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(57) Abstract: We used Accutase™, a commercially available cell detachment solution, for single cell propagation of pluripotent hESCs. Unlike trypsin dissociation, Accutase treatment does not significantly affect the plating efficiency of hESC dissociation into single cells. Cultures dissociated with Accutase to single cells at each passage maintain a higher proportion of pluripotent cells as compared to collagenase-passaged hESCs. Accutase-treated hESCs can be grown to a high density as monolayers, and yet retain their pluripotency.



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A. CLASSIFICATION OF SUBJECT MATTER IPC: C12N 5/00(2006.01) USPC: 435/325 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) U.S. : 435/325 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)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,675,063 (KNIGHT) 7 October 1997 (07.10.1997), column 12, lines 19-44.	1-6, 8, 11-19, 24-29 ----- 7, 9, 10, 20-23
X --- Y	US 5,690,926 (HOGAN) 25 November 1997 (25.11.1997), column 11, lines 20-49.	1-6, 8, 11-19, 24-29 ----- 7, 9, 10, 20-23
X --- Y	US 2004/0014210 A1 (JESSELL et al) 22 January 2004 (22.01.2004), paragraph 99.	1-9, 11-21, 23-29 ----- 7, 9, 10, 2-23
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Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201		Authorized officer Lora E. Barnhart Telephone No. 571-272-1600