„Novel eukaryotic cells and methods for recombinantly expressing a product of interest"

FIELD OF THE DISCLOSURE

[1] The present disclosure concerns the field of recombinant expression technologies. It *inter alia* provides altered eukaryotic cells which are capable of enhanced production of a product of interest as well as their use in recombinant expression methods. Furthermore, tools are provided which allow early in the selection process the identification of eukaryotic cells that express a recombinant product with high yield and improved stability based on the expression profile of the eukaryotic cell. The eukaryotic cell preferably is a mammalian cell.

BACKGROUND OF THE DISCLOSURE

[2] The market for biopharmaceuticals continues to grow at a high rate as biopharmaceuticals become more and more important for today's medicine. Currently, an increasing number of biopharmaceuticals is produced in eukaryotic cells such as in particular mammalian cells. Successful and high yield production of biopharmaceuticals in mammalian cells is thus crucial. The time to generate such a cell line producing a therapeutic protein of interest is an essential part of the time needed to bring any biopharmaceutical in the clinic. Furthermore, also considering the production costs for biopharmaceuticals it is important to have high and stably expressing recombinant eukaryotic cell lines, in particular mammalian cell lines.

[3] For biopharmaceutical production efficiency's sake in particular on industrial scale, a tremendous effort is put into the clone selection process, with the goal to identify high producing clones with good expression stability and growth characteristics in a short amount of time. The generation of recombinant cell clones for production of therapeutic proteins usually comprises excessive screening of individual clones to detect and isolate high expressing clones. However, even if high expressing clones are identified in the course of the screening process, these initially high expressing clones often lose their advantageous expression characteristics and the expression yield decreases over time. This gradual loss of recombinant protein expression in cell clones during prolonged subculture is a common issue with many cell lines such as CHO cell lines and is referred to as instability. This instability seriously affects the industrial production process of recombinantly produced polypeptides. Therefore, care must be taking in order to identify within the population of successfully expressing cells and even among the cell clones which express the protein of interest initially with a good yield those cells, respectively cell clones, which also have a high production stability during prolonged cultivation and therefore, are not prone to a gradual loss of recombinant protein expression. Such clones are also called "stable" clones. During prolonged culturing periods, stable clones should not lose more than 30%, preferably 25% of their initial productivity within a period of 8-12 weeks. Productivity is defined as volumetric productivity, which is the expressed amount of protein per volume (e.g. g/L) at a certain time

point of cultivation, respectively as cell specific productivity, which is the specific amount of expressed protein per cell per day (e.g. pg/cell/day). In order to avoid that a cell clone is selected for subsequent large scale production which is prone to instability and therefore will lose titer during prolonged culturing, usually extensive stability analyses are performed over several weeks up to several months in order to eliminate those cell clones which become unstable during that time period and to identify the stable clones. Therefore, the generation of recombinant cell clones for production of therapeutic proteins and other recombinant polypeptides that are produced on large scale usually comprises excessive and time-consuming screening of individual clones in order to identify the high expressing cell clones that also show the expression stability necessary for large scale production. This practice prolongs the development of biotechnological such as biopharmaceutical production processes. Even when using highly stringent selection systems that favour survival of high expressing cells under the used selection conditions, finding a suitable production clone within the surviving population which combines a high expression rate with good growth and stability characteristics is difficult.

[4] It is an object of the invention to improve recombinant production of a product of interest in eukaryotic cells such as in particular mammalian cells. In particular, it is an object of the present invention to provide a novel eukaryotic cell line which upon transfection with a polynucleotide encoding a product of interest express the product of interest with improved yield. Furthermore, it is one object to provide a selection method that allows identifying successfully transfected cells with improved expression characteristics. Additionally, it is an object to provide an improved method for recombinantly producing a product of interest. Furthermore, it is one object to provide analysis tools that allow discriminating between high and low producing recombinant cell clones and/or between stable and unstable cell clones at an early stage of the development process.

SUMMARY OF THE DISCLOSURE

[5] The present disclosure is *inter alia* based on the unexpected finding that impairing the effect of the expression product of gene C12orf35 in an eukaryotic cell, e.g. by reducing or eliminating the functional expression of gene C12orf35 in said cell, allows to significantly increase the expression of a recombinant product of interest in said cell. Thus, a key gene was identified that influences recombinant expression. Impairing the effect of the expression product of gene C12orf35 in the cell as described herein allows to significantly improve the recombinant production of a product of interest by increasing the expression yield. Therefore, the present invention makes an important contribution to the prior art.

[6] According to a first aspect, the present disclosure provides an isolated eukaryotic cell, wherein the effect of the expression product of gene C12orf35 is impaired in said cell. Impairment can be achieved e.g. by reducing or eliminating functional expression of endogenous gene C12orf35 in said cell, e.g. by gene silencing, gene deletion or by mutating the gene so that a non- or less functional protein is expressed. As is shown by the examples, upon transient or stable transfection respectively altered eukaryotic cells are surprisingly

capable of producing a recombinant product of interest with higher yield. Thus, these eukaryotic cells are particularly suitable as host cells for recombinant production technologies and can be used for recombinant production of a product of interest.

[7] According to a second aspect, a method is provided for selecting a host cell which recombinantly expresses a product of interest, comprising

(a) providing eukaryotic cells according to the first aspect as host cells, wherein said host cells comprise at least one heterologous polynucleotide encoding the product of interest; and
(b) selecting one or more host cells expressing the product of interest.

[8] According to a third aspect, a method is provided for recombinantly producing a product of interest, comprising using a eukaryotic cell according to the first aspect as host cell for recombinant expression of the product of interest. As described above, due to their increased production capacity, these novel eukaryotic cells are particularly suitable as host cells for recombinant production.

[9] According to a fourth aspect, a method is provided for producing a eukaryotic cell suitable for recombinant production of a product of interest, comprising impairing the effect of the expression product of an endogenous gene C12orf35 in the eukaryotic cell. This can be achieved e.g. by reducing or eliminating functional expression of gene C12orf35 in said cell.

[10] According to a fifth aspect, a method is provided for analyzing eukaryotic cells for their suitability as host cells for recombinant expression of a product of interest, comprising analyzing directly or indirectly whether the effect of the expression product of gene C12orf35 is impaired in said cells. This method can be advantageously used e.g. in combination with the method according to the fourth aspect in order to identify e.g. whether a eukaryotic cell was obtained, wherein the effect of the expression product of gene C12orf35 is impaired. Furthermore, this method can be used as analytical tool in order to discriminate between high and low expressing clones and in embodiments between stable and unstable clones that express the product of interest.

[11] According to a sixth aspect, the present disclosure pertains to the use of an isolated eukaryotic cell for recombinantly expressing a product of interest, wherein the effect of the expression product of gene C12orf35 is impaired in said cell.

[12] Other objects, features, advantages and aspects of the present application will become apparent to those skilled in the art from the following description and appended claims. It should be understood, however, that the following description, appended claims, and specific examples, while indicating preferred embodiments of the application, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following.

BRIEF DESCRIPTION OF THE FIGURES

[13] **Fig. 1** provides a schematic overview of the telomeric region of chromosome 8 of Chinese hamster ovary (CHO) cells and genes located in said telomeric region. The genomic region depicted in the figure is the result of the merged scaffolds number 6 and 25 on chromosome 8. An overview over genes and putative genes on chromosome 8 of CHO cells can be found using the gene bank annotation file associated with the assembly of Brinkrolf et al. (Nature Biotechnology Volume 31, 694–695 (2013); see gene bank: APMK00000000, version APMK01000000 as is described in said publication). Furthermore, the Beijing Genomics Institute also provided an annotation of this region (Xu et al, Nature Biotechnology, Volume 29, number 8, 735-741 (2011); see gene bank: AFTD00000000, version AFTD01000000). Annotations which are marked with an * in Fig. 1 are from gene bank file AFTD01000000.

[14] A corresponding overview over the telomeric region of chromosome 6 of mouse can be found e.g. in the Ensembl database. Chromosome 6 of mouse has a structure which corresponds to chromosome 8 of Chinese hamster. The subsequent link of the Ensemble database shows the telomeric region of chromosome 6 of mouse which contains the C12orf35 gene (here named 2810474O19Rik):

http://www.ensembl.org/Mus_musculus/Location/View?db=core;g=ENSMUSG00000032712;r=6:149309414-149335658

[15] The subsequent Table 1 provides an overview over abbreviations and alternative names (aliases) of genes and encoded products shown in Fig. 1 and indicates the corresponding annotation in mouse and Chinese hamster (according to Brinkrolf et al, 2013 and/or Xu et al, 2011) where feasible. Table 1 also lists alternative names used e.g. in different species. Wherein the present disclosure refers to a specific protein or gene name, this also refers to and encompasses any alternative names of said protein or gene e.g. used to characterize the corresponding gene or protein in a different species. In particular, homologs and orthologs having the same function are encompassed thereby.

Table 1: Abbreviations and alternative names (aliases) of products encoded by genes located in chromosome 8 of Chinese hamster or chromosome 6 of mouse.

Abbreviation	**In public annotation of Chinese hamster, the gene product is annotated as**	**In public annotation of mouse the gene product is annotated as**	**Aliases (see www.genecards.org)**
Ccdc91	Coiled-coil domain-containing protein 91	Coiled-coil domain containing 91	Coiled-Coil Domain Containing 91 P56 GGA-Binding Partner P56 Accessory Protein

			DKFZp779L1558 FLJ11088 Coiled-Coil Domain-Containing Protein 91 GGA Binding Partner GGABP
Far2	Fatty acyl CoA reductase 1 isoform 1	Fatty acyl CoA reductase 2	Fatty Acyl CoA Reductase 2 MLSTD1 SDR10E2 Male Sterility Domain-Containing Protein 1 EC 1.2.1.N2 FLJ10462 Male Sterility Domain Containing 1 Fatty Acyl-CoA Reductase 2 Short Chain Dehydrogenase/Reductase Family 10E, Member 2
Ergic2	Endoplasmic reticulum-Golgi intermediate compartment protein 2	ERGIC and golgi 2	ERGIC And Golgi 2 PTX1 Erv41 Cd002 CD14 Protein Endoplasmic Reticulum-Golgi Intermediate Compartment Protein 2 ERV41 CDA14
RPS4Y2	40S ribosomal protein S4, X isoform like	Ribosomal protein S4, Y linked 2	Ribosomal Protein S4, Y-Linked 2 RPS4Y2P Ribosomal Protein S4, Y-Linked 2 Pseudogene 40S Ribosomal Protein S4, Y 40S Ribosomal Protein S4, Y Isoform 2
Tmtc1	Transmembrane and TPR repeat-containing protein 1	Transmembrane and tetratricopeptide repeat containing 1	Transmembrane And Tetratricopeptide Repeat Containing 1 OLF ARG99 FLJ31400 FLJ41625 TMTC1A Transmembrane And Tetratricopeptide Repeat Containing 1A Transmembrane And TPR Repeat-Containing Protein 1
Zfp 1	Zinc finger HIT domain-containing protein1		ZFP1 Zinc Finger Protein ZNF475 Zinc Finger Protein 475 FLJ34243 Zinc Finger Protein 1

			Zinc Finger Protein 1 Homolog (Mouse) Zfp-1 Zinc Finger Protein 1 Homolog
IPO8	Importin-8-like	Importin-8	Importin 8 RANBP8 RAN Binding Protein 8 Ran-Binding Protein 8 IMP8 Imp8 Importin-8 RanBP8
Caprin2	Caprin-2-like protein	Caprin family member 2	Caprin Family Member 2 C1QDC1 EEG1 RNG140 Caprin-2 Cytoplasmic Activation/Proliferation-Associated Protein 2 Gastric Cancer Multidrug Resistance-Associated Protein C1q Domain-Containing Protein 1 RNA Granule Protein 140 FLJ11391 FLJ22569 C1q Domain Containing 1 EEG-1 KIAA1873 Protein EEG-1
FAM60A	Protein FAM60A-like	Family with sequence similarity 60, member A	Family With Sequence Similarity 60, Member A C12orf14 TERA Tera Protein Homolog Chromosome 12 Open Reading Frame 14 Protein FAM60A
Dennd5b	Denn domain-containing protein 5B-like	DENN/MADD domain containing 5B	DENN/MADD Domain Containing 5B Rab6IP1-Like Protein MGC24039 DENN Domain-Containing Protein 5B
METTL20	Methyltransferase-like protein 20	4833442J19Rik	METTL20 C12orf72 DKFZp451L235 MGC50559 Chromosome 12 Open Reading Frame 72 Methyltransferase-Like Protein 20 EC 2.1.1.
AMN1	Putative protein	Antagonist of mitotic	Antagonist Of Mitotic Exit Network 1

	AMN1 like protein	exit network 1	Homolog (S. Cerevisiae) Protein AMN1 Homolog
	Opioid growth factor receptor-like protein	Opioid growth factor receptor-like protein	
C12orf35	Uncharacterized protein C12orf35 homolog	likely orthologue of H. sapiens chromosome 12 open reading frame 35 (C12orf35); 2810474O19Rik	KIAA1551, C12orf35 FLJ10652, FLJ20696 Chromosome 12 Open Reading Frame 35 Uncharacterized Protein C12orf35 Uncharacterized Protein KIAA1551
Bicd 1	Putative protein bicaudal D	Bicaudal D homolog 1	Bicaudal D Homolog 1 (Drosophila) Bic-D 1 Bicaudal D (Drosophila) Homolog 1 BICD Cytoskeleton-Like Bicaudal D Protein Homolog 1 Protein Bicaudal D Homolog 1

[16] **Fig. 2** shows the relative expression levels of genes located in the telomeric region of chromosome 8 in a CHO cell line, namely TMTC1 (1), RPS4Y2 (2), IPO8 (3), CAPRIN2 (4), FAM60A (5), Dennd5b (6), METTL 20 (7), AMN1 (8), C12orf35 (9), Bicd1 (10).

[17] **Fig. 3A to L** show FACS profiles obtained after reducing the expression of different target genes located in the telomeric region of chromosome 8 of Chinese hamster (CHO) cells using siRNAs. Cells that were stably transfected with an expression vector and expressed the encoded antibody as product of interest were fluorescently stained to detect the amount of recombinantly expressed antibody. The higher the intensity in the FACS profile, the more the antibody is expressed by the stained cell. The left peak shown in the FACS profile corresponds to the parental cell line (not transfected and hence not expressing the antibody) which was included for comparative purposes. The two other curves represent results obtained for a cell clone that was stably transfected with the expression vector and which recombinantly expresses the antibody. This cell clone was transfected with either a siRNA negative control (dark curve; no effect on expression of any gene) or with a siRNA that reduces the expression of a target gene (light grey curve). If silencing of the target gene does not have an effect on recombination expression of the antibody, the fluorescent curve for the siRNA control and the target siRNA overlap and remain the same. If silencing of the target gene increases the expression rate of the recombinantly expressed antibody, the intensity of the corresponding FACS profile increases and shifts to the right. **A**: gene Mettl20_1, 125 pmol, 24.9%; **B**: gene C12orf35_1, 125 pmol, 30.6%; **C**: gene C12orf35_2, 150 pmol, 31.7%; **D**: gene Caprin2_6, 100 pmol, 53.3%; **E**: FAM60A_3, 150 pmol, 48%; **F**: Ipo8_1, 125 pmol, 20.3%; **G**: Ipo8_2, 150 pmol, 57.5%; **H**: Ipo8_3, 150 pmol, 21.5%; **I**: Dennd5b_2, 100 pmol, 36.9%; **J**: Amn1_4, 125 pmol, 30.8%; **K**: TMTC1_1, 150 pmol, 60.6%; **L**: TMTC1_2, 150 pmol, 53.4% (percentage values correspond to mRNA expression

of the target gene between reference siRNA versus ctrl siRNA). **Fig. 3B** and **C** show that a downregulation of gene C12orf35 significantly increases expression of the recombinant antibody and thus results in a higher productivity as is indicated by the clear shift of the FACS profile to the right (see light grey curve on the right, also marked with an arrow).

[18] **Fig. 4 and 5** show the mRNA expression levels of the antibody light and heavy chains of two different model polypeptides of interest (antibody 1 and 2) in different clones and pools in each case after reducing expression of gene C12orf35 in CHO cells by RNAi. The mRNA levels of the antibody chains are upregulated, if expression of gene C12orf35 is reduced by gene silencing. Thus, reduction of C12orf35 expression surprisingly leads to higher mRNA levels of HC and LC.

[19] **Fig. 6** shows that upon silencing of gene C12orf35 using siRNA, significantly higher volumetric titers were obtained.

[20] **Fig. 7** also demonstrates that silencing of gene C12orf35 leads to higher specific productivities (calculated from days 3, 4, 5 and 6 of cultivation). siRNA-1 had a more pronounced silencing effect than siRNA-2 and hence lead to higher expression rates.

[21] **Fig. 8** shows that the 46 highest producing clones (black) derived from a CHO cell line wherein the telomeric region comprising gene C12orf35 on chromosome 8 (q arm) is deleted (C8DEL) have higher titers compared to the 45 highest producing clones obtained from the parental cell line which were tested to be IPO8 positive (grey).

[22] **Fig. 9** shows FACS profiles of stably transfected C8DEL cell pools after selection using a folate receptor/DHFR system. The concentration of MTX was increased from A to E (A: no MTX; B: 1nM MTX; C: 5nM MTX; D: 10nM MTX; E: 50nM MTX). Recombinant antibody expression was detected based on fluorescence. At 50nM MTX, predominantly high producing cells were comprised in the obtained pool as is demonstrated by FACS analysis. The obtained pool profile remarkably resembled the profile of a cell clone. This supports the extraordinary impact the technology described herein has on the expression yield.

[23] **Fig. 10** shows the results of stability tests performed for 7/8 weeks with three different cell clones (CHO wildtype and two FAM60A knock-out clones s16 and s23 derived from said wildtype) following stable transfection with an expression vector encoding an antibody as product of interest. (1) shows the stability results obtained with the parental wildtype cells (derived from CHO-K1); (2) shows the stability results with the FAM60A knock-out clone s16; (3) shows the stability results with the FAM60A knock-out clone s23. As can be seen, the expression stability was significantly increased in the cell clones that derived from the FAM60A knock-out cells (see (2) and (3)). The number of stable clones was significantly increased when using the FAM60A knock-out cells for recombinant expression. Thus, impairing the effect of FAM60A in the host cell, here by gene knock-out, significantly improved the expression stability. Therefore, according to one embodiment, the effect of protein FAM60A is additionally impaired in the eukaryotic cell, preferably by reducing or

eliminating functional expression, to increase the expression stability upon stable transfection.

DETAILED DESCRIPTION OF THE DISCLOSURE

[24] The present disclosure is *inter alia* based on the surprising finding that eukaryotic cells, in which the effect of the expression product of gene C12orf35 is impaired, e.g. by reducing or eliminating functional expression of the endogenous gene C12orf35, by deleting said gene or by introducing mutations, are capable of expressing a recombinant product of interest with significantly improved yield. Based on this surprising finding that gene C12orf35 has a strong impact on the expression of a recombinant product of interest, the present disclosure also provides novel selection and production methods and associated technologies which allow improving recombinant production of a product of interest. Therefore, the present disclosure makes an important contribution to the prior art.

[25] The individual aspects and suitable and preferred embodiments thereof will now be described in detail.

A. Modified eukaryotic cells

[26] According to a first aspect, the present disclosure provides an isolated eukaryotic cell, wherein the effect of the expression product of gene C12orf35 is impaired in said cell. As is shown by the examples for mammalian cells, respectively modified eukaryotic cells show a significant higher productivity following stable as well as following transient transfection with an expression vector comprising a polynucleotide encoding a product of interest as is demonstrated by the examples. Furthermore, the abundance of high expressing cells in the population of transfected cells is improved when using respectively altered eukaryotic cells. In embodiments, it was also found that clone stability is improved. The improved expression stability allows shortening or even skipping time consuming stability studies of high expressing cell clones. Further advantages are also described in the following and are also apparent from the examples. Thus, using these advantageous novel eukaryotic cell lines for recombinant production of a product of interest reduces screening efforts for identifying high expressing cells or cell clones and in particular reduces the time needed for obtaining high expressing cell clones suitable for producing the product of interest on large scale. Thus, these eukaryotic cell lines have important advantages when being used as host cells for recombinant production technologies.

[27] The C12orf35 gene is endogenously expressed in eukaryotic cells such as e.g. mammalian species such as human, mouse and hamster. The expression product of the C12orf35 gene is a rather large protein. The sequence listing shows exemplary amino acid sequences or putative amino acid sequences of the protein encoded by the endogenous C12orf35 gene of different mammalian species such as hamster (SEQ ID NO: 1 and 2), human (SEQ ID NO: 3 and 4), mouse (SEQ ID NO: 5), cattle (SEQ ID NO: 6) and wild boar (SEQ ID NO: 7). The CDS (Coding DNA Sequence) of C12orf35 from Chinese hamster is shown as SEQ ID NO: 8. Furthermore, a section of the 5'UTR (see SEQ ID NO: 9) and of the

3'UTR (see SEQ ID NO: 10) of the C12orf35 mRNA from Chinese hamster was sequenced. Gene C12orf35 is also referred to as C12orf35like or C12orf35 homolog in hamster or 2810474O19Rik in mouse. Information about the gene, the coding sequence and the predicted C12orf35 protein is also disclosed for Cricetulus griseus in NCBI: XM_003512865, herein incorporated by reference. In human, it also referred to as KIAA1551. Different names can be assigned in different species for the protein or the gene and non-limiting alternative names (aliases) are also listed above in Table 1. The term "C12orf35" as used herein also encompasses any homologs and orthologs of C12orf35 which have the same function as C12orf35. Accordingly, the term "C12orf35 gene" as used herein in particular encompasses any endogenous gene which encodes a protein that shares at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% homology or identity to one or more of the amino acid sequences shown in SEQ ID NO: 1 to 7 or the protein encoded by SEQ ID NO 8. The protein encoded by such gene preferably has the same function as the protein having an amino acid sequence as is shown in SEQ ID NO: 1 or one or more of SEQ ID NO: 2 to 7 or the protein encoded by SEQ ID NO: 8. Said gene can be modified as described herein in order to impair the function of the expression product that is expressed by the unmodified cell. The protein expressed by gene C12orf35 has not been described in detail in the literature. The terms "C12orf35 protein" or "expression product of endogenous C12orf35 gene" and similar expressions as used herein encompass homologs and orthologs of C12orf35 and in particular encompass any protein that shares at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% homology or identity to one or more of the amino acid sequences shown in SEQ ID NO: 1 to 7 or the protein encoded by SEQ ID NO 8. Such C12orf35 protein preferably has the same function as the protein having an amino acid sequence as is shown in SEQ ID NO: 1 or as is shown in one or more of SEQ ID NO: 2 to 7 or the protein encoded by SEQ ID NO: 8. Homology, respectively identity may be calculated over the entire length of the reference protein.

[28] The present disclosure *inter alia* pertains to modified eukaryotic cells, such as preferably mammalian cells, wherein the effect of the expression product of the C12orf35 gene, which usually is endogenously expressed by a corresponding unmodified eukaryotic cell, is impaired in said cell. As is demonstrated by the examples, this modification of the cell which can be transient or permanent allows to increase the expression of a recombinant product of interest.

[29] There are several possibilities to modify a eukaryotic cell to impair the effect of the expression product of gene C12orf35 in said cell. The effect of the expression product of gene C12orf35 and hence the C12orf35 protein may be impaired e.g. on the gene level or on the protein level. The effect of C12orf35 can be impaired, for example, by modification of the structure/sequence of the C12orf35 protein, the transcription and/or translation. Non-limiting options are described in the following.

[30] According to one embodiment, the effect of the expression product of gene C12orf35 is impaired in the eukaryotic cell because functional expression of gene C12orf35 is reduced or eliminated in said cell. As is shown by the examples, altering the expression of gene C12orf35, e.g. by gene silencing or by deleting said gene, is a very efficient measure to provide eukaryotic cells capable of expressing a recombinant product of interest with high yield. When the expression level of gene C12orf35 is reduced or eliminated in an eukaryotic cell and hence less or no functional C12orf35 protein is produced by the respectively altered cell, the expression yield of a recombinant product of interest is significantly increased. This correlation between functional C12orf35 expression and yield of recombinant protein expression is an unexpected finding.

[31] Reduction or elimination of functional expression of gene C12orf35 may be achieved by various means. Functional expression can be reduced for example by reducing the expression level of gene C12orf35 or by disrupting the function of C12orf35 or by a combination of such methodologies. According to one embodiment, the cell is altered so that functional expression of gene C12orf35 is reduced or eliminated by gene knock-out, gene mutation, gene deletion, gene silencing or a combination of any of the forgoing. According to one embodiment, functional expression of gene C12orf35 is reduced or eliminated in the cell by gene knockout. A gene knockout is a genetic technique by which a gene is made inoperative by disrupting its function. E.g. a nucleic acid can be inserted into the coding sequence, thereby disrupting the gene function. Furthermore, the complete C12orf35 gene or a portion thereof can be deleted, whereby no or no functional protein is expressed by the respectively altered cell. Another option is to introduce one or more knock-out mutations into the coding sequence, which renders a non- or a less functional expression product. E.g. one or more frameshift mutations can be introduced that result in a non- or less-functional protein. Alternatively or additionally, one or more stop codons can be introduced into the coding sequence so that a truncated, non- or less functional protein is obtained. Hence, according to one embodiment, gene C12orf35 comprises one or more mutations which provide a non- or less functional expression product. Other options include but are not limited to one or more mutations in the promoter, in the 5'- and/or 3' UTR or other regulatory elements. According to one embodiment, the promoter function of gene C12orf35 is disrupted, e.g. by introducing a promoter deletion or by introducing a construct between the promoter and the transcription start. Methods for achieving a gene knockout to suppress or eliminate expression of the target gene are also well-known to the skilled person and thus, do not need any detailed description herein. Some non-limiting examples are nevertheless described below.

[32] According to one embodiment, the C12orf35 gene is functionally knocked out by genetic engineering. Examples include but are not limited to genome editing, such as genome editing with engineered nucleases (GEEN). This is a type of genetic engineering in which DNA is inserted, replaced or removed from a genome using artificially engineered nucleases, or "molecular scissors." The nucleases create specific double-stranded breaks (DSBs) at desired locations in the genome, and harness the cell's endogenous mechanisms to repair the induced break by natural processes of homologous recombination (HR) and

nonhomologous end-joining (NHEJ). There are at least four families of engineered nucleases that can be used: Zinc finger nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), CRISPR, and engineered meganuclease re-engineered homing endonucleases.

[33] According to one embodiment, one or more copies of gene C12orf35 present in the genome of the eukaryotic cell are altered, e.g. knocked-out or deleted, to reduce or eliminate and hence impair the effect of the expression product of gene C12orf35 in the eukaryotic cell. Thus, according to one embodiment, at least one copy of gene C12orf35 is deleted or functionally inactivated in the genome of the eukaryotic cell. For example, one or more mutations may be inserted into the copy or copies of gene C12orf35 (if more than one copy is present) to provide a non- or less functional expression product or to eliminate or reduce expression in toto and, hence impair the effect of C12orf35 in the mammalian cell. Thereby, gene C12orf35 is basically inactivated in the genome. According to one embodiment, in case more than one copy is present, all copies of gene C12orf25 are respectively altered in the eukaryotic cell, which preferably is a mammalian cell.

[34] According to one embodiment, the eukaryotic cell is a metazoan cell, a vertebrate cell or preferably, a mammalian cell. According to one embodiment, a portion of a chromosome is deleted in said cell, wherein the deleted portion comprises gene C12orf35. According to one embodiment, a chromosomal portion comprising gene C12orf35 is deleted in all chromosomes which comprise a copy of gene C12orf35. Thereby, all copies of gene C12orf35 are deleted from the genome.

[35] According to one embodiment, a portion of the telomeric region of a chromosome is deleted, wherein the deleted portion comprises gene C12orf35. According to a preferred embodiment, the altered cell is a rodent cell. According to one embodiment, the cell is a hamster cell such as e.g. a CHO cell and at least a portion of the telomeric region of chromosome 8 is deleted in the genome, wherein said deleted portion comprises gene C12orf35. The meaning of the term "C12orf35" is explained above and non-limiting alternative names of homologs and orthologs that are also encompassed by the scope of said term are also indicated in Table 1. According to one embodiment, such deletion occurs in the q arm of the chromosome 8 of a hamster cell, in particular a Chinese hamster cell, which comprises the FAM60A gene. As is shown by the examples, a CHO cell comprising a respective deletion in the telomeric region of chromosome 8 is particularly suitable as host cell for recombinant expression. After stable or transient transfection with an expression vector, these cells show a significant higher productivity compared to cells wherein said portion in the telomeric region of chromosome 8 is not lost. Furthermore, the abundance and thus the amount respectively proportion of high expressing cells in the transfected cell population is significantly increased. In case of stable transfection, the stability of recombinant expression is significantly increased e.g. in such hamster cells which have lost said portion of the telomeric region in chromosome 8. Further important advantages are described in detail in the examples wherein CHO cells in which a respective portion of the telomeric region of chromosome 8 is deleted due to chromosome breakage are further

characterized. The advantageous properties render these hamster cells particularly suitable as industrial production cell lines. Alternatively, the altered rodent cell may be a mouse cell wherein at least a portion of the telomeric region of chromosome 6 is deleted in the genome, wherein said deleted portion comprises gene C12orf35. The telomeric region of chromosome 6 of mouse is highly similar to the telomeric region of chromosome 8 of hamster.

[36] According to one embodiment, at least a portion of the telomeric region is deleted or not present in both chromosomes of chromosome pair 8 of hamster (or chromosome pair 6 in case of mouse cells), wherein the deleted portions comprise the C12orf35 gene.

[37] According to one embodiment, at least a portion of the telomeric region is deleted in one chromosome of chromosome pair 8 of hamster (or chromosome pair 6 in case of mouse), wherein said deleted portion comprises the C12orf35 gene and the expression of gene C12orf35 in the other chromosome is reduced or eliminated. Suitable ways to reduce or eliminate the expression of a gene are known to the skilled person and non-limiting examples are also described herein. According to one embodiment, such deletion occurs in the q arm of chromosome 8 of hamster, in particular Chinese hamster.

[38] According to one embodiment, the deleted chromosomal region comprises the C12orf35 gene and additionally one or more or all genes selected from the group consisting of Bicd1, Amn1, methyltransferase-like protein 20, Dennd5b, FAM60A, Caprin2 and Ipo8. According to one embodiment, all of the aforementioned genes are deleted. According to one embodiment the deleted chromosomal region additionally comprises at least a portion of or the full gene Tmtc1. In hamster cells such as CHO cells, these genes are also located in the telomeric region of chromosome 8. According to one embodiment, the deleted chromosomal region additionally comprises gene RPS4Y2 if present. An overview over the telomeric region of chromosome 8 of the Chinese hamster genome wherein the location of aforementioned genes is shown is provided as Fig. 1. As is shown by the examples, CHO cells comprising a respective deletion in the telomeric region of chromosome 8 (q arm) have particular advantageous properties with respect to expression yield and expression stability. In mouse cells the aforementioned genes are located in the telomeric region of chromosome 6. Non-limiting alternative names of the aforementioned individual genes and/or encoded proteins including homologs and orthologs are also indicated in Table 1 above and the respective genes are encompassed by the scope of the terms used above for the individual genes.

[39] According to one embodiment, the deletion of the C12orf35 gene is due to a chromosome breakage. A chromosome breakage can be induced e.g. by treating the eukaryotic cells with a toxic agent that promotes chromosome breakage, such as e.g. MTX, aphidicolin or hygromycin. Other options for inducing chromosome breakages include but are not limited to radiation, irradiation, mutagens, cancerogenic substances and bleomycin. Chromosome breakages may also occur spontaneously during transfection e.g. electroporation. Methods for inducing chromosome breakage are also known to the skilled person and thus, do not need any detailed description here. After inducing chromosome breakage, eukaryotic cells having the desired breakpoint (which results in a deletion of gene

C12orf35) can be identified e.g. be analyzing the DNA or by using the method according to the fifth aspect of the present disclosure. For example, the expression profile of the treated cells can be analyzed to determine whether gene C12orf35 or genes located centromeric of gene C12orf35 are expressed, whether the expression is reduced or whether the genes are not expressed. For example, in case of mouse or hamster cells it can be analysed whether gene C12orf35 is expressed and alternatively or in addition thereto, it can be analyzed whether one or more genes selected from the group consisting of methyltransferase-like protein 20, Dennd5b, FAM60A, Caprin2, Ipo8, Tmtc1 or genes that are located telomeric of the aforementioned genes (wherein telomeric in this respect means into the direction of the telomeric end) are expressed by the cell and/or whether the expression is reduced or eliminated. If the induced breakpoint is located centromeric of the respective gene(s) (wherein centromeric in this respect means further into the chromosome and hence further away from the telomeric end), the telomeric end comprising said genes is deleted which eliminates or reduces (if other copies of the gene exist elsewhere that are expressed) their expression. As is evident from Fig. 1, gene C12orf35 is located telomeric of the aforementioned genes, i.e. it is located further into the direction of the telomeric end. Thus, if the aforementioned genes are deleted by a chromosome break, the deleted region also includes gene C12orf35. Thus, the above genes can be validly used as markers in order to basically indirectly determine whether the induced chromosome breakage resulted in a deletion of a chromosome portion that includes gene C12orf35. Furthermore, it was found that even though located telomeric of gene C12orf35, also gene Bicd1 can be used as marker to determine whether a chromosome breakage was induced which resulted in a deletion of gene C12orf35. It was found in CHO cells that if gene Bicd1 is deleted because of a chromosome breakage, the deletion usually also includes gene C12orf35. It was confirmed by analyzing the expression characteristics of several hundred clones that the aforementioned genes can be validly used as markers in order to discriminate cell clones with high and stable expression characteristics from cell clones having low and unstable expression characteristics. The relative expression of the aforementioned genes in CHO cells is shown in Fig. 2. As can be seen from Fig. 2, genes Ipo8 (3), FAM60A (5) and C12orf35 (9) are relatively highly expressed in comparison to other genes that are located in the telomeric region of chromosome 8 in normal CHO cells such as CHO-K1 cells, which do not comprise a deletion in the telomeric region of chromosome 8. Thus, it is advantageous to include one or more of the aforementioned genes in the analysis, as this simplifies the detection that their expression is eliminated or reduced. Non-limiting alternative names of the aforementioned individual genes and encoded proteins, including homologs and orthologs, are also indicated in Table 1 above and the respective genes are encompassed by the scope of the terms used above for the individual genes.

[40] According to one embodiment, the breakpoint on chromosome 8 is located centromeric of the methyltransferase-like protein 20 gene, centromeric of the Dennd5b gene, centromeric of the FAM60A gene, centromeric of the Caprin2 gene, centromeric of the Ipo8 gene or centromeric of gene RPS4Y2. It was found that the breakpoint on chromosome 8 of the hamster genome is often located centromeric of the Ipo8 gene. According to one embodiment, the breakpoint on chromosome 8 is located within the Tmtc1 gene and said

gene is not expressed or its expression is low. According to one embodiment, the Ergic2 gene, which is located centromeric of the Tmtc1 gene, is not deleted on chromosome 8. Thus, according to this embodiment, the breakpoint is telomeric of the Ergic 2 gene (wherein telomeric in this respect means downstream into the direction of the telomeric end) and the Ergic 2 gene is present.

[41] According to one embodiment, functional expression of gene C12orf35 is reduced or eliminated in the eukaryotic cell which preferably, is a mammalian cell. Functional expression of gene C12orf35 can be influenced by various means, for example by altering the promoter and/or an enhancer of gene C12orf35 so that less or no transcript is produced, or by gene silencing technologies such as transcriptional or post-transcriptional gene silencing. According to one embodiment, the isolated eukaryotic cell comprises one or more mutations in the promoter region of gene C12orf35. For example, the promoter region may be altered to provide a less functional or non-functional promoter, the promoter may also be completely eliminated. Alternatively or in addition, it is possible to add a polynucleotide sequence encoding a polypeptide including a stop codon between the promoter and the start codon of gene C12orf35 which leads to the expression of the other polypeptide instead of C12orf35. Respective methods are well-known to the skilled person and thus, do not need any detailed description here.

[42] Reduction of functional gene expression may achieve a level wherein expression is even eliminated. Post-transcriptional gene silencing can be achieved e.g. by antisense molecules or molecules that mediate RNA interference. Non-limiting examples will be briefly described in the following.

[43] Antisense polynucleotides may be designed to specifically bind to RNA, resulting in the formation of RNA-DNA or RNA-RNA hybrids, with an arrest of reverse transcription or messenger RNA translation. Many forms of antisense have been developed and can be broadly categorized into enzyme-dependent antisense or steric blocking antisense. Enzyme-dependent antisense includes forms dependent on RNase H activity to degrade target mRNA, including single-stranded DNA, RNA, and phosphorothioate antisense. Antisense polynucleotides are typically generated within the cell by expression from antisense constructs that contain the antisense strand as the transcribed strand. Trans-cleaving catalytic RNAs (ribozymes) are RNA molecules possessing endoribonuclease activity. Ribozymes may be specifically designed for a particular target and may be engineered to cleave any RNA species site-specifically in the background of cellular RNA. The cleavage event renders the mRNA unstable and prevents protein expression. The genome of the eukaryotic cell can be altered so that a respective antisense molecule is e.g. permanently expressed.

[44] Another suitable option for reducing functional expression of gene C12orf35 on a post-transcriptional level is based on RNA interference (RNAi). As is shown by the examples, reducing the expression of gene C12orf35 by RNAi is effective in order to increase the productivity of the host cell. Significantly more recombinant product is produced upon

silencing of gene C12orf35 by RNAi. Furthermore, as is demonstrated by the examples, the mRNA expression levels of the product of interest are increased upon silencing of gene C12orf35. Methods for silencing genes by RNAi are well known to the skilled person and thus, do not need any detailed description here. Examples of RNAi inducing compounds that can be used to silence the expression of gene C12orf35 include but are not limited to short interfering nucleic acids (siNA), short interfering RNA (siRNA), microRNA (miRNA), short hairpin RNAs (shRNA) as well as precursors thereof which are processed in the cell to the actual RNAi inducing compound. According to one embodiment, a siRNA is used for silencing. The siRNA may be provided as double-stranded molecule having 3' overhangs on each strand. Blunt ended molecules may also be used. Said siRNA may comprise desoxy- as well as ribonucleotides and furthermore, may comprise modified nucleotides. Several embodiments and variations of siRNA compounds are known in the prior art and can be used to reduce expression of gene C12orf35. Suitable siRNAs targeting the chosen/identified target sequences of the target genes on the RNA level can be identified by using proper computational methods, applying certain design-algorithms. In order to obtain a siRNA against the target transcript, the double-stranded molecule can be transfected directly into the cell. As is shown by the examples, such transient methods for reducing expression of gene C12orf35 are effective to increase recombinant expression of a product of interest. Alternatively, the siRNA may result from processing by dicer, an enzyme that converts either long dsRNAs or small hairpin RNAs (shRNAs) into siRNAs. These precursors or the final siRNA molecules can be produced exogenously (artificially) and can then be introduced into the eukaryotic cells by various transfection methods. According to a further embodiment, the RNAi inducing compound is expressed by a vector that is transfected into the eukaryotic cell. For siRNA, this can be done e.g. by the introduction of a loop between the two strands, thus producing a single transcript, which can be then processed into a functional siRNA in the eukaryotic cell. Such transcription cassettes typically use an RNA polymerase 3 promoter (for example U6 or H1) which usually direct the transcription of small nuclear RNAs (shRNAs). It is assumed that the resulting shRNA transcript from the vector is then processed by dicer, thereby producing the double-stranded siRNA molecules, preferably having the characteristic 3' overhangs. According to one embodiment, such shRNA providing vector is stably integrated into the genome of the eukaryotic cell. This embodiment is advantageous, as the downregulation of gene C12orf35 is due to the constantly produced siRNA rather stable and not transient. Cells comprising a respective shRNA providing vector can then be transfected with an expression vector comprising a polynucleotide encoding the product of interest. Alternatively, co-transfection strategies can be used, wherein the vector generating the shRNA is co-transfected with the expression vector comprising the polynucleotide encoding the product of interest.

[45] Transcriptional gene silencing may e.g. include epigenetic modifications. According to one embodiment, expression of gene C12orf35 is reduced by epigenetic silencing. Mammalian cells were identified wherein the expression of gene C12orf35 is significantly reduced by epigenetic silencing, presumably DNA methylation, and the recombinant productivity of said cells is extraordinarily high. Furthermore, the sequence of the gene can

be changed to reduce the half-life of the mRNA. This also achieves a reduction in the effect of the C12orf35 protein in the respectively altered cell.

[46] According to one embodiment, functional expression of gene C12orf35 is reduced or eliminated by targeting a regulatory element involved in the regulation of expression of gene C12orf35. E.g. a transcription factor, promoter (see also above), enhancer, UTRs, or other regulatory elements can be targeted e.g. by knock-out, deletion, down-regulation or any other alteration that inactivates or reduces the activity of said regulatory element, thereby preventing or reducing functional expression of gene C12orf35 and thereby impairing the effect of the endogenous expression product.

[47] According to one embodiment, the genome of the eukaryotic cell is altered to impair the effect of C12orf35 by heterologous expression of a mutant C12orf35 which is non- or less functional than the endogenously expressed C12orf35 protein. In this embodiment, the isolated eukaryotic cell comprises in addition to the heterologous polynucleotide encoding the polypeptide of interest a further heterologous polynucleotide encoding the mutant C12orf35. By overexpressing a respective non- or less functional mutant form of C12orf35, a dominant negative phenotype can be created. A further option to impair and hence reduce the effect of C12orf35 in the cell is the heterologous expression of a protein such as an antibody which neutralizes C12orf35 and hence impairs the effect of C12orf35 in the cell. According to one embodiment, the effect of C12orf35 is impaired in the cell by reducing or eliminating functional expression of molecules that functionally interact with C12orf35.

[48] According to another embodiment, a low molecular weight compound is used that inhibits expression of the C12orf35 gene by specifically inhibiting binding of a transcription factor to a regulatory region in the promoter, or by inhibiting an activator of transcription required for transcription of the target gene.

[49] According to one embodiment, expression of gene C12orf35 is reduced by at least 3 fold, at least 5 fold, at least 10fold, at least 20fold, at least 30fold, at least 40fold, at least 50fold, at least 60fold, at least 70fold, at least 75fold, at least 80fold, at least 90fold, at least 100fold or at least 125fold, at least 250fold, at least 500 fold, at least 750fold, at least 1000 fold, at least 1250fold, at least 1500fold, at least 1750fold or at least 2000fold. This can be determined e.g. by using real-time RT-PCR or other sensitive RNA detection methods. Such reduction can be achieved e.g. in comparison with the unmodified reference cell wherein the expression of gene C12orf35 is not reduced. According to one embodiment, expression of gene C12orf35 is 0.05% or less, 0.0475% or less, 0.045% or less, 0.0425% or less, 0.04% or less, 0.0375% or less, 0.035% or less, 0.0325% or less, 0.03% or less, 0.0275% or less, 0.025% or less, 0.0225% or less, 0.02% or less, 0.0175% or less, 0.015% or less compared to the expression of the 18S RNA (set as 100%) in the same cell. According to one embodiment, expression of gene C12orf35 is even less such as 0.001% or less, 0.0001% or less or even 0.00001 or less compared to the expression of the 18S RNA (set as 100%) in the same cell. The functional expression of gene C12orf35 is reduced such that it results in an increase in the expression of a recombinant product of interest if said modified eukaryotic

cell is transfected with an expression vector encoding the product of interest compared to a corresponding cell wherein the functional expression of gene C12orf35 is not reduced or eliminated. According to one embodiment, expression of the recombinant product or interest is at least 1.5 times higher, at least 1.75 times higher, at least 2 times higher, at least 2.5 times higher, at least 3 times higher, at least 4 times higher or at least 5 times higher than the expression of a corresponding cell wherein the functional expression of gene C12orf35 is not reduced or eliminated. According embodiments, expression rates are obtained that are at least 8 times higher, at least 10 times higher or at least 15 times higher than the expression of a corresponding cell wherein the expression of gene C12orf35 is not reduced or eliminated.E.g. expression rates were determined after selection with G418 that were up to 35 fold higher than the rates of a corresponding cell wherein the expression of gene C12orf35 is not reduced or eliminated. The expression rate of the recombinant product can be tested using e.g. the assays described in the examples.

[50] According to a further embodiment, the effect of the expression product of the C12orf35 gene is reduced or eliminated by using a compound which counteracts or inhibits the effect of the expression product of the C12orf35 gene. Respective inhibitory compounds can be of any nature and include but are not limited to chemical compounds such as in particular small molecules, proteins and peptides. Another possibility is to use of compounds such as low molecular weight compounds stimulating degradation of the protein product, for example by stimulating ubiquitination of the protein.

[51] According to one embodiment, additionally the effect of the expression product of one or more genes selected from the group consisting of Bicd1, Amn1, methyltransferase-like protein 20, Dennd5b, FAM60A, Caprin2, Ipo8, RPS4Y2 and Tmtc1 or one or more genes located telomeric of the aforementioned genes is impaired. Non-limiting alternative names of the aforementioned individual genes and/or the encoded proteins, including homologs and orthologs, are also indicated in Table 1 above and the respective genes encoding the respective proteins are encompassed by the scope of the terms used above for the individual genes. Impairment of the effect can likewise be achieved e.g. by reducing or eliminating the functional expression of the respective genes. Suitable technologies and embodiments are described above in conjunction with the C12orf35 gene and likewise apply to any other target gene. As described above, said genes are located in the telomeric region of chromosome 8 of Chinese hamster and chromosome 6 of mouse. If a portion of said telomeric region is deleted, e.g. by inducing a chromosome breakage as described above, the deleted region usually comprises one or more of the aforementioned genes.

[52] According to a preferred embodiment, in said cell wherein the effect of C12orf35 is impaired, additionally the effect of protein FAM60A is impaired, preferably by reducing or eliminating functional expression of the FAM60A gene. A further unexpected finding was that impairing the effect of FAM60A in a eukaryotic cell, for example by reducing or eliminating functional expression of the FAM60A gene, results upon stable transfection with a polynucleotide encoding a product of interest in a significantly increase in the abundance of obtained cell clones that express the recombinant product with improved stability

characteristics. Thus, with the FAM60A gene, a key gene was identified that influences the stability of recombinant expression. Additionally impairing the effect of FAM60A in the cells allows to significantly improve the recombinant production of a product of interest by increasing the expression stability. As is shown in the examples, impairing the effect of FAM60A, e.g. by gene knock-out, significantly improves the stability characteristics. Pronounced losses in expression stability during prolonged culturing are rarer with respective host cells and furthermore, if occurring, result in a less dramatic decrease in the productivity compared to cells wherein the genome is not altered to impair the effect of protein FAM60A in the cell. The abundance of stable clones is increased upon stable transfection. Therefore, stability analyses for identifying host cells that are not or less prone to instability during prolonged cultivation may be shortened or even skipped. This is an important advantage as it shortens the time that is required for obtaining stably expressing cell clones that express the recombinant product of interest with good yield over a prolonged time period and which accordingly, are suitable for large scale production purposes. This significantly reduces the screening effort. Therefore, impairing the effect of FAM60A and C12orf35 in the same cell is particularly advantageous, because eukaryotic host cells are provided which show improved characteristics with respect to expression stability and yield. Thereby, eukaryotic host cells having particularly advantages properties for the production of a recombinant product of interest are provided. As described herein, the eukaryotic cell preferably is a mammalian cell.

[53] FAM60A is a sub-unit of the SIN3-histone deacetylase (HDAC) complex (SIN3/HDAC complex) that functions in transcriptional repression (Munoz et al., 2012, THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 39, pp. 32346-32353; Smith et al., 2012, Mol Cell Proteomics 11 (12): 1815-1828). Histone deacetylases (HDAC) catalyze the removal of acetyl groups from histones. Acetylation of histones on lysines is a major mechanism for modulating chromatin conformation. Histone acetylation promotes a relaxed, transcriptionally active chromatin state whereas deacetylation catalyzed by histone deacetylases (HDACs) favor a silent, inactive state. Database analysis revealed the presence of at least one FAM60A ortholog in most metazoans, but not in nematodes. The FAM60A gene is conserved in metazoans and can be found in all vertebrate and most invertebrate genomes that have been completely sequenced. E.g. a 100% sequence identity of FAM60A protein can be found between human, rat, mouse and cow. Sequence similarity research of FAM60A homologs indicates that predominantly, there is only a single representative member of this family in the genome. There are only few exceptions. According to the Smith et al, 2012, the FAM60A protein has a unique sequence lacking any known protein domains. Moreover, it was described by Smith et al 2012, that it does not exhibit any sequence homology to other known proteins in the human proteome. Sequence comparison between FAM60A proteins from different species showed that the FAM60A protein generally comprises three regions: (1) an N-terminus comprising highly conserved segments in all metazoans (2) a middle region which is highly conserved across vertebrates whereas in invertebrates it consists of a non-conserved spacer of a variable length (3) a C-terminus comprising highly conserved segments in all metazoans. Thus, highest conservation was observed in the FAM60A N- and C- terminal regions. As described above, research indicates that FAM60A associates with SIN3/HDAC complexes in various eukaryotic cell types such as in particular mammalian

cells. However, to date, functional information about FAM60A is quite restricted. Recent functional studies (see Smith et al, 2012) indicate that FAM60A may repress gene expression and regulates a specific subset of genes. Smith et al 2012 report a role of FAM60A in the regulation of the TGF-beta signaling pathway, which plays a pivotal role in processes like cancer progression, metastasis, cell migration and immune surveillance. There are findings indicating that FAM60A acts as a transcriptional repressor of components of the TGF-beta signaling pathway whereas this FAM60A function seems to be permitted via its role in the SIN3-HDAC complex. Depletion of FAM60A in different cancer cell lines using siRNA against FMA60A resulted in a change of normal cancer cell morphology. Furthermore, it was found that FAM60A protein levels do periodically change within the course of the cell cycle in U2OS cells (Munoz et al, 2012). FAM60A knock-down experiments using FAM60A siRNA in U2OS human bone osteosarcoma cells revealed that FAM60A restrains cyclin D1 gene expression. Against this scientific background, it was highly surprising to find that impairing the effect of protein FAM60A in a eukaryotic cell such as preferably a mammalian cell, significantly increases the stability of heterologous gene expression in said cell without negatively affecting other characteristics of the cell that are important for recombinant expression. This correlation between the effects of protein FAM60A and the expression stability during prolonged culturing of the cells was unexpected.

[54] As described, the FAM60A gene is endogenously expressed in metazoan and hence in mammalian species such as human, mouse, rat and hamster and the amino acid sequence of FAM60A is highly conserved in mammalian species as well as in vertebrates. The altered eukaryotic cell in which the effect of FAM60A is impaired is derived from a eukaryotic cell which endogenously expresses FAM60A. For the ease of simplicity, the protein FAM60A as well as the FAM60A gene encoding protein FAM60A is spelled herein in capital letters even though in some species a different spelling is used for the gene and/or the protein. The sequence listing shows exemplary amino acid sequences of known and/or predicted FAM60A proteins of different vertebrate species, namely Homo sapiens (SEQ ID NO: 11), Rattus norvegicus (SEQ ID NO: 12), Mus musculus (SEQ ID NO: 13), Cricetulus griseus (SEQ ID NO: 14), Gallus gallus (SEQ ID NO: 15), Pan troglodytes (SEQ ID NO: 16), Pongo abelii (SEQ ID NO: 17) and Bos taurus (SEQ ID NO: 18). The predicted FAM60A cDNA of Cricetulus griseus is shown in SEQ ID NO: 19 (coding sequence from 14-679; see also NCBI Reference Sequence: XM_003505482.1). Different names can be assigned for protein FAM60A or the FAM60A gene in different species and non-limiting alternative names (aliases) are also listed above in Table 1. The term "FAM60A" as used herein also encompasses any homologs and orthologs of FAM60A which have the same function as FAM60A. According to one embodiment the term "FAM60A" as used herein in particular refers a protein that shares at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% homology to one or more of the amino acid sequences shown in SEQ ID NO: 11 to 18. According to one embodiment, the foregoing percentage values refer to polypeptide identity instead of homology. Homology, respectively identity, may be calculated over the entire length of the reference protein. A respective protein preferably has the same function as the protein having an amino acid sequence that is shown in SEQ ID NO: 11 or one or

more of SEQ ID NO: 12 to 18, preferably SEQ ID NO: 14. The FAM60A protein has not been described in detail in the literature. Thus, it was highly surprising that the expression stability of a recombinant host cell can be improved, if the genome of the host cell is altered so that the effect of endogenous protein FAM60A is impaired in the cell, as can be e.g. achieved by reducing or eliminating the functional expression of the FAM60A gene in said cell. It was unexpected that FAM60A influences the expression stability of a recombinant product of interest. According to one embodiment, the FAM60A gene encoding the FAM60A protein is altered in order to impair the effect of FAM60A in the cell. This can be achieved e.g. by genetic engineering technologies such as gene knock-out technologies as are e.g. described herein. The genomic gene sequence of different mammalian species is known, and is e.g. decribed in Homo sapiens (NCBI Gene-ID: 58516); Rattus norvegicus (NCBI Gene-ID: 686611); Mus musculus (NCBI Gene-ID: 56306); Bos Taurus (NCBI Gene-ID: 538649) and others. Transcript variants may exist in a species-dependent manner and in different number. E.g. the human FAM60A gene expresses 3 putative transcript isoforms which differ in the UTRs but encode the same protein.

[55] According to one embodiment, expression of gene FAM60A is reduced by at least 3 fold, at least 5 fold, at least 10fold, at least 20fold, at least 30fold, at least 40fold, at least 50fold, at least 60fold, at least 70fold, at least 75fold, at least 80fold, at least 90fold, at least 100fold or at least 125fold, at least 250fold, at least 500fold, at least 750fold, at least 1000fold, at least 1250fold, at least 1500fold, at least 1750fold, at least 2000fold, at least 2500fold, at least 3000fold or at least 3500fold. Expression can be determined e.g. by using real-time RT-PCR or other sensitive RNA detection methods. Such reduction can be achieved e.g. in comparison with the unmodified reference cell wherein the expression of endogenous gene FAM60A is not reduced. According to one embodiment, expression of gene FAM60A is 0.05% or less, 0.04% or less, 0.03% or less, 0.02% or less, 0.01% or less, 0.005% or less or 0.0025% or less compared to the expression of the 18S RNA (set as 100%) in the same cell. According to one embodiment, expression of gene FAM60A is even less, such as 0.001% or less, 0.0005% or less or even 0.0002 or less compared to the expression of the 18S RNA (set as 100%) in the same cell.

[56] According to one embodiment, the isolated eukaryotic cell originates from a population of eukaryotic cells which are altered so that additionally the effect of protein FAM60A is impaired in said cells and wherein said cells comprise stably integrated into their genome a heterologous polynucleotide encoding a product of interest, wherein on average at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85% or at least 90% of the cells originating from said population do not lose more than 30%, preferably not more than 25%, of their product of interest expression titer over a time period of at least 8 weeks, preferably 10 weeks, more preferably over a time period of 12 weeks. As is shown in the examples, after transfection and identification of stably transfected cells, the amount of cells which do not show a gradual loss in productivity during prolonged culturing is increased when using the altered cells described herein, i.e. more stable cell clones are obtained from a selected cell population. The stability property can be tested, by cultivating individual cells from said population as cell clones and determining the titer over the indicated time period.

The stability can be tested using e.g. the assays described in the examples. The stability rates can vary from project to project depending on the expressed protein and whether it is e.g. codon-optimized. However, with the altered eukaryotic cells according to the present disclosure, in all projects analyzed, a significant increase in stably expressing clones was observed compared to the wildtype cells wherein the effect of FAM60A is not impaired. The abundance of cells with stable expression characteristics was significantly increased in the population of successfully transfected host cells. Therefore, the risk that an instable clone which gradually loses productivity during prolonged culturing is chosen for large scale production is significantly reduced with this embodiment wherein the effects of FAM60A and C12orf35 impaired in the cell. This important advantage allows to significantly reduce or even completely skip long terms stability analyses that are usually performed in order to eliminate instable clones.

[57] The eukaryotic cell is derived from a species which endogenously expresses the C12orf35 gene. Furthermore, the eukaryotic cell is derived from a cell type that usually expresses said gene. Examples are described below. The term "isolated" is used to render clear that the eukaryotic cell is not contained in a living organism such as an animal or human. As described herein, the cell can be provided as cell culture, cell line, cell clone and the like. Examples are also described below. As is described above, said eukaryotic cell is modified to impair the effect of the expression product of gene C12orf35 in said cell compared to a corresponding unmodified eukaryotic cell which endogenously expresses C12orf35. Impairment is preferably achieved by reducing or eliminating functional expression of gene C12orf35. Embodiments are described above. In order to provide production cell lines with uniform and thus predictable characteristics, it is preferred to alter the genome of the eukaryotic cell to achieve impairment. Suitable embodiments are described above. The respectively altered eukaryotic cell can then be transfected with an expression vector comprising a polynucleotide encoding a product of interest. The eukaryotic cell may be a metazoan cell such as a vertebrate cell, preferably a mammalian cell. Thus, all embodiments described herein for eukaryotic cells in general apply to the preferred embodiment wherein mammalian cells are used. The eukaryotic cell may be e.g. selected from the group consisting of rodent cells, human cells and monkey cells. Preferred eukaryotic cells are rodent cells such as e.g. cells derived from hamster or mouse. They can be selected from the group consisting of a Chinese hamster cell such as a CHO cell, a BHK cell, a NS0 cell, a C127 cell, a mouse 3T3 fibroblast cell, and a SP2/0 cell. Particularly preferred is a CHO cell. Examples for CHO cells are CHO-K1, CHO-S, CHO-K1SV, CHO-SSF3, CHO-DG44, CHO-DUXB11, or a cell line derived therefrom. As is shown in the examples, reducing or eliminating the expression of gene C12orf35 in a CHO cell provides CHO cells which are capable of producing a recombinant product with significant increased yield. The C12orf35 gene is also expressed in human cells. Thus, according to one embodiment, the eukaryotic cell is derived from a human cell, which may be e.g. selected from the group consisting of a HEK293 cell, a MCF-7 cell, a PerC6 cell, a CAP cell, hematopoietic cells and a HeLa cell. Another alternative are monkey cells, which, e.g. may be selected from the group consisting of a COS cells, COS-1, a COS-7 cell and a Vero cell. According to one embodiment the eukaryotic cell, which preferably is a mammalian cell, is provided as cell clone or cell line.

[58] According to one embodiment, the eukaryotic cell does not comprise a heterologous polynucleotide encoding a product of interest, a heterologous polynucleotide encoding a selectable marker and/or a heterologous polynucleotide encoding a reporter polypeptide that is/are expressed, in particular secreted from said cell. A respective "empty" eukaryotic cell can be used e.g. as cloning cell line for recombinant production technologies. A respective cell can be transfected with a heterologous polynucleotide encoding a product of interest, e.g. using an appropriate expression vector. Such "empty" eukaryotic cells in which the effect of the expression product of gene C12orf35 is impaired and which do not yet express and in particular do not secrete a recombinant product can thus be transfected with different expression vectors, depending on the desired product of interest that is supposed to be recombinantly produced. Thus, such eukaryotic cell line can be used for different projects, i.e. for the production of different products of interest, in particular secreted polypeptides of interest. Transfection can be transient or stable and as is demonstrated by the examples, improved expression yields are observed in both embodiments.

[59] According to one embodiment, the eukaryotic cell comprises a heterologous polynucleotide encoding a product of interest. The product of interest is the recombinant product that is supposed to be expressed by the eukaryotic cell in large quantity. Preferably, the product of interest is a polypeptide. Furthermore, the eukaryotic cell may additionally comprise a heterologous polynucleotide encoding a selectable marker and/or a heterologous polynucleotide encoding a reporter. This simplifies the selection of host cells which are successfully transfected and thus express the product of interest. Furthermore, the eukaryotic cell may comprise several polynucleotides encoding different selectable markers and/or reporter polypeptides. According to one embodiment, the heterologous polynucleotide encoding the product of interest is stably integrated into the genome of the eukaryotic cell according to the first aspect.

[60] A "heterologous polynucleotide" or "heterologous nucleic acid" and likewise expressions used herein in particular refer to a polynucleotide sequence that has been introduced into the eukaryotic cell e.g. by the use of recombinant techniques such as transfection. A "polynucleotide" in particular refers to a polymer of nucleotides which are usually linked from one deoxyribose or ribose to another and refers to DNA as well as RNA, depending on the context. The term "polynucleotide" does not comprise any size restrictions.

[61] An expression vector can be used to introduce heterologous polynucleotides. The polynucleotides can be comprised in an expression cassette. The polynucleotide(s) encoding the product of interest and the polynucleotide(s) encoding a selectable marker or reporter polypeptide may be located on the same or different expression vectors. Introduction into the eukaryotic cell may be achieved e.g. by transfecting a suitable expression vector comprising the polynucleotide encoding the product of interest into the host cells. The expression vector preferably integrates into the genome of the host cell (stable transfection). In case the heterologous nucleic acid is not inserted into the genome, the heterologous nucleic acid can be lost at the later stage e.g. when the cells undergo mitosis (transient transfection). As is

shown by the examples, the novel eukaryotic cells described herein are advantageous for both embodiments, i.e. transient as well as stable transfection. Stable transfection is preferred for generating high expressing cell clones for producing a product of interest such as a polypeptide of interest on industrial scale. This is particularly important for therapeutic or diagnostic polypeptides of interest. Several appropriate methods are known in the prior art for introducing a heterologous nucleic acid such as an expression vector into eukaryotic such as mammalian host cells and thus, do not need any detailed description herein. Respective methods include but are not limited to calcium phosphate transfection, electroporation, lipofection, biolistic- and polymer-mediated genes transfer and the like. Besides traditional random integration based methods also recombination mediated approaches can be used to transfer the heterologous polynucleotide into the host cell genome. As respective methods are well known in the prior art, they do not need any detailed description here. Non-limiting embodiments of suitable vector designs are also described subsequently and it is referred to the respective disclosure.

[62] Expression vectors used to achieve expression of a recombinant product of interest usually contain transcriptional control elements suitable to drive transcription such as e.g. promoters, enhancers, polyadenylation signals, transcription pausing or termination signals usually as element of an expression cassette. If the desired product is a polypeptide, suitable translational control elements are preferably included in the vector, such as e.g. 5' untranslated regions leading to 5' cap structures suitable for recruiting ribosomes and stop codons to terminate the translation process. The resultant transcripts harbour functional translation elements that facilitate protein expression (i.e. translation) and proper translation termination. A functional expression unit, capable of properly driving the expression of an incorporated polynucleotide is also referred to as an "expression cassette". It is well-known to the skilled person how an expression cassette shall be designed in order to allow the expression in a eukaryotic cell, such as preferably in a mammalian cell.

[63] The polynucleotide(s) encoding the product of interest and the polynucleotides encoding the selectable marker(s) and/or reporter polypeptide(s) as described herein are preferably comprised in expression cassettes. Several embodiments are suitable. For example, each of said polynucleotide(s) can be comprised in a separate expression cassette. This is also referred to as monocistronic setting. It is also within the scope of the present invention that at least two of the respective polynucleotides are comprised in one expression cassette. According to one embodiment, at least one internal ribosomal entry site (IRES) element is functionally located between the polynucleotides that are expressed from the same expression cassette. Thereby, it is ensured that separate translation products are obtained from said transcript. Respective IRES based expression technologies and other bi- and polycistronic systems are well known and thus need no further description here.

[64] As described, the expression vector may comprise at least one promoter and/or promoter/enhancer element as element of an expression cassette. Promoters can be divided in two classes, those that function constitutively and those that are regulated by induction or derepression. Both are suitable. Strong constitutive promoters which drive expression in

many cell types include but are not limited to the adenovirus major late promoter, the human cytomegalovirus immediate early promoter, the SV40 and Rous Sarcoma virus promoter, and the murine 3-phosphoglycerate kinase promoter, EF1a. According to one embodiment, the promoter and/or enhancer is either obtained from CMV and/or SV40. The transcription promoters can be selected from the group consisting of an SV40 promoter, a CMV promoter, an EF1alpha promoter, a RSV promoter, a BROAD3 promoter, a murine rosa 26 promoter, a pCEFL promoter and a β-actin promoter. Also other promoters can be used if they result in expression of the product of interest in the eukaryotic cell which preferably is a mammalian cell.

[65] Furthermore, an expression cassette may comprise at least one intron. Usually, introns are placed at the 5' end of the open reading frame but may also be placed at the 3' end. Said intron may be located between the promoter and or promoter/enhancer element(s) and the 5' end of the open reading frame of the polynucleotide encoding the product of interest to be expressed. Several suitable introns are known in the state of the art that can be used in conjunction with the present disclosure.

[66] The product of interest can be any biological product capable of being produced by transcription, translation or any other event of expression of the genetic information encoded by the polynucleotide encoding the product of interest. The product of interest may be selected from the group consisting of polypeptides and nucleic acids, in particular RNA. The product can be a pharmaceutically or therapeutically active compound, or a research tool to be utilized in assays and the like. Preferably, the product of interest is a polypeptide. Any polypeptide of interest can be expressed with the method of the present invention. The term "polypeptide" refers to a molecule comprising a polymer of amino acids linked together by a peptide bond(s). Polypeptides include polypeptides of any length, including proteins (e.g. having more than 50 amino acids) and peptides (e.g. 2 – 49 amino acids). Polypeptides include proteins and/or peptides of any activity, function or size, and may include e.g. enzymes (e.g. proteases, kinases, phosphatases), receptors, transporters, bactericidal and/or endotoxin-binding proteins, structural polypeptides, membrane-bound polypeptides, glycoproteins, globular proteins, immune polypeptides, toxins, antibiotics, hormones, growth factors, blood factors, vaccines or the like. The polypeptide may be selected from the group consisting of peptide hormones, interleukins, tissue plasminogen activators, cytokines, immunoglobulins, in particular antibodies or functional antibody fragments or variants thereof and Fc-fusion proteins. The polypeptide of interest that is expressed according to the teachings described herein may also be a subunit or domain of a polypeptide, such as e.g. a heavy chain or a light chain of an antibody or a functional fragment or derivative thereof. The terms "product of interest" or "polypeptide of interest" may refer to such individual subunit or domain or the final protein that is composed of the respective subunits or domains, depending on the context. In a preferred embodiment the polypeptide of interest is an immunoglobulin molecule, more preferably an antibody, or a subunit or domain thereof such as e.g. the heavy or light chain of an antibody. The term "antibody" as used herein particularly refers to a protein comprising at least two heavy chains and two light chains connected by disulfide bonds. The term "antibody" includes naturally occurring antibodies as

well as all recombinant forms of antibodies, e.g., humanized antibodies, fully human antibodies and chimeric antibodies. Each heavy chain is usually comprised of a heavy chain variable region (VH) and a heavy chain constant region (CH). Each light chain is usually comprised of a light chain variable region (VL) and a light chain constant region (CL). The term "antibody", however, also includes other types of antibodies such as single domain antibodies, heavy chain antibodies, i.e. antibodies only composed of one or more, in particular two heavy chains, and nanobodies, i.e. antibodies only composed of a single monomeric variable domain. As discussed above, the polynucleotide encoding the polypeptide of interest may also encode one or more subunits or domains of an antibody, e.g. a heavy or a light chain or a functional fragment or derivative thereof, as polypeptide of interest. Said subunits or domains can be expressed either from the same or different expression cassettes. A "functional fragment or derivative" of an antibody in particular refers to a polypeptide which is derived from an antibody and is capable of binding to the same antigen, in particular to the same epitope as the antibody. It has been shown that the antigen-binding function of an antibody can be executed by fragments of a full-length antibody or derivatives thereof. Examples of fragments or derivatives of an antibody include (i) Fab fragments, monovalent fragments consisting of the variable region and the first constant domain of each the heavy and the light chain; (ii) $F(ab)_2$ fragments, bivalent fragments comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) Fd fragments consisting of the variable region and the first constant domain CH1 of the heavy chain; (iv) Fv fragments consisting of the heavy chain and light chain variable region of a single arm of an antibody; (v) scFv fragments, Fv fragments consisting of a single polypeptide chain; (vi) $(Fv)_2$ fragments consisting of two Fv fragments covalently linked together; (vii) a heavy chain variable domain; and (viii) multibodies consisting of a heavy chain variable region and a light chain variable region covalently linked together in such a manner that association of the heavy chain and light chain variable regions can only occur intermolecular but not intramolecular. According to one embodiment, the eukaryotic cell secretes the recombinant polypeptide of interest into the cell culture medium. According to one embodiment, the polypeptide of interest is not or does not comprise a C12orf35 protein.

[67] The eukaryotic cell may or may not comprise an endogenous polynucleotide corresponding to, respectively being identical to the polynucleotide encoding the product of interest. According to one embodiment, the eukaryotic cell does not comprise an endogenous gene corresponding to the product of interest.

[68] As described, in preferred embodiments, the eukaryotic cell comprises at least one heterologous polynucleotide encoding a selectable marker and/or a heterologous polynucleotide encoding a reporter polypeptide in addition to the heterologous polynucleotide encoding the product of interest.

[69] A “selectable marker” allows under appropriate selective culture conditions the selection of host cells expressing said selectable marker. A selectable marker provides the carrier of said marker under selective conditions with a survival and/or growth advantage. Thereby, host cells successfully transfected with the expression vector can be selected under

appropriate selection conditions. Typically, a selectable marker gene will confer resistance to a selection agent such as a drug, e.g. an antibiotic or other toxic agent, or compensate for a metabolic or catabolic defect in the host cell. It may be a positive or negative selection marker. For selecting successfully transfected host cells a culture medium may be used for culturing the host cells comprises a selection agent that allows selection for the selectable marker used. In other embodiments, the selection marker enables the host cell to survive and proliferate in the absence or reduction of a compound which is essential for survival and/or proliferation of the host cells lacking the selection marker. By cultivating the host cells in a medium which does not comprise the essential compound in a concentration high enough for survival and/or proliferation of the host cell or comprises a reduced amount of said essential compound, only host cells expressing the selection marker can survive and/or proliferate. According to one embodiment, the selectable marker is a drug resistance marker encoding a protein that confers resistance to selection conditions involving said drug. A variety of selectable marker genes have been described (see, e.g., WO 92/08796, WO 94/28143, WO2004/081167, WO2009/080759, WO2010/097240). E.g. at least one selectable marker may be used which confers resistance against one or more antibiotic agents. The selectable marker may according to one embodiment be an amplifiable selectable marker. An amplifiable selectable marker allows the selection of vector containing host cells and may promote gene amplification of said vector in the host cells. Selectable marker genes commonly used with eukaryotic cells such as in particular mammalian cells include the genes for aminoglycoside phosphotransferase (APH), hygromycin phosphotransferase (hyg), dihydrofolate reductase (DHFR), thymidine kinase (tk), glutamine synthetase, asparagine synthetase, and genes encoding resistance to neomycin (G418), puromycin, hygromycin, zeocin, ouabain, blasticidin, histidinol D, bleomycin, phleomycin and mycophenolic acid. According to one embodiment, a folate receptor is used as selectable marker in conjunction with the novel eukaryotic cells described herein (see e.g. WO2009/080759), which preferably are mammalian cells. According to one embodiment, the eukaryotic cell comprises a heterologous polynucleotide encoding a folate receptor as selectable marker and/or comprises a heterologous polynucleotide encoding a dihydrofolate reductase (DHFR) as selectable marker. This embodiment will also be described in detail below in conjunction with the selection method according to the second aspect. The eukaryotic cell may express endogenously DHFR and a folate receptor.

[70] A "reporter polypeptide" allows the identification of a cell expressing said reporter polypeptide based on the reporting characteristics (e.g. fluorescence). Reporter genes usually do not provide the host cells with a survival advantage. However, the expression of the reporter polypeptide can be used to differentiate between cells expressing the reporter polypeptide and those cells which do not. Therefore, also a reporter gene enables the selection of successfully transfected host cells. Suitable reporter polypeptides include but are not limited to as e.g. green fluorescence protein (GFP), YFP, CFP and luciferase. According to one embodiment, the reporter polypeptide has characteristics that enable the selection by flow cytometry.

[71] As described, the expression vector comprising the polynucleotide encoding the product of interest may also comprise more than one selectable marker and/or reporter gene. Furthermore, the one or more polynucleotides encoding the selectable marker(s) and/or the one or more polynucleotides encoding the reporter polypeptide(s) may also be provided on one or more different expression vectors which are co-transfected with the expression vector which comprises the polynucleotide encoding the product of interest. Such co-transfection strategies likewise enable selection as is well-known in the prior art.

[72] The expression vector or the combination of at least two expression vectors comprised in the eukaryotic cell may additionally comprise further vector elements. E.g. at least one additional polynucleotide encoding a further product of interest can be comprised. As explained above and as becomes apparent from the above described examples of polypeptides that can be expressed according to the present teachings, the final polypeptide that is to be produced and preferably secreted by the host cell can also be a protein that is composed of several individual subunits or domains. A preferred example of a respective protein is an immunoglobulin molecule, in particular an antibody that comprises e.g. heavy and light chains. There are several options for producing a respective protein that is composed of different individual subunits or domains and appropriate vector designs are known in the art. According to one embodiment, two or more subunits or domains of said protein are expressed from one expression cassette. In this embodiment, one long transcript is obtained from the respective expression cassette that comprises the coding regions of the individual subunits or domains of the protein. According to one embodiment, at least one IRES element (internal ribosomal entry site) is functionally located between the coding regions of the individual subunits or domains and each coding region is preceded by a secretory leader sequence. Thereby, it is ensured that separate translation products are obtained from said transcript and that the final protein can be correctly assembled and secreted. Respective technologies are known in the prior art and thus, do not need any detailed description herein.

[73] For some embodiments such as the expression of antibodies it is even preferred to express the individual subunits or domains from different expression cassettes. According to one embodiment, the expression cassette used for expressing the product of interest is a monocistronic expression cassette. All expression cassettes comprised in the expression vector or combination of expression vectors may be monocistronic. According to one embodiment, accordingly, each expression cassette designed for expressing a product of interest comprises a polynucleotide encoding one subunit or domain of the protein to be expressed as polypeptide of interest. E.g. in case of antibodies, one expression cassette may encode the light chain of an antibody and another expression cassette may encode the heavy chain of the antibody. After expression of the individual subunits or domains from the individual expression cassettes, the final protein such as an antibody is assembled from said subunits or domains and secreted by the host cell. This embodiment is particularly suitable for expressing immunoglobulin molecules such as antibodies. In this case, a first heterologous polynucleotide encoding a product of interest encodes e.g. the heavy or the light chain of an immunoglobulin molecule and a second heterologous polynucleotide

encoding a product of interest encodes the other chain of the immunoglobulin molecule. According to one embodiment, the expression vector or combination of at least two expression vectors used for transfecting the mammalian host cell comprises at least one expression cassette comprising a polynucleotide encoding the heavy chain of an immunoglobulin molecule or a functional fragment thereof and at least one expression cassette comprising a polynucleotide encoding the light chain of an immunoglobulin molecule or a functional fragment thereof. Said polynucleotides may be located on the same or on different expression vectors in case a combination of at least two expression vectors is used. Upon expression of said polynucleotides in the transfected host cell, a functional immunoglobulin molecule is obtained and preferably is secreted from the host cell. As is shown by the examples, using the novel cells and cell lines described herein wherein the effect of the expression product of gene C12orf35 is impaired, preferably by reducing or eliminating functional expression of said gene, are particularly suitable for expressing proteins, in particular proteins that are composed of several subunits or domains such as antibodies. As is shown by the examples, the overall amount of antibody that is expressed by said eukaryotic cells is increased. Furthermore, in embodiments, in particular wherein the effect of FAM60A is additionally impaired in the cell, also the expression stability was improved. Therefore, the novel eukaryotic cell lines described herein have particular advantages when being used for recombinant expression of polypeptides, including proteins that are composed of several subunits of domains such as e.g. antibodies. Further advantages are also described in conjunction with the examples.

B. Selection method

[74] According to a second aspect, a method for selecting a host cell which recombinantly expresses a product of interest is provided, comprising

(a) providing eukaryotic cells according to the first aspect as host cells, wherein said host cells comprise at least one heterologous polynucleotide encoding the product of interest; and
(b) selecting one or more host cells expressing the product of interest.

[75] The eukaryotic cells according to the first aspect, including suitable and preferred embodiments, as well as their advantages are described in detail above and it is referred to the respective disclosure which also applies here. As described, vertebrate cells, in particular mammalian cells, are preferably used as host cells. The advantageous properties of said eukaryotic cells simplify e.g. the selection and thus identification of suitable production clones. Furthermore, as the proportion of high producing cells in the transfected cell population is significantly increased when using the eukaryotic cells described herein, less clones need to be analysed and screened for their production characteristics in order to identify suitable production clones. This saves time and furthermore, allows handling more projects in parallel. Furthermore, as is demonstrated by the examples, the number respectively proportion of high expressing cells is increased after transfection and selection steps when using said novel eukaryotic cells. Such expressing cells, also called cell pools, produce significant quantities of the product of interest. Thus, such cell pools comprising high

expressing cells can be e.g. used in order to produce a polypeptide of interest within a short timeframe. Therefore, the product of interest can be produced rapidly in respective cells.

[76] According to one embodiment, stage (a) of the selection method according to the second aspect comprises transfecting eukaryotic cells according to the first aspect, which preferably do not yet comprise a heterologous polynucleotide encoding the product of interest, with a heterologous polynucleotide encoding the product of interest, thereby providing eukaryotic cells which comprise a heterologous polynucleotide encoding the product of interest. As described, mammalian cells are preferably used as eukaryotic host cells. The polynucleotide encoding the product of interest may be comprised in an expression vector that is then transfected into the eukaryotic cell.

[77] Selection stage (b) may be a multi-step selection process comprising several selection steps in order to select and thus identify host cells that express a product of interest with high yield. For example, stage (b) may include one or more selection steps to identify cells that were successfully transfected as well as one or more subsequent selection steps to select high expressing cells from the pool of successfully transfected cells. The appropriate selection strategy depends on the design of the expression vector that is used for introducing the polynucleotide encoding the product of interest and in particular depends on the used selection marker(s) and/or reporter(s). Non-limiting embodiments will be described in the following.

[78] As described above, the eukaryotic host cells may comprise at least one heterologous polynucleotide encoding a selectable marker. The polynucleotide encoding the selectable marker can be introduced into the host cell together with the polynucleotide encoding the product of interest using either the same or a different co-transfected expression vector. Stage (b) then comprises culturing said plurality of host cells under conditions providing a selection pressure to the host cells to identify successfully transfected host cells, e.g. using an appropriate selection medium. As used herein, a "selection medium" in particular refers to a cell culture medium useful for the selection of host cells that express the selectable marker. It may include e.g. a selection agent such as a toxic drug which allows to identify successfully transfected host cells. Alternatively, an essential compound can be absent or its concentration can be reduced in the selection medium. According to one embodiment, host cells which were not successfully transfected and hence, do not express the selection marker(s) or wherein expression is low cannot proliferate or die under the selective cultivation conditions. In contrast, host cells which were successfully transfected with the expression vector(s) and which express the selection marker(s) (and accordingly the co-introduced product of interest) with sufficient yield are resistant to or are less affected by the selection pressure and therefore, can proliferate, thereby outgrowing the host cells which were not successfully transfected or wherein the integration site into the genome of the cell is not favourable. According to one embodiment, the selectable marker is selected from the group consisting of antibiotic resistance markers, drug resistance markers and metabolic markers. Suitable examples for selectable markers and selection principles are described above in conjunction with the first aspect and appropriate selection conditions for the

individual selectable markers are also well-known to the skilled person. Non-limiting but advantageous embodiments will be described briefly subsequently.

[79] As is shown by the examples, one advantage of the novel eukaryotic cells described herein, such as in particular a CHO cell line comprising a deletion in the telomeric region of chromosome 8 which includes gene C12orf35, is that already after one standard selection step, such as e.g. a G418/neo selection, the percentage of high-producing cells is significantly increased. For example, when transfecting normal CHO cell lines, wherein the expression of gene C12orf35 is not reduced or eliminated, often 60% or even up to 80% of the cells surviving G418/neo selection are non-producers. The number of non- or low producers is significantly reduced when using the novel eukaryotic cell lines according to the present disclosure. This allows to efficiently perform the selection in stage (b) by using only one selectable marker and thus only one selection step, such as for example a G418/neo selection, if desired. This increases the speed of selection and accordingly, reduces the time required for obtaining transfected cells that express the product of interest. Furthermore, because the number of high producers is increased, it was found that even in the absence of a specific selection for high producing cells cell populations can be generated with clone like performance and thus, within very short time. Therefore, the product of interest can even be manufactured from such pools. Thus, for applications wherein e.g. only certain amounts of protein are needed quickly, for example for research or testing purposes, such a rapid selection system which quickly renders good producing cells or producing cell pools is highly advantageous.

[80] However, if it is desired to further increase the productivity, a multi-step selection in stage (b) is preferred. This is particularly the case when intending to establish a clonal cell line for producing the product of interest on large scale, in particular industrial scale.

[81] As described above, the expression vector or expression vectors introduced into the eukaryotic cells may comprise two or more selectable marker genes and selection for the different selectable markers may be done simultaneously or sequentially for selecting host cells which were successfully transfected with the expression vector(s) and express the product of interest with high yield. The selection medium used for cultivation may comprise selection agents for all selectable markers used. In another embodiment, cultivation can be performed first with a selection medium only comprising the selection agent(s) of one or a subset of the selectable marker genes used, followed by the addition of one or more of the selection agents for the remaining selectable marker genes. In another embodiment, the host cells are cultivated with a first selection medium only comprising the selection agent(s) of one or a subset of the selectable marker genes of the vector(s), followed by cultivation with a second selection medium comprising the selection agent(s) of one or more of the selection agents of the remaining selectable marker genes. According to one embodiment, the second selection medium does not comprise the selection agent(s) used in the first selection medium.

[82] According to one embodiment, the eukaryotic cells provided in stage (a) comprise a heterologous polynucleotide encoding a dihydrofolate reductase (DHFR) as selectable marker and stage (b) comprises performing a selection step for DHFR. A respective selection step usually involves a selection medium comprising a DHFR inhibitor. Several suitable DHFR enzymes and accordingly DHFR genes are known in the prior art that can be used as selectable marker. The term DHFR refers to wild type DHFR as well as to DHFR enzymes having one or more amino acid sequence exchanges (e.g. deletions, substitutions or additions) with respect to the amino acid sequence of the corresponding wildtype DHFR enzyme, fusion proteins comprising a DHFR enzyme and DHFR enzymes which have been modified to provide an additional structure and/or function, as well as functional fragments of the foregoing, which still have at least one function of a DHFR enzyme. Such embodiments are well-known in the prior art and thus, do not need to be described in detail. For example, a DHFR enzyme may be used as selectable marker that is more or less sensitive to antifolates such as MTX than the wild type DHFR enzyme and/or the DHFR enzyme endogenously expressed by the host cell if expressed. Respective DHFR enzymes are well-known in the prior art and e.g. are described in EP 0 246 049 and other documents. The DHFR enzyme can be derived from any species as long as it will be functional within the present invention, i.e. compatible with the mammalian host cell utilised. E.g. a mutant mouse DHFR with a major resistance to MTX has been extensively used as a dominant selectable marker in eukaryotic cells. A DHFR enzyme may be used as selectable marker which is less susceptible to a DHFR inhibitor such as MTX than the DHFR enzyme endogenously expressed in a DHFR $^{+}$ (plus) host cell and thus a host cell which comprises a functional endogenous DHFR gene. According to one embodiment, an intron or a fragment thereof is placed at the 3' end of the open reading frame of the DHFR gene. The intron used in the DHFR expression cassette is leading to a smaller, non-functional variant of the DHFR gene (Grillari et al., 2001, J. Biotechnol. 87, 59-65). Thereby, the expression level of the DHFR gene is lowered which further increases the stringency of selection. Alternative methods making use of an intron to reduce the expression level of the DHFR gene are described in EP0 724 639 and could also be used.

[83] A "DHFR inhibitor" in particular refers to a compound which inhibits the activity of dihydrofolate reductase (DHFR). A respective inhibitor may for example compete with the DHFR substrate for binding to DHFR. Suitable DHFR inhibitors are for example antifolates such as methotrexate (MTX). Thus, according to one embodiment, stage (b) comprises performing a selection step for DHFR by cultivating said plurality of host cells in a selective culture medium which comprises at least one DHFR inhibitor, such as preferably an antifolate. Respective selection conditions are known to the skilled person. According to one embodiment, a selective culture medium is used in stage (b) which comprises an antifolate in a concentration of 1500nM or less, 1250nM or less, 1000nM or less, 750nM or less, 500nM or less, 250nM or less, 200nM or less, 150nM or less, 125nM or less, 100nM or less or 75nM or less. The used concentration of said inhibitor in the selective culture medium (which may also be increased gradually), determines the stringency of the selection conditions. Preferred concentration ranges for the antifolate may be selected from 500nM – 0.1nM, 350nM – 1nM, 200nM – 2.5nM, 150nM – 5nM, 100nM – 7.5nM and 75nM – 10 nM. According to one

embodiment, the selective culture medium comprises MTX as antifolate. As is shown by the examples, low MTX concentrations can be used in conjunction with the novel eukaryotic cells described herein for performing a DHFR selection, in particular if selection is performed in combination with a limiting concentration of folate.

[84] According to one embodiment, the host cells according to the present disclosure provided in stage (a) comprise a heterologous polynucleotide encoding a folate transporter as selectable marker. A folate transporter based selection system has several advantages when being used in conjunction with eukaryotic cells that are dependent on folate uptake such as mammalian cells. A folate transporter allows to import at least one folate from the culture medium into the host cell which preferably is a mammalian cell. According to one embodiment, the folate transporter is or comprises the reduced folate carrier (RFC). RFC is a ubiquitously expressed membrane glycoprotein that serves as the major transporter for the uptake of reduced folates such as 5-methyl-THF and 5-formyl-THF into the cell. However, RFC displays a very poor affinity for the oxidized folate, folic acid. Hence, cells that lack the expression of RFC or have been deleted from the genomic RFC locus can serve as recipients for the transfection of the selectable marker gene RFC under conditions in which reduced folates such as 5-formyl-THF are gradually deprived from the growth medium, thereby allowing to identify cells that express increased levels of the this folate transporter and accordingly the product of interest. Suitable selection conditions when using RFC as selectable marker are known to the skilled person and are e.g. described in WO2006/059323.

[85] According to one embodiment which is also described in detail below and in the example section, the folate transporter used as selectable marker is a folate receptor. A folate receptor based selection system has several advantages. For example for selection, no toxic substances are needed (even though they can be used) and furthermore, the endogenous folate receptor of the host cell does not need to be knocked out. Furthermore, this expression system works particularly well in conjunction with the novel eukaryotic cell line described herein, wherein the effect of the expression product of gene C12orf35 is reduced or eliminated, preferably by reducing or eliminating the expression of the C12orf35 gene. One explanation is that introduced genes, such as the polypeptide of interest but also selectable marker genes, are expressed at a higher rate which simplifies the selection.

[86] A "folate receptor" as used herein refers to a folate receptor that is functional and thus capable of import or uptake of a folate or derivative thereof into the eukaryotic cell. Preferably, the receptor is capable of unidirectional import or uptake of folate or a derivative of folate into the eukaryotic cell. Furthermore, a folate receptor as used herein is membrane-bound. Thus, the folate receptors described herein are functional membrane-bound folate receptors. Membrane anchorage can be achieved e.g. by a transmembrane anchor or by a GPI anchor. A GPI anchor is preferred as it corresponds to the natural setting of membrane-bound folate receptor. Folate receptors (FRs) are high-affinity folate-binding glycoproteins. They are encoded by three distinct genes FR alpha, FR beta and FR gamma. FR alpha and FR beta are glycosylphosphatidylinositol (GPI)-anchored, cell surface glycoproteins, whereas

FR gamma is devoid of a GPI anchor and is a secreted protein. However, it can be genetically altered to include a transmembrane anchor or a GPI anchor. Such an altered form of a FR gamma that includes a membrane anchor is also considered a membrane-bound folate receptor if it is capable of import or uptake of a folate or derivative thereof into a eukaryotic cell. The term "folate receptor" also includes membrane-bound mutants of wildtype folate receptors that are capable of folate uptake and fusion proteins comprising a respective folate receptor.

[87] The folate receptor utilized can be derived from a folate receptor of any species as long as it will be functional within the present invention, i.e. it is compatible with the host cell that is utilized and when being expressed in the transfected host cell, incorporates folate, in particular folic acid, from the culture medium into the host cell that is dependent on folate uptake. In general, the folate receptor that is introduced into the host cell as selectable marker can be homologous or heterologous to an endogenous folate receptor of the host cell (if an endogenous folate receptor is present what is preferred). If it is homologous, it will be derived from the same species as the host cell. If it is heterologous, it will be derived from another species than the host cell. For example a human folate receptor may be used as selectable marker for a rodent host cell, e.g. a hamster cell such as a CHO cell. Preferably, a folate receptor derived from a mammalian species is used, for example derived from a rodent, such as mouse, rat or hamster, or, more preferred, derived from a human. The membrane-bound folate receptor used as selectable marker can be selected from the group consisting of a folate receptor alpha, a folate receptor beta and a folate receptor gamma. According to one embodiment, a human folate receptor alpha is used as selectable marker.

[88] The use of a membrane-bound folate receptor as folate transporter is advantageous because cells can be used, which endogenously express their own folate receptors and folic acid can be processed by the folate receptor. Suitable membrane-bound folate receptors that can be used as selectable markers for eukaryotic cells that are dependent on folate uptake such as mammalian cells and suitable selection conditions are also described in WO 2009/080759, herein incorporated by reference. According to one embodiment, the mature wild type human folate receptor alpha is used which comprises the following amino acid sequence shown in SEQ ID NO: 20 (1-letter code, shown in direction from N-terminus to C-terminus).

[89] Folate receptor alpha is naturally anchored to the cell membrane by a GPI anchor. The signal sequence for a GPI anchor is not shown in SEQ ID NO: 20. According to one embodiment, the folate receptor alpha which is derived from or comprises SEQ ID NO: 20 comprises a GPI anchor signal at the C-terminus. Any suitable GPI anchor signal may be used. The natural GPI anchor signal sequence of human folate receptor alpha is shown in SEQ ID NO: 21 (1-letter code, shown in direction from N-terminus to C-terminus).

[90] Membrane anchorage may alternatively be achieved by using a membrane anchor, e.g. a transmembrane anchor. In this embodiment, the folate receptor comprises a membrane anchor at its C-terminus. Suitable anchors are known in the prior art. The folate receptor

alpha which is derived from or comprises SEQ ID NO: 20 may comprise a leader sequence at the N-terminus. Any suitable leader sequence can be used which ensures functional expression of the folate receptor.

[91] The full amino acid sequence including the natural leader sequence (at the N-terminus) and the natural GPI anchor signal sequence (at the C-terminus) of the wild type human folate receptor alpha is shown in SEQ ID NO: 22 (1-letter code, shown in direction from N-terminus to C-terminus).

[92] According to one embodiment, the membrane-bound folate receptor has or comprises the amino acid sequence of SEQ ID NO: 20, or 22 or is a functional variant of the foregoing which has at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% homology or identity to SEQ ID NO: 20 or 22, is membrane-bound and is capable of folic acid uptake into the cell.

[93] When using a eukaryotic cell that is dependent on folate uptake such as a mammalian host cell, which comprises a heterologous polynucleotide encoding a folate transporter, preferably a folate receptor, stage (b) comprises a selection step wherein said plurality of host cells are cultured in a selective culture medium comprising folate in a limiting concentration. A "limiting concentration of folate" as used herein in particular refers to a concentration of folate(s) in the selective culture medium which provides a selective pressure on the host cell. Under such selection conditions, only host cells grow and proliferate that have incorporated the expression vector and thus, express the folate transporter as selectable marker. Accordingly, folates are not comprised in the selective culture medium in affluence, and this limitation of folate(s) in the culture medium provides a selection pressure on the host cells. The folate comprised in the selective culture medium in a limiting concentration is capable of being taken up into and being processed by the host cell, in particular by host cells that have incorporated the folate transporter that is used as selectable marker. Folates and in particular derivatives of folate which would not or cannot be processed by the host cell do not contribute to the selection pressure that is exerted to select host cells that have incorporated the folate transporter as selectable marker and accordingly do not contribute to the limiting concentration of folate. However, respective folates, such as e.g. antifolates, may be present and even preferably are present, if a combined selection with DHFR is performed as will be described subsequently. The folate present in the selective culture medium in a limiting concentration can e.g. be an oxidized folate or a reduced folate or a derivative thereof. Oxidized folates, such as folic acid, as well as reduced derivatives of folic acid, known as reduced folates or tetrahydrofolates (THF), are a group of B-9 vitamins that are essential cofactors and/or coenzymes for the biosynthesis of purines, thymidylate and certain amino acids in eukaryotic cells that are dependent on folate uptake such as mammalian cells. THF cofactors are particularly crucial for DNA replication and hence cellular proliferation. Preferably, the folate that is comprised in a limiting concentration in the selective culture medium is folic acid. Suitable concentration ranges for providing a limiting concentration of folate are described below.

[94] The folate transporter based selection system is based on the limited availability of folate, preferably folic acid, in the cell culture medium. Host cells that have not successfully incorporated the expression vector(s) and hence do not express sufficient quantities of the folate transporter, which preferably is a folate receptor, die or are impaired in growth under the selective culture conditions compared to host cells that have successfully incorporated the expression vector(s). As is shown by the examples, using a folate receptor based selection in combination with the novel eukaryotic cells described herein allows an accelerated selection, screening and establishment of eukaryotic cell clones that overexpress high levels of recombinant products of interest such as polypeptides.

[95] The selective culture medium that is used in stage (b), respectively used in a sub-step of stage (b), for selection against a folate receptor as selectable marker may comprise folate and in particular folic acid in a concentration selected from:

(a) about 5000nM – 0.1nM;
(b) about 2500nM – 0.1nM;
(c) about 1500nM - 0.1nM;
(d) about 1000nM- 0.1nM;
(e) about 750nM – 0.1nM;
(f) about 500nM – 0.1nM;
(g) about 250nM – 0.2nM; preferably about 250nM – 1nM or about 250nM – 2.5nM;
(h) about 150nM – 0.3nM; preferably about 150nM – 1nM or about 150nM – 2.5nM;
(i) about 100nM – 0.5nM; preferably about 100nM – 1nM or about 100nM – 2.5nM;
(j) about 75nM – 0.6nM, preferably about 75nM – 1nM or about 75nM – 2.5nM;
(k) about 50nM – 1nM; preferably about 50nM – 2.5nM or about 50nM – 5nM;
(l) about 35nM – 0.75nM; and
(m) about 25nM – 1nM or about 25nM – 2.5nM, about 20nM – 3nM about 15nM – 4nM or 10nM - 5nM.

[96] The concentrations and concentration ranges as described above are particularly suitable for fast growing suspension mammalian cells, such as CHO cells, which is a preferred embodiment for commercial production cell lines. However, different cell lines may have different folic acid consumption properties. Suitable concentrations, however, can easily be determined experimentally by the skilled person. The folate comprised in the selective culture medium is preferably folic acid and according to one embodiment, folic acid is the only folate comprised in the selective culture medium that contributes to the limiting concentration of folate.

[97] According to one embodiment, the host cells provided in stage (a) comprise a heterologous polynucleotide encoding a folate transporter as first selectable marker and a heterologous polynucleotide encoding a second selectable marker which processes a substrate which is a folate, a derivative of a folate and/or a product that can be obtained by the processing of folate. E.g. the second selectable marker may be a dihydrofolate reductase (DHFR) or an enzyme operating downstream or in conjunction with DHFR such as

thymidylate synthase (TS) and serine hydroxymethyltransferase (SHMT). Preferably, a membrane-bound folate receptor is used as first selectable marker and the second selectable marker is a dihydrofolate reductase (DHFR). According to this embodiment, stage (b) comprises culturing the host cells in a selective culture medium which comprises folate, preferably folic acid, in a limiting concentration and comprises at least one DHFR inhibitor such as e.g. MTX. As is shown by the examples, when using the novel eukaryotic cell lines described herein as host cells for recombinant expression in combination with such a folate receptor/DHFR based selection, high and homogeneous expression results are obtained already on the cell pool level. As is shown in the example section, a 7 to 8-fold higher expression on pool level could be obtained already with low DHFR inhibitor concentrations. This significant increase in pool titer is advantageous e.g. when intending to produce smaller amounts of a polypeptide of interest quickly as one may use the selected cell pools for production which saves the time for establishing a clonal cell cline. Furthermore, as the number of high expressing cells in the pool is significantly increased, obtaining high producing cell clones from said cell pool is simplified. Less cell clones need to be made what reduces the screening effort. Suitable embodiments of the folate receptor and DHFR as well as suitable selection conditions and concentrations are described above and it is referred to the above disclosure which also applies here. The described concentrations and concentration ranges for folate and antifolate described above can be combined with each other. In one embodiment, a folate concentration of about 0.1nM – 150nM, 1nM – 125nM, 5nM – 100nM or 7.5nM to 75nM is used in combination with an antifolate concentration of 0.1nM – 150nM, 1nM to 125nM, 2.5nM to 100nM, 5nM to 75nM or 7.5nM to 50nM in the selective culture medium. As described, folic acid is preferably used as folate and MTX as antifolate.

[98] Using a respective combination of a folate receptor and DHFR as selectable marker and applying in stage (b) the selection conditions for both markers simultaneously by using an appropriate selective culture medium, results in a very stringent selection system allowing the efficient enrichment of high producing cells from the transfected host cell population. The host cell's viability is considerably increased under selective conditions, if both selectable markers are strongly expressed. Thereby, eukaryotic cells dependent on folate uptake such as mammalian cells can be selected which show an increased expression rate of the product of interest. This concept of using a folate receptor as selectable marker in combination with a further selectable marker involved in the folate metabolism such as preferably a DHFR is disclosed in WO 2010/097240, herein incorporated by reference. As is shown by the examples, the high stringency of the selection system according to this embodiment can be combined advantageously with the novel eukaryotic cells described herein wherein the effect of the expression product of gene C12orf35 is impaired, preferably by reducing or eliminating the expression of said gene. This combination further lowers the number of low producers and a more homogenous population of high producing cells can be obtained after selection. This *inter alia* simplifies single cell cloning of high producing cells. Furthermore, this combination allows to significantly reduce the MTX concentration necessary for DHFR selection.

[99] According to one embodiment, two or more selection steps are performed in stage (b), wherein the two or more selection steps are based on the same or a different selection principle. For example, if an additional selectable marker is used in addition to the folate receptor and/or DHFR, the selective conditions for said additional selectable marker can be applied prior to (e.g. in a pre-selection step) or simultaneously with applying the selective conditions for the folate receptor and/or DHFR. For example, in case the neomycin phosphotransferase gene (neo) is used as additional selectable marker, stage (b) may comprise first growing cells in a medium e.g. containing G418 in order to select cells that have incorporated the expression vector or the combination of at least two expression vectors and then performing a selection step using a selective culture medium comprising a limiting concentration of folate and an inhibitor of the second selectable marker, such as e.g. MTX when using DHFR as second selectable marker.

[100] According to one embodiment, a flow cytometry based selection is performed in stage (b). A selection step employing flow cytometry, in particular fluorescence activated cell sorting (FACS), has the advantage that large numbers of cells can be screened rapidly for the desired characteristic expression yield.

[101] According to one embodiment, said flow cytometry based selection is performed in addition to, preferably after, one or more selection steps against one or more selectable marker gene(s) were performed. Thereby, high expressing cell clones can be identified in the population of successfully transfected cells and separated out.

[102] According to one embodiment, high expressing cells are identified by detecting the expression of a co-expressed reporter polypeptide such as e.g. green fluorescence protein (GFP), CFP, YFP, luciferase or other common reporter polypeptide that can be detected by flow cytometry. According to this embodiment, stage (a) comprises providing a plurality of host cells comprising at least one heterologous polynucleotide encoding a reporter and stage (b) comprises identifying host cells expressing the reporter based on at least one characteristic of said reporter polypeptide using flow cytometry. In such reporter based selection, the expression of the reporter gene correlates with the expression of the product of interest. The reporter polypeptide may be intracellularly located, thereby marking the expressing cell. According to one embodiment, the expression of the reporter polypeptide is tightly linked to the expression of the product of interest which is a polypeptide. E.g. the reporter polypeptide and the polypeptide of interest may be expressed as separate polypeptides but from the same expression cassette, however, separated by an IRES element (internal ribosomal entry site). Furthermore, the reporter polypeptide and the polypeptide of interest may be expressed as fusion protein. According to one embodiment, in the expression cassette for expressing the polypeptide of interest, the polynucleotide encoding the polypeptide of interest is separated by at least one stop codon from the polynucleotide encoding the reporter. A fusion protein comprising the reporter polypeptide is only expressed if translation reads over the stop codon. As stop codon readthrough occurs only to a certain extent, which can be influenced by the number and design of the stop codon and the culture conditions, a certain proportion of the polypeptide of interest is produced as

fusion protein comprising the reporter polypeptide which can be detected by flow cytometry. Using a respective strategy allows to tightly link the expression of the reporter polypeptide to the expression of the polypeptide of interest. The principle of obtaining fusion proteins by stop codon read through will also be explained subsequently in conjunction with an embodiment wherein a fusion protein (not necessarily comprising a reporter) is displayed on the cell surface and e.g. is stained by using a detection compound. For expressing a secreted polypeptide of interest one can additionally include a polynucleotide encoding a membrane anchor either between the stop codon and the polynucleotide encoding the reporter or downstream of the polynucleotide encoding the reporter. The membrane anchor ensures that the reporter polypeptide remains associated with the expressing cell. Thereby, the reporter polypeptide comprised in the fusion protein provides the expressing cells with a trait that is selectable by flow cytometry. The polypeptide of interest is expressed into the culture medium. The higher e.g. the fluorescence of the reporter polypeptide, the more fusion protein is produced and accordingly, the higher is the expression rate of the polypeptide of interest. A respective method is e.g. disclosed in WO 03/014361 to which it is referred.

[103] According to one embodiment, stage (b) comprises:

(i) performing at least one selection under conditions selective for one or more selectable markers expressed by transfected host cells; and
(ii) performing a flow cytometry based selection.

[104] Optionally, one or more further selection steps can be performed prior to or after step (i) and/or step (ii).

[105] According to one embodiment, the flow cytometry based selection comprises selecting a plurality of host cells expressing the polypeptide of interest with a desired yield based upon to the presence or amount of the polypeptide of interest. Preferably, the polypeptide of interest is a secreted polypeptide. According to one embodiment, the polypeptide of interest is detected on the cell surface using a detection compound that binds to the polypeptide of interest. According to one embodiment, the secreted polypeptide of interest is detected while it passes the cell membrane and accordingly is transiently associated with the plasma membrane during polypeptide secretion. A respective flow cytometry based selection system is e.g. disclosed in WO03/099996 to which it is referred.

[106] According to one embodiment, a portion of the polypeptide of interest is expressed fused to a membrane anchor and thus is expressed as membrane-bound fusion polypeptide. Thereby, a portion of the polypeptide is displayed as fusion polypeptide on the cell surface and can be stained using a detection compound. No reporter polypeptide is required for this type of selection even though it may be additionally used. Due to the presence of the membrane anchor, the polypeptide of interest is tightly anchored to the expressing cell. As the amount of produced fusion polypeptide correlates with the overall expression rate of the expressing cell, host cells can be selected via flow cytometry based upon the amount of fusion polypeptide displayed via the membrane anchor on the cell surface. This allows a

rapid selection of high producing host cells. To allow efficient selection using flow cytometry, preferably by using FACS, it is advantageous to use special expression cassette designs for expressing the polypeptide of interest. Thus, according to one embodiment, the polynucleotide encoding the polypeptide of interest is comprised in an expression cassette that is designed such that a portion of the expressed polypeptide of interest comprises a membrane anchor. The polypeptide of interest which is fused to a membrane anchor is also referred to a fusion polypeptide or fusion protein. Several options exist to achieve that result.

[107] According to one embodiment, the host cells provided in stage (a) comprise
(i) a heterologous expression cassette comprising
aa) the polynucleotide encoding a polypeptide of interest,
bb) at least one stop codon downstream of the polynucleotide encoding the polypeptide of interest, and
cc) a further polynucleotide downstream of the stop codon encoding a membrane anchor and/or a signal for a membrane anchor;
and
(ii) at least one heterologous expression cassette comprising a polynucleotide encoding a selectable marker;

and selection in stage (b) comprises
(i) culturing said the host cells under conditions selective for the at least one selectable marker and allowing expression of the polypeptide of interest wherein at least a portion of the polypeptide of interest is expressed as fusion polypeptide comprising a membrane anchor, wherein said fusion polypeptide is displayed on the surface of said host cell;
(ii) performing a flow cytometry based selection, comprising selecting a plurality of host cells expressing the polypeptide of interest with a desired yield based upon the presence or amount of the fusion polypeptide displayed on the cell surface using flow cytometry.

[108] Transcription of the polynucleotide encoding the polypeptide of interest comprised in the above described expression cassette results in a transcript comprising in consecutive order at least
aa) a polynucleotide, wherein translation of said polynucleotide results in the polypeptide of interest;
bb) at least one stop codon downstream of said polynucleotide;
cc) a polynucleotide downstream of said stop codon, encoding a membrane anchor and/or a signal for a membrane anchor.

[109] At least a portion of the transcript is translated into a fusion polypeptide comprising the polypeptide of interest and the membrane anchor by translational read-through of the at least one stop codon. This design of the expression cassette that is used in this embodiment has the effect that through translational read-through processes (the stop codon is "leaky") a defined portion of the polypeptide of interest is produced as a fusion polypeptide comprising a membrane anchor. Thus, at least a portion of the transcript is translated into a fusion polypeptide comprising the polypeptide of interest and the membrane anchor by translational

read-through of the at least one stop codon. Translational read-through may occur naturally due to the choice of the stop codon/design of the translation termination signal or can be induced by adapting the culturing conditions, e.g. by using a termination suppression agent. This read-through level results in a certain proportion of fusion polypeptides. These fusion polypeptides comprise a membrane anchor, which tightly anchors the fusion polypeptides to the cell surface. As a result, the fusion polypeptide is being displayed on the cell surface and cells displaying high levels of membrane-anchored fusion polypeptide can be selected by flow cytometry, preferably by FACS. Thereby, host cells are selected that express the polypeptide of interest with high yield. Details and preferred embodiments of this stop codon readthrough based technology are described in WO2005/073375 and WO 2010/022961 and it is referred to this disclosure for details.

[110] According to one embodiment, the expression cassette additionally comprises iv) a polynucleotide encoding a reporter polypeptide, such as e.g. GFP. Said polynucleotide encoding the reporter polypeptide is located downstream of the stop codon. Upon stop codon read-through a fusion polypeptide is obtained which comprises the reporter, thereby allowing selection by flow cytometry based on the characteristics of the reporter polypeptide such as e.g. its fluorescence. Details of said embodiment are already described above and it is referred to the above disclosure. Preferably, the polynucleotide encoding the reporter polypeptide is located downstream of the polynucleotide encoding a membrane anchor.

[111] According to an alternative embodiment, a portion of the polypeptide of interest is expressed as cell surface displayed fusion polypeptide using the technology described in WO2007/131774. Here, through transcription and transcript processing at least two different mature mRNAs (mRNA-polypeptide of interest) and (mRNA-polypeptide of interest-anchor) are obtained from the expression cassette encoding the polypeptide of interest. Translation of the mRNA-polypeptide of interest results in the polypeptide of interest. Translation of the mRNA-polypeptide of interest-anchor results in a fusion polypeptide comprising the polypeptide of interest and a membrane anchor. As a result, this fusion polypeptide is again displayed on the cell surface and cells displaying high levels of membrane-anchored fusion polypeptide can be selected by flow cytometry, preferably FACS. Thereby, again host cells can be selected that have a high expression rate. According to one embodiment, the expression cassette additionally comprises a polynucleotide encoding a reporter polypeptide, such as e.g. GFP. Said polynucleotide encoding the reporter polypeptide is located downstream of the intron. Thereby, a fusion polypeptide is obtained which comprises the reporter polypeptide, thereby allowing selection by flow cytometry based on the characteristics of the reporter polypeptide such as e.g. its fluorescence. Preferably, the polynucleotide encoding the reporter polypeptide is located downstream of the polynucleotide encoding a membrane anchor. Thereby, the reporter is located inside the host cell.

[112] Both exemplary embodiments described above result in that a portion of the polypeptide of interest is expressed as fusion polypeptide that is displayed at the surface of the host cells, and cells displaying high levels of membrane-anchored fusion polypeptides

(indicating a high level of secreted polypeptide) can be selected e.g. by flow cytometry, in particular by fluorescence activated cell sorting (FACS). Here, different embodiments are available. E.g. if a reporter polypeptide is comprised in the fusion polypeptide, high expressing host cells can be selected based upon a characteristic of the reporter polypeptide, e.g. its fluorescence. Furthermore, appropriately labelled detection compounds can be used as will be described briefly in the following.

[113] According to one embodiment, stage b) comprises selecting a plurality of eukaryotic host cells expressing the polypeptide of interest with a desired yield based upon the presence or amount of the fusion polypeptide displayed on the cell surface using flow cytometry by contacting the host cells with a detection compound binding the fusion polypeptide displayed on the cell surface and selecting a plurality of host cells expressing the polypeptide of interest with a desired yield based upon the presence or amount of the bound detection compound using flow cytometry. Thus, cells may be contacted with an appropriately labelled detection compound that binds the fusion protein, e.g. the portion corresponding to the polypeptide of interest. The detection compound used for binding to the fusion polypeptide may have at least one of the following characteristics: said compound may be labelled, in particular fluorescently labelled, it may be an antigen, it may be an immunoglobulin molecule or a binding fragment thereof or it may be protein-A, -G, or -L. The detection compound used for binding the fusion polypeptide at the cell surface can for example be an immunoglobulin molecule or a fragment thereof such as an antibody or antibody fragment, recognising the fusion polypeptide. Basically all accessible portions of the fusion polypeptide can be detected, thereunder also the portion corresponding to the polypeptide of interest which is secreted in parallel to the fusion polypeptide in soluble form. In order to allow detection and selection, said detection compound used for binding the fusion polypeptide may be labelled. The labelled detection compound that binds the fusion polypeptide displayed on the cell surface thereby labels, respectively stains the cell surface. The higher the amount of fusion polypeptide that is expressed by the host cell, the more labelled detection compound is bound and the more intensive is the staining. This has the advantage that the flow cytometry based selection of the host cells can be easily performed as not only the presence but also the amount of the bound detection compound can be determined. To select high producing host cells, those host cells can be selected from the population of host cell which are most effectively, respectively intensively labelled by the detection compound. The label is suitable for flow cytometry based selection, in particular FACS selection. A fluorescent label is preferred as this allows easy detection by flow cytometry.

[114] According to one embodiment, the expression cassette is constructed such that approximately ≤ 50%, ≤ 25%, ≤ 15%, ≤ 10%, ≤ 5%, ≤ 2.5%, ≤ 1.5%, ≤ 1% or less than ≤ 0.5% fusion polypeptide is obtained. The remaining portion is produced as the secreted polypeptide form not comprising the membrane anchor.

[115] The membrane anchor may be of any kind as long as it enables anchorage of the polypeptide of interest to the cell membrane and thus allows the display of the fusion

polypeptide on the cell surface. Suitable embodiments include but are not limited to a GPI anchor or a transmembrane anchor. A transmembrane anchor is preferred to ensure tight binding of the fusion polypeptide to the cell surface and to avoid shedding of the fusion protein. Particularly preferred, in particular when expressing antibodies as polypeptide of interest, is the use of an immunoglobulin transmembrane anchor. Other membrane anchors and preferred embodiments of an immunoglobulin transmembrane anchor are described in WO2007/131774, WO2005/073375 and WO 2010/022961.

[116] According to one embodiment, the host cells express an immunoglobulin molecule such as an antibody as polypeptide of interest. The polynucleotide encoding the heavy chain of an immunoglobulin molecule and the polynucleotide encoding the light chain of an immunoglobulin molecule may be comprised in the same expression cassette or preferably, are comprised in separate expression cassettes as is described above in conjunction with the first aspect. When using an expression cassette design as described above, wherein a portion of the polypeptide of interest is produced as membrane-anchored fusion polypeptide by translational readthrough or alternative splicing, such design is used for expressing the antibody heavy chain.

[117] According to one embodiment, two or more flow cytometry based selection cycles may be performed in stage (b) to select and enrich high expressing host cells.

[118] In one embodiment, host cells expressing a high amount of polypeptide of interest which accordingly depict a high signal are sorted using fluorescence-activated cell sorting (FACS). FACS sorting is advantageous, since it allows rapid screening of large numbers of host cells to identify and enrich those cells which express the polypeptide of interest with a high yield. This embodiment is particularly suitable if the cells are selected based upon the expression of a fusion protein as described above. Those cells, showing the highest fluorescence rate can be identified and isolated by FACS. A positive and statistically significant correlation between fluorescence, as determined by FACS, and the amount of produced polypeptide is found. Therefore, FACS sorting can be used not only for a qualitative analysis to identify cells expressing a polypeptide of interest in general, but can actually be used quantitatively to identify those host cells that express high levels of the polypeptide of interest. Thereby the best producing cells can be selected/enriched in stage (b).

[119] According to one embodiment, cells which express the polypeptide of interest with the desired yield, e.g. above a certain threshold and/or the top 15%, top 10%, top 5% or the top 2% of the host cells, are selected as pool. E.g. several high expressing cells, e.g. at least 10, at least 20, at least 30, at least 50, at least 100, at least 200, at least 300, at least 500, at least 1000 or at least 5000 high expressing cells can be selected in stage (b) and sorted into a cell pool. This cell pool comprising a plurality of different high expressing cells is also referred to as high expressing cell pool. Said cell pool comprising different individual high expressing cells can then be used in order to obtain individual cell clones for large scale production of the polypeptide of interest. As is shown by the examples, using the mammalian

host cells of the invention wherein the expression of gene C12orf35 is reduced or eliminated provides after selection against one or more selectable markers and FACS selection a cell pool with advantageous characteristics. E.g. the determined FACS profiles show that such high expressing cell pools often have a FACS profile that closely resemble that of cells that were obtained from a cell clone. The reduction or elimination of expression of gene C12orf35 provides after transfection and selection as described host cells that rather uniformly express the product of interest with high yield. This leads to a significant reduction of low- or non-producing clones in the selected cell population. Furthermore, these cell pools may also directly be used for producing the polypeptide of interest e.g. for analytical purposes or if smaller quantities are needed, as significantly higher pool titers are achieved when using the novel eukaryotic cells according to the invention. However, it was found that even without FACS selection for enriching high expressing cell pools, cell populations with clone like performance could be generated in very short time when using the cell lines of the present disclosure. This is an important advantage e.g. if a polypeptide of interest needs to be produced quickly. The obtained pools were suitable for production purposes. Another advantage is that the selection times are reduced as well.

[120] According to one embodiment, the eukaryotic cells provided in stage (a) are mammalian cells, preferably rodent cells, more preferably hamster cells such as CHO cells. Suitable and preferred embodiments are described above in conjunction with the first aspect and it is referred to the above disclosure. As described above, according to one embodiment, said CHO cells have lost a portion of the telomeric region of chromosome 8 wherein said lost portion comprises gene C12orf35. Alternative embodiments are also described above. Particular advantageous is the embodiment wherein said lost portion additionally comprises gene FAM60A. These cells have particularly favorable characteristics with respect to yield and stability. According to one embodiment, the method according to the second aspect comprises as step of analyzing whether expression of gene C12orf35 is reduced or eliminated. Such analysis may be performed after selection, in particular DHFR selection. Details of such analytical method are already described above in conjunction with the first aspect of the present disclosure and are also described below in conjunction with the method according to the fifth aspect and it is referred to the respective disclosure.

[121] Further preferred embodiments in particular with respect to the eukaryotic cells according to the first aspect which preferably are mammalian cells, the expression vector, or combination of expression vectors are described in detail above. It is referred to the above disclosure.

[122] Cells obtained as a result of the selection method according to the second aspect can be isolated and cultured as individual cells. It is, however, also possible to use an enriched population of different host cells, i.e. a cell pool, in the downstream process. The obtained host cells can also be subjected to additional qualitative or quantitative analysis, or can be used e.g. in the development of a clonal cell line for protein production. A clonal cell line may be established from a selected host cell which stably expresses the product of interest with high yield.

[123] Thus, according to one embodiment, selected cells are cultivated to provide cell clones, in particular in the form of clonal cell cultures. A clonal cell culture is a cell culture derived from one single ancestral cell. In a clonal cell culture, all cells are clones of each other. Preferably, all the cells in a cell culture contain the same or substantially the same genetic information. In certain embodiments, the amount or concentration of the polypeptide of interest in the cell culture is determined to determine the productivity. E.g. the titer can be measured by analysing the culture supernatant. According to one embodiment, after determining the productivity performance of each individual clone, a titer ranking is made to select the best producing clones as production clones. Furthermore, a stability study can be performed with the obtained cell clones. However, as is shown in the examples, using the novel eukaryotic cells described herein as host cells and in particular CHO cells which have lost a portion of the telomeric region of chromosome 8 which comprises the C12orf35 gene and the FAM60A gene provide after selection recombinant cell clones showing a significantly improved stability. Thus, losses in expression stability are rare with respective host cells and furthermore, if occurring, often only results in a less dramatic decrease in the productivity compared to when using cells wherein functional expression of gene C12orf35 and FAM60A is not reduced or eliminated. Therefore, in embodiments, in particular those wherein the effect of C12orf35 and FAM60A are impaired, e.g. because at least a portion of the telomeric region which comprises the C12orf35 gene and the FAM60A gene is lost, stability analyses may be shortened or even skipped which is an important advantage as it again shortens the time that is required for obtaining stably expressing cell clones that can be used for large scale production of the product of interest.

C. Method for producing a product of interest

[124] According to a third aspect, a method is provided for producing a recombinant product of interest, comprising using a eukaryotic cell according to the first aspect as host cell for recombinant expression of a product of interest.

[125] As described above, the novel eukaryotic cells provided herein are particularly suitable as production host cells for recombinantly producing a product of interest such as a polypeptide of interest. Suitable and preferred examples of said eukaryotic cell, wherein the effect of the expression product of gene C12orf35 in said cell impaired, preferably by reducing or eliminating the functional expression of gene C12orf35, as well as suitable and preferred examples of the product of interest are described in detail above and it is referred to the above disclosure which also applies here. As described above, the eukaryotic cell preferably is a vertebrate cell, more preferably a mammalian cell. Particularly advantageous is the embodiment wherein in addition to C12orf35, the effect of FAM60A is impaired in said cell, for example by reducing or eliminating functional expression of the FAM60A gene as it was found that thereby, the expression stability can be significantly improved. The abundance of stably expressing clones increases. By impairing the effect of protein FAM60A as well as of protein C12orf35, eukaryotic host cells, preferably mammalian host cells, can be provided which show improved characteristics with respect to both features, long-term

stability as well as production yield, and hence key features important for the large-scale production of a product of interest, in particular a polypeptide of interest.

[126] According to one embodiment, the method comprises introducing into said eukaryotic cell a polynucleotide encoding a product of interest and selecting a host cell which expresses the product of interest. Introduction can be achieved by transfection as described above. Selection may occur using the method according to the second aspect. Preferably, host cells are selected wherein the heterologous polynucleotide encoding the product of interest is stably integrated into the genome of the host cell.

[127] According to one embodiment, the method comprises
(a) culturing eukaryotic cells according to the first aspect which comprise a heterologous polynucleotide encoding a product of interest and/or a host cell selected according to the method according to the second aspect under conditions that allow for the expression of the product of interest;
(b) isolating the product of interest from said cell culture medium and/or from said host cells; and
(c) optionally processing the isolated product of interest.

[128] According to one embodiment, said host cells are cultured under serum-free conditions. The expressed product of interest may be obtained by disrupting the host cells. Preferably, the product of interest is a polypeptide. The polypeptide is preferably expressed, e.g. secreted, into the culture medium and can be obtained therefrom. For this purpose, an appropriate leader peptide is provided in the polypeptide of interest. Leader sequences and expression cassette designs to achieve secretion are well known in the prior art. Also a combination of the respective methods is possible. Thereby, polypeptides such as proteins can be produced and obtained/isolated efficiently with high yield.

[129] The product of interest which preferably is a polypeptide of interest that is produced may be recovered, further purified, isolated, processed and/or modified by methods known in the art. For example, the polypeptide may be recovered from the nutrient medium by conventional procedures including, but not limited to, centrifugation, filtration, ultra-filtration, extraction or precipitation. Further processing steps such as purification steps may be performed by a variety of procedures known in the art including, but not limited to, chromatography (e.g. ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (e.g., preparative isoelectric focusing), differential solubility (e.g. ammonium sulfate precipitation) or extraction. Furthermore, the isolated and purified polypeptide of interest may be further processed, such as e.g. formulated, into a composition, e.g. a pharmaceutical composition.

D. Method for producing a eukaryotic cell line

[130] According to a fourth aspect, a method is provided for producing a eukaryotic cell suitable for recombinant production of a product of interest, comprising impairing the effect of the expression product of gene C12orf35 in said cell. Suitable and preferred embodiments to

achieve that result are described above in conjunction with the eukaryotic cells according to the first aspect and it is referred to the above disclosure which also applies here. Non-limiting embodiments are again briefly described in the following.

[131] According to one embodiment, the method comprises reducing or eliminating the functional expression of gene C12orf35 thereby impairing the effect of the expression product of the C12orf35 gene in said cell. Suitable ways are described above in conjunction with the eukaryotic cells according to the first aspect of the present disclosure and it is referred thereto. According to one embodiment, the genome of the eukaryotic cell is altered to reduce or eliminate the functional expression of gene C12orf35. For example, a gene knock-out may be introduced into the C12orf35 gene. According to one embodiment, such gene knock-out is introduced in all copies of the C12orf35 gene. According to one embodiment, the C12orf35 gene is deleted. All copies of the C12orf35 gene may be deleted in the genome. According to one embodiment, the method comprises deleting a portion of a chromosome, wherein the deleted portion comprises gene C12orf35. The deleted portion may correspond to a telomeric region. Such deletion can be induced e.g. by using an agent that induces chromosome breakages. Here, the cells can be repeatedly treated with such agent in order to obtain cells in which the functional expression of gene C12orf35 is reduced or eliminated, e.g. because all copies of said gene are deleted because of induced chromosome breaks.

[132] According to one embodiment, the eukaryotic cell is a metazoan cell, preferably a vertebrate cell, more preferred a mammalian cell. According to one embodiment, the mammalian cell is a rodent cell. Preferably, the rodent cell is a hamster cell such as a CHO cell, e.g. a CHO cell derived from CHO-K1. According to one embodiment, the method comprises deleting at least a portion of the telomeric region of chromosome 8 in a hamster cell, wherein said deleted portion comprises the C12orf35 gene. As is shown by the examples, CHO cells comprising a respective deletion in the telomeric region of chromosome 8, here the q arm, have particularly favorable expression characteristics and thus are particularly suitable as host cells for recombinant production. According to one embodiment, the deleted telomeric region comprises the C12orf35 gene and one or more or all genes selected from the group consisting of Bicd1, Amn1, methyltransferase-like protein 20, Dennd5b, FAM60A, Caprin2, Ipo8 and RPS4Y2. According to one embodiment, the deleted region additionally comprises at least a portion of or all of the Tmtc1 gene. According to one embodiment, the method comprises deleting at least a respective portion of the telomeric region in both chromosomes of chromosome pair 8. Non-limiting alternative names of the aforementioned individual genes and encoded proteins are also indicated in Table 1 above and are, as well as homologs and orthologs, encompassed by the scope of the terms used above for the individual genes and/or encoded proteins.

[133] As described above, the telomeric region of chromosome 6 in mouse corresponds to the telomeric region of chromosome 8 in Chinese hamster. Thus, the above disclosure with respect to the telomeric region of chromosome 8 of hamster correspondingly applies to the telomeric region of chromosome 6 in mouse.

[134] According to one embodiment, the method comprises causing a chromosome break in the telomeric region of chromosome 8 of the hamster genome (or chromosome 6 of the mouse genome), wherein the breakpoint on chromosome 8 (or chromosome 6 of the mouse genome) is located centromeric of the methyltransferase-like protein 20 gene (referred to as 4833442J19Rik in mouse), centromeric of the Dennd5b gene, centromeric of the FAM60A gene, centromeric of the Caprin2 gene, centromeric of the Ipo8 gene or centromeric of the RPS4Y2gene. Thereby, all genes that are present telomeric thereof, i.e. which lie further to the direction of the telomeric end, are deleted. Non-limiting alternative names of the aforementioned individual genes are also indicated in Table 1 above and the respective genes and encoded proteins are encompassed by the scope of the terms used above for the individual genes. According to one embodiment, the obtained cells comprising a chromosome break in the telomeric region have one or more of the following characteristics:

a) the breakpoint is located centromeric of the Ipo8 gene;
b) the breakpoint is located within the Tmtc1 gene.

[135] As discussed above, all genes that are located telomeric of the breakpoint, i.e. further into the direction of the telomeric end, are comprised in the deleted region. This includes gene C12orf35. Methods for identifying respective eukaryotic cells having such breakpoint are described above are also described in the following in conjunction with the fifth aspect of the present disclosure. According to one embodiment, the Ergic2 gene is not deleted.

[136] According to one embodiment, a CHO cell, preferably derived from the cell line K1, is used in order to produce a eukaryotic cell line wherein the effect of the expression product of the C12orf35 gene is impaired, preferably by reducing or eliminating functional expression of gene C12orf35. According to one embodiment, a telomeric region on the q arm of chromosome 8 comprising gene C12orf35 is deleted. Details how to achieve that result are known to the skilled person and suitable embodiments are also described herein and it is referred to the respective disclosure. Particularly advantageous is the embodiment wherein additionally, the effect of FAM60A is impaired in said cell, for example by reducing or eliminating functional expression of the FAM60A gene as it was found that thereby, the expression stability can be significantly improved. Details are explained above and it is referred to the above disclosure. Mammalian host cells that are altered so that the effect of of both C12orf35 and FAM60A is impaired in said cells have particularly favorable expression characteristics. Thereby, mammalian host cells are provided which show improved characteristics with respect to both features, long-term stability as well as production yield, and hence key features important for the large-scale production of a product of interest, in particular a polypeptide of interest.

[137] According to one embodiment, the method according to the fourth aspect comprises introducing into the eukaryotic cell in which the expression of gene C12orf35 is reduced or eliminated at least one polynucleotide encoding a product of interest and preferably at least one polynucleotide encoding a selectable marker. According to one embodiment, the

polynucleotide encoding a product of interest and the polynucleotide encoding a selectable marker are located on the same or on separate expression vectors. The expression vectors can be introduced by transfection, stable transfection is preferred. Suitable and preferred embodiments are also described above and it is referred to the respective disclosure which also applies here. Host cells that were successfully transfected and express the product of interest can be selected using the method according to the second aspect. It is referred to the above disclosure.

E. Method for analysing eukaryotic cells

[138] According to a fifth aspect, a method is provided for analyzing eukaryotic cells for their suitability as host cells for recombinant expression of a product of interest, comprising analyzing directly or indirectly whether the effect of the expression product of gene C12orf35 is impaired in said cells. As described above, the eukaryotic cell is preferably a mammalian cell.

[139] This analytical method can be advantageously used e.g. in combination with the method according to the fourth aspect of the present disclosure in order to identify whether a eukaryotic cell was produced, wherein the effect of the expression product of gene C12orf35 is impaired in said cell. Furthermore, said method can be used in order to discriminate between high and low producing clones. Furthermore, in embodiments, this method can additionally be used to discriminate between stable or unstable clones during the selection/screening process. By using this analytical method, clones can be identified early in the selection process, which have favorable expression characteristics. This increases the probability to select stable and high producing clones resulting in a higher proportion of high and stable producing clones. Therefore, this analytical method has important applications and provides a further improvement of recombinant expression technologies.

[140] According to a preferred embodiment, the method comprises analyzing whether the functional expression of gene C12orf35 is reduced or eliminated in said cells. The analysis whether functional expression of gene C12orf35 is reduced or eliminated can be performed directly or indirectly. Non-limiting embodiments are described in the following. Which analytical method is suitable also depends on how the cells are altered to achieve the reduction or elimination of functional expression of endogenous gene C12orf35.

[141] For example, when introducing a gene knock-out into the C12orf35 gene in order to reduce or eliminate expression of gene C12orf35, one can amplify the corresponding DNA section and sequence the amplified DNA in order to confirm that the gene knock-out was introduced in gene C12orf35. If functional expression of gene C12orf35 is reduced or eliminated by completely or partly deleting said gene, one can detect the deletion on the DNA level, e.g. using suitable amplification based detection methods to detect the deletion (such methods are known to the skilled person).

[142] According to one embodiment, the expression profile of the eukaryotic cells is analyzed to determine whether functional expression of gene C12orf35 is reduced or

eliminated. For example, the analysis may comprise performing a qualitative or quantitative RT (reverse transcription) PCR in order to detect the presence, absence, amount or length of C12orf35 mRNA. This would be an example of a direct analysis, as the analysis directly involves the transcript of gene C12orf35. Indirect analyses, wherein the expression status of gene C12orf35 is indirectly determined by analyzing the expression profile of genes different from gene C12orf35 and wherein accordingly, the analysis does not directly involve the analysis of gene C12orf35 or its transcript are also suitable and thus encompassed by the term "analyzing whether the functional expression of gene C12orf35 is reduced or eliminated". Such indirect analysis are e.g. suitable if a chromosomal portion comprising gene C12orf35 (and other genes) is deleted by chromosome breakage and will be described subsequently. For a quantitative analysis, a comparison with a reference (e.g. unaltered corresponding cell) can be performed.

[143] According to one embodiment, it is additionally analyzed directly or indirectly whether the effect of protein FAM60A is impaired in said cells. This can be analyzed by determining whether the functional expression of the endogenous FAM60A gene is reduced or eliminated in said cells. This analysis can be performed *mutatis mutandis* as was described for gene C12orf35. It is referred to the above discussion.

[144] According to one embodiment, prior to analysis, cells are treated with an agent that induces chromosome breaks in order to delete a portion of the chromosome which comprises the C12orf35 gene. As described above, all copies of gene C12orf35 could be deleted thereby. The analysis may then comprise analyzing whether treatment with said agent resulted in a deletion of a portion of a chromosome which includes gene C12orf35. The cells are treated with an agent that induces chromosome breakage in an appropriate concentration so that chromosome breakage occurs. Here, also several treatment rounds can be performed. Chromosome breakage may be induced during the selection process if selection involves the use of an agent that induces chromosome breakages in a sufficiently high concentration. A non-limiting example of a suitable agent is e.g. MTX. In this case the cells may comprise a heterologous polynucleotide encoding DHFR as selectable marker in order to be able to survive MTX treatment. However, as discussed above also other agents can be used such as e.g. hygromycine and this was confirmed by examples.

[145] After treating the cells to induce chromosome breaks, the obtained cells can be analysed using the method according to the fifth aspect to determine whether treatment with said agent resulted in a deletion of a portion of a chromosome which includes gene C12orf35. Different embodiments are suitable for that purpose. According to one embodiment, the expression profile of the treated cells is analyzed. For example, it can be analysed whether gene C12orf35 or one or more genes located centromeric of gene C12orf35 (i.e. further away from the telomeric end into chromosome) are expressed. For example, in case of mouse or Chinese hamster cells it can be analysed whether gene C12orf35 is expressed and accordingly, if its mRNA can be detected (example of a direct analysis) and alternatively or in addition thereto, it can be analyzed whether one or more genes selected from the group consisting of methyltransferase-like protein 20 (referred to as

4833442J19Rik in mouse), Dennd5b, FAM60A, Caprin2, Ipo8, Tmtc1 or genes that are located telomeric of the aforementioned genes are expressed by the cell (example of indirect analysis). Non-limiting alternative names of the aforementioned individual genes are also indicated in Table 1 above and the respective genes are encompassed by the scope of the terms used above for the individual genes and encoded products. If the induced breakpoint is located centromeric of the respective gene(s), said genes are deleted which eliminates or reduces (if other copies of the gene exist elsewhere that are expressed) their expression. As is evident from Fig. 1, gene C12orf35 is located telomeric of the aforementioned genes. Thus, if the aforementioned genes are deleted, the deleted region also includes gene C12orf35. Thus, the above genes can be validly used as markers in order to basically indirectly determine whether the induced chromosome breakage resulted in a deletion of gene C12orf35 and thus resulted in a reduction or elimination of expression of gene C12orf35. Thus, the analysis of whether expression of gene C12orf35 was reduced or eliminated does not necessarily have to be based on a direct analysis of e.g. the C12orf35 mRNA and such indirect methods are also encompassed by the method of the fifth aspect. Furthermore, it was found that even though located telomeric of gene C12orf35 (i.e. further down into the direction of the telomeric end), also gene Bicd1 can be used as marker to determine whether a chromosome breakage was induced which resulted in a deletion of gene C12orf35. It was found in conjunction with the analysis of Chinese hamster cells such as CHO cells that if gene Bicd1 is deleted because of a chromosome breakage, the deleted telomeric region usually also includes C12orf35. It was confirmed by analyzing the expression characteristics of several hundred clones that the aforementioned genes can be validly used as markers in order to discriminate cell clones with high and stable expression characteristics from cell clones having low and unstable expression characteristics. The relative expression of the aforementioned genes in CHO cells is shown in Fig. 2.

[146] According to one embodiment, the method according to the fifth aspect is for analyzing hamster cells, preferably CHO cells, and the method comprises analyzing whether expression of gene C12orf35 is reduced in said cells by analyzing whether the expression of one or more genes located in the telomeric region of chromosome 8 and being selected from the group consisting of the Tmtc1 gene and genes located telomeric of the Tmtc1 gene is reduced or eliminated in said cells. As described above, respective cells which after treatment with the agent that introduces chromosome breakage do no longer express the Tmtc1 gene and/or genes located telomeric of the Tmtc1 gene usually comprise a deletion in the telomeric region of chromosome 8 which comprises said genes and in particular comprises the C12orf35 gene. Genetic material telomeric of the breakpoint is lost.

[147] According to one embodiment, the selected host cells having the above described characteristics are transfected with an expression vector comprising at least one polynucleotide encoding a product of interest and preferably comprising at least one selectable marker. Suitable embodiments are described above in conjunction with the other aspects and it is referred to the above disclosure.

[148] According to one embodiment, prior to performing the analysis using the method according to the fifth aspect, the eukaryotic cells are transfected with at least one heterologous polynucleotide encoding a product of interest and at least one heterologous polynucleotide encoding a selectable marker, and prior to analysis at least one selection step is performed to identify successfully transfected host cells. Suitable embodiments are described in detail above. According to one embodiment, selection involves the use of a selection agent that induces chromosome breakages. In this embodiment, the selectable marker may be DHFR and the selection agent may be MTX. Alternatively, the selection agent may be hygromycin and the selectable marker may be a gene that confers resistance against hygromycin such as e.g. the hph gene. In such applications, wherein the method is performed during or after the selection process, the method according to the fifth aspect can be used as analytical tool in order to identify within the selected cell population such cells, wherein the expression of gene C12orf35 is reduced or eliminated. As described, such reduction or elimination may be caused or supported by the selection conditions, if e.g. a chromosome breakage is induced thereby which results in a deletion of gene C12orf35 and the method according to the fifth aspect allows to identify such cells e.g. based on their expression profile. This allows the identification of such cells or cell clones which because of their expression profile, in particular due to the reduced or eliminated expression of gene C12orf35, are particularly suitable for establishing a recombinant production cell line as it can be expected that the high expression of the product of interest remains stable. As described, in case of hamster cells such as CHO cells wherein gene FAM60A is located in the telomeric region of chromosome 8 it is preferred that the cells have lost the telomeric region of chromosome 8, preferably the q arm, thereby reducing or eliminating expression of gene C12orf35. The analytical method can be performed after generating cell clones from cells comprised in the population of high expressing cells. According to one embodiment, a plurality of cell clones is analysed for discriminating between stable and unstable and/or between high and low producing cell clones.

[149] According to one embodiment, the method according to the fifth aspect comprises selecting at least one cell wherein the effect of the expression product of gene C12orf35 is impaired, preferably by reduction or elimination of functional expression of gene C12orf35, for recombinant expression of a product of interest, preferably a polypeptide of interest. Cells having the respective characteristics are particularly suitable for recombinant expression as is shown by the examples. Further embodiments of respective cells are also described in detail above. As described, preferably vertebrate cells such as most preferably mammalian cells are used as eukaryotic host cells.

F. Use of altered eukaryotic cells for recombinant production of a product of interest

[150] According to a sixth aspect, the present disclosure pertains to the use of an isolated eukaryotic cell for recombinantly expressing a product of interest, wherein the effect of the expression product of gene C12orf35 is impaired in said cell. Details with respect to the respective eukaryotic host cells and embodiments suitable for achieving impairment of the effect of the expression product of gene C12orf35 in said cells, preferably by reducing or eliminating functional expression of gene C12orf35 were described in detail above and it is

referred to the above disclosure which also applies here. Non-limiting embodiments are described briefly below.

[151] Preferably, a eukaryotic host cell according to the first aspect is used as eukaryotic host cells. Said cells are described in detail above and it is referred to the above disclosure. The eukaryotic cell may be selected from a metazoan, a vertebrate or mammalian cell. Preferably, the eukaryotic cell is a mammalian cell such as a rodent cell. Preferred are CHO cells. The genome of the eukaryotic cell may be altered as described in detail above to achieve impairment. According to one embodiment, additionally the effect of protein FAM60A is impaired in said cell, preferably by reducing or eliminating functional expression of endogenous gene FAM60A. Details with respect to this embodiment and the associated advantages were described in detail above and it is referred to the above disclosure.

[152] According to one embodiment, the product of interest is a polypeptide. Preferably, the polypeptide of interest is upon expression in the eukaryotic cell secreted into the cell culture medium. Details with respect to the polypeptide of interest are described above and it is referred to the respective disclosure. To allow expression of the product of interest, the eukaryotic host cell may be transiently or preferably stably transfected with an expression vector comprising a polynucleotide encoding the polypeptide of interest. Details are described above and it is referred to the above disclosure.

[153] Numeric ranges described herein are inclusive of the numbers defining the range. The headings provided herein are not limitations of the various aspects or embodiments of this disclosure which can be read by reference to the specification as a whole. According to one embodiment, subject-matter described herein as comprising certain elements also refers to subject-matter consisting of the respective elements. In particular, the polynucleotides described herein as comprising certain sequences may also consist of the respective sequences. It is preferred to select and combine preferred embodiments described herein and the specific subject-matter arising from a respective combination of preferred embodiments also belongs to the present disclosure.

[154] The present application claims priority of prior US provisional application no. US 61/919313 filed on 20 December 2013, the entire disclosure of which is incorporated herein by reference.

EXAMPLES

[155] The following examples serve to illustrate the present invention without in any way limiting the scope thereof. In particular, the examples relate to preferred embodiments of the present invention.

Example 1: Reducing C12orf35 gene expression by RNA interference (RNAi) in CHO cells increases expression yield

[156] In order to demonstrate that reducing expression of gene C12orf35 results in an increase in volumetric or specific productivity, siRNAs were designed against different target genes located in the telomeric region of chromosome 8 of the Chinese hamster genome analysed (CHO-K1). siRNAs were designed against the following target genes listed in Table 2:

Table 2: siRNAs against different target genes

Target gene	Sense	Antisense
METTL20_1 (4833442J19Rik)	CCCUGAUGUUGUUAGAGGATT (SEQ ID NO: 23)	UCCUCUAACAACAUCAGGGTT (SEQ ID NO: 24)
C12orf35_1	CAUCCAGACAAAUCUUACATT (SEQ ID NO: 25)	UGUAAGAUUUGUCUGGAUGTG (SEQ ID NO: 26)
C12orf35_2	CCAGAAAGAUAAAUCUACATT (SEQ ID NO: 27)	UGUAGAUUUAUCUUUCUGGTA (SEQ ID NO: 28)
Caprin2_6	UGACCUGCCCUGAAAGAAATT (SEQ ID NO: 29)	UUUCUUUCAGGGCAGGUCAGT (SEQ ID NO: 30)
FAM60A	GCUUCCAGCUCUAACAGAATT (SEQ ID NO: 31)	UUCUGUUAGAGCUGGAAGCCA (SEQ ID NO: 32)
IPO8_1	GACCCGAACUUUGACCCUATT (SEQ ID NO: 33)	UAGGGUCAAAGUUCGGGUCTG (SEQ ID NO: 34)
IPO8_2	CGGAGACUCUUCAAAUUGATT (SEQ ID NO: 35)	UCAAUUUGAAGAGUCUCCGGA (SEQ ID NO: 36)
IPO8_3	GCCUGAUUGAAGACGAGGATT (SEQ ID NO: 37)	UCCUCGUCUUCAAUCAGGCTT (SEQ ID NO: 38)
Dennd5b_2	GGGUCUCCCUUAUUCAAGATT (SEQ ID NO: 39)	UCUUGAAUAAGGGAGACCCTG (SEQ ID NO: 40)
Amn1_4	GCUGCUUAAGUAUUACUGATT (SEQ ID NO: 41)	UCAGUAAUACUUAAGCAGCCA (SEQ ID NO: 42)
TMTC1_1	GUAUACCUGUGAUAAAACATT (SEQ ID NO: 43)	UGUUUUAUCACAGGUAUACAT (SEQ ID NO: 44)
TMTC1_2	CGGUGAAUGUCAUUCUACATT (SEQ ID NO: 45)	UGUAGAAUGACAUUCACCGCA (SEQ ID NO: 46)
siRNA negative control (no effect on expression; referred to as siRNA control)	Silencer Negative Control siRNA #5 (50uM) (Ambion, Cat#AM4642)	

[157] The used siRNAs were validated using real-time RT-PCR to confirm that they reduce the expression of the target genes by gene silencing. Gene expression was normalized to 18S RNA. The gene expression observed when transfecting the siRNA negative control was set as 100%. The relative reduction of expression of the target gene is shown in the subsequent Table 3 for the two different siRNAs against target gene C12orf35:

Table 3

Concentration	Gene expression (siRNA 1)	Gene expression (siRNA 2)
100pmol	Approx. 28%	Approx. 37%
125pmol	Approx. 25%	Approx. 38%
150pmol	Approx. 35%	Approx. 30%

[158] Furthermore, it was confirmed that the target siRNAs used in this experiment does not inhibit growth of the transfected cells. Furthermore BLAST (Basic Local Alignment Search Tool) analysis based on the available Chinese hamster genome data does not indicate any off-target effects.

[159] The following cell lines were transfected: A CHO cell line derived from CHO-K1 was used as parental cell line. Said cell line expresses the above genes as is shown in Fig. 2. This parental cell line was not transfected with an expression vector and served as control. CHO cells (clones and pools) derived from said parental cell line which comprised an expression vector encoding an antibody as protein of interest stably integrated into the genome was used to determine the effect of the siRNAs. The expression vector comprised in said cell clone comprised selectable marker genes and the antibody heavy chain and the antibody light chain were expressed from different expression cassettes. The expression cassette used for the heavy chain was designed such that a portion of the heavy chain was expressed due to stop codon read through as fusion polypeptide comprising a membrane anchor. The fusion protein was displayed on the cell surface, thereby simplifying FACS analysis (see description). Said CHO cells expressed the above-mentioned siRNA target genes similar to the parental cell line which was determined by microarrays for hundreds of clones and pools. A CHO clone which recombinantly expressed the antibody was used in order to determine whether a downregulation of one or more of the above target genes results in an increase of the expression of the polypeptide of interest. If this was the case, an increase of the antibody volumetric productivity of said cell clone would be seen which is detectable using FACS analysis.

[160] The CHO clone comprising the expression vector stably integrated into the genome was transfected either with a siRNA control (not having an effect on gene expression) or with one of the above-mentioned siRNAs against the target genes. After transfection of the siRNAs it was analyzed whether the reduction of the expression of the target gene results in an increase of the expression of the antibody. *Inter alia*, the transfected cells were stained by using a fluorescent detection compound and analysed by FACS in order to determine the expression rate of the antibody. The more antibody is produced, the more displayed fusion protein can be stained using a labeled compound and accordingly, the higher is the fluorescence signal detected by FACS. Therefore, the higher the intensities in the FACS profiles, the more antibody is expressed.

[161] The results are shown in Fig. 3A to L for the different siRNAs tested. The left peak in the profiles corresponds to the signal obtained for the parental cell line, which does not express the antibody. The two other curves represent the results obtained with the antibody expressing cell clone which was transfected with either the siRNA-negative control (no effect on expression) or with the tested siRNA which reduces the expression of its target gene. If the tested siRNA and accordingly, the downregulation of the target gene does not have an effect on the expression of the antibody (i.e. no upregulation of volumetric productivity), the obtained fluorescence curve for the siRNA-negative control and the fluorescent curve obtained for the target siRNA overlap and thus are basically identical. This was in essence

the case for all tested target genes on clone level, except for gene C12orf35. For FAM60A, however, a slight shift was seen on pool level which is assumed to be attributable to the increased expression stability (data not shown).

[162] As can be seen from the results obtained with the siRNAs against C12orf35 (see Fig. 3B and C), the fluorescence peaks obtained for the siRNA-negative control and the siRNA against gene C12orf35 clearly separate upon silencing gene C12orf35 using RNAi. The fluorescent peak obtained for the cell clone transfected with the siRNA against gene C12orf35 clearly shifts to the right (marked by arrows), which means that the fluorescence is significantly increased. This observed increase in fluorescence is attributable to a higher expression of the antibody as more fusion protein is present and thus is stained on the cell surface. Therefore, this experiment clearly shows that a downregulation of the functional expression of gene C12orf35 directly results in a significant upregulation of the recombinant expression of the antibody (yield of antibody). The same remarkable shift in the FACS profiles was observed when using said siRNAs against C12orf35 in all three concentrations. A prolonged reduction of the expression of gene C12orf35 by RNA interference can be achieved if for example stably integrated in an expression vector which expresses an RNAi inducing transcript, as is for example described in the description. Furthermore, a reduction or elimination of the expression of gene C12orf35 can be achieved by gene knockout or gene deletion/mutation, according to one embodiment by deleting a portion of the telomeric region of chromosome 8 in case of hamster cells such as CHO cells, as described in the description. Furthermore, as is described therein, it is also feasible to reduce or eliminate the effect of the expression product e.g. by introducing one or more mutations which result in a non-functional or less functional protein.

[163] The achieved increase in the expression of the recombinant polypeptide of interest was also confirmed by analysis of the mRNA expression level of the heavy and the light chain (which were expressed from separate expression cassettes, see above). The results are shown for two different polypeptides of interest (antibody 1 and 2) in Fig. 4 and 5. The data shown is normalized to the siRNA negative control (125pmol). It was found that reduction of expression of gene C12orf35 leads to significantly higher mRNA levels of the heavy chain and the light chain of the expressed antibody. In comparison, reduction of expression of other tested target genes did not have an impact on the mRNA expression level of the heavy and the light chain. Thus, reduction of the expression of gene C12orf35 results in a significant increase in the mRNA level of the recombinant polypeptide of interest. Furthermore, it was observed that also other introduced genes such as selection markers are upregulated if gene C12orf35 is silenced. An experiment, in which the gene silencing effect was measured over time starting on day 3, showed that siRNA1 (see siRNA C12orf35_1 in Table 4) had a longer effect compared to siRNA2 (see siRNA C12orf35_2 in Table 4). In addition, cell numbers and titer were determined throughout the time course experiment and revealed that downregulation of C12orf35 leads to significantly higher specific and volumetric productivities (see Figs. 6 and 7).

[164] Furthermore, expression of the C12orf35 gene relative to 18S RNA was analysed in an antibody expressing clone upon repression with siRNA 1 and 2 and compared to different controls. The results are shown in the subsequent Table 4:

Table 4

	18S	C12orf35	Heavy Chain	Light Chain
siRNA C12orf35_1	97%	0.0129%	0.8517%	3.4639%
siRNA C12orf35_2	91%	0.0186%	1.0643%	2.9623%
siRNA control	93%	0.0617%	0.3098%	0.9195%
cells untreated	**100%**	0.0782%	0.2713%	0.7840%
cells lipofectamin	114%	0.0648%	0.2633%	0.8019%

Example 2: Generation of a CHO cell line which comprises a deletion in the telomeric region of chromosome 8

[165] A novel CHO cell line (C8DEL) was generated, which comprises a deletion in the telomeric region of the q arm of chromosome 8. The deletion was induced by chromosome breakage. The deleted portion comprised gene C12orf35 as well as among others gene FAM60A (see Fig. 1). Said novel cell line was obtained from a parental cell line derived from CHO-K1. Said cell line was prepared as follows. The parental CHO cells were split at 2E5 cells/ml in culture medium comprising 0.5 µM, 1 µM or 2 µM MTX. After six days the cell viabilities were around 30-40%. Cells were centrifuged at 180xg for 5 min and cultivated in culture medium without MTX to allow the cells to recover until viabilities were above 95% (after ca. 21 days). This procedure was repeated two more times. Single cell clones were obtained from cell pools. Overall 561 cell clones were grown and DNA was isolated using the "Extract-N-Amp Blood PCR Kit". PCR screening using primers detecting the gene Ipo8 was performed. Three out of the 561 clones were "IPO8 negative" indicating the loss of telomeric region of chromosome 8 which includes the Ipo8 gene. The Ipo8 gene is located centromeric of the C12orf35 gene (see Fig. 1). Thus, if the Ipo8 gene is deleted due to chromosome breakage, all genes located telomeric of the Ipo8 gene (and accordingly also the C12orf35 gene and the FAM60A gene) are deleted as well. These three clones were expanded and further evaluated. One of these clones is referred to as "C8DEL" cell line. Using PCR technique the breakpoint of the telomeric region of chromosome 8 from the C8DEL cell line could be determined. The breakpoint was determined between two PCRs, called PCR20 and 28:

PCR20: fwd 5'-ACC AGT GAA TAA TCG TGT TT-3' (SEQ ID NO: 47), rev 5'-CTA TGA GTC AAT GTC CCA AG-3' (SEQ ID NO: 48);

PCR28: fwd 5'-CAC ACA CAA CCT CCT AAC AAC CC-3' (SEQ ID NO: 49), rev 5'-TTC CGC ACC GAC TCA GTT CT-3' (SEQ ID NO: 50)

[166] The breakpoint lies within the Tmtc1 gene. Additionally it could be demonstrated that the identified breakpoint of C8DEL cell line is stable over several weeks of cultivation

(determined via PCR). Transfecting this novel eukaryotic cell line with an expression vector encoding a product of interest results in significant higher volumetric product titers in comparison with the parental cell line (not comprising said deletion in the telomeric region) as well as a higher probability of selecting stable producing clones as is shown in the following. Transfection and MTX treatment of C8DEL cell line apparently has no effect on the breakpoint (no further genetic material is lost) as was determined based on the analysis of transfected clones.

Example 3: Analysis of the characteristics of the cell line C8DEL

[167] The cell line C8DEL was analyzed for its performance when recombinantly expressing a polypeptide of interest and compared to the parental cell line from which the cell line C8DEL was derived. As described above, said parental cell line does not comprise a corresponding deletion in the telomeric region of chromosome 8.

3.1. Analysis of productivity

[168] The volumetric productivity of C8DEL was evaluated in comparison to the parental cell line from which it was derived. Stable as well as transient transfections were performed.

Stable transfection

[169] Cell cultivation, transfection and screening were carried out in shake flasks using suspension growing CHO cells in a chemically defined culture medium. Cells were transfected by electroporation with different expression vectors encoding a variety of antibodies and therapeutic proteins. Expression vectors used comprised an expression cassette comprising a polynucleotide encoding a neomycin phosphotransferase as selectable marker and an expression cassette encoding a DHFR as selectable marker. The expression vectors used for expressing antibodies additionally comprised an expression cassette comprising a polynucleotide encoding the light chain of an antibody and an expression cassette comprising a polynucleotide encoding the heavy chain of an antibody so that a complete antibody was expressed from said expression vector. The expression vectors used for expressing a polypeptide which was not an antibody, comprised an expression cassette comprising a polynucleotide encoding the polypeptide in addition to the selection markers. All expression cassettes in the expression vectors were oriented in the same direction. The vector was suitable for FACS selection and details of such vector are described above.

[170] Depending on the cell viability, the first selection step was started 24-48 h after transfection by adding G418 selective medium to the cells. As soon as cells recovered to a viability of above 80%, a second selection step was applied by passaging the cells to 500nM MTX or 1µM MTX.

[171] Volumetric productivity of the selected cell populations was analyzed after G418 and MTX selection steps via overgrown shake flask batch cultures in medium with G418 or MTX.

The G418 batch was done in 30ml (125ml flask) and MTX fed-batch in 100ml (500flask). G418 batch cultures were seeded at 1E5 vc/ml in shake flask and cultivated in a shaker cabinet (not humidified) at 150 rpm and 10% CO2. Fed-batches were seeded at 4E5 vc/ml. Viability of cells had to be >90% when starting the assay. Titer determination took place at day 14. Antibody titers in the cell culture supernatant were determined by protein-A HPLC 14 days after starting the culture.

[172] After the first selection step (G418 selection) a massive volumetric titer increase (12-35 fold) could be detected for the stably transfected C8DEL pools in comparison to the stably transfected parental pools. After the second selection step (MTX selection) the C8DEL fed-batch pools were expressing 4-7 fold more polypeptide of interest in comparison to the transfected parental cells, as exemplified by 2 antibodies (antibody 1 and antibody 2). Volumetric G418 and MTX (fed-batch) titers of C8DEL in comparison to the parental CHO cell line not comprising the deletion in the telomeric region of chromosome 8 are exemplarily displayed for two antibody projects in the following Tables 5.a and 5.b. Tables 5.a and 5.b show the volumetric G418 and MTX fed-batch pool titer (antibody) produced in C8DEL in comparison to the parental pools (average of 4 pools/condition are shown).

Table 5.a: Pool titers of example antibody 1

Cell line	Pool titer after G418 selection	Pool titer after G418 and MTX selection
Parental cell line derived from CHO-K1	0.02 g/L	0.62 g/L
C8DEL	0.78 g/L	4.41 g/L

Table 5.b: Pool titers of example antibody 2

Cell line	Pool titer after G418 selection	Pool titer after G418 and MTX selection
Parental cell line derived from CHO-K1	0.02 g/L	0.32 g/L
C8DEL	0.2 g/L	1.21 g/L

[173] The results support the conclusion that a deletion in the telomeric region of chromosome 8 which includes the C12orf35 gene is correlated with higher volumetric productivity. Already with respect to volumetric pool titer, the C8DEL cell line is outperforming the parental cell line that does not comprise a respective deletion in the telomeric region of chromosome 8. Analysis of the frequency and distribution of the integration sites within the karyotype of C8DEL pools and parental pools was analyzed. Both pools show more than 100 different integration sites in 200 analyzed cells highlighting that a huge variability in both pools is existent with no difference between the two cell lines. This indicates that the higher volumetric productivity is not due to any integration artefacts.

Transient transfection

[174] C8DEL cells and parental cell line cells grown in culture medium were transiently transfected in triplicates with expression plasmids encoding either eGFP or an Fc fusion protein as model protein of interest. Polyethylenimine (PEI) was used as transfection reagent. The titer of the model protein in the medium supernatant was measured by Protein A HPLC on day 3 and day 6 after transfection. The expression of the model protein was approx. 3 fold higher in C8DEL. The percentage of eGFP-expressing cells was measured 48 h after transfection by flow cytometry with non-transfected cells acting as negative control. Cells exhibiting a fluorescence level greater than 99% of the negative control cells were regarded as "transfected". Cells exhibiting fluorescence level of more than 1000-fold the intensity of the negative control cells were regarded as "highly fluorescent". The number of high fluorescent cells was 2-3 fold higher using the C8DEL cell line compared to the parental cell line from which C8DEL was derived.

[175] This example shows that the advantages of increased volumetric productivity are also achieved when performing a transient transfection with the C8DEL cell line wherein a portion of the telomeric region of chromosome 8 is lost due to chromosome breakage.

3.2. Stability analysis

[176] The stability characteristics of 46 C8DEL derived clones and 37 clones derived from the parental cell line (which were tested to be IPO8 positive and hence did not lose the telomeric region of chromosome 8) was analysed after stable transfection. All clones recombinantly expressed the same antibody as product of interest and were classified as stable if they were not losing more than 25% antibody titer (volumetric) in 12 weeks. 76% of the analyzed clones from the parental cell line lost more than 25% of titer (volumetric) within 12 weeks of cultivation. Only 24% of the analysed clones were classified as stable. Thus, instability rates were high. In comparison, 67% of the C8DEL clones could be classified as stable and only 33% went instable as is shown in Table 6:

Table 6: Results of stability studies

Cell line	**Stable clones**	**Unstable clones**
Parent cell line (12 weeks)	24%	76%
C8DEL (deletion of gene FAM60A and C12orf35 due to chromosome breakage) (12 weeks)	67%	33%

[177] Using a $\chi 2$-test with a Yates-correction a p-value of 0.0002 could be calculated which supports that C8DEL derived clones have a significant higher tendency to be stable producers. Thus, a significant higher number of stable producing clones for the C8DEL cell line were found. This further supports in particular in conjunction with the knock-out experiments of example 5 that hamster cells such as CHO cells, wherein a portion of the telomeric region of chromosome 8 comprising also gene FAM60A is deleted, show superior stability characteristics. Thus, using such cell line for recombinant expression increases the chance that high and stable producing recombinant cells are identified. Furthermore, the volumetric productivity of said clones was analysed (see 3.3.).

3.3. Further analyses of the characteristics of C8DEL

[178] The characteristics of the CHO cell line comprising a deletion in the telomeric region of chromosome 8 wherein said deletion comprises gene C12orf35 were analysed in additional experiments which demonstrate further advantages of said cell line.

Less single cell cloning required for selecting high producers

[179] An advantageous characteristic of the C8DEL cell line is the greater proportion of high producing clones after single cell cloning. It was found that the C8DEL pools contain an enlarged proportion of high producing cells (resulting in an increased volumetric pool titer) compared to the parental cell line derived from CHO-K1. After single cell cloning of C8DEL pools using FACS technology, a significantly greater proportion of clones expressing high quantities of antibody were selected compared to pools derived from the parental cell line. Table 7 shows that most clones derived from the parental cell line had a volumetric "96 well titer" of 0-20 mg/L. In contrast, the majority of clones derived from C8DEL cell line had an average volumetric titer of 80-100 mg/L what is a significant improvement. One advantage of using C8DEL pools is the reduced number of clones which have to be generated to obtain a comparable amount of high producing clones. This significantly reduces the screening effort.

Table 7

96 well titer (mg/L)	Parental cell line	C8DEL
0-20	80.3%	0.8%
20-40	6.1%	3.1%
40-60	5.4%	5.3%
60-80	6.1%	28.2%
80-100	1.4%	32.1%
100-120	0.0%	17.6%
120-140	0.7%	7.6%
140-160	0.0%	3.1%
160-180	0.0%	1.5%
180-200	0.0%	0.8%

[180] Using the C8DEL cell line as production cell line results not only in an enlarged proportion of high producing clones, also the volumetric titer of the individual C8DEL clones is higher. Fig. 8 shows the volumetric titer of the 45 highest producing clones from the parental cell line derived from CHO-K1 (all Ipo8 positive) and C8DEL from an antibody project (results of the stability analysis of said clones is shown in 3.2). As can be seen, the average volumetric titer for the clones derived from C8DEL is higher compared to the parental cell line, and additionally, also the highest antibody producer clones are originated from C8DEL cell line.

Bioreactor suitability

[181] Additional tests to evaluate C8DEL cell line in comparison to the parental cell line derived from CHO-K1 were performed *inter alia* to determine their suitability for upscaling. Bioreactor runs have shown that C8DEL cell line is suitable for upscaling. C8DEL cell line cultivated in bioreactors had a viable cell density that is suitable for large scale production. Furthermore, it was found that the viability was better than that for the parental cell line. Overall, the C8DEL cell line is suitable for upscaling and is outperforming the parental cell line from which it is derived regarding viability. The viability of the C8DEL cell line stays longer at a higher level.

Improved time lines from transfection to stable pool production

[182] Another advantage of the C8DEL cell line is the faster recovery from MTX selection. The recovery of pools after MTX incubation was accomplished 7-8 days faster compared to the parental cell line wherein no portion of the telomeric region of chromosome 8 comprising gene C12orf35 is deleted. Overall, it was found that the cell crisis is significant lower with the cells wherein expression of gene C12orf35 is reduced or eliminated. Without wishing to be bound in theory, it is believed that this is very likely due to the fact that heterogenous genes, i.e. exogenes, including the selection marker are higher expressed.

Example 4: Selection using a folate receptor as selectable marker

[183] The C8DEL cell line was used in different settings in conjunction with the folic acid receptor as selectable marker and shows particular advantages in conjunction with said selection system. In particular, a combined selection against the folate receptor and DHFR as selectable markers is beneficial. Here, the transfected cells comprised a human folate receptor alpha and DHFR as selectable markers and expressed an antibody. Whereas selection of the parental cell line with very low amounts of folic acid (50 nM folic acid (FA)/50nM MTX) encountered difficulties due to the selection stringency (cells did not always recover), the combination of C8DEL and the folate receptor as selectable marker is very powerful under such stringent conditions and resulted in a significant volumetric titer increase. Table 8 highlights the volumetric titer differences between the parental cell line and C8DEL as well as the additional volumetric titer increase that is achieved when using the folate receptor as a selection marker in combination with low amounts of folic acid instead of 500nM MTX selection step. Thus, the use of the eukaryotic cells described herein wherein the expression of gene C12orf35 and gene FAM60A is reduced or eliminated allows in combination with the folate receptor/DHFR selection system to use very stringent selection conditions that do not require the use of high amounts of toxic agents.

Table 8

Cell line	Selection conditions	Pool titer (mAb g/L) – shake flask batch culture
Parental cell line derived from CHO-K1	0.8 g/L G418/500nM MTX	Approx. 0.07
C8DEL	0.8 g/L G418/500nM MTX	Approx. 0.83
C8DEL	50 nM FA/50nM MTX	Approx. 1.61

[184] Furthermore, C8DEL cells were transfected (nucleofection) with an expression vector which comprised an expression cassette comprising a polynucleotide encoding a human folate receptor alpha and an expression cassette comprising a polynucleotide encoding DHFR. Thus, both selectable markers FRalpha and DHFR were on the same expression vector. Furthermore, the expression vector comprised an expression cassette comprising a polynucleotide encoding the light chain of an antibody and an expression cassette comprising a polynucleotide encoding the heavy chain of an antibody. The expression cassette for the antibody heavy chain was designed such that a portion of the heavy chain was due to stop codon readthrough produced as membrane-anchored fusion, thereby facilitating FACS selection (see above). Five different selection conditions using 100nM folic acid (FA) and different concentrations of MTX were tested. The selection media are summarized in subsequent Table 9. After selection, the selected cell pools were transferred to complete medium and grown in shake flask batch cultures. At day 13 of the culture, samples of the culture medium were taken and analyzed for antibody content by Protein-A HPLC. The results are also shown in Table 9.

Table 9

Selection condition	Approx. antibody concentration [g/L]
100nM FA/no MTX	0.13
100nM FA/1nM MTX	0.12
100nM FA/5nM MTX	0.46
100nM FA/10nM MTX	1.44
100nM FA/50nM MTX	1.57

[185] As can be seen, a MTX concentration already as low as 5nM provided a selection advantage. Increasing the selection stringency also increases the volumetric pool titer. Thus, the volumetric antibody productivities are significantly increased. Furthermore, compared to standard MTX selections, significantly lower concentrations of MTX can be used during

selection. This is an important advantage considering that MTX is a toxic agent. Furthermore, it was analyzed how the selection stringency influences the volumetric pool titers and the time for selection. It was found that increasing the selection stringency by increasing the concentration of MTX prolongs the recovery time. Thus, the selection stringency can be adjusted according to the needs of different applications (time versus volumetric titer). For certain applications, wherein smaller overall amounts of protein of interest are sufficient, less stringent selection conditions can be used, thereby still obtaining pools with a sufficiently high production rate to allow the purification of the respective protein in sufficient quantities. For establishing a clonal cell line that can be used for production of the protein of interest on industrial scale, it is preferred to apply a higher selection stringency, in order to obtain single-cell clones which have a very high expression rate combined with a good stability rate.

[186] Furthermore, analysis of the pools obtained after folic acid/MTX selection for surface expression of the antibody by FACS, show that using this selection system in combination with the novel cell line significantly increases the abundance of high producers in the cell pool as is apparent from the obtained fluorescent profiles shown as Fig. 9A to E. The concentration of MTX was increased from A to E (A: no MTX; B: 1nM MTX; C: 5nM MTX; D: 10nM MTX; E: 50nM MTX). When increasing the MTX concentration, the number of high expressing cell clones in the cell pool was increased as can be derived from the increase of the peak size on the right hand side (higher fluorescence correlating with a higher antibody expression rate). Using 50nM folic acid in combination with 10nM MTX (see Fig. 9D) already resulted in a cell pool predominantly comprising high producing cell clones (one dominant peak on the right hand side). Furthermore, when increasing the MTX concentration to 50nM (see Fig. 9E), basically exclusively high-producing cells were comprised in the obtained pool. These results are remarkable, because when using the C8DEL cell line in combination with the folate receptor/DHFR selection system, one obtains a pool profile after FACS analysis, which closely resembles more that of a cell clone (comprising genetically identical cells) than that of a cell pool (comprising genetically different cells). It appears that the deletion of the C12orf35 gene comprised in the lost telomeric region of cell line C8DEL results in a significant increase of the volumetric productivity, so that the majority of the cells in the cell pools obtained after folic acid/MTX selection under appropriate conditions were high producers according to the FACS profiles.

[187] Furthermore, it was found that when cultivating stably transfected clones obtained from the C8DEL cell line (gene FAM60A is lost, see above) in selective medium (50nM folic acid, 10nM MTX), stability rates of up to 80% and up to almost 100% could be obtained in projects. Significant high stability results were also achieved in a semi-selective medium, which only comprised a limited concentration of folic acid (50nM), however, no MTX. Here, stability rates up to 87% were achieved with this cell line. In certain projects, stability rates of up to almost 100% were obtained.

Example 5: Knock-out of FAM60A in CHO cells using TALEN technology

[188] Two cell clones on the basis of CHO cells derived from the cell line CHO-K1 were made that comprise a knock-out mutation in the FAM60A gene. For creating the FAM60A mutant cells, TALEN (Transcription Activator-Like Effector Nucleases) technology was used. For the knock-out of FAM60A, a coding region (presumed exon 1) of the gene FAM60A was targeted. The CHO-K1 cells used as parental cell only contain one copy of FAM60A. Thus, a single knock-out per cell is sufficient to impair the effect of FAM60A in said cell.

5.1. Design/production and use of TALENs which are specific for FAM60A

[189] The following genomic DNA exon sequence of gene FAM60A of the CHO parental cell line was targeted:

atgtttggttttcacaagccaaaga**tgtaccgaagtatagagggctgctg**tatctgcagagccaag**tcctccagctctcggttc acggaca**gtaaacgttatgaaaaggacttccagagctgttttgg (SEQ ID NO: 51)

[190] The nucleotides of the TALEN binding sites are marked in bold. Two TAL Fok I targeting the coding sequence of FAM60 shown above (SEQ ID NO: 51) were designed. TALEN TAL-L targets and binds the marked 25 nucleotides on the 5' (forward) DNA strand and TALEN TAL-R targets and binds to the marked 25 nucleotides on the 3' (reverse) DNA strand of gene FAM60A as is shown above for SEQ ID NO: 51 (see also Table 10, which additionally shows primer sequences that were subsequently used for identifying knock-outs). The two binding sites are separated by the sixteen nucleotides of the cutting site. Plasmids coding for the two TALENS TAL-L and TAL-R were obtained.

Table 10: TALEN target sequences for FAM60A gene knockout and primer sequences

TAL-L	TGTACCGAAGTATAGAGGGCTGCTG (SEQ ID NO:52)
TAL-R	TGTCCGTGAACCGAGAGCTGGAGGA (SEQ ID NO: 53)
Primer 1:	GTCCCAGCACTCATGAGGAT (SEQ ID NO: 54)
Primer 2:	CCTCCTAGCTCCAGGTATTT (SEQ ID NO:55)
Primer 3:	GAGGACTTGGCTCTGCAGAT (SEQ ID NO: 56)
Primer 4:	TTCCACAGAGCACAGCCGAT (SEQ ID NO: 57)

[191] A portion of the genomic DNA sequence of gene FAM60A which encompasses the targeted coding sequence shown in SEQ ID NO: 51 and which also extends over the primer binding sites for primers 1, 2 and 4 which are located in intron sections is shown as SEQ ID NO: 58.

5.2.Transfection of the TALEN plasmid

[192] Transfection was carried out the using a standard transfection protocol involving electroporation using the parental CHO cells in exponential growth phase with viability over 95% and 5 µg of the each of the TALEN plasmids.

5.3. Cel-I-Assay and cell sorting

[193] The Cel-I-assay was performed according to the manual of SAFC Biosciences. The Cel-I-assay is a standard assay in order to determine the cutting efficiency. In brief, after several days of cultivation, genomic DNA was isolated from the cells and a PCR was performed using primers 1 and 2 (see Table 10). The amplification product was denatured and allowed to renature. Then, nuclease S and nuclease S enhancer were added and incubated. The digested product was analyzed. If TALEN activity occurred, two smaller bands are present indicating TALEN activity within that region of the genome and therefore, supporting that cells wherein the FAM60A gene is knocked-out are present in the analysed cell pool. From the positive cell pools, single cells were sorted in 96 well plates by limiting dilution.

5.4. Screening strategy

[194] Genomic DNA (gDNA) was extracted from each clone in 96 well plates. The gDNA was analyzed by standard procedure to identify knock-out clones by PCR analysis. For this purpose, primers 3 and 4 (see Table 10) were used. In case of a mutation in the cutting region, primer 3 will not bind so that no PCR product is generated. The PCR products resulting from PCR with primers 1 and 2 of gDNA (see above) of positive clones were sequenced in order to analyze the introduced mutation.

[195] Two cell clones with a knock-out mutation were obtained: FAM60A_ko_s16 (s16), with a deletion of 14 nucleotides and FAM60A-ko_s23 (s23) with a deletion of 5 nucleotides. The mutated sequences of the cell clones are shown in Table 11. Each of the deletions results in a frame-shift. Due to the frame-shift within the targeted sequence of FAM60A, stop codons are provided within the reading frame (highlighted in Table 11 by nucleotides in italic letters and underlining). Thus, it is expected that an abnormally short and less-or non-functional FAM60A expression product is expressed by the obtained FAM60A knock-out clones.

Table 11: DNA sequence of presumed exon 1 of FAM60A in CHO wildtype (WT - derived from CHO-K1) and two knock-out cell clones (s16 and s23) derived from said WT. Nucleotides of TALEN binding sites are highlighted in bold and nucleotides of premature stop codons in italic letters and underlining.

WT	atgtttggttttcacaagccaaaga**tgtaccgaagtatagagggctgctg**tatctgcagagccaag**tcctcca gctctcggttcacggaca**gtaaacgttatgaaaaggacttccagagctgttttgg (SEQ ID NO: 51)
s16 (del14)	atgtttggttttcacaagccaaaga***tgtaccgaagtatagagggctgctg***ta***tcctccagctctcggttcacg gacag taa***acgtta*tga*aaaggacttccagagctgttttgg (SEQ ID NO: 59)
s23: (del5)	atgtttggttttcacaagccaaaga***tgtaccgaagtatagagggctgctg***tatctgccaag***tcctccagctctc ggttcacggacag***taa*acgtta*tga*aaaggacttccagagctgttttgg (SEQ ID NO: 60)

5.5. Stability analysis

[196] The parental WT cell line from which the FAM60A knock-out clones were obtained and the obtained FAM60A knock-out cells were stably transfected with an expression vector encoding an antibody as polypeptide of interest. The transfected expression vector comprised an expression cassette comprising a polynucleotide encoding a neomycin phosphotransferase as selectable marker, an expression cassette encoding DHFR as selectable marker, an expression cassette comprising a polynucleotide encoding the light chain of an antibody and an expression cassette comprising a polynucleotide encoding the heavy chain of an antibody so that a complete antibody was expressed from said expression vector. All expression cassettes in the expression vectors were oriented in the same direction. The expression cassette used for the heavy chain was designed such that a portion of the heavy chain was expressed due to stop codon read through as fusion polypeptide comprising a membrane anchor. The fusion protein was displayed on the cell surface, thereby simplifying FACS analysis (see description). The transfected cells were selected for recombinant expression using G418 and MTX (1μM) selection. From the selected pools of each stably transfected cell line (CHO WT, s16 and s23), cell clones that expressed the product of interest with good yields were obtained and were cultured for several weeks (7 weeks for WT CHO parental cell line (45 clones) and 8 weeks for the FAM60A knock-out cells (13 clones for s16 and 18 clones for s23)) in order to analyse their expression stability during prolonged culturing. To ensure that production cell lines can be upscaled to high volume bioreactors, stability studies of 12 weeks, in particular additional analysis of the expression stability during 12 week culturing, were also performed. Clones were classified as unstable if they lost more than 25% of their initial volumetric expression titer over the stability period analyzed. Within the usual level of variation some clones are just above or under the border line of 25%. The higher proportion of unstable clones in week 7/8 compared to week 12 for parental cell line can be explained with the variation in the productivity assay for clones which are close to the 25% threshold.

[197] Table 12 compares the stability results that were obtained with the cell lines. As can be seen, the percentage of clones with stable volumetric titer is considerably higher in the clones derived from either FAM60A knock-out cell line compared to the clones that are derived from the wildtype cell line. This demonstrates that impairing the effect of endogenous FAM60A in the cell, here by introducing a gene knock-out, significantly improves the stability results during prolonged culturing. The ratio of stable versus unstable clones is significantly increased when using the cells of the invention so that more stable clones are obtained that maintain their favorable high expression characteristics during prolonged culturing. The antibody that was recombinantly expressed in this example was not codon-optimized and showed in the parental cell line a very high degree of instability. Because of this significant instability, this project was chosen as example for comparison because it demonstrates the significant benefits that are achieved with the present invention even when being confronted with difficult projects wherein instability rates are with the wildtype unmodified cell line high. However, as discussed above, with other projects, the instability rates are with the parental CHO wildtype cell line less high. However, in all cases analysed, the host cells according to

the present disclosure, wherein the effect of FAM60A is impaired in said cells, achieve in comparison with the unmodified wildtype a significant increase in the amount of stable cells. The stability rates can reach up to 60% or more, 70% or more, 80% or more, 85% or more, or even 90% or more, depending on the project. With the cells according to the present disclosure, which e.g. comprise a gene knock-out in the FAM60A gene or wherein a portion of the telomeric region comprising gene FAM60A is lost due to chromosome breakage, instable clones appeared irrespective of the project analysed significantly less frequently and even if appearing, the loss in volumetric productivity was less pronounced compared to corresponding cells wherein the effect of FAM60A is not impaired in the cell. Therefore, due to the increased percentage of stable clones that are obtained after transfection and selection, the cells according to the present disclosure allow to significantly shorten or even skip long-term stability studies. The stability studies of the FAM60A knock-out clones confirm the beneficial results that are achieved with the technology of the present disclosure.

Table 12: Results of stability studies

Cell line	Stable clones	Unstable clones	Stable clones	Unstable clones
	7/8 weeks		12 weeks	
Parental cell line (7 and 12 weeks)	13.3%	86.7%	24.3%	75.7%
FAM60A knock-out cell line s16 (8 and 12 weeks)	61.5%	38.5%	61.5%	38.5%
FAM60A knock-out cell line s23 (8 and 12 weeks)	44.4%	55.6%	47.1%	52.9%

[198] The results are also shown in Fig. 10 and demonstrate the important advantages that are achieved when impairing the effect of FAM60A in the cells, here by gene knock-out. Therefore, it is advantageous to additionally impair the effect of protein FAM60A in the eukaryotic cell, e.g. by reducing or eliminating functional expression of the FAM60A gene by altering the genome of the eukaryotic cell, as thereby, the expression stability can be improved.

Example 6: Validation tool to identify high and stable producers based on the expression profile

[199] A real-time RT-PCR analytical tool was developed to predict clone productivity and stability at an early stage of development pipeline process. Real-time RT-PCR was implemented for four genes: C12orf35, Dennd5b, FAM60A and Ipo8 (all localized at telomeric region on the q arm of chromosome 8). After selection and clone generation, several hundred clones stably expressing an antibody as polypeptide of interest were analyzed with respect to the presence and expression level of these four genes at telomeric region of chromosome 8 and the expression yield. A clear correlation was found between volumetric antibody productivity and a loss in the telomeric region of chromosome 8. Furthermore, it was found that these cells show higher levels of heavy chain and light chain mRNA. Thus, high expressing cell clones can be identified using said analytical method.

[200] Furthermore, a study was conducted to determine if there is a correlation between stability and presence of telomeric region of chromosome 8. Clones were classified as stable if they were not losing more than 25% volumetric titer in 12 weeks. A significant correlation between loss of telomeric region of chromosome 8 and clone stability is existent (p-value: 4.67E-06 based on $\chi 2$-test). Consequently, the loss of telomeric region on chromosome 8 can be used as a prediction tool for stability. Analysing the presence of telomeric region of chromosome 8 via real-time RT-PCR increases the probability to select a higher proportion of stable clones in pipeline projects.

[201] Furthermore, it was found by analyzing several hundred clones that have lost a portion of the telomeric region of chromosome 8, that there appear to be several breakpoints in the telomeric region of chromosome 8 existent that can be induced. In most analysed cases, the breakpoint was located centromeric of the Ipo8 gene. Breakpoints were also detected between the genes methyltransferase-like protein 20 (referred to as 4833442J19Rik in mouse) and Dennd5b, between Dennd5b and FAM60A, between FAM60A and Ipo8. The deleted region comprised in all cases gene C12orf35 (which is located telomeric of gene encoding methyltransferase-like protein 20). All breakpoints were associated with high volumetric productivity, thereby confirming the relevance of gene C12orf35 for the volumetric productivity.

Example 7: Reducing C12orf35 gene expression cells by RNA interference (RNAi) in a HEK293-derived cell line increases expression yield

[202] In order to demonstrate that reducing expression of gene C12orf35 results also in other mammalian cell lines in an increase in volumetric productivity, two siRNAs were designed against C12orf35 of Homo sapiens. siRNAs sequences are listed in Table 13. As RNAi negative control (siRNA negative control), the Silencer® Negative Control No.1 siRNA (AM4611) was used.

Table 13: siRNAs against C12orf35 and control gene

Target gene	Sense	Antisense
C12orf35_3	CAGTGTATCCCGTTATTAA (SEQ ID NO: 61)	TTAATAACGGGATACACTG (SEQ ID NO: 62)
C12orf35_5	GCAACTGTATCTCATCAAA (SEQ ID NO: 63)	TTTGATGAGATACAGTTGC (SEQ ID NO: 64)

[203] The used siRNAs were validated using real-time RT-PCR to confirm that they reduce the expression of the target genes by gene silencing. Gene expression was normalized to 18S RNA. The gene expression observed when transfecting the siRNA negative control was set as 100%. The relative reduction of expression of the target gene is shown in the subsequent Table 14 for the two different siRNAs against target gene C12orf35:

Table 14

Concentration	Gene expression (siRNA 1)	Gene expression (siRNA 2)
150pmol	Approx. 30%	Approx. 26%

[204] Furthermore BLAST (Basic Local Alignment Search Tool) analysis based on Homo sapiens genome data does not indicate any off-target effects.

[205] The following cell lines were transfected: A HEK293 derived cell line was used as parental cell line. Said cell line stably expresses endogenous C12orf35. A pool derived from the stable transfection of a HEK293 derived cell line with an expression vector encoding a recombinant therapeutic protein of interest stably integrated into the genome was used to determine the effect of the siRNAs. The expression vector in said cell pool comprised selectable marker genes and the recombinant therapeutic protein. The pool which recombinantly expressed the recombinant protein was used in order to determine whether a downregulation of C12orf35 results in an increase of the expression of the polypeptide of interest. If this was the case, an increase of the target protein volumetric productivity of said cell clone would be seen.

[206] The HEK-derived pool comprising the expression vector stably integrated into the genome was transfected either with a siRNA control (not having an effect on gene expression) or with one of the above-mentioned siRNAs against C12orf35. After transfection of the siRNAs it was analyzed whether the reduction of the expression of C12orf35 results in an increase of the expression of the recombinant protein.

[207] The results are shown in Table 15 for the different siRNAs tested. The HEK293-derived cells were transfected in 6-well scale with the respective siRNAs using Lipofectamin2000 as transfection reagent. Five days after transfection the concentration of the recombinant target protein in the cell culture supernatant was measured using affinity HPLC. For this a short proprietary tag on the recombinant protein of interest was used. An increase of the protein of interest could be detected independent which of the two evaluated siRNA was used. A prolonged reduction of the expression of gene C12orf35 by RNA interference can be achieved if for example stably integrated in an expression vector which expresses an RNAi inducing transcript. Furthermore, a reduction or elimination of the expression of gene C12orf35 can be achieved by gene knockout or gene deletion/mutation. Furthermore, as is described therein, it is also feasible to reduce or eliminate the effect of the expression product e.g. by introducing one or more mutations which result in a non-functional or less functional protein.

Table 15: Volumetric titer increase after transfection of siRNA against C12orf35

	Titer recombinant protein
siRNA C12orf35_3	37.3 mg/L
siRNA C12orf35_5	28.9 mg/L
siRNA control	16.1 mg/L
cells untreated	18.5 mg/L

[208] Furthermore, expression of the C12orf35 gene was analysed upon repression with siRNA 3 and 5 at day 3 and day 5. The results are shown in the subsequent Table 16:

Table 16

	Day 3	Day 5
	C12orf35	
siRNA C12orf35_3	35 %	42 %
siRNA C12orf35_5	27 %	52 %
siRNA control	100 %	100 %
cells untreated	72 %	122 %

Example 8: Generation of CHO cell lines which comprise knock-out of C12orf35 gene by frameshift mutations within C12orf35 gene

[209] Three C12orf35 knock-out ("KO") cell clones on the basis of CHO-K1 derived cells were generated using TALEN (Transcription Activator-Like Effector Nucleases) technology. For the knockout, C12orf35 was targeted at 5-prime region. Adding frameshift mutations at 5 prime region has the advantage that the truncated protein will be short.

8.1 Design/production and use of TALENs

[210] Two truncated TAL FokI nucleases targeting 5 prime region were designed. Each TALEN is targeting and binding to 19 nucleotides on either the 5' (on the forward) or the 3' (on the reverse) DNA strand, respectively. The two binding sites are separated by the sixteen nucleotides of the cutting site. Each designed TAL was synthetized and cloned in a suitable entry vector and subcloned in a pcDNA3.3_DEST_A343 destination vector. Product description and methods are available from Life Technology/GeneArt.

8.2 Transfection of TALEN plasmids

[211] Parental CHO cells in exponential growth phase with viability over 95% were used for transfection. Electroporation (nucleofection) was performed using the Amaxa™, Nucleofector™ Technology according to the instructions of the manufacturer (Lonza). The transfected cells were expanded at day 5 after transfection and single cells separated at day 8 in 20 x 96-well plates. Monoclonality and confluence were controlled with the CloneSelect™ Imager (Genetix).

8.3 Cel-I-assay and screening strategy

[212] The Cel-I-assay was performed according to the manual of SAFC Biosciences. The Cel-I-assay is a standard assay in order to determine the cutting efficiency. In brief, after 6 days of cultivation, genomic DNA was isolated from the cells and a PCR was performed using the following primers:

Fwd: GCATCCAGTGAACTTACTTATCCAGAT (SEQ ID NO: 65)

Rev: GCTCTGCCACTGCTGTTGAAAG (SEQ ID NO: 66)

[213] The amplification product was denatured and allowed to renature. Then, nuclease S and nuclease S enhancer were added and incubated. The digested product was analyzed. Two smaller bands were present indicating TALEN activity within that region of the genome and therefore, supporting that cells wherein the C12orf35 gene is altered by mutation were present in the analysed cell pools. From the most positive cell pools (stronger intensity of the two smaller bands), single cells were sorted in 96 well plates.

[214] Genomic DNA (gDNA) was extracted from each clone in 96 well plates using the Extract-N-Amp™ Blood PCR Kits (cat. XNAB2R, Sigma). The gDNA extracts were used to screen for mutated clones, in a PCR assay using the Forward Primer (Fwd) (SEQ ID NO 67: TGCTGGGATTAAAGGGGAAAGCTTT) in combination with a primer ("Cut. Primer") binding on the cutting site which had the following sequence:

Cut. Primer: TCTAGAAACAGACTGAGAATTTTGCAC (SEQ ID NO: 68)

[215] Clones with mutations were transferred in 125ml shake-flasks and sequenced. In said screening assays 10 clones were identified and three clones selected for further analysis. The genotypes of the three clones are shown in Table 17 highlighting the 5-prime C12orf35 sequences referring to the wildtype and the respective mutations. The respective amino acid sequences are shown in Table 18.

Table 17: C12orf35 gene sequences encoding 5-prime end from CHO-K1 derived WT and the different KO clones. Δ indicates a deletion. The number behind the Δ indicates how many nucleotides were deleted. In parentheses, the deleted nucleotides are shown and furnished with a separate SEQ ID NO as indicated. TALEN binding site and cutting site are marked in bold. Sequences around the TALEN binding and cutting site have been confirmed by Sanger sequencing in KO clones and the rest of the sequence is based on the assumption to be the same as the wild type sequence.

WT	atgaattggaatgcaaaaccagagaatgctgccccaaacccaccatattctaaaagccagtcgtctct tttgcagcagtttttaatgccttccacaacttctcaaagttctttcagctgtctcccacataaccaagaagc atgcatatatcccactaattcaaattcagtttcacagccacttctgaacgtcaggagtttcataaatcctcc gatctctgtttctaatgtgcataataggacagttgtggcctcacagacctcagtagaaagagtcacatat acaaatgttaaaggagcccaacaaccaaaccacaatttgcaaacagtgtcttctggagttgtgcaaa atgcctggatgaattcaacaatgaggaattttatgccttctcttacagaggcaaccatatctcataaacct gatggtgggcctagtatgccatatatgcatgcaccacagagtcatcttgtcacatcagacacctactctg tgcaactacagatgactccttcaaactctgtaagaggccctgtaacttaccaaggaaattatcaagga aatccgggacttaaccactcgatggcaggtgagcttggctgggtacaatgtgcatccagtgaacttact tatccagattacagaccacctccaaagcaatatccttatttaccacaaagctttgtgcaagacacttctgt tcagaaacaaaactttgtgtcatctacatcattacaagttaaaaataatcagcttccaccttctacacaga ccttaccatcaaagcgccctgtacctgtgtcgtcatatcagtatgctgcagaaaccagcaaaagactcc ctccccccccttacagctgtagatat**ggaagccaacatgtgcaaaattctcagtctgtttctagaca** **cttgcctgtgga**agttcctcagagttcagaaatgcactcgtctgaaaaaaagaaagatgcttacaaa

	gtctttcaacagcagtggcagagcactagtaaaaatgtcagtacaataggaaaattctgtgagttgaa aattaatacaaaacagtcttacaatgactctgctggctcttctggggatggtgttcatactcttgttcaaaat aatcaagaagaaagaaagtattcttataatccaagtacaaatcaaatactagacacaaatgtcacaa aagaaaag (SEQ ID NO: 69)
Δ8	atgaattggaatgcaaaaccagagaatgctgccccaaacccaccatattctaaaagccagtcgtctct tttgcagcagtttttaatgccttccacaacttctcaaagttctttcagctgtctcccacataaccaagaagc atgcatatatcccactaattcaaattcagtttcacagccacttctgaacgtcaggagtttcataaatcctcc gatctctgtttctaatgtgcataataggacagttgtggcctcacagacctcagtagaaagagtcacatat acaaatgttaaaggagcccaacaaccaaaccacaatttgcaaacagtgtcttctggagttgtgcaaa atgcctggatgaattcaacaatgaggaattttatgccttctcttacagaggcaaccatatctcataaacct gatggtgggcctagtatgccatatatgcatgcaccacagagtcatcttgtcacatcagacacctactctg tgcaactacagatgactccttcaaactctgtaagaggccctgtaacttaccaaggaaattatcaagga aatccgggacttaaccactcgatggcaggtgagcttggctgggtacaatgtgcatccagtgaacttact tatccagattacagaccacctccaaagcaatatccttatttaccacaaagctttgtgcaagacacttctgt tcagaaacaaaactttgtgtcatctacatcattacaagttaaaaataatcagcttccaccttctacacaga ccttaccatcaaagcgccctgtacctgtgtcgtcatatcagtatgctgcagaaaccagcaaaagactcc ctccccccccttacagctgtagata**tggaagccaacatgtgcaa(Δaattctca)gtctgtttctaga cacttgcctgtgga**agttcctcagagttcagaaatgcactcgtctgaaaaaaagaaagatgcttaca aagtctttcaacagcagtggcagagcactagtaaaaatgtcagtacaataggaaaattctgtgagttg aaaattaatacaaaacagtcttacaatgactctgctggctcttctggggatggtgttcatactcttgttcaa aataatcaagaagaaagaaagtattcttataatccaagtacaaatcaaatactagacacaaatgtca caaaagaaaag (SEQ ID NO: 70)
Δ20	atgaattggaatgcaaaaccagagaatgctgccccaaacccaccatattctaaaagccagtcgtctct tttgcagcagtttttaatgccttccacaacttctcaaagttctttcagctgtctcccacataaccaagaagc atgcatatatcccactaattcaaattcagtttcacagccacttctgaacgtcaggagtttcataaatcctcc gatctctgtttctaatgtgcataataggacagttgtggcctcacagacctcagtagaaagagtcacatat acaaatgttaaaggagcccaacaaccaaaccacaatttgcaaacagtgtcttctggagttgtgcaaa atgcctggatgaattcaacaatgaggaattttatgccttctcttacagaggcaaccatatctcataaacct gatggtgggcctagtatgccatatatgcatgcaccacagagtcatcttgtcacatcagacacctactctg tgcaactacagatgactccttcaaactctgtaagaggccctgtaacttaccaaggaaattatcaagga aatccgggacttaaccactcgatggcaggtgagcttggctgggtacaatgtgcatccagtgaacttact tatccagattacagaccacctccaaagcaatatccttatttaccacaaagctttgtgcaagacacttctgt tcagaaacaaaactttgtgtcatctacatcattacaagttaaaaataatcagcttccaccttctacacaga ccttaccatcaaagcgccctgtacctgtgtcgtcatatcagtatgctgcagaaaccagcaaaagactcc ctccccccccttacagctgtagata**tggaagccaacatgt(Δgcaaaattctcagtctgttt** =SEQ IDNO:71)**ctagacacttgcctgtgga**agttcctcagagttcagaaatgcactcgtctgaaaaaaaa gaaagatgcttacaaagtctttcaacagcagtggcagagcactagtaaaaatgtcagtacaatagga aaattctgtgagttgaaaattaatacaaaacagtcttacaatgactctgctggctcttctggggatggtgtt catactcttgttcaaaataatcaagaagaaagaaagtattcttataatccaagtacaaatcaaatacta gacacaaatgtcacaaaagaaaag (SEQ ID NO: 72)
Δ59 plus nucleotide insertions "C"	atgaattggaatgcaaaaccagagaatgctgccccaaacccaccatattctaaaagccagtcgtctct tttgcagcagtttttaatgccttccacaacttctcaaagttctttcagctgtctcccacataaccaagaagc atgcatatatcccactaattcaaattcagtttcacagccacttctgaacgtcaggagtttcataaatcctcc

and "A"	gatctctgtttctaatgtgcataataggacagttgtggcctcacagacctcagtagaaagagtcacatat acaaatgttaaaggagcccaacaaccaaaccacaatttgcaaacagtgtcttctggagttgtgcaaa atgcctggatgaattcaacaatgaggaattttatgccttctcttacagaggcaaccatatctcataaacct gatggtgggcctagtatgccatatatgcatgcaccacagagtcatcttgtcacatcagacacctactctg tgcaactacagatgactccttcaaactctgtaagaggccctgtaacttaccaaggaaattatcaagga aatccgggacttaaccactcgatggcaggtgagcttggctgggtacaatgtgcatccagtgaacttact tatccagattacagaccacctccaaagcaatatccttatttaccacaaagctttgtgcaagacacttctgt tcagaaacaaaactttgtgtcatctacatcattacaagttaaaaataatcagcttccaccttctacacaga ccttaccatcaaagcgccctgtacctgtgtcgtcatatcagtatgctgcagaaaccagcaaaagactcc ctcccccccCttacagctgtagata**tggaagc(Δcaacatgtgcaaaattctcagtctgtttctag** **acacttgcctgtgga**agttcctcagag=SEQIDNO:73)Attcagaaatgcactcgtctgaaaaa aagaaagatgcttacaaagtctttcaacagcagtggcagagcactagtaaaaatgtcagtacaatag gaaaattctgtgagttgaaaattaatacaaaacagtcttacaatgactctgctggctcttctggggatggt gttcatactcttgttcaaaataatcaagaagaaagaaagtattcttataatccaagtacaaatcaaatac tagacacaaatgtcacaaaagaaaag (SEQ ID NO: 74)

Table 18: Partial C12orf35 amino acid sequences of CHO WT and KO clones. A star * represents the first stop codon occurring due to the respective frameshift mutations. Due to the deletion of 8 bp respective 20 bp for clones "Δ8" respective "Δ20" only parts of the in frame translations of the TALEN binding sites are still present and are marked in bold. Clone "Δ59 plus nucleotide insertion "C" and "A"" shows no TALEN binding site anymore due to the insertion of the nucleotide C before the TALEN binding site and the resulting frameshift.

WT	MNWNAKPENAAPNPPYSKSQSSLLQQFLMPSTTSQSSFSCLPHN QEACIYPTNSNSVSQPLLNVRSFINPPISVSNVHNRTVVASQTSVER VTYTNVKGAQQPNHNLQTVSSGVVQNAWMNSTMRNFMPSLTEAT ISHKPDGGPSMPYMHAPQSHLVTSDTYSVQLQMTPSNSVRGPVTY QGNYQGNPGLNHSMAGELGWVQCASSELTYPDYRPPPKQYPYLP QSFVQDTSVQKQNFVSSTSLQVKNNQLPPSTQTLPSKRPVPVSSY QYAAETSKRLPPPPYSCRY**GSQHVQNSQSVSRHLPV**EVPQSSEM HSSEKKKDAYKVFQQQWQSTSKNVSTIGKFCELKINTKQSYNDSA GSSGDGVHTLVQNNQEERKYSYNPSTNQILDTNVTKEK (SEQ ID NO: 75)
Δ8	MNWNAKPENAAPNPPYSKSQSSLLQQFLMPSTTSQSSFSCLPHN QEACIYPTNSNSVSQPLLNVRSFINPPISVSNVHNRTVVASQTSVER VTYTNVKGAQQPNHNLQTVSSGVVQNAWMNSTMRNFMPSLTEAT ISHKPDGGPSMPYMHAPQSHLVTSDTYSVQLQMTPSNSVRGPVTY QGNYQGNPGLNHSMAGELGWVQCASSELTYPDYRPPPKQYPYLP QSFVQDTSVQKQNFVSSTSLQVKNNQLPPSTQTLPSKRPVPVSSY QYAAETSKRLPPPPYSCRY**GSQHVQ**VCF* (SEQ ID NO: 76)
Δ20	MNWNAKPENAAPNPPYSKSQSSLLQQFLMPSTTSQSSFSCLPHN QEACIYPTNSNSVSQPLLNVRSFINPPISVSNVHNRTVVASQTSVER VTYTNVKGAQQPNHNLQTVSSGVVQNAWMNSTMRNFMPSLTEAT ISHKPDGGPSMPYMHAPQSHLVTSDTYSVQLQMTPSNSVRGPVTY QGNYQGNPGLNHSMAGELGWVQCASSELTYPDYRPPPKQYPYLP QSFVQDTSVQKQNFVSSTSLQVKNNQLPPSTQTLPSKRPVPVSSY QYAAETSKRLPPPPYSCRY**GSQHV*** (SEQ ID NO: 77)
Δ59 plus nucleotide insertions "C"	MNWNAKPENAAPNPPYSKSQSSLLQQFLMPSTTSQSSFSCLPHN QEACIYPTNSNSVSQPLLNVRSFINPPISVSNVHNRTVVASQTSVER VTYTNVKGAQQPNHNLQTVSSGVVQNAWMNSTMRNFMPSLTEAT

and "A"	ISHKPDGGPSMPYMHAPQSHLVTSDTYSVQLQMTPSNSVRGPVTY QGNYQGNPGLNHSMAGELGWVQCASSELTYPDYRPPPKQYPYLP QSFVQDTSVQKQNFVSSTSLQVKNNQLPPSTQTLPSKRPVPVSSY QYAAETSKRLPPPPLQL* (SEQ ID NO: 78)

[216] In order to confirm the results from the siRNA experiments, an antibody candidate was expressed in three C12orf35 knock-out cell clones in a batch and fed-batch cultivation and the volumetric antibody titers were analyzed and compared to the results obtained with a corresponding CHO wildtype cell line which endogenously expresses intact C12orf35.

8.4 Transfection of the mAb coding vector

[217] The CHO wild-type cell line, the above described C8DEL and the C12orf35 knock-out CHO cell clones (see Tables 17 and 18) were transfected with expression vectors comprising polynucleotides encoding monoclonal antibodies (mAb) and two selectable marker genes, namely neo and DHFR. Two vectors encoding two different exemplary antibodies (antibody 1 and antibody 2) were evaluated. For transfection, cells were grown to exponential phase and 5 x 10^6 cells were transfected with 3µg vector DNA. Three transfection replicates were performed. Selection of cells stably transfected with the vectors encoding the protein of interest was performed with G418 (G418 concentration 0.8 mg/ml) followed by MTX selection (500nM). The selection conditions were identical for all pools. During the selection process, volumetric titers of expressed mAb in the medium were determined. The volumetric titer in the supernatant of the C12orf35 knock-out clones were compared to the volumetric titer of the CHO wild-type. The cells were cultivated in shakers containing a chemical defined medium. The batch cultivation was performed at temperature of 37°C and under shaking conditions. During the fed-batch cultivation cells were cultivated in shakers containing a chemical defined medium enriched in amino acids. The fed-batch cultivation was performed at temperature of 37°C and shaking, and a temperature shift was performed at day 5 to 33°C. Additionally a feed containing glucose and amino acids was regularly added along the process. During the fed-batch cultivation process, samples of the fed-batch culture material were regularly collected to determine the viable cell density (vcd) using a Vi-Cell cell viability analyzer (Beckman Coulter) and to determine the protein titers in the cell culture medium. At the end of the batch and fed-batch (day 14), the cultivation process was stopped. Analysis of the batch and fed-batch cultures revealed that over the 14 days cultivation period, the volumetric titer of expressed mAb in the culture medium of the C12orf35 KO pools was similar to the volumetric titer of C8DEL and significant higher than in the culture medium of CHO wild-type cell pools.

[218] After the first selection step (G418 selection) a massive volumetric titer increase (4-10 fold) could be detected for the stably transfected C12orf35 KO pools in comparison to the stably transfected parental pools. After the second selection step (MTX selection) the C12orf35 KO pools were expressing 4-7 fold more polypeptide of interest in comparison to the transfected parental cells (batch) and 2-6 fold more polypeptide (fed-batch). Volumetric G418 and MTX (fed-batch) titers of C12orf35 KO pools in comparison to the parental CHO cell line are displayed for the exemplary antibody projects 1 and 2 in the following Tables 19

and 20. Tables 19 and 20 show the G418 and MTX batch and fed-batch volumetric pool titer (antibody) produced in C12orf35 KO pools in comparison to the parental pools (average of 3 pools/condition are shown).

Table 19: Pool titers as exemplified by antibody 1

Cell line	Pool titer after G418 selection	Pool titer after G418 and MTX selection (batch)	Pool titer after G418 and MTX selection (fed-batch)
Parental cell line derived from CHO-K1	0.04 g/L	0.28 g/L	1.49 g/L
C8DEL	0.72 g/L	1.12 g/L	4.18 g/L
C12orf35 KO 1	0.41 g/L	1.16 g/L	4.38 g/L
C12orf35 KO 2	0.41 g/L	1.19 g/L	3.65 g/L
C12orf35 KO 3	0.45 g/L	1.16 g/L	4.25 g/L

Table 20: Pool titers as exemplified by antibody 2

Cell line	Pool titer after G418 selection	Pool titer after G418 and MTX selection (batch)	Pool titer after G418 and MTX selection (fed-batch)
Parental cell line derived from CHO-K1	0.03 g/L	0.06 g/L	0.27 g/L
C8DEL	0.22 g/L	0.39 g/L	1.37 g/L
C12orf35 KO 1	0.15 g/L	0.39 g/L	1.51 g/L
C12orf35 KO 2	0.14 g/L	0.40 g/L	1.63 g/L
C12orf35 KO 3	0.12 g/L	0.30 g/L	1.26 g/L

[219] The results support the conclusion that the loss of function of C12orf35 is correlated with higher volumetric productivity.

[220] Additionally a shorter selection time (G418 and MTX steps) could be detected for the stably transfected C12orf35 KO pools in comparison to the stably transfected parental pools. The selection time was in average 7 days shorter for the C12orf35 KO pools.

CLAIMS

1. An isolated eukaryotic host cell for recombinant production of a product of interest, wherein the effect of the expression product of gene C12orf35 is impaired in said cell because functional expression of gene C12orf35 is reduced or eliminated by gene knock-out, gene mutation, gene deletion, gene silencing or a combination of any of the foregoing, wherein the C12orf35 gene is a gene encoding a protein that shares at least 70% identity with one or more of the amino acid sequences shown in SEQ ID NO: 1 to 7 or the protein encoded by SEQ ID NO: 8, and wherein the cell comprises at least one heterologous polynucleotide encoding the product of interest, wherein the product of interest is a polypeptide.

2. The isolated eukaryotic host cell according to claim 1, wherein the eukaryotic host cell has one or more of the following characteristics:

a) the C12orf35 gene comprises as gene mutation one or more mutations in the C12orf35 gene which provide a non- or less functional expression product;
b) at least one or all copies of gene C12orf35 is deleted or functionally inactivated in the genome of the eukaryotic cell;
c) a portion of a chromosome is deleted, wherein the deleted portion comprises gene C12orf35.

3. The isolated eukaryotic host cell according to claim 1 or 2, wherein the eukaryotic cell has one or more of the following characteristics:

a) the eukaryotic cell is a metazoan cell;
b) the eukaryotic cell is a vertebrate cell;
c) the eukaryotic cell is a mammalian cell;
d) the eukaryotic cell is a rodent cell;
e) the eukaryotic cell is a hamster cell;
f) the eukaryotic cell is a CHO cell;
g) the eukaryotic cell is provided as cell clone or cell line;
h) the eukaryotic cell expresses endogenously DHFR and a folate receptor.

4. The isolated eukaryotic host cell according to claim 2, wherein a portion of a chromosome is deleted, wherein the deleted portion comprises gene C12orf35, and wherein:

a) the eukaryotic cell is a hamster cell and at least a portion of the telomeric region of chromosome 8 is deleted, wherein said deleted portion comprises gene C12orf35; or
b) the eukaryotic cell is a mouse cell and at least a portion of the telomeric region of chromosome 6 is deleted, wherein said deleted portion comprises gene C12orf35.

5. The isolated eukaryotic host cell according to claim 4, wherein the isolated eukaryotic cell has one or more of the following characteristics:

a) the deleted telomeric region comprises gene C12orf35 and comprises one or more genes selected from the group consisting of Bicd1, Amn1, methyltransferase-like protein 20, Dennd5b, FAM60A, Caprin2, Ipo8 and RPS4Y2;
b) the deletion is induced by chromosome breakage and the breakpoint is located centromeric of the Ipo8 gene;
c) the deletion is induced by chromosome breakage and the breakpoint is located centromeric of the Ipo8 gene within the Tmtc1 gene;
d) at least a portion of the telomeric region is deleted in both chromosomes of the respective chromosome pair, wherein the deleted portions comprise gene C12orf35.

6. The isolated eukaryotic host cell according to one or more of claims 1 to 5, wherein the C12orf35 gene is a gene encoding a protein that shares at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity with one or more of the amino acid sequences shown in SEQ ID NO: 1 to 7 or the protein encoded by SEQ ID NO: 8, and/or wherein additionally the effect of protein FAM60A is impaired in said cell, wherein the FAM60A protein shares at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity with one or more of the amino acid sequences shown in SEQ ID NO: 11 to 18.

7. The isolated eukaryotic host cell according to one or more of claims 1 to 6, wherein:

a) the functional expression of gene C12orf35 is reduced or eliminated in said cell and wherein the reduction or elimination results in an increase in the volumetric production of a recombinant product of interest compared to a corresponding cell wherein the functional expression of gene C12orf35 is not reduced or eliminated; and/or
b) the genome of the eukaryotic cell is altered to impair the effect of the expression product of gene C12orf35 in said cell.

8. The isolated eukaryotic host cell according to one or more of claim 1 to 7, wherein the heterologous polynucleotide encoding the product of interest is comprised in an expression cassette that is integrated into the genome of the eukaryotic cell.

9. The isolated eukaryotic host cell according to one or more of claims 1 to 8, having one or more of the following characteristics:

a) the eukaryotic cell comprises integrated into its genome at least one heterologous polynucleotide encoding the product of interest and at least one heterologous

polynucleotide encoding a selectable marker or reporter polypeptide and wherein said heterologous polynucleotides are located on the same or different expression vectors;

b) the eukaryotic cell comprises a heterologous polynucleotide encoding a folate receptor as selectable marker and/or comprises a heterologous polynucleotide encoding a dihydrofolate reductase (DHFR) as selectable marker;

c) the eukaryotic cell secretes said polypeptide of interest into the cell culture medium; and/or

d) the product of interest is a polypeptide selected from a therapeutic polypeptide and a diagnostic polypeptide.

10. A method for selecting a host cell which recombinantly expresses a product of interest, wherein the product of interest is a polypeptide, comprising

(a) providing of eukaryotic cells according to one or more of claims 1 to 9 as host cells; and

(b) selecting one or more host cells expressing the product of interest.

11. The method according to claim 10, having one or more of the following characteristics

a) said host cells provided in stage (a) are mammalian cells;

b) said host cells provided in stage (a) additionally comprise at least one heterologous polynucleotide encoding a selectable marker and stage (b) comprises culturing said plurality of host cells under conditions selective for the selectable marker;

c) said host cells provided in stage (a) comprise at least two heterologous polynucleotides each encoding a selectable marker wherein the first selectable marker is a folate receptor and wherein the second selectable marker is a DHFR and wherein stage (b) comprises culturing said plurality of host cells in a selective culture medium which comprises folate in a limiting concentration and a DHFR inhibitor

d) heterologous polynucleotides are introduced into the eukaryotic cells by transfecting one or more expression vectors;

e) stage (b) comprises multiple selection steps;

f) stage (b) comprises performing a flow cytometry based selection;

g) the selected host cells recombinantly express an immunoglobulin molecule.

12. A method for recombinantly producing a product of interest, comprising utilizing a eukaryotic host cell according to one or more of claims 1 to 9 as host cell for recombinant expression of the product of interest and comprising

(a) culturing the host cells under conditions that allow for the expression of the product of interest;

(b) isolating the product of interest from the cell culture medium and/or from said host cells; and
(c) optionally processing the isolated product of interest.

13. A method for producing a eukaryotic cell according to one or more of claims 1 to 9, comprising impairing the effect of the expression product of an gene C12orf35 in said cell, wherein the C12orf35 gene is a gene encoding a protein that shares at least 70% identity with one or more of the amino acid sequences shown in SEQ ID NO: 1 to 7 or the protein encoded by SEQ ID NO: 8.

14. A method comprising analyzing eukaryotic cells for their suitability as host cells for recombinant expression of a product of interest by analyzing directly or indirectly whether the effect of the expression product of endogenous gene C12orf35 is impaired in said cells, wherein the C12orf35 gene is a gene encoding a protein that shares at least 70% homology or identity with one or more of the amino acid sequences shown in SEQ ID NO: 1 to 7 or the protein encoded by SEQ ID NO: 8,
wherein the method further comprises selecting at least one cell wherein the functional expression of C12orf35 is impaired because functional expression of gene C12orf35 is reduced or eliminated by gene knock-out, gene mutation, gene deletion, gene silencing or a combination of any the foregoing, for recombinant expression of the product of interest.

15. The method according to claim 14, comprising one or more of the following features:

a) directly analyzing comprises analyzing whether the functional expression of gene C12orf35 is reduced or eliminated in said cells;
b) prior to analysis, the eukaryotic cells are treated with an agent that induces chromosome breakage and wherein the analysis comprises analyzing whether treatment with said agent resulted in a deletion of a portion of a chromosome which includes gene C12orf35;
c) prior to analysis, the eukaryotic cells are transfected with a heterologous polynucleotide encoding a product of interest and a heterologous polynucleotide encoding a selectable marker, and wherein prior to analysis at least one selection step is performed to identify successfully transfected host cells;
d) the method comprising additionally analyzing directly or indirectly whether the effect of FAM60A is impaired in said cells, wherein the FAM60A protein shares at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity with one or more of the amino acid sequences shown in SEQ ID NO: 11 to 18;
e) the eukaryotic cells are hamster cells and the method comprises analyzing whether expression of one or more genes located in the telomeric region of chromosome 8 and being selected from the group consisting of the Tmtc1 gene and genes located telomeric of the Tmtc1 gene is eliminated or reduced thereby

analyzing whether the expression of gene C12orf35 is reduced or eliminated in said cells;

f) a plurality of cell clones is analyzed for discriminating between stable and unstable and/or between high and low producing cell clones and wherein one or more cell clones are selected as production clone wherein the expression of gene C12orf35 and optionally FAM60A is reduced or eliminated, wherein the FAM60A protein shares at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity with one or more of the amino acid sequences shown in SEQ ID NO: 11 to 18.