General Protocol

for nucleofection® of adherent cell lines

Chapter	Contents
1	Procedure outline & important advice
2	Optimization guidelines
3	Experimental set-up
4	Protocol
4.1	› Cell culture
4.2	› DNA preparation and quality
4.3	› Important controls and vector information
4.4	› Nucleofection® protocol
5	Recommended literature

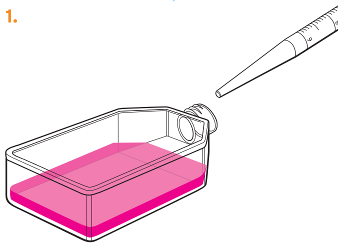
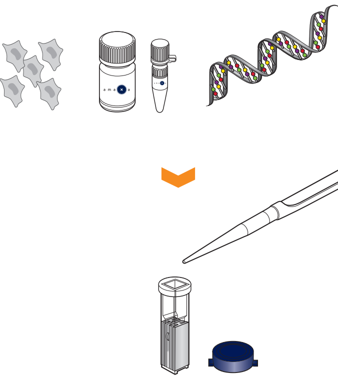
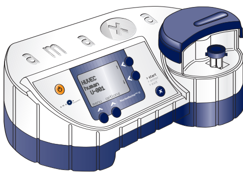
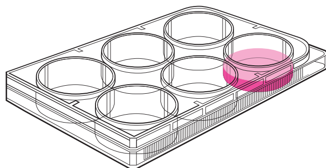
Please check the cell line database on our website:
www.amaxa.com/products/celldatabase
to see if an Optimized Protocol or any customer data exists for your specific cell type. If no such data exists we recommend using our Cell Line Optimization Nucleofector® Kit.

amaxa AG
Europe/World
Scientific Support
+49 (0)221-99199-400
scientific-support@amaxa.com

› www.amaxa.com

amaxa Inc.
USA
Scientific Support
(888) 632-9110 (toll free)
scientific-support.US@amaxa.com

1 Procedure outline and important advice

	Procedure outline	Important advice
1. 	Culturing of cells before nucleofection®. (For details see 4.1). Prewarm culture medium to 37°C.	› For culturing, follow instructions of the cell line supplier. › Passage 2 - 3 days before nucleofection®. › Cells should be nucleofected at 70 - 85% confluency.
2. 	Combine the cells of inte-rest, DNA or siRNA and the appropriate cell-type specific Nucleofector® solution and transfer to an amaxa certified cuvette. (For details see 4.4.)	**Contents of one nucleofection® sample:** › 1 x 10^6 - 5 x 10^6 cells › 1 - 5 µg highly purified plasmid DNA (in max. 5 µl) or 2 µg maxGFP® or 2 nM - 2 µM siRNA (final concentration 3 ng - 3 µM / sample) › 100 µl Nucleofector® Solution Process each sample separately to avoid storing the cells longer than 15 min in Nucleofector® Solution.
3. 	Choose the cell-type specific program. Insert the cuvette into the Nucleofector® and press the start button »X«. (For details see 4.4.)	› Select the appropriate Nucleofector® program in Optimized Protocol or as per optimization guidelines indicated on page 3. Software requirements: › *version **V 2.4** or higher for Nucleofector® I Device › **version **S 3.5** or higher for Nucleofector® II Device
4. 	Rinse the cuvette with culture medium using an amaxa certified pipette. Transfer the cells into the culture dish. (For details see 4.4.)	› Using an amaxa certified pipette, imme-diately remove sample from the cuvette with 500 µl prewarmed medium. › Transfer directly to 37°C.

amaxa AG
Europe/World
Scientific Support
+49 (0)221-99199-400
scientific-support@amaxa.com

› www.amaxa.com

amaxa Inc.
USA
Scientific Support
(888) 632-9110 (toll free)
scientific-support.US@amaxa.com

2 Optimization guidelines

Note We recommend contacting Scientific Support to discuss the best way of establishing the Nucleofector® technology with your cell line.

Step 1 The first set of experiments is comprised of 9 reactions:
The Cell Line Nucleofector® solution is tested in combination with 7 different Nucleofector® programs plus 2 controls.

Step 2 To maximize nucleofection® efficiency, we recommend establishing a second set of experiments based on the best results obtained. For this purpose submit your complete results to our Scientific Support Team and within one work day we will suggest additional programs to be tested in combination with the best Nucleofector® solution.

3 Experimental set-up

Sample	maxGFP®	program
		Nucleofector® I / Nucleofector® II
sample 1	+	A-20 / A-020
sample 2	+	T-20 / T-020
sample 3	+	T-30 / T-030
sample 4	+	X-01 / X-001
sample 5	+	X-05 / X-005
sample 6	+	L-29 / L-029
sample 7	+	D-23 / D-023
sample 8	+	- / -
sample 9	-	T-20 / T-020

4 Protocol

4.1 › Cell culture

For commercially available cell lines we recommend following the instructions of the supplier regarding culture medium and supplements as well as passaging and seeding conditions. Best nucleofection® results will be obtained with standardized cell culture conditions.

For cells grown in high-calcium medium, such as Dulbecco's Modified Eagle Medium (DMEM), you may use a low-calcium medium like RPMI for the transfer from the cuvette into the plate (see 4.4 steps 3 and 13).

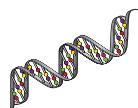
Culture conditions before nucleofection®

- The cells should be passaged 2 - 3 days before nucleofection®.
- Cells should be nucleofected after reaching 70 - 85% confluency.
  Higher cell densities may cause lower nucleofection® efficiencies.

Note

Contamination of cell culture with mycoplasma is a wide spread phenomenon that might negatively influence experimental results. We recommend the use of Normocin™ [Cat. No. VZA-1001], a new antibiotic formulation specifically developed to protect cell lines from mycoplasma infection and microbial contaminations. For more information and ordering info see **www.amaxa.com/antibiotics**

4.2 › DNA preparation and quality



The quality and the concentration of DNA used for nucleofection® plays a central role for the efficiency of gene transfer. We strongly recommend the use of high quality products for plasmid purification like EndoFree® Plasmid Kits [Qiagen, Cat. No. 12391 Giga Kit, 12362 Maxi Kit, 12381 Mega Kit]. The purified DNA should be resuspended in deionized water or TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) with a concentration between 0.2 - 1 µg/µl. Please check the purity of each plasmid preparation by measurement of the A260:A280 ratio, according to Qiagen protocol.

amaxa AG
Europe/World
Scientific Support
+49 (0)221-99199-400
scientific-support@amaxa.com

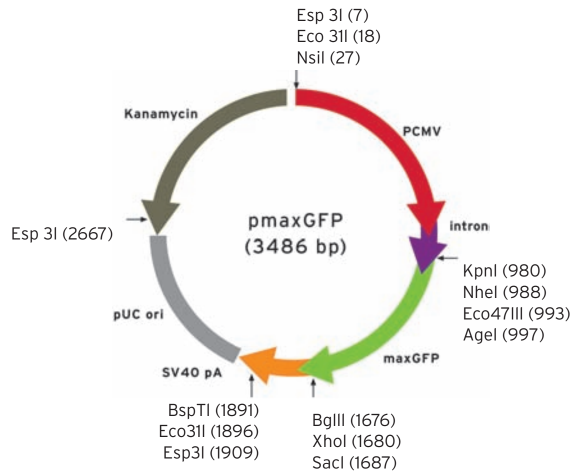
› www.amaxa.com

amaxa Inc.
USA
Scientific Support
(888) 632-9110 (toll free)
scientific-support.US@amaxa.com

4.3 › Important controls and vector information

Positive control

We strongly recommend establishing the Nucleofector® technology with the positive control vector **pmaxGFP®** as provided in this kit. pmaxGFP® encodes the green fluorescent protein (GFP) from copepod *Pontellina p.* Just like eGFP expressing cells, maxGFP® expressing cells can easily be analyzed by fluorescence microscopy or flow cytometry to monitor transfection efficiency.



Negative control

We recommend you always perform two control samples to assess the initial quality of cell culture and the potential influences of nucleofection® or amount/purity of DNA on cell viabilty.

control 1 Recommended amount of cells in Nucleofector® solution with DNA but without application of the program (alternatively: untreated cells) **(Cells + Solution + DNA - program)**

control 2 Recommended amount of cells in Nucleofector® solution without DNA with application of the program **(Cells + Solution - DNA + program)**

Vector information

If using IRES sequences in your vectors, please remember that the gene encoded 3' of the IRES sequence is usually expressed to a lesser extent than the upstream gene, and in some cell types may not be expressed at all. As alternatives we suggest either: co-transfecting two (or more) plasmids, using one plasmid with each gene under the control of its own promoter, or making a GFP fusion

4.4 › Nucleofection® protocol

Preparation of Nucleofector® Solution	Add 0.5 ml Supplement to 2.25 ml Nucleofector® solution and mix gently. The Nucleofector® solution is now ready to use and is stable for 3 months at 4°C. **Note the date of addition on the vial**.
One nucleofection® sample contains	› **1 x 10^6 - 5 x 10^6 cells** › **1 - 5 µg plasmid DNA (in 1 - 5 µl H_2O or TE) or 2 µg pmaxGFP® or 2 nM - 2 µM siRNA** › **100 µl Nucleofector® solution**
	For more details about the nucleofection® of siRNA: **www.amaxa.com/RNAi**

Preparation of samples

1. Cultivate the required number of cells.
2. Prepare **1 - 5 µg DNA** for each sample.
3. Pre-warm the supplemented Nucleofector® solution recommended by amaxa to room temperature. Pre-warm an aliquot of culture medium containing serum/supplements at 37°C in a 50 ml tube (500 µl per sample).
4. Prepare 6-well plates by filling the appropriate number of wells with 1.5 ml of culture medium containing serum and supplements and pre-incubate plates in a humidified 37°C/5% CO_2 incubator.
5. Remove the medium from the cultured cells. Wash cells once with PBS. Aspirate and discard PBS.
6. Harvest the cells, e.g. with trypsin/EDTA and stop the trypsinization with culture medium containing serum and supplements (see Nucleofector® Manual for details).
7. Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.
8. Centrifuge the required number of cells (**1 x 10^6 - 5 x 10^6** cells per nucleofection® sample) at **90 x g** at **room temperature** for **10 min**. Discard supernatant completely so that no residual medium covers the cell pellet.
9. Resuspend the pellet in room temperature Nucleofector® solution recommended by amaxa to a final concentration of **1 x 10^6 - 5 x 10^6** cells/100 µl. Avoid storing the cell suspension longer than **15 min** in Nucleofector® solution, as this reduces cell viability and gene transfer efficiency.

Important: Steps 10-14 should be performed for each sample separately.

Nucleofection®

10. Mix 100 µl of cell suspension with **1-5 µg DNA**.

11. Transfer the sample into an amaxa certified cuvette. Make sure that the sample covers the bottom of the cuvette, avoid air bubbles while pipetting. Close the cuvette with the blue cap.
12. Select the appropriate Nucleofector® program (see Nucleofector® Manual for details). Insert the cuvette into the cuvette holder (Nucleofector® I: rotate the turning wheel clockwise to the final position). Press the »X« button to start the program.
13. **To avoid damage to the cells remove the sample from the cuvette immediately after the program has finished (display showing »OK«).** Take the cuvette out of the holder. Add 500 µl of the pre-warmed culture medium containing serum and supplements (please see 8.1) and transfer the sample into the prepared 6-well plates. Alternatively, transfer the sample into a 1.5 ml microcentrifuge tube and place it in a 37°C heat block. To transfer the cells from the cuvettes, we strongly recommend using the plastic pipettes provided in the kit to prevent damage and loss of cells.
14. Press the »X« button to reset the Nucleofector®.
15. If you have incubated the samples in 1.5 ml microcentrifuge tubes, transfer all samples into the prepared 6-well plates.

Cultivation post nucleofection®

16. Incubate cells in a humidified 37°C/5% CO_2 incubator. Following transfection, gene expression should be analyzed at different times. Depending on the gene, expression is often detectable after 3 - 8 hours. If this is not the case, the incubation period may be prolonged to 24 hours.

5 Recommended literature

For an up-to-date list of all Nucleofector® references, please refer to:
www.amaxa.com/citations

* amaxa's Nucleofector® process, Nucleofector® device and Nucleofector® solutions are covered by PCT applications PCT/EP01/07348, PCT/DE02/01489, PCT/DE02/01483 and other pending patents and domestic or foreign applications corresponding thereto.
* amaxa, Nucleofector, nucleofection and maxGFP are trademarks of amaxa AG.
* This kit contains a proprietary nucleic acid coding for a proprietary copepod fluorescent protein intended to be used as a positive control with this amaxa product only. Any use of the proprietary nucleic acid or protein other than as a positive control with this amaxa product is strictly prohibited. USE IN ANY OTHER APPLICATION REQUIRES A LICENSE FROM EVROGEN. To obtain such a license, please contact Evrogen at license@evrogen.com.
* The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.
* QIAGEN and EndoFree are trademarks of QIAGEN.
* All other product and company names mentioned herein are the trademarks of their respective owners.