Exhibit 5
Part 44
To Third Declaration of
Joseph N. Hosteny

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Furthermore, the Patent Owner has failed to provide a statement indicating how the copy of the declaration from the prosecution of the related patent 5,843,780 meets even one of the six requirements of 37 CFR §1.111(a)(2)(i).

The Third Party Requester's comments concerning the Patent Owner's supplemental amendment of October 4, 2007 has also been entered into the record.

Status of Claims

Claims 1 - 3 were originally present in U.S. Patent No. 7,029,913 undergoing reexamination.

In the Response of March 30, 2007, claims 1 - 3 have been amended in the following manner. The phrase "derived from a pre-implantation embryo, the culture comprising cells" was inserted into claim 1 to further define "A replicating in vitro cell culture of human embryonic stem cells." In both claims 2 and 3 the word "preparation" has been replaced by the phrase "in vitro cell cultures."

In the supplemental amendment filed October 4, 2007, the Patent Owner inserted the phrase "will proliferate" in place of "are capable of proliferation" in part (i) of claim 1. Additionally, the phrase "in an undifferentiated state" was inserted into part (i) of claim 1 to further describe characteristics of the human embryonic stem cells growing in culture for over one year. Finally, the word "pluripotent" was inserted into the first line of claim 1 to modify "human embryonic stem cells."

Information Disclosure Statement (IDS)

The Patent Owner submitted an Information Disclosure Statement (PTO-1449) on May 30, 2007. The references listed on this document have been fully considered and an initialed, signed copy of this IDS is attached to this Office action.

The Third Party Requester also submitted an information disclosure statement (PTO/SB/08b) on June 29, 2007. The references listed on this document have been fully considered and an initialed, signed copy of this IDS is attached to this Office action.

Continuing Duty to Disclose

The Patent Owner is reminded of the continuing responsibility under 37 CFR §§ 1.555, 1.565(a) and 1.933 to apprise the Office of any litigation activity, or other prior or concurrent proceeding, involving Patent No. 7,029,913 throughout the course of this reexamination proceeding. The Third Party Requester is also reminded of the ability to similarly apprise the Office of any such activity or proceeding throughout the course of this reexamination proceeding. See MPEP §§2684, 2280, and 2282.

Erratum

The Patent Owner correctly points out on page 7 of the Response that there are no joint inventors as suggested at page 8 of the non-final Office action of March 30, 2007. Dr. James A. Thomson is the sole inventor of U.S. Patent No. 7,029,913.

STATUS: GROUNDS OF REJECTION

a. Proposed by 3rd Party Requester: not adopted

GROUNDS #1: Requester submits that claims 1 - 3 are unpatentable under 35 U.S.C. §103(a) as being obvious over Robertson '83 in view of the Declaration by Dr. Jeanne F. Loring (Request, pages 4 - 7). This rejection was **not adopted** by the Examiner. See Office action of March 30, 2007 at page 7.

The Requester indicates in the first paragraph on page 6 of the Comments that the Loring Declaration was submitted in order to explain "what the prior art disclosed to, and what motivations or suggestions it provided to those of ordinary skill in the art." However, only patents or printed publications can be used in the reexamination proceeding to raise a substantial new question of patentability. See MPEP §§2658(I.), 2258, and 2258.01. The Loring declaration cannot by itself provide the necessary motivation to combine references; however this declaration has been given full consideration as evidence supporting obviousness in each rejection under 35 USC §103(a) in the non-Office action of March 30, 2007. Accordingly, this proposed rejection continues to be **not adopted** for all pending claims.

GROUNDS #2: Requester submits that claims 1 - 3 are unpatentable under 35 U.S.C. §103(a) as being obvious over Piedrahita in view of the Declaration by Dr. Jeanne F. Loring (Request, pages 7 - 11). This rejection was **not adopted** by the Examiner. See Office action of March 30, 2007 at page 7.

The Requester indicates in the first paragraph on page 6 of the Comments that the Loring Declaration was submitted in order to explain "what the prior art disclosed to,

and what motivations or suggestions it provided to those of ordinary skill in the art." However, only patents or printed publications may be used in the reexamination proceeding to raise a substantial new question of patentability. See MPEP 2658(I.), 2258. and 2258.01). The Loring declaration cannot by itself provide the necessary motivation to combine references; however this declaration has been given full consideration as evidence supporting obviousness in each rejection under 35 USC §103(a) in the non-final Office action of March 30, 2007. Accordingly, this proposed rejection continues to be **not adopted** for all pending claims.

GROUNDS #3: Requester submits that claims 1 - 3 are unpatentable under 35 U.S.C. §103(a) as being obvious over Robertson '83, Robertson '87 and Piedrahita in view of the Declaration by Dr. Jeanne F. Loring (Request, pages 7 - 11) This rejection was **not adopted** by the Examiner. See Office action of March 30, 2007 at page 8.

The Requester indicates in the first paragraph on page 6 of the Comments that the Loring Declaration was submitted in order to explain "what the prior art disclosed to, and what motivations or suggestions it provided to those of ordinary skill in the art." However, only patents or printed publications may be considered in reexamination proceedings as raising a substantial new question of patentability. See MPEP 2258(I.), 2258, and 2258.01. The Loring declaration cannot by itself provide the necessary motivation to combine references; however this declaration has been given full consideration as evidence supporting obviousness in each rejection under 35 USC §103(a). Accordingly, this proposed rejection continues to be **not adopted** for all pending claims.

b. Raised by Patent Examiner in Office Action of March 30, 2007

GROUNDS #4: Claims 1 - 3 are rejected under 35 U.S.C. §102(b) as being anticipated by, or in the alternative, under 35 USC §103(a) as obvious over Williams et al. (5,166,065) alone, or as further evidenced by the instant patent disclosure for demonstrating inherency. The complete explanation of this rejection at pages 9 - 12 of the Office action of March 30, 2007 is hereby incorporated by reference. As explained below, the preponderance of the evidence of record supports the conclusion that the Williams '065 patent is not an enabling reference under 35 USC §102(b) and also fails as a reference under 35 USC §103(a) because of the unpredictability in the art at the time of the invention which leads to the lack of a reasonable expectation of success. Therefore, **this rejection has been withdrawn**.

GROUNDS #4 (102(b)): Patent Owner's Response, Third Party Requester's Comments, and Examiner's Decision

Patent Owner: (Response, page 6, line 19 to page 7, line 13)

A. Williams '065 does not anticipate invention (Response, p. 6, l. 13 - p. 7, l. 13)

Human ES cells, let alone a replicating in vitro cell culture of the same, were not known in the art prior to the landmark invention of Dr. Thomson.

According to *In re Best*, 562 F.2d 1252, 1254 - 55, 195 U.S.P.Q. 430 (C.C.P.A. 1977), where *In re Best* cites *In re Swinehart*, 439 F.2d 210 (1971);

> "[I]t is elementary that the mere recitation of a newly discovered function or property, inherently possessed by *things* in the prior art, does not cause a claim drawn to those *things* in the prior art, to distinguish over the prior art. Additionally, where the Patent Office has reason to believe that a functional limitation asserted to be critical for establishing novelty in the claimed subject matter may, in fact, be an inherent characteristic of the prior art, it possesses the authority to require the applicant to prove that the subject matter shown to be in the prior art does not possess the characteristic relied on. *In re Swinehart*, 439, F.2d at 212-213.

[Emphasis added.]

Here, this standard for inherent anticipation does not apply. The invention claimed by Dr. Thomson, namely, a replicating in vitro cell culture of human ES cells, is not in the prior art. The applied prior art neither explicitly nor inherently teach a replicating in vitro cell culture of human ES cells. Simply stated, the replicating in vitro cell culture of human ES cells disclosed in the present patent did not previously exist. They differ markedly from the prior art murine ES cells (Williams '065) and Hogan's EG cells (Hogan '926) and the cells of the other prior art references, which cells do not share the claimed features. There is no composition in the cited prior art that is a replicating in vitro cell culture of human ES cells that are capable of proliferation for over one year without the application of exogenous LIF as claimed in present claim 1.

Third Party: (Comments, page 6, line 13 to page 7, line 2)

A. Williams '065 anticipates the invention of Thomson '913

In the Office Action, the Examiner rejected all three claims as being anticipated by or obvious over Williams et al. (U.S. Patent No. 5,166,065) ("Williams '065"). Office Action at 9. The Examiner found that Williams '065 disclosed human embryonic stem cells and a method for preparing such embryonic stem cells that were "essentially the same procedure" as described in the pending patent's specification. *Id.* at 10. Further, the Examiner concluded that, "there is no structural difference between pluripotent human ES cells disclosed by Williams '065 and the ES cells instantly claimed." *Id.* at 11 - 12. The Patent Owner made several arguments in its Response as to why Williams '065's teaching of human embryonic stem cells does not invalidate the pending claims, but each of the Patent Owner's arguments are without merit. Thus, the Examiner's rejection of the pending claims based on Williams '065 was and remains appropriate.

Examiner:

A. Williams '065 does not anticipate the invention of Thomson '913

The Examiner finds the arguments of the Patent Owner to be persuasive with respect to the inapplicability of *In re Best* to the inherency of the characteristics of the human ES cells disclosed in Williams '065 at column 4, lines 17 - 23:

> The ES cells in accordance with this aspect of the invention include cells derived from humans, mice, birds (e.g. chickens), sheep, pigs, cattle, goats, and fish. As with the isolation of ES cells from animal embryos, the LIF used in the aforementioned

> process is preferably recombinant LIF. The culture medium may or may not contain feeder cells.

This disclosure by Williams is without any accompanying specific working example other than the mouse. Without such an exemplification of human ES cells, this disclosure provides only for the possibility of obtaining human ES cells. Proper application of *In re Best* requires that the examiner establish a *prima facie* case of anticipation. This means that Williams '065 must disclose an actual in vitro culture of human ES cells with a set of defining characteristics that would be consistent with those of the pending claims. If one or two of these requisite traits of human ES cells were missing from the Williams '065 disclosure, then according to *In re Best,* it would be appropriate to shift the burden back to the Patent Owner to demonstrate that such additional missing characteristics were not inherent. However, in this instance, the burden has not been shifted to the Patent Owner because the examiner has failed to establish a *prima facie* case of anticipation.

Patent Owner: (Response, page 7, lines 15 - 19)

B. Williams '065 lacks critical elements of invention

Williams lacks all of the claimed elements. Williams does not disclose a replicating in vitro cell culture of human ES cells capable of proliferation without addition of LIF. Williams' cells require LIF (Stewart, ¶ 23).

Third Party: (Comments, page 7, lines 4 -16)

B. All of the elements are expressly or inherently present

The Patent Owner first argues in the Response that Williams '065's cells require LIF. Response at 7. While it may be true that Williams '065 was principally directed towards researching the ability to use LIF to maintain ES cells without feeder layers, its

teachings did not exclude cultures maintained with only feeder cells in the absence of LIF. Rather, Williams '065 expressly states that LIF can "substitute" for feeder layers in supporting the maintenance of pluripotential ES cells. Williams '065 at 1:58 - 62 and 3:62 - 64 ("LIF may be used to substitute for, or add to, feeder cells."). Contrary to the Patent Owner's interpretation, a skilled artisan would not understand that Williams '065 is "directed to the *advantages* of LIF in isolating and maintaining ES cells." Response at 15 (emphasis added). Rather, those of skill in the art understood Williams '065 to merely be directed to showing the *capability* of LIF to be used in isolating and maintaining ES cells. Loring Declaration at 3 - 4 ("Williams '065's discovery was merely that LIF could be used . . . , not that it was an improvement over feeder layers"). Thus, the Patent Owner's proposed interpretation of Williams '065 as requiring LIF should be rejected.

Examiner:

B. Williams discloses ES cells with feeder cells and/or LIF

The examiner finds the Third Party Requester's position persuasive with regard to the teachings of Williams '065 about LIF and feeder cells.

The primary purpose of Williams '065 is to demonstrate that LIF can substitute for feeder cells in the maintenance of mouse ES cells (column 2, lines 30 - 40; Examples 1 & 2). And as the Third Party Requester observes, Williams '065 does not teach that maintaining the mouse ES cells with LIF is an improvement over the use of feeder cells. However, Williams '065 also discloses to the artisan that ES cells can be maintained on feeder layers alone, on LIF alone, and on a combination of feeder cells and LIF. These three conditions of ES cells are disclosed either explicitly or implicitly at column 1, lines 58 - 61:

> In work leading to the present invention, it has been found that LIF has the capacity to substitute for, or be added to, feeder layers (or conditioned medium) in supporting the maintenance of pluripotential ES cells in vitro.

Furthermore, independent claims 1 and 10 of Williams '065 encompass a method for

isolating maintaining animal ES cells on feeder layers with LIF and with LIF alone. This is emphasized by dependent claims 2 and 11 which specify that the culture medium is free of feeder cells. This further limitation makes it clear that the preceding claims encompass the isolation of animal ES cells in a culture media of LIF or LIF and feeder layers. In other words, Williams '065 can still implicitly disclose animal ES cells on feeder layers even though its primary intent is the disclosure of animal ES cells on feeder layers and/or LIF. Consequently, an artisan reading the Williams '065 patent would immediately envision human ES cells in LIF alone, in feeder layers alone, or in a combination of feeder layers plus LIF.

Patent Owner: (Response, page 7, line 18 - page 9, line 22)

C. Williams suggests isolating ES cells from other species and later retracts this suggestion

Williams makes the mere suggestion, later retracted by Williams himself (Cherny et al., 1994, Reprod. Fertil. Dev. 6: 569 - 575: "[t]he murine model for totipotential stem cell isolation is yet to prove applicable to domestic animals", page 574), that his method is applicable to the isolation of ES cells from other species, including humans. Williams described a method of making mouse ES cells and merely suggested its efficacy for humans, which proved to be wrong in practice. This does not rise to the legal standard for anticipation.

It is hornbook law that "[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." MPEP §2131 (quoting *Verdegaal Bros. V. Union Oil Co. of Calif.*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987)). "The identical invention must be shown in as complete detail as is contained in the . . . claim." Id. (quoting *Richardson v. Suzuki Motor Co..*, 9 USPQ2d 1913, 1920 (Fed. Cir 1989) (emphasis added). Therefore, Williams must describe each and every element of claims 1 - 3 in order to anticipate there claims under 35 U.S.C. §102(b). The Williams reference does not satisfy this requirement.

Even the Examiner's efforts to string together a disclosure that includes all of the elements of the present claims fails. The Examiner refers to specific sections in Williams that are simply incorrect. For example:

On page 10 of the Office action, the Examiner cites Williams column 2, lines 30 - 40; column 3, lines 35 - 47, column 4, lines 18 - 19; and column 6, lines 50 - 66 as disclosing "human embryonic stem cells . . . method for preparing such embryonic stem cells . . . which is essentially the same procedure as disclosed by Thomson." This is not correct. At, column 2, lines 30 - 40, Williams states:

> Accordingly, a first aspect of the present invention relates to a method for the isolation of embryonic stem (ES) cells from animal embryos in vitro which method comprises deriving ES cells from said embryos in culture medium, said culture medium containing an effective amount of leukaemia inhibitory factor (LIF), for a time and under conditional sufficient for the development of said ES cells. (Emphasis added.)

Williams does not disclose the same or even the essentially same procedure encompassed by the present claim 1 which explicitly states that the human ES cell culture grows in the absence of LIF.

The Examiner continues on page 10 of the Office Action to cite to sections of Williams that simply do not disclose what the Examiner says Williams discloses. For example:

- (1) column 3 lines 1 - 3 does not describe plating inner mass cells on embryonic fibroblasts, but does describe plating the cells in LIF:
- (2) column 8, lines 29 - 31 does not disclose dissociating the mass into associated cells, in fact, Williams nowhere discloses dissociation of the cells at all.
- (3) column 8, lines 29 - 31 does not disclose replating the dissociated cells on embryonic feeder cells (or LIF alone);
- (4) column 6, lines 65 - 66 does not disclose culturing ES cell colonies on embryonic feeder layers or with LIF alone;
- (5) last paragraph on page 10 - column 4, line 65 - to column 5, line 5 of Williams does not disclose proliferation for over one year without the application of exogenous leukemia inhibitory factor. The only references in this section to propagation of cells discloses that they are propagated in LIF; not in its absence. Moreover, the disclose of 20 passages in this section is simply not over one year as the Examiner alleges.
- (6) on page 11 of the Office Action, the Examiner states that William's cells maintain karyotype. The words karyotype or chromosomes nowhere appear in Williams.

Williams simply does not disclose what the Examiner says Williams discloses. Even if one looks elsewhere in Williams, one cannot find what the Examiner says Williams discloses. This goes to the heart of the Examiner's inherency argument which fails under the correct interpretation of the facts.

One skilled in the art cannot consider Williams to teach an all-purpose recipe for isolating and culturing embryonic stem cells from all species so that a replicating in vitro cell culture of human ES cell is obtained. Instead, Williams's contribution to the art is one discrete advancement to the then-current state of the art, namely that feeder layers, previously required in murine embryonic stem cell cultures, can be replaced by leukemia inhibitory factor (LIF) when isolating and maintaining ES cells. Even Williams speculation that his methods are applicable to non-murine species have proven untrue for primate/human ES cells because derivation and maintenance of primate/human ES cells is independent of LIF. (Steward, ¶23).

<u>**Third Party:**</u>

C. Williams does not retract teaching toward isolating animal ES cells
(Comments, page 7, line 16 to page 8, line10)

Next, the Patent Owner argues that Williams '065 "merely suggests" that its method for deriving embryonic stem cells could be used to isolate ES cells of other species and that one of the inventors of Williams '065, Dr. Robert Lindsay Williams, "later retracted" that suggestion. Response at 7 (citing Cherny et al., 6 Reprod. Fertil. Dev. 569 - 575 (1994)) ("Cherny '94"). However, Dr. Williams did not retract the teaching in Williams '065 that methods for isolating ES cells in one species could be used for other species. In fact, Cherny '94 reiterated this understanding by saying, "the ability to culture murine ES cells to produce unlimited numbers of cells while still retaining their development potential provides a strong incentive for the isolation of domestic animal ES cells." Cherny '94 at 569. Further Cherny '94 also said that although the murine model for stem cell isolation has "yet" to prove applicable to domestic animals, "criteria used in the identification of murine ES cells can serve as guidelines." *Id.* at 574.

Thus, contrary to the Patent Owner's characterization, Dr. Williams never "retracted" the teaching in Williams '065 that methods of deriving and maintaining mouse ES cells could be used to isolate and culture human embryonic stem cells. Instead, he actually reiterated it.

<u>**Examiner:**</u>

C. Williams suggests isolating ES cells of other animals by method used for mouse, but this is later contradicted by Cherny '94

The Examiner finds the position of the Patent Owner to be persuasive with respect to the teachings Williams '065 in view of Cherny '94.

Williams '065 teaches the isolation of ES cells from a variety of species and maintenance of said cells in the presence of LIF at column 4, lines 17 - 22.

> The ES cells in accordance with this aspect of the invention include cells derived from **human,** mice, birds (e.g., chickens), sheep, pigs, cattle, goats, and fish. As with the isolation of animal embryos, the LIF used in the aforementioned process is preferably recombinant LIF. (Emphasis added.)

This disclosure of Williams '065 at column 3, lines 6 - 8 regarding human ES cells is negated by the Cherny '94 article (Williams is also a co-author) which was published about four years after the effective filing of the application which issued as Williams '065. The critical passage from Cherny '94 is found at page 571, left column, lines 1 - 15:

> Initial research into the isolation of domestic animal ES cells in our and other laboratories attempted to repeat the work carried out in mice by isolating cell lines directly from cultured preimplantation embryos. Published reports of such studies in pigs, cattle and sheep, together with our own research, indicated that cells which displayed some ES cell characteristics could be identified but **the isolation of proven pluripotential ES cells remained elusive.** (Handside et al., 1987; Piedrahita et al., 1990; Notarianni et al. 1991 ; Sims and First 1993.) One reason for the limited success of these strategies may be the substantial differences in the early embryonic development of murine and domestic species. (Emphasis added.)

The failure or limited success of the procedure for isolating mouse ES cells to yield ES cells of domestic animals was the precise impetus for the Cherny '94 paper--to find an alternative way to isolate animal ES cells, e.g., the preparation of bovine priomordial germ cell-derived (PGCd) cells. It is within this context that the authors draw their

conclusion at page 574, first sentence under "Discussion."

> The murine model for totipotential stem cell isolation is yet to prove applicable to domestic animals.

The next sentence does not provide an affirmation of the statement from Williams '065 as the Requester asserts, but only acknowledges that the criteria used to isolate murine ES may be generally useful without being a precise protocol.

> However, criteria used in the identification of murine cells can serve as guidelines.

Consequently, the disclosure of Williams '065 cannot be viewed as enabling the isolation of any domestic animal, much less a preparation of primate/human ES cells.

Patent Owner:

D. Williams not enabled for isolating and maintaining primate/human ES cells
(Response, pages 9, line 24 to page 12, line 7)

1. Cherny '94 contradicts teaching of Williams '065

Williams does not enable a replicating in vitro cell culture of human ES cells that are capable of proliferation for over one year in the absence of exogenous LIF.

In a recent case, the Federal Circuit has reaffirmed that "to be anticipating, a prior art reference must be enabling so that the claimed subject matter may be made or used by one skilled in the art." *Impax Laboratories, Inc. v. Aventis Pharmaceuticals Inc.*, 468 F.3d 1366, 1381 (Fed. Cir. 2006). The legal standard for an enabling anticipatory reference, requires that the prior art reference teach one of ordinary skill in the art to make and carry out the claimed invention without undue experimentation. [Italics added.] *Amgen Inc. v. Hoechst Marion Roussel, Inc.* 457 F.3d 1293, 1306 - 1307 (Fed. Cir. 2006); *Etan Pharmaceuticlas, Inc. V. Mayo Foundation*, 346 F.3d 1051, 1053 (Fed. Cir. 2003). Enablement is a question of law based on underlying facts. *Minnestota Mining & Mfg. v. Chemque, Inc.*, 303 F.3d 1294, 1301 (Fed. Cir. 2002). Enablement is clearly a necessity for anticipation and Williams is not an enabling reference for human ES cells.

Anticipatory reference must place the public in possession of the invention. *In re Donohue*, 766 F.2d 531, 533 (Fed. Cir. 1985). It is not enough for a reference to simply suggest that the disclosure contained therein can be used in a different way that is

neither described not enabled in the prior art reference, and be effective as an anticipatory reference for the unsupported suggestion.

The Examiner applies Williams as anticipating a reference to an in vitro cell culture of human ES cells based only on the suggestion that the methods disclosed therein for murine cells would be applicable to human ES cells. It is not enough to simply note that Williams states that his method would "extend to the generation and maintenance of ES cells from humans, mice, birds (e.g., chickens), sheep, pigs, cattle, goats, and fish . . ." (column 3, lines 6 - 8), without more. Williams does not disclose any means for derivation of human or primate ES cells and if the skilled artisan followed Williams and applied the methods disclosed therein to human ES cell isolation, the artisan would fail (Steward, ¶24). Even Williams himself could not extend his methods to the isolation of ES cells from other non-murine mammals (Cherny et al., 1994, Reprod. Fertil. Dev. 6: 569 - 575: "[t]he murine model for totipotential stem cell isolation is yet to prove applicable to domestic animals", page 574; and Stewart, ¶22). Here, if the Williams method was followed, according to Williams in Cherny/Williams (*Id*), ES cells would not be isolated from any non-murine species.

<u>**Third Party:**</u> (Comments, page 8, line 16 to page 9, line 16)

1. Cherny '94 reiterates teaching of Williams '065

The Patent Owner next argues that Williams '065 does not invalidate the pending claims because it was not a sufficiently enabling disclosure. Response at 9 - 12. On this point, the Patent Owner again refers to Cherny '94 and claims that it shows Dr. Williams "could not extend his method to the isolation of ES cell from other non-murine animals. *Id*. However, Cherny '94 was expressly directed towards '[the] isolation, culture and preliminary characterization of bovine primordial germ cell-derived (PGDc) cells," not the derivation of human embryonic stem cells that are the subject of the instant claims. Cherny '94 at 569 (Abstract). Thus, Cherny '94 is not relevant to the issue of whether Williams '065 was an enabling disclosure.

Regardless, the Patent Owner read Williams '065 disclosure too narrowly, limiting it to its preferred embodiment and not taking into account all of its teachings, suggestions and motivations as identified by the Examiner in the Office Action.

<u>**Examiner:**</u>

1. Cherny '94 contradicts teaching of Williams '065

The Examiner finds the comments of the Patent Owner persuasive with regard to

the teachings of Cherny '94. Cherny '94 establishes that the Willaims '065 patent is not enabling for isolating and maintaining primate/human ES cells in culture. See the comments in the Examiner's Decision at pages 14 - 15 of this Office Action.

Patent Owner:

2. Undue experimentation was required to practice the invention
(Response, page 10, line 26 to page 12, line 7)

Some considerations that aid in determining the enabling character of a reference are (1): the quantity of experimentation necessary, (2) the amount of direction or guidance presented [in the reference], (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. *In re Wands*, 838 F.2d 731, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988)

Williams fails as an enabling anticipatory reference for the claims invention under the Wands analysis for the following reasons: (1) the quantity of experimentation necessary was great as evidenced by how many others tried and failed to isolate human ES cells (Steward, ¶22, 27 - 31); (2) there is simply no guidance for isolating human ES cells in the Williams reference (Stewart, ¶22 - 25); (3) there are no working examples of isolation of human ES cells in the references; (4) the nature of the invention by Dr. Thomson is the first isolation and culture of human ES cells, for which he is widely acclaimed (See attachment A); (5) there were no examples of methods for isolating human ES cells in the art; in light of Williams own subsequent statements, the skilled artisan would conclude that Williams' method cannot be used to isolate a human ES cell line, (6) even where the relative skill level of those skilled in the art is very high (Ph. D. level) (Stewart, ¶12); (7) in a very difficult and unpredictable art. Further, (8) the claims specifically recite "a replicating in vitro cell culture of human embryonic stem cells," are not overly broad and do not read on mouse ES cells. A practitioner reading the cited references could not arrive at the presently claimed invention. Williams does not place the public in possession of the claimed invention.

Williams does not teach an all-purpose recipe for isolating and culturing embryonic stem cells from all species. Williams requires undue experimentation in order to practice the present invention and does not place the public in possession of the claimed invention as evidenced by Williams in Cherny/Williams, Reprod. Fertil. Dev. 6; 569 - 575 (1994).

Consistent with the state of the art and what Cherny/Williams published, even the Patent Office viewed the disclosure of Williams as not being enabled for anything more than murine ES cells and the methods of isolating/culturing them. Throughout the file history of the Williams patent (and related patent applications), Williams was never able

to overcome the Examiner's enablement rejection of claims encompassing non-murine ES cell culture. Since the application that matured into U.S. patent number 5,166,065 was filed in 1990, the consistent position of the U.S. Patent and Trademark Office and of the Examiner's handling the Williams' patent applications over 17 years is that Williams et al. enabled only murine ES cell isolation and culture. The Patent Owner finds no basis in the Examiner's comments for a change in the Patent Office's position now and respectfully maintains that Williams does not enable human embryonic stem cell isolation and, as such, cannot anticipate the present claims.

The failure by Williams eviscerates the "teaching" in his patent reference, - and relied on by the Examiner - that his mouse ES cell method was equally applicable to a list of animals (cows, pigs, sheep, etc.) when, in fact, it was not. Its application to humans was never shown either. This failure further evidences the unpredictability of this art (Steward ¶31, 32).

Third Party: (Comments, page 8, line 16 to page 9, line 17)

2. Undue experimentation was not required to practice invention

The Patent Owner next argues that Williams '065 does not invalidate the pending claims because it was not a sufficiently enabling disclosure. Response at 9 - 12. On this point, the Patent Owner again refers to Cherny '94 and claims that it shows Dr. Williams "could not extend his method to the isolation of ES cell from other non-murine animals." *Id.* However, Cherny '94 was expressly directed towards "]t\he isolation, culture and preliminary characterization of bovine primordial germ cell-derived (PGCd) cells," not the derivation of human embryonic stem cells that are the subject of the instant claims. Cherny '94 at 569 (Abstract). Thus, Cherny '94 is not relevant to the issue of whether Williams '065 was an enabling disclosure.

Regardless, the Patent Owner reads Williams '065's disclosure too narrowly, limiting it to its preferred embodiment and not taking into account all of its teaching, suggestions and motivations as identified by the Examiner in the Office Action. Office Action at 12 ("Williams human ES cells will contain, either expressly or inherently, all of the characteristics of human ES cells of the instant invention"). When read fully, Williams '065's disclosure was indeed sufficient to enable one or ordinary skill in the art to isolate and maintain human embryonic stem cells, especially when one considers the high level of skill in this field and the general knowledge, common sense and creative ability that they would posses[s]. For example, although Williams '065 is primarily directed to the use of LIF to maintain ES cell cultures, one of ordinary skill in the art would have also attempted to use both LIF in combination with feeder cells and feeder cells without LIF, because they would have seen Williams '065 as a guide to be followed loosely, not as a recipe requiring strict adherence to its exact teachings. Loring Declaration at 4.

Examiner:

2. Undue experimentation was required to practice invention

The Examiner finds the remarks of the Patent Owner persuasive with respect to the undue experimentation required to practice the method of Williams '065 for isolating primate/human ES cells. While the artisan at the time of the invention may well have been motivated to try to isolate primate/human ES cells using the known technique for isolating murine ES cells, there was no **reasonable expectation of success**, especially in view of the remarks by Cherny/Williams at page 571, left column, lines 1 - 65. The fact that the murine procedure using feeder layers apparently worked when Dr. Thomson isolated primate/human ES cells, does not change the fact that there was no reasonable expectation of success at the time of the invention. Only in hindsight can one argue that there was no burden of undue experimentation created by the unpredictability in the art.

Patent Owner:

3. Failure of others is evidence of non-enablement
(Response, page 12, line 8 to page 13, line 14)

Besides Williams, many others failed to isolate ES cells from any non-murine species prior to Dr. Thomson's invention. In fact, Dr. Stewart recites a long list of publications that disclose clear failure by others to isolate ES cell lines from non-murine species including rat, hamster, sheep, pig, and even human (Stewart, 12 - 15). Evidence of failure by others is found in:

a. Brook and Gardner and Brook et al. demonstrate failure of others

Brook and Gardner, 1997, PNAS 94: 5709 - 5712, Brook et al., 2003, Diabetes 52: 205-208--methods used to isolate mouse ES cells are not universally predictable across different strains of mice.

Third Party:

3. Others did not fail to make the claimed invention:
(Comments, page 9, line 17 to page 17, line 18)

In its Response, the Patent Owner cites the work of other embryonic stem cell scientists as "failures of others" to isolate mammalian embryonic stem cells. Response at 12. However, the pending claims are to human embryonic stem cells *cultured on fibroblast feeder layers and without the application of exogenous LIF*. Response at 2. The Federal Circuit has repeatedly declared that, to be relevant, evidence of the "failure of others" must show that others failed to "develop the claimed invention." See, e.g., *Ormco Corp. v. Align Tech., Inc.* 463 F.3d 1299, 1313 (Fed. Cir 2006).

Upon inspection, it is immediately recognized that not a single piece of evidence provided by the Patent Owner on this point shows the failure of other stem cell scientists to develop *human* embryonic stem cells cultured on fibroblast feeder layers and *without the application of exogenous LIF*, as all but one reference relates to species other than humans (or even primates) and the only piece of evidence related to humans did not attempt to culture its successfully isolated human embryonic stem cells on fibroblast feeder layers Therefore, since none of the evidence proffered by the Patent Owner is relevant, it does not rebut the Examiner's *pima facie* obviousness determination.

Even putting aside the issue of the applicability of the proffered evidence to the pending claims, a deeper review of that evidence shows that the Patent Owner's characterizations are inaccurate.

a. Brook and Gardner and Brook et al. do not evidence failure of others

First, the Patent Owner argues that Brook and Gardner, 94 Proc. Natl. Acad. Sci. 5709 - 5712 (1997) ("Brook"), and Brook et al., 52 Diabetes 205 - 208 (2003) ("Brook '03"), show that "methods used to isolate mouse ES cells are not universally predictable across different strains of mice." Response 12. However, Brook '97 actually repeatedly suggested applying its method for deriving mouse embryonic stem cells in other mammals. 94 Proc. Natl. Acad. Sci. at 5709 and 5712 ("[T]his approach to the derivation of germline-competent ES cell lines may not only prove generic for the mouse but also worth pursuing in other species of mammal." "Here we describe a simpler and more direct approach to the problem of devising a generic technique for deriving ES cell lines in the mouse and hence, possible, in other mammals" and "the present approach may be not only of general utility for the mouse but also applicable to other mammals")

Further, Brook '03 was directed to the derivation of "highly germline-competent embryonic stem cells containing [nonobese diabetic]-derived genome," a much more specific type of embryonic stem cell than that currently claimed, which is more difficult to derive. Loring Declaration at 8 - 9. Therefore, whether Brook '03 successfully accomplished that more difficult task is irrelevant to any analysis of whether the pending

claims, or even broader claims to primate embryonic stem cells, were obvious. As such, neither Brook '97 nor Brook '03 provide any support for the Patent Owner's arguments regarding the validity of the pending claims. *Id.*

Examiner:

3. Failure of others evidences non-enablement of Williams '065

a. Gardner and Brook and Brook et al. teach the unpredictability of the method for isolating mouse ES cells

The Examiner finds the remarks of the Patent Owner to be persuasive with regard to the teachings of Gardner & Brook and Brook et al. Neither Gardner & Brook nor Brook et al. demonstrates the failure of others in an attempt to isolate human ES cells on fibroblast feeder layers as observed by the Third Party Requester. However each of these references does document the difficulty and unpredictability in applying the technique developed for isolating murine ES cells to all strains of mice and to other species of mammals.

First of all, Gardner & Brook teach that the prior art procedure for isolating murine ES cells varies across the different strains of mice at page 5709, left column, last full sentence:

> ES cell lines of proven ability to colonize the germ-line have been obtained at a very low frequency in only a few mouse strains other than 129, as yet, in other species of mammal (1). These strain and species limitations severely restrict the scope of transgenesis via ES cell for elucidating gene function and for obtaining appropriate animal models for human genetic diseases.

Nearly seven years after the filing of the Thomson '161 patent and two years after the issuance of this patent, Gardner & Brook recognized the inadequacy

of the prior art methods for isolating murine ES cells to yield high efficiency across all strains of mice and other species of mammals. Furthermore, it was the limitations of the prior art procedure for isolating and maintaining mouse ES cells that was the motivation for their research paper. At page 5709, right column, lines 23 to 25, Gardner & Brook state:

> Here we describe a simpler and more direct approach to the problem of devising a generic technique for deriving ES cell lines in the mouse, and hence, possibly in other mammals.

The Brook et al. article further supports the unpredictability in the art (as late as eight years after the filing of the Thomson '161) to isolate ES cells from different strains of mice even though the non-obese diabetic strains used by Brook et al. were known to be particularly resistant to such procedures.

Patent Owner: (Response, page 12, lines 17 - 21)

b. Iannaccone et al. failed to isolate rat ES cells

Brenin et al. 1997, Transplant Proc. 29: 1761 - 1765 -- rat ES cells were not isolated by Iannaccone et al., 1994, Dev. Biol. 163: 288 - 292, rather Iannaccone's cells were contaminating mouse ES cells; and Ouhibi et al., 1995, Mol. Reprod. & Dev. 40: 311 - 324 -- rat ES cells that can be passaged beyond passage four could not be isolated.

Third Party:

b. Brenin '97 and Ouhibi '95 succeeded in isolating rat ES cells

Second, the Patent Owner argues that Brenin et al., 29 Transplant Proc. 1761-1765 (1997) ("Brenin '97") shows that "rat ES cells were not isolated by Iannaccone et al. 1994, Dev. Biol. 163: 288 - 292 ('Iannaccone et al.'), rather Iannaccone's cells were contaminating mouse ES cells." Response at 12. However, Brenin '97 actually shows the opposite of what the Patent Owner claims, a successful derivation of rat ES cells:

> We have subcloned the original RESC-01 cell line and obtained from it rat ES cell subclones. The PCR of subclone 5 shows no evidence of mouse in cell culture preparations from this subclone. Karyotypes showed 100/100 metaphases to be rat. . . .We have begun injecting cells from this subclone, and so far out of 18 viable offspring there are no chimeras.
> 1764.

Further, Brenin '97 stated that out of ten mice and two rats injected with the rat embryonic stem cell subclones, there was only one isolated incident of a mouse developing a tumor later determined to be of mouse origin. *Id.* However, Brenin '97 believed that isolated incident was "spurious", or - at worst - implied that there was possibly a "stable low level" contamination resulting from contaminated cells being carried into the culture during the physical cloning. Id. Benin '97 also said that , "[I]t is important to recognize that there are many possible explanations for this preliminary result." 1765. Thus, Brenin '97 did not - in fact - fail to derive mammalian embryonic stem cells as the Patent Owner claims. Loring Declaration at 9.

The Patent Owner attempts to support its position on this piece of evidence by claiming that Ouhibi et al., 40 Mol. Reprod. & Dev. 311 - 324 (1995) ("Ouhibi 95"),shows that "rat ES cells that can be passaged beyond passage four could not be isolated." Response at 12. However, as the Patent Owner's own expert, Dr. Colin Stewart, concedes in his declaration, "Ouhibi et al. . . . succeeded in isolating rat cells from rat embryos." Declaration of Colin Stewart, D. Phil. ("Steward Declaration") at 4. And while Ouhibi '95 may not have maintained those cell lines for an extended period of time, it suggested that such was the result of the culture conditions, not the method followed. 317. Further, Ouhibi '95 stated that it was well known that embryonic stem cell work was being done on "other animal species, including sheep, hamster, pig, cow, mink, and rabbit," and than, in fact, "various embryo-derived cell lines have been isolated." 311. Ouhibi '95 even discussed LIF and found that it did not need to be used in the process of deriving embryonic stem cells. Therefore, not only did Ouhibi '95 actually succeed at deriving mammalian embryonic stem cells, it actually suggested not applying exogenous LIF, which the Patent Owner argues is an inventive aspect of the pending claims.

Examiner:

b. Iannaccone '94 and Ouhibi '95 document the difficulty and unpredictability in the art of isolating and maintaining rat ES cells

The examiner does not find the remarks of either the Patent Owner or the Third Party Requester to be persuasive with respect to the state of rat ES cell research at the time of the invention as set forth by Brenin '97, Ouhibi '95, and Iannaccone '94.

The Third Party Requester (TPR) states that Brenin '97 and Ouhibi '95 did isolate rat ES cells but is silent with regard to the teachings of Iannaccone '94. The TPR evaluates these prior art references with respect to whether they tried and failed to definitively isolate and maintain primate/human ES cells in vitro. Based upon this criteria, the TPR asserts that Iannaccone '94 and Ouhibi '95 do not directly teach away from the primate/human ES cell of Thomson '913.

On the other hand, the Patent Owner dismisses any effort to isolate and maintain animal ES cells as a failure unless the reference completely establishes that said ES cells meet all of the traditional criteria: (1) will proliferate in an in vitro culture indefinitely (over one year) in an undifferentiated state; (2) maintains a karyotype in which the chromosomes are euploid through prolonged culture; (3) maintain the potential to differentiate into derivatives of endoderm, mesoderm, and ectoderm tissue throughout the culture, and (4) are inhibited from differentiation when cultured on a fibroblast feeder layer.

The Examiner takes a third position. When the reference isolates rat ES cells or rat ES-like cells but fails to establish that said ES cells meet all of the required criteria for ES cells, then this reference documents the difficulty and unpredictability of isolating and maintaining ES cells in culture.

The date at the time of the invention is the effective filing date of the Thomson '913, January 18, 1996. Therefore, in order to define the state of the art at the time of the invention, all prior art accomplishments must pre-date January 18, 1996. The Iannaccone et al. reference was published in 1994, i.e., prior to Thomson's invention.

The putative rat ES cells isolated by Iannaccone '94 were contaminated by mouse ES cells (Brenin '97, page 1762, right column "Results" to page 1764, left column, line 3). It was not until after Thomson's invention that Brenin '97 reported that they further subcloned the contaminated rat ES cells of Iannaccone '94 (RESC-01) and, in this way, were able to remove the contaminating mouse ES cells (page 1764, left column, lines 4 - 12). Furthermore, Brenin '97 admitted that they were unable to establish the critical criteria for ES cells: pluripotency (page 1765, last paragraph). Brenin '97 acknowledges that their rat ES cells could be grown with undifferentiated morphology and ES markers for at least 12 passages. However, twelve passages falls far short of the "for at least one year" characteristic of Thomson's primate/ human ES cell lines.

Another effort to isolate rat ES cells just prior to the date of the invention was made by Ouhibi et al. (Molec. Reprod. Dev. 40: 311 - 324, 1995). Ouhibi '95 isolated ES-like cells that were positive for alkaline phosphatase and SSEA-1 (Abstract). However, these ES-like cells became arrested in proliferation at the fourth passage (page 317, left column, lines 9 - 14).

Thus, Iannaccone '94 and Ouhibi '95 document further the difficulty of isolating and maintaining ES cells and the unpredictability in the art of ES cell isolation and maintenance.

Patent Owner: (Response, page 12, lines 23 - 23)

c. Doetschman et al. failed to isolate hamster ES cells

Doetschman et al., 1988, Dev. Biol. 127 : 224 - 227 - failure to isolate hamster ES cells capable of long term proliferation.

Third Party: (Comments, page 13, lines 4 - 11)

c. Doetschman succeeded in isolating hamster ES cells

Third, the Patent Owner argues that Doetschman et al., 127 Dev. Biol. 224 - 227 (1988) ("Doetschman '88"), "fail[ed] to isolate hamster ES cells capable of long term proliferation." Response at 12. However, this is a mischaracterization of Doetschman '88, which actually succeeded at establishing "highly pluripotent" hamster ES cell lines and maintaining them "for over 3 months" without loss of undifferentiated state." As such, Doetschman '88 does not evidence a failure, as the Patent Owner suggests, but is instead further proof that the known methods for deriving mouse embryonic stem cells could be used to derive embryonic stem cells of other species. Loring Declaration at 9.

Examiner:

c. Doetschman succeeded in isolating hamster ES cells

The Examiner finds the remarks of the Third Party Requester persuasive with regard to the teaching of Doetschman '88. Doetschman '88 specifically discloses hamster ES cells which were highly pluripotent and were maintained in the undifferentiated state for over three months. The Patent Owner argues that Doetschman '88 failed to isolate a hamster ES cell line because these cells were not in culture for at least one year in the undifferentiated state. However, there is no reason to believe from Doetshman '88 that the hamster ES cells would not have allowed continued passaging for over to one year. Thus, Doetschman '88 appears to have successfully isolated and maintained hamster ES cell in culture in the undifferentiated state for substantial period of time. However, these hamster ES cells technically do fail the Patent Owner's strict definition of "long term proliferation," as being greater than one year. Finally, Doetschman '88 indicates in the last two sentences of the article that their laboratory is carrying out experiments to determine if the hamster ES cells can colonize

the germ line when introduced back into the hamster blastocysts. "If this proves to be possible, there will then exist a second ES cell system for small animal disease model" (last sentence of Doetschman). The authors apparently never published the results of this final experiment.

It is noteworthy that Thomson himself acknowledges in the specification of his patent that "Strong evidence of these required properties have been published only for rodents ES cells including mouse (Evans & Kaufman, Nature 292: 154-156, 1981; Martin, Proc. Natl. Acad. Sci. USA 78: 7634-7638, 1981) hamster (Doetschman et al. Dev. Biol. 127: 224-227, 1988), and rat (Iannaccone et al. Dev. Biol. 163: 288-292, 1994) . . ." (Thomson '913, column 3, line 67 to column 4, line 6). However, Thomson '913 did not have the benefit of the information in Brenin '97 because this reference was published later.

Thus, Doetschman '88 teaches the successful isolation of hamster ES cells and maintenance of the ES cell line for at least three months using mouse fibroblasts as the feeder layer. In suspension culture, the hamster ES cells differentiated into endoderm and ectoderm (page 226, right column, line 3 to page 227, left column, line 26) and maintained normal karyotype (page 227, left column, lines 27 - 40). This was only the second rodent species (mouse being the first) for which an ES cell line was established (Abstract, line 5).

Patent Owner: (Response, page 12, lines 24 - 28)

d. Piedrahita failed to isolate ovine and porcine ES cells

Piedrahita et al., 1990, Theriogenology 34 : 879-901 --failure to isolate ovine ES cells and doubtful isolation of porcine ES cells; and a later publication by Moore et al. (including Piedrahita), 1997, In Vitro Cell Dev Biol. 33: 62 - 71 showing porcine ES cells were not in fact isolated in the earlier publication.

Third Party: (Comments, page 13, line 12 to page 14, line 6)

d. Piedrahita succeeded in isolating ovine and porcine ES cells

As its fourth offer of evidence of the failure of others to derive mammalian embryonic stem cells, the Patent Owner claims that Piedrahita et al., 34 Theriogenology 879 - 901 (1990) ("Piedrahita"), showed a "failure to isolate ovine ES cells and doubtful isolation of porcine ES cells," and that Moore et al., 33 In Vitro Cell. Dev. Biol. 62 - 71 (1997) ("Moore '97"), confirmed that failure. Response at 12. However, the Examiner was completely correct in the Office action in finding that, "Piedrahita discloses murine, porcine and ovine ES cells." Office Action at 18. While Piedrahita '90 may not have actually isolated such ES cells, that does not make it evidence of a "failure," because its disclosure was sufficient to enable one of ordinary skill in the art to do so. Loring Declaration at 7.

Further, contrary to the Patent Owner's assertion, Moore '97 actually confirmed the successful isolation of embryonic stem cell lines in various species, including rat, mink, rabbit hamster, primates, sheep, cattle and swine. Moore '97 at 62 ("varying degree of pluripotentiality have been demonstrated for each.") While it is true that Moore '97 states that the inability to maintain porcine ES cell lines was common, it did not attempt to isolate porcine ES cells itself, nor did it use feeder layers.

Examiner:

d. Piedrahita failed to isolate ovine and porcine ES cells

The Examiner finds the comments of the Patent Owner persuasive with respect to the interpretation of Piedrahita '90. Piedrahita '90 used the art accepted procedure for isolating and maintaining mouse ES cells to isolate and maintain porcine and ovine ES cells. As a control Piedrahita '90 also isolated mouse ES cells alongside the porcine and ovine ES cells. Piedrahita '90 observed that mouse ES cells that survived greater than five passages became permanently established (page 896, lines 3 - 4). Piedrahita

was unsuccessful in isolating porcine and ovine ES cells. "From our results it appears that expanded blastocysts and early hatched blastocyst stage embryos are not conducive to isolation of ES-like cells with a high rate of proliferation" (page 897, lines 10 - 12).

The Third Party Requester argues that the disclosure of porcine and ovine ES cells by Piedrahita '90 combined with the knowledge in the art placed these cells within the possession of the person of ordinary skill in the art. However, this argument fails because the authors of Piedrahita '90 themselves must be considered as persons of ordinary skill in the art. As persons of skill in the art, Piedrahita '90 failed to isolate and maintain porcine and ovine ES cells with demonstrated (1) pluripotential to differentiate into endoderm, mesoderm, or ectoderm, (2) ability to proliferate indefinitely in vitro in undifferentiated state, and (3) able to maintain a normal karyotype through prolonged culture.

One year after the date of the invention, Piedrahita published another article (Moore et al., In vitro Cell Dev. Biol.--Animal 33:62-71, 1997) in which he acknowledged that he had never isolated and maintained true porcine ES cells (Moore '97, page 62, right column, line 14 to page 63, left column, line 13). Piedrahita also summarizes the state of the prior art in the introduction at page 62, left column, line 16 to right column, line 2:

> Isolation of ES cell lines has been attempted in the rat (16) mink (34), rabbit (12), hamster (6,25), primates (37), sheep (14,24), cattle (9,27,28,32), and swine (1,9,11,21,23,24,33,35). Varying degrees of pluripotentiality have been demonstrated for each, yet only the mouse and rat have produced chimeric animals, with mouse ES cell being the only cell lines to date conferring

germline transmission.

Thus Piedrahita '90 further establishes both the difficulty in isolating and maintaining ES cells and the unpredictability in this art.

Patent Owner: (Response, page 12, last two lines)

e. Talbot failed to isolate bovine ES cells

Talbot et al., 1995, Mol. Reprod. & Dev. 42: 35 - 52 -- failure to isolate bovine ES cells.

Third Party: (Comments, page 14, lines 7 - 13)

e. Talbot succeeded in isolating bovine epiblast cells

Fifth, the Patent Owner claims that Talbot et al., 42 Mol. Reprod. & Dev. 35 - 52 (1995) ("Talbot '95"), showed a "failure to isolate bovine ES cells." Response at 12. However, Talbot is not directed to the isolation of embryonic stem cells. Further, Talbot '95 expressly "did not address the issue of the sustainable culture of undifferentiated bovine epiblast cells as ES cells," although it did expressly "demonstrate[ ] the pluripotency of bovine epiblasts in culture." Talbot '95 at 49. Thus, since Talbot '95 was not focused on isolating and maintaining embryonic stem cell cultures, it is disingenuous to claim that it "failed" to do so. Loring Declaration at 9 - 10.

Examiner:

e. Talbot documents difficulty and unpredictability of the art

The Examiner finds the remarks of the neither the Patent Owner nor the Third Party Requester persuasive with regard to the teachings of Talbot '95.

Third Party Requester correctly notes that the purpose of the Talbot '95 paper was not to address the issue of the sustainable culture of undifferentiated bovine epiblast in culture (Talbot, page 49, left column, lines 1 - 4). Instead, Talbot '95 achieved their goal of isolating bovine epiblasts with demonstrated pluripotency.

However, the Talbot '95 reference does emphasize the technical difficulties and unpredictability in the art of ES cell isolation and maintenance for domestic animals. At page 49, left column, second and third sentence:

> The main problems identified for the sustainable culture of bovine epiblast cells as ES cell lines were their extreme sensitivity to dissociation and the uncontrolled differentiation of the cells. These were also seen as the overriding problems in establishing ES cells from pig and sheep epiblast cells (Talbot et al, 1993a).

Talbot '95 continues to delineate the problems in isolating and maintaining ES cells of domestic animals at page 51, left column, second paragraph:

> The dissociation of the epiblast into viable single cells is probably crucial to the successful establishment of ES cell lines of the cow. The epiblast cells of the cow, like those of the pig and sheep, epiblast cells could not be viably separated from one another by physical means or with various enzymes such as pancreatin, trypsin, elastase, hyaluronidase, collagenase, pronase, dispase I and thermolysin (Talbot et al. 1993a; unpublished observations).

And in the last sentence of the article, Talbot '95 summarizes the obstacle to isolating and maintaining bovine ES cells:

> Until a method is found to separate the cells in a viable state, or to promote a "maturation" of the epiblast cells so that they can tolerate dissociation, it will be difficult to test medium constituents and substrate conditions that will enable the growth of undifferentiated bovine epiblast cells in continuous culture.

Thus, at the time of the invention (1995-1996) the prior art clearly could not isolate and maintain bovine, ovine, or porcine ES cells in continuous culture, further emphasizing the difficulty and unpredictability of the ES cell art.

Patent Owner: (Response, page 13, lines 1 - 4)

f. Bongso failed to isolate human ES cell line

Bongso, 1994, Human Reprod. 9: 2110 -- failure to isolate long term cultures of human ES cells, and Rubinoff et al. (including Bongso), 2000, Nature Biotech. 18: 399 - 404 -- acknowledgement of the earlier failure and Dr. Thomson's success.

Third Party: (Comments, page 14, lines 14 - page 16, line 7)

f. Bongso succeeded in isolating human ES stem cells

Sixth, the Patent Owner argues that Bongso et al., 9 Human Reprod. 2110 - 2017 (1994) ("Bongso '94"), showed a "failure to isolate long term cultures of human embryonic stem cells," and that Reubinoff et al., 18 Nature Biotech. 399 - 404 (2000) ("Reubinoff '00"), showed "acknowledgement of the earlier failure and Dr. Thomson's success." Response 13. It should be first noted that human embryonic stem cells isolated and cultured by Bongso '94 are identical to those of the instant claims except that Bongso '94 cultured their cells using LIF and not feeder layers, while the instant claims use feeder layers and not LIF. Bongso '94 at 2110; Response at 2. Bongso '94 addressed the issue of feeder layer selection specifically at page 2116.

> Since STO fibroblasts were not used in this study [,] it is not possible to conclude whether or not they would be equally effective as feeder layers. A feeder cell type similar to the species of the embryo may be more ideal than that of the heterologous species.

Thus, Bongso '94 expressly suggested that using a feeder cell from a species similar to the embryo might be better and motivated those of ordinary skill in the art to modify the disclosed process by using feeder layers in order to achieve better cell proliferation.

Also, Reubinoff '00 actually shows that two of the authors of Bongso '94, Dr. Ariff Bongso and Chui-Yee Fong, along with other human embryonic stem cell researchers recognized--before Dr. Thomson publicized his accomplishment - that using feeder layers instead of LIF would work better:

> Since [Bongso '94] did not use embryonic feeder cell support (required for proliferation of pluripotent human EC and nonhuman primate ES cells) but relied instead on LIF supplementation of the culture medium, these cells eventually underwent differentiation or death. Therefore, we subsequently employed a culture system incorporating embryonic fibroblast feeder cell layers to derive human ES cells from blastocysts. While this work was in progress,