USDA

United States Department of Agriculture

Food Safety and Inspection Service

Protecting Public Health and Preventing Foodborne Illness

FSIS Webinar on Reporting of Whole Genome Sequencing Results in Quarterly Establishment Information Letters

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Meryl Silverman, PhD
Glenn Tillman, PhD

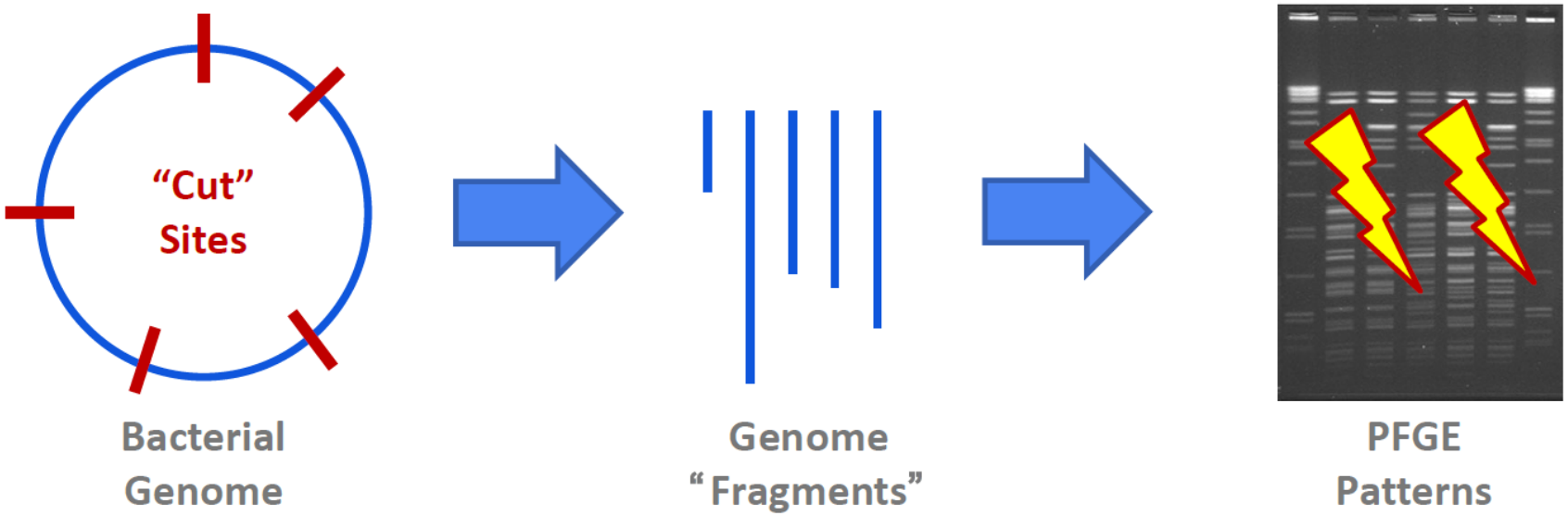
Outline

- Whole genome sequencing (WGS) or pulsed-field gel electrophoresis (PFGE)? What's the difference?
- Transition from PFGE to WGS for *Listeria monocytogenes* (*Lm*) analysis
- Current status of WGS characterization of *Lm* isolates
- Overview of Quarterly Establishment Letters
- Future plans for transitioning to WGS for other pathogens

What is PFGE?

- Laboratory technique that can be used to help characterize bacterial pathogens.
- Enzyme is used to cut DNA.
- Electrical field periodically changes direction (pulsed field) to separate DNA fragments on a gel matrix.
- A photo is taken of the pattern the DNA fragments make when separated.

PFGE Conceptual Framework

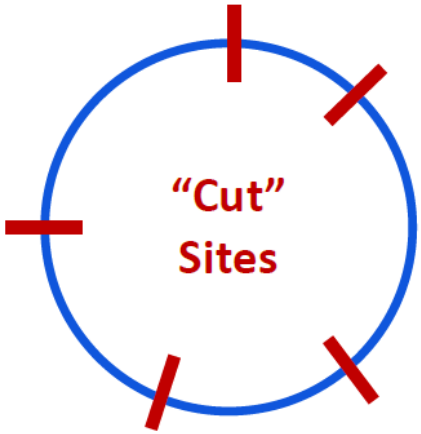


Source: CDC

What is WGS?

- DNA sequencing technology that can be used to help characterize bacterial pathogens.
- Advances in DNA sequencing technology have made WGS a powerful tool to characterize isolates with greater accuracy and detail than PFGE.

PFGE-WGS Comparison



PFGE only gives information at a "cut" site via the banding pattern

WGS has the ability to give us information at nearly every position in the genome

Source: CDC

What are the Benefits of WGS?

- WGS has a number of uses that benefit FSIS and its mission to protect public health.

- These uses include:
  - identifying harborage and cross-contamination of pathogens in FSIS-regulated facilities,
  - tracing human illness outbreak data to regulated food products, and
  - identifying unique genes related to virulence and pathogenicity, survival and adaptation, and resistance to biocides (sanitizers, metal, etc.) and antimicrobials.

Transition from PFGE to WGS

- FSIS began performing WGS in parallel with PFGE for *Lm* starting in FY13 and for all pathogens starting in early FY16.
- Centers for Disease Control and Prevention (CDC) PulseNet partners are transitioning away from using PFGE as the primary molecular characterization tool toward using WGS.
- In coordination with CDC PulseNet, FSIS suspended PFGE for *Lm* and as of January 15, 2018, now generates *Lm* characterization through WGS only.

How is WGS data analyzed?

- FSIS uses different tools to analyze WGS information including:
  - Multi-locus Sequence Typing (MLST) - resulting in
    - Public Sequence Type and
    - Allele Code
  - High-quality Single Nucleotide Polymorphisms (hqSNP)

Multi-locus Sequence Typing (MLST)

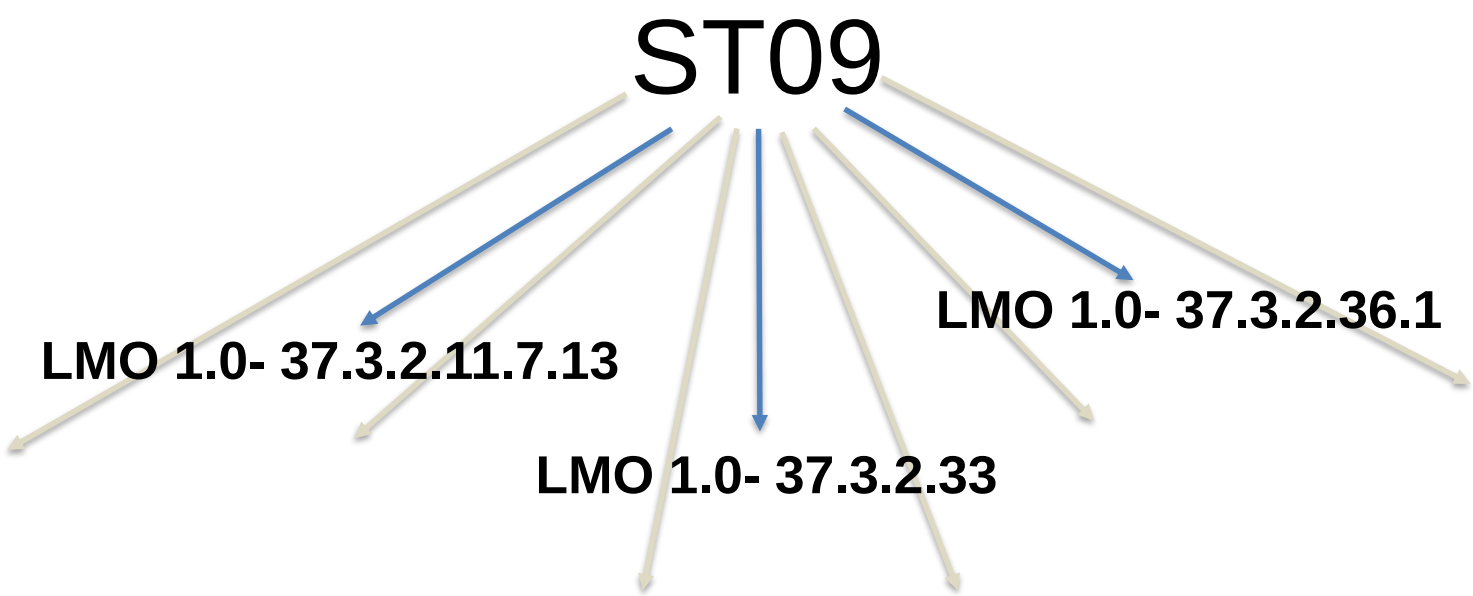
MLST can generate a **pattern name or designation** (similar to a PFGE pattern name) based on differences in a pre-defined set of genes.

MLST Results will be Provided by FSIS as Follows:

- **Public Sequence Type** (“MLST ST”, “ST”, or “pubST”)
  - small number of genes (i.e., 6-12)
  - named using the publicly available database developed by Jolley & Maiden (2010) (e.g., publicST09)
- **Allele Code**
  - compares over 1,800 genes
  - named by using CDC PulseNet numerical code (e.g., LMO 1.0-5.1.1.2.5.1)

Jolley K. A., & Maiden M. C. (2010). BMC Bioinformatics. 11: 595.

MLST: pubST & Allele Codes Example



Allele Code is more specific than public Sequence Type; one public Sequence Type can be inclusive of many Allele Codes.

Reading Allele Codes

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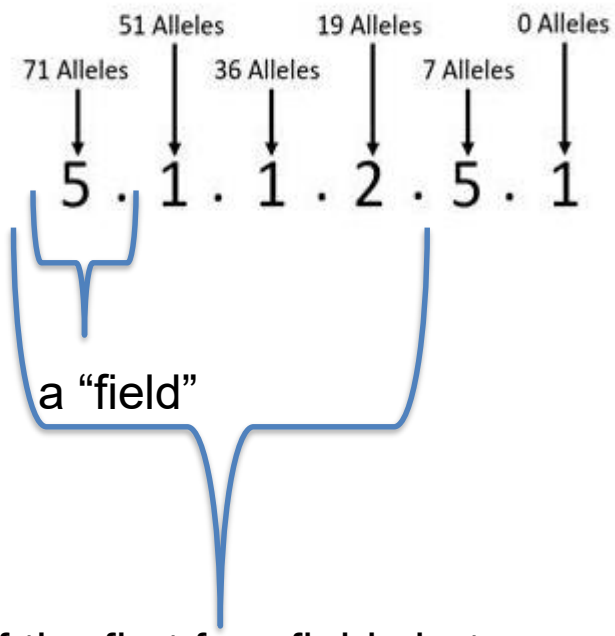
Example: LMO 1.0-5.1.1.2.5.1

LMO – *L. monocytogenes*
Version 1.0



If the first four fields between two isolates match, the isolates may be closely related

Source: CDC

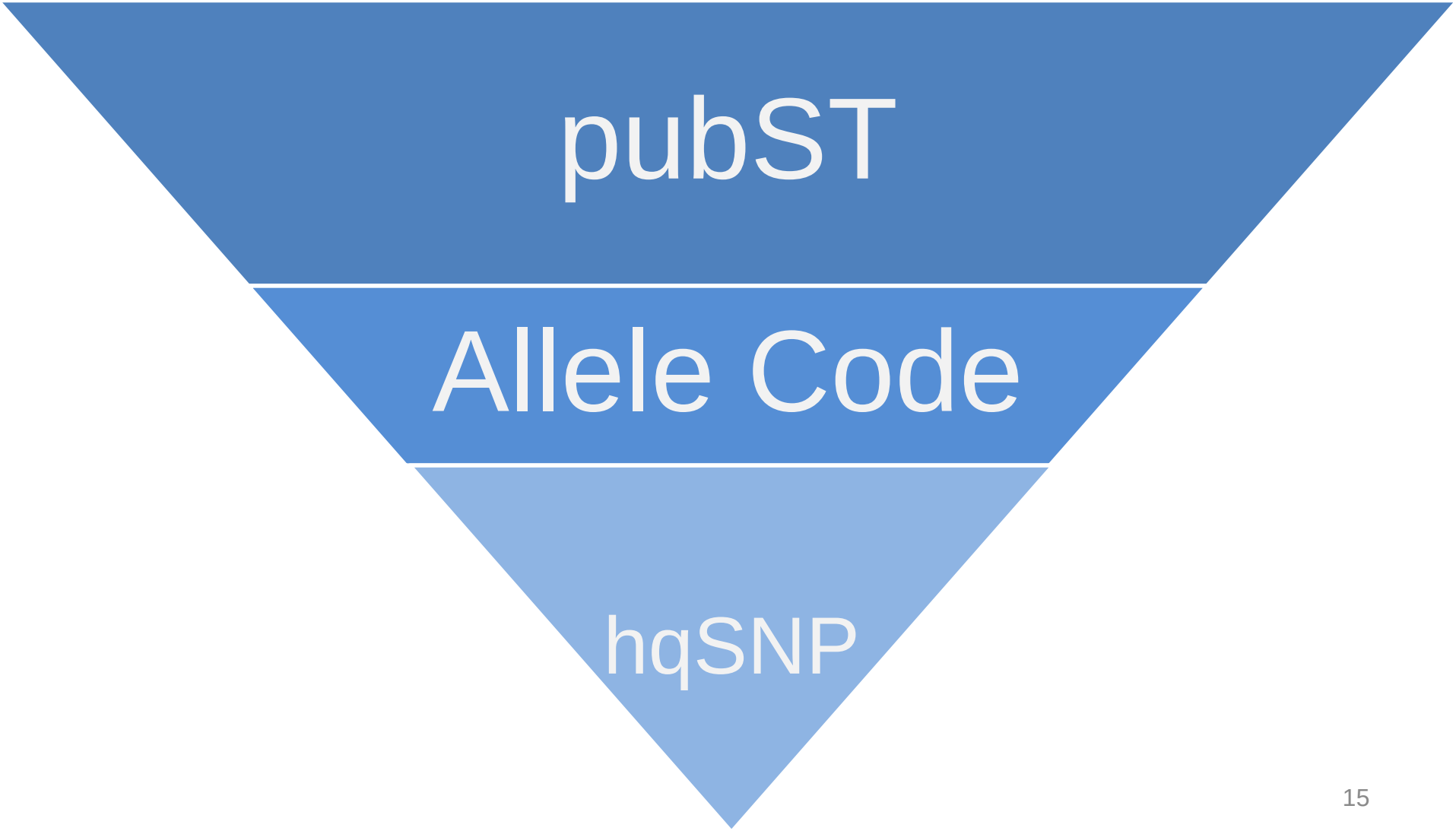
What's a SNP?

- Single Nucleotide Polymorphism (SNP)

ATGTT**C**CTC isolate A
ATGTT**G**CTC isolate B

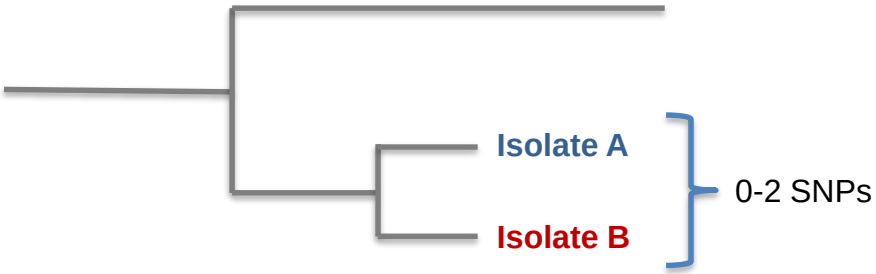
This is a single SNP difference!

WGS tools: resolution



Harborage Example

- Isolate A:
  - ST09
  - Allele code: LMO1.0-1.2.3.4.5.6
  - 2 SNP difference to nearest isolate (Isolate B)
- Isolate B:
  - ST09
  - Allele code: LMO1.0-1.2.3.4.5.6
  - 2 SNP difference to nearest isolate (Isolate A)



Epidemiological link: Isolate A and Isolate B were collected from Establishment A during two different RTE collection events 2 years apart.

Conclusion: Consistent with harborage or reintroduction

Overview of Quarterly Establishment Letters

- FSIS began nationwide implementation of Quarterly Establishment Information Letters in August, 2017.

- Letters include
  - All sampling results for an establishment and
  - Industry averages.

Quarterly Establishment Letters – *Lm* Results

- With the transition to WGS, FSIS plans to include the MLST designation (Allele Code) in place of the PFGE pattern name for *Lm* results starting with the next quarterly letters set to issue by September 30th.

- FSIS plans to include the MLST designation for all isolates reported in the 12-month window in the letters. This includes isolates where FSIS may have reported the PFGE pattern name in a previous quarterly letter.

Quarterly Establishment Letters – Harborage

- **Harborage or repeated introduction** is indicated when:
  1. two or more isolates from any RTE sampling project,
  2. collected over multiple days, weeks, months, or years,
  3. have at least the first four fields of the MLST Allele Code designation match

Form ID	Collection Date	Project	Product	Analysis	MLST Designation/ (#Recurrence)*	Consistent with Harborage†	Consistent with Cross-Contamination†
100000010	05/10/18	RLMCONT	RLm Food Contact Surface	Listeria monocytogenes	LMO1.0-1.2.3.4.5.6 (1)	Yes	No
100000000	09/04/17	RTEPROD _RAND	RTE Product Sample	Listeria monocytogenes	LMO1.0-1.2.3.4.6.7 (1)	No	No

*MLST designations characterizing the isolate (number of recurrences in establishment samples with available designations over the past five years). Note: Two or more isolates with MLST designations where at least the first four fields after the "-" match may be closely related; however, further analysis by the OPHS Laboratories is needed to determine the degree of relatedness and whether results are consistent with harborage and cross-contamination.

Quarterly Establishment Letters – Cross-contamination

- **Cross-contamination** is indicated when**:**
  1. two or more isolates from any RTE sampling project,
  2. collected during the same sampling event,
  3. have at least the first four fields of the MLST Allele Code designation match

Form ID	Collection Date	Project	Product	Analysis	MLST Designation/ (#Recurrence)*	Consistent with Harborage†	Consistent with Cross-Contamination†
100000020	06/10/18	RLMCONT	RLm Food Contact Surface	Listeria monocytogenes	LMO1.0-1.2.3.4.5.6 (1)	No	Yes
100000030	06/10/18	RLMCONT	RLm Food Contact Surface	Listeria monocytogenes	LMO1.0-1.2.3.4.6.7 (1)	No	Yes

*MLST designations characterizing the isolate (number of recurrences in establishment samples with available designations over the past five years). Note: Two or more isolates with MLST designations where at least the first four fields after the "-" match may be closely related; however, further analysis by the OPHS Laboratories is needed to determine the degree of relatedness and whether results are consistent with harborage and cross-contamination.

Future Plans for Transitioning to WGS for Other Pathogens

- Although FSIS has transitioned away from PFGE to using only WGS for characterizing *Lm*, PFGE analysis, in addition to WGS will continue to be performed for other pathogens, consistent with CDC PulseNet.

- FSIS anticipates that in FY2019, in continued coordination with CDC PulseNet, it will replace PFGE with WGS as the primary subtyping tool for *Campylobacter*, Shiga toxin-producing *Escherichia coli* (STEC), and *Salmonella*.

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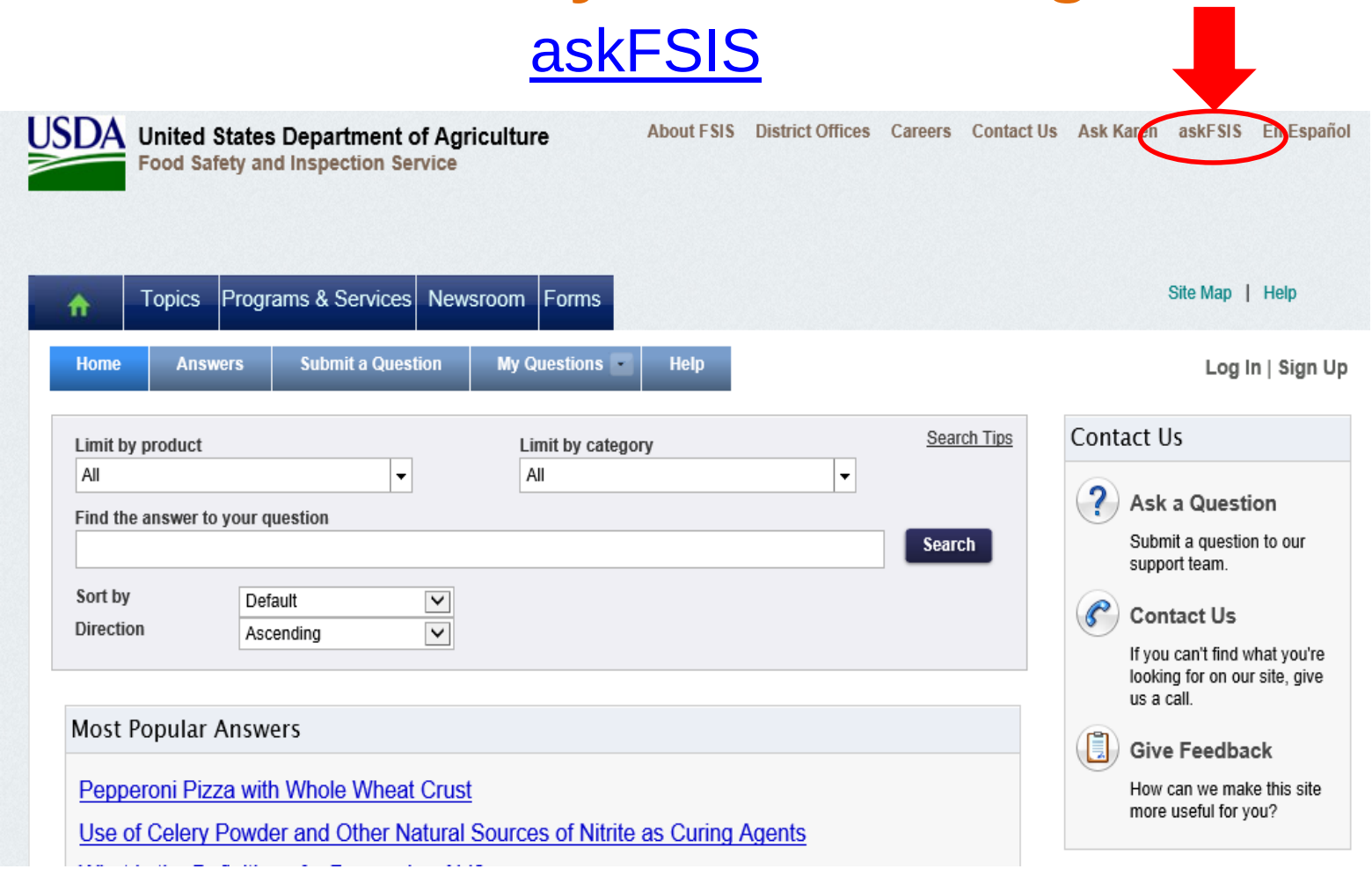
Can't find what you are looking for?

askFSIS