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[Continued on next page]

(54) Title: METABOLIC ENGINEERING OF A GALACTOSE ASSIMILATION PATHWAY IN THE GLYCOENGINEERED YEAST *PICHA PASTORIS*

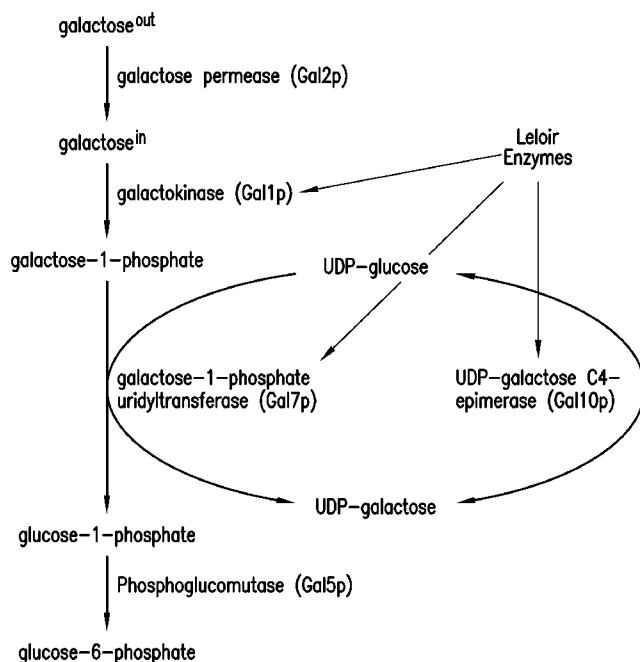


FIG.4

(57) Abstract: Lower eukaryotic cells such as *Pichia pastoris* that normally cannot use galactose as a carbon source but which have been genetically engineered according to the methods herein to use galactose as a sole source of carbon are described. The cells are genetically engineered to express several of the enzymes comprising the Leloir pathway. In particular, the cells are genetically engineered to express a galactokinase, a UDP-galactose-C4-epimerase, and a galactose-1-phosphate uridylyltransferase, and optionally a galactose permease. In addition, a method is provided for improving the yield of glycoproteins that have galactose-terminated or -containing N-glycans in cells that have been genetically engineered to produce glycoproteins with N-glycans having galactose residues but which normally lack the enzymes comprising the Leloir pathway comprising transforming the cells with one or more nucleic acid molecules encoding a galactokinase, a UDP-galactose-C4-epimerase, and a galactose-1-phosphate uridylyltransferase. The methods and host cells described enable the presence or lack of the ability to assimilate galactose as a selection method for making recombinant cells. The methods and host cells are shown herein to be particularly useful for making immunoglobulins and the like that have galactose-terminated or containing N-glycans.



TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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