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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte SCOTT C. FAHRENKRUG and DANIEL F. CARLSON[1]

Appeal 2017-002215
Application 13/025,373
Technology Center 1600

Before TONI R. SCHEINER, JEFFREY N. FREDMAN, and DAVID COTTA, *Administrative Patent Judges*.

SCHEINER, *Administrative Patent Judge*.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134(a) from the final rejection of claims directed to a transgenic swine and a transfected swine cell line. The Examiner rejected the claims as obvious. We have jurisdiction under 35 U.S.C. § 6(b).

We affirm.

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[1] Appellants identify Recombinetics, Inc. as the real party in interest. Appeal Br. 3.

BACKGROUND

"Swine are an important agricultural commodity and biomedical model" and "[g]enetic engineering can also expand the utility of pigs for modeling human disease, developing clinical treatment methodologies, or donating tissues for xenotransplantation." Spec. 1.

The Specification describes a stably transfected swine cell "with a knockout for the low density lipoprotein receptor [gene] *(LDLR)* . . . in male and female domestic and miniature swine cells." *Id.* at 2. The cells may be fetal or adult fibroblasts, and "[a] transgenic swine may be prepared by nuclear transfer of such a cell." *Id.* at 3. "An embodiment of the invention is a transgenic swine comprising a genomic disruption of an endogenous gene," e.g., "a Low-Density Lipoprotein Receptor gene *(LDLR)* . . . for preventing expression of a functional protein and/or preventing expression [of] any protein." *Id.* at 2. "The swine may be homozygous or heterozygous for said disrupted gene" (*id.*), and "may exhibit a phenotype chosen from the group consisting of hypercholesterolemia, atherosclerosis, and atherosclerotic lesions" (*id*).

The Specification teaches that transgenic swine may be produced by exposing swine cells *in vitro* to "a transfection agent that comprises an exogenous nucleic acid [construct] during a first culture time period" (*id.* at 3), and "subsequently adding a second group of swine cells to the first group for a second culture time period, wherein the second group of cells have not been exposed to the transfection agent" (*id.*). The Specification also teaches that "[o]ther elements that can be included on a nucleic acid construct" include "a sequence encoding a selectable marker . . . flanked by loxP

recognition sequences for a recombinase such as, e.g., Cre or Flp . . . such that the selectable marker can be excised from the construct." *Id.* at 30.

The Specification discloses that:

> [T]ransgenic swine cells were made with a defect in LDLR expression. . . . In brief, the production of a functional *LDLR* gene product was disrupted by introduction of a stop cassette within *LDLR* exon 4 by Adeno-associated virus (rAAV) homologous recombination (HR). An rAAV HR cassette (rAAVLDLR-E4-stop) was generated with a PGK-Neo selection cassette inserted within LDLR exon 4 at the *XhoI* restriction site (Figure I). Replacement of *LDLR* exon 4 with rAA V-LDLR-E4-stop resulted in a truncated, non-functional *LDLR* protein product. Several AAV constructs were designed and created that either targeted other exons or that avoided certain repetitive elements around exon 4. Colonies with LDLR nonexpression were identified from three separate transductions.

Id. at 7–8.

In addition to disruption of *LDLR* exon 4, the Specification teaches that "frame-shift mutation, truncation, or introduction of single amino acid changes throughout the LDLR gene are expected to disrupt LDLR function." The Specification teaches that "[t]argeting such changes would simply require the acquisition of the pig LDLR sequences available in Genbank, ENSEMBLE, or as described in SEQ ID NO:1"[2] (*id.* at 11), and "the application of methods for homologous recombination, allele conversion, or the introduction of an indel using zinc finger nucleases, meganucleases, or

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[2] The Specification indicates that SEQ ID NO:1 is "LDLR Partial CDS [coding sequence] from MARC library est sequenced by Applicants." Spec. 6. "SEQ ID NO:1 was used to generate a probe for . . . screening and recovery of a genomic clone containing the pig LDLR gene." *Id.* at 12.

TAL effector nucleases or any other targeted method for DNA breakage/modification" (*id.*).

According to the Specification, "tests verified that cells were made with *LDLR* knockouts." *Id.* at 10. The Specification does not exemplify a transgenic pig comprising a genomic knockout of the endogenous low-density lipoprotein receptor gene, but teaches that the knockout "cells may be cloned into male and female pigs" (*id.*), and "[t]hese founders may then be bred to create pigs homozygous for knockout of the *LDLR* gene" (*id.*). Further according to the Specification, "[v]arious techniques known in the art can be used to introduce nucleic acid constructs into non-human animals to produce founder lines, in which the nucleic acid construct is integrated into the genome" (*id.* at 24), including "somatic cell nuclear transfer" (*id.* at 24–25).

STATEMENT OF THE CASE

Claims 1, 3, 6, 7, 19–22, 26, 27, 35, and 39 are on appeal; claims 2, 4, 5, 8–18, 23–25, 28–34, and 36–38 have been canceled. Claims 1 and 21 are representative and read as follows:

> 1. A transgenic swine comprising a genomic knockout of an endogenous Low-Density Lipoprotein Receptor gene (*LDLR*), wherein the animal is free of selectable marker genes.
>
> 21. An isolated transfected somatic swine cell comprising a gene knockout of an endogenous Low-Density Lipoprotein Receptor gene (*LDLR*), wherein the transfected cell is free of selectable marker genes.

The Examiner relies on the following evidence:

Bentzon et al. ("Bentzon et al.")	WO 2008/106982	Sept. 12, 2008
Bentzon et al. ("Bentzon '643")	US 8,546,643	Oct. 1, 2013

S. Ishibashi et al., *Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery*, 92 J. CLIN. INVEST. 883–893 (1993) ("Ishibashi").

Katalin Banki et al., *Cloning and Expression of the Human Gene for Transaldolase*, 269 J. BIOL. CHEM. 2847–2851 (1994) ("Banki").

Xiaoling Xu et al., *Direct Removal in the Mouse of a Floxed Neo Gene From a Three-loxP Conditional Allele by Two Novel Approaches*, 30 GENESIS 1–6 (2001) ("Xu").

Christopher S. Rogers et al., *Production of CFTR-null and CFTR-ΔF508 heterozygous pigs by adeno-associated virus-mediated gene targeting and somatic cell nuclear transfer*, 118 J. CLIN. INVEST. 1571–1577 (2008) ("Rogers I").

Li Li et al., *Production of a reporter transgenic pig for monitoring Cre recombinase activity*, 382 BIOCHEM. BIOPHYS. RES. COMM. 232–235 (2009) ("Li").

Appellants rely, in relevant part, on the following additional evidence:

Declaration of Dr. Eric A. Hendrickson, dated June 25, 2015, originally entered into the record with the Amendment of September 17, 2015 ("Hendrickson Decl.").

Declaration of Dr. Daniel F. Carlson, dated August 3, 2015, originally entered into the record with the Amendment of September 17, 2015 ("Carlson Decl.").

Liangxue Lai et al., *Production of α-1,3-Galactosyltransferase Knockout Pigs by Nuclear Transfer Cloning*, 295 SCIENCE 1089–1092 (2002) ("Lai").

Christopher S. Rogers et al., *Disruption of the CFTR Gene produces a Model of Cystic Fibrosis in Newborn Pigs*, 321 SCIENCE 1837–1841 (2008) ("Rogers II").

J. Ramsoondar et al., *Targeted disruption of the porcine immunoglobulin kappa light chain locus*, Transgenic Res. Published online: 26 Sept. 2010 ("Ramsoondar").

The claims stand rejected under 35 U.S.C. § 103(a) as follows:[3]

Claims 1, 3, 6, 19–22, 26, 27, 35, and 39 as unpatentable over Bentzon et al. or Bentzon '643,[4] in view of Xu and Li; and

Claim 7 as unpatentable over Bentzon et al. or Bentzon '643, in view of Xu, Li, and Ishibashi.

THE PRIOR ART

Bentzon '643

Bentzon '643 "relates to a genetically modified pig as a model for studying atherosclerosis, wherein the pig model expresses at least one phenotype associated with said disease and/or a modified pig comprising at

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[3] According to the Examiner, rejections of previously pending claims 1–3, 5, 6, 8, 19–23, 26, and 27 as anticipated by Bentzon et al. under 35 U.S.C. § 102(b), and of the same claims as anticipated by Bentzon '643 under 35 U.S.C. § 102(e), "were withdrawn due to the amendment of the claims to recite that the transfected swine cell or transgenic pig is free of a selectable marker gene" "in the Non-Final Office Action mailed . . . on 8/1/14." Ans. 2–3.

[4] Throughout the Answer, the Examiner relies on both Bentzon et al. and Bentzon '643 for the same disclosures. Ans., *passim*. Appellants characterize these references, as well US 2010/0138939 (the initially published version of Bentzon '643), as "counterparts of each other," and indicate that their "[r]eferences to 'Bentzon' herein refer to the group of them." Appeal Br. 9. For the sake of simplicity, our citations in this decision refer to Bentzon '643, unless otherwise indicated.

least one mutation in the . . . endogenous ApoE gene and/or . . . endogenous LDL receptor gene." Bentzon '643, 3:41–48. Bentzon '643 further "relates to a genetically modified porcine donor cell." *Id.* at 4:49–50.

Bentzon '643 teaches that the donor cells may be somatic cells "originat[ing] from fetal or adult tissue . . . and [e]specially preferred somatic cells are those of foetal origin." *Id.* at 19:25–31. "Preferably, the somatic cells are fibroblast cells as the[y] can be obtained both from developing fetuses and adult animals in large quantities . . . [and] may furthermore be easily propagated in vitro." *Id.* at 19:53–56.

Bentzon '643 teaches that "[t]he genetic modifications are introduced in the somatic cell prior to cell nuclear transfer." *Id.* at 9:39–40. Bentzon '643 further teaches that the modifications "may be targeted to a specific region in the porcine genome by homologous recombination of a targeting construct or by gene editing procedures," and "could be inactivation (e.g. knock-out) of specific genes that will cause a disease or phenotype" (*id.* at 9:65–10:3). In addition, although Bentzon '643 does not explicitly disclose transfected cells or transgenic pigs that are free of selectable marker genes, the reference does teach that such genes are removable. For instance, Bentzon '643 discloses that "the recombinant vector construct may also comprise at least one site for Cre recombinase" (*id.* at 21:11–12), and "[t]he presence of two Cre recombination sites allows Cre recombinase-mediated cassette exchange . . . thereby facilitating, if needed, removal of plasmid sequences and selection genes" (*id.* at 49:9–13).

According to Bentzon '643,

> [I]mproved procedures [are provided] for cloning pigs by nuclear transfer which refers to the introduction of a full complement of nuclear DNA from one cell to an enucleated

> cell. The genetically modified pig of the present invention may be produced using any technique in which modified genetic material, transcriptional product and/or translational product or part thereof is transferred from [a] donor cell to a host cell, such as an enucleated oocyte. A number of techniques exist such as introducing genetic material from a genetically modified somatic cell into an enucleated oocyte by for example microinjection or by nuclear transfer.
>
> In cloning, the transfer of the nucleus of a somatic (body) cell or somatic cell into an egg cell (oocyte) which has had its own nucleus removed (denucleated or enucleated) is called somatic cell nuclear transfer. The new individual will develop from this reconstructed embryo and be genetically identical to the donor of the somatic cell.

Id. at 16:17–32.

Example 2 of Bentzon '643 demonstrates production of ApoE knockout porcine fibroblasts by adeno-associated virus-mediated homologous recombination, initial screening of locus-specific targeting events by PCR, and verification of locus-specific events by Southern blot. *Id.* at 38:25–41:29. According to Bentzon '643, "[t]he resulting transgenic ApoE knockout porcine fibroblasts were subsequently used for somatic cell nuclear transfer (SCNT) by handmade cloning" (*id.* at 41:30–32), but no results are reported regarding production of ApoE knockout pigs.

Example 4 of Bentzon '643 describes a method of making LDLR knockout porcine fibroblasts pig by homologous recombination, for use in producing LDLR knockout pigs:

> Part of the pig LDL receptor coding sequence has been sequenced (GenBank accession no. AF065990). A targeting vector containing part of the gene sequence is created with a promoter-less neomycin resistance gene cassette inserted into one of the exons to disrupt gene function. The targeting vector is linearized and transfected into Yucatan fetal fibroblasts.

> Fibroblasts are cultured in the presence of G418. Resistant clones are screened for homologous recombination by PCR. Yucatan minipigs with knockout of one or both apoE alleles are created from recombinant cells by "hand-made" cloning.

Id. at 43:20–43.

Xu

Xu teaches that "[c]onditional knockouts in mice have been used successfully to overcome embryonic lethality." Xu, 1.

> To produce conditional knockouts, loxP sites are placed by homologous recombination using a cotransfer type-targeting construct . . . into two introns that surround the coding sequence or a critical protein domain. One of the loxP sites is associated with a positive selection marker, most commonly a neo gene, which is flanked by two loxP sites (floxed neo) to allow its removal. Such a three lox construct can be directly introduced into the desired locus by homologous recombination in ES cells and then into the germline by standard techniques. However, the presence of the neo gene in the intron often affects endogenous gene expression and results in the reduction or complete inactivation of the floxed genes. . . . Thus, it is important to be able to remove the floxed-neo gene from targeted loci whenever it is necessary.

Id.

Xu discloses two different approaches for direct removal of the ploxP-neo-loxP cassette from introns in mice. *Id.*, Abstract.

Li

Li discloses a reporter pig strain containing the EGFP gene driven by the CMV promoter, where the EGFP gene is expressed only after Cre-mediated excision of loxP-flanked stop sequences. Li, 232.

DISCUSSION

Rejection I

Claims 1, 3, 6, 19–22, 26, 27, 35, and 39 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Bentzon et al. or Bentzon '643, Xu, and Li. Ans. 2–5.

As Appellants do not argue the claims separately, we focus our analysis on independent claims 1 and 21, and claims 3, 6, 19, 20, 22, 26, 27, 35, and 39 will stand or fall with those claims. 37 C.F.R. § 41.37 (c)(1)(iv); *see also* Appeal Br. 22–24 (indicating that the dependent claims are patentable "for the same reasons as set forth with respect to claim 1").

Issue

The dispositive issue raised by this rejection is whether the Examiner has established by a preponderance of the evidence that the teachings of Bentzon et al. or Bentzon '643, together with the teachings of Xu and Li, render obvious the production of the claimed transfected swine cells and transgenic swine.

Discussion

The Examiner finds, in relevant part, that Bentzon '643 describes a method of making a transfected swine cell comprising a knockout of an endogenous *LDLR*, where the transfected cell is a fetal or adult pig fibroblast, as well as a method of using the transfected cell to produce a transgenic miniature pig comprising a knockout of an endogenous Low-Density Lipoprotein Receptor Gene (*LDLR*). Ans. 3 (citing Bentzon '643, cols. 3, 4, 19, 43 (Example 4)). The Examiner finds that Bentzon '643 teaches that the transgenic pig can be produced by somatic cell nuclear transfer of the transgenic donor cell. *Id.* (citing Bentzon '643, cols. 19, 43).

The Examiner also finds that Bentzon '643 "teaches to delete/disrupt an exon of the endogenous LDL receptor gene in a pig, and . . . the use of a cre-lox system to genetically modify pigs and in particular a system which allows for removal of a selection marker" (*id.* at 4 (citing Bentzon '643, 21, 49)), but acknowledges that the reference "does not specifically teach to use the cre/lox system to delete/disrupt an exon in the LDL receptor gene in the pig wherein the resulting pig is free of selectable marker genes." Ans. 4.

Nevertheless, the Examiner finds:

> [A]t the time of filing, the prior art reported a number of strategies for disrupting/deleting coding exons in genes of interest in a transgenic animal, including pigs. Xu et al., for example, teaches several inducible cre-loxP based knockout approaches of a gene allele, Brca 1, in mice which further result in the removal of the selection marker (Xu et al., pages 1–6). Although the strategies taught by Xu et al. were exemplified in mice, Li et al. demonstrates that Cre-loxP strategies can be used successfully in the generation of transgenic pigs (Li et al., page 232).

Ans. 4.

The Examiner concludes that "it would have been *prima facie* obvious . . . to delete/disrupt an exon in the LDL receptor gene in a pig using an inducible cre-loxP system, and to further remove any inserted selection marker gene with a reasonable expectation of success" (*id.*), "in view of the teachings of Bentzon ['643] to delete/disrupt an exon of the endogenous LDL receptor gene in a pig, the further teachings of Bentzon ['643] for the use of a cre-lox system for targeted genetic modification of pigs and for the removal of selectable markers" (*id.* at 7), together with "the teachings of Xu et al. for cre-loxP based knockout approaches of a gene allele which result in removal of selectable marker genes, and the demonstration by Li et al. that

Cre-loxP strategies can be used successfully in the generation of transgenic pigs" (*id.*).

Appellants do not dispute the Examiner's representation of Bentzon '643's disclosure. Rather, Appellants contend "the evidence in the Record plainly demonstrates that the prior art was not enabling for the claimed inventions." Appeal Br. 9. Based on an analysis of the *Wands* factors[5] (Appeal Br. 10–15), and relying on the Carlson and Hendrickson Declarations, Appellants contend "there [was] no reasonable expectation of success when using the references or state of the art at the time of filing for making the claimed invention." *Id.* at 10. In addition, Appellants contend "the claimed invention is not merely a predictable use of the prior art" (*id.*), and the claimed invention would have required undue experimentation (*id.* at 11).

With respect to the *Wands* factors, Appellants contend "[t]he state of knowledge of the art and its predictability pointed to a general expectation of non-success for gene knockouts in pigs." *Id.* at 11. Appellants contend "although cloning was well known (*q.v.* Dolly the Sheep), it effectively required a primary somatic cell" and "[w]orking with primary cells is difficult because genetic changes had to be made and verified . . . before the

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[5] *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). Factors to be weighed in determining whether a disclosure is in compliance with the enablement requirement include: (1) the quantity of experimentation necessary to practice the invention; (2) the amount of direction or guidance presented; (3) the presence or absence of working examples; (4) the nature of the invention; (5) the state of the prior 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.

cells senesced." *Id.* (citing Carlson Decl. ¶ 7). Appellants contend, in practice, "some gene knockouts are fatal; primary cells have to be edited and screened quickly but prior art processes were severely limiting; processes commonly used in mice are not useful in the pig; and customary selection processes were severely limiting." *Id.* at 12 (citing Carlson Decl. ¶ 8).

In addition, Appellants contend that "chromosomal DNA is the starting point for making a knockout" (*id.* at 13), but, according to Dr. Hendrickson, "[a]t the time of Bentzon et al. there was no more than a partial cDNA sequence available for the LDLR gene" (Hendrickson Decl. 3:86–87). Moreover, Appellants contend that "conventional processes used homology arms that have homology to target DNA . . . typically 3000–8000 bases long" (*id.* at 14 (citing Hendrickson Decl. 2:60–71, 3:110–111)), but these conventional processes failed—probably because repetitive elements located within the homology arm promoted promiscuous hybridization (Hendrickson Decl. 3:101–105). "[I]nstead, a short arm of 298 bases" was unexpectedly effective and successful. Appeal Br. 14 (citing Hendrickson Decl. 3:116–117).

Furthermore, Appellants contend it was well known that the efficiency of targeting DNA sequences varies from locus to locus (*id.* at 12 (citing Carlson Decl. ¶ 8)), and "whether or not a knockout at a particular locus would be successful or not was unknown" (*id.* at 13 (quoting Hendrickson Decl. 4:127–134)).

Finally, Appellants contend "[t]here are no working examples of the experimental procedure in the cited references" with respect to the pig LDLR gene (*id.* at 14)—that is, Bentzon '643 merely "aspires to knockout LDLR but does not demonstrate making an animal with an LDLR knockout

or a cell that could be used to make such an animal" (*id.*). Appellants acknowledge that Bentzon '643 reports production of somatic cells with "knockouts of a different gene, ApoE," but Appellants contend "by the time the experiments were done, the somatic cells were high-passage cells, which are not expected to be useful for cloning." Reply Br. 4. Indeed, Appellants contend "knockouts in pigs were almost unknown—despite very many attempts" (Appeal Br., 12), and "[o]nly a very few genes had been knocked out in swine; roughly 4 of 20,000, which is about 0.02%" (*id.* at 13). In this regard, Dr. Carlson states he is "aware of only four genes for which there had been a report of a successful creation of a knockout pig by methods such as those taught in the [Bentzon '643] reference" (Carlson Decl., 10), e.g., α-1,3-galactosyltransferase, cystic fibrosis transmembrane receptor, and IgG kappa light chain, disclosed by Lai, Rogers II, and Ramsoondar,[6] respectively (*id.*).

According to Appellants, "[t]his lack of efficiency very quickly translates into undue experimentation and a discouragement to artisans to make such attempts." *Id.* at 11. Furthermore, "[t]he success of targeting a particular locus by the [Bentzon '643] reference methods is not expected because it cannot be predicted." *Id.* at 12–13.

During patent prosecution, an examiner is entitled to reject claims over a prior art publication or patent without conducting an inquiry into whether or not that prior art reference is enabling, and, "[a]s long as an examiner makes a proper prima facie case . . . by giving adequate notice under § 132, the burden shifts to the applicant to submit rebuttal evidence of

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[6] Lai, Rogers II, and Ramsoondar accompanied Dr. Carlson's Declaration.

nonenablement." *In re Antor Media Corp.*, 689 F.3d 1282, 1289 (Fed. Cir. 2012).

Here, Bentzon '643 explicitly, albeit prophetically, teaches knocking out the *LDLR* gene in miniature pig fetal fibroblasts by homologous recombination, and using those transfected cells to produce a knockout pig to serve as a model for atherosclerosis. Accordingly, we find that the Examiner has established a proper prima facie case with respect to enablement of Bentzon '643, shifting the burden to Appellants to establish that Bentzon '643 is not enabling for producing a transfected somatic swine cell comprising a gene knockout of an endogenous *LDLR* gene, or a transgenic swine comprising a genomic knockout of an endogenous *LDLR* gene. We determine that Appellants have not carried that burden.

An enabling specification "must teach those skilled in the art how to make and use the full scope of the claimed invention without 'undue experimentation.'" *In re Wright*, 999 F.2d 1557, 1561 (Fed. Cir. 1993). "That *some* experimentation may be required is not fatal; the issue is whether the amount of experimentation required is 'undue.'" *In re Vaeck*, 947 F.2d 488, 495 (Fed. Cir. 1991). Some experimentation, even a considerable amount, is not "undue" if, for example, the specification provides a reasonable amount of guidance as to the direction in which the experimentation should proceed. *Wands*, 858 F.2d at 737.

Again, Bentzon '643 explicitly teaches knocking out the LDLR gene in miniature pig fetal fibroblasts by homologous recombination in order to produce a knockout pig to serve as a model for atherosclerosis. Moreover, we note that Bentzon '643 exemplifies the successful production of fetal pig

fibroblasts with a knockout of the ApoE gene (*see* Example 2, discussed above).[7]

Although Appellants argue persuasively that transfecting somatic pig cells is difficult, time consuming, and costly, that many gene knockouts are fatal (and therefore unsuccessful), and that "the artisan must contemplate a need to 'burn' through many many pigs with a feedback loop of success/failure/modify/retry of at least four months per round of cloning" (Appeal. Br. 11), these considerations relate principally to the amount and cost of experimentation likely required, rather than the direction in which the experimentation should proceed. As discussed above, even a considerable amount of experimentation is not "undue" if the specification provides a reasonable amount of guidance as to the direction in which the experimentation should proceed. *Wands*, 858 F.2d at 737.

With respect to Appellants' contention that Bentzon '643 discloses cDNA for the pig LDLR, rather than genomic DNA, and that Appellants' initial attempts to obtain the genomic sequence through PCR failed because of repetitive elements in the pig LDLR sequence, we find that the Examiner has provided persuasive evidence that "there were many methods considered routine in the art for obtaining genomic sequence using partial cDNA sequence that do not use PCR." Ans. 11. Specifically, the Examiner provides evidence that "Southern blot cloning of cDNA and genomic DNA

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[7] We note Appellants' argument that "the distinctions between a Gain-of-Function versus a Knockout have to be appreciated" (Appeal Br. 11), but we are not persuaded the distinctions are relevant here, as Bentzon '643 discloses gene knockouts as well as gain-of-function modifications (*see* Example 2 of Bentzon '643).

libraries was considered routine at the time of filing and did not require foreknowledge of a complete cDNA sequence," and "has been further demonstrated to work successfully in isolating genomic clones for genes with highly repetitive elements." *Id.* (citing Banki[8]). Appellants have not addressed this evidence.

With respect to Appellants' assertion that only about four of 20,000 swine genes had been knocked out at the time of filing, we agree with Appellants that the calculation has little or no meaning absent some evidence of the number of pig genes subjected to knockout attempts (*see* Reply Br. 3). Appellants, through Dr. Carlson, submitted several references (e.g., Lai, Rogers II, and Ramsoondar (Carlson Decl. 10–11)) as evidence of the rarity of knockouts in pigs. Nevertheless, as we find the Examiner has established a prima facie case that Bentzon '643 is enabling, it is Appellants' burden to establish that number of successful knockouts was or was not reasonably proportional to the number of attempts (*see Antor Media*, 689 F.3d at 1289). Appellants have not done so.

Moreover, we agree with the Examiner that the art cited by Appellants shows that "unrelated and substantially different genes were successfully targeted in pig donor cells which were then used to make transgenic knockout pigs" using somatic cell nuclear transfer (Ans. 15), and that neither the Carlson Declaration nor the Hendrickson Declaration "provides any specific evidence that the LDLR gene differs sign[i]ficantly from any of these other gene loci such that the skilled artisan would not have had a

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[8] The Examiner provided Banki as rebuttal evidence with the non-final Office Action of October 6, 2015.

reasonable expectation of success in making an LDLR knockout pig at the time of filing" *(id.* at14–15). By way of example, we note that Rogers II "used homologous recombination in fibroblasts of outbred domestic pigs to disrupt the *CFTR* gene and somatic cell nuclear transfer to generate *CFTR*+/- pigs." Rogers II, 2. Similarly, Lai discloses production of α-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning using clonal fetal fibroblasts (Lai, 1089), and also teaches that "[t]he ability to use cryopreserved donor cells without further culture . . . [is] advantageous, as it extends the number of potential donor lines available for use in nuclear transfer" (*id.* at 1091).

Appellants have not established persuasively that producing a transfected somatic swine cell comprising a gene knockout of an endogenous LDLR gene, or a transgenic swine comprising a genomic knockout of an endogenous LDLR gene, would have required undue experimentation, given Bentzon '643's teachings and the state of the art at the time of filing. Therefore, Appellants have not carried their burden of establishing that Bentzon '643 does not enable production of transfected swine cells and transgenic swine.

Turning to the claims' requirement that the transfected cells and transgenic animals are "free of selectable marker genes," Appellants argue that "the Cre/loxP or FRT/FLP systems . . . are not directed to specific target sites." Appeal Br. 16. Rather, "the loxP or FRT site is inserted without specificity and then the CRE or FLP reacts specifically with the loxP or FRT sites." *Id.* Appellants argue that the Examiner's "rationale errs in fact by incorrectly assuming that cre-lox can be used to target a specific gene such

as LDLR and knock it out," but "the *lox* site is necessarily inserted *randomly*." Reply Br. 6. Appellants contend:

> For instance, in Li et al., a gene cassette flanked by lox is *randomly* inserted. Then cre can be used to remove the gene cassette. Cre acts with specificity towards lox but for nothing else. Xu does not cure these defects because it uses the Cre-lox systems in mice, which is useless for the claimed pig, in part because embryonic stem (ES) cells in mice can be easily manipulated but there are no comparable techniques in swine.

Id.

Appellants' arguments are not persuasive. The Examiner's rationale is not, as Appellants' argue, that cre-lox can be used to target a specific gene . . . and knock it out." Reply Br. 6. Rather the Examiner contends Bentzon '643, in addition to "teach[ing] knocking out . . . gene alleles using homologous recombination in the donor cell followed by nuclear transfer" (Ans. 3), "also teaches that site-specific modification can be utilize[d] [for] the insertion of recombination sites for . . . enzymes such as FLP or CRE, and can be further combined to allow removal of selection genes" (*id.* at 3–4 (citing Bentzon '634, 21, 49)). Indeed, the Examiner confirms that "Li was not cited for using cre-loxP to knock out a gene in a pig," but "for teaching that cre-loxP systems can be successfully introduced into the genome of pigs and that expressed cre is functional in mediating loxP excision in transgenic pigs" (Ans. 17). The Examiner's findings are consistent with the teaching in Bentzon '643 that the Cre-Lox system may be used for removal of selection genes. *See* Bentzon '643, 49:9–13.

Accordingly, the rejection of claims 1, 3, 6, 19–22, 26, 27, 35, and 39 as unpatentable over Bentzon et al. or Bentzon '643, in view of Xu and Li is affirmed.

Rejection II

Claim 7 stands rejected under 35 U.S.C. § 103(a) as unpatentable over Bentzon '643, Xu, Li, and Ishibashi (Ans. 5–7).

Claim 7 depends from claim 1 and specifies that the *LDLR* gene is disrupted at exon 4.

Appellants contend that "Claim 7 is patentable for the same reasons as set forth with respect to claim 1," and "Ishibashi does not make up for the defects of the other references." Appeal Br. 24.

As we are not persuaded that claim 1 is patentable over the cited art for the reasons discussed above, the rejection of claim 7 under 35 U.S.C. § 103(a) as unpatentable over Bentzon '643, Xu, Li, and Ishibashi is affirmed for the same reasons.

SUMMARY

The rejection of claims 1, 3, 6, 19–22, 26, 27, 35, and 39 as unpatentable over Bentzon et al. or Bentzon '643, in view of Xu and Li is affirmed; and

The rejection of claim 7 as unpatentable over Bentzon et al. or Bentzon '643, in view of Xu, Li, and Ishibashi is affirmed.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED