A. Program Description

Program Overview, Objectives, and Priorities
The Borlaug International Agricultural Science and Technology Fellowship Program (Borlaug Fellowship Program) advances USDA’s agricultural research goals of promoting collaborative programs among agricultural professionals of eligible countries, agricultural professionals of the United States, the international agricultural research system, and United States entities conducting research by providing fellowships to individuals from eligible countries who specialize or have experience in agricultural education, research, extension, or other related fields. Fellowships promote food security and economic growth in eligible countries by educating a new generation of agricultural scientists, increasing scientific knowledge and collaborative research to improve agricultural productivity, and extending that knowledge to users and intermediaries in the marketplace. The collaborative nature of the training and research programs not only benefits the Fellow, his or her home institution, and partner country; the U.S. host institution, its professors, researchers, and students; and the global agricultural sector by improving agricultural productivity, systems, and processes in partnering nations through the transfer of new science and agricultural technologies.

USDA will identify Borlaug Fellows based on country-specific topics of importance to international, agricultural trade. USDA then places Fellows with U.S. research institutions for 10-12 week, intensive programs. These programs are expected to contribute to the strategic goals and objectives of the fellow and those institutions through a hands-on experience in a “real-world” agricultural research scenario, providing opportunity for application of research agendas where they can have a direct impact on food security and economic growth in an emerging economy. It is hoped that host institutions will share the knowledge gained through the program in their classroom and extension work with their faculty, students, extension officers, and constituents; and that they will continue to maintain professional contacts with the fellows after their departure from the United States.

Borlaug fellows may be identified for the topic listed below:

(A) Food safety

PLACE OF PERFORMANCE

- The applicant is expected to host fellows at a research facility on their campus in the United States.
- The mentor is expected to make a reciprocal visit of up to two weeks to the fellow’s home institution, which may be in a developing country.
EXPECTATIONS:

(1) Assignment of a Principal Investigator (Training Coordinator)
The host institution will designate a contact person as the Principal Investigator (PI) responsible for coordinating all administrative and programmatic arrangements.

(2) Assignment of a Mentor
A key component of the program is matching the Fellow with a mentor. The host institution will select an appropriate mentor for one-on-one work with the Fellow for the duration of the program.

- The mentor will establish a professional relationship, providing guidance and training in the Fellow’s research and studies.
- The mentor will work with the Fellow before arrival to discuss appropriate work plan, site visits, and other arrangements. A work plan should be agreed upon and finalized no later than 2 weeks after the program start date.
- The mentor will provide draft of work plan through the PI to USDA/FAS for consultation and approval approximately 2 weeks before the commencement of the program.
- The mentor agrees to commit a significant amount of time each week for one-on-one work with the Fellow during the program.
- The mentor will continue communicating with the Fellow beyond the end of the program in the U.S. through the mentor visit.
- Mentor will submit quarterly progress reports that indicate all program activities conducted (form SF-PPR).
- The mentor may assign other faculty members to assist with Fellow’s training and research activities.
- Mentor may not be assigned to multiple Fellows during the same time frame.

(3) Mentor Follow-up Visit
- The mentor visit is a required component of the Borlaug Fellowship Program.
- The mentor will work with the Fellow to plan a follow-up visit to the Fellow’s home country. The trip should occur within 6 months to 1 year after the program ends.
- The PI should provide USDA/FAS with an agenda for mentor’s travel, including goals and objectives. The mentor’s travel information must be provided for emergency contact purposes and country clearance (if required by the cognizant FAS Overseas Office).
- The mentor will provide a trip report highlighting the trip’s activities and results through the PI to USDA/FAS within 30 days after the visit.
- The mentor should plan to meet with the USDA/FAS Attaché or staff from the U.S. Embassy while they are traveling, if feasible. USDA/FAS can assist with coordination prior to the trip.

(4) Visa
- USDA/FAS will provide a DS-2019 for the Fellow to request and obtain a J-1 Visa. USDA/FAS will provide instructions to the Fellow regarding the application process, the amount of lead-time needed, and any paperwork required. The visa start and end date
will be coordinated with the host institution who will be responsible for purchasing round trip plane tickets for the fellow to come to the U.S. for his or her program.

- Fellows, including that already in possession of another valid U.S. visa, must still obtain a J-1 visa to participate in the program. Fellows will be refused entry if they arrive in the United States without the appropriate category of visa.

(5) **Travel and Transportation**
- The host institution must comply with the Federal Travel Regulations (41 CFR 300 et seq.).
- The host institution will provide round trip, economy class, international airfare from the Fellow’s home to the university.
- The host institution is responsible for arranging and purchasing all domestic travel related to the Fellow’s training program.
- The host institution will provide housing for the Fellow for the duration of the training program, taking into account gender and cultural norms.
- The host institution will pay lodging fees directly. The host institution will not require the Fellow to pay for his or her lodging expenses, whether through reimbursement or advance payment.
- Lodging will include a private bedroom, private or shared bathroom, access to a laundry room, and access to a kitchen with pots, pans, and utensils.
- Basic necessities, such as sheets, towels, and cleaning supplies (if not already provided), will be provided for Fellow’s use. The Fellow should not have to pay for these items.
- Lodging will be within walking distance to the campus/training location or easily accessible by public transportation.
- If public transportation is required to access campus/training location, the host institution will provide the Fellow with a bus pass or proper allowance for transportation expenses.
- When planning lodging options, the host institution should check with the Fellow and account for any special dietary restrictions or preferences.

(6) **Meals and Incidentals (M&IE)**
- The host institution will provide each Fellow with meal and living allowances for the duration of stay.
- Daily M&IE allowance may not exceed current GSA per diem rates.
- The host institution can determine the frequency of per diem allotments, but the Fellow must receive per diem within the first week of the Fellowship. The PI must inform the Fellow and USDA/FAS immediately if this cannot be accommodated.

(7) **Emergency Health Insurance**
- The host institution will purchase emergency health insurance for the Fellow for the duration of stay, as required for all J-1 Visa holders (22 CFR 62.14).
- The Fellow will not be required to purchase his or her health insurance and then be reimbursed.
• The host institution will educate the Fellow as to what is covered under health insurance policy, especially highlighting that pre-existing medical conditions are not covered.
• The host institution will alert USDA/FAS staff if any health/medical conditions arise during the Fellowship.

(8) Communication
• The host institution will initiate contact with the Fellow as soon as possible.
• The host institution will develop the training program in consultation with USDA/FAS and the Fellow.
• The host institution will keep USDA/FAS informed regarding any logistical or program planning.
• The host institution will notify USDA/FAS immediately upon Fellow’s physical arrival and departure from the U.S. to comply with U.S. Department of Homeland Security requirements.
• The host institution will provide USDA/FAS with the Fellow’s temporary U.S. address and phone number, and emergency contact numbers for the PI, mentor, or other appropriate institution personnel. This information is required so that Fellow can be reached in the event of an emergency.

(9) Fellowship Program
• The host institution will provide educational materials and supplies to each Fellow necessary for their full participation in the fellowship.
• The host institution will pay for all fees related to the Fellow’s training program, such as (but not limited to) technology fees, administrative fees, laboratory fees, etc.
• The host institution will arrange relevant field visits as applicable to the Fellow’s training program.
• The host institution will ensure the Fellow submits an interim and final report (2-3 pages each) to USDA/FAS before the Fellow leaves the United States.

(10) Orientation
• The PI/Training Coordinator will communicate directly with the Fellow at least 4-8 weeks before his or her arrival in the U.S. to ensure that all pertinent information is provided, including:
  o Name and contact information of PI/Training Coordinator
  o Name and contact information of mentor
  o Institution information, weather information, and clothing needs
  o Housing and M&IE allowance
  o Program plan and anticipated site visits
  o Professional development expectations
  o Reminder to bring any necessary prescription medications
  o Explain what is and is not covered under emergency health insurance policy (e.g. no pre-existing conditions, no dental, etc.)
• Institution will provide an orientation upon the Fellow’s arrival to acquaint them with campus and community resources, such as:
  o Explanation and demonstration of local bus/transportation options
  o Explanation of cultural and legal expectations

• USDA will provide a welcome and orientation packet for mentors

Issued By
Foreign Agricultural Service, Office of Capacity Building & Development, Trade & Scientific Exchanges Division, Scientific Exchanges Branch

Catalog of Federal Domestic Assistance (CFDA) Number and Title
10.777
Norman E. Borlaug International Science and Technology Fellowship Program

Notice of Funding Opportunity Title
Borlaug Fellowship Program

NOFO Number
USDA-FAS-10777-0700-10.-18-0054; Fellow 1 Mexico
USDA-FAS-10777-0700-10.-18-0055; Fellow 2 Mexico
USDA-FAS-10777-0700-10.-18-0056; Fellow 3 Mexico
USDA-FAS-10777-0700-10.-18-0057; Fellow 4 Mexico
USDA-FAS-10777-0700-10.-18-0058; Fellow 5 Mexico

Authorizing Authority for Program
The legislative authority for the Borlaug Fellowship Program is provided in Sec. 7139 of the Food, Conservation, and Energy Act of 2008 (PL 110-234), as incorporated in to the National Agricultural Research, Extension, and Teaching Policy Act of 1977, as amended.

Appropriation Authority for Program
Consolidated Appropriations Act, 2017 (PL 115-31)

Program Type
New

B. Federal Award Information
   Award Amounts, Important Dates, and Extensions

Available Funding for the NOFO: Each award (for one fellow) is up to $50,000.

Projected number of Awards: 5

Number of Project Budget Periods: 1
Projected First Budget Period:  N/A

Projected Period of Performance Start Date(s):  Subject to the availability of implementer and Fellows.

Projected Period of Performance End Date(s):  18 months after the start date

Extensions are allowable, please see Section H. Additional Information to see how to requests one should the need arise.

Pre-Award costs:  Not Allowable

Cost Share or Match requirements:  A cost match or cost share is not required.

Funding Instrument
USDA will enter into a cost reimbursable agreement under 7 USC § 3319a with selected universities.

C.  Eligibility Information

Eligible Applicants
Proposals may be received from U.S. State Cooperative Institutions or other colleges and universities, including minority serving institutions (MSIs).

A single mentor may not host two fellows simultaneously. Both the PI and mentor must hold positions at an eligible U.S. institution.

Eligibility Criteria
All applicants must have an active registration in the SAM database at www.sam.gov – pending or expired registrants are not eligible. This requirement must be met by the closing date of the announcement and will not be waived. Please contact the program officer listed if you have questions about this requirement.

In addition to obtaining a DUNS number and registering in SAM, you must also obtain Level 2 eAuthentication to apply for this funding opportunity in ezFedGrants (eFG). You must submit an online form requesting access. Normally you will receive an email within 24 hours of your submission, if your request is approved. After this occurs, you will need to schedule an appointment with an LRA. Once you meet with the LRA, your Level 2 eAuthentication should be granted within 2 to 3 days after that meeting. See Section D of this NOFO for detailed information.

Maintenance of Effort (MOE)
MOE is not allowable.
D. **Application and Submission Information**

### Key Dates and Times

- **Application Start Date:** 06/06/2018
- **ezFedGrants Posting Date:** 07/06/2018
- **Application Submission Deadline:** 07/06/2018 at 11:59PM EST
- **Anticipated Funding Selection Date:** Approximately 2-3 weeks after the submission deadline, subject to the availability of funding
- **Anticipated Award Date:** Approximately 2-3 weeks after selection, subject to the availability of funding

### Address to Request Application Package

This NOFO represents the full application information.

Applications will be processed through the ezFedGrants portal at [https://grants.fms.usda.gov](https://grants.fms.usda.gov) – prospective applicants are encouraged to register for this portal. Applicants that are unable to access the ezFedGrants portal should contact the program manager for alternative submission instructions. Note that if selected, registration is a requirement of performance.

### Content and Form of Application Submission

Institutions must be able to host multiple groups over the period of performance and should submit a proposal following the guidelines below:

- Required forms and certifications, including:
  - SF-424 version 2.1, with an OMB Expiration Date of 10/31/2019
  - SF-424A version 1.0, revised July 1997. This should be accompanied by a detailed budget worksheet and a detailed budget narrative (NOTE: A budget narrative must be provided). All line items should be described in sufficient detail that would enable FAS to determine that the costs are reasonable and allowable for the project per federal regulations. An example budget narrative is included in the appendix, but is not required.
  - AD-3030, revised February 2016
  - AD-3031, revised February 2016

- Indicate the name of the institution applying to host the Fellows.
- Indicate the country, research interest, and reference number.
- Identify a Primary Investigator.
• Identify a Mentor. A Mentor may not be assigned to multiple Fellows who are in the U.S. at the same time.

• Provide a tentative research plan based on the Fellow’s research proposal and action plan, including topics covered, field visits, and other activities.

• Include a narrative description of the proposed fellowship, how it will be administered, and the role of the university faculty and support staff.

• Provide a summary of relevant institutional capabilities for hosting international scientists and policymakers in the proposed field.

• Briefly describe the research expertise and international experience of the mentor in the Fellow’s field of interest.

• Provide a one to two page curriculum vitae for the mentor and other collaborating researchers involved in the proposed program.

• Identify the expected skills or knowledge to be acquired by the Fellow at the end of the program.

• If attending the World Food Prize, the budget should include time and funding for the Fellow and Mentor to attend. An adjustment to the Fellow’s M&IE must be made for the time spent in Iowa.

The SF-424 and SF-424 A can be completed within the ezFedGrants platform. However, the other required forms must be downloaded from the Forms sections on Grants.gov. The Certification regarding Lobbying and the Grants and Agreement Coversheet will be sent to you along with this NOFO.

**Unique Entity Identifier and System for Award Management (SAM)**

The link below provides information on 2 CFR §25.110. Please read.

https://www.ecfr.gov/cgi-bin/retrieveECFR?gp=1&SID=7a45f973880240465cd255471f1380ef&ty=HTML&h=L&mc=true&n=pt2.1.25&r=PART

FAS is using ezFedGrants to post NOFO’s and issue agreements, which is an electronic grants management system. Applicant(s) with electronic access are to submit their applications electronically through:

https://grants.fms.usda.gov

Before you can apply, you must have a DUNS number, be registered in SAM, and have access to the ezFedGrants website.

**Applicants are encouraged to register early. Due to recent changes in the SAM platform, the registration process can take 6-8 weeks to be completed. Therefore, registration should be done in sufficient time to ensure it does not impact your ability to meet required submission deadlines.**
**DUNS number.** Instructions for obtaining a DUNS number can be found at the following website: [http://www.dnb.com/duns-number.html](http://www.dnb.com/duns-number.html)

The DUNS number must be included in the data entry field labeled "Organizational DUNS" on the Standard Forms (SF)-424 forms submitted as part of this application.

**System for Award Management.** In addition to having a DUNS number, applicants applying electronically through ezFedGrants must register with SAM. Step-by-step instructions for registering with SAM can be found here: [www.sam.gov](http://www.sam.gov)

Failure to register with SAM will result in your application being rejected during the submissions process.

**ezFedGrants System Access and Electronic Signature**

**Level 2 eAuthentication.** The next step in the registration process is to obtain a Level 2 eAuthentication account that will allow access to the ezFedGrants system. Instructions for getting a Level 2 eAuthentication account can be obtained by emailing [ezFedGrants@cfo.usda.gov](mailto:ezFedGrants@cfo.usda.gov).

You may also request Level 2 eAuthentication online at: [https://www.eauth.usda.gov/MainPages/index.aspx](https://www.eauth.usda.gov/MainPages/index.aspx)

If you experience any issues with self-registration or have eAuthentication-related questions, please contact the eAuthenticationHelpDesk for assistance: By email to [eAuthHelpDesk@ftc.usda.gov](mailto:eAuthHelpDesk@ftc.usda.gov)

**Requesting a role in ezFedGrants.**

After obtaining eAuthentication, users will need a role in the system. Descriptions of the roles available and instructions on how to request a role can be obtained by emailing [ezFedGrants@cfo.usda.gov](mailto:ezFedGrants@cfo.usda.gov)

You may also go into the link below for instructions on requesting eFG access. The document is called “External Portal Access Request Submission”.

[https://www2.nfc.usda.gov/FSS/Training/Online/ezFedGrants/access_user_roles.hp](https://www2.nfc.usda.gov/FSS/Training/Online/ezFedGrants/access_user_roles.hp)

**Electronic Signature.** Applications submitted through ezFedGrants constitute a submission as electronically signed applications. When you submit the application through ezFedGrants, the name of your Signatory Official on file will be inserted into the signature line of the application.

If you experience difficulties accessing information or have any questions please email the Helpdesk at [ezFedGrants@cfo.usda.gov](mailto:ezFedGrants@cfo.usda.gov).
The Federal awarding agency may not make a Federal award to an applicant until the applicant has complied with all applicable DUNS and SAM requirements and, if an applicant has not fully complied with the requirements by the time the Federal awarding agency is ready to make a Federal award, the Federal awarding agency may determine that the applicant is not qualified to receive a Federal award and use that determination as a basis for making a Federal award to another applicant.

**Intergovernmental Review**

This program is not subject to E.O. 12372.

**Funding Restrictions**

This will be a cost reimbursable agreement issued under 7 USC § 3319a. University indirect costs for cost reimbursable agreements are limited to 10% of modified total direct costs (MTDC).

**Allowable Costs:**

1. **Salaries and Fringe Benefits:**
   Requested funds may be allocated toward salaries, fringe benefits, or the combination thereof. No more than 20% of the requested funds may be allocated toward salaries, consultant fees, fringe benefits, or the combination thereof. Only individuals that hold positions at eligible U.S. institutions should be listed in this category.

2. **Travel:**
   For domestic travel, provide the purpose of the travel and information used in calculating the estimated cost, such as the destination, number of travelers, and estimated cost per trip. There are several restrictions associated with traveling on federal funds. In most cases, airfare must be purchased in economy class from a U.S. carrier. Travelers must also adhere to federally mandated domestic per diem guidelines. Additional information may be found in the circulars listed in the “Legislative Authority” section of this announcement.

3. **Supplies:**
   All personal property excluding equipment, intangible property, and debt instruments as defined in this section.

4. **Other Direct Costs:**
   Other Direct Costs are those anticipated charges not included in other budget categories, including materials and supplies, lab fees, publication costs, reasonable consultant fees, computer services, sub-awards (the level of detail required for the sub-award budget is the same as the recipient organization), equipment rental, facility rental, conferences and meetings, speaker fees, honorariums.

5. **Indirect Costs:**
   Indirect Costs may not exceed 10% of direct costs (7 USC 3319a).
6. Tax Withholding:
Borlaug Fellows (as trainees, not students) are considered EXEMPT INDIVIDUALS under the IRS Substantial Presence Test for tax purposes. The exemption falls under one or both of the following categories: either the Foreign Government-Related Individuals standard or the Closer Connection Exception. Tax treaties might also exist between the U.S. and the Fellow’s home country. The only requirement is to complete IRS Form 8843 (Sections 1 and 2). No taxes should be withheld from Borlaug Fellows since they are exempt.

Unallowable Costs:

General purpose equipment (no particular scientific, technical, or programmatic purpose) and scientific equipment exceeding $5,000 or more; entertainment; any stipend or remuneration for the fellow, other than ordinary allowances for meals and supplies; capital improvements; thank you gifts, and other expenses not directly related to the project are not allowed. “Please note, Borlaug Fellows (as trainees, not students) are considered EXEMPT INDIVIDUALS under the IRS Substantial Presence Test for tax purposes. The exemption falls under one or both of the following categories: either the Foreign Government-Related Individuals standard or the Closer Connection Exception. The only requirement is to complete IRS Form 8843 (Sections 1 and 2). These funds are for federal financial assistance; as such no taxes should be withheld from Borlaug Fellows since they are exempt.”

Management and Administration (M&A) Costs:
M&A costs are not allowable.

Indirect Facilities & Administrative (F&A) Costs.
By statute, indirect costs for cost reimbursable agreements cannot exceed 10% of direct costs.

Other Submission Requirements
All applications must be submitted electronically as indicated above.

E. Application Review Information
Application Evaluation Criteria

Prior to making a Federal award, the Federal awarding agency is required by 31 U.S.C. 3321 and 41 U.S.C. 2313 to review information available through any OMB-designated repositories of government-wide eligibility qualification or financial integrity information. Therefore application evaluation criteria may include the following risk based considerations of the applicant: (1) financial stability; (2) quality of management systems and ability to meet management standards; (3) history of performance in managing federal award; (4) reports and findings from audits; and (5) ability to effectively implement statutory, regulatory, or other requirements.
**Technical Expertise and Experience (40 points)**

Mentor must have appropriate technical background to provide the desired, advanced training. If necessary, other appropriate collaborating scientists should be identified to meet any of the objectives which the mentor cannot address. Mentor’s experience and knowledge of relevant agricultural conditions within the Fellow’s country or a similar location will be considered as appropriate. The trainer’s experience with international training and adult-education will also be considered.

**Overall Program (35 points)**

The overall program plan and design should be relevant to the Fellow’s objectives background. The program plan should be thorough, and it should help achieve the desired post-program deliverables and the Fellow’s research goals and objectives. Relevant agricultural practices within the region of the university will be considered as appropriate. Relevant university resources should be identified. Additional resources/organizations should be identified as appropriate. Site visits and meetings should be meaningful to the content of the program, if included.

**Budget (25 points)**

The proposed budget should be appropriate for the number of Fellows and length of the program. The budget should include appropriate cost savings where available and narrative should accompany each line item. Host is strongly encouraged to use the Budget Worksheet provided in this NOFO.

**Review and Selection Process**

In all cases, the Program Manager will ensure application is submitted on time as specified in this announcement. Also, the Program Manager will ensure the organization is capable of delivering the program/activities as described in the announcement based on the applicant’s project narrative.

Qualified applications will be referred to a panel of 2-3 program staff and/or technical experts, and adjudicated among the criteria described above. In general, the highest-rated proposal will be selected, however, FAS may occasionally select out of score order for policy reasons, such as geographic distribution, incorporation of minority-serving institutions, past experience, etc.

**Confidentiality and Conflict of Interest**

Technical and cost proposals submitted under this funding opportunity will be protected from unauthorized disclosure in accordance with applicable laws and regulations. FAS may use one or more support contractors in the logistical processing of proposals. However, funding recommendations and final award decisions are solely the responsibility of FAS personnel.

FAS screens all technical reviewers for potential conflicts of interest. To determine possible conflicts of interest, FAS requires potential reviewers to complete and sign conflicts of interest and nondisclosure forms. FAS will keep the names of submitting institutions and individuals as
well as the substance of the applications confidential except to reviewers and FAS staff involved in the award process. FAS will destroy any unsuccessful applications after three years following the funding decision.

F. Federal Award Administration Information

Notice of Award
Notice of award will be given to the institution via email. This email is not an authorization to begin performance. The notice of Federal award signed by the grants officer (or equivalent) is the authorizing document through electronic means. It should also indicate if there are any pass-through obligations that successful applicants are required to meet upon receiving award funds, including specific timeline requirements.

Administrative and National Policy Requirements
All successful applicants for all grant and cooperative agreements are required to comply with Standard Administrative Terms and Conditions for Overseas Federal Assistance Awards, which can be found on the FAS website:

https://www.fas.usda.gov/grants/general_terms_and_conditions/default.asp

The applicable Standard Administrative Terms and Conditions will be for the last year specified at that URL, unless the application is to continue an award first awarded in an earlier year. In that event, the terms and conditions that apply will be those in effect for the year in which the award was originally made.

Before accepting the award the Recipient should carefully read the award package for instructions on administering the grant award and the terms and conditions associated with responsibilities under Federal Awards. Recipients must accept all conditions in this NOFO as well as any Special Terms and Conditions in the Notice of Award to receive an award under this program.

Reporting

Federal Financial Reporting Requirements. The Federal Financial Reporting Form (FFR), as known as the SF-425, must be submitted semi-annually (the reporting period ending every 6 months after the start date of the agreement) within 30 days of the end of the reporting period, with the final FFR submitted within 90 days of the end of the agreement. The required form is available online at:

https://www.grants.gov/web/grants/forms/post-award-reporting-forms.html#sortby=1

At the top of the website select FORMS, and from the drop down box select POST AWARD REPORTING FORMS.
Program Performance Reporting Requirements.
Performance Progress Reporting must be submitted semi-annually (the reporting period ending every 6 months after the start date of the agreement) within 30 days of the end of the reporting period, with the final PPR submitted within 90 days of the end of the agreement, and should include details the activities undertaken and progress made during the reporting period.

Program Performance Requirements.
- Ensure that each Fellow completes the Borlaug Fellowship Program Evaluation.
- A brief Fellow final report before the fellow departs the U.S. (Template will be provided).
- The Principal Investigator or Mentor will submit a final report to USDA/FAS within 30 days after the Mentor visit. (Template will be provided).
- The Principal Investigator or Mentor will submit semi-annual progress reports.
- Reports should include the following:
  - Summary of activities, accomplishments, and any problems encountered or overcome
  - Photographs, when possible
  - Completed program evaluations and action plan
- An invoice/claim cannot be paid if a progress report is past due, and will not be paid until the required report has been received.

Close Out Reporting Requirements.
Within 90 days after the end of the period of performance, or after an amendment has been issued to close out a grant, whichever comes first, recipients must submit a final FFR and final progress report detailing all accomplishments and a qualitative summary of the impact of those accomplishments throughout the period of performance.

After these reports have been reviewed and approved by OCBD, a close-out notice will be completed to close out the grant. The notice will indicate the period of performance as closed, list any remaining funds that will be de-obligated, and address the requirement of maintaining the grant records for three years from the date of the final FFR.

The recipient is responsible for returning any funds that have been drawn down but remain as unliquidated on recipient financial records.

G. Awarding Agency Contact Information
Contact and Resource Information
For all general questions, contact:
Tim Sheehan, Branch Chief
Hours of operation: 9:00 AM – 4:30 PM Eastern Standard Time
Telephone: (202) 690-1940
E-mail address: BorlaugProposals@fas.usda.gov
1400 Independence Ave, SW #3226-South
Washington, DC 20250-1031
H. **Additional Information**

1. **Extensions**
   Extensions to this program are allowed. Applicants may request a no-cost extension in order to complete all project activities. The request must be submitted 60 days prior to the expiration of the performance period. Requests for extensions are subject to approval by FAS.

2. **Prior Approval**
   The Recipient shall not, without the prior written approval of the FAS Program Manager, request reimbursement, incur costs or obligate funds for any purpose pertaining to the operation of the project, program, or activities prior to the approved Budget Period/Performance Period.

3. **Budget Revisions**
   a. Transfers of funds between direct cost categories in the approved budget when such cumulative transfers among those direct cost categories exceed ten percent of the total budget approved in this Award require prior written approval by the FAS Program Manager.

   b. The Recipient shall obtain prior written approval from the FAS Program Manager for any budget revision that would result in the need for additional resources/funds.

   c. The Recipient is not authorized at any time to transfer amounts budgeted for direct costs to the indirect costs line item or vice versa, without prior written approval of the FAS Program Manager.
## Appendix A

### Borlaug Fellowship Program for Mexico

#### Index of Fellowships

<table>
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<tr>
<th>Fellow Reference Number</th>
<th>Country</th>
<th>Gender</th>
<th>Fellowship Length (weeks)</th>
<th>Research Focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>USDA-FAS-10777-0700-10.-18-0054 Fellow 1</td>
<td>Mexico</td>
<td>Female</td>
<td>12</td>
<td>Escherichia coli prevalence in different spinach tissues and assignation of control issues</td>
</tr>
<tr>
<td>USDA-FAS-10777-0700-10.-18-0055 Fellow 2</td>
<td>Mexico</td>
<td>Female</td>
<td>12</td>
<td>Implement a new method to identify food pathogen fungi and create a database</td>
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<tr>
<td>USDA-FAS-10777-0700-10.-18-0056 Fellow 3</td>
<td>Mexico</td>
<td>Female</td>
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<td>Maximum Residue Limits as a Phytosanitary Measure that Impacts Trade</td>
</tr>
<tr>
<td>USDA-FAS-10777-0700-10.-18-0057 Fellow 4</td>
<td>Mexico</td>
<td>Male</td>
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<td>Criteria to assess the risk in controlling organic operations</td>
</tr>
<tr>
<td>USDA-FAS-10777-0700-10.-18-0058 Fellow 5</td>
<td>Mexico</td>
<td>Female</td>
<td>12</td>
<td>Comparison between genotypic prediction (DNA, RNA) and phenotypic evidence of Antimicrobial Resistance of different samples of Salmonella</td>
</tr>
</tbody>
</table>
Individual Proposals and Action Plans

Proposal and Action Plan

Fellow #1, Mexico, Female/ NOFO: USDA-FAS-10777-0700-10.-18-0054

Proposal:
1. The goal of my research is the study Escherichia coli prevalence on different spinach tissues and assignation of control measures.
2. Objectives:
   a) Evaluation of the conditions of colonization (internalization and adherence) of E. coli on different spinach tissues
   b) Assignation of control measures on prevalence of Escherichia coli on spinach tissues

3. Background information:
There is currently an increase in human consumption of young seedlings or sprouts, due to their higher nutritional value as compared to the fully developed plants (Perez-Balibrea et al., 2011). Commercially-available baby vegetables include cherry tomato and baby carrot, lettuce, and spinach. However the consumption of young seedlings and sprouts has been linked to some foodborne diseases over the last few years. One reason for this may be that most of these vegetables are consumed raw, usually to preserve their natural flavor and to prevent degradation of some nutrients during the cooking process. The Centers for Disease Control and Prevention has linked these products to Escherichia coli. For example, in 2006, Escherichia coli O157 was isolated from 13 spinach packages; in 2012, the New York Department Health Wadsworth, Center Laboratories isolated STEC O157:H7 from Organic Spinach produced by State Garden, Chelsea, Massachusetts, affecting five states; and the most recent report was in 2014, was linked to raw clover sprouts with E. coli O121 (Centers for Disease Control and Prevention, 2006, 2012, 2014).

Furthermore, some investigations report that spinach seed germination varies from 3 to 4 days, when seeds are more susceptible to contamination. Castro-Rosas and Escartin (2000) observed that Escherichia coli O157:H7 may develop during the first hours of germination. After the early stage, once colonization has taken place, cells can form a biofilm of immobilized microorganisms and polymers (Iturriaga et al., 2006).

The risk for microbial contamination of sprouts is not limited to the presence of bacteria. Other factors, such as the microorganisms ability to survive and proliferate on soil, moisture, temperature, soil type, and sun exposure, have a huge impact on microbial behavior (Luna-Guevara et al., 2012) and increase the risk to consumers. It is thus important to study the behavior of microorganisms associated to sprout and baby vegetables. It is thus important to study the behavior of microorganisms associated to sprout and baby vegetables.

For this reason, the present program plan evaluates the effect of E. coli inoculated in irrigation water and substrate on the colonization of baby spinach and assignation of control measures related to sources contamination (irrigation water and substrate).
4. During the fellowship, I want to accomplish the following tasks:

a) Preparation of the microorganism in study (E.coli)
b) Inoculate and numbering of E. coli in irrigation water and substrate
c) Crop growing of spinach
d) Analyze samples of different tissues of spinach and numbering of E. coli
e) Confirmation of internalization and adherence
f) Propose the control measures related to the results obtained in the microbiological analyze

I am waiting to reach the following results:
- Confirmation of E. coli growth (Log10 CFU/g) in spinach plant inoculated with the irrigation water and substrate.
- Our work will show that E. coli adhere and/or internalize in spinach tissue, these mechanisms will allow the survival of this bacteria.
- Recommend control measures related with the risk of colonization of E. coli associated with the consumption of spinach.

About the contribution of my participation to this program to enhanced agricultural productivity and food security of my country, also I am sure that I enhance my understanding of food and agricultural science. I would like to highlight the following.

Recent data reveal that 80% of Mexican exports are shipped to the US, therefore, food safety practices with a preventive approach are required. A large part of these exported products correspond to vegetables among those that find the sprouts. However the agricultural sector in Puebla, Mexico, presents several difficulties related to the handling, processing and postharvest conditions that prevent ensuring the safety of agricultural products. Therefore, it is necessary to propose control measures related to avoiding contamination of vegetables with pathogens such as E. coli.

Action Plan

Week 1 Activities:
- Arrival, Introduction, orientation, discussions and re (planning) of work detail
- Outcomes(s) Accommodation issues settled. Final detailed plan made with Mentor
- Materials/Requirements, contact with the lab group

Week 2-3 Activities:
- Literature search (reviews of the Mentor’s publications)
- Selection of E. coli strain.
- Methods of inoculation, isolation, numbering of E. coli strain.
- Results. Evaluation of E. coli growth in different conditions (irrigation water and substrate)

Week 4-8 Activities:
- Laboratory work: a) Analysis of recovery and counting of E. coli will be conducted during the spinach plant growth. b) Development of methods to confirm the presence and internalization of E. coli in the samples surface and tissues of spinach (root, stem and leaf).
- Results. Recounts of E. coli growth in spinach plant, confirmation of the presence and internalization of E. coli in tissues of spinach.

Week 9-12 Activities:
• Laboratory works continue.
• Analyze the results and discussion with Mentor.
• Propose the control measures and elaboration of a farmer training program on the sources of contamination E.coli in vegetables.
• Finalizing of the report.
• Results
• Final report/ results submitted to mentor. Review of work done and way forward for implementation of outcomes and follow-ups.
Proposal and Action Plan

Fellow #2, Mexico, Female/ NOFO: USDA-FAS-10777-0700-10.-18-0055

Proposal:
1. The goal of my project is to implement a new method in order to identify food pathogen fungus and start to have a database for these organisms.

2. Objectives:
   a) To identify the most common fungi in food (or the cases recently presented in the world) and from those fungi select three to start the analysis.
   b) To test different DNA extraction methods in order to have the best protocol for seeds samples.
   c) To analyze the 28S gene (gen specific for fungi organisms), in order to assure the organism is a fungus, using PCR.
   d) To analyze by whole genome sequencing (WGS), these three fungi and with bioinformatics tools identify those genes related to the cause of the disease.
   e) To research specific genotyping markers in the three selected fungi with database and information on Internet.
   f) To test the markers in sequencing tests using molecular biology techniques (as PCR, and Sanger sequencing, …)
   g) To validate this method using another food samples.

3. Background information:
   The “food poisoning” includes symptoms as diarrhea, vomiting, cramps, nausea and fever but these symptoms are not really a poison but an infectious organism that we can ingest through contaminated food or drink (Science Media Centre of Canada, 2014). Foodborne illnesses or “food poisoning” caused 128,000 hospitalized people and 3,000 deaths each year (CDC, 2018). The main cause of these foodborne illnesses are bacteria, viruses, parasites, harmful toxins and chemicals.

   The fungi are microorganisms from Fungi domain, they habit in plants and animals, and the most of them are filamentous. They are characterized by spore production, which can be transported by air, water or insects. There are millions of different fungal species on Earth, but only 300 of those are known to make people sick (Garcia-Solache, 2010). Besides some fungi can produce aflatoxins inside or on food surface, the aflatoxins are poisons which cause cancer. Aflatoxins can be in animal food and in grains as corn and peanut (USDA, 2010).

   Currently, Mexican regulations are concentrate on verification of aflatoxins maximum allowable limits (NOM-188-SSA1-2002, NOM-247-SSA1-2008), because the presence of these toxins are related to the pathogen fungi as aflatoxicosis in livestock from domestic animals and humans throughout the world (USDA, 2010), and studies have evaluated fungi as food-borne pathogens and assessing associated risks are limited and the impact of fungi in foodborne pathogens is largely underestimated (Lee et al., 2014).

   The fact is the fungi can be fatal and kill an estimated 1.5 million people globally each year (CNN, 2016), this is more than twice the number of women who die from breast cancer. Crypstococcus, Candida and Aspergillus are probably the fungi which cause more than a million of deaths each year.
In September 2013, contaminated yogurts were sold to more 200 consumers who became ill (FDA, 2013), and the responsible was identified as Mucor circinelloides, a fungus, using whole genome sequencing (WGS) technology.

The information obtained from WGS helps investigators quickly identify the responsible organisms for foodborne illness outbreaks, helps in identification of related cases, in monitoring/tracking the transmission route and in determining intervention strategies.

As this case, there is another around the world, thus in terms of prevention, this project proposes the first research in order to identify fungus in food, from the soils to the final food products.

4. During the fellowship, I want to accomplish the following:

   a) I would like to have an efficient protocol for identification of specie and genre of three selected fungus related to foodborne pathogen diseases. I already know how to identify the foodborne pathogen bacteria but I think it is important to have the tools for all the cases of foodborne illnesses, and we can start with the fungus cases. The funguses are completely different organisms to bacteria but the procedure for this identification could be similar. I have the background about the foodborne pathogen bacteria but with the help of an expert in fungus (experience in isolated fungus, DNA fungus extraction, fungus characteristic genes analysis), we can development the protocol for identification at least the genre of fungus, and after we could identify those genetic markers for each genre.

   b) Food trade regulations are specific and strict because they are focus on the people’s health but when there are customs conflicts, each country must demonstrate with technic-scientific evidence the harmlessness of food and then, with this resolution, the food can be accepted or rejected. This project wants to generate the information to support the resolutions in the food trade regulations in terms of fungus related to agrifood products. Also it is important to have information as reference in order to have a tracking of fungi through the food chain.

   c) A Borlaug Fellowship can contribute to enhanced agricultural productivity, having the analysis which allows the commerce (export) of national products, updating the regulation of the national products and also the imported ones. With the analysis of fungus, it can be assurance the harmlessness of products and the people’s health (by food consumption).

**Action Plan**

**Week 1 Activities:**
- Research and exchange of ideas for the project
- Initial visits
- Presentation of the project Exchange ideas for the project Research and flowchart of work plan
- Materials: Classroom Internet access Library access Computer

**Week 2 Activities:**
- Solutions preparation for isolated fungus and DNA extraction
- Read protocols Medium preparation Sterilization
- Materials: Autoclave Microbiology instruments (Petri dishes, pipettes, etc.) Reagents to prepare medium
Week 3 Activities:
- Collect samples and DNA extraction
- Define the samples to work with Collect samples
- Treat samples (freeze or cool) DNA extraction (at least test two protocols for DNA extraction)
- Materials: Reagents for DNA extraction Equipment for DNA extraction

Week 4 Activities:
- Design and test on PCR for the 28S gene primers
- Design primers for 28S gene amplification
- PCR in silico and in vitro for testing primers
- Materials: Equipment of molecular biology (micro tubes, micropipettes, tips, etc.)

Weeks 5-7 Activities:
- Genome Sequencing
- Sequencing of three fungus
- Materials: Illumina platform Reagents for sequencing Molecular biology equipment

Weeks 8-9 Activities:
- Genetic markers research in genome
- Bioinformatic analysis (alignments, use of different software for the research of markers specifically for each genre of fungus)
- Materials: Computer Processor to analyze the sequencing data

Week 10-12 Activities:
- Primers design and test for markers
- Design primers specifically for markers PCR with controls (negatives and positives), to test the primers
- Materials: Equipment of molecular biology
Proposal and Action Plan

**Fellow #3, Mexico, Female/ NOFO:** USDA-FAS-10777-0700-10.-18-0056

Proposition:
1. **Maximum Residue Limits as a Phytosanitary Measure that Impacts Trade**

2. Objectives:
   a) The objective of my project is to generate a proposal for a pilot procedure for the extrapolation of internationally adopted MRLs for crops that are related to crops of economic importance for export to Mexico, and for which there are no authorized MRLs; generate a theoretical basis on the problem of MRLs in Mexico, prior to the entry into force of the new Mexican regulation called "Official Mexican Standard NOM-082-SAG-FITO / SSA1-2017. Maximum residue limits."

   b) Technical guidelines and authorization and revision procedure”; to have an analysis of the MRLs adopted by the *Codex Alimentarius* of the FAO and by the EPA (USA) for some products of import and export in Mexico that could affect the trade of fruits and vegetables.

3. Background information:
   At the international level, the *CODEX Alimentarius* standards are based on safety, for the protection of public health, and have the Codex Committee on Pesticide Residues (CCRP), which is charged with preparing MRLs ("concentration Maximum pesticide residue allowed in or on products intended for human or animal consumption") for pesticides. However, more and more countries are determining their own MRLs, a situation that is generating several problems that affect export and import, especially in cases where a country adopts CODEX MRLs that are generally high, and the importing country has a much lower MRL authorized. If we add to this the fact that buyers or accreditation bodies also request different MRLs, an increasingly international disharmonization is encouraged.

In the case of pesticides, it is increasingly common for countries to generate their own regulations for MRL determination or for countries to adopt default values, when the corresponding scientific studies are not available. Such is the case of Japan, where a modification of the legislation in 2006 comes into force, a "positive list" of substances enters into force, in which, if the substance is not listed, an MRL value is automatically applied. equal to the lowest level of laboratory detection, which is 0.01ppm. This generated for the case of Mexico and the substance acephate and its metabolite methamidophos, that shipment of Mexican avocados were rejected in Japan when exceeding the default value of 0.01ppm.

From 2011 to 2013 the frequency of monitoring of a regulatory level from 10% to 100% of Mexican shipments in search of acephate was increased, since for Mexico the MRL of acephate is 0.1ppm. This led to the adoption of strict control measures for production so that prior to the shipment of the product, it was certain that no residues were found or were within the allowed limit in Japan.

This example and others, represent a phytosanitary measure that affects to trade because the entry of food with minimum levels higher than certain limits is not allowed. In the worst case, they are not backed up on the date of issuance of the measure, by analysis of dietary risk, and others that sustain it, departing from the international standards of *CODEX Alimentarius*. 
In the case of Mexico, on October 4th, 2017, NOM-082-SAG-FITO / SSA1-2017 was published, which contemplates a scenario in which Mexico can develop its own studies in order to determine MRLs.

Therefore, there is an interest in analyzing the situation of the MRLs of the CODEX Alimentarius recommended for a group of Mexican crops with high economic export value, against MRLs from countries such as the USA, Canada and Japan, and the impact of the disharmonization of MRLs among the countries of destination of the products. Also, generate a pilot proposal for the procedure for the extrapolation of internationally adopted MRLs for crops that are related to crops of economic importance for export to Mexico, and for which there are no authorized MRLs.

During the project, it is expected to know more in depth the processes of generation of MRLs worldwide, the role of bodies or technical-scientific councils in charge of evaluating the data packages for approval purposes, criteria of the EPA in Mexico to carry out the monitoring of pesticide residues in fresh fruits and vegetables from Mexico to the USA and some countries of other commercial blocks and allow the commercialization of the product.

4. During the fellowship, I want to accomplish the following tasks:

a) The interest of the research is related to my academic training and work experience, because I have collaborated in the generation of knowledge about pathogenic organisms for the human being, and now in the governmental scope I am in charge of the execution of diverse activities on agricultural pesticides, the which if they are misused in the field generate food contamination problems.

b) Having the assistance of a tutor will allow directing the documentary research towards documents and key factors.

c) As exemplified above, the authorization of different MRL values worldwide is causing trade barriers and, consequently, economic losses to the productive sectors.

d) A missing element in the regulation on MRLs of pesticides in Mexico has to do with import tolerances. For the attention of this item was counted more than 10 years ago with collaboration with the United States and Canada for the preparation of a trilateral reference document. It is in the interest of SENASICA to resume this participation, having new and more current knowledge regarding the theoretical and technical bases for the Development of a Mexican proposal. We believe that participating in the Borlaug Scholarship can help guide the technical staff involved in the field of agriculture, to the generation of robust and specific information and procedures for Mexico on the subject of MRLs.
Action Plan

Week 1 Activities:
- Backgrounds on MRL determination in the USA and CODEX Alimentarius
- Do literature research on regulatory frameworks for MRLs mainly USA and Codex Alimentarius
- Materials: Access to internet, computer and printer, technical advisory

Week 2 Activities:
- Backgrounds on MRL determination in the USA and CODEX Alimentarius
- Do literature research on regulatory frameworks for MRLs mainly USA and Codex Alimentarius
- Materials: Access to internet, computer and printer, technical advisory

Week 3 Activities
- Summary of documentary findings
- Based on the analyzed documents, writing a summary of main aspects
- Access to internet, computer and printer, technical advisory

Week 4 Activities:
- Backgrounds on monitoring systems for pesticides residues in the USA and CODEX
- To know general principles of monitoring mechanisms for pesticide residues in fresh fruit and vegetables, especially what regulations apply mainly
- Materials: Access to internet, computer and printer, technical advisory

Weeks 5-6 Activities:
- Preparation of a first draft of the general procedure on monitoring pesticide residues in Mexico
- Based on the information already collected and analyzed, prepare a draft procedure with the general bases for monitoring waste, including normative basis
- Materials: Access to internet, computer and printer, technical advisory

Week 7 Activities
- Review of draft procedure for residues monitoring, including normative basis
- Revision by the tutor of the project, identify if it has the general base and begin to outline specific aspects for Mexico
- Materials: Access to internet, computer and printer, technical advisory

Week 8-9 Activities
- Preparation of draft procedure for extrapolation of MRLs for crops related to Mexican crops that do not have authorized MRLs
- Based on the information already collected and analyzed, prepare a draft procedure for extrapolation of MRLs for crops related to Mexican crops that do not have authorized MRLs, previous analysis and selection of crops of economic interest for Mexico
- Materials: Access to internet, computer and printer, technical advisory

Week 10 Activities:
- Review of draft procedure for extrapolation of MRLs for crops related to Mexican crops that do not have authorized MRLs
- Revision by the tutor of the project, identify if the proposed procedures has the main criteria to consider for MRL extrapolation for crops lacking and MRL in Mexico
- Materials: Access to internet, computer and printer, technical advisory
Weeks 11-12 Activities:

- Reviewing of all documents proposed to final adjustments and validation
- Having concluded the proposed documents, subject them to review by the tutor as well as the hierarchical superiors for final validation
- Materials: Access to internet, computer and printer, technical advisory
Proposal and Action Plan

Fellow #4, Mexico, Male/ NOFO: USDA-FAS-10777-0700-10.-18-0057

Proposal:

1. The objective of the project is to increase efficiency in organic inspections, as part of the National Control System for organic production in México the establishment of criteria to assess the risk by certification bodies in certified operations under the Law of Organic Products.

2. Objectives:
   a) To identify the critical control points of the Mexican for organic production.
   b) To perform the analysis of organic integrity loss risk in the critical control points.
   c) To classify critical control points regarding the level of risk.

3. Background information:
   The risk assessment to control organic operators is a requirement that has been extended among Mexico's main trading partners. In this sense, the European Commission is based on the ISO 17065: 2012 and 17020: 2012 norms, as well as the European Regulation (ER) 882/2004, 834/2007 and 889/2008, to establish the risk assessment as a requirement for the operation of the control system.

   In this way, each Member State must implement a risk assessment system for organic operations, in such a way that the certification bodies execute effective controls in the organic operations under their monitoring. Although, in general, the ER establishes the minimum number of announced, non-announced and random inspections, including sampling, this number can increase due to the risk assessment in each operation. In this regard, every operation is affected by its very specific conditions: crop production, livestock, wild harvesting, processing; even social and geographical conditions, among others.

   The Mexican organic regulation does not include a risk assessment in the control system for organic products. Therefore, it becomes necessary to develop the criteria that will lead the certification bodies to categorize each kind of operation according to the level risk, establishing the number of additional or unannounced inspections that allow greater efficiency in the operation of the national control system.

   With these actions, such criteria could be included in the national organic regulation, increasing the confidence on the national standard, and therefore, facilitating the international recognitions with other countries and assuring the confidence of national and international consumers.

   One of the duties of SENASICA is to implement the National Control System for organic products, which includes to approve certification bodies as well as to develop guidelines for their operation; furthermore, it is up to SENASICA to establish the equivalence recognition with other countries. In this regard, there is the necessity to receive the methodological advice from the academy through an expert researcher in the subject, in order to implement a robust and effective control system.
4. During the fellowship, I want to accomplish the following:

   a) The proposed project is conceived as a solution to eliminate technical barriers to trade by increasing the confidence on Mexican organic products that are exported to our main markets, including the US, and facilitating the recognition of equivalence among other standards.

   b) As an output of the project, it is expected to identify the critical control points in the organic operations and to perform the corresponding risk assessment that will allow increase and develop the general criteria for the organic control.

   c) The Barlaug Fellowship may contribute to improve the effectiveness of the Mexican control system, by increasing control of the organic operation, thus offering a greater guarantee of organic integrity to consumers. Likewise, it can help to achieve the equivalence recognition of the regulations and the control system between Mexico and other countries. With these actions, production costs may be reduced to the organic operators that depend on the import and export of products and raw material, by eliminating the necessity to certify their products under another regulation different from the Mexican one.

**Action Plan**

**Weeks 1-2 Activities:**
- Finish work plan and identify the nature and characteristics of the organic operation under Mexican regulations
- Identify the production processes on organic products and the conditions that represent risks to loss organic integrity
- Materials: Polls applied to the organic sector: certifiers, producers, handlers and consumers

**Week 3-4 Activities:**
- Establish risk profiles for organic operations
- Gather information related to specific conditions and products that may loss organic integrity
- Materials: N/A

**Weeks 5-6 Activities**
- Design criteria to assess the risk
- Describe current measures of control applied to organic production including international regulations
- Materials: Mexican and international organic regulations

**Weeks 7-8 Activities:**
- Decision on the necessity to assess the risk
- Identify processes, situations and operations in which a risk assessment is needed
- Materials: Discussion and analysis with mentor

**Week 9 Activities**
- Selection of the method to assess the risk
- Analyze the factors surrounding organic operation including institutional resources, to select the best method to assess the risk
- Materials: Literature on risk assessment
Weeks 10-11 Activities:

- Design the method to assess the risk
- Establish the criteria to assess the risk on organic operations
- Materials: N/A

Week 12 Activities:

- Risk assessment matrix
- Elaborate the matrix including for risk assessment
- Materials: N/A
Proposal and Action Plan

Fellow #5, Mexico, Female/ NOFO: USDA-FAS-10777-0700-10.-18-0058

Proposal:

1. The aim of the project I am proposing for this program is to compare the Antimicrobial resistance data obtained by the RNA-seq analysis with the DNA prediction information and with the phenotypic evidence of this resistance proved by inhibition.

2. Objectives:
   a) Select Salmonella spp. samples (at least 12) with known resistance to antibiotics and with previous identification of genes by DNA sequencing or phenotypic proved inhibition.
   b) Extract and purify specifically the RNA of these selected samples.
   c) Perform a RNA sequencing run, preferably in an Illumina sequencing technology (MiSeq), in order to obtain the transcriptome of the bacteria.
   d) Analyze by bioinformatic software the obtained data to determine the expression profile related to the AMR genes.
   e) Compare the expression results with the previous AMR available data from the samples to have the % of concordance between the different methodologies.

3. Background information:
   Infections caused by pathogen bacteria has always been a priority talking about human and agriculture health, but nowadays antimicrobial-resistant (AMR) infections have become one of the most important threats to human health, with a conservative estimate of 700 000 directly attributed deaths per year worldwide. If we do not start strategies to fight this issue, is estimated to rise to 10 million by 2050. AMR arises when the micro-organisms which cause infection survive exposure to a medicine that would normally kill them or stop their growth. The uncontrolled use of this drugs, is leading bacteria to become more resistant than ever, including clinical treatments but also the excessive use of it in agriculture and the environment.

   This problem has to be treated globally, involving governments and health institutions worldwide in order to provide a joint solution. An important component of any strategy to stop this growing antimicrobial resistance is having rapid and accurate methods for identifying markers of resistance and an accurate surveillance data. The detection of this resistance, used to be performed by measurement of resistance phenotype (antibiogram). In recent years, genome and metagenome sequencing is increasingly being used for surveillance of AMR genotypes, revealing important information on the relative roles of intrinsic resistance, mutation, horizontal gene transfer, in addition to the underlying mechanisms of resistance. Our understanding of the mechanisms and diversity of AMR is improving, in part due to the increased availability of genome sequence data, with the use of genome sequencing in personalized medicine set to become one key tool in the fight against AMR.

   In the Sequencing and Bioinformatics Unit from the National Service for Agrifood Health, Safety and Quality (SENASICA), we are currently performing the DNA sequencing analysis of bacteria of agriculture interest including the genres Salmonella spp., Escherichia coli, Listeria spp, Shigella spp among others, but according to the WHO, Salmonella is one of the four main causes of diarrheal diseases. While most cases of salmonellosis are mild, sometimes the disease can be fatal. The severity of the disease depends on factors specific to the host and Salmonella serotype and, together with its demonstrated resistance to antimicrobials, it has become a global public health problem and it also could affect the food chain.
In my research group, we analyze the sequencing data in order to predict genes related to the antimicrobial resistance but we do not have the phenotypic data of our resistance profiles. One of our future projects includes the comparison between our DNA sequencing AMR predictions and a phenotypic analysis applying the VITEK 2 system for resistance, which can determine by culture and identification of inhibition of antimicrobials, in other words, the expression of this resistance.

In the unit, we do not have this equipment, so this study will be done as a collaboration with other institutes, but we have the sequencing machines so there is another alternative we can perform. In recent years, the development of RNA sequencing provided a chance to analyze levels of expression of these genes, so the approach of new technologies is revealing advanced focus in these kind of analysis that will allow us to save resources and time in future researches.

4. During the fellowship, I want to accomplish the following:

   a) This fellowship program, will establish a close relationship between both countries and the institutions involved in the surveillance and control of antimicrobial resistance to strengthen the strategies that will help to stop this global threat, as well as allowing us to implement a new analysis methodology that at this moment is not available in our institution to corroborate the analysis we have previously done.

   b) My interest in this fellowship program is to know how our scientific peers conduct their research and learn new analysis methodologies like RNA sequencing and its respective Bioinformatics analysis. I’ve been working with sequencing techniques for about 6 years but this field is always moving and implementing new technology so I would like to provide this knowledge to my research group in Mexico and establish these techniques to verify the previous studies we have already done, taking advantage of the expertise that other international institutions have. This is a great opportunity to exchange experiences, knowledge but also learn about other traditions and cultures.

Action Plan

Week 1 Activities:
- Initial Visit U.S. fellow institution.
- Present the project to the U.S. fellow institution, feasibility review.
- Select 12 (proposed) Salmonella samples with previous serotyping, DNA sequencing and phenotypic proved AMR
- Materials: Salmonella samples and its AMR previous information

Week 2-3 Activities:
- Sample conditioning and RNA extraction and purification
- Isolation and Culture of bacteria
- RNA extraction and purification could be performed by RNA Extraction Kit QIAGEN or Promega and review RNA quality with RIN in the Bioanalyzer 2100
- Materials: Selective agar for Salmonella and microbiological lab infrastructure, RNA extraction kit, Agilent Bioanalyzer 2100 with RNA Chip kit.

Week 4 Activities
- Sequencing library preparation
- The RNA libraries could be done with True seq kit from Illumina
• Materials: TrueSeq Illumina Kit  Thermalcycler Agilent 2100 Bioanalyzer with High Sensitivity Chip Kit Qubit Fluorometer Molecular Biology Lab Infrastructure

Weeks 5 Activities:
• Sequencing Run
• Sequencing run could be done in a Miseq-Illumina platform
• Materials: Illumina MiSeq sequencing machine

Weeks 6-9 Activities
• Bioinformatics Analysis
• Review read’s quality control and initial analysis
• Mapping reads against reference
• Expression analysis
• Materials: Workstation or Server at least with 32 Gb in RAM and 8 processing cores, internet connectivity

Weeks 10-11 Activities:
• Data comparison between available methodologies (DNA Seq, RNA Seq and Phenotypic resistance)
• Bioinformatics comparison, statistics analysis if possible
• Materials: Workstation or Server at least with 32 Gb in RAM and 8 processing cores. Internet connection

Week 12 Activities:
• Present final project results
• Materials: PC