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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

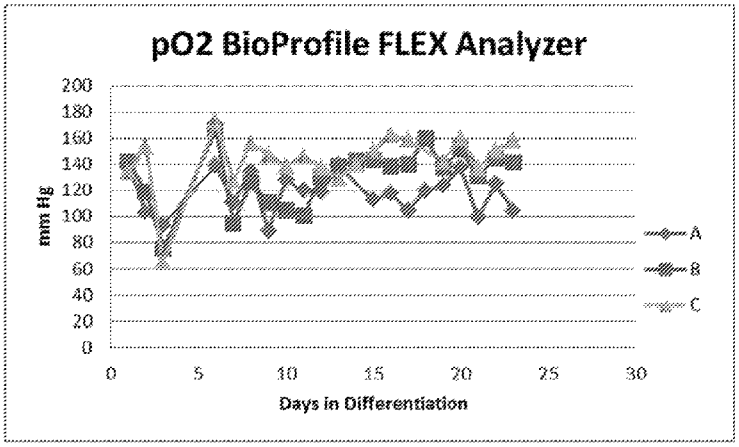
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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(54) Title: SUSPENSION CULTURING OF PLURIPOTENT STEM CELLS

(57) Abstract: The present invention provides methods of differentiating pluripotent cells into beta cell using suspension clustering. The methods of the invention use control of one or more of pH, cell concentration, and retinoid concentration to generate a nearly homogenous population of PDX1/NKX6.1 co-expressing cells by suppressing precocious NGN3 expression and promoting NKX6.1 expression. Also, the nearly homogenous population of PDX1/NKX6.1 co-expressing cells may be further differentiated *in vitro* to form a population of pancreatic endocrine cells that co-express PDX1, NKX6.1, insulin and MAFA.

Figure 1A



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