



# Andrew Alliance

APPLICATION NOTE  
No.9 20160909 v3

## An improved method for magnetic bead separation: the BeadTender™

*Magnetic separation of biomolecules from a mixed sample is a complex manipulation significantly affected by variability. The BeadTender method automates magnetic separation of beads inside conventional pipette tips and produces biomolecules of high purity with a reproducibility up to eight times better than that of the most experienced human operators.*

Isolation and purification of biomolecules (double- and single-stranded DNA, total RNAs, mRNAs, miRNA, proteins), as well as specific cells and organs, facilitate a wide array of downstream applications in gene and protein expression studies, cloning, transfection, protein-protein interactions, immunology, clinical diagnostics, cDNA library synthesis, PCR and qPCR, Sanger and NGS sequencing, and more. Due to its simplicity, recovery efficiency and purity, magnetic separation has found its place in this bio-separation arena and gradually replaced the traditional liquid phase and solid phase separation methods, which require extensive centrifugation or vacuum filtration procedures that are difficult to automate.

However, when reproducibility plays a vital role in obtaining the results, manual performance varies greatly between different operators and even for the same operator. Automation can standardize different procedures, significantly reducing this variability, but may be limited in other aspects such as liquid evacuation or efficient bead collection. The BeadTender method (**Figure 1**), is an automatic solution to manual magnetic bead manipulation

exploiting the Andrew robot. It consists of a specific manipulation and magnetic design enabling beads separation, washing, and elution fully inside a conventional pipette tip. The proprietary method (patents pending) improves the reproducibility of the purified biomolecules, with the highest efficiency and flexibility in sample and elution volumes.

The entire process of the BeadTender method is executed within a conventional tip connected to a conventional pipette. It encompasses sample aspiration, active bead mixing, bead washing, bead pelleting, incubation, drying, and finally sample elution. Thus, users are totally spared from manual operations and the repetitive stress injury caused by the manual bead resuspension at various steps. Completely independent of the consumables holding the samples, the BeadTender method is scalable for all common volume ranges of biological applications, with adjustable and optimizable parameters for bead manipulation adaptable to each and every protocol or bead type. Therefore, the BeadTender provides a flexible and universal solution to the small-scale automation of magnetic bead manipulation.



**Figure 1:** In-tip separation of beads from samples by the BeadTender method and the Andrew robot

## THE BEADTENDER METHOD IMPROVES THE REPRODUCIBILITY OF DNA PURIFICATION

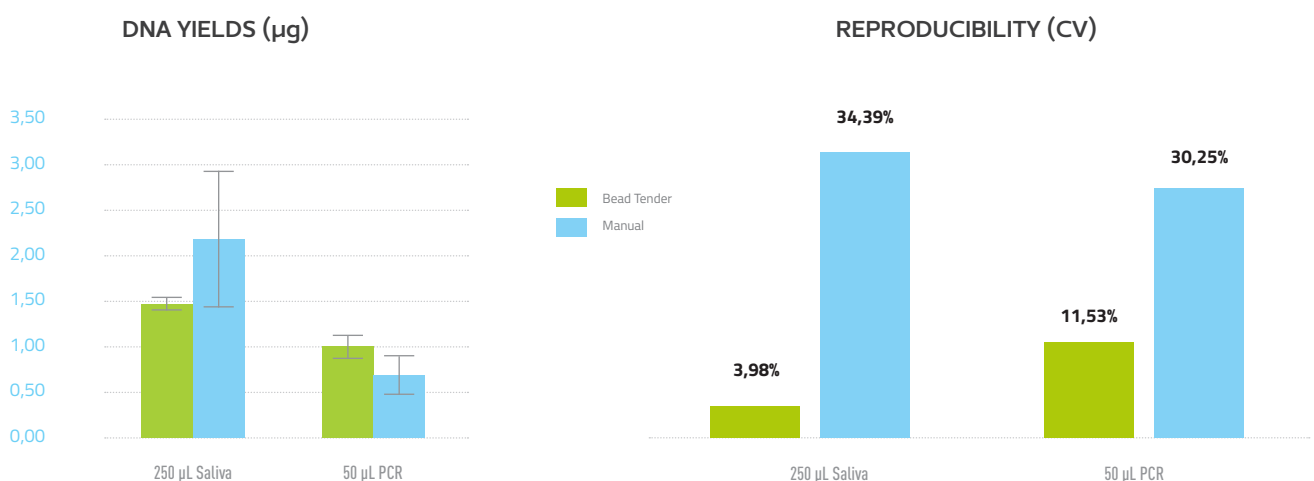
The BeadTender method performance was assessed with two different procedures: (1) genomic DNA extraction from 250  $\mu\text{L}$  human saliva samples contained in 2mL microcentrifuge tubes, and (2) purification of 50  $\mu\text{L}$  PCR products contained in 0.2mL PCR tubes. The saliva sample treated at 50°C overnight was divided into 8 aliquots of 250  $\mu\text{L}$ . The 700bp PCR products were generated with the KAPA HiFi HotStart ReadyMix PCR Kit (KAPA). Multiple PCR reactions were pooled and split into ten 50  $\mu\text{L}$  samples. Five replicates of PCR purification and four replicates of saliva genomic DNA extraction were performed by a pipetting robot Andrew model 1000G. The 250 $\mu\text{L}$  tips from Biotix were used with the P200 pipette for PCR purification, and 1000 $\mu\text{L}$  Diamond tips (Gilson D1000) with the P1000 pipette for genomic DNA extraction. The same number of replicates were also carried out by an experienced human operator performing the process using the same material.

For PCR purification, 90  $\mu\text{L}$  of AMPure XP magnetic beads (Beckman Coulter) were mixed by Andrew with 50  $\mu\text{L}$  PCR samples. The bead pellets were washed twice with 140  $\mu\text{L}$  80% EtOH and the tips holding the bead pellets were washed once with 50  $\mu\text{L}$  water. The purified PCR products were eluted in 50  $\mu\text{L}$  10mM Tris buffer (pH 8).

The saliva genomic DNA was extracted with reagents from the Mag-Bind Blood and Tissue DNA HDQ Kit (Omega Biotek). 20  $\mu\text{L}$  of Proteinase K (20 mg/mL) and 290  $\mu\text{L}$  of lysis buffer AL were mixed with each sample, incubated at room temperature for 5 minutes, then mixed by pipetting up and down 200 times with

400  $\mu\text{L}$  HDQ binding buffer and 20  $\mu\text{L}$  HDQ magnetic beads. The bead pellet was washed twice with the VHB buffer, once with the SPM buffer, and once with water, all in 980  $\mu\text{L}$ , and air dried for 5 min before being resuspended in 200  $\mu\text{L}$  of elution buffer to elute DNA. For the automatic procedure, all resuspension and pelleting steps were done inside the BeadTender by Andrew, while the 96S magnetic plate (Alpaqua) and the Chemagic Stand 2x12 (Perkin Elmer) were used as magnetic separator for manual pelleting. The manual bead resuspension steps were done by vortexing for 60 seconds except for the final step with the elution buffer, where manual pipetting up and down 50 times were carried out. Samples were processed simultaneously with the manual method and sequentially with BeadTender. The hands-on time for designing the protocols took few minutes by exploiting the specific Wizard of Andrew Lab V1.5 (under release), and preparing samples for Andrew and BeadTender of 4 saliva DNA extractions and 5 PCR clean-ups took 7 and 5 minutes, respectively, while manual operation (including protocol execution) took 38 and 35 minutes.

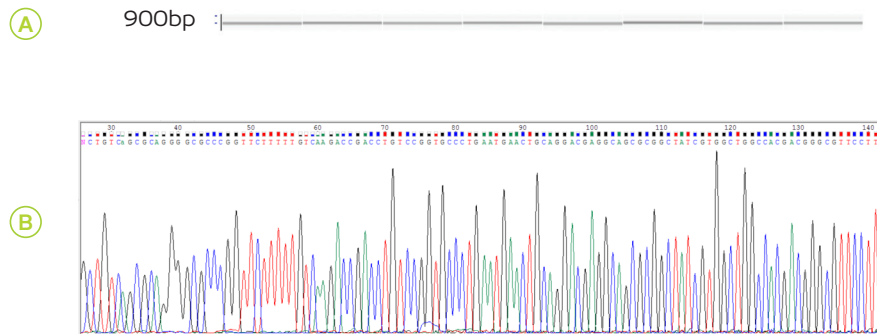
The purified DNA samples were diluted 50x or 100x for quantification by the Qubit HS DNA assay (Thermo Fisher Scientific). The coefficient of variations (CV) of the DNA yields from saliva and PCR products performed by BeadTender and Andrew are eight times and three times lower than those of the experienced human operator, indicating a significant improvement of reproducibility (**Figure 2**).



**Figure 2:** Reproducibility of DNA extraction by BeadTender vs. human operator

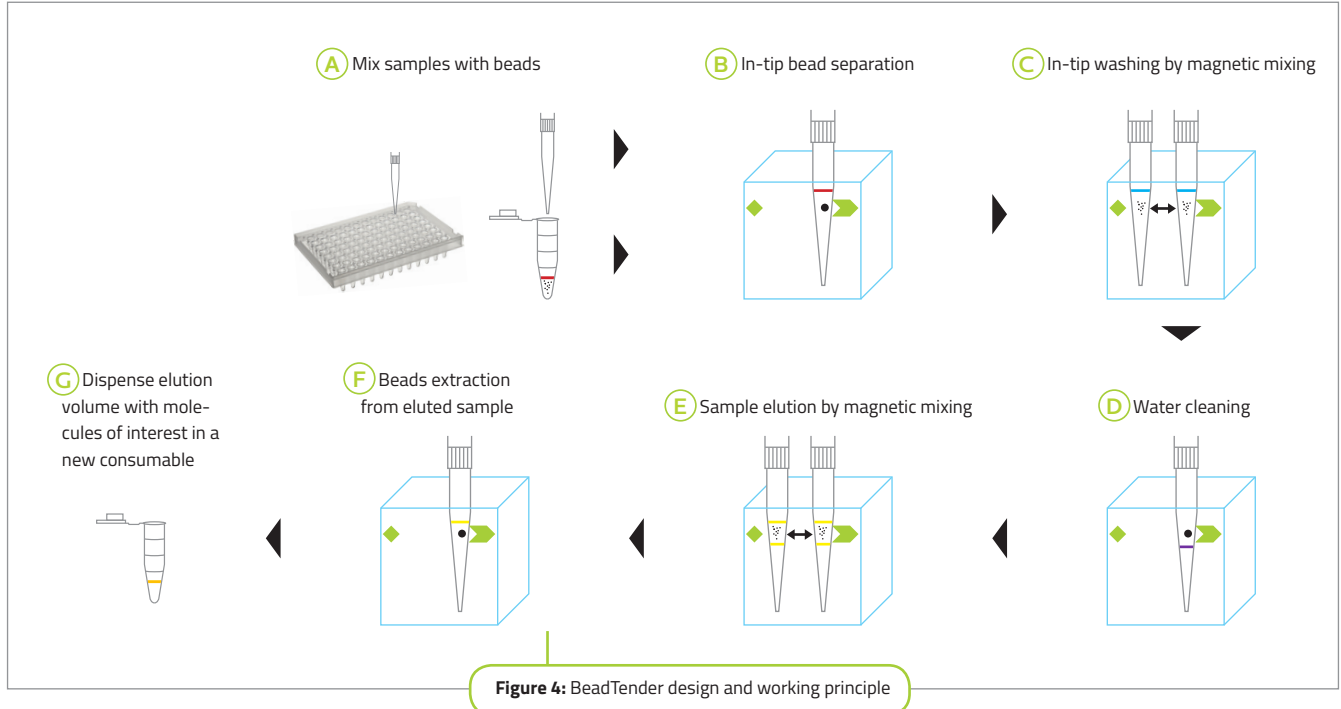
The purity of the DNA samples extracted by BeadTender and manually was evaluated by two downstream applications: For the saliva genomic DNA, a 50  $\mu$ L PCR reaction was performed with 2  $\mu$ L template DNA and the primers for the human gene Hemoglobin Beta; for the cleaned-up PCR products, 4  $\mu$ L was sequenced by the Sanger sequencing method. An amplicon of the expected size of ~900bp was successfully amplified from

all 8 genomic DNA samples (**Figure 3A**), and all 10 Sanger-sequenced cleaned-up PCR products showed clean chromatograms with single sharp peaks for all 700 nucleotide positions (**Figure 3B**). These results demonstrate that the BeadTender method removes all contaminants (PCR inhibitors, unincorporated nucleotides and primers, by-product primer dimers, left-over salt and enzymes) with excellent efficiency.



**Figure 3:** Purity of genomic DNA (A) and cleaned-up PCR products (B) extracted by BeadTender and manually

## BEADTENDER – CONSUMABLE-INDEPENDENT MAGNETIC SEPARATION



**Figure 4:** BeadTender design and working principle

The workflow of magnetic separation by BeadTender is contained entirely within a pipet tip, which can accommodate samples as low as 5  $\mu$ L and as high as 5 mL depending on the applications. The process starts in any consumable of users' choice where samples have been mixed with the appropriate magnetic

bead type for capturing the biomolecules of interest (**Figure 4A**). The sample-bead mixture is aspirated into a pipet tip, which is moved into a region where a suitably designed magnetic field can sweep and collect the beads into a pellet (**Figure 4B**). After the supernatant is physically separated from the beads by the

combined use of magnetic field and pipetting action, it is discarded while the beads are kept intact inside the tip; an ethanol mixture is then aspirated in the same tip, and magnetic mixing is performed to enhance the bead washing quality, followed by pelleting of the beads (**Figure 4C**). Next, ethanol is discarded and water is aspirated into the tip (without touching the bead pellet) to remove any ethanol trace, and the pellet inside the tip is dried (**Figure 4D**). Finally, the elution buffer of user's choice is drawn inside the tip at the desired volume, and the biomolecules are released from the beads by resuspending the beads in the pellet through active magnetic mixing inside the BeadTender (**Figure 4E**). The beads that have released the molecules of interest are now pelleted inside the tip and separated from the elution buffer

## SUMMARY

The BeadTender allows isolation and purification of biomolecules inside a conventional pipette tip with flexibility of sample and elution volumes, independently from source and destination

containing the biomolecules (**Figure 4F**). The eluted solution is then transferred to a new destination consumable (**Figure 4G**) and the tip containing the used beads is discarded. The complete process is carried out by the Andrew pipetting robot without any user intervention; the operator only needs to supply the samples, beads, wash buffers, elution buffer of choice, and clean consumables for the final purified products.

All parameters of the procedure (for example, pelleting time, re-suspension time, buffer volume) are entirely adjustable in the graphical user interface of the software Andrew Lab. Users can therefore fine-tune the method to optimize efficiency and purity according to the bead types, sample viscosity, ionic force or pH of the buffers, for example.

consumables. The reproducibility of the method improves by up to eight times the performance of a skilled human operator, and it is all accomplished totally hands-off by the Andrew robot.

Get in touch for more information



[andrewalliance.com](http://andrewalliance.com)

or



+41 22 518 03 57  
+1 781 761 0119



**Quy A. Ngo**

Senior Application Scientist, Andrew Alliance  
[Applications@AndrewAlliance.com](mailto:Applications@AndrewAlliance.com)

21 Chemin Grenet ■ 1214 Vernier, Switzerland  
185 Dartmouth St. ■ 02116 Boston, USA