

## National Predictive Modeling Tool Initiative (NPMTI) 2021 Statement of Work (SOW) National Agricultural Genotyping Center (NAGC)

OVERVIEW – NAGC is a not-for-profit testing facility that provides services to NPMTI-recognized Research Area Committees (RAC), which represent the interests of specific commodity/crop groups and associations. These services are paid for by NPMTI, thus allowing each RAC to focus its resources on field-based research, data collection, and analysis.

NAGC testing includes screening of a variety of substrates including, but not limited to, plant tissue, seed, soil, residue and spore traps, nucleic acid purification, and analysis. NAGC's assays have been thoroughly validated by the highest standards as established by the International Organization for Standardization (ISO) criteria including, but not limited to the following studies: Annealing Temperature, Chemical Reagent Titrations, Specificity, Sensitivity, Mixture Studies, Standard Specimen, and Proficiency Testing.

NAGC services to RACs fall into one of three categories: 1) development of validated, quantifiable assays for specific diseases of concern to each RAC; 2) spearheading the proficiency testing effort to assure standardization across all labs involved in NPMTI; and 3) in-lab high-throughput testing of field samples of crop residues and/or soil to quantify pathogen loads. Then during the growing season, we can test seed and plant tissue as well as field-supplied air samples to detect and quantify spores of various diseases, assuming the genetic markers exist for the spores in question.

1) Assay Development The RAC for corn identified its two top disease priorities in 2020 as: grey leaf spot (GLS, *Cercospora zeaemaydis*, *C. zeina*), and northern corn leaf blight (NCLB, *Exserohilum turcicum*). NAGC added bacterial leaf streak (BLS, *Xanthomonas vasicola* pv. *Vasulorum*) because it is often misdiagnosed as GLS. NAGC has quantifiable assays for these three diseases.

During 2021, NAGC will assist corn researchers on the development of an assay for *Phyllachora maydis*, causal agent of tar spot, and on *Gibberella zea*, causal agent for Gibberella ear rot. NAGC also will be working with university labs affiliated with the Corn RAC to help validate assays for airborne spore sampling.

The Cotton RAC has identified its top priority for NAGC assistance in 2021 as grey or areolate mildew (*Ramularia gossypii*). The *Ramularia* spore sample detection methodology is expected to come out of peer review during the Spring 2021, at which point NAGC will help validate the methodology for quantification of this disease. Other pathogens of interest to the Cotton RAC in 2021 include *Fusarium* spp., *Pythium* spp., *Rhizoctonia solani*, and *Thielaviopsis basicola*.

During 2020, NAGC developed assays for the three forms of wheat rust – stem or black rust (*Puccinia graminis*), stripe or yellow rust (*Puccinia striiformis*), and leaf or brown rust (*Puccinia triticina*). During 2021, NAGC will finish the validation of these three assays, then combine them into a single triplex assay. Also, during 2021, NAGC will develop assays for three species of wheat blotch, including *Parastagonospora nodorum* (formerly *Stagonospora nodorum* and *Septoria nodorum*), causal agent of Septoria nodorum blotch (SNB); *Zymoseptoria tritici* (formerly *Septoria tritici*), causal agent of Septoria leaf blotch or Septoria tritici blotch (STB); and *Pyrenophora tritici-repentis*, causal agent of tan spot (also called yellow leaf blotch).

2) Proficiency Testing NAGC has led the effort to establish standardization among and across all labs that are currently (or may someday) be involved in testing services for the NPMTI. Standardization is important because it helps consolidate research and research interpretation in a comparable fashion. It is not a one-time exercise. It is an ongoing effort that must be reviewed on a regular basis. As an ISO-17025:2017 certified lab, NAGC can provide proficiency testing of other labs as needed. NAGC will team with Assured Bio, also ISO-17025 certified and a Cotton RAC subcontractor, to insure consistency of effort. Standardizing and proficiency testing of detection methods for cotton leafroll dwarf virus (CLRVD) is a priority.

3) In-Lab Testing In order to help researchers focus resources on field-based study, data collection, and analysis, NAGC will test up to 6,000 samples at no cost to the researchers. This equates to 2,000 samples for each RAC, although this number is simply a guideline and not a limit. These testing services will be provided to each researcher on a confidential basis. Follow-up reports will go to the researcher and any other parties the researcher may designate. It will be up to the researcher to submit data to Los Alamos National Laboratory (LANL) for aggregation and to help further the development of predictive modeling.

The validated, quantifiable assays that are ready to use are the highest in-lab testing priority. This includes the assays for northern corn leaf blight and gray leaf spot in corn. NAGC can accept samples at any time. Next are those assays that will be in development during the 2021 season. This includes tests for tar spot and Gibberella ear rot in corn, and areolate mildew in cotton. And in wheat, validating assays for leaf, stem, and stripe rust, and developing assays for the three forms of blotch, including *Septoria nodorum* blotch (SNB), *Septoria tritici* blotch (STB); and tan spot or yellow leaf blotch. Although not a formal part of predictive modeling, the lab will also accept and provide quantitative testing for Goss's wilt, bacterial leaf streak, *Diplodia* leaf streak, and *Diplodia* ear and stalk rot due to diagnostic issues with these diseases (i.e., they can be confused with similar looking diseases).

It is expected that the majority of samples received will be for soil and/or crop residue testing. NAGC can also provide quantifiable testing for plant tissue and seed if the researcher so desires. Regardless of the substrate, molecular assays rely on quality sampling procedures in order to produce quality results. To this end, NAGC has developed separate sampling procedures that the researcher can follow for soil, crop residue, plant tissue, and seed.

Quantification of airborne pathogens via spore sampling will be more difficult since the capture efficiency and gain factors vary for different spore monitor designs, hence numerical values in a given field could vary based on what kind of spore monitor is used. Because of these differences, standardized numerical values may not be possible for the next couple of years, but it may be possible to establish numerical values for each monitor design. NAGC can assist the RACs in this area.