World J Microbiol Biotechnol (2011) 27:1167–1173
DOI 10.1007/s11274-010-0563-8

ORIGINAL PAPER

# Conditions optimized for the preparation of single-stranded DNA (ssDNA) employing lambda exonuclease digestion in generating DNA aptamer

**Marimuthu Citartan · Thean-Hock Tang · Soo-Choon Tan · Subash C. B. Gopinath**

Received: 18 July 2010 / Accepted: 6 September 2010 / Published online: 18 September 2010


**Abstract** The generation of DNA aptamer by Systematic Evolution of Ligands by Exponential Enrichment requires a good method of ssDNA generation. There are various methods developed to generate ssDNA such as streptavidin-biotin based separation techniques, asymmetric PCR and strand separation of the PCR product containing primer with a terminator and an extension of 20 nucleotides on denaturing urea-polyacrylamide gel. In the present investigation, we have shown the possible improvements for the regular lambda nuclease digestion under optimized conditions. Optimization of the PCR cycles, time course studies on lambda nuclease digestion and purification of the ssDNA from the lambda exonuclease digestion mixture was found to be able to recover ssDNA amounting up to 39.19 ± 2.48 % of the starting amount of dsDNA. These strategies can be applied to the techniques involving essential usage of ssDNA.

**Keywords** Lambda exonuclease · Single stranded DNA · Optimization · Aptamer

**Electronic supplementary material** The online version of this article (doi:10.1007/s11274-010-0563-8) contains supplementary material, which is available to authorized users.

M. Citartan · T.-H. Tang (✉)
Infectious Disease Cluster, Advanced Medical & Dental Institute (AMDI), Universiti Sains Malaysia, 13200 Kepala Batas, Penang, Malaysia
e-mail: tangth.amdi@usm.edu.my

S.-C. Tan
Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia

S. C. B. Gopinath (✉)
Photonics Research Institute, Nanodevice Research Center, National Institute of Advanced Industrial Science & Technology, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8562, Japan
e-mail: gopi-subashchandrabose@aist.go.jp

## Introduction

The discovery of the catalytic RNA molecules (Gilbert 1986) has dramatically changed the perspective on the function of the nucleic acid, which was initially considered as linear carrier of genetic information to the fact that single stranded nucleic acid can also carry out intracellular functions owing to the remarkable ability to form a wide variety of three dimensional structures (Hermann and Patel 2000). By using combinatorial approach, these prominent capabilities of the single stranded nucleic acid molecules to form an array of tertiary structure has been harnessed to isolate high affinity ligands from initial randomized pool of nucleic acid against a wide variety of target molecules such as small inorganic molecules (Stojanovic et al. 2000), nucleotides (Sassanfar and Szostak 1993), cofactors (Saran et al. 2003), amino acids (Majerfeld et al. 2005), antibiotics (Lato et al. 1995) and viruses (Gopinath et al. 2006a, b). These high affinity ligands are termed aptamers while the process that generates these aptamers is called Systematic Evolution of Ligands by Exponential Enrichment (SELEX) (Ellington and Szostak 1990).

The SELEX process starts with a large library of randomized nucleic acid sequences. Following the incubation of the library with the target molecule, those sequences that bind to the target are partitioned from those that do not by numerous methods such as nitrocellulose filter membrane (Gopinath et al. 2005, 2006a, b, c), column matrices (Skrypina et al. 2004 and Brockstedt et al. 2004), capillary electrophoresis (Mendonsa and Bowser 2004; Schou and Heegaard 2006) and other methods of separation (Gopinath

2007). The retained ligands are then amplified to enrich the target bound ligand molecules and these processes of selection and amplification are reiterated until the ligands with the highest binding affinity are obtained.

In the SELEX involving DNA aptamer, dsDNA has to be converted from dsDNA to ssDNA whereas in the selection process associated with RNA aptamer, the dsDNA generated has to be transcribed to RNA by in vitro transcription using T7 RNA Polymerase. DNA aptamer and RNA aptamer does not differ much in the affinity and specificity (Breaker 1997). However, in comparison to the DNA aptamer, the RNA aptamer is less stable due to the hydroxyl group at the 2′-OH position that is particularly reactive at higher than neutral pH leading to the generation of cyclic 2′,3′-phosphate that leads to the degradation of the RNA (Wiegand et al. 1996). This makes DNA aptamer molecules suitable choice for subsequent application that involves clinical trial and in vivo application.

There are several methods available to generate ssDNA from dsDNA in the SELEX involving DNA aptamer generation. One of the methods is asymmetric PCR amplification which generates ssDNA due to the usage of two primers at unequal concentration (Gyllensten and Erlich 1988). The next method is streptavidin biotin separation, in which one of the primers is attached with biotin at the 5-′end. Following PCR amplification, the incubation of the PCR product with the streptavidin beads which has affinity to the avidin will bind the strand containing the biotin at the end, therefore resulting in ssDNA generation (Hultman et al. 1989). William and Bartel (1995) have explained the strand separation on the denaturing-urea PAGE following PCR amplification using one of the primers containing hexaethylene glycol with an extension of 20-nucleotides length of a string of adenosine.

Lambda-exonuclease is an enzyme of the bacteriophage l that assists in the repair of double stranded breaks of the viral DNA (Dapprich 1999). As an exodeoxyribonuclease, it selectively digests the phosphorylated strand(s) of double stranded DNA (dsDNA) from 5′ to 3′ end. In fact, Lambda exonuclease has 20 times more affinity for a phosphorylated 5′-end than a hydroxylated 5′-end, thus leaving non-phosphorylated single stranded DNA (Fig. 1). However, this enzyme has greatly reduced activity on single-stranded DNA and non-phosphorylated DNA and no activity against nicked DNA and gapped DNA (Little 1967). Since the ssDNA preparation is the most important step in the standard SELEX procedure, a fast and efficient method of generating ssDNA with high quality and yield by lambda exonuclease digestion was carried out. In this study, lambda exonuclease digestion of the PCR product followed by purification of ssDNA by phenol:chloroform extraction have been shown to be completed under the total amount of time of 60–68 min, which greatly reduces the time spent on

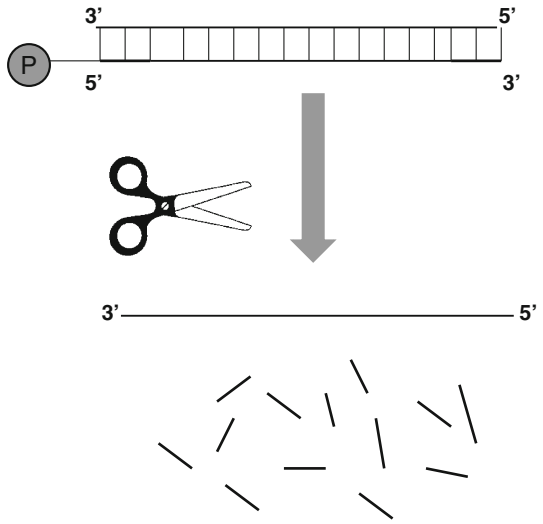


**Fig. 1** Schematic representation of lambda exonuclease digestion of the purified PCR product containing phosphorylated end at one of the strand. Lambda exonuclease selectively digests the phospharylated strands of double stranded DNA from 5′ to 3′ ends and leaves non-phosphorylated single stranded DNA

SELEX experiment, whereby up to one SELEX cycle can be carried out in a single day towards generating DNA aptamer.

## Materials and methods

### PCR amplification of random ssDNA

The template DNA used in this experiment is random ssDNA pool (5′-AGCTTAGGATCCAACCTGATCT$(N)_{40}$ GGTACCAACTGCATACCGAGCT-3′) consisting a randomized sequence region of 40 nucleotides (N), flanked by fixed primer binding regions. The sense primer is 5′-AG CTTAGGATCCAACCTGATCT-3′ while the antisense primer is 5′-AGCTCGGTATGCAGTTGGTACC-3′ which is phosphorylated at the 5′-end. These ssDNA pool and the primers were purchased from Biobasic Inc. (Toronto, Canada). PCR conditions were an initial denaturation at 95°C for 1 min followed by 6–15 amplification cycles (45 s denaturation at 94°C; 1 min annealing at 55°C; and 1 min elongation at 72°C) and final elongation at 72°C for 5 min. PCR reactions were conducted in 100 µL reaction with 20 ng of template in 1× PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl) containing 1.5 mM $MgCl_2$, 200 µM each of four dNTPs (dATP, dGTP, dCTP and dTTP) and 2.5 U Taq DNA polymerase (MBI Fermentas, Lithuania). Initially, PCR reactions were run in 6, 8 and 10 cycles in which 10µL of the final PCR products were analyzed on 3% agarose gel electrophoresis. The PCR cycles that give the best amplification with the minimum

background amplification were then used to perform large scale PCR amplification in batches of 10 reactions.

### Nucleospin gel extract II

Nucleospin gel extract II kit (Macherey-Nagel, Duren, Germany) was used to purify the PCR product. For every 100 μL of the PCR product, 200 μL of the Buffer NT1 was added and transferred into nucleospin column. The nucleospin columns were centrifuged at 11,000×*g* for 1 min, and the flow-through were discarded. Around 700 μL of buffer NT3 was added into each column, centrifuged at 11,000×*g* for 1 min and the flow-through were discarded. The columns were centrifuged at 11,000×*g* for another 2 min to further dry the column. 60 μL of buffer NE was added into the columns, left at RT for 1 min, followed by centrifugation at 11,000×*g* for 1 min. The flow-through of the columns which were collected into new eppendorf tubes were quantified by spectrophotometer at wavelength of 260 nm and around 2 μg of the dsDNA was used for lambda exonuclease digestion.

### Lambda exonuclease digestion of the PCR product

Lambda exonuclease digestion was performed in 50 μL of reaction in 1× lambda exonuclease reaction buffer (67 mM glycine–KOH, pH 9.4, 2.5 mM $MgCl_2$, 0.01% Triton X–100). For every 2 ug of purified dsDNA, 10 units of enzyme lambda exonuclease were employed following manufacturer's instruction (MBI Fermentas, Lithuania). This reaction mixture was then incubated at 37°C for 30 min followed by heat deactivation at 80°C for 10 min. However, this heat deactivation step can be omitted if the lambda exonuclease digestion mixture is to be purified immediately. An aliquot of this final lambda exonuclease digestion mixture was loaded onto 3% agarose gel electrophoresis to ensure complete digestion of the PCR product to ssDNA prior to the following cycle of SELEX.

In this experiment, 2 μg of the purified PCR product was used for the lambda exonuclease digestion. This final lambda exonuclease digestion mixture was then phenol:chloroform:isoamyl alcohol extracted followed by ethanol precipitation to a suitable volume. Similary, lambda exonuclease digestion experiment was also performed on 40 μL of the unpurified PCR product to find the efficiency of ssDNA production from the unpurified PCR product. On the other hand, time course analysis of lambda exonuclease digestion was performed on 2 μg of purified PCR product and an aliquot of 5 μL was taken from the lambda exonuclease digestion mixture following incubation at 10, 20, 30, 40, 50, 60, 70 and 80 min to be analyzed on 10%, 8 M denaturing-urea PAGE. Aliquots of the lambda exonuclease digestion mixture at different incubation time were analyzed on 10%, 8 M denaturing-urea PAGE (19 acrylamide: 1 bis-acrylamide, 7 M urea) which was run in TBE buffer (89 mM Tris-borate, 2 mM EDTA, pH 8.3) and stained with 0.5 μg $mL^{-1}$ of ethidium bromide in 1× TBE buffer for 7 min. Upon loading, the lambda exonuclease digested products were mixed with equal volume of 2× RNA loading dye (8 M urea, 0.25% bromophenol blue, 1× TBE) heat-denatured at 95°C for 3 min, followed by snap-cooling on ice.

### Extraction of ssDNA from lambda exonuclease digestion mixture

The final lambda exonuclease digestion mixture of the purified PCR product was added with an equal volume of phenol:chloroform:isoamyl alcohol, mixed thoroughly and centrifuged at maximum speed (21,000×*g*) for 5 min at 4°C. The upper layer of the mixture was carefully collected and added up to a final volume of 400μL followed by addition of 40μL of 3 M NaOAc and 1 mL of 100% ethanol. The mixture was then placed at −80°C for 15 min, followed by centrifugation at maximum speed of 21,000×*g* for 8 min at 4°C. The supernatant was then discarded and the pellet was washed with 1 mL of 70% ethanol. After centrifugation at maximum speed (21,000×*g*) for 2 min, supernatant was discarded and the pellet was air-dried for 2 min at 37°C. The resuspended pellet was then quantified by spectrophotometer at wavelength of 260 nm.

## Results and discussion

Although SELEX methodology is successful for generating high-affinity molecules, these methods are time consuming and repetitive (Gopinath 2007). Moreover, single stranded DNA generation is the most time consuming step in a SELEX experiment which adds to the tediousness of this procedure. Methods such as hexaethylene glycol (terminator) with an extension of 20 adenine nucleotides followed by subsequent purification from polyacrylamide gel is very time consuming and results in poor yield of potential binders of ssDNA. On the other hand, the usage of streptavidin-biotin system, in which the non-biotinylated ssDNA is separated from biotinylated strand by alkaline denaturation often, leads to the loss of the tertiary structure of the ssDNA which is imperative for the binding of the ssDNA to the target molecule (Paul et al. 2009). This necessitates a fast and efficient method of generating ssDNA to ensure the production of aptamer with high binding affinity. Among all the ssDNA generation methods available, lambda exonuclease digestion is a routine method of producing sufficient ssDNA for SELEX cycles

(Kujau and Wolfl 1997). Moreover, lambda exonuclease enzyme is a processive enzyme, which remain bound to the DNA molecule until complete removal of the phosphorylated strand has been achieved in comparison to other exonucleases such as T7 exonuclease, spleen exonuclease, *E. coli* exonuclease III, 3′–5′ exonuclease of T4 DNA polymerase, and both the 3′–5′ and the 5′–3 ‘ exonucleases of *E. coli* DNA polymerase I, which frequently dissociates from the substrate during the course of digestion, subtantiating the efficiency of the enzyme lambda exonuclease in generating ssDNA from the dsDNA over the other exonucleases (Thomas and Olivera 1978). The overall steps involve for the optimization of lambda nuclease digestion are shown in the supplementary Fig. 1.

## Optimization of PCR cycles

PCR cycle optimization is the most important factor to consider in preparing dsDNA for lambda exonuclease digestion in generating optimum yield of ssDNA. In SELEX during PCR amplification of the eluted bound DNA, it is important not to over-amplify the sequences. Over-amplification will lead to background amplification and would require more selection cycles in overcoming the background noise to obtain high affinity molecules. Therefore in this study, in amplifying the eluted bound DNA from each SELEX cycle, amplification was started with 6, 8 and 10 PCR cycles and an aliquot of 10-uL of the PCR product from each PCR cycles was loaded onto the agarose gel to check for the amplification to ensure band at the correct size was obtained with minimum aberrant molecular weight product as seen in Fig. 2a. From the agarose gel electrophoresis, it was evident that PCR cycle 8 gave the best amplification with minimum background amplification with 20 ng of template. This is further supported by ImageJ analysis that showed PCR cycle 8 constituting the highest arbitrary value of 250 in comparison to the rest of the PCR cycles (Fig. 2b). On the other hand, in order to measure the background amplification of these PCR reactions, 3D plot view of ImageJ analysis was performed (Fig. 2c). From this analysis, it was known that the background amplification of the PCR cycle 6 and 8 was almost similar, however collectively; PCR cycle 8 was chosen to carry out large scale amplification due to the intensity of the band of interest. The intensity of the band was calculated as more than 25% increment from PCR products with 6 cycles. In general, in any SELEX experiment, the PCR cycles in each SELEX round is increased by 2 cycles if the band obtained is very faint and another 4 additional PCR cycles if there is no band observed in order to get good amplification of the eluted bound DNA to ensure successful outcome of any SELEX experiment. Utilizing these PCR products, the optimal conditions for lambda nuclease digestion were determined as described below. Even though in this SELEX experiment, 6 PCR cycles seems to be sufficient in generating enough PCR product for lambda exonuclease activity, a total of 8 PCR cycles was chosen for amplification, as higher number of PCR cycles will produce higher amount of PCR products, taken into consideration factors such as background amplification as well.

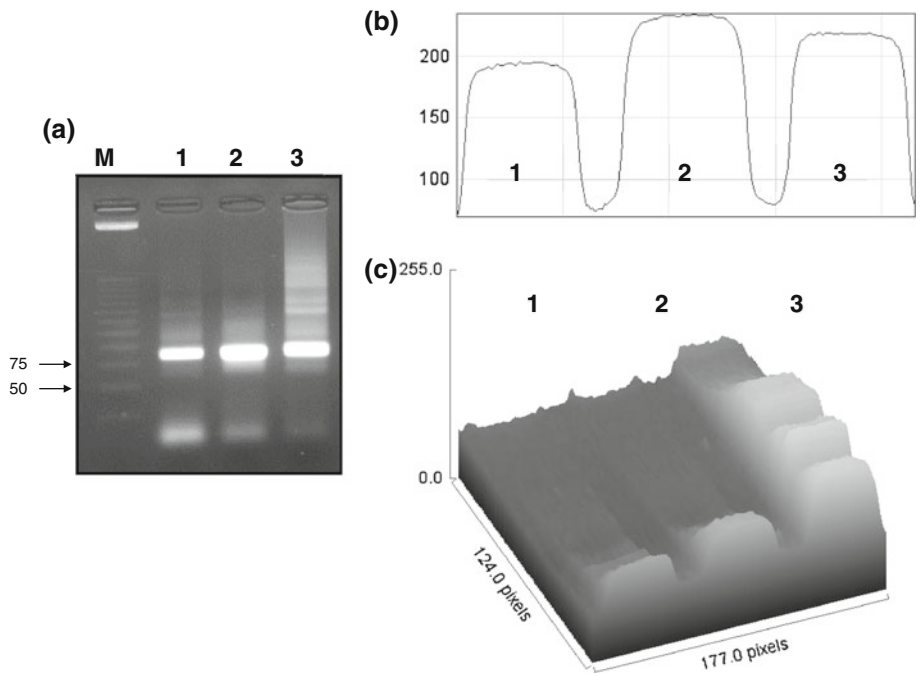


**Fig. 2** Analyses of PCR products with different PCR cycles. **a** *Lane M*: 25-bp DNA ladder (*Lanes 1–3*): Mobility of PCR products with PCR cycles 6, 8 and 10 respectively on 3% agarose gel **b** Band intensities of dsDNA and ssDNA as measured using ImageJ software **c** 3-D plot view of background amplification

## Optimization of lambda exonuclease digestion by time-course analysis

In order to determine the progress of lambda exonuclease digestion on the dsDNA, time-course analyses of lambda nuclease digestion were performed. This can provide useful information on optimal incubation period of the enzyme and the substrate dsDNA. In this experiment, the progress of the lambda exonuclease digestion was measured by taking out an aliquot of the reaction mixture every 10 min intervals with incubation at 37°C and analyzing them on the 8%, 7 M denaturing-urea PAGE gel up to 80 min. The extent of the digestion can be determined as the band of double-stranded DNA becomes lighter with the increase of the incubation time, implicating that the double-stranded DNA has been converted into single-stranded DNA. From Fig. 3a, it can be said that the optimum time of incubation for efficient generation of ssDNA is 30–40 min at 37°C, with the increase by another additional 10 min will not result in any increase of the yield of ssDNA, corroborated by ImageJ analysis as in Fig. 3b. This is because after a certain period of time, lambda exonuclease will act on unphosphorylated ssDNA due to the fact that lambda exonuclease also has a greatly reduced activity on ssDNA, thus reduces the final yield. Therefore, the use of excess amount of enzyme lambda exonuclease should also be avoided (Zha et al. 2003). This test can be run once in any SELEX experiment involving lambda exonuclease digestion in generating ssDNA to determine how the activity of the enzyme lambda exonuclease on the purified PCR product differs with the time of incubation. This extent of the lambda exonuclease digestion can then be used to determine the optimum time required to produce maximum amount of ssDNA from the corresponding dsDNA.

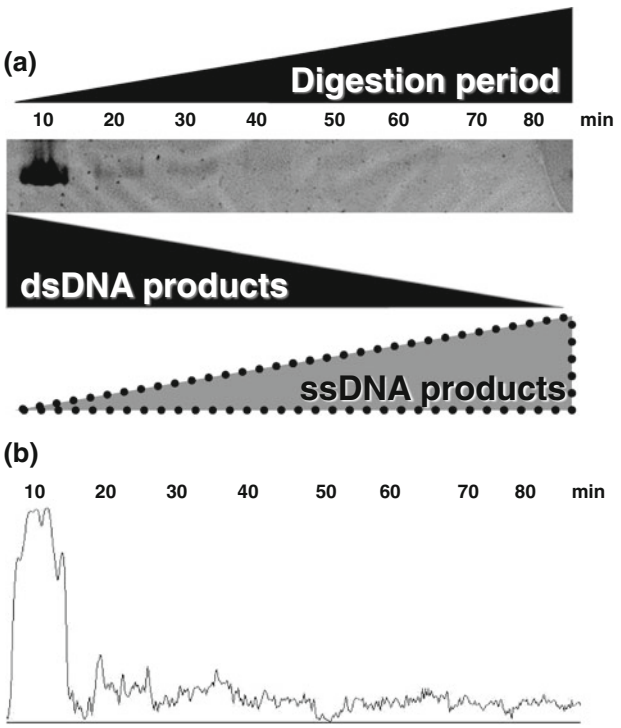


**Fig. 3** Denaturing Urea-Polyacrylamide gel analysis of Lambda exonuclease digestion mixture. **a** 5 μL of the lambda exonuclease digestion mixture was analyzed on the gel after incubation at 37°C. *Lane 1*: 10 min. *Lane 2*: 20 min. *Lane 3*: 30 min, *Lane 4*: 40 min, *Lane 5*: 50 min, *Lane 6*: 60 min, *Lane 7*: 70 min, *Lane 8*: 80 min. **b** Extent of lambda exonuclease digestion as measured by ImageJ software

## Lambda exonuclease digestion of the purified and unpurified PCR products

The lambda exonuclease activity on both unpurified and purified PCR product was conducted to see the efficiency of the ssDNA generation in both of these PCR reactions. As is evident in Fig. 4a, purified PCR product (lane 2) gave a better yield of ssDNA compared to the unpurified PCR product (lane 1). ImageJ analysis on the band intensities (Fig. 4b) further supported this, as the band intensity of the purified PCR product was with the value of 120, higher than the unpurified PCR product which was around 85. This showed the increment in ssDNA generation with pure PCR product was about 40%, which was higher than unpurified PCR product. Therefore, it can be concluded, the PCR product should always be purified before lambda exonuclease digestion in removing the primer dimers, excess primers, dNTPs and Taq Polymerase.

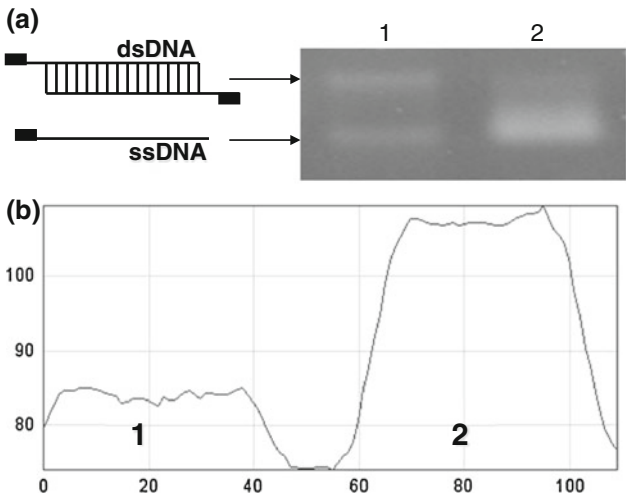


**Fig. 4** Analyses of lambda exonuclease digestion mixture of purified and unpurified PCR product. **a** *Lane 1*: Mobility of lambda exonuclease digested of unpurified PCR product on 3% agarose gel, *Lane 2*: Mobility of lambda exonuclease digested of purified PCR product on 3% agarose gel **b** Band intensities of ssDNA as measured using ImageJ software

The lambda exonuclease generated ssDNA should always be analyzed on the agarose gel to check for the presence of the undigested double stranded DNA. In this investigation, we found that the lambda exonuclease digestion of the PCR product was complete as there was no traces of PCR product present in the digestion mixture. As expected, the resulting ssDNA generated migrated faster compared to the corresponding dsDNA (Fig. 5a). To further substantiate this, surface plot measurement of ImageJ analysis was performed to gauge the distance between the ssDNA and dsDNA. This analysis clearly revealed that there was a difference in the migration between the ssDNA and the dsDNA, with ssDNA migrated faster than the dsDNA due to the lower molecular weight of the ssDNA compared to that of the dsDNA (Fig. 5b) (Kujau and Wolfl 1997). However, the band intensity of the ssDNA was lower than that of the dsDNA, as the amount of Ethidium bromide incorporated into ssDNA is lower than that of the dsDNA (Fig. 5c) (Wooddell and Burgess 2010).

Apart from all these optimization, degree of phosphorylation of the primer also plays a big role in complete digestion of the phosphorylated PCR product, as some of the unphosphorylated primer will render the PCR product incompletely digested to ssDNA (Null et al. 2000). Consequently, in some cases there will be some undigested PCR product remaining in the lambda exonuclease digestion mixture. Moreover, further incubation with excess enzyme lambda exonuclease with extended time to remove the undigested dsDNA will only result in the reduction of the purified single stranded DNA. Furthermore, to ensure complete lambda exonuclease digestion of phosphorylated DNA strand, the manufacturing of phosphorylated primer must be given thorough consideration. In addition, phosphate groups are temporarily protected by blocking groups until the oligonucleotide synthesis is completed and these groups must be completely removed from the phosphate groups after the completion of the synthesis to ensure entire lambda exonuclease digestion of phoshorylated DNA strands (Adali et al. 2001).

### Purification of the ssDNA from lambda exonuclease digested mixture

For the subsequent SELEX cycle, it is of utmost important to ensure that the lambda exonuclease generated ssDNA does not contain any enzyme lambda exonuclease and truncated nucleotides. Therefore, phenol:chloroform:isoamyl alcohol (25:24:1) extraction was performed followed by ethanol precipitation to eliminate the lambda exonuclease from the ssDNA. Thereby, the lambda exonuclease molecules that can serve as the target for SELEX experiment can be removed. Although this step eliminates lambda exonuclease, it also resulted in some loss of ssDNA (Adali et al. 2001). However, we can still generate an amount of 39.19 ± 2.48% of the starting amount of dsDNA (Supplementary Table 1). This amount of ssDNA is very suitable for the subsequent cycles of SELEX experiment (Tang et al. 2006).

## Conclusion

The preparation of high-quality ssDNA is of paramount important to ensure the success of SELEX technology. In

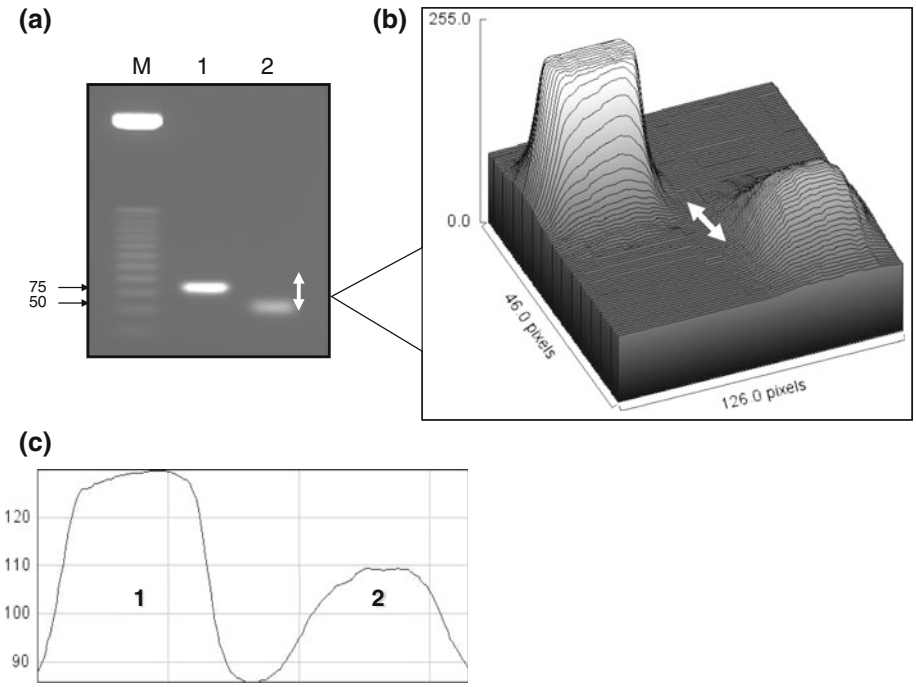


**Fig. 5** Analyses of lambda exonuclease generated ssDNA. **a** *Lane M*: 25-bp DNA ladder. *Lane 1*: Mobility of purified PCR product on 3% agarose gel, *Lane 2*: mobility of ssDNA production of lambda exonuclease digestion on 3% agarose gel electrophoresis **b** Surface plot measurement of migration difference between ssDNA nand dsDNA **c** Band intensities of dsDNA and ssDNA as estimated by ImageJ software

this study, we have shown that Lambda exonuclease digestion offered a method for ssDNA preparation, in which up to 5 cycles of SELEX can be done in less than one week by using lambda exonuclease digestion followed by phenol:chloroform extraction, which greatly reduces the time of the preparation of ssDNA up to 1 h. This strategy of ssDNA generation can also be applied in other applications that require ssDNA generation such as sequencing.

**Acknowledgments** We would like to thank Universiti Sains Malaysia for awarding postgraduate fellowship to Citartan for this work and the support rendered by the Ministry of Science, Technology and Innovation (MOSTI), under Brain Gain Malaysia, for the travelling and subsistence of Dr. Subash C.B. Gopinath to Malaysia.

## References

Adali AM, Paul A, Wilhem N, Ziemer G, Wendel HP (2001) Upgrading SELEX technology by using lambda exonuclease digestion for single-stranded dna generation. Molecules 15:1–11

Breaker RR (1997) DNA aptamers and DNA enzymes. Curr Opin Chem Biol 1:26–31

Brockstedt U, Uzarowska A, Montpetit A, Pfau W, Labuda D (2004) In vitro evolution of RNA aptamers recognizing carcinogenic aromatic amines. Biochem Biophys Res Commun 313:1004–1008

Dapprich J (1999) Single-molecule DNA digestion by lambda-exonuclease. Cytometry 36:163–168

Ellington AD, Szostak JW (1990) In vitro selection of RNA molecules that bind specific ligands. Nature 346:818–822

Gilbert W (1986) The RNA world. Nature 319:618

Gopinath SCB (2007) Methods developed for SELEX. Anal Bioanal Chem 1:171–182

Gopinath SCB, Kawasaki K, Kumar PK (2005) Selection of RNA-aptamer against human influenza B virus. Nucleic Acids Symp Ser (Oxf) 49:85–86

Gopinath SCB, Balasundaresan D, Akitomi J, Mizuno H (2006a) An RNA aptamer that discriminates bovine factor IX from human factor IX. J Biochem 140:667–676

Gopinath SCB, Misono TS, Kawasaki K, Mizuno T, Imai M, Odagiri T, Kumar PK (2006b) An RNA aptamer that distinguishes between closely related human influenza viruses and inhibits haemagglutinin-mediated membrane fusion. J Gen Virol 87:479–487

Gopinath SCB, Yuriko S, Kazunori K, Kumar PKR (2006c) An efficient RNA aptamer against human influenza B virus hemagglutinin. J Biochem 139:837–846

Gyllensten UB, Erlich HA (1988) Generation of single-stranded DNA by the polymerase chain reaction and its application to direct sequencing of the HLA-DQA locus. Proc Natl Acad Sci USA 85:7652–7656

Hermann T, Patel DJ (2000) Adaptive recognition by nucleic acid aptamers. Science 287:820–825

Hultman T, Stahl S, Hornes E, Uhlen M (1989) Direct solid phase sequencing of genomic and plasmid DNA using magnetic beads as solid support. Nucleic Acids Res 17:4937–4946

Kujau MJ, Wolfl S (1997) Efficient preparation of single-stranded DNA for in vitro selection. Mol Biotechnol 7:333–335

Lato SM, Boles AR, Ellington AD (1995) In-vitro selection of RNA Lectins using combinatorial chemistry to interpret ribozyme evolution. Chem Biol 2:291–303

Little JW (1967) An exonuclease induced by bacteriophage lambda. II. Nature of the enzymatic reaction. J Biol Chem 242:679–686

Majerfeld I, Puthenvedu D, Yarus M (2005) RNA affinity for molecular Lhistidine; genetic code origins. J Mol Evol 61: 226–235

Mendonsa SD, Bowser MT (2004) In vitro selection of high-affinity DNA ligands for human IgE using capillary electrophoresis. Anal Chem 76:5387–5392

Null AP, Hannis JC, Muddiman DC (2000) Preparation of single-stranded PCR products for electrospray ionization mass spectrometry using the DNA repair enzyme lambda exonuclease. Analyst 125:619–626

Paul A, Avci-Adali M, Ziemer G, Wendel HP (2009) Streptavidin-coated magnetic beads for DNA strand separation implicate a multitude of problems during cell-SELEX. Oligonucleotides 19:243–254

Saran D, Frank J, Burke DH (2003) The tyranny of adenosine recognition among RNA aptamers to coenzyme A. BMC Evol Biol 3:26

Sassanfar M, Szostak JW (1993) An RNA motif that binds ATP. Nature 364:550–553

Schou C, Heegaard NH (2006) Recent applications of affinity interactions in capillary electrophoresis. Electrophoresis 27:44–59

Skrypina NA, Savochkina LP, Beabealashvilli RS (2004) In vitro selection of single-stranded DNA aptamers that bind human pro-urokinase. Nucleos Nucleot Nucl 23:891–893

Stojanovic MN, de Prada P, Landry DW (2000) Fluorescent sensors based on aptamer self-assembly. J Am Chem Soc 122: 11547–11548

Tang J, Xie J, Shao N, Yan Y (2006) The DNA aptamers that specifically recognize ricin toxin are selected by two in vitro selection methods. Electrophoresis 27:1303–1311

Thomas KR, Olivera BM (1978) Processivity of DNA exonucleases. J Biol Chem 253:424–429

Wiegand TW, Williams PB, Dreskin SC, Jouvin MH, Kinet JP, Tasset D (1996) High-affinity oligonucleotide ligands to human IgE inhibit binding to Fc epsilon receptor I. J Immunol 157:221–230

Williams KP, Bartel DP (1995) PCR product with strands of unequal length. Nucleic Acids Res 23:4220–4221

Wooddell CI, Burgess RR (2010) Use of asymmetric PCR to generate long primers and single-stranded DNA for incorporating cross-linking analogs into specific sites in a DNA probe. Genome Res 6:886–892

Zha W, Zhu T, Zhao H (2003) Family shuffling with single-stranded DNA. Methods Mol Biol 231:91–97