APPLICATION NOTE

# Extraction of Genomic and Plasmid DNA from Mammalian Cultured Cells and Tissue Samples

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In this application note we describe how we assessed the suitability and effectiveness of two kits from BioEcho Life Sciences, the EchoLUTION Cell Culture DNA Kit and the EchoLUTION Tissue DNA Micro Kit. We validated the extraction of genomic DNA (gDNA) from a representative variety of human cells and tissue samples. DNA samples from cultured human cells, ranging from 15 × $10^3$ to 2 × $10^6$, were successfully amplified and displayed a linear correlation between DNA yield and number of cells. Total DNA isolated from transfected cells was effectively transformed into *E. coli*, demonstrating that plasmid DNA can be also isolated with the EchoLUTION Cell Culture DNA Kit. In addition, we compared the EchoLUTION Cell Culture DNA Kit, on several measures of performance, to an equivalent kit from another vendor that is based on the silica (*bind-wash-elute*) method. Data reveal that EchoLUTION Cell Culture Kit results in higher DNA yield than the silica method.

## Introduction

The extraction of genomic and plasmid DNA is needed for certain experiments and assays like antibody epitope mapping, cell surface display or characterization of stably transfected cell lines. While DNA -and especially plasmid DNA- extraction from bacteria is well understood with various solutions available on the market, DNA extraction from mammalian cells remains a challenging and comparatively expensive procedure. For optimal quality of the extracted DNA, the process must meet certain requirements in terms of the number of cells, procedure preparation, and reagents composition.

In this application note, we describe the extraction of genomic DNA from various cultivated cell lines and human tissue samples with the EchoLUTION technology. Eluates were analyzed and compared to the results obtained with a silica-based method. Moreover, total DNA has been isolated from transiently transfected cells including plasmids and used to successfully transform *E. coli*, demonstrating that plasmid DNA can also be isolated with the EchoLUTION Cell Culture DNA Kit. Further, we assessed the EchoLUTION Cell Culture DNA Kit efficiency and DNA quality for downstream applications.

## Experimental Protocol

### *Amplification of genomic DNA extracted from cell culture or tissue*

We used the EchoLUTION Cell Culture DNA Kit to extract the genetic material of 1 × $10^6$ cells from HEK293 (adherent), Expi-293 (suspension), and JeKo (suspension), as well as pre-sorted, transiently transfected HEK293 (HEK293+) human cell lines, following the kit protocol. In parallel, we extracted DNA from a total of 1 × $10^6$ HEK293 and transfected HEK293 cells (HEK293+) using a comparable kit from another vendor based on the silica (*bind-wash-elute*) method. We assessed the correlation between DNA concentration and number of cells via a titration assay with cultured HEK293 cells ranging from 15 × $10^3$ and 2 × $10^6$. Furthermore, we used the EchoLUTION Tissue DNA Kit to extract gDNA from two samples of human adenoid tissue (10 mg each), one fresh and the other one stabilized in RNAlater® (Merck®, Germany).

We characterized all DNA samples regarding yield and purity with a NanoDrop™ spectrophotometer (Thermo Fisher Scientific®, Germany). Corresponding volumes of the elution fractions from HEK293 and HEK293+ cells, obtained with the EchoLUTION and the silica-based kits respectively, were visualized on a 0.8 % agarose gel via GelRed® (Merck®, Germany) staining. 25 ng of template DNA was used as initial material for standard PCR (30 cycles). The PCR products (or amplicons) were visualized on a 1 % agarose gel.

### *Extraction of plasmid DNA from mammalian cells*

Since total DNA is extracted from the cells using the EchoLUTION Cell Culture DNA Kit, plasmid DNA should also be found in the extracted DNA fraction. To verify this, we transiently transfected HEK293 cells with a vector carrying an ampicillin resistance cassette (AmpR). After DNA extraction from 3.4 × $10^4$ cells, we transformed chemically competent *E. coli* XL1-Blue with 10, 20, 40, and 80 ng of DNA, respectively. Transformation was performed by heat shock procedure at 42°C for 45 s, followed procedure incubation in SOC medium for min at 37° C. Afterwards, bacteria were centrifuged at 650 rpm and plated onto 2 × YT agar plates with ampicillin. Colonies were counted after overnight incubation at 37 °C.

***Table 1:*** *Spectrophotometric analyses of DNA elution fractions isolated with EchoLUTION kits and a Silica-based extraction kit.*

| Extraction kit | Sample | | DNA concentration [ng/µL] | Total DNA yield [µg] | A260/A280 (ideal: 1.8 – 1.9) |
|---|---|---|---|---|---|
| | ID | Amount | | | |
| **Silica-based kit (another vendor)** | HEK293 | 1 × $10^6$ | 86 | 17.1 | 1.99 |
| | HEK293 + (transfected) | 1 × $10^6$ | 126 | 25.2 | 1.93 |
| **EchoLUTION Cell Culture DNA Kit (BioEcho)** | HEK293 | 1 × $10^6$ | 368 | 36.8 | 1.84 |
| | HEK293 + (transfected) | 1 × $10^6$ | 437 | 43.7 | 1.83 |
| | HEK293 + (transfected) | 3.4 × $10^4$ | 26 | 2.6 | 1.53 |
| | Expi – 293 | 1 × $10^6$ | 370 | 37.0 | 1.84 |
| | Jeko | 1 × $10^6$ | 139 | 13.9 | 1.81 |
| | gDNA extraction control | - | (14) | (1.4) | 1.32 |
| **EchoLUTION Tissue DNA Micro Kit (BioEcho)** | Human adenoid tissue (fresh) | 10 mg | 114 | 11.4 | 1.91 |
| | Human adenoid tissue (stabilized) | 10 mg | 73 | 7.3 | 1.78 |

## Results

We extracted DNA from various mammalian cell lines (HEK293, Expi-293, and Jeko) and from both fresh and stabilized human adenoid tissue to evaluate the efficacy of the EchoLUTION Cell Culture DNA Kit and EchoLUTION Tissue DNA Micro Kit, respectively. DNA concentrations, total yield, and purity (A260/A280) of each eluate are shown in ***Table 1***. Over several experiments, we extracted up to 40 µg of DNA from 1 × $10^6$ cultured human cells and up to 11 µg of gDNA from human adenoid tissues.

We visualized DNA extracted from non-transfected and transiently transfected HEK293 cells with two preparation technologies (EchoLUTION Cell Culture DNA Kit vs. silica-based kit) in an agarose gel. Intact, high-molecular weight DNA was obtained in both cases, though the yield was higher with the EchoLUTION Kit (***Figure 1*** and ***Table 1***). Next, we wanted to know whether the extracted DNA from various samples were suitable for PCR. The AAVS-1 locus could be amplified effectively by 2-step nested PCR (30 cycles each), as expected. The amplified DNA bands from HEK293, Expi-293, and JeKo cells, as well as from human adenoid tissues are shown in ***Figure 2***.

To study the influence of the initial number of cells on DNA quality and quantity, we prepared a series of cell numbers ranging from 15 × $10^3$ to 2 × $10^6$ HEK293 cells. ***Table 2*** displays DNA concentrations, yield, and purity of the eluates from each fraction.

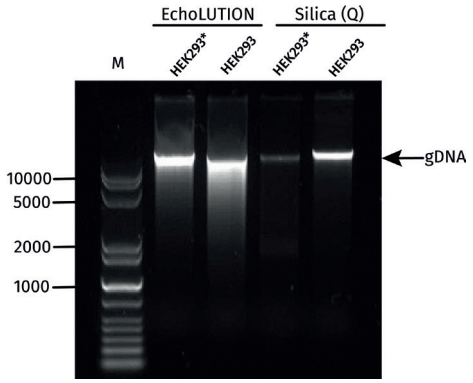


***Figure 1:*** *Visualization of whole DNA extracted from human cells. Corresponding volumes were loaded per lane. M: DNA ladder GelPilot® 1kb Plus (QIAGEN®, Germany).*


***Figure 2:*** *Agarose gel electrophoresis of DNA after nested PCR on gDNA from different human cell samples. M: DNA ladder GeneRuler®1kb Plus.*

The data demonstrate that DNA yield increases linearly over the titration range of sample input (***Figure 3***). We found that purity ratios were below the optimal value when less than 12.5 × $10^4$ cells were processed. A similar observation was made when DNA was extracted with the silica method (data not shown). The eluates obtained in the assay shown in ***Table 2*** were used in a standard PCR to find out whether genomic loci could still be amplified from DNA with a reduced $A_{260}/A_{280}$ ratio.

***Table 2:*** *Spectrophotometric analyses of DNA elution fractions from a titration assay.*

| HEK293 cell number | DNA concentration [ng/µL] | Total DNA yield [µg] | A260/A280 (ideal: 1.8—1.9) |
|---|---|---|---|
| **15,000** | 10 | 1.0 | 1.13 |
| **30,000** | 14 | 1.4 | 1.22 |
| **60,000** | 21 | 2.1 | 1.41 |
| **125,000** | 40 | 4.0 | 1.57 |
| **500,000** | 44 | 4.4 | 1.55 |
| **1,000,000** | 140 | 14.0 | 1.80 |
| **2,000,000** | 238 | 23.8 | 1.84 |

***Figure 4*** demonstrates that the reduced absorption ratio had no effect on PCR amplification of the AAVS-1 genomic locus.

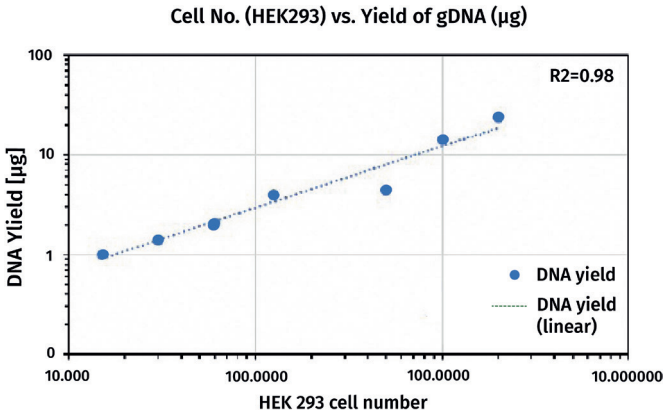


***Figure 3:*** *Standard correlation between DNA yield and initial number of HEK293 cells.*

Following the genetic material extraction from 3.4 × $10^4$ transiently transfected HEK293 cells with a vector carrying an AmpR cassette, the resulting DNA was directly transformed into chemically competent *E. coli* XL1-Blue bacteria. After overnight culture in a media containing ampicillin for positive colonies selection, a total of 20 could be recovered and plasmid containing the AmpR gene was proven to be correct by DNA sequencing.

The validity of A260/A280 ratios at lower DNA concentrations is questionable because of the limitations of optical density (OD) measurement. Under small DNA concentrations the ratio determination could be not reliable (1), although we found that even small amounts of isolated DNA can be used for PCR applications. Our conclusion is that OD measurements should generally be complemented with functional studies to accurately assess DNA quality (1). This is underlined by the results shown in ***Figure 4***, in which PCR performance was consistent over the entire titration range. This data demonstrate the absence of potentially inhibitory reagents in the extracted DNA sample obtained with EchoLUTION technology, which certainly contributes to the robustness of the results.

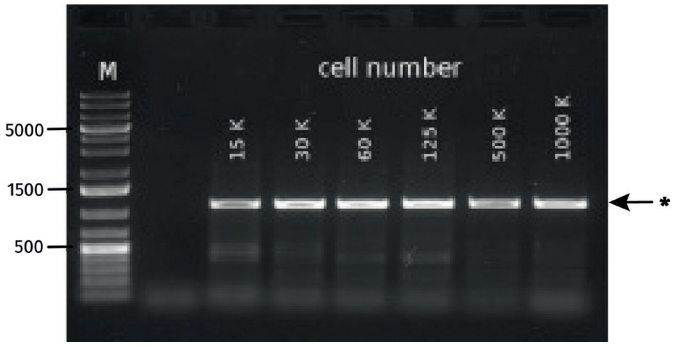


***Figure 4:*** *Agarose gel electrophoresis of DNA after PCR on gDNA extracted from different numbers of HEK293 cells (lanes 2-7). Lane 1, no-template amplification control; M: DNA ladder GeneRuler® 1kb Plus * 1099 bp PCR fragment from AAVS genomic locus.*

## Discussion

The BioEcho EchoLUTION product line with its novel isolation technology offers the fastest and easiest DNA extraction from a wide range of mammalian cells and tissues. We achieved a considerable yield of DNA from HEK293, Expi-93, and JeKo cells, as well as human adenoid tissue. Significantly, the DNA concentration obtained with EchoLUTION Cell Culture DNA Kit was about 76 % higher in HEK293 cells and 71 % in HEK293 transfected cells compared to a silica method. Furthermore, the DNA concentration and yield were consistently positively correlated with the number of cells subject to extraction.

Though the transformation efficiency might be further enhanced by treatment with exonucleases or other methods that increase the ratio of plasmid DNA to genomic DNA, or by changing transformation parameters (e. g., using electroporation), the EchoLUTION Cell Culture Kit offers a real advantage by not requiring any additional preparation steps for the extraction of plasmid DNA from mammalian cells. Even small amounts of DNA can be used for direct transformation of extracted DNA into bacterial cells as well as for amplification reactions.

# Ordering Information

| EchoLUTION Cell Culture DNA Kits | Reactions | Product No. |
|---|---|---|
| EchoLUTION Cell Culture DNA Kit (10)<br>EchoLUTION Cell Culture DNA Kit (50)<br>EchoLUTION Cell Culture DNA Kit (250) | 10 rxn<br>50 rxn<br>250 rxn | 010-006-010<br>010-006-050<br>010-006-250 |
| EchoLUTION Cell Culture DNA 96 Kit (2 × 96)<br>EchoLUTION Cell Culture DNA 96 Kit (8 × 96)<br>EchoLUTION Cell Culture DNA 96 Core Kit (8 × 96) | 2 × 96 rxn<br>8 × 96 rxn<br>8 × 96 rxn | 010-106-002<br>010-106-008<br>010-106-108 |

| EchoLUTION Tissue DNA Kits | Reactions | Product No. |
|---|---|---|
| EchoLUTION Tissue DNA Kit (10)<br>EchoLUTION Tissue DNA Kit (50)<br>EchoLUTION Tissue DNA Kit (250) | 10 rxn<br>50 rxn<br>250 rxn | 010-002-010<br>010-002-050<br>010-002-250 |
| EchoLUTION Tissue DNA 96 Kit (2 × 96)<br>EchoLUTION Tissue DNA 96 Kit (8 × 96)<br>EchoLUTION Tissue DNA 96 Core Kit (8 × 96) | 2 × 96 rxn<br>8 × 96 rxn<br>8 × 96 rxn | 010-102-002<br>010-102-008<br>010-102-108 |


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**Reference**


*(1)* **B. Matlock.** *B. Matlock. Assessment of nucleic acid purity. Technical Note 52646, ThermoFisher Scientific. https://tools.thermofisher.com/content/sfs/brochures/ TN52646-E-0215M-NucleicAcid.pdf*