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# Genes of the Pig, *Sus scrofa*, reconstructed with EvidentialGene

Donald G Gilbert Corresp. <sup>1</sup>

<sup>1</sup> Indiana University, Bloomington, IN, United States

Corresponding Author: Donald G Gilbert  
Email address: gilbertd@indiana.edu

The pig is a well studied model animal of biomedical and agricultural importance. Genes of this species, *Sus scrofa*, are known from experiments and predictions, and collected at the NCBI Reference Sequence database section. Gene reconstruction from transcribed gene evidence of RNA-seq now can accurately and completely reproduce the biological gene sets of animals and plants. Such a gene set for the pig is reported here, including human orthologs missing from RefSeq and other improvements to the current NCBI pig gene set. Methodology for accurate and complete gene set reconstruction from RNA is used: the automated SRA2Genes pipeline of EvidentialGene project.

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# Genes of the Pig, *Sus scrofa*, reconstructed with EvidentialGene

Author: Donald G. Gilbert

Affiliation: Indiana University, Bloomington, IN, USA

Email address: gilbertd@indiana.edu or gilbert.bionet@gmail.com

## Abstract

The pig is a well studied model animal of biomedical and agricultural importance. Genes of this species, *Sus scrofa*, are known from experiments and predictions, and collected at the NCBI Reference Sequence database section. Gene reconstruction from transcribed gene evidence of RNA-seq now can accurately and completely reproduce the biological gene sets of animals and plants. Such a gene set for the pig is reported here, including human orthologs missing from RefSeq and other improvements to the current NCBI RefSeq pig gene set. Methodology for accurate and complete gene set reconstruction from RNA is used: the automated SRA2Genes pipeline of EvidentialGene project.

## 24 Introduction

25

26 Precision genomics is essential in medicine, environmental health, sustainable agriculture, and  
27 research in biological sciences (eg. Goldfeder et al. 2016). Yet the popular genome informatics  
28 methods lag behind the high levels of accuracy and completeness in gene construction that are  
29 attainable with today's accurate RNA-seq data.

30

31 To demonstrate the accuracy and completeness of gene set reconstruction from expressed gene  
32 pieces (RNA-seq) alone, excluding chromosome DNA or other species genes, the pig is a good  
33 choice. The pig has a well constructed, partly curated NCBI RefSeq gene set, one of 7 RefSeq  
34 top-level model organisms, and is based on extensive expressed sequences dating from the  
35 1990s. Pig has a well-assembled chromosome set from BAC-clone Sanger + Illumina hybrid  
36 sequencing in the 2000s (Groenen et al. 2012), and contributions of experimental gene evidence  
37 from many projects of agricultural and biomedical focus. As well, published RNA-seq of the  
38 pig from over 2,000 studies weighs in the top 10 of model animals and plants. Yet there is just  
39 one public transcript assembly from these many pig studies, from blood samples only.

40

41 If successful, this demonstration can be used to improve RefSeq genes for this species. It will  
42 demonstrate to others how to produce reliably accurate gene sets. Unreliability is a continuing  
43 problem in gene set reconstructions, whether from RNA assembly or chromosome-based gene  
44 modeling. Reasons for this failure are many; the EvidentialGene project aims to provide a  
45 solution that others can use, simple in concept, always obtaining an accurate gene set picture  
46 from a puzzle box full of gene pieces.

47

48 Gene sets reconstructed by the author are more accurate by objective measures of homology and  
49 expression recovery, than those of the same species produced by popular methods, including  
50 NCBI Eukaryotic Genome Annotation Pipeline (EGAP, Thibaud-Nissen et al. 2013), Ensembl  
51 gene annotation pipeline, MAKER genome-gene modeling (Holt & Yandell 2001), Trinity RNA  
52 assembly (Grabherr et al 2001), Pacific Biosciences long RNA assemblies, and others. These  
53 improved gene sets include popular and model animals and plants such as *Arabidopsis*, *Zea mays*  
54 corn, *Th. cacao* chocolate tree, *Danio zebrafish*, *Fundulus* killifish, *Aedes* and *Anopheles*  
55 mosquitos, *Nasonia* jewel wasp, *Daphnia* water fleas (Gilbert 2012, 2013, 2016, 2017).

56

57 Gene sets reconstructed by others using EvidentialGene methods are also more accurate  
58 (Nakasugi et al. 2014, Mamrot et al. 2017), in independent assessments. However some  
59 investigators do not apply necessary details of EvidentialGene methodology, or modify portions  
60 in ways that reduce accuracy. One impetus for this work is engineering a full, automated  
61 pipeline that others can more readily use, for these validated methods.

62

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65

## 66 **Methods**

67

68 EvidentialGene uses several gene modeling and assembly methods, annotates their results with  
69 evidence, then classifies and reduces this over-assembly to a set of loci that best recovers the  
70 gene evidence. Each modeling method has qualities that others lacks, and produces models with  
71 better gene evidence recovery.

72

73 Gene reconstruction steps are (Gilbert 2012): 1. produce several predictions and transcript  
74 assembly sets with quality models. 2. annotate models with available gene evidence (transcript  
75 introns, exons, protein homology, transposon and other). 3. score models with weighted sum of  
76 evidence. 4. remove models below minimum evidence score. 5. select from overlapped models  
77 at each locus the highest score, and alternate isoforms, including fusion metrics (longest is not  
78 always best). 6. evaluate resulting best gene set (i.e. compare to other sets, examine un-  
79 recovered gene evidence). 7. re-iterate the above steps with alternate scoring to refine.

80 Evidence criteria for genes are, in part, protein homology, coding/non-coding ratio, RNA read  
81 coverage, RNA intron recovery, and transcript assembly equivalence.

82

83 For RNA-only assembly, this paradigm is refined at step 2-4 to introduce a coding-sequence  
84 classifier (Gilbert 2013), which reduces large over-assembly sets (e.g., 10 million models of  
85 100,000 biological transcripts) efficiently, using only the self-referential evidence of coding  
86 sequence metrics (protein length and completeness, UTR excess).

87

88 CDS overlap by self-alignment identifies putative gene loci and their alternate transcripts,  
89 similarly to how CDS overlap by alignment to chromosomal DNA is used in traditional genome-  
90 gene modeling to classify loci. This CDS classifier, in tr2aacds.pl pipeline script, uses the  
91 observed high correlation between protein completeness and homology completeness, making a  
92 computationally efficient classifier that will reduce the large over-assembly set to one small  
93 enough that the additional evidence classifications are feasible to refine this rough gene set to a  
94 finished one, using evidence of protein homology, expression validity, chromosomal alignment,  
95 and others.

96

97 A fully automated pipeline that includes the above, SRA2Genes, is introduced for this pig gene  
98 reconstruction. It includes RNA-seq data fetching from NCBI SRA, over-assembly of these data  
99 by several methods and parameters, transcript assembly reduction with coding-sequence  
100 classifier, protein homology measurement, sequencing vector and contamination screening, gene  
101 annotation to publication quality sequences, and preparation for submission to transcript shotgun  
102 assembly archive (TSA).

103

104 For pig gene set reconstruction, four RNA source projects were selected, from public RNA in  
105 SRA, based on tissue sampling, methodology (all paired-end reads of recent Illumina  
106 sequencers), and other factors. See “Data and Software Citations” section for details. Each  
107 project RNA set was assembled using SRA2Genes, to the step of non-redundant gene set with  
108 alternate isoforms. Then a superset of the best of these projects is produced, using these four  
109 reduced assemblies as input over-assembly to SRA2Genes, stepping from assembly reduction to

110 gene set annotation and publication. Assemblies were done in stages, assessing completeness  
111 then adding source data to improve that. Many more RNA source projects are available to  
112 improve this set. There are data choice problems for this and other large vertebrates, where  
113 most RNA samples are for specific tissues, often for mutant strains, with limited sample  
114 documentation. Collecting from public RNA samples to include all expressible genes can be  
115 difficult, with some tissue, stage or stress-specific genes missing or weakly expressed.

116  
117 RNA data source projects are *pig1a*: PRJNA416432 (China Agricultural University), *pig2b*:  
118 PRJNA353772 (Iowa State University, USDA-ARS), *pig3c*: PRJEB8784 (Univ. Illinois), and  
119 *pig4e*: PRJNA255281 (Jiangxi Agricultural University, Nanchang, China). These comprising 26  
120 read sets of 1,157,824,292 read pairs, or 106,654 megabases. All these are paired-end reads,  
121 from Illumina, ranging from 75bp to 150bp read length. PRJEB8784 includes adult female and  
122 male tissues of muscle, liver, spleen, heart, lung, kidney. PRJNA416432 includes adult female  
123 tissues of two sample types. PRJNA353772 includes tissue samples of brain, liver, pituitary,  
124 intestine, and others. PRJNA255281 provides embryonic tissue RNA. Notably missing were  
125 head sensory organs, one result being that some eye, ear, nose and taste receptor genes are under-  
126 represented or fragmented in this reconstruction.

127  
128 Assembler software used includes Velvet/Oases (Schulz et al. 2012), idba\_trans (Peng et al.  
129 2013), SOAPDenovoTrans (Xie et al. 2013), Trinity (Grabherr et al. 2011), and rnaSPAdes  
130 (Bankevich et al. 2012). K-mer shred sizes were selected to span the read sizes, and as observed  
131 in many RNA assemblies, 1/2 read-size produces the single most complete set, however most k-  
132 mer sizes produce some better gene assemblies, due to wide variation in expression levels and  
133 other factors (strongly expressed, long genes tend to assemble well with large k-mer). Both non-  
134 normalized and digitally normalized RNA sets were used; each way produces a different set of  
135 accurate genes.

136  
137 Additional assemblies with rnaSPAdes were targeted to unfinished genes, after reference  
138 homology measurements identified gene models that were incomplete. Prior work with several  
139 methods of assembly extension have proved unreliable, including assemblers Oases, SOAP and  
140 idba. These typically work to extend fragments by sequence overlap alone, but rarely produce  
141 longer coding sequences, instead indel errors and fused genes are frequent artifacts. rnaSPAdes,  
142 unlike the others, uses a graph of paired reads to extend partial transcripts, and may prove more  
143 successful.

144  
145

## 146 Results

147  
148 **Data and software result public access:** An open access, persistent repository of this annotated  
149 pig gene data set is at <https://scholarworks.iu.edu/> with DOI 10.5967/K8DZ06G3.  
150 Transcriptome Shotgun Assembly accession is DQIR01000000 at DDBJ/EMBL/GenBank,  
151 BioProject PRJNA480168, for these annotated transcript sequences. Preliminary gene set is at  
152 <http://eugen.es.org/EvidentialGene/vertebrates/pig/pig18evigene/>. EvidentialGene software  
153 package is available at <http://eugen.es.org/EvidentialGene/> and at  
154 <http://sourceforge.net/projects/evidentialgene/>.

155

156 The results of gene assembly for each of 4 data sources are summarized as *pig1a* 11,691,549  
157 assemblies reduced to 595,497 non-redundant coding sequences (5%), *pig2b* 3,984,284  
158 assemblies reduced to 404,908 (10%), *pig3c* 8,251,720 assemblies reduced to 564,523 (7%), and  
159 *pig4e*, a smaller embryo-only RNA set, of 1,955,018 assemblies to 134,156 (7%). These 4  
160 reduced assemblies are then used in secondary runs of SRA2Genes, stepping from assembly  
161 inputs. Several secondary runs were performed, with reference homology assessment, to ensure  
162 all valid homologs are captured. Additional assemblies with rnaSPAdes of incomplete genes  
163 improved some of the fragment models (16,168 or 5% of final transcripts, including 1571 loci  
164 with best homology).

165  
166 The final gene set is summarized in Table 1 by categories of gene qualities and evidences. The  
167 number of retained loci include all with measurable homology to 4 related vertebrate species  
168 gene sets, and a set of non-homolog, but expressed with introns in gene structure, two forms of  
169 gene evidence that provide a reliable criterion. The number with homology is similar to that of  
170 RefSeq genes for pig. The expressed, multi-exon genes add 15,000 loci, which may be  
171 biologically informative in further studies. The pig RefSeq gene set has 63,586 coding-sequence  
172 transcripts at 20,610 loci, of which 5,177 have exceptions to chromosome location (indels, gaps  
173 and RNA/DNA mismatch).

174  
175 The extended gene data set includes culled transcript sequences, which do not meet criteria for  
176 homology or unique expression, but which pass other criteria for unique transcripts: 92627 culled  
177 loci, and 175,793 culled alternate transcripts. Further evidence may indicate some of these are  
178 valid. The published gene data set includes mRNA, coding and protein sequences in FastA  
179 format for the public set (*pig18evigene\_m4wf.public.mrna.cds.aa*), and the culled set  
180 (*pig18evigene\_m4wf.xcull.mrna.cds.aa*). There are two sequence object-annotation tables,  
181 *pig18evigene\_m4wf.pubids* (gene locus and alternate public ids, object ids, class, protein and  
182 homology attributes), and *pig18evigene\_m4wf.mainalt.tab* (locus main/alternate linkage for  
183 original object ids). A gene annotation table *pig18evigene\_m4wf.ann.txt* contains public ids,  
184 name, protein, homology, database cross references, and chromosome location annotations.  
185 Chromosome assembly locations to RefSeq pig genome are given in  
186 *pig18evigene\_m4wf.mrna.gmap.gff* in GFF version 3 format.

187  
188 [insert **Table 1.** *Sus scrofa* (pig) gene set numbers, version Susscr4EVM ]  
189

190 The table 2a scores are measured against vertebrate conserved BUSCO subset of OrthoDB v9,  
191 and are counts relative to 2586 total conserved genes, but for the Align average in aminos. Full  
192 is the count of pig genes completely aligned to conserved proteins. Table 2b has scores for  
193 human gene alignments, percentages relative to all reference genes found in either pig set (n=  
194 37,883), calculated from table of “blastp -query human.proteins -db two\_pigsets.proteins -  
195 evaluate 1e-5”. These proteins include 19122 of 20191 (95%) of human gene loci. NCBI has  
196 25% of best match, Evigene 20% of best, and 55% of comparisons are equal for the human  
197 proteins that are found in either pig gene set. Scores are Align = alignment to reference proteins,  
198 as percent of human gene, Frag = percent with fragment alignment, size < 50% of reference,  
199 Short = percent with size < 95% of reference, Miss = percent with no alignment, Best = percent  
200 or count of greater alignments in pairwise match to each reference gene. Supplemental tables 1  
201 and 2 have the pair-wise pig gene alignment scores of summary tables 2a and 2b.  
202

203 [insert **Table 2.** *Sus scrofa* gene sets compared for gene evidence recovery]

204

205 Average homology scores are nearly same for both of these gene sets, but they differ for  
206 individual loci. The “Best” columns in Table 2 indicate a subset of Evigene that can usefully  
207 improve the RefSeq gene set: 4,521 proteins have improved human gene homology to greater or  
208 lesser extent. 283 of Evigene improvements have no pig RefSeq equivalent, including the 9  
209 vertebrate conserved BUSCO genes missing from the NCBI set. 121 of the improved coding  
210 genes are modeled as non-coding in RefSeq ([NX]R\_ IDs), that can be better modeled as coding  
211 genes with exceptions in chromosome mapping. 548 have a RefSeq mRNA that is co-located  
212 with an Evigene model, but notably deficient in human gene alignment (i.e. a fragment or  
213 divergent model), while a majority of 1048 improvements have small, exon-sized differences, as  
214 alternate transcripts to existing RefSeq loci.

215

216 Many of the 15,000 putative genes that lack homology to human, cow, mouse or fish RefSeq  
217 genes do have homology by other measures. With non-redundant NCBI protein database, 11%  
218 of these have a significant match, to uncharacterized genes in other mammals or vertebrates, or  
219 endonuclease/reverse transcriptase transposon-like proteins, or as fragment alignments to  
220 characterized proteins. Coding alignment of these putative genes to the cow (*Bos taurus*)  
221 chromosome set, and calculation of synonymous/non-synonymous substitutions (Ka/Ks),  
222 identifies from 13% to 28% have coding sequence conservation, the majority not identified as  
223 having protein homology in the other tests. These putative genes may include recently  
224 duplicated and modified coding genes, ambiguous non-coding/coding genes, as well as  
225 fragments of other genes, putative transposon residue, and untranslated but expressed genome  
226 regions.

227

228 [insert **Table 3.** Assembler method effects on Human reference gene recovery in Pig gene sets]

229 The table 3a scores are for alignments to human gene with blastp, subset by assembler method  
230 for data of Bioproject PRJNA416432. Table 3b scores for a second pig project are also subset by  
231 methods for alignment to human genes. This second project collected both Illumina RNA-seq  
232 (75bp paired reads) and PacBio (<1-2kb, 2-3kb, 3-5kb, >5kb single reads from Pacific  
233 Biosciences instrument) from the same set of tissue samples. This PacBio assembly, which  
234 includes improvement using the Illumina RNA with Proovread, was done by the project authors,  
235 and published in SRA, under Bioproject ID PRJNA351265, while the Illumina RNA is under  
236 Bioproject PRJNA353772.

237

238 The major option used for these various assemblies is k-mer size, the sub-sequence length for  
239 placing reads in the assembly graph structure. Different genes are best assembled with different  
240 k-mer sizes, depending on expression level, gene complexity, and other factors, that indicates  
241 why many assemblies of the same data but different options result in a larger set of accurate gene  
242 reconstructions. For Table 3a sample, with read size of 150 bp, k-mers from 25 to 125 were  
243 used. k-mer of 105 returned the most accurate genes, for both velvet and idba methods. The  
244 range k70..k125 produced 5/10 of best models, range k40..k65 produced 4/10, and range  
245 k25..k35 the remaining 1/10 of best models. The popular Trinity method underperforms all  
246 others, due part to its limited low k-mer option.

247

248 Sample 2 (Table 3b) demonstrates the value of assembling accurate gene pieces (Illumina, 80%  
249 of reads have highest quality score in SRA), over inaccurate but longer sequences (PacBio, 15%



250 of reads have highest quality score in SRA). This project sequenced pig RNA with both  
251 technologies, and PacBio assembly software plus Illumina RNA to improve PacBio sequence  
252 quality, to produce a gene set that is less accurate than that produced from the Illumina-only  
253 RNA, assembled with a competent short-read assembler.

254

## 255 **Discussion**

256

257 The main result of this demonstration compared with the NCBI RefSeq pig gene set is, on  
258 average, they are equally valid by homology measures, but differ at many gene loci, with  
259 Evigene adding many alternate transcripts. The Evigene set also retains more putative loci,  
260 lacking measured homology but with other evidence, that further study will clarify their value.  
261 Improvements to the pig gene set are numerous enough to warrant updating RefSeq with those  
262 from this work. These include 1,500 missing or poorly modeled genes with homology to human,  
263 and improved vertebrate conserved genes. Between RefSeq and Evigene sets, all highly  
264 conserved vertebrate genes of the BUSCO set exist in pig. The another 3,000 improvements are  
265 mostly alternate transcripts with greater alignment to other species, by changes in an exon or  
266 two.

267

268 This Evigene set has demonstrated objectively accurate gene assemblies that improve the  
269 reference gene set of the pig model organism. It has been submitted for that purpose to NCBI as  
270 a third party annotation/assembly (TPA) of a transcriptome shotgun assembly (TSA), which are  
271 International Nucleotide Sequence Database Collaboration (INSDC) classifications. There are  
272 policy reasons to limit inferential or computational TPA entries, and there are also policy reasons  
273 to accept these. On one hand, objectively accurate gene and chromosome assemblies of  
274 experimental RNA and DNA fragments are the desired contents of public sequence databases.  
275 On the other hand, having many assemblies of the same RNA or DNA fragments is confusing  
276 and could overwhelm databases devoted to experimentally derived genome sequences. This pig  
277 gene set adheres to the described policy of TPA in that (a) it is assembled from primary data  
278 already represented in the INSDC databases (SRA sequence read section); (b) it is indirectly  
279 experimentally supported by reference gene homology measures; (c) it is published in a peer-  
280 reviewed scientific journal. Additionally this gene set provides thousands of improvements to  
281 the reference gene set. The author produced no wet-lab experimental evidence, but has  
282 assembled gene sequence evidence from several sources into a gene set that substantially  
283 improves upon NCBI EGAP and Ensembl gene sets. Review of this data set, by NCBI and  
284 independent peers, weighs the above dilemma: improve public genome sequences or limit  
285 independent computational assemblies.

286

287 Combining and selecting by evidence criteria the assemblies of several methods improves gene  
288 reconstruction to a higher level of accuracy. The individual methods return from 77% (Velvet)  
289 down to 50% (Trinity) of the best gene models, and a hybrid PacBio+Illumina assembly is  
290 intermediate at 66%. K-mer sizes are an important parameter, as noted by others: "smaller  
291 values of k collapse more repeats together, making the assembly graph more tangled. Larger  
292 values of k may fail to detect overlaps between reads, particularly in low coverage regions,  
293 making the graph more fragmented." (SPAdes, Bankevich et al 2012). Alternate isoforms of  
294 each gene, which share exons and differ in expression levels, are more accurately distinguished

295 from other genes at large k-mer sizes (idba\_tran, Peng et al. 2013). These results are consistent  
296 with multi-method reconstructions for arabidopsis, corn, zebrafish, mosquitos, and water fleas.

297  
298 The main flaw in this Evigene pig set is incomplete reconstruction of many genes, especially  
299 longer ones. While this is not always a problem with RNA-only assemblies, it is a common one.  
300 Importantly, there does not appear to be a reliable method for improving gene assemblies  
301 identified as fragmentary, using de-novo RNA assembly. While there are several methods that  
302 attempt to address this, those tested by the author are unreliable. A trial of rnaSPAdes to extend  
303 fragments did improve some genes, but not as many as the RNA data warrants.

304  
305 A second flaw in EvidentialGene's method of classifying loci from self-referential alignment of  
306 coding sequences, is that some paralogs are confused as alternate transcripts of the same locus.  
307 With high sequence identity, paralogs align to each other similarly to transcripts of one locus (a  
308 class termed "altpar" or "paralt"), though with mismatches that chromosome alignment can  
309 resolve. This has been measured at a rate of about 5% for reference gene sets of mouse and  
310 zebrafish, and 3% for arabidopsis; a smaller 0.5% portion of alternates at one locus are  
311 misclassified as paralog loci. Several de-novo gene assembly methods that classify loci have  
312 similar altpar confusion, as RNA-seq reads are often shared among paralogs as well as alternate  
313 transcripts. These altpar transcripts have not been resolved for this pig gene set, though it is an  
314 improvement in development.

315  
316 This demonstration excluded the use of chromosomes and other species genes to assemble or  
317 extend assemblies. Both methods can be employed to advantage to reconstruct genes, where  
318 there are few errors in these additional evidences. An important reason to limit initial gene  
319 reconstruction to RNA-only assembly is to avoid compounding errors from several sources. This  
320 limited-palette reconstruction is validated with independent evidence from genomic DNA and  
321 other species sources; genes identified as mis-assembled, or missing, in such RNA-only sets can  
322 be improved with these other methods. Many discrepancies between RNA-only reconstruction  
323 and the other evidences are from flaws in chromosome assemblies or other species genes that can  
324 be identified with careful evaluations.

325  
326 Gene transcripts from any source, such as EST and PacBio, may be added into SRA2Genes  
327 pipeline. Excluded from this reconstruction are the extensive public set of pig ESTs, and the  
328 PacBio+Illumina assembly of sample *pig2b*. These contribute a small number of improved  
329 transcripts not in this EvidentialGene set (8 missed human orthologs in ESTs, 12 EST and 24  
330 PacBio with significant improvements), and are used in the RefSeq set. However as these are  
331 already in the public databases, this demonstration reconstruction adds no value to them.

332  
333 While these gene data and paper were in review at repositories, Zhao P, X Zheng, Y Yu et al  
334 (2018) pre-published a reconstruction of pig genes, with newly sampled proteomic and  
335 transcriptomic sequences. The authors provide public access to these under BioProject  
336 PRJNA392949 for SRA RNA-Seq, and a bioRxiv preprint with sequences of 3,703 novel protein  
337 isoforms. The experimental design of this work is well suited to gene set reconstruction, as it  
338 sampled 34 tissues of adult male, female and juvenile pigs. Unlike the samples winnowed from  
339 prior SRA entries by this author, each from a pig portion, this new work is comprehensive in  
340 collecting expressed and translated genes.

341  
342 Zhao and colleagues compare their results to the same RefSeq gene set and chromosome  
343 assembly as this paper. In brief, of the 3,700 novel proteins, most align to other gene sets and  
344 chromosome assembly: 74% are contained in this paper's transcripts, 65% are contained in the  
345 RefSeq transcript set, and 61% are contained in the pig chromosome set, at 75% or greater  
346 alignment (protein to RNA/DNA aligned with tBLASTn). None the less, most of these novel  
347 proteins do not have a protein equivalent in the gene sets: about 800 novel proteins align to  
348 Evigene proteins, and about 600 to Refseq proteins. A main difference here lies in measures  
349 from RNA to protein, including new alternate transcripts and discrepancies in RNA to protein  
350 reconstruction, rather than in newly identified gene loci, and is beyond scope of this note to  
351 resolve. A rough draft with SRA2Genes of this recent RNA-Seq, assembling only well-  
352 expressed genes, contains about the same 74% of novel proteins as for this paper's set. An  
353 application suited to SRA2Genes is to update with these completely sampled pig genes,  
354 including depositing an improved version to Transcriptome Shotgun Assembly public database  
355 for further uses.

356

## 357 **Conclusions**

358

359 The SRA2Genes pipeline is demonstrated, for the pig model organism, as a reliable gene  
360 reconstruction method, useful to other projects and for improving public reference gene sets.  
361 The resulting complete transcriptome assembly of pig fills a void at public repositories.  
362 Reconstruction from RNA only provides independent gene evidence, free of errors and biases  
363 from chromosome assemblies and other species gene sets. Not only are the easy, well known  
364 ortholog genes reconstructed well, but harder gene problems of alternate transcripts, paralogs,  
365 and complex structured genes are usually more complete with EvidentialGene methods.

366

367

## 368 Acknowledgements

369 XSEDE/TeraGrid shared computational resources, for a decade of development and  
370 implementation, Award# MCB100147, to Genome Informatics for Animals and Plants, D.G.  
371 Gilbert. IUScholarWorks staff, including Richard Higgins, for providing a permanent open-  
372 access repository of EvidentialGene animal and plant gene sets. NCBI GenBank submissions  
373 staff for reviewing effort to deposit TPA/TSA gene data sets.  
374

## 375 Supplemental Information

376  
377 Supplemental Table 1. Conserved vertebrate genes recovered in Pig Evigene vs NCBI gene sets,  
378 as computed with vertebrate conserved genes of OrthoDB v9, BUSCO and hmmer software.  
379 Columns include gene ids of BUSCO\_ID, Evigene\_ID, and NCBI RefSeq ID. Other columns:  
380 Cmp, the qualitative comparison (evgain, same, evloss) of alignment difference; Diff, numeric  
381 difference in alignment score to conserved protein; dEvg-Ncb, the two alignment scores; BC,  
382 the BUSCO complete/fragment/missing quality score; and Product\_Name, the vertebrate protein  
383 product. File name: pig18evg\_ncbi\_busco.xlsx  
384

385 Supplemental Table 2. Human genes recovered in Pig Evigene vs NCBI gene sets, as computed  
386 with human and pig RefSeq and Evigene proteins and NCBI BLASTP software. This includes  
387 only unique alignments of isoforms of both pig gene sets to isoforms of human genes. Columns  
388 include gene ids for Human RefSeq ID, Evigene\_pig\_ID, NCBI\_pig\_ID; AAsize, human protein  
389 size; EvAlign, NcAlign, alignment scores to Evigene and NCBI proteins; DiffA, difference in  
390 alignments; and Human\_Gene\_Name. File name: pig18evg\_ncbi\_human.xlsx  
391

## 392 Data and Software Citations

393  
394 NCBI pig gene set used in comparison, from [ftp://ftp.ncbi.nlm.nih.gov/refseq/  
395 S\\_scrofa/mRNA\\_Prot/pig.1.rna.gbff.gz](ftp://ftp.ncbi.nlm.nih.gov/refseq/S_scrofa/mRNA_Prot/pig.1.rna.gbff.gz), accessed on 27 Apr 2018.  
396 Ensembl pig gene set used in comparison, from  
397 [ftp://ftp.ensembl.org/pub/current\\_fasta/sus\\_scrofa/pep/Sus\\_scrofa.Sscrofa11.1.pep.all.fa.gz](ftp://ftp.ensembl.org/pub/current_fasta/sus_scrofa/pep/Sus_scrofa.Sscrofa11.1.pep.all.fa.gz),  
398 accessed on 28 Jul 2018.  
399 NCBI RefSeq pig chromosome assembly Sscrofa11.1, accession: GCF\_000003025.6, dated  
400 2017-2-7, is used for chromosome mapping.  
401 NCBI RefSeq gene sets used as reference genes are H\_sapiens, M\_musculus, B\_taurus, and  
402 D\_rerio, accessed at same location and date as pig genes.  
403 RNA data sources with NCBI BioProject ID are  
404 SRA data *pig1a*: PRJNA416432 (China Agricultural University),  
405 SRA data *pig2b*: PRJNA353772 (Iowa State University, USDA-ARS),  
406 SRA data *pig3c*: PRJEB8784 (Univ. Illinois),  
407 SRA data *pig4e*: PRJNA255281 (Jiangxi Agricultural University, Nanchang, China).  
408 The SRA read table of these data sets is the starting point for SRA2Genes, and provided at  
409 <http://eugenesis.org/EvidentialGene/vertebrates/pig/pig18evigene/>

410 Expressed sequences of the pig from dbEST, by Sanger and 454 sequencing (max length 900  
411 bases), from projects reported in PubMedID:14681463, dbEST n=304,418, and PubMedID:  
412 17407547, dbEST n=716,260.  
413 Vertebrate conserved single-copy genes, of OrthoDB v9 (<http://www.orthodb.org>), BUSCO.py  
414 software, with hmmer (v3.1, <http://hmmer.org/>).  
415  
416 Software components of EvidentialGene SRA2Genes:  
417 fastq-dump, of sratoolkit281, <https://www.ncbi.nlm.nih.gov/sra/docs/toolkitsoft/>  
418 blastn, blastp of <https://blast.ncbi.nlm.nih.gov/>  
419 vecscreen, tbl2asn of <http://ncbi.nlm.nih.gov/tools/vecscreen/>, [/genbank/tbl2asn2/](http://genbank/tbl2asn2/)  
420 fastanrdb, of exonerate, <https://www.ebi.ac.uk/about/vertebrate-genomics/software/exonerate>  
421 cd-hit, cd-hit-est, of <https://github.com/weizhongli/cdhit/>  
422 normalize-by-median.py, of khmer, <https://github.com/ged-lab/khmer>  
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427 rnaSPAdes, of SPAdes assembler, <http://cab.spbu.ru/software/spades/>  
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429 International Nucleotide Sequence Database Collaboration (INSDC) policy documents  
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431 About TSA, <https://www.ncbi.nlm.nih.gov/genbank/TSA>  
432 About TPA, <https://www.ncbi.nlm.nih.gov/genbank/TPA>  
433 TPA FAQ, <https://www.ncbi.nlm.nih.gov/genbank/tpafaq>  
434 TPA-Inferential, <https://www.ncbi.nlm.nih.gov/genbank/TPA-Inf>  
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**Table 1** (on next page)

pig18evg\_datadesc.pages *Sus scrofa* (pig) gene set numbers, version Susscr4EVm

1 **Table 1.** *Sus scrofa* (pig) gene set numbers, version Susscr4EVm

39879 gene loci, all supported by RNA-seq, most also have protein homology evidence  
39879 (100%) are protein coding, 0 are non-coding  
All genes (100%) are assembled from RNA evidence, 0 are genome-modeled  
25383/39879 (64%) have protein homology to other species genes.  
316491 alternate transcripts are at 25512 (64%) loci, with 5 median, 12.4 ave, transcripts  
per locus, with 756 alts maximum, 1079 loci have 50+ alts, 8453 have 10+ alts,  
27473 (69%) have complete proteins, 12406 have partial proteins, of 39879 coding genes  
37918 (95%) are properly mapped to chromosome assembly ( $\geq 80\%$  align),  
1144 partial-mapped coverage (  $10\% < \text{align} < 80\%$ ),  
817 are ~un-mapped genes (  $\text{align} < 10\%$  ),  
6746/37918 (18%) are single-exon loci of those mapping  $\geq 50\%$  to genome,  
3274 of these have homology to other species genes.

92627 are culled loci, not in public gene set, but with some unique sequences.  
99 culls are multi-exon, well aligned; 87515 are single exon, well aligned,  
1082 are partially mapped, and 3931 are poorly aligned to chromosomes.  
13658 culls have protein homology, 78969 lack it.  
175793 are culled alternate transcripts, at both public and culled loci, redundant  
in splicing patterns to public alternates, or lacking in alignment or evidence.

Gene locus IDs: Susscr4EVm000001t1 .. Susscr4EVm137575t1, Alternate transcripts have  
ID suffix t2 .. t100. EVm000001 is the longest protein, ID numbers are ordered by protein  
size, mostly. Culled transcripts are those initially classed as unique coding sequences, but  
re-classified as redundant, or lacking sufficient evidence, by chromosome alignment and  
homology evidence. These are separate from the public gene set as low quality, but are  
available as expressed transcripts, that may be recovered with further evidence.



**Table 2**(on next page)

pig18evg\_datadesc.pages *Sus scrofa* gene sets compared for gene evidence recovery:  
2a. Conserved vertebrate genes in pig gene sets (BUSCO), 2b. Human reference genes (Homo\_sapiens RefSeq).

1 **Table 2.** *Sus scrofa* gene sets compared for gene evidence recovery: 2a. Conserved vertebrate  
 2 genes in pig gene sets (BUSCO), 2b. Human reference genes (Homo\_sapiens RefSeq).

**2a. Vertebrate conserved genes**

4

Geneset	Full	Align	Miss	Best
Evigene	2568	447 aa	8	776
NCBI	2567	440 aa	17	80
Ensembl	2552	431 aa	14	na

**2b. Human reference genes**

Geneset	Align	Miss	Frag	Best
Evigene	96.0%	0.7%	1.7%	20%
NCBI	97.2%	0.7%	0.6%	25%

6

**Table 3**(on next page)

pig18evg\_datadesc.pages Assembler method effects on Human reference gene recovery in Pig gene sets: 3a. Sample set 1 (PRJNA416432), 3b. Sample set 2 (PRJNA353772)

1 **Table 3.** Assembler method effects on Human reference gene recovery in Pig gene sets: 3a.  
2 Sample set 1 (PRJNA416432), 3b. Sample set 2 (PRJNA353772).

**3a. Sample set 1**

<b>Method</b>	<b>Miss</b>	<b>Frag</b>	<b>Short</b>
Velvet	5%	7%	23%
Idba	8%	12%	30%
Soap	12%	16%	36%
Trinity	20%	28%	49%

3

**3b. Sample set 2**

<b>Method</b>	<b>Miss</b>	<b>Frag</b>	<b>Short</b>
Illumina_all	4%	6%	20%
Illum_velvet	5%	7%	23%
PacBio+	12%	15%	33%

4

