DNA Preparation and Linker Amplification for Pyrosequencing

Bonnie Poulos, Melissa Duhaime, Li Deng

Abstract

Here, we adapt the linker amplified shotgun library (LASL) approach to next generation sequencing by offering an alternate polymerase for challenging samples, developing a more efficient sizing step, integrating a “reconditioning PCR” step to increase yield and minimize late-cycle PCR artifacts. Our optimized linker amplification method requires as little as 1 pg of DNA and is the most precise and accurate available, with amplification biases less than 1.5-fold, even for complex samples as diverse as a wild virus community. While optimized here for 454 sequencing, this linker amplification method can be used to prepare metagenomics libraries for sequencing with next-generation platforms, including Illumina and Ion Torrent.

Adapted from the Broad’s LASL Sanger Seq’g Protocol, and optimized for low template work.

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Guidelines

Ultrasonic shearing of DNA

Samples in 1x TE, pH 7.5, <5 µg in 130 µl. Send to Covaris for shearing, turn around time about 1 week. Shear to 400-600 bp size. Information at http://covarisinc.com/products/laboratory-services/. UAGC Covaris S2 settings: Duty cycle=5%, Intensity=3, 200 cycles/burst, 6-8°C, 120sec.

Concentrate sheared DNA

Centrifugal concentrators, Amicon Ultra-0.5 or Nanosep, with a 30 kDa cut-off are used to concentrate the sample. Dispense up to 500 µl into reservoir and centrifuge 14,000 g for 10 min 15°C (smaller starting volumes require less time). The concentrators should retain 10µl but sometimes look dry – can add back 10-15 µl TE or flow through to membrane and rinse up and down. Invert unit and place into fresh tube. Centrifuge 1000 g for 1 min to elute concentrated sample. Should have ≤ 35µl.

End Repair DNA

Use End-Repair Kit (Epicentre Biotechnologies Cat. #ER0720 or ER81050, containing 20 µl or 50 µl enzyme, respectively; 6 reactions per ER0720 kit). For each DNA sample prepare:

Sheared DNA	≤35 µl
10x End Repair Buffer	7.5 µl
2.5 mM dNTP mix	7.5 µl
10 mM ATP	7.5 µl

nuclease-free H_2O	to 72 µl (use 14.5 µl if DNA at 35 µl)
End-repair enzyme mix	3 µl

Cycle 1:	4°C	2 min
Cycle 2:	25°C	45 min
Cycle 3:	4°C	Hold

When reaction is complete immediately purify the DNA using MinElute Reaction Clean Up kit protocol (Qiagen #28204) and elute samples with 33 µl EB buffer.

MinElute Reaction Clean up using centrifuge
Label Min-Elue columns in collection vessel. Warm EB buffer to 55°C in heat block. Mix endrepaired DNA with 300 µl ERC buffer. Apply to column. Centrifuge 16,100 g for 1 min. Discard flow thru. Wash column with 750 µl PE buffer (ethanol added); wait 3 min then centrifuge 16,100 g for 1 min. Discard flow thru and spin column to remove residual buffer for 2. 5min at 16,100 g. Transfer column to fresh, labeled 1.5 ml centrifuge tube. Elute sample using 33 µl warm (55°C) EB buffer applied directly onto membrane. Wait 2 min. Centrifuge 1min at 16,100 g to collect DNA (caps open, so point caps inward toward rotor spindle and use cover if provided with centrifuge). Discard column. Note: EB buffer is 10 mM TrisCl, pH 8.5.

Anneal Adaptors (Linker A)
Purchase 100nm amount of forward and reverse linker A with forward 5’ phosphorylated.

Linker A forward: 5'-phosphorylated-GTA TGC TTC GTG ATC TGT GTG GGT GT-3'
Linker A reverse: 5'-CCA CAC AGA TCA CGA AGC ATA C-3'

Resuspend each linker to 570 µM concentration in 1xTE plus 50 mM NaCl. Mix in equal volumes. If your cycler has step-down function, cycle 93°C, 2 min, ramp down to 4°C at 0.1°C/sec. Otherwise, use a boiling water bath: boil the mixture for 2 min in a 250-500 ml beaker, then turn off the heat and allow the water bath to cool slowly to room temperature. Then place the annealed sample at 4°C or on ice for 5 min. This stock annealed linker is now 570 µM concentration for the annealed linker.

Dilute stock annealed Adapter A to 10 µM in nuclease-free water: dispense 5 µl stock into 280 µl water and mix. This can be stored at -20°C

Use Fast-Link Ligation kit (Epicentre Biotechnologies #LK0750H or LK6201H which contain 50 µl or 100 µl ligase, respectively; 10 reactions per LK0750 kit).

End-repaired sheared DNA	30 µl
Adapter A (10 pmol/µl=10 µM)	7.5 µl
10x Fast-Link Ligation buffer	5.0 µl
10 mM ATP	2.5 µl
Fast-Link DNA Ligase (2U/µl)	5.0 µl

Total Volume (µl)	50.0 µl

Cycle 1:	4°C	2 min
Cycle 2:	23.5°C	2 hr
Cycle 3:	4°C	Hold

Immediately remove sample from thermal cycler and add to 300 µl ERC buffer (Qiagen kit 28204).

MinElute Reaction Clean up using centrifuge

* Label Min-Elue columns in collection vessel. Warm 1x TE pH 7.5 buffer to 55°C in heat block. Mix end-repaired DNA with 300 µl ERC buffer. Apply to column. Centrifuge 16,100 g for 1 min. Discard flow thru. Wash column with 750 µl PE buffer (ethanol added); wait 3 min then centrifuge 16,100 g for 1 min. Discard flow thru and spin column to remove residual buffer for 2.5 min at 16,100 g. Transfer column to fresh, labeled 1.5ml centrifuge tube. Elute sample using 20 µl warm (55°C) 1x TE pH 7.5 applied directly onto membrane. Wait 2 min. Centrifuge 1 min at 16,100 g to collect DNA (caps open, so point caps inward toward rotor spindle and use cover if provided with centrifuge). Discard column. Note: final volume will be ~19 µl.

* **Size Fractionation: the procedure described here uses gel electrophoresis for sizing. Solid Phase Reversible Immobilization (SPRI) beads can also be used. Use of Pippin Prep apparatus (Sage Science), a gel electrophoresis-based method, yields most accurate sizing and does not require additional clean-up. See Notes for details**

Prepare 1% SeaKem GTG agarose in 1x TAE buffer. For some samples, it is best to electrophorese only one sample per gel, leaving 2-4 lanes in between ladders on either end and the sample. For other samples, each sample should be separated by at least 2-3 lanes from other samples and ladders, being sure not to overload any of the samples. Gel trays, combs and unit should be treated with 10% bleach for 10 min and rinsed prior to use.

* In sterile bottle dispense enough agarose for the size gel tray you are using. Add appropriate volume of autoclaved 1x TAE buffer for 1% gel. Tare the bottle on the balance. Cover loosely and melt agarose in microwave until all particles are fully dissolved. Un-cap and place bottle on tared balance. Add additional 1x TAE or R/O water to bring volume back to starting volume. Allow to cool 10-15 min and then slowly pour into gel tray with combs in place. Samples can be loaded once the gel has solidified.

* For lower MW DNA, use 100 bp or 250 bp DNA ladder in 6x Blue Orange Gel Loading Dye (Promega #G1881). This loading dye has a yellow marker dye that runs just in front of the 300 bp fragments, making it easier to cut out 300-800 bp sized DNA.

* Mix 4 µl 6x GLB to each DNA sample and load into 1-2 wells of the gel. Load the DNA ladder in the first and last well of the gel for sizing later. Run gel at 80V for 1-2 hr depending on size of the gel.

* Place gel tray on sheet of white paper with straight horizontal line drawn the length of the gel. Use this line to cut out the DNA marker lanes with a clean scalpel blade. Mark upper right and lower left edges of marker lane to help with orientation later. Place the marker gel lanes into a stain solution (ethidium bromide, 20 µl per 100 ml 1x TAE, or SYBR green I gel stain, 30 µl in 150 ml ddH_2O). Stain for 10 min. Cover remaining gel with Saran Wrap to keep from drying out and place at 4°C.

* Observe stained marker gel pieces under UV transillumination and determine migration distance of 300-800 bp markers with cm fluorescent ruler; use this distance to measure cutting distance of samples in the unstained gel that is placed on plastic wrap or UV-transparent gel carrier sheet. Use a fresh scalpel blade for excising each sample. Alternatively, can place the stained marker gel pieces back into the gel carrier and carefully slip the entire gel onto the UV-transparent sheet cut so that it will fit back into gel tray. Photograph the entire gel with marker lanes. Measure the total gel size in cm. In Adobe Photoshop, crop gel photo to include just the gel. Print with preview in landscape mode, negative, adjusting size of photo to exact size of gel. Can print up to a 20x20 cm gel on a single sheet of 8 ½" x 11" paper. Draw a very straight line on the paper between the 300 bp and the 800 bp markers at either end. Put the gel on the transparent sheet back into the carrier and place on top of the printed gel image so that the sides are completely aligned. Using a fresh scalpel blade for each sample, cut out the 300-800 bp gel pieces (use the lower yellow dye to orient sample lane – it should be just below the 300 bp line). Place gel slices into 2 ml centrifuge tubes).

MinElute Gel Extraction

Elute DNA from gel slices using MinElute Gel Extraction kit (Qiagen #28604). Tare balance to empty 2 ml tube. Weigh each sample and record (weight=volume). Add 3 volumes QG buffer to each sample. Incubate at room temperature 20 min, inverting to mix several times, until gel is completely dissolved (do not heat to dissolve). Sample in QG buffer should be yellow in color. If the color is orange or violet add 5-10 µl 3M sodium acetate pH 5 and mix. Add 1 volume isopropanol to each sample. Load 750 µl per column and centrifuge 16,100 g, 1.5 min. Discard flow thru and repeat until all of sample is loaded onto the column. Wash with 0.5ml QG buffer 16,100 g, 1.5 min. Add 750 µl buffer PE (to which ethanol has been added). Wait 3 min, then centrifuge 2min at 16,100 g. Discard flow-thru and spin for 2.5 min 16,100 g to remove residual wash buffers. Transfer column to fresh, labeled 1.5 ml centrifuge tube. Elute sample using 35µl warm (55oC) EB buffer applied directly onto membrane. Wait 2 min. Centrifuge 1min at 16,100 g to collect DNA (caps open, so point caps inward toward rotor spindle and use cover if provided with centrifuge). Discard column.

Small-scale PCR Titration using LA Takara HS

Prepare PCR A primer: 5'-phosphorylated-CCA CAC AGA TCA CGA AGC ATA C-3'. This primer can contain a 5-10 bp bar code at the 5' end using rules that there are no runs of nucleotides and the nucleotide at the 3'end of the bar code is not a C. Only one primer is needed as it primes off both the forward and reverse strand of the linker-ligated sheared DNA. Prepare 100 µM stock of the primer in nuclease-free water. Dilute to 10 µM in nuclease-free water for working dilution. Use LA Takara HS (Clontech #RR042 or RR042A) for high-fidelity PCR.

For each sample, prepare a master mix. For each 25 µl {or 5x25 µl} reactions:

Nuclease-free water	16.75µl {83.75 µl }
LA Takara HS 10x Buffer	2.5 µl {12.5 µl}

2.5mM dNTP mix	4.0 µl {30 µl}
10µM PCR A primer	0.5 µl {2.5 µl}
5U/µl LA Takara HS	0.25 µl {1.25 µl}
Linker-ligated sheared DNA	1.0 µl {5.0 µl} (may want to test several dilutions)
Total	25 µl {125 µl}

Dispense into 0.2 ml thin-walled PCR tubes.

Cycle 1: 95°C 2 min
Cycle 2: 95°C 30 sec
60°C 1 min
72°C 1.5 min
Set cycles to pause after 18, 22, 25, 28 & 30 cycles; remove one sample after each pause with last sample going through final 10 min extension step.
Cycle 3: 72°C 10 min

To determine optimal number of cycles (and dilution), mix 5 µl of sample with 1 µl 6x Blue Orange gel loading dye and examine in 1.2% agarose gel in 1xTAE. Look for the minimum number of cycles (and dilution) yielding maximum amount of desired size fragments, without too much lower MW products.

Large Scale PCR using LA Takara HS
For each sample, prepare a master mix. For each 25 µl reaction:

Nuclease-free water	16.75 µl
LA Takara HS 10x Buffer	2.5 µl
2.5mM dNTP mix	4.0 µl
10µM PCR A primer	0.5 µl
5U/µl LA Takara HS	0.25µl
Linker-ligated sheared DNA	1.0 µl
Total	25 µl

Dispense into 0.2 ml thin-walled PCR tubes.

Cycle as above with cycles set to determined optimum number followed by 10 min extension step. Pool all replicate reactions and run 5µl in 1.2% agarose gel in 1x TAE to determine quality and yield. Note: 50 µl reactions can be run instead of 25 µl if desired.

Reconditioning PCR: if more DNA is required than obtained with standard PCR or if heteroduplex formation may be an issue, then a "reconditioning PCR" step can be run to increase final yield, and to minimize heteroduplex formation.

For each large scale PCR reaction, prepare enough master mix for 10x reaction. Assuming 20 µl large scale, prepare enough master mix for 200 µl:

Nuclease-free water	15.5 µl {155 µl }
LA Takara HS 10x Buffer	2.5 µl {25 µl}
2.5mM dNTP mix	4.0 µl {40 µl}
10µM PCR A primer	0.5 µl {5.0 µl}
5U/µl LA Takara HS	0.25 µl {2.5 µl}
Large scale DNA product	2.0 µl {20 µl}
Total	25 µl {200 µl}

Dispense into 0.2 ml thin-walled PCR tubes. Note: 50 µl reactions can be run if desired.

Cycle 1: 95°C 2min
Cycle 2: 95°C 30 sec
60°C 1 min 3 Cycles (yes, only 3 cycles)
72°C 1.5 min
Cycle 3: 72°C 10 min

Pool all replicate reactions and run 5 µl in 1.2% agarose gel in 1x TAE to determine quality and approximate yield.

PCR Cleanup

Use either MinElute PCR Clean-up kit (Qiagen #) or QiaQuick PCR Clean-up kit (Qiagen #28104). The MinElute columns hold a maximum of 5 µg DNA and minimum elution volume of 10 µl whereas QiaQuick columns hold a maximum of 10 µg and minimum elution volume of 30 µl. If reconditioning volume is greater than 500 µl, the pooled reaction can be concentrated using Amicon Ultra-0.5 or Nanosep, with a 30kDa cut-off using manufacturer's directions.

Add 5 volumes of buffer PB to each pooled sample. Be sure to have pH indicator in the buffer as Takara PCR seems to be a bit alkaline and you will have to adjust pH with 2-5 µl 3M sodium acetate pH 5 as indicated in Qiagen protocol. Apply to column and centrifuge 16,100 *g*, 1 min. Discard flow-through (can run it back over column one time and may want to save until final analysis is complete). Add 750 µl buffer PE (with ethanol added), wait 3 min, then centrifuge 1.5 min at 16,100 *g*. Spin again to dry, 16,100 *g* for 2 min. Add 10-40 µl warm (55°C) buffer EB to each column (smaller amounts if using MinElute column), wait 2 min, then spin 1 min at 16,100 *g*. If you choose, you can elute a second time (can recover an additional 10%) using 1030 µl warm EB. Do not pool second elution with first until quantification.

Final Analysis

Quantify DNA using Quant-it PicoGreen dsDNA kit (Invitrogen). Run 1-2 µl in 1.2% agarose gel in 1x TAE to determine quality. Sample should also be analzyed using Agilent DNA 7500 kit and Agilent 2100 bioanalyzer to determine size range of PCR products.

NOTES:

Amicon Ultra-0.5-30K (MilliporeUFC503024) or Nanosep-30K (OD030C33), 1 per sample (Note: Microcon YM concentrators have been discontinued by the manufacturer).

1x TE, pH 7.5

End-It DNA End Repair Kit (3 µl enzyme per reaction) Epicentre #ER0720 (contains 20 µl enzyme; available through Fisher as NC9807549) or ER81050 (contains 50 µl enzyme)

Nuclease-free Water Promega #P1193 (available through Fisher as PRP1193))

Minelute Reaction Clean-up kit (2 columns per sample) Qiagen 28204

Minelute Gel Extraction Kit (1-2 columns per sample) Qiagen 28604

QiaQuick PCR Purification Kit (1 column per sample) Qiagen 28104

Fast Link DNA Ligation Kit (5 µl enzyme per sample) Epicentre #LK0750H (contains 50 µl enzyme; available through Fisher as NC9804176) or LK6201H (contains 100 µl enzyme; available through Fisher as NC9527092)

SeaKem GTG Agarose Cambrex #50074 or from Fisher #138319 (which is Lonza #50071)

Blue Orange 6X Loading Dye Promega #G1881 (this is a great loading dye as the yellow marker migrates just ahead of the 300 bp size; available through Fisher as PRG1881)

DNA ladder, but we found the 250 bp or 100 bp ladder to be better for sizing the fractions: 1Kbp Invitrogen #15615-06, 250 bp Invitrogen #10596-013, 100bp Invitrogen #15628-019

Takara LS Taq HS, sold by Fisher for Clontech as RR042 (25µl) and RR042A (4x25µl). Also order

additional dNTP mix as you will run out at these reaction conditions, Clontech #TAK4030

Quant-it PicoGreen dsDNA kit, Invitrogen #P7589

Broad also recommends using Sybr Green Gel Stain (Invitrogen 40412) but ethidium bromide was sensitive enough for our samples

E Gels as suggested with Sybr Safe (not very sensitive; regular gel worked fine) - first time order needs starter kit which has the electrical hookup (not avalaible alone) Starter kit Invitrogen G620601; 1.2% gels only with Sybr Safe G521801. These E-gels also come with EtBr stain.

In addition to all of this you also need to order the linker and the primer:

Linker A forward: 5'-phosphorylated -GTA TGC TTC GTG ATC TGT GTG GGT GT-3'
Linker A reverse: 5'-CCA CAC AGA TCA CGA AGC ATA C-3'
PCR-A Primer: 5'-phosphorylated -[5bp bar code] CCA CAC AGA TCA CGA AGC ATA C-3'
Order 100 nmol amount of each oligo. For the Linker A annealing, the lyophilized material was diluted to 1.14 mM in 1xTE plus 50 mM NaCl. Equal amounts of the forward and reverse oligo were mixed, boiled it for 2 min and removed the beaker from the heat but the tube was left in the water until it cooled down to room temperature then placed at 4C. This stock should be 570 µM concentration which is then diluted to 10 µM for the working dilution.

The PCR-A primer was diluted to 100 µM in nuclease-free water and the working stock was diluted to 10uM in nuclease-free water. You only need one primer since it primes off of both strands.

Based on first 454 sequencing results, we have decided to use DNase treatment of the resuspended iron chloride precipitated viral preparation, followed by CsCl gradient purification and subsequent Wizard Prep DNA extraction prior to shearing.

The new additions to version 3 are use of LS Takara HS enzyme system, testing both # PCR cycles and template dilution, and use of the “reconditioning step” to obtain final DNA for sequencing. Reasons:

1. Higher product yield with LS Takara HS than with PFU Turbo. Takara Taq has proof-reading ability. This protocol uses higher amount of dNTP and reducing that has not yet been tested. We have been able to amplify ~100fg DNA/25 µl reaction with this enzyme.
2. Experiments comparing Takara and PFU demonstrated 2 things: less template in the reaction tends to yield more higher MW products so diluting templates (eg, 1:10, 1:100) when testing optimal number of cycles can improve percent of higher MW products and reduce amount of

linkered DNA need to get enough product for sequencing; and as cycle number increases, the MW of the products tends to decrease so we test routinely from 20-30 cycles and no more unless template simply will not amplify with fewer cycles. We chose both dilution of template and # of PCR cycles for final PCR based on titration results.

3. Reconditioning step is taken from J.R. Thompson et al, 2002 (Nucleic Acid Res. 30(9):2083-2088; http://www.ncbi.nlm.nih.gov/pmc/articles/PMC113844/): this step not only increases product yield significantly but should also reduce heteroduplex formation that occurs with mixed template samples.

Version 4 has been modified to account for discontinued Microcon YM-100 centrifugal concentrators. The alternative products, Amico Ultra and Nanosep, both recommend a 30kDa cut-off for DNA concentration, therefore, they are included in this version.

Version 5 notes that SPRI beads may be used for size fractionation instead of gel-sizing. If the DNA shearing has produced fragments all below the needed maximum size, then the use of beads is quicker, less prone to contamination, and results in much higher yields than gel sizing. The protocol for AMPure® bead (A 63880, Agencourt, Beckman Coulter) calibration is described by Roche (GS FLX Titanium General Library Preparation, section 5.3 to determine the proper ratio of beads to DNA to eliminate fragments less than 400 bp). In general, a ratio of 55-60 µl beads to 100 µl DNA was optimal for recovering DNA above 400 bp.

Version 6 notes that Pippin Prep (made by Sage Science) is most accurate method for sizing and does not require downstream clean-up to remove agarose. This protocol uses Sage Science 2% agarose gel with ethidium bromide was run with settings to recover 400-600bp fragments. DNA is recovered in 40-50 µl final volume.

In preparing manuscript realized that LA Takara may not be the best Taq polymerase to use as it appears to produce more “rare” sequences and may not amplify optimally when DNA is limiting. The original, Pfu Turbo 2x Master Mix (Stratagene 600600-51) may be a better choice and yield more product with fewer cycles. Follow manufacturer’s directions for PCR.

Manuscript citation is: *MB Duhaime, L Deng, BT Poulos, MB Sullivan. 2012. Towards quantitative metagenomics of wild viruses and other ultra-low concentration DNA samples: a rigorous assessment and optimization of the linker amplification method. Environmental Microbiology 14(9):2526-2537.*

Steve Giovannoni (Nov. 2012) has cautioned about using Tris-based gels or mildly acid conditions on natural bacterial DNA samples. Apparently a significant amount of natural bacterial DNA may be phosphorothiolated (see L. Wang et al., PNAS 108(7):2963-2968, 2011), which makes the DNA fall apart under certain common laboratory preparation conditions resulting in their loss prior to DNA sequencing.

Version 8 has a correction made to the Annealing of the Linker A: previously made stock up at 1.14

mM of each oligo and mixed together: this is actually a final concentration of 1.14mM with respect to the annealed linker, not 570 µM, as stated previously. To prepare 570 µM concentration of each oligo: divide nmol x 0.57 = 570 µM.

Small-scale PCR Titration with PFU Hotstart PCR Master Mix instead of LA Takara HS

Prepare PCR A primer: 5'-phosphorylated-CCA CAC AGA TCA CGA AGC ATA C-3'. This primer can contain a 5-10 bp bar code at the 5' end using rules that there are no runs of nucleotides and the nucleotide at the 3'end of the bar code is not a C. Only one primer is needed as it primes off both the forward and reverse strand of the linker-ligated sheared DNA. Prepare 100 µM stock of the primer in nuclease-free water. Dilute to 10 µM in nuclease-free water for working dilution. Use PFU Hotstart PCR Master Mix (Stratagene 600600) for high-fidelity PCR.

For each sample, prepare a master mix. For each 25 µl {or 5x25 µl} reactions:

Nuclease-free water	10.5 µl {52.5 µl}
LA Takara HS 10x Buffer	12.5 µl {625.0 µl}
10µM PCR A primer	1.0 µl {5.0 µl}
Linker-ligated sheared DNA	1.0 µl {5.0 µl} (may want to test several dilutions
Total	25 µl {125 µl}

Dispense into 0.2 ml thin-walled PCR tubes.

Cycle 1:	95°C 2 min	
Cycle 2:	95°C 30 sec	Set cycles to pause after 18, 22, 25, 28 & 30 cycles; remove one sample after each pause with last sample going through final 10 min extension step.
	60°C 1 min	
	72°C 1 min	
Cycle 3:	72°C 10 min	

To determine optimal number of cycles (and dilution), mix 5 µl of sample with 1 µl 6x Blue Orange gel loading dye and examine in 1.2% agarose gel in 1xTAE. Look for the minimum number of cycles (and dilution) yielding maximum amount of desired size fragments, without too much lower MW products.

AMPure (aka SPRI) Bead clean-up of reactions instead of Qiagen

AMPure XP beads (Beckman Coulter PN #A6880)

70% Ethanol (freshly prepared: 3 ml Mo.Bio. water plus 7 ml 100% ethanol)

Dyna-Mag2 Magnetic Particle Concentrator (MPC; Invitrogen PN #123-21D)

Bring beads to room temperature. Just prior to dispensing, vortex beads vigorously. Ratios of beads to DNA varies depending on what size DNA one is working with; anything above 1.2X will get rid of

DNA less than 100bp; this protocol uses 2X beads (i.e., 2 volumes beads to 1 volume DNA). To reduce amount of beads needed, can concentrate the DNA to 50-100 µl using Amicon 30kDa centrifugal concentrators.

Mix 2 volumes of vortexed beads to 1 volume of DNA in 1.5 ml tubes. Allow to incubate at room temperature for 15 min. Open tops and put the tubes into the MPC and allow the beads to adhere to the wall of the tube for 2-5 min. Carefully remove the supernatant fluid (save this flow through until you calculate DNA yield – it is possible that some DNA will not bind if concentration is high enough or if you use less than 1X beads). Wash the bound beads 2X with 500 µl fresh 70% ethanol. Pipet on for about 1 min and then remove and discard. Try to draw off all of the last wash with a 10 µl pipet.

Remove the tubes from the MPC and place in a 37^{o}C heat block for 10 min to evaporate any residual ethanol. Mix the beads with TE, ultrapure water or other elution buffer and pipet up and down to completely resuspend the beads. Allow DNA to elute off beads for 5 min at room temperature. Open tops and put the tubes back into the MPC and allow the beads to adhere to the wall for the tube for 2-5 min. Carefully, with a fine tip pipet tip, remove the eluted DNA (best to set pipettor to 2-4 µl less than the volume added so as not to draw up any of the magnetic beads). Transfer to a low-DNA bind tube. You may elute a second time if desired. Calculate yield using Pico Green analysis. Yields can be quite good compared to column clean-up methods.

Protocol