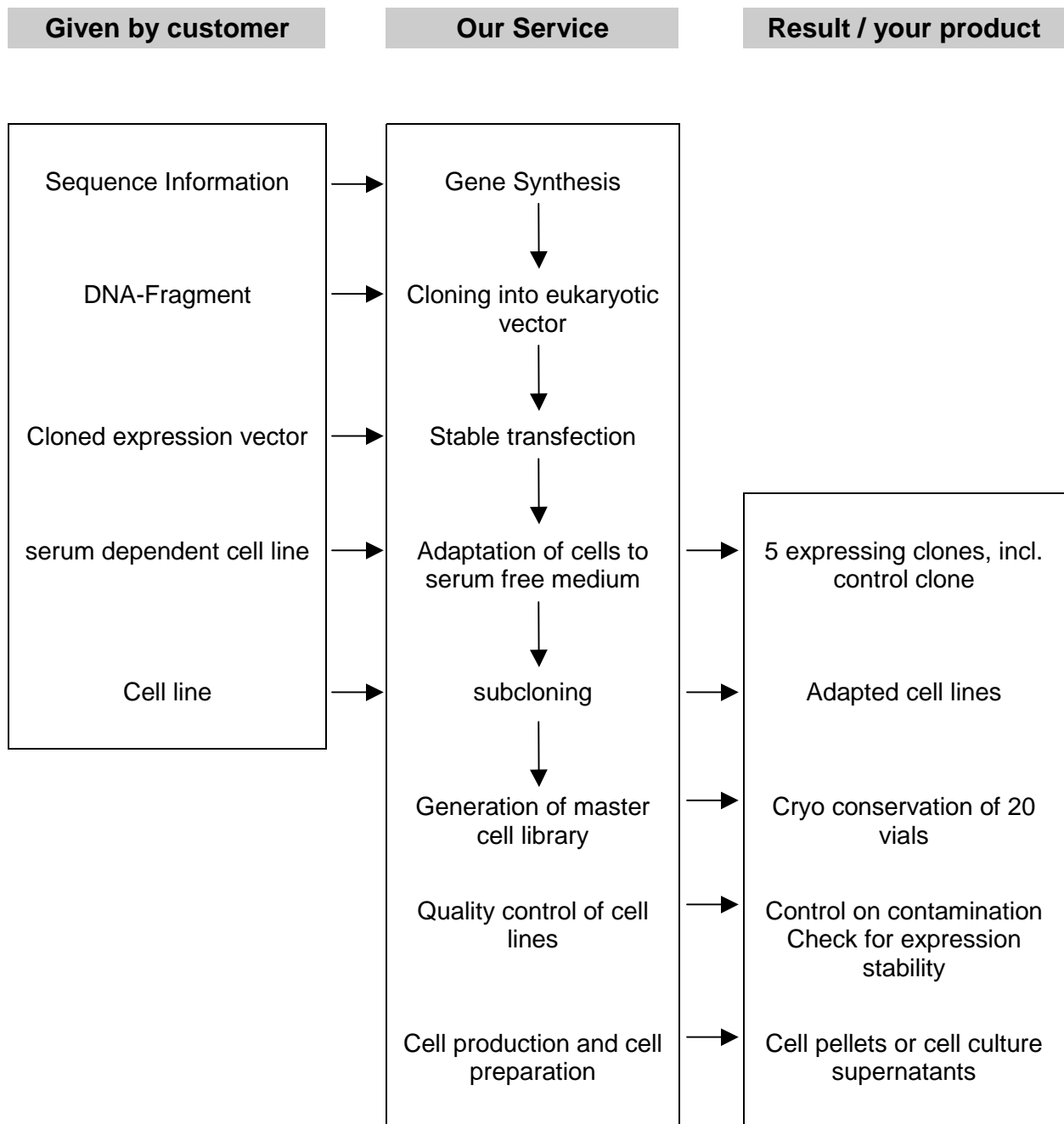


Cell Line Service



Cell Line Service

Gene Synthesis

Cloning of a gene from genomic DNA or cDNA is time consuming, tedious and a source for mistakes. By a close cooperation Genaxxon can offer the synthesis of genes with 100% sequence accuracy. Coding sequences from 100bp up to complete genes with more than 10 kb can be synthesised according to your needs. This way a targeted amino acid exchange in the recombinant protein can be performed very easily.

(Time schedule: 1 – 3 weeks)

Cloning of eukaryotic vectors

We are cloning the DNA-fragment of customers choice into a eukaryotic vector. For this service customers can choose between several expressions- and reporter systems. The DNA-fragment provided by customer (vector, gDNA or cDNA) will be amplified by PCR technique. On request we will add Tags or sequences to the gene sequence usable for the purification or the detection of the protein, as protease cutting or signal recognition sites. The constructed vector will be purified by ion exchange chromatography and the quality of the preparation checked. The identity of the insert will be verified by sequencing **and** an appropriate restriction digest. For reference reasons we will provide our customers with 10 ug of the purified vector. The rest of the DNA will be used for transfection of cells.

(Time schedule: 1 – 2 weeks)

Stable transfection

Often used Cell lines are on stock at Genaxxon and can be provided for immediate transfection. A list of all cells on stock is available at our service department. Beyond that we will use every suitable cell line, provided by customer, for the transfection. If requested by customer, we will also contact ATCC to ask for a specific cell line.

The original cells will be cultured and transfected with the cloned vector. For control reasons we will additionally transfect cells with an "empty" vector for control reasons.

The antibiotic resistance transferred by the vector is used to select for stable cell lines.

We will isolate 40 resistant cell clones, take aliquots as retained samples that can be used later if necessary. The expression of the transgenic protein will be checked in all 40 clones. The appropriate antibody for Immuno-Western-Blot or a suitable assay for the activation of a reporter gene should be provided by customer.

Five positive clones will be chosen according the expression strength or taken as being representative for transfection. The transgenic cell lines will be delivered as growing cultures or as cryo cultures on dry ice.

(Time schedule: 8 - 9 weeks)

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Adapting cells on serum free medium

Most cell lines can be adapted for growth on serum free medium. This is not only saving money, but is also increasing reproducibility of experimental results by minimizing effects of unknown impurities of serum containing medium.

Before adapting the cells, the most relevant features of the cell line as proliferation rate, or the expression of characteristic markers, will be documented. Subsequently cells will be adapted to a serum free, chemically defined medium. After each adaptation step, we will take an aliquot used as retained samples to be stored in liquid nitrogen.

After the successful adaptation of the cells has been documented, the growing behaviour of the cells and the expression of the specific marker protein will be checked again and compared with their original behaviour.

Customer will get adapted cells as growing culture or as cryo culture stored and shipped on dry-ice.

(Time schedule: 3 – 6 weeks)

Subcloning

Productivity of a cell line or the sensitivity of an assay, lost by frequent passaging, can be restored by subcloning.

The first step is the documentation of the status of the heterogeneous cell line. Subcloning is done by separating cells into 96-well plates by cell sorting (one cell per well). 48 subclones will be isolated, expanded and stored in liquid nitrogen. By performing Immuno-Western-Blots or another appropriate assay subclones will be characterised and compared with the original cell line.

We will choose 5 optimized subclones, shipped to customer as growing cultures or as cryo cultures on dry-ice, together with a comprehensive documentation.

(Time schedule: 3 – 4 weeks)

Construction of a master cell library

We will use cell lines for the construction of a master cell library without respect to the origin of the source of the cells (established by us, or supplied by customer).

We will culture cells, characterise their relevant features (e.g. expression of a transgenic protein) and check the culture for contamination(s). Afterwards we will expand cells, establishing a cell library of 20 vials with 1×10^6 cells each.

Freezing will be done in an optimised freezing medium according to a specific protocol. After freezing cells will be stored in liquid nitrogen. One cell sample will be thawed to be checked for vitality and their specific features.

As an additional service we offer to store your cell library in our storage facility (liquid nitrogen) that is frequently controlled. Vials will be shipped on dry-ice on request

(Time schedule: 1 – 2 weeks)

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Quality check of cell lines

We are performing phenotypic characterisation of cell lines. Based on Immuno-Western-Blots, immuno fluorescence microscopy and flow cytometry we check the expression of characteristic marker and transgenic proteins. Customer should provide appropriate antibodies.

Stability tests are performed over a period of 20 cell cycles. After each passage a sample will be taken and tested for the expression of relevant proteins. If marker proteins are not secreted homogeneity of a cell line is checked by immuno fluorescence microscopy and flow cytometry. Results are documented. Additionally we will check your cell lines for contamination by, e.g. mycoplasma or viruses. At Genaxxon we do have different assays available that are established and validated. Depending on the test procedure we need samples from cells or the cell culture supernatant. Detailed information about the test procedures performed at Genaxxon are available from our service department.

Cell production and cell processing

We produce and process cells according your individual needs. Most common cell lines are available at Genaxxon and will be provided by us. Additionally we will cultivate every cell line (up to L2/S2) that we get from customer or which we can get from ATCC. All incoming material will be quality checked before entering our laboratories.

Adherent cells can be cultivated in scale as small as few cell culture dishes up to 80 roller flasks. Cells in suspension can be cultivated either in spinner flasks or bioreactors up to a 20L volume.

We can produce batches with high amounts of cells preparing these for your trials. On request we deliver each amount of cell material you need actually. That way you get the highest flexibility possible, being able to use cell material from the same batch while performing long lasting trials.

Cells can be pre-processed according your needs (e.g. in vivo marking) with a final refinement.

Additionally we can provide customer with vital cells in each amount requested, used e.g. for inoculation of bioreactors or for cell based assays.

For the time being we are working on a technique to prepare and ship cells in 96- and 384-well plate format. Using these ready-to-use plates will enable you to use cells that are, after a short revitalisation phase, ready for immediate use in your experiments.

(Time schedule: 1 – 4 weeks)

Cell Line Service

Price list

Catalog No.	Service description		Price (Euro)
P2041.0001	Cloning of eukaryotic vectors,	Set-up	890,00
	<ul style="list-style-type: none"> • PCR amplification of the requested DNA-fragment. • Cloning into expression- or reporter vector. • Production and purification of vector. • Sequencing and restriction digest for quality check. 		plus costs for sequencing and primer
P2042.0001	Stable transfection		
	<ul style="list-style-type: none"> • Quality control of original cell lines. • Transfection of cells with purified vector DNA. • Control transfection with empty vector. • Selection of stable transfected cells. • Isolation of 40 single cell clones (plus 8 control clones). • Expansion of clones and storage of retained samples. • Expression controls of transgenic cell lines (westernblot or reporter gene assay). • Selection of 5 positive (expressing) clones. • Delivery of clones as growing culture or as Cryo stock. 		
	Projects in established systems	Set-up	8500,00
P2042.0002	Stable transfection	Set-up	13500,00
	Projects with uncertain outcome in non-established systems		
P2042.0003	Stable transfection	Set-up	On request
	Complex projects that need highly sophisticated approaches/strategies.		
P2043.0001	Cell adaptation to serum free medium	Set-up	2700,00
	<ul style="list-style-type: none"> • Quality control and characterisation of original cell line. • Subsequent adaptation to serum free medium. • Control and securing of cells after each adaptation cycle. • Final characterisation of cell line. Delivery of adapted cell line as cryo stock. 		

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P2043.0002	Subcloning	Set-up	3600,00
	<ul style="list-style-type: none"> • Quality check and characterisation of original cell line. • Separation of cells in 96-well plates (single cells). • Isolation and expansion of 48 subclones. • Storage of retained samples). • Characterisation of subclones including comparison with original cell line. • Selection of 5 optimised subclones. • Delivery of subclones as cryo stock on dry-ice. 		
P2043.0003	Master cell library	Set-up	3400,00
	<ul style="list-style-type: none"> • Quality check and characterisation of cell line. • Freezing of 20 vials a 1x10(6) vital cells in liquid nitrogen. • Thawing control (vitality check, contamination test, characterisation). 		
P2043.0004	Cell line storage in safe deposits	Per year	100,00
P2044.0001	Quality check of cell lines	Set-up	180,00
	Immuno phenotyping (per antigen, excl. Antibody)		
P2044.0002	Stability check	Set-up	2900,00
	Stability control of expression for a period of 20 cell cycles (per antigen, excl. Antibody).		
P2044.0003	Mycoplasma test	Set-up	40,00
	Check for mycoplasma DNA in cell culture supernatant.		
P2044.0004	Virus test	Set-up	On request
	Check for virus contamination (antigen detection)		

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P2045.0011	Cell production and cell processing Production of adherent cells in roller flasks (1x10 ⁶) cells per pellet.	5 pellets	1500,00
P2045.0012	Cell production and cell processing Production of adherent cells in roller flasks (1x10 ⁶) cells per pellet.	10 pellets	2400,00
P2045.0013	Cell production and cell processing Production of adherent cells in roller flasks (1x10 ⁶) cells per pellet.	50 pellets	6500,00
P2045.0021	Cell production and cell processing Production of cells in suspension. Production in spinner flasks or bioreactor (1x10 ⁶) cells per pellet.	10 pellets	1200,00
P2045.0022	Cell production and cell processing Production of cells in suspension. Production in spinner flasks or bioreactor (1x10 ⁶) cells per pellet.	50 pellets	3000,00
P2045.0023	Cell production and cell processing Production of cells in suspension. Production in spinner flasks or bioreactor (1x10 ⁶) cells per pellet.	100 pellets	4500,00
P2045.0031	Cell production and cell processing Cell culture supernatant from adherent growing cells or suspension cells. Sterile filtered.	1 L	800,00
P2045.0032	Cell production and cell processing Cell culture supernatant from adherent growing cells or suspension cells. Sterile filtered.	5 L	2500,00
P2045.0033	Cell production and cell processing Cell culture supernatant from adherent growing cells or suspension cells. Sterile filtered.	10 L	4000,00

For more information, please contact Genaxxon at: www.genaxxon.com