]. Electrocompetent *E. coli* and plasmid DNA were electroporated in 1 mm cuvettes at 1.8 kV using the MicroPulser electroporator (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Plasmid mini- and maxi-preparation protocols were adapted from Sambrook and Russell [11], with the addition of RNase A to cell lysis buffers. The QIAGEN-tip 500 was used to further purify crude plasmid extract (#10063; QIAGEN, Hilden, Germany).

Ladder assembly

Detailed protocols for ladder assembly from purified pHAPE DNA can be found in the Supplementary Data File.

Gel electrophoresis

Agarose gels were composed of agarose in 1× sodium borate (SB) buffer (36.4 mM boric acid, 10 mM NaOH, pH 8) with 0.5 μg/ml of ethidium bromide. Where post-staining was performed, gels were submerged in 1 μg/ml ethidium bromide in dH_2O for 15–30 min before being rinsed with water. Electrophoresis was performed in 1× SB at 90–120 V for 1 h. All agarose gels were 6 × 10 cm (width × length) and 4 mm thick, with 3 × 1 × 3 mm (width × length × depth) wells.

Polyacrylamide (PA) gels were prepared from 40% acrylamide and bis-acrylamide solution (19:1) (#1610146. Bio-Rad Laboratories, Inc.). A detailed protocol on casting the gradient gel can be found in the Supplementary Data File. Electrophoresis was performed using 0.5–1× tris-borate EDTA (TBE) buffer (1×: 45 mM tris-borate, 1 mM EDTA) at 90–150 V for 2 h using the Mini Gel Tank (#A25977. Invitrogen, Thermo Fisher Scientific, Inc.). Following electrophoresis, the gels were post-stained with 1 μg/ml of ethidium bromide in dH_2O for 15 min before rinsing with water.

Table 1. Fragment lengths from restriction enzyme cleavage of pHAPE

HAPE				BamHI		
		Proportion				
Length (bp)	Copies	Plasmid	Ladder	Length (bp)	Copies	Proportion
ApaI			**0.035**	1178	1	0.1178
10000	1	1.00	0.035	979	1	0.0979
EcoRI			**0.088**	943	1	0.0943
6000	1	0.60	0.053	800	1	0.0800
4000	1	0.40	0.035	700	1	0.0700
PstI			**0.175**	600	1	0.0600
3000	1	0.30	0.053	500	2	0.1000
2500	1	0.25	0.044	450	1	0.0450
2000	1	0.20	0.035	400	1	0.0400
1500	1	0.15	0.026	350	1	0.0350
1000	1	0.10	0.018	300	2	0.0600
HindIII			**0.702**	250	1	0.0250
1151	1	0.12	0.081	200	5	0.1000
1004	1	0.10	0.070	150	2	0.0300
945	1	0.09	0.066	100	2	0.0200
800	1	0.08	0.056	50	5	0.0250
700	1	0.07	0.049			
600	1	0.06	0.042			
500	5	0.25	0.175			
400	2	0.08	0.056			
300	2	0.06	0.042			
200	2	0.04	0.028			
100	5	0.05	0.035			

Notes: The number of fragments of particular lengths produced by cleavages of one plasmid molecule are shown for the 'HAPE' ladder (left) and the 'BamHI' ladder (right). For the HAPE ladder, the fragments produced by particular restriction enzymes are listed beneath each enzyme's name. Numbers in the HAPE 'Plasmid' and BamHI 'Proportion' columns represent the fractions of the total plasmid mass comprised by fragments of particular lengths. Numbers in the HAPE 'Proportion' column represent the fractions of the total mixture mass comprised by fragments of particular lengths when HindII, ApaI, PstI, and EcoRI digests of equal masses of plasmid are mixed at a ratio of 20:1:5:2.5 (HindIII, ApaI, PstI, EcoRI) to produce the HAPE ladder. This mass fraction information can be useful for visual estimation (by comparison) of DNA mass in other electrophoresis bands of interest (when the total mass of pHAPE loaded into a gel size marker lane is known). The mass proportion of the HAPE ladder comprised by DNA from each digest in the 20:1:5:2.5 mixture is indicated by numbers in bold.

All gels were imaged with the ChemiDoc XRS+ system and adjustments were made using Image Lab (Bio-Rad Laboratories, Inc.). The 1 kb (#G5711) and 100 bp (#G2101) reference DNA ladders were purchased from Promega Corporation, Madison, WI, USA.

Results and discussion

Design considerations

The pHAPE plasmid is based on the pBluescript II SK+ phagemid, a high copy number vector conferring ampicillin resistance [9]. To produce a plasmid with a final size of exactly 10 000 bp, a 7141 bp insert based on a random sequence generated by the FaBox online toolbox [10] and constrained to contain equal quantities of A, C, G, and T nucleotide residues was cloned between the KpnI and SacI sites of a mutated form of this vector. We designed the insert sequence with the intention that it should possess no evolved or designed function in cells. The random sequence generated initially was then modified to possess the desired restriction enzyme recognition sequences only at appropriate positions. The final sequence inserted into the modified form of pBluescript II SK+ to construct pHAPE can be found in the Supplementary Data File.

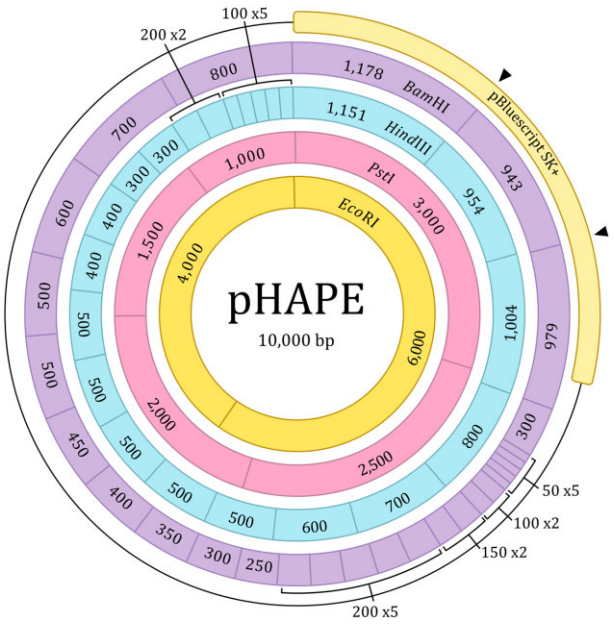

Figure 1. Structural map of pHAPE, showing the locations of restriction sites and fragment lengths (in bp) produced by each restriction enzyme digest: EcoRI in yellow, PstI in pink, HindIII in blue, and BamHI in purple. The modified pBluescript II SK+ backbone is indicated, with arrowheads indicating the positions where HindIII and BamHI restriction sites were introduced.

Initially, we intended that pHAPE would allow production of two ladders encompassing different fragment length ranges: (1) a versatile 'HAPE ladder' with fragments ranging from 10,000 bp down to 100 bp and including 100 bp increments between 1 kb and 100 bp. (2) A ladder including 25–100 bp increments, specifically for use on high percentage agarose gels for estimating the lengths of low molecular weight DNA fragments of less than 1 kb (now referred to as the 'BamHI ladder', Table 1). Ultimately, the BamHI ladder was modified to include only 50–100 bp increments for reasons of stability (described later). An additional factor constraining our design was that the visibility of a stained DNA 'band' in an electrophoresis gel varies with the total DNA mass in the band. Therefore, our restriction digests of the pHAPE plasmid should produce more of the smaller DNA fragments of any particular size than of the larger fragments, thereby increasing the visibility of the lower molecular weight bands. It is also desirable to have 'landmark' bands representing particular known lengths that are significantly brighter than neighbouring bands to facilitate size identification (Table 1). The copy numbers necessary to achieve the desired band intensities were calculated based on the fragment masses seen in commercial ladders using this formula (assuming an average of 650 Da per base-pair):

$$\text{copies} = \frac{\text{ng} \cdot 6.022 \cdot 10^{23}}{\text{length (bp)} \cdot 650 \cdot 10^{9}}.$$

In designing the locations of restriction enzyme cleavage sites in pHAPE, fragments of similar sizes were allocated to single enzymes to allow versatility in the combination of the resulting fragments (Fig. 1). For example, the HindIII digest (which produces the smaller fragments of the HAPE ladder) can produce a ladder in its own right with 100 bp increments up to 1 kb. A final design consideration was to utilize restriction sites for only common, inexpensive restriction enzymes. We chose HindIII, ApaI, PstI, EcoRI, and BamHI. Of course, for single-site cleavage of pHAPE, numerous alternatives to ApaI exist in the pBluescript II SK+ derived sequence.

In order that restriction enzyme digests of a single 10 kb plasmid be capable of producing all the desired fragments, the DNA of the pBluescript II SK+ vector (2859 bp) was modified to include

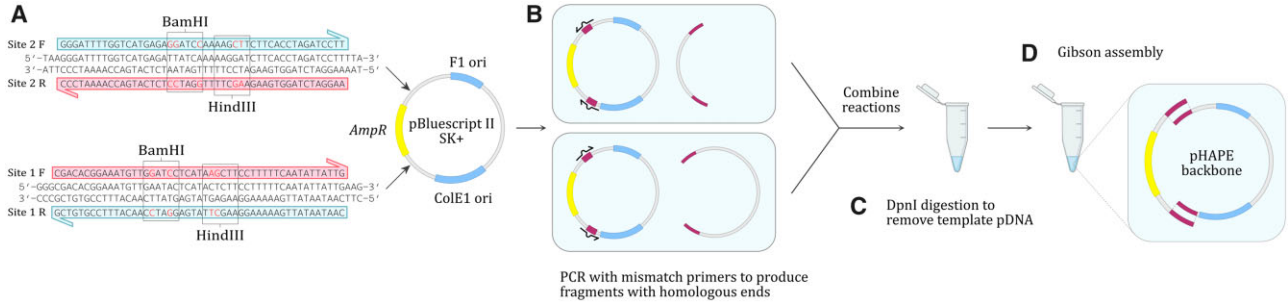

Figure 2. Site-directed mutagenesis of pBluescript II SK+ before insert inclusion to form pHAPE. Mismatch primers were used to introduce two BamHI sites and two HindIII sites into the pBluescript II SK+ vector on either side of the AmpR gene (**A**). Phusion high-fidelity DNA polymerase was used to amplify two fragments with ~50 bp of homology at their ends (**B**). *Escherichia coli*-derived plasmid template pDNA was fragmented using DpnI digestion (**C**) before Gibson (isothermal) assembly of the modified form of pBluescript II SK+ (**D**).

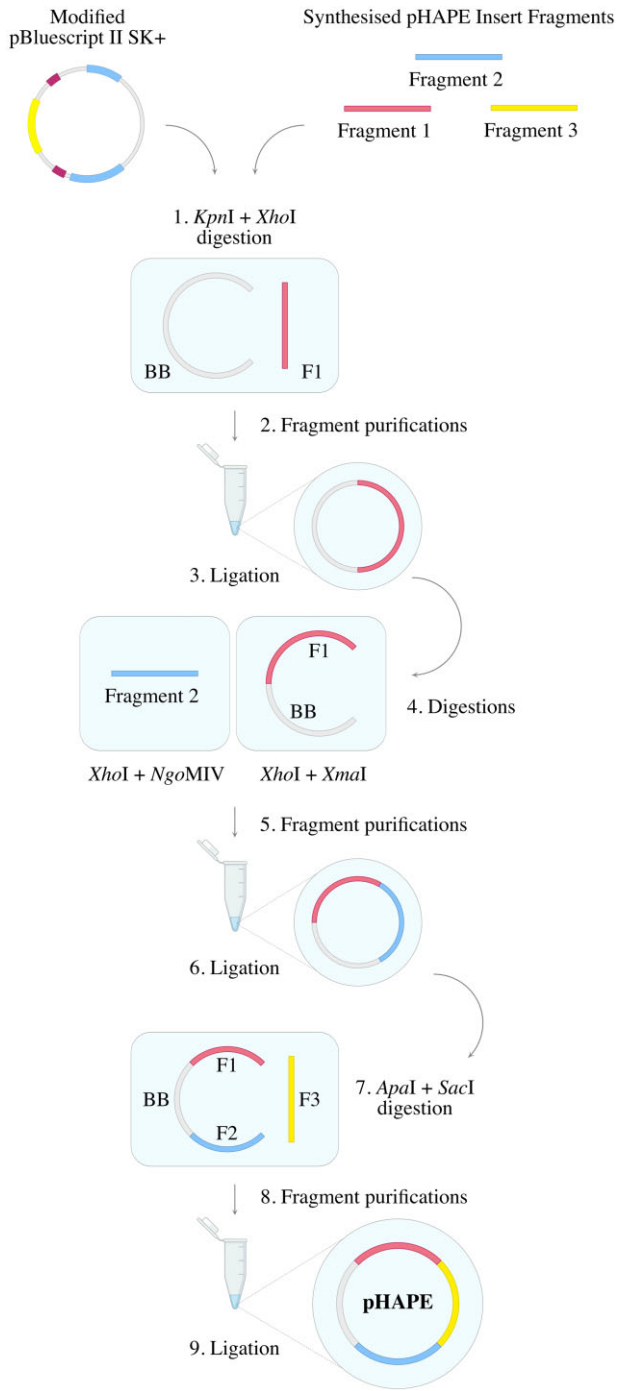

Figure 3. Cloning scheme for assembly of pHAPE. BB is the modified pBluescript II SK+ backbone (see Fig. 2), F1 is Insert Fragment 1b, F2 is Insert Fragment 2, and F3 is Insert Fragment 3. The plasmid was transformed into *E. coli* following each ligation reaction to allow for purification of sufficient DNA for the subsequent assembly step.

additional BamHI and HindIII cleavage sites flanking the vector's AmpR gene (Fig. 2). To reduce the likelihood of these modifications affecting propagation of the plasmid, we aimed to alter as few bases as possible. Ultimately, we made five and four substitutions each at two sites flanking AmpR. The modifications were achieved by PCR amplification using mismatched primers (sequences and PCR conditions are provided in the Supplementary Data File) followed by DpnI digestion and Gibson (isothermal) assembly [13]. The introduced BamHI and HindIII restriction sites are placed sufficiently close together that a single pair of mismatched primers for each site can be used to incorporate the required mutations (Fig. 2).

Construction of pHAPE

Initially, we attempted to assemble the designed insert sequence from three synthetic fragments of ~2500 bp each flanked by unique restriction sites for directional cloning (see the Supplementary Data File). To preserve and amplify the synthetic sequences, these were first cloned individually and separately into pBluescript II SK+ to form 'Insert Fragment Plasmids'. Despite each insert fragment harbouring restriction sites with unique sticky ends, a four-component simultaneous ligation of the purified fragments with the modified pBluescript II SK+ backbone vector (described in Fig. 2) proved unsuccessful. Therefore, we assembled the pHAPE plasmid through a series of sequential ligations and transformations, adding one fragment to the vector at a time, as shown in Fig. 3. However, we discovered subsequently that the reason for our lack of success with four-component simultaneous ligation may have been plasmid instability due to one of the fragments (Insert Fragment 1a—see the Supplementary Data File). The suspected cause of instability was a regularly spaced series of 13 BamHI sites intended to produce 25 bp fragments [14, 15]. Removal of these sites would not significantly affect the design of the ladder (as the region of DNA allocated to the 25 bp fragments would, instead, form an additional 300 bp fragment). Therefore, a 305-bp section of the sequence was redesigned to abolish those BamHI sites. A new DNA fragment was synthesized and used as a 'mega-primer' in site-directed mutagenesis of the Insert Fragment 1a plasmid to form the Insert Fragment 1b plasmid (see the Supplementary Data File for the DNA sequence of the mega-primer, the conditions used for mutagenesis, and the intended sequence of Insert Fragment 1b).

When cultured side-by-side, colonies harbouring Insert Fragment 1b appeared to grow faster than those with the original sequence (Insert Fragment 1a), and transformation efficiency also appeared to be higher for the Insert Fragment 1b plasmid (data not shown). Note that the *E. coli* strain DH5α was used in all the cloning work described above except that strain Stbl3^{TM} was used when performing the cloning experiments with Insert Fragment 1a and 1b (due to the relatively closely spaced repetitive restriction sites harboured by Insert Fragment 1a) as Stbl3^{TM} is RecA deficient (RecA13). We have found that the assembled pHAPE plasmid can be maintained in *E. coli* DH5α with no apparent instability.

Since pHAPE is approximately three times the mass of pBluescript II SK+, it transforms bacteria at a somewhat lower rate than the latter plasmid. For example, in one side-by-side comparison, the transformation of chemically competent DH5α cells with pHAPE yielded 5.70e^5 cfu/μg of DNA while transformation with pBluescript II SK+ produced 1.43e^7 cfu/μg of DNA. More than 1 mg of plasmid DNA can be obtained from 200 ml of a culture in Lysogeny Broth medium containing 100 μg/ml ampicillin. For a typical preparation of the HAPE ladder in our laboratory, 28.5 μg of pHAPE DNA can be digested (20, 1, 5, and 2.5 μg per H, A, P, and E digest) to produce sufficient ladder for 250 electrophoresis gel lanes (assuming 114 ng of ladder is loaded per lane).

Ladder assembly

The pHAPE plasmid should be digested separately with HindIII, ApaI, PstI, and EcoRI before combination of the digest products to

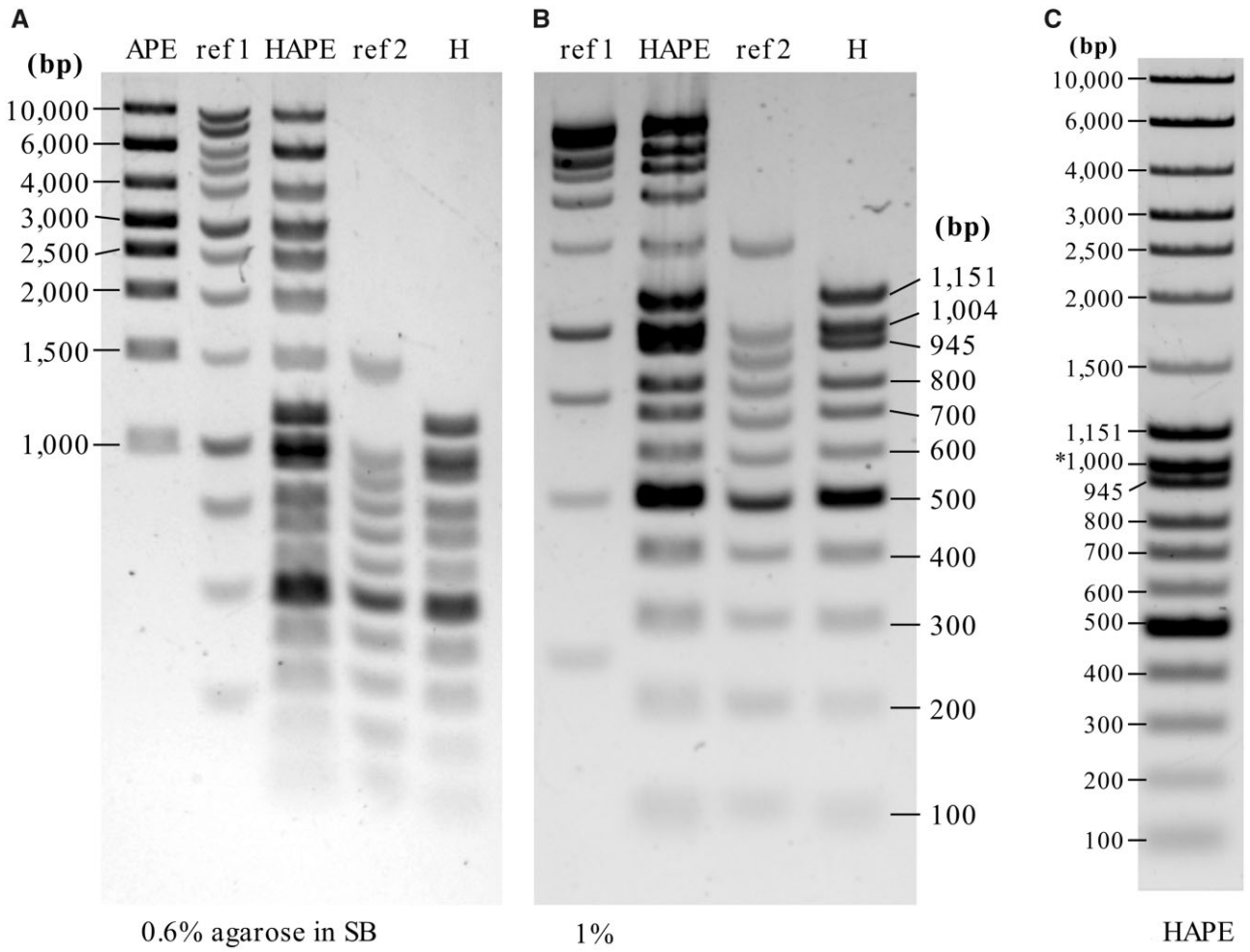

Figure 4. Electrophoresis of pHAPE-derived DNA ladders through 0.6% and 1% agarose gels in SB or TAE buffers. Gels A and B contain 0.5 μg/ml ethidium bromide and were run at 90 V for 1 h. Gel C is 1% agarose in TAE and was run at 90 V for 1 h before staining in a solution of 1 μg/ml ethidium bromide. The '*1000 bp' band is comprised of both the 1000 and 1004 bp fragments from the PstI and HindIII digests, respectively. Letters indicate the restriction enzyme digests of pHAPE used to make each ladder: H, HindIII; A, ApaI; P, PstI; and E, EcoRI. Reference ladders are the Promega 1 kb (ref 1) and 100 bp (ref 2) ladders.

assemble the HAPE ladder of fragments ranging from 10 kb to 100 bp. It should be noted that longer digestion times than typically recommended may be needed due to the large number of restriction sites present and the supercoiled nature of the purified plasmid [16]. Assuming an equal concentration of pHAPE in each digest, these can subsequently be combined in a volume ratio of 20:1:5:2.5 (HindIII, ApaI, PstI, EcoRI) to produce a visually favourable combination of band intensities. Alternatively, the fragment mass proportions provided in Table 1 can be used to tailor the ladder to the specific needs of a laboratory.

The resulting combination of digests can be used for gel electrophoresis without any purification (i.e. by addition only of a gel loading buffer). However, the inclusion of a purification step followed by resuspension of the ladder in 1× TE appears to improve its storage longevity and gives less distortion of electrophoresis bands due to dissolved salts (data not shown). Organic extraction of DNA (e.g. with phenol:chloroform) is recommended for protein removal as we observed the loss of some fragments when silica-based columns were used for purification. Lower molecular weight bands (<200 bp) were poorly retained by the columns and a slight reduction in the concentration of the 10 kb band of the HAPE ladder was seen (Supplementary Fig. S1, see the Supplementary Data File).

The pHAPE-derived 'BamHI' ladder (with fragments ranging from 50 bp to 1 kb) is produced with a single BamHI digest. Note that the BamHI enzyme cannot be heat inactivated so protein removal after digestion (eg, by phenol–chloroform extraction, followed by precipitation using ethanol, and then redissolution in 1× TE) is recommended.

Another advisable practice to reduce distortion of fragment bands during electrophoresis is to limit the mass of DNA loaded in a well to a maximum of 15 ng of DNA per 1 mm^2 of that surface of the well into which the DNA migrates (~150 ng for 3 × 3 mm wells).

Gel electrophoresis

Examples of pHAPE-derived DNA ladders are shown in Figs 4 and 5. Both the HAPE and BamHI ladders migrate true-to-size compared with commercial reference ladders (Figs 4A and B and 5A and B). All fragments of the HAPE ladder were successfully resolved on a 1% gel after 1 h at 120 V. Distinct bands were observed for the BamHI ladder at gel concentrations >1% (Fig. 5A). To visualize the lower molecular weight bands (<200 bp), we found that post-staining of the gel was necessary (Fig. 5C).

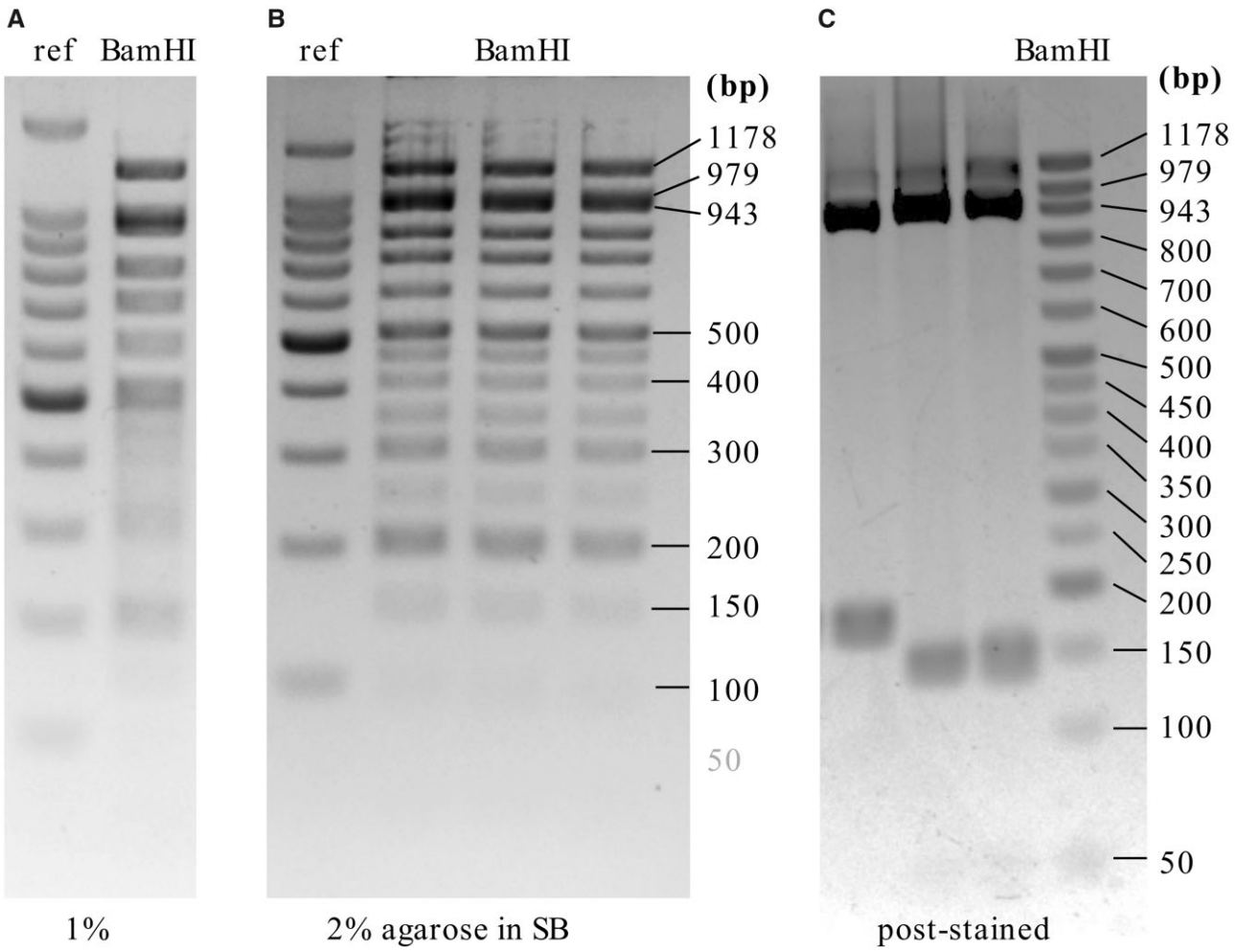

Figure 5. Electrophoresis of the BamHI ladder through 1% (**A**) and 2% (**B**, **C**) agarose gels in SB buffer. Gels (A, B) contain 0.5 μg/ml ethidium bromide and were run at 90 V for 1 h. The Promega 100 bp ladder was used as a reference (ref). Gel (C) shows the BamHI ladder in use to estimate the sizes of PCR and restriction digest products (not described) and was run at 200 V for 20 min before staining in a solution of 1 μg/ml ethidium bromide for 15 min.

DNA page

A common observation is that some DNA fragments do not migrate according to their size during PAGE [17]. We ran the HAPE and BamHI ladders on PA gels to determine whether they would migrate true-to-size relative to a commercial reference ladder and to fragments of synthetic DNA (Fig. 6). The migration of most bands in the HAPE ladder appeared to be consistent with the reference ladder, with the exception of the 1.5 kb, 1 kb, and 500 bp bands (Fig. 6A). Both the ~1 kb (comprised of 1000 and 1004 bp fragments) and 500 bp bands of the HAPE ladder are 'landmarks', and therefore consist of multiple fragments of the same size, but of differing sequences. Multiple bands are observed for the ~1 kb fragment, with only the band resulting from PstI digestion (as it does not appear in the 'HindIII ladder') aligning with the commercial ladder. The other ~1 kb fragments of the HAPE ladder, the 979 bp fragment of the BamHI ladder, and some 500 bp fragments (both ladders) appeared to migrate more slowly than equivalent bands in the commercial reference ladder (Fig. 6A). Additionally, there appeared to be a slight difference in the migration of the 400 bp bands between the HAPE and BamHI ladders. As the HAPE ladder 400 bp is composed of two unique sequences that did not separate, we believe it likely that the BamHI ladder 400 bp fragment migrates slightly slower than expected (Fig. 6A).

To assess the migration of fragments smaller than 250 bp (the smallest band in the reference ladder) in the HAPE and BamHI ladders, we generated a random sequence of 790 bp (with the FaBox tool, constrained to equal quantities of A, C, G, and T) and incorporated restriction sites to produce bands of 50, 100, 150, and 200 bp. Additionally, we modified the sequence to allow investigation of the effects of 'blunt' or 5′ overhang ('sticky') restricted DNA ends on fragment migration. To achieve this, restriction sites for EcoRV (that produces blunt ends), HindIII, and BamHI (both producing 5' overhang ends) were incorporated.

We observed faster migration of fragments with blunt ends than of fragments with 5' overhand ends (Fig. 6B). However, the 100 and 150 bp bands of the BamHI digest (5' overhang ends) did align with the EcoRV digest products (blunt ends), suggesting that sequence differences rather than the form of the DNA fragment end may have affected migration. Consistent with this, the 200 bp fragments of both the HAPE and BamHI ladders appeared as two separate bands, once again likely due to sequence differences (see the arrowhead in Fig. 6B).

Conclusion

In summary, the pHAPE plasmid can be generated by mutagenesis of the commonly used pBluescript II SK+ phagemid followed

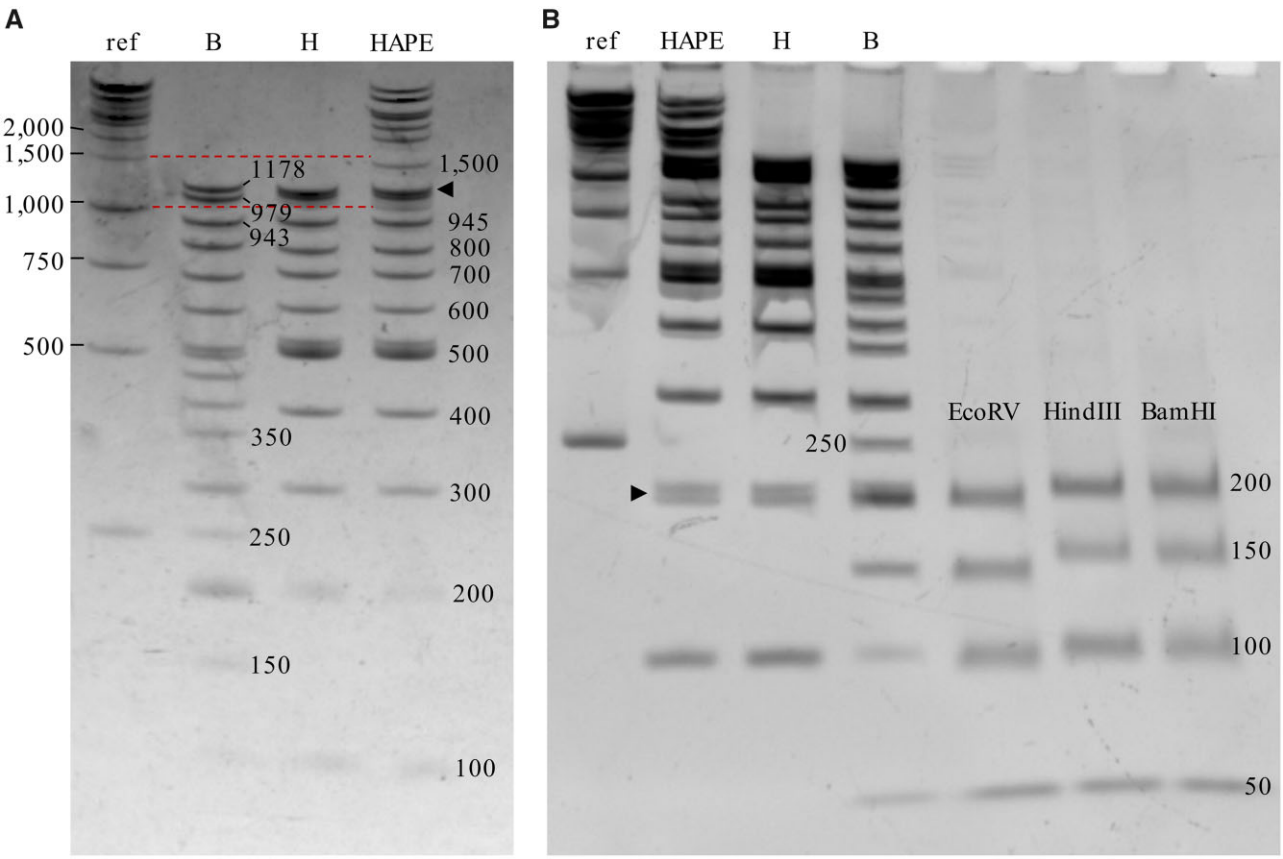

Figure 6. PAGE of pHAPE-derived DNA ladders. (**A**) 5% PA in 1× TBE buffer, run for 2 h at 150 V. Migration inconsistent with the reference is seen at ~1 kb for the HAPE (arrowhead) and BamHI ladders, with the majority of fragments migrating slower than the reference (lower dotted red line). The HAPE ladder 1.5 kb fragment displayed faster migration than expected from the reference ladder (upper red line). Slower than reference migration of some 500 bp fragments of plasmid-derived ladders is visible. (**B**) 4–12% PA gradient gel in 0.5× TBE, run for 2 h at 150 V. A 790 bp synthetic DNA fragment with restriction sites for EcoRV (blunt end), HindIII (5′ overhang end), and BamHI (5′ overhang end) was digested and run alongside these ladders to determine whether differences in migration may occur. Separated 200 bp bands (arrowhead) can be observed in the HAPE, H (HindIII), and B (BamHI) ladders. Both gels were stained in a solution of 1 μg/ml ethidium bromide after electrophoresis. Letters indicate the restriction enzyme digests of pHAPE used to make each ladder: H, HindIII; A, ApaI; P, PstI; E, EcoRI. All fragment sizes displayed are in base pairs. The reference ladder (ref) is the Promega 1 kb ladder.

by insertion of 7141 bp between its KpnI and SacI sites. pHAPE can be used for generation of a variety of DNA size marker ladders via simple restriction enzyme digestions.

A protocol for generation of the HAPE ladder from pHAPE can be found in the Supplementary Data File. Alternatively, the fragment mass proportion data provided in Table 1 can be used to tailor ladders to the specific needs of a laboratory.

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Author contributions

Angel G. Allen (Conceptualization [equal], Investigation [lead], Methodology [equal], Project administration [equal], Visualization [lead], Writing—original draft [lead], Writing—review & editing [equal]), Karissa Barthelson (Conceptualization [equal], Investigation [supporting], Resources [supporting], Supervision [supporting], Validation [lead], Writing—original draft [supporting], Writing—review & editing [supporting]), and Michael Lardelli (Conceptualization [equal], Investigation [supporting], Methodology [equal], Project administration [equal], Resources [lead], Supervision [lead], Writing—review & editing [lead])

Supplementary data

Supplementary data are available at *Biology Methods and Protocols* online.

Conflict of interest statement

None declared.

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Data availability

Sequence data for pHAPE are available through GenBank (accession: OQ538031). All other data underlying this article are available in the article and in its Supplementary Data File.

References

1. Helling RB, Goodman HM, Boyer HW. Analysis of endonuclease R-EcoRI fragments of DNA from lambdoid bacteriophages and other viruses by agarose-gel electrophoresis. *J Virol* 1974;**14**:1235–44. https://doi.org/10.1128/JVI.14.5.1235-1244.1974.
2. Livshits MA, Amosova OA, Lyubchenko YL. Flexibility difference between double-stranded RNA and DNA as revealed by gel electrophoresis. *J Biomol Struct Dyn* 1990;**7**:1237–49. https://doi.org/10.1080/07391102.1990.10508562.
3. Maniatis T. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, 1982.
4. Henrici RC, Pecen TJ, Johnston JL, Tan S. The pPSU plasmids for generating DNA molecular weight markers. *Sci Rep* 2017;**7**:2438.https://doi.org/10.1038/s41598-017-02693-1.
5. Abbasian M, Seyedi HAE, Boroujeni ZK *et al.* Easy method for production of a home-made DNA ladder in every laboratory. *Adv Biomed Res* 2015;**4**:70.https://doi.org/10.4103/2277-9175.153894.
6. Gopalakrishnan R, Joseph S, Sellappa S. Constructing a DNA ladder range for lambda phage by multiplex PCR. *Iran J Microbiol* 2010;**2**:210–2.
7. Mostaan S, Ajorloo M, Khanahmad H *et al.* A novel combined method for cost–benefit production of DNA ladders. *Adv Biomed Res* 2015;**4**:15.https://doi.org/10.4103/2277-9175.148298.
8. Zhang J-H, Yang R, Wang T-Y *et al.* A simple and practical method that prepares high molecular weight DNA ladders. *Mol Med Rep* 2012;**6**:1211–3. https://doi.org/10.3892/mmr.2012.1061.
9. Alting-Mees MA, Short JM. pBluescript II: gene mapping vectors. *Nucleic Acids Res* 1989;**17**:9494.
10. Villesen P. FaBox: an online toolbox for fasta sequences. *Mol Ecol Notes* 2007;**7**:965–8. https://doi.org/10.1111/j.1471-8286.2007.01821.x.
11. Sambrook J, Russell DW. *Molecular Cloning: A Laboratory Manual*, 3rd edn. Cold Spring Harbor Laboratory, 2001.
12. Tu Q, Yin J, Fu J *et al.* Room temperature electrocompetent bacterial cells improve DNA transformation and recombineering efficiency. *Sci Rep* 2016;**6**:24648.https://doi.org/10.1038/srep24648.
13. Gibson DG, Young L, Chuang R-Y *et al.* Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat Methods* 2009;**6**:343–5. https://doi.org/10.1038/nmeth.1318.
14. Lovett ST, Gluckman TJ, Simon PJ *et al.* Recombination between repeats in *Escherichia coli* by a recA-independent, proximity-sensitive mechanism. *Mol Gen Genet* 1994;**245**:294–300. https://doi.org/10.1007/BF00290109.
15. Oliveira PH, Lemos F, Monteiro GA *et al.* Recombination frequency in plasmid DNA containing direct repeats—predictive correlation with repeat and intervening sequence length. *Plasmid* 2008;**60**:159–65. https://doi.org/10.1016/j.plasmid.2008.06.004.
16. Snounou G, Malcolm ADB. Supercoiling and the mechanism of restriction endonucleases. *Eur J Biochem* 1984;**138**:275–80. https://doi.org/10.1111/j.1432-1033.1984.tb07912.x.
17. Stellwagen NC. Electrophoresis of DNA in agarose gels, polyacrylamide gels and in free solution. *Electrophoresis* 2009;**30(Suppl. 1)**:S188–95. https://doi.org/10.1002/elps.200900052.