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What has PSR Genetics discovered?

We identified a gene, designated as the *PSR* gene, which leads to significant levels of *Salmonella* and *E. coli* O157:H7 resistance in non-black cattle (regardless of breed). This gene also leads to *Salmonella* and *E. coli* O157:H7 resistance in some black cattle (about 2%), while this gene leads to partial resistance in most black cattle (about 98%).



PSR → full resistance (100%)*



PSR → full resistance (2%)*



PSR → partial resistance (98%)*

* when an animal has two copies of the *PSR* gene

Why will these findings revolutionize the beef industry?

Salmonella and *E. coli* O157:H7 are the two most important food safety concerns in beef. Both of these bacteria cause diarrheal diseases that can be fatal in humans. This may not seem to be economically important but:

(1) Our research has shown that these resistant cattle are healthier and perform better in the feedlot.

(2) Identifying and propagating resistant cattle will dramatically reduce the incidence of beef-associated outbreaks of *Salmonella* and *E. coli* O157:H7, thus increasing the demand for beef. Owners of these cattle could even market their beef as “safer”. If desired, beef from these animals can be marketed as such:

Tarentaise Beef

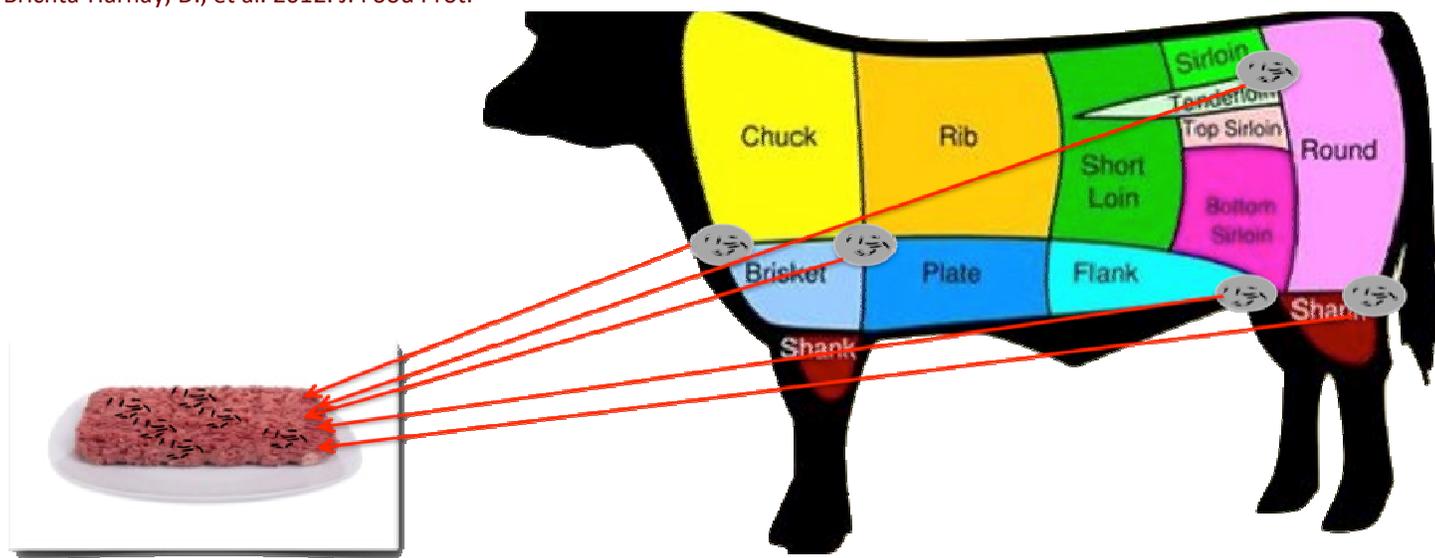
Tender.Leaner.Safer®

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The story behind PSR Genetics LLC

PSR Genetics LLC is a biotechnology company founded in western Kansas, with research facilities in Iowa. The company was started with the goal of identifying cattle that resist *Salmonella* and *E. coli* O157:H7 infections. *Salmonella* was targeted because recent USDA studies¹ revealed an alarming and emerging trend in which many cattle now harbor *Salmonella* in their lymph nodes. Each animal has about 20 lymph nodes (●) that can contain *Salmonella* (🦠). These lymph nodes are closely associated with muscles and thus inevitably get incorporated into hamburger.

¹ Brichta-Harhay, D., et al. 2012. J. Food Prot.



Unfortunately, most beef is not tested for the presence of *Salmonella*. The Table below highlights the recent *Salmonella* infections associated with ground beef²:

Year	Number of cases	Number of states	Number of hospitalizations
2013	22	6	7
2012	46	9	12
2011	20	7	8

² <http://www.cdc.gov/salmonella/outbreaks.html>

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How PSR Genetics was able to identify cattle that resist *Salmonella* & *E. coli* O157:H7

The approach to identifying these cattle was based on:

- (a) observations that, within groups of cattle infected with *Salmonella*, there is a subpopulation of animals that are not susceptible to *Salmonella* infection;
- (b) observations that certain cattle are less likely to harbor *E. coli* O157:H7 in their intestinal tracts;
- (c) a fundamental knowledge of the specific cattle proteins that are exploited by bacterial pathogens during the intestinal infection and/or colonization processes;
- (d) unique and novel perspectives on alternative proteins functions that can be identified genetically using a proprietary platform.

Scientists at PSR Genetics focused on small genetic differences, i.e. single nucleotide polymorphisms (SNPs), as the bases for identifying cattle that naturally resist *Salmonella* and *E. coli* O157:H7 infections. SNPs underlie many unique and desirable genetic traits, including the myostatin-inactivating SNP found in Piedmontese cattle and resistance to bovine spongiform encephalopathy (“mad cow disease”) identified in certain cattle breeds.

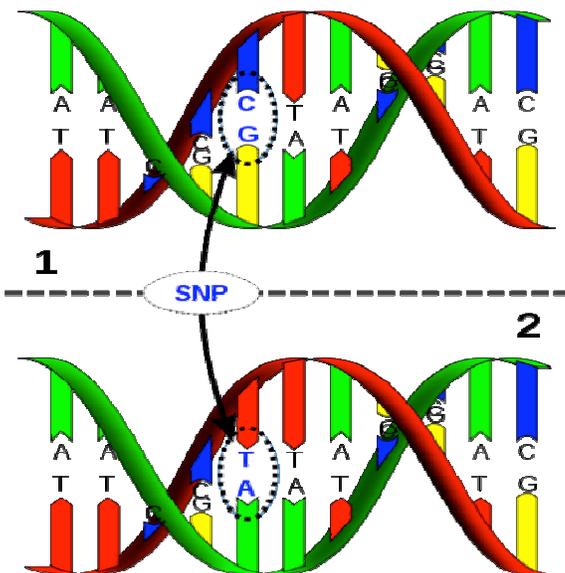
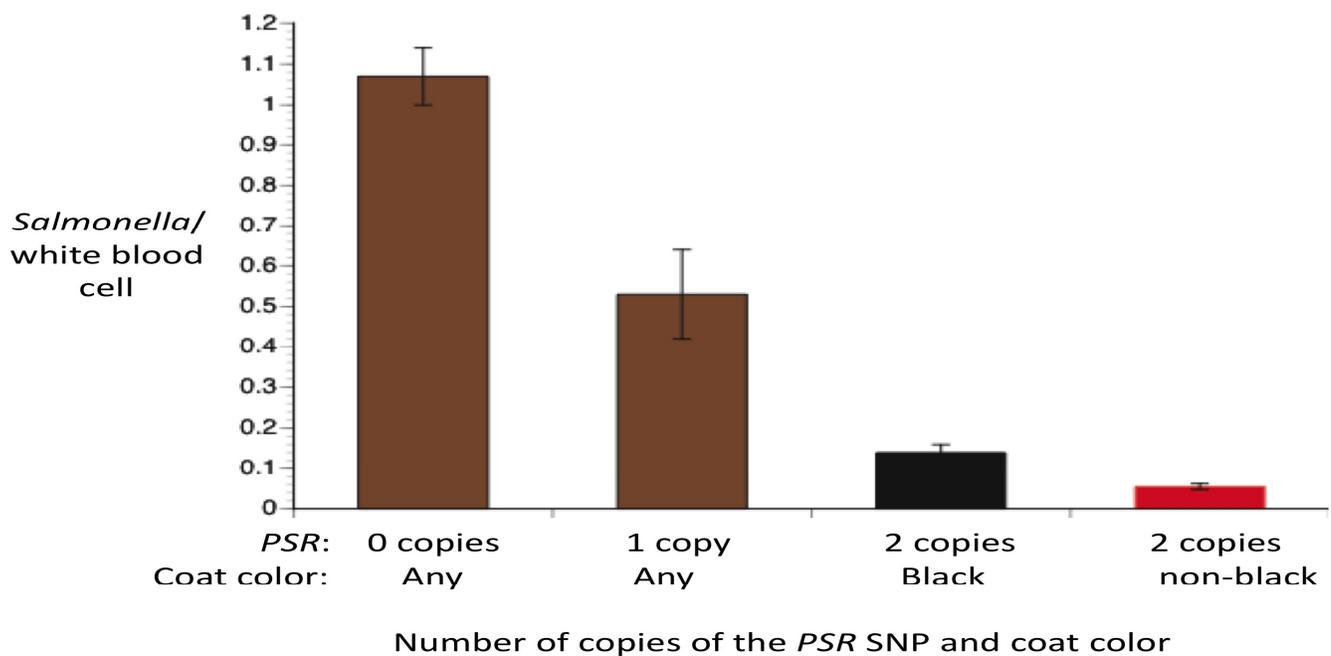


Illustration of a single nucleotide polymorphism.

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Using the proprietary platform, a search of the bovine genome identified a small number of candidate SNPs that could lead to *Salmonella* resistance. To investigate each SNP, blood was obtained from cattle and this blood was subjected to a laboratory test that determines the ability of *Salmonella* to infect cattle white blood cells. This test identified a SNP in the *PSR* gene as a marker of *Salmonella* resistance in cattle.

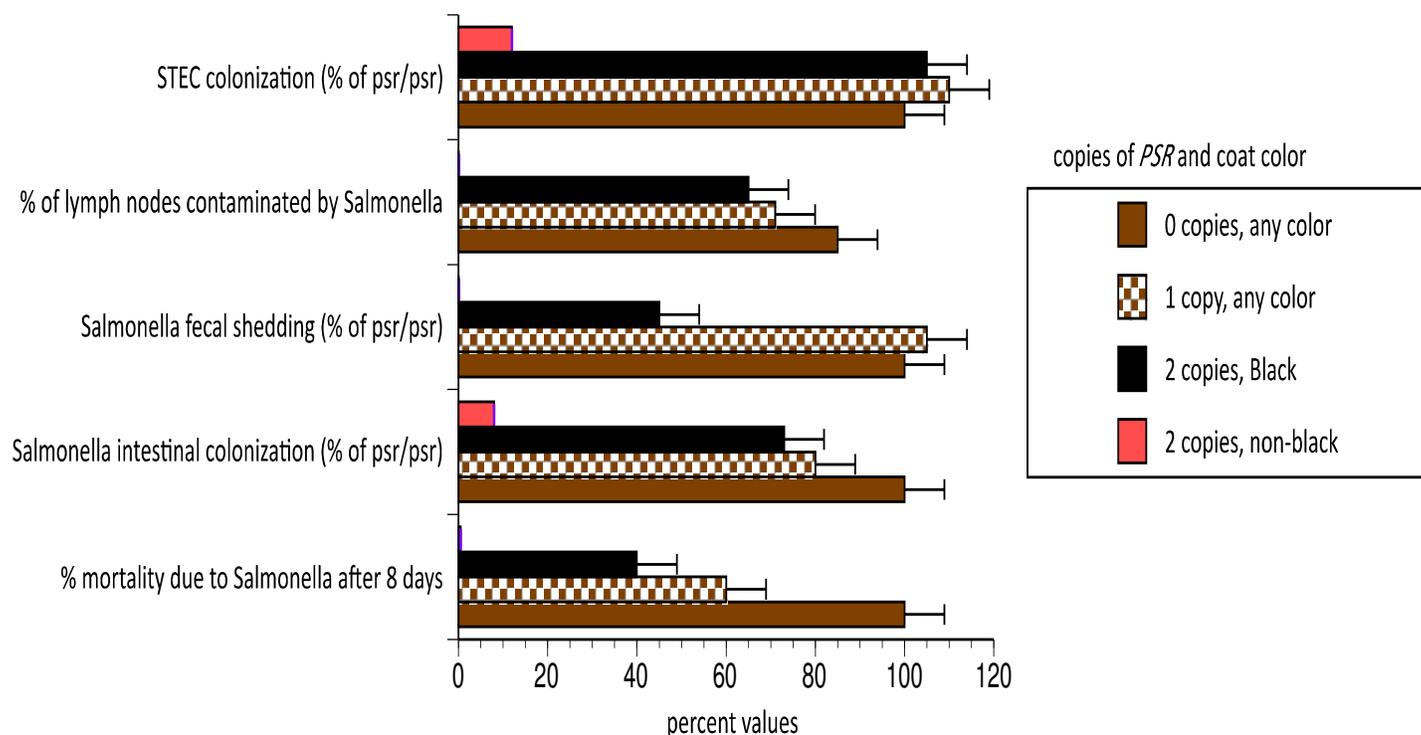
As shown the figure below, *Salmonella* resistance was observed in cattle with two copies of the *PSR* SNP (i.e. the *PSR/PSR* genetic profile). Interestingly, the greatest degree of *Salmonella* resistance was observed in non-black cattle bearing two copies of the *PSR* SNP. These data represent a sampling of the following breeds: Angus, Barzona, Braunvieh, Hereford, Holstein, Piedmontese, Pinzgauer, Simmental, Shorthorn, and Tarentaise.



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Subsequent studies (next figure) with live cattle revealed the following:

- (1) Non-black *PSR/PSR* cattle are resistant to doses of *Salmonella* that are lethal to other cattle.
- (2) Non-black *PSR/PSR* cattle are resistant to *Salmonella* colonizing their intestinal tracts.
- (3) Non-black *PSR/PSR* cattle do not shed *Salmonella* in their feces.
- (4) Non-black *PSR/PSR* cattle are resistant to lymph node infiltration by *Salmonella*.
- (5) Non-black *PSR/PSR* cattle harbor 90% fewer *E. coli* O157:H7 (STEC) than cattle of other genetic profiles.



Importance of non-black *PSR/PSR* cattle for the beef industry

The reduction of *E. coli* O157:H7 and other Shiga-toxin-producing *E. coli* (STECs) in cattle is of paramount importance to the industry. Studies by PSR Genetics, using live cattle, revealed that non-black *PSR/PSR* cattle harbor 90% fewer *E. coli* O157:H7 than cattle of other genetic profiles. It is also of note that laboratory tests revealed that non-black *PSR/PSR* cattle are significantly less likely to be contaminated with *E. coli* O145, another STEC that was recently implicated in a multi-state beef-associated outbreak in which one child died.

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While STECs have been the scourge of the beef industry for almost 20 years, an emerging trend indicates how important *Salmonella* is to the safety of beef. Recent studies from the Meat Animal Research Center (Clay Center, NE) indicate that up to 30% of cattle lymph nodes contain *Salmonella* (Brichta-Harhay, D., et al. 2012. J. Food Prot.). These lymph nodes are difficult to excise at slaughter and are located in regions that become part of the “grind”. Thus the lymph nodes are a reservoir for *Salmonella* microbes that end up in hamburger. This is underscored by recent hamburger-associated outbreaks of *Salmonella* in the Northeast, Canada, and at Taco Bell.

Because of these issues with *Salmonella* and lymph nodes, it is anticipated that the Food Safety and Inspection Service will soon begin mandatory testing of *Salmonella* in beef and this pathogen will be designated as an adulterant. Therefore it will be highly advantageous to own, raise, and sell non-black *PSR/PSR* cattle in order to curb this problem that the beef packers are not currently able to mitigate. This value-added genetic profile will be desired by beef packers, especially considering the additional benefit of the diminished presence of *E. coli* O157:H7 (and other STECs) in non-black *PSR/PSR* cattle. This trait will also be desired by feedlots since it appears that *PSR/PSR* cattle have superior performance characteristics and marbling attributes, as evidenced by our small ongoing study with *PSR/PSR* cattle in a non-black herd:

Genotype	Average marbling scores at slaughter (rank with in the herd)	Average daily gain (rank within the herd)	Average longevity (rank with the herd)	Average fertility (rank within the herd)
<i>PSR/PSR</i>	1st	1st	1st	1st
<i>PSR/psr</i>	2nd	2nd	2nd	2nd
<i>psr/psr</i>	3rd	3rd	3rd	3rd

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Other producer benefits of the natural genetic resistance of the *PSR/PSR* genetic profile in non-black cattle

- (1) This type of natural resistance does not require immunization or prior exposure, i.e., no additional handling is required to instill the resistance.
- (2) This type of natural resistance is not compromised when the animal is stressed, unlike immunity that can be compromised during shipping and co-mingling.
- (3) Non-black cattle have higher pain thresholds, indicating that they are less likely to go off-feed because of an illness or injury.

Summary of the importance of non-black *PSR/PSR* cattle

Given the food safety advantages of *PSR/PSR* genetic profile and the potential economic benefits of these healthier cattle, non-black *PSR/PSR* cattle have the potential to revolutionize the beef industry. Testing for the *PSR/PSR* genotype is the start of this revolution that will need to be initiated and supported by important groups such as your breed association.

Genotype and coat color	Resistance to lethal doses of <i>Salmonella</i>	Resistance to <i>Salmonella</i> shedding in feces	Resistance to <i>Salmonella</i> infiltration into lymph nodes	Resistance to STEC colonization
<i>PSR/PSR</i> , non-black	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>PSR/PSR</i> , black*	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>PSR/PSR</i> , black**	_____	_____	_____	_____
<i>PSR/psr</i> , any color	_____	_____	_____	_____
<i>psr/psr</i> , any color	_____	_____	_____	_____

* about 2% of the population tested

** about 98% of the population tested

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Identifying cattle that are *PSR/PSR*

For the past 18 months we have been trying to make available a genetic test for the *PSR/PSR* genotype. We are now at a crossroads for this endeavor, and need your assistance.

Our findings have implications for pharmaceutical and vaccine development. Thus we must chose between selling the information to a pharmaceutical company or making a genetic test available through Neogen/GeneSeek.

In order to make this test available and match the economic potential of selling the information to a pharmaceutical company, we are asking breed associations to consider becoming partners with PSR Genetics. Ultimately we would like to create a consortium of breed associations serving as stakeholders of PSR Genetics.

To initiate this process, it is prudent for each breed association to know the approximate frequency of the *PSR* SNP in their breed. Therefore, we are now offering a test in which the prevalence of the *PSR* SNP is determined in a large population of animals.

If interested, please print and fill out the enclosed form and submit a composite hair sample to the address on form. The composite sample can be from up to 100 animals. Please include a check for \$950 with your sample.

Additional Information about PSR Genetics

Ongoing research to identify *PSR/PSR* black cattle genotypes conferring resistance to *Salmonella* and STECs

As shown herein, we found *Salmonella* and *E. coli* O157:H7 resistance in a very small percentage of *PSR/PSR* black cattle. It thus appears that the maximal resistance to *Salmonella* and *E. coli* O157:H7 is due to a complementary genotype in *these animals*. PSR Genetics is currently seeking equity investors for this new line of research that will identify the complementary genotype. For more information about becoming an equity

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investor in this research, please contact the principle scientist responsible for this project. Dr. Steve Carlson can be reached at psrdairygenetics@yahoo.com or 515-460-1224.

Other research from PSR Genetics

PSR Genetics is currently developing an on-farm test that predicts which calves will succumb to bovine respiratory disease (BRD), the costliest beef cattle disease in the U.S. Calves will be tested as they enter the feedlot, and susceptible calves will be fed an inexpensive drug that prevents BRD. PSR Genetics is currently seeking equity investors for this new line of research. For more information about becoming an equity investor in this research, please contact the principle scientist responsible for this project. Dr. Steve Carlson can be reached at psrdairygenetics@yahoo.com or 515-460-1224.

Profiles of the senior personnel at PSR Genetics

Duane Ramsey is the CEO and founder of PSR Genetics. He has over 40 years experience as an agribusinessman in the cattle and swine industries. He owns banks in western Kansas and was a founder of US Premium Beef. He is on the Board of Directors for National Beef and also owns feedlots in Iowa.

Dr. Steve Carlson received his DVM from Iowa State University. After three years as a private practice large animal veterinarian, Dr. Carlson returned to school and received a PhD in Pharmacology. Following postdoctoral training in pathogenic bacteriology focusing on *Salmonella*, Dr. Carlson worked as a Veterinary Medical Officer with the USDA where he solved a major problem regarding antibiotic resistant *Salmonella* with enhanced disease-causing capabilities. He now is an Associate Professor at the College of Veterinary Medicine at Iowa State University. He has published over 40 papers and is considered the international expert on the virulence of multiple antibiotic resistant *Salmonella* in livestock. His involvement with PSR Genetics stems from numerous observations of cattle that naturally resist bacterial infections.

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Dr. Tim Day received a B.Sc. in Biology from Kansas State University, and a PhD in Pharmacology from Michigan State University. Dr. Day received postdoctoral training in Zoology prior to serving as an Assistant Professor at Michigan State University. Dr. Day has been on the faculty at Iowa State University for 12 years and currently is a Professor of Pharmacology at the College of Veterinary Medicine. He has published over 50 papers and is considered an international expert on schistosomiasis, a disease involving parasitic worms in tropical and subtropical regions. His involvement with PSR Genetics stems from extensive experiences with molecular determinants of infections and unique approaches that identify genetic-based alterations in protein functions. Uncovering these altered functioning proteins are at the core of identifying cattle that naturally resist bacterial infections.

Mark Nelson is a consultant who has worked in the beef industry for over 20 years. He has worked as a cattle buyer for several companies (including Cargill) and he has a network of contacts in the industry, including breed associations and branded beef companies.

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