

Which Little Piggy Went to Market? Bioinformatics and Meat Science

by

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Part I—Pigs Is Pigs?

Sue was busy doing what scientists do most of the time. She was waiting for something to happen. In this case the “what” was a sequencing gel. The phone rang. It was an old friend of hers currently working at the meat processing plant in town. He had a problem for her. He needed a way to determine if the pork he was using to make a new gourmet sausage came solely from gilts.

“Why do you care?” Sue asked. “What difference does it make what sex the meat came from? Pig meat by any other name is still pig meat.”

“Not really,” John replied. “Boar meat has a definite very disagreeable odor. The phenomenon is called boar taint. Having boar meat in the mix isn’t a problem if you’re making a product that is heavily spiced. The spices cover it up. But this new sausage recipe I’m working with has very few spices.”

“I see what you mean. Having my breakfast smell bad doesn’t sound too appetizing.”

“My company pays top dollar for gilt meat,” John continued. “But this last batch had a funny smell. I think it has some boar in it. I have no way of knowing if the distributor is cheating me. That’s why I called you. I need a reliable, quick, and sensitive way to check if the meat that I’m receiving contains any boar meat.”

Sue heard the timer go off telling her that the sequencing gel was done. She needed to finish the experiment and get the gel under film as soon as possible. John’s problem would have to wait.

“Listen, John, I have to finish up some work here at the lab. But I’ll call you back later and we can talk about it.”

Sue got home late that night and decided to wait until the next afternoon to call John.

Questions

1. What is the specific problem John is asking Sue to help him with?
2. What information would Sue need to know before trying to solve the problem?



Part II—Boar Taint

Sue's thoughts kept returning to John's problem throughout the day. She knew very little about pigs—only that she liked her brats boiled in beer and served with sauerkraut. She decided to do some research on boar taint on the Internet before she called John the next day.

Questions

3. What is the difference between a gilt, a sow, a barrow, and a boar?
4. What causes boar taint?
5. What are some methods of detecting the chemicals causing boar taint in pork?
6. In mammals, how are males different from females?



Part III—Test Wanted

The next day Sue called John.

“Hi, it’s me, Sue. I did some research last night on boar odor. I had no idea this was such a problem in the pork industry.”

“Yeah,” said John. Sue noticed he sounded a little tired. “There are a couple of research groups trying to figure out how to lower or eliminate the chemicals responsible for causing the odor. There are benefits in using intact males instead of barrows, namely lower feed costs and the meat is leaner. But the problem is that the meat stinks, literally.”

“Isn’t there some biochemical test you could use to detect for levels of skatole and 16-androsterone steroids in the meat?” asked Sue.

“Sure,” said John. “I could send out samples of the meat and have a lab run it through a gas chromatograph or have an ELISA performed on it. But I want a test that is quick and sensitive.”

“Well, let me think about it. I’ll call you in a couple of days.”

“Thanks. I appreciate your help.”

Question

7. Design an experiment that will show the presence of boar meat in a mixed sample, keeping in mind that biochemical tests are too slow and relatively insensitive.



Part IV—Sue’s Solution

Sue began to think about how she might detect the presence of boar meat in a mixed sample. Assaying for skatoles and male steroids was out since they can also be found in female tissue. She asked herself what else makes male tissue different from female tissue. Sue suddenly remembered her genetics. The one thing males have that females don't is the Y chromosome. She could use the presence of some gene on the Y chromosome as a marker for boar meat. The problem would be to detect the specific DNA. She then remembered her favorite TV program—CSI. She could use PCR to amplify the DNA. DNA is very stable. Even if the meat had been sitting around for a while or been cooked, there should be enough DNA to run a PCR amplification producing enough DNA to detect on a gel. Once you have the primer, PCR is relatively quick and moderately inexpensive.

Questions

8. Generally, what genes are found on the Y chromosome?
9. Has the swine sex-determining gene on the Y chromosome been sequenced? Go to GenBank at <http://www.ncbi.nlm.nih.gov/entrez/query/> to determine this. Select nucleotide and then enter “swine SRY gene.” If the sequence is present, print a copy of it.
10. How does PCR work? What is the role of each item listed below in the process?
 - a. Taq polymerase
 - b. Primers
 - c. Temperature cycling
11. How are primers selected and made?

Websites

- “Genetics of Boar Taint: Implications for the Future Use of Intact Males,” by E.J. Squires, Dept. of Animal and Poultry Science University of Guelph, Guelph, Ontario, Canada.
<http://mark.asci.ncsu.edu/nsif/99proc/squires.htm>
- “Use of Entire Males for Pig Production,” by M. Bonneau and E.J. Squires, Conferencia Virtual Internacional sobre Qaulidade de Carne Suina.
http://www.cnpsa.embrapa.br/pork/anais00cv_bonneau_en.pdf
- “Principle of the PCR,” by Andy Vierstraete.
<http://allserv.rug.ac.be/~avierstr/principles/pcr.html>
- “What the Heck is PCR?”
<http://people.ku.edu/~jbrown/pcr.html>



Part V—Success?

After finding the swine sex-determining region gene at GenBank, Sue selected two sequences to act as primers. She sent the sequence to a company in California and had them make the primers for her. Both primers should create a fragment 158 base pairs long. She ran a PCR reaction on samples of boar and sow meat as well as a mixture of both. The results are shown in **Figure 1**.

Questions

12. What do you conclude from **Figure 1**?
13. Why were lanes 1, 2, 3, 5, and 6 included in this experiment?

Sue was excited. The PCR amplification worked. She could detect the presence of the sex-determining gene in samples of boar meat but not in sow. She also saw the same size band after PCR of a sausage sample containing both boar and sow. Sue remembered that John needed a sensitive assay. She next wanted to know the sensitivity of her assay.

Questions

14. Why does Sue want to know the sensitivity of her assay?
15. **Figure 2** illustrates the results of such an experiment. What do you conclude from this figure?
16. Why were lanes 2, 3, and 4 included in this experiment?

Sue was getting even more excited. Her calculations indicated that the sensitivity of her assay was down to a dilution of boar meat to sow meat of at least 1:10,000. She had one more question to answer before she could call John. She needed to know if processing the meat would affect the efficacy of PCR amplification of the swine sex-determining gene.

Questions

17. **Figure 3** illustrates the effect of spices and cooking on the efficacy of PCR to amplify the sex-determining gene. What can you conclude from this figure?
18. When Sue calls John with her results, do you think she has solved John's problem? Why or why not?

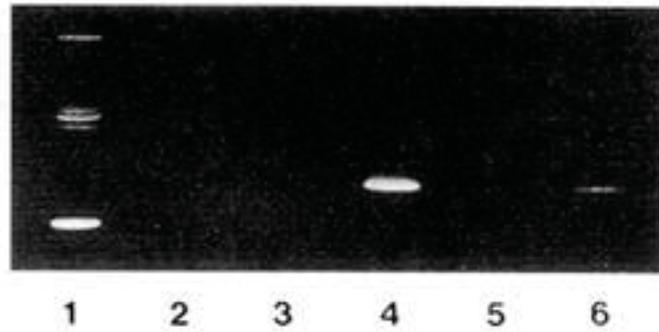
Assignment

Your assignment is to write an abstract (summary) of Sue's results.

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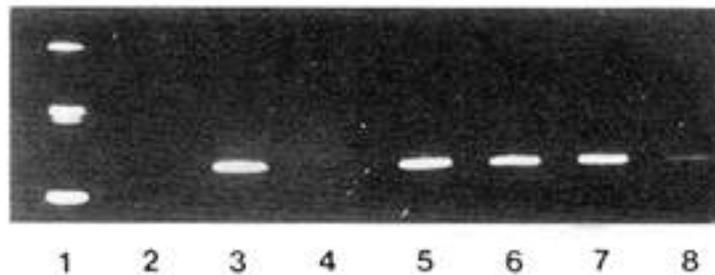
Figure 1



Identification of unique sex-determining region Y gene in male tissue. Presence of 158 bp sex-specific fragment from male tissue or sausage containing male tissue on 8% polyacrylamide gel. Lane 1: 123 bp DNA ladder, 123, 246, and 369 bp fragments from bottom to top. Lane 2: DNA omitted from the PCR reaction. Lane 3: Primers were omitted from the PCR. Lane 4: 158 bp fragment following PCR of boar tissue. Lane 5: PCR of sow tissue. Lane 6: PCR of sausage sample containing some male tissue.

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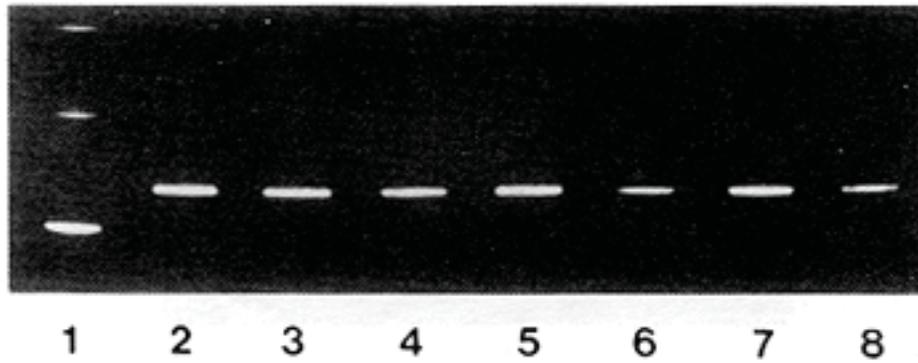
Figure 2



Sensitivity of PCR reaction for identifying male tissue in mixed tissue samples. Eight percent polyacrylamide gel showing bands from tissue samples using PCR following serial dilution. Lane 1: 123 bp DNA ladder; 123, 246, and 369 bp fragments from bottom to top. Lane 2: DNA omitted from PCR reaction. Lane 3: 158 bp fragment following PCR from boar muscle. Lane 4: PCR of sow muscle. Lanes 5–8: Boar muscle DNA diluted with sow muscle DNA at 1:10, 1:100, 1:1000, and 1:10,000 prior to PCR, respectively.

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Figure 3



Lack of effect of spices or cooking on efficacy of PCR to amplify male-specific region of DNA. Eight percent polyacrylamide gel. Lane 1: 123 bp DNA ladder; 123, 246 and 369 bp fragments from bottom to top. Lane 2: 158 bp DNA fragment PCR amplified from male muscle sample. Lanes 3, 5 and 7 are from frozen boar muscle. Lanes 4, 6 and 8 are frozen bratwurst samples made with a mixture of boar and sow tissue. Lanes 3 and 4, samples cooked to 50°C for 15 min prior to PCR. Lanes 5 and 6, samples cooked to 70°C for 15 min prior to PCR. Lanes 7 and 8, samples cooked to 100°C for 15 min prior to PCR.

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