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C07K 1/00 (2006.01)
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[Continued on next page]

(54) **Title:** CELLS FOR PRODUCING RECOMBINANT IDURONATE-2-SULFATASE

FIG. 2A

I2S and SUMF1 co-expression options

Expression units on separate vectors (co-transfection or subsequent transfections)

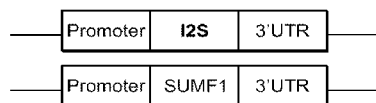


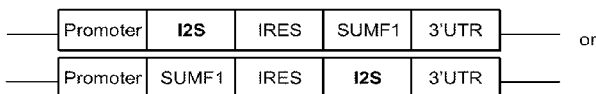
FIG. 2B

Expression units on the same vector (one transfection)

1) Separate cistrons



2) Transcriptionally linked cistrons



**(57) Abstract:** The present invention provides, among other things, methods and compositions for production of recombinant I2S protein with improved potency and activity using cells co-express I2S and FGE protein. In some embodiments, cells according to the present invention are engineered to simultaneously over-express recombinant I2S and FGE proteins. Cells according to the invention are adaptable to various cell culture conditions. In some embodiments, cells of the present invention adaptable to a large-scale suspension serum-free culture.

WO 2014/005019 A3

- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
  - *with sequence listing part of description (Rule 5.2(a))*
- (88) **Date of publication of the international search report:**  
27 February 2014

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US13/48571

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/46; C07K 1/00; C12P 21/06 (2013.01)

USPC - 435/68.1, 69.1, 325

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): C12P 21/06; C07K 1/00, 16/00; A61L 33/00; A61K 38/46, 38/17; A61P 31/12; C12Q 1/68; C07H 21/04 (2013.01)

USPC: 435/68.1, 69.1, 325, 350, 41, 196, 320.1; 530/350, 387.1; 522/87; 436/6; 536/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google/Google Scholar; Proquest; ScienceDirect; Search Terms Used: 'iduronate sulfatase', formylglycine, enzyme, cysteine, recombinant, bioreactor, perfusion, 'roller bottle'

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2004/072275 A2 (VON FIGURA, K et al.) August 26, 2004; page 4, lines 30-32; page 13, line 29; page 14, lines 3-6; page 54, lines 17-25; page 54, lines 30-33; page 66, lines 29-32; page 71, lines 30-35 to page 72, line 1; page 87, lines 26-30; page 87, lines 31-34 to page 88, lines 1-12; page 91, lines 16-21; Claims 2, 7, 9	1, 3/1 ----- 2, 3/2
Y	WO 2011/044542 A1 (PARDRIDGE, WM et al.) April 14, 2011; paragraphs [0003], [0099], [00161], [00183]; figure 4	1, 2, 3/1, 3/2
Y	WO 2005/113765 A2 (ZANKEL, T et al.) December 1, 2005; page 25, lines 5-11; page 25, lines 26-33 to page 26, lines 1-8	2, 3/2

 Further documents are listed in the continuation of Box C.

* Special categories of cited documents:	"I" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

27 November 2013 (27.11.2013)

Date of mailing of the international search report

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US13/48571

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.: 4-33  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.