FIG. 21 is a photograph illustrating RT-PCR analysis of fucosylation pathway genes. Total RNA was isolated from CHO-S (C) and ITL-LF2 (LF) cells and subjected to RT-PCR utilizing polydT followed by gene specific primers. The resulting products were run on an agarose gel and detected under UV light by SyberSafe™ staining. Expected sizes: Fut8 – 1.7 Kb; GMD – 1.1 Kb; FX – 1.2 Kb; GFPP – 1.7 Kb; M – DNA size marker.

FIGs. 22A-B are protein sequence comparisons between full length GMD and splice variants. cDNAs prepared by RT PCR from RNA extracted from CHO-S and ITL-LF2 cells were run on gels, isolated and sequenced. The DNA sequence was then translated to protein by the CloneManager™ software. The protein sequence of splice variant 1 (SV1; Figure 22A; SEQ ID NO: 23) and splice variant 2 (SV2; Figure 22B, SEQ ID NO: 24) were compared to the full length GMD gene (SEQ ID NO: 25). Dashed lines denote areas of deletion. Text indicates which exons are deleted in each splice variant.

FIGs. 23A-B illustrate restriction enzyme analysis of the two GMD splice variants (SEQ ID Nos: 26 and 27, respectively). Figure 23A - Location of unique restriction enzyme sites within GMD splice variants; Figure 23B – Agarose gel showing the bands after restriction. Sizes and their explanation are detailed in the Table 7 of the Examples section below.

FIG. 24 is a graph illustrating the analysis of fucosylation pathway gene expression levels in ITL-LF2 cells *vs*. CHO-S cells by Q-PCR. cDNA was prepared from ITL-LF2 and CHO-S (control) RNA and subjected to Q-PCR analysis utilizing gene specific primers and probes that are situated on exons 5-6 (which are present in the full length GMD mRNA and both splice variants). The results for each gene were obtained from four separate RNA extractions, from cells plated on four different dates. From each extraction date, cDNAs were prepared and used for detection of all genes by Q-PCR. All samples were run in triplicates and normalized against a Beta-Actin endogenous control. Numbers above bars indicate P-values for each gene as calculated utilizing the paired Ttest statistical analysis. Significance is indicated by P-values < 0.05.

RQ – Fold of change of expression from control.

FIG. 25 is a schematic view of vector MB-129 (pCMV-P-GMD). The full length GMD cDNA from CHO-S wt was cloned into vector pCMV-P. pCMVpr – human CMV promoter. IVS – intron A of hCMV IE gene. PAC – puromycin resistance gene.

FIG. 26 is a graph illustrating that ITL-LF2 regains fucosylation upon transfection with wild type GMD cDNA. ITL-LF2 cells were transfected with vector containing GMD cDNA (pCMV-P-GMD) in C6614 medium in the presence of fucose. The transfected cells were transferred to ProCHO5 medium in the absence of fucose. The cells were analyzed by FACS following labeling with biotinylated AAL and fluorescent streptavidin. CHO-S, not labeled (□), CHO-S (○), ITL-LF2 (Δ), GMD transfected ITL-LF2 cells (◊).

FIG. 27 is a diagram illustrating the anti EGFR mAb containing plasmid-PGL3 anti EGFR mAb-EMCV-PAC1-DHFR Tandem. Linear plasmid containing the LC and HC of anti EGFR mAb was used for transfection of CHO-S and ITL-LF2. mCMV-murine cytomegalovirus promoter, EMCV-encephalomyocarditis virus, IRES-internal ribosome entry site, PAC- puromycin N-acetyltransferase, DHFR-dihydrofolate reductase, HC-heavy chain, LC-light chain, SV40-simian virus 40, pA-polyadenylation.

FIG.28 is a diagram illustrating stable expression of anti EGFR mAb in ITL-LF2 and CHO-S pools. ITL-LF2 and CHO-S were transfected with anti EGFR mAb containing vector (PGL3 anti EGFR mAb EMCV-PAC1-DHFR Tandem). After recovery, productivity was evaluated in pools.

FIG. 29 is a graph illustrating FACS analysis of fucose levels on ITL-LF2 anti EGFR mAb transfected cells. Analysis of fucose levels on the membrane of anti EGFR mAb transfected cells propagated in C6614 medium before and after removal of fucose. CHO-S, not labeled (□), CHO-S in ProCHO5 (○), ITL-LF2 in C6614 (◊), ITL-LF2 anti-EGFR mAb transfected in C6614 in the presence of fucose (▼), ITL-LF2 anti-EGFR mAb transfected in C6614 in the absence of fucose (Δ).

FIG. 30 is a graph illustrating FACS analysis of sialic acid levels on ITL-LF2 anti EGFR mAb transfected cells. Analysis of sialic acid levels on the membrane of anti EGFR mAb transfected cells propagated in ProCHO5 medium. The cells were labeled with FITC conjugated sialic acid binding lectin (MAA) and analyzed by

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BAR-SHIMON, MEIRAV
TOISTER-ACHITUV, MIRA

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            20                  25                  30

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        35                  40                  45

Glu Val His Gly Ile Val Arg Arg Ser Ser Ser Phe Asn Thr Gly Arg
    50                  55                  60

Ile Glu His Leu Tyr Lys Asn Pro Gln Ala His Ile Glu Gly Asn Met
65                  70                  75                  80

Lys Leu His Tyr Gly Asp Leu Thr Asp Ser Thr Cys Leu Val Lys Ile
                85                  90                  95

Ile Asn Glu Val Lys Pro Thr Glu Ile Tyr Asn Leu Gly Ala Gln Ser
            100                 105                 110

His Val Lys Ile Ser Phe Asp Leu Ala Glu Tyr Thr Ala Asp Val Asp
        115                 120                 125

Gly Val Gly Thr Leu Arg Leu Leu Asp Ala Ile Lys Thr Cys Gly Leu
    130                 135                 140

Ile Asn Ser Val Lys Phe Tyr Gln Ala Ser Thr Ser Glu Leu Tyr Gly
145                 150                 155                 160

Lys Val Gln Glu Ile Pro Gln Lys Glu Thr Thr Pro Phe Tyr Pro Arg
                165                 170                 175

Ser Pro Tyr Gly Ala Ala Lys Leu Tyr Ala Tyr Trp Ile Val Val Asn
            180                 185                 190
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Phe Arg Glu Ala Tyr Asn Leu Phe Ala Val Asn Gly Ile Leu Phe Asn
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    210                 215                 220

Arg Ser Val Ala Lys Ile Tyr Leu Gly Gln Leu Glu Cys Phe Ser Leu
225                 230                 235                 240

Gly Asn Leu Asp Ala Lys Arg Asp Trp Gly His Ala Lys Asp Tyr Val
                245                 250                 255

Glu Asp Phe Leu Gln Gly Asp Cys Ser Lys Ala Gln Gln Lys Leu Asn
            260                 265                 270

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Gly Gln Asp Gly Ser Tyr Leu Ala Glu Phe Leu Leu Glu Lys Gly Tyr
        35                  40                  45

Glu Ile Ser Phe Asp Leu Ala Glu Tyr Thr Ala Asp Val Asp Gly Val
    50                  55                  60

Gly Thr Leu Arg Leu Leu Asp Ala Ile Lys Thr Cys Gly Leu Ile Asn
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                85                  90                  95

Gln Glu Ile Pro Gln Lys Glu Thr Thr Pro Phe Tyr Pro Arg Ser Pro
            100                 105                 110

Tyr Gly Ala Ala Lys Leu Tyr Ala Tyr Trp Ile Val Val Asn Phe Arg
        115                 120                 125

Glu Ala Tyr Asn Leu Phe Ala Val Asn Gly Ile Leu Phe Asn His Glu
    130                 135                 140

Ser Pro Arg Arg Gly Ala Asn Phe Val Thr Arg Lys Ile Ser Arg Ser
145                 150                 155                 160

Val Ala Lys Ile Tyr Leu Gly Gln Leu Glu Cys Phe Ser Leu Gly Asn
                165                 170                 175

Leu Asp Ala Lys Arg Asp Trp Gly His Ala Lys Asp Tyr Val Glu Ala
            180                 185                 190

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        195                 200                 205

Thr Gly Glu Val His Ser Val Arg Glu Phe Val Glu Lys Ser Phe Met
    210                 215                 220

His Ile Gly Lys Thr Ile Val Trp Glu Gly Lys Asn Glu Asn Glu Val
225                 230                 235                 240

Gly Arg Cys Lys Glu Thr Gly Lys Ile His Val Thr Val Asp Leu Lys
                245                 250                 255

Tyr Tyr Arg Pro Thr Glu Val Asp Phe Leu Gln Gly Asp Cys Ser Lys
            260                 265                 270

Ala Gln Gln Lys Leu Asn Trp Lys Pro Arg Val Ala Phe Asp Glu Leu
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20 25 30

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35 40 45

Glu Val His Gly Ile Val Arg Arg Ser Ser Ser Phe Asn Thr Gly Arg
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Ile Glu His Leu Tyr Lys Asn Pro Gln Ala His Ile Glu Gly Asn Met
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Lys Leu His Tyr Gly Asp Leu Thr Asp Ser Thr Cys Leu Val Lys Ile
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Ile Asn Ser Val Lys Phe Tyr Gln Ala Ser Thr Ser Glu Leu Tyr Gly
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Lys Val Gln Glu Ile Pro Gln Lys Glu Thr Thr Pro Phe Tyr Pro Arg
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Ser Pro Tyr Gly Ala Ala Lys Leu Tyr Ala Tyr Trp Ile Val Val Asn
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Phe Arg Glu Ala Tyr Asn Leu Phe Ala Val Asn Gly Ile Leu Phe Asn
        195                 200                 205

His Glu Ser Pro Arg Arg Gly Ala Asn Phe Val Thr Arg Lys Ile Ser
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Arg Ser Val Ala Lys Ile Tyr Leu Gly Gln Leu Glu Cys Phe Ser Leu
225                 230                 235                 240

Gly Asn Leu Asp Ala Lys Arg Asp Trp Gly His Ala Lys Asp Tyr Val
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Glu Ala Met Trp Leu Met Leu Gln Asn Asp Glu Pro Glu Asp Phe Val
            260                 265                 270

Ile Ala Thr Gly Glu Val His Ser Val Arg Glu Phe Val Glu Lys Ser
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Phe Met His Ile Gly Lys Thr Ile Val Trp Glu Gly Lys Asn Glu Asn
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Glu Val Gly Arg Cys Lys Glu Thr Gly Lys Ile His Val Thr Val Asp
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Leu Lys Tyr Tyr Arg Pro Thr Glu Val Asp Phe Leu Gln Gly Asp Cys
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Ser Lys Ala Gln Gln Lys Leu Asn Trp Lys Pro Arg Val Ala Phe Asp
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cagaaagaga ccaccccttt ctatccaagg tcgccctatg gagcagccaa actttatgcc 360

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Arg Ser Ile Met Thr Asp Leu Tyr Tyr Leu Ser Gln Thr Asp Gly Ala
                165                 170                 175

Gly Glu Trp Arg Glu Lys Glu Ala Lys Asp Leu Thr Glu Leu Val Gln
            180                 185                 190

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Lys Leu Val Cys Asn Ile Asn Lys Gly Cys Gly Tyr Gly Cys Gln Leu
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Val Glu Leu Pro Ile Val Asp Ser Leu His Pro Arg Pro Pro Tyr Leu
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Gly Asp Pro Ala Val Trp Trp Val Ser Gln Phe Val Lys Tyr Leu Ile
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Leu Gly Phe Lys His Pro Val Ile Gly Val His Val Arg Arg Thr Asp
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His Val Glu Glu His Phe Gln Leu Leu Glu Arg Arg Met Lys Val Asp
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Lys Lys Arg Val Tyr Leu Ala Thr Asp Asp Pro Ser Leu Leu Lys Glu
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Ala Lys Thr Lys Tyr Ser Asn Tyr Glu Phe Ile Ser Asp Asn Ser Ile
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Ser Trp Ser Ala Gly Leu His Asn Arg Tyr Thr Glu Asn Ser Leu Arg
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Gly Val Ile Leu Asp Ile His Phe Leu Ser Gln Ala Asp Phe Leu Val
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Cys Thr Phe Ser Ser Gln Val Cys Arg Val Ala Tyr Glu Ile Met Gln
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Thr Leu His Pro Asp Ala Ser Ala Asn Phe His Ser Leu Asp Asp Ile
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Tyr Tyr Phe Gly Gly Gln Asn Ala His Asn Gln Ile Ala Val Tyr Pro
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His Gln Pro Arg Thr Lys Glu Glu Ile Pro Met Glu Pro Gly Asp Ile
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Ile Gly Val Ala Gly Asn His Trp Asn Gly Tyr Ser Lys Gly Val Asn
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WHAT IS CLAIMED IS:

1. A method of selecting a Chinese hamster ovary (CHO) cells having a zero fucose level useful as a host cell for expressing a recombinant protein, the method comprising:

(a) introducing a genetic mutation into a coding region for a GDP-mannose 4,6-dehydratase (GMD) in a CHO-cell of a population of CHO cells by contacting the cells with methotrexate (MTX), thereby generating a CHO cell with a mutated GMD protein,

(b) contacting the population of CHO cells comprising of at least one CHO cell with a mutated GMD protein with a non-toxic fucose binding agent for an amount of time that allows binding of the fucose binding agent to a fucose moiety on a cell membrane of one or more cells of the population of cells, wherein the amount of time does not allow killing of the cells; and

(c) depleting from the population of cells comprising mutated cells, a subpopulation of one or more cells which bind the fucose binding agent, thereby selecting cells useful as host cells for expressing recombinant proteins, the selected cells having zero fucose content in the absence of external fucose, wherein the expressed mutated GMD protein lacks amino acids encoded by exons 3 and 4 or lacks amino acids encoded by exons 8 and 9, and further wherein the ells exhibit a stable fucose phenotype for more than 100 population doublings.

2. The method of claim 1, wherein the CHO cells are selected from CHO-K1, CHO-S, DUXB11, CHO-1E5, CHO3F, CHO/DG44 and CHO-2.6.

3. The method of claim 1, wherein the recombinant protein is an antibody or a Fc protein.

4. The method of claim 1, wherein said fucose binding agent is attached to a detectable moiety or an affinity moiety.

5. The method of claim 1, wherein said population of CHO cells comprising mutated cells is in contact with the non-toxic fucose binding agent for an amount of time that is no longer than 60 minutes.

6. The method of claim 4, wherein when said fucose binding agent is attached to an affinity moiety, the method further comprises contacting said mutated population of cells with an additional agent, such additional agent comprising a cognate binding moiety for said affinity moiety.

7. The method of claim 4, wherein said affinity moiety comprises biotin.

8. The method of claim 4, wherein said cognate binding moiety is attached to a detectable moiety.

9. The method of claim 8, wherein said detectable moiety comprises a fluorescent moiety or a magnetic moiety.

10. The method of claim 1, wherein said depleting is effected by FACS sorting.

11. The method of claim 1, wherein said depleting is effected by magnetic separation.

12. The method of claim 1, wherein said depleting is effected by at least three rounds of sequential depleting.

13. The method of claim 1, wherein said fucose binding agent is aleuria aurantia lectin (AAL) or Aspergillus oryzae 1-fucose-specific lectin (AOL).

14. An isolated CHO cell comprising an expressed mutated GDP-mannose 4,6-dehydratase (GMD), wherein the cell has a zero fucose content in the absence of external fucose, and wherein the expressed mutated GMD lacks amino acids encoded

b exons 3 and 4 or lacks amino acids encoded by exons 8 and 9, and further wherein the cell exhibits a fucose phenotype for more than 100 population doublings.

15. The isolated CHO cell of claim 14 wherein the cell is genetically modified to express a mutated GDP-mannose 4,6-dehydratase (GMD) having an amino acid sequence as set forth in SEQ ID NO: 23 or 24.

16. Th isolated CHO cell of claim 14 wherein the cell is genetically modified such that an amount of fucosylation of a protein expressed therein is linearly dependent on a concentration of external fucose.

17. The isolated CHO cell of claim 14, having a 20% higher integral viable cell concentration (IVCC) than non-mutated CHO cells.

18. The isolated CHO cell of claim 14, expressing a wild-type fucosyl transferase-8 (Fut8).

19. The isolated CHO cell of claim 14, expressing a wild-type GDP-keto-6-deoxymannose 3, 5-epimerase, 4-reductase (FX).

20. The isolated CHO cell of claim 14, having a population doubling time of no more than 20 hours.

21. The isolated CHO cell of claim 14, expressing a recombinant antibody or Fc fusion protein of interest.

22. A CHO cell line comprising the isolated cell of claim 14.

23. The isolated CHO cell of claim 21, deposited under CNCM Accession Number I-4449.

24. A cell culture which comprises the isolated CHO cell of claim 14 and a cell culture medium being devoid of animal-derived contaminants.

25. The cell culture of claim 24, wherein said cell culture medium comprises fucose.

26. The cell culture of claim 24, wherein said cell culture medium is devoid of fucose.

27. A method for the production of a recombinant protein comprising transfecting the selected CHO cell of claim 1 with a polynucleotide comprising a nucleic acid sequence which encodes the recombinant protein, culturing the transfected CHO cell under conditions suitable for expression of the recombinant protein, and isolating the protein.

28. The method of claim 27, wherein the recombinant protein is an antibody or a Fc fusion protein.

29. The method of claim 27, wherein said transfecting is effected in the presence of exogenous fucose.

30. The method of claim 27, further comprising culturing the selected CHO cell in a culture medium comprising fucose following said transfecting.

31. The isolated CHO cell of claim 14, wherein the mutated GMD lacks amino acids encoded by exons 3, 4, 8 and 9.

32. The isolated CHO cell of claim 14 wherein the mutated GDP-mannose 4,6-dehydratase (GMD) has the amino acid sequence set forth in SEQ ID NO: 25 at positions 1-49, 151-257, and 330-372.