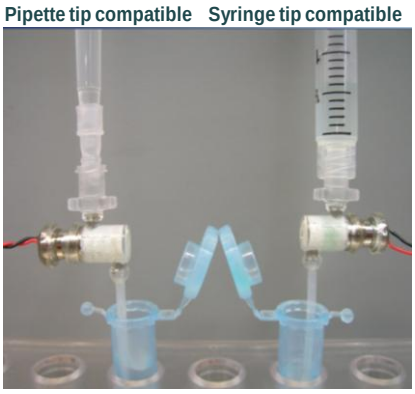
OmniLyse®/PureLyse® DNA Extraction

The **PureLyse®** rapid DNA extraction technology from **ClaremontBio** was tested for capture and elution efficiency of E. coli DNA from a known number of cells. For each cartridge 1 ml of binding buffer containing 1 x 10^8 cells was injected at a rate of 200ul/min using a 1 ml syringe and syringe pump. The initial flow-through fraction was collected, which contains unbound DNA along with cell debris. The DNA was then eluted in 4 x 150ul successive fractions. DNA was quantitated for the flow-through and elution fractions using PicoGreen™ staining (Figure 1). A total of 82% of the theoretical DNA was eluted in the four elution fractions, with nearly 50% eluted within the first elution fraction.



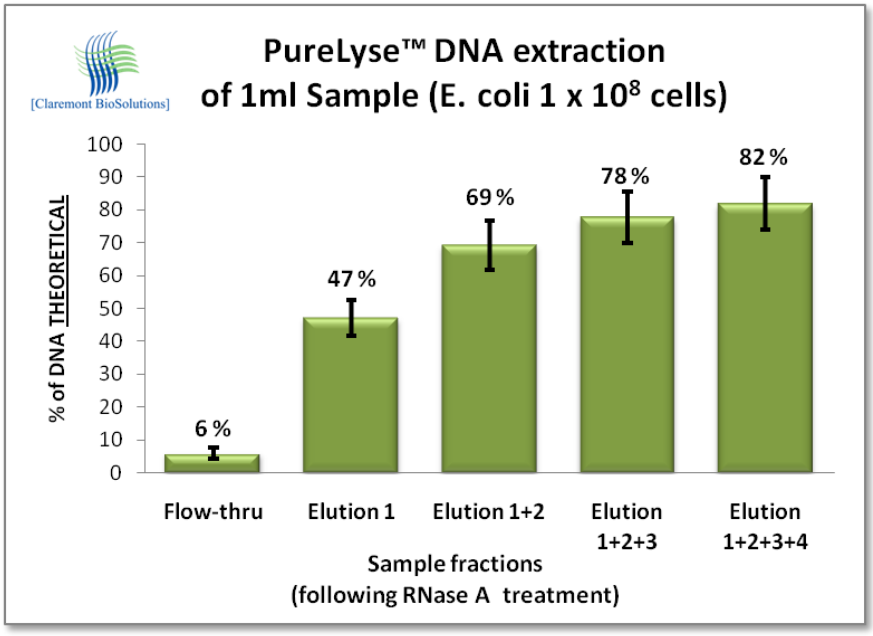


Figure 1. Yield of DNA from each fraction, the sample flow-through and four 150 ul elutions, as percent of DNA predicted from the cell count. Each fraction was treated with 1ug RNase A. The control Lambda DNA used for quantitation was also stained with and without with RNase A treatment. The average yield from all fractions totaled 88% of the theoretical amount of total DNA with 82% of the DNA in the sum of the four elutions. Replicates: n=5.

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Due to the presence of RNA, which has been shown to be co-captured and co-eluted with the DNA using PureLyse® technology, the raw PicoGreen™ fluorescence of the samples is typically twice that predicted for the number of cells processed, which is easily corrected with RNase A treatment (Figure 2). The fluorescence signals obtained are consistent with the known relative load of RNA to DNA in bacterial cells (approx 5:1) and the relative PicoGreen™ staining efficiency of RNA to DNA (approx 1:5). It is known that there is approximately five-fold more RNA (~20 fg/cell) than DNA (~4.2 fg/cell) of DNA in bacterial cell during log growth phase. To eliminate the fluorescence due to the presence of RNA each fraction was treated with 1ug RNase A, then both treated and untreated fractions were stained with PicoGreen™. The control Lambda DNA used for quantitation was also stained with and without with RNase A. All results shown in Figures 1, 2, and 3 were processed using the RNase A treatment method. This data shows that RNA is successfully captured and eluted using the PureLyse® technology.

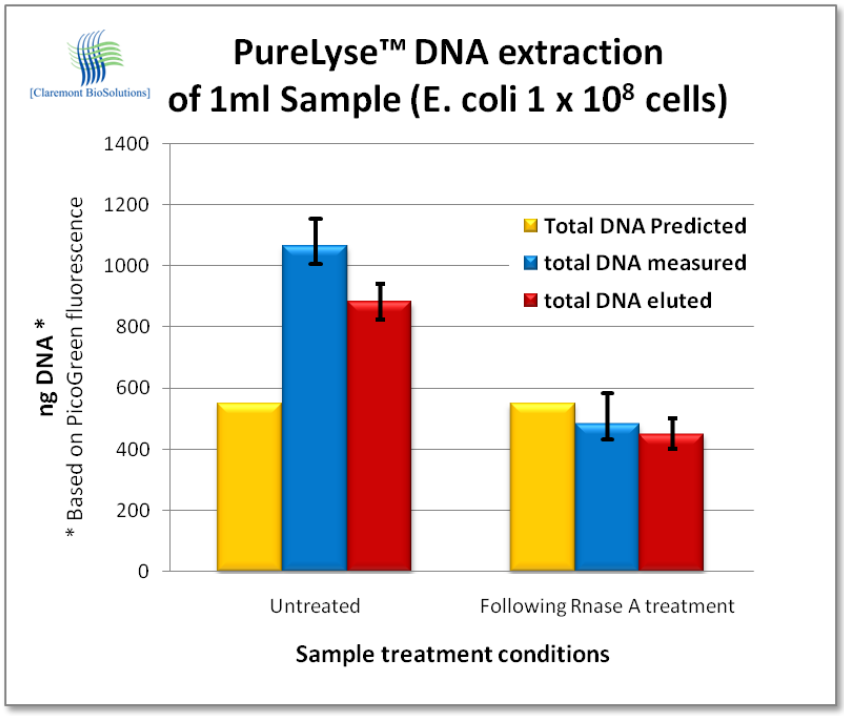

Figure 2. Nucleic acid yields from 5 PureLyse® preparations, measured by PicoGreen™ staining before and after RNase A treatment. On average the sum of the yields in all fractions was reduced by 54% by RNase A treatment. The Gold bars represent the amount of DNA expected from the carefully counted E.coli cells. Cells were quantitated by optical density and colony counts.

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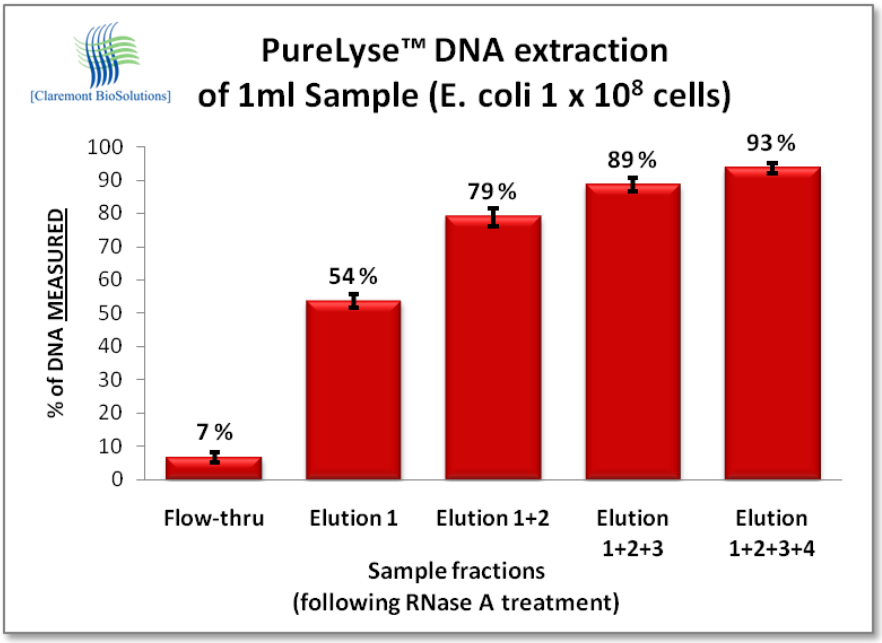

Figure 3. Percent yield of total measured DNA after RNase A treatment. 7% of the DNA has been measured in the sample flow through (the unbound DNA in the sample discard) and 79% of the DNA is eluted in the first two fractions.

The performance of the PureLyse® system was also compared to a leading DNA extraction kit currently on the market used for the extraction bacterial genomic DNA from E. coli cells. Identical 1ml sample volumes of K12 E. coli were processed at different concentrations and DNA was eluted in 200ul. The PureLyse® protocol took 3 minutes per sample, whereas the protocol from Company Q took at least 4 hours to perform. Samples were tested by PCR and the results are compared by plotting critical threshold values versus cell concentration (Figure 4). Samples were set-up at 10 fold dilutions from $1x10^9$ cells/ml to $1x10^4$ cells/ml (respectively, $5x10^6$ copies to 50 copies of target DNA were transferred to the PCR reaction. It is important to note that the ClaremontBio PureLyse® cartridge (with its ~ 5 minute processing time) essentially co-plots with the more cumbersome Company Q kit (in terms of steps required, equipment needs, and time required totaling at least 4 hours). However, the errors bars are tighter using the PureLyse® system due having a simple 2-step protocol without multiple transfer steps which can introduce more variation. The trend generated by the PureLyse® system is a cleaner and more monotonic trend throughout a wide range of cell concentrations than the other system used.

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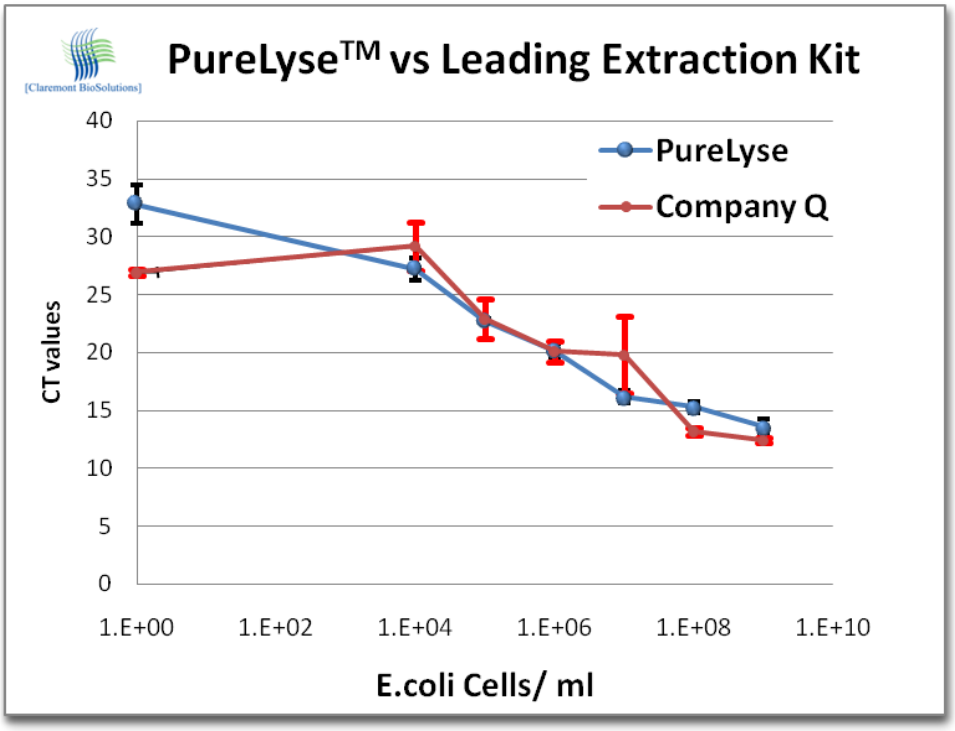

Figure 4. PureLyse® to PCR. E.coli cells were titrated in 1 ml samples of binding buffer from $1x10^9$ to $1x10^4$ cells/ml. Each PureLyse® sample was processed under five minutes. The samples processed by the kit from Company Q took 3-4 hours. Samples were eluted in 200ul and then 5ul of each sample was transferred to a PCR reaction tube for amplification using a Roche LightCycler™. 3 replicates per titer.

A more recent titration of E.coli cells has been tested using the Purelyse® system down to 200 cells in ½ ml samples. Samples were lysed within 90 seconds and eluted within one minute. Each titer was tested in triplicate. PCR was performed with primers designed to generate a 246 base pair fragment in the presence of E. coli DNA. Gel analysis of the amplified products reveals that the positive samples are true positives and that the material amplified in the negatives is primarily nonspecific material (primer-dimers).

ClaremontBio Solutions

MAKING THE EXTRACTION OF DNA EASY™

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