Application Note

Extraction of genomic and plasmid DNA from mammalian cells

Maximilian Ruschig and Wieland Fahr, Institute for Biotechnology, Technical University Braunschweig, Germany
(m.ruschig@tu-bs.de, w.fahr@tu-bs.de)

In this application of BioEcho's EchoLUTION CellCulture DNA Kit, genomic DNA as well as plasmid DNA has been extracted from cultured cells as part of a complex antibody display project. Up to 40 µg of gDNA was extracted from 1x10^6 cultured human cells. Genomic DNA from increasing numbers of cells was successfully amplified to demonstrate the functionality of nucleic acid fractions obtained from low to high sample input amounts. DNA yields increased linearly between 15.000 and 2x10^6 cells. DNA isolated from transfected cells was successfully transformed into *E. coli* showing that also plasmid DNA can be isolated using the kit. The EchoLUTION Tissue DNA Micro Kit was used, too, and up to 11 µg of genomic DNA were purified from fresh and stabilized human adenoid tissues.

Introduction

While DNA – and especially plasmid DNA – extraction from bacterial cells is well investigated and many kit solutions are available, DNA extraction from mammalian cells remains a challenging and comparably expensive procedure. For optimal quality of the product DNA, the cells must meet certain requirements in terms of number, preparation and buffer ingredients.

The extraction of genomic and plasmid DNA can be necessary for various different experiments and assays. Applications like antibody epitope mapping, cell surface display or characterization of stably transfected cell lines often require the extraction of DNA from mammalian cells.

In this application note, we describe the extraction of whole genomic DNA from various cell lines cultivated in cell culture and from human tissue samples. We also extracted plasmid DNA that had been transiently transfected into the cells. We assess DNA quality, reproducibility and workload.

Experimental Protocol

Amplification of genomic DNA after extraction from cultured or tissue cells

Cells of various mammalian cell lines were subjected to DNA extraction using the BioEcho EchoLUTION CellCulture DNA Kit. DNA was extracted from 1x10^6 cells of human cell lines HEK293 (adherent), Expi-293 (suspension) and JeKo (suspension), as well as pre-sorted, transiently transfected HEK293 (HEK293+) according to the kit protocol. Here, a titration between 15.000 and 2.000.000 cultured cells was run. In parallel, DNA from 1x10^6 HEK293 and transfected HEK293 cells (HEK293+) was extracted using a Silica kit.

Furthermore, genomic DNA was extracted from 10 µg each of fresh or RNAlater-stabilized human adenoid tissue using the EchoLUTION Tissue gDNA Micro Kit.

Extracted DNA was characterized with respect to purity and yield using a NanoDrop spectrophotometer. Corresponding volumes of the elution fractions from HEK293 and HEK293+ extraction obtained with the EchoLUTION and the Silica kits were visualized on a 0.8% agarose gel via GelRed staining.

Subsequently, a 2-step nested PCR was conducted, amplifying the genomic AAVS-1 locus. Resulting DNA was visualized in a 1% agarose gel.

Extraction of plasmid DNA from mammalian cultured cells

Since total DNA is extracted from the cells using the kit, plasmid DNA should also be found in the extracted DNA fraction. Therefore, we transiently transfected HEK293 cells with a vector carrying an Ampicillin resistance cassette (AmpR). After DNA extraction from 1.000.000 cells, we transformed 10, 20, 40, and 80 ng of extracted DNA, respectively, into *E. coli* XL1-Blue chemically competent bacteria using heat shock at 42°C for 45 sec, incubated the bacteria for 30 min at 37°C, 650 rpm in SOC media and plated them onto 2xYT-GA Agar plates. After overnight incubation at 37°C, colonies were counted.

Results

DNA concentrations, total yield and purity (A_{260} / A_{280}) are shown in Table 1.

DNA extracted from untransfected and transiently transfected HEK293 cells with two preparation technologies (EchoLUTION vs. Silica) was visualized in an agarose gel (Figure 1). Intact high-molecular weight

Table 1. Spectrophotometric analyses of obtained DNA elution fractions. * Silica kit extraction.

Sample		DNA concentration [ng/µL]	Total DNA yield [µg]	A_{260} / A_{280} (ideal: 1.8-1.9)
ID	Amount			
Expi-293	1x10^6	370	37.0	1.84
JeKo	1x10^6	139	13.9	1.81
Human Adenoid tissue (fresh)	~10 mg	114	11.4	1.91
Human Adenoid tissue (stabilized)	~10 mg	73	7.3	1.78
gDNA extraction control	-	(14)	(1.4)	1.32
HEK293+ (transfected cells)	34.000	26	2.6	1.53
HEK293	1x10^6	368	36.8	1.84
HEK293+ (transfected cells)	1x10^6	437	43.7	1.83
HEK293 *	1x10^6	86	17.1	1.99
HEK293+ (transfected cells) *	1x10^6	126	25.2	1.93

DNA was obtained in both cases whereas the yield was higher with the EchoLUTION kit.

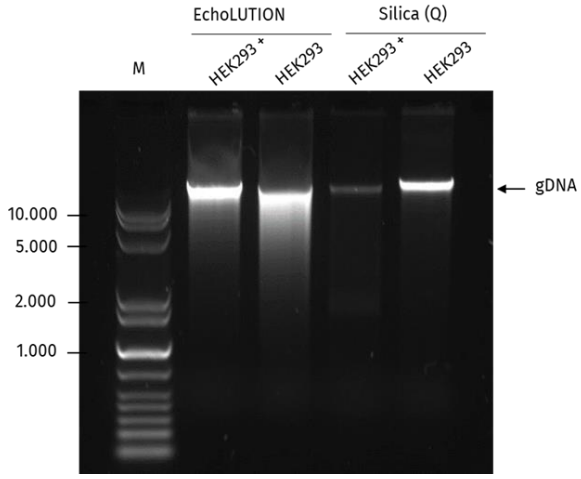

Figure 1. Visualization of whole DNA extracted from human cells. Corresponding volumes were loaded per lane. M: DNA ladder *GelPilot 1kb Plus*.

Next, we wanted to know whether the extracted DNA could be used for PCR. The AAVS-1 locus could be amplified by 2-step nested PCR (30 cycles each) as expected. Figure 2 shows the resulting DNA.

In order to study DNA quality when working with a low amount of cells, we prepared a cell number series ranging from 15.000 to 2.000.000 HEK293 cells. Table 2 shows DNA concentration, yield and purity.

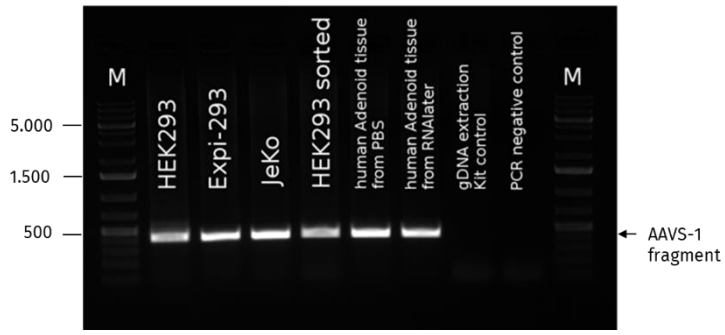

Figure 2. Agarose Gel Electrophoresis of DNA after nested PCR on gDNA extract from different human cell samples. M: DNA ladder *GeneRuler 1kb Plus*.

DNA yield increased linearly over the titration range of sample input (Fig. 3). Absorption ratios were below the optimal value when less than 125.000 cells were processed. A similar observation was made when DNA was extracted with Silica methods (data not shown).

For the experiment shown in Table 2, a standard 30-cycle PCR was conducted to see whether genomic loci could still be amplified from DNA with a reduced A_{260} / A_{280} ratio. 25 ng of template DNA were used in each

Table 2. Spectrophotometric analyses of obtained DNA elution fractions from titration of sample input amount.

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

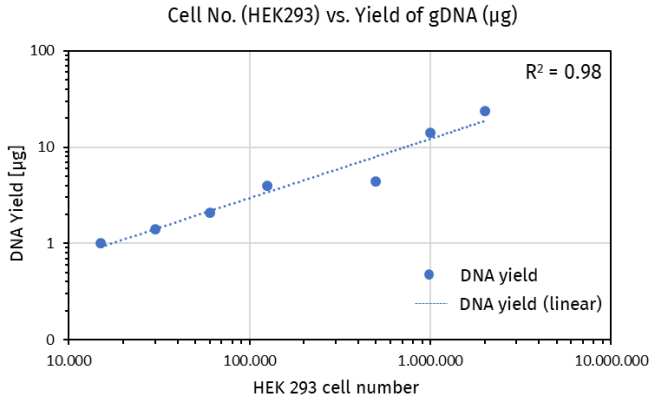

Figure 3. DNA extracted from increasing numbers of cells.

sample. Results are shown in Figure 4. The reduced absorption ratio had no effect on PCR amplification.

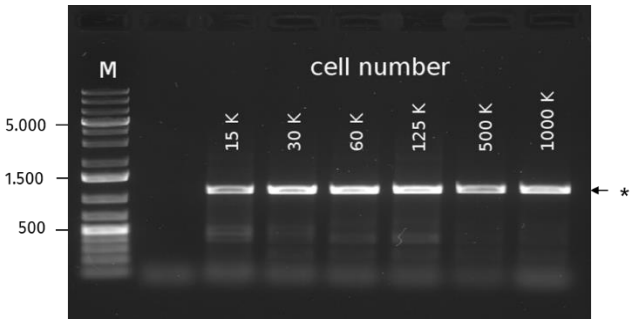

Figure 4. Agarose Gel Electrophoresis of DNA after PCR on gDNA extracted from different numbers of HEK293 cells. Lane 1, no-template amplification control; M: DNA ladder *GeneRuler 1kb Plus*; * 1099 bp PCR fragment from AAVS genomic locus.

After DNA extraction from 34.000 transiently transfected HEK293 cells, resulting DNA was directly transformed into chemically competent *E. coli* XL1-Blue bacteria. After overnight culturing, a total of 20 clones could be recovered. Plasmid content was correctly proven by DNA sequencing.

Discussion

Of the kits tested, the BioEcho EchoLUTION series and its novel isolation technology offers the fastest and easiest DNA extraction from a wide range of mammalian cell preparations and tissues. The amount of extracted DNA correlates with cell number when the same cell line is used in all samples. We found that even low amounts of DNA can be used for PCR-type applications. The validity of A_{260} / A_{280} ratios at lower DNA concentrations is somewhat questionable because of limitations and variances of OD values of diluted DNA solutions (1). This is confirmed by the higher concentrated DNA preparation of the same experimental series where DNA concentrations are at a range that can be safely measured even though the sample mass load was significantly higher than with the initial titration points. In conclusion, OD measurements should generally be complemented with functional studies in order to accurately assess DNA quality (1). This is underlined by the results shown in Figure 4 where PCR performance was robust over the entire titration range of amount of sample input. The absence of potentially inhibitory reagents in the kit used probably contributes to this robustness.

Additionally, the extraction of plasmid DNA from mammalian cells is possible with the EchoLUTION CellCulture DNA Kit without any additional preparation steps. Even low amounts of DNA can be used for direct transformation of extracted DNA into bacterial cells as well as for amplification reactions. This 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).

Reference

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