BILL & MELINDA GATES foundation

Progress Narrative

Use this form to provide updates to your foundation program officer regarding progress made toward achieving your project's stated outputs and outcomes.

The Progress Narrative must be submitted in Word, as PDFs will not be accepted.

General Information

Investment Title	NextGen Phytosanitation: Rapid Elimination of Viruses from RTB Plants for Crop Improvement and Seed Systems		
Grantee/Vendor	International Potato Center		
Primary Contact	Dave Ellis	**Investment Start Date**	September 29th, 2016
Feedback Contact[1]	Dave Ellis	**Investment End Date**	September 30th, 2019
Feedback Email[1]	d.ellis@cgiar.org	**Reporting Period Start Date**	September 29th, 2016
Program Officer	Jim Lorenzen	**Reporting Period End Date**	October 31st, 2017
Program Coordinator	Emily Zuberi	**Reporting Due Date**	November 30th, 2017
Investment Total	$2,385,906.00	**Opportunity/Contract ID**	OPP1130216
Scheduled Payment Amount (If applicable)	$828,688.00		

[1] Feedback Contact/Email: The full name and email of the contact whom foundation staff queries for various surveys.

Submission Information

By submitting this report, I declare that I am authorized to certify, on behalf of the grantee or vendor identified on page 1, that I have examined the following statements and related attachments, and that to the best of my knowledge, they are true, correct and complete. I hereby also confirm that the grantee or vendor identified on page 1 has complied with all of the terms and conditions of the Grant Agreement or Contract for Services, as applicable, including but not limited to the clauses contained therein regarding Use of Funds, Anti-Terrorism, Subgrants and Subcontracts, and Regulated Activities.

Date Submitted	November 30th, 2017	**Submitted by Contact Name**	Maria Teresa Bellido
		Submitted by Contact Title	Grants and Contracts Manager
		Submitted by Contact Email	m.bellido@cgiar.org
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Progress and Results

1. Progress Details

Provide information regarding the current period's progress toward achieving the investment outputs and outcomes as well as the work planned or anticipated for the next period. In addition, submit the Results Tracker with actual results as requested.

SUMMARY

Overall, the project is slightly behind schedule but making substantial progress. Sweetpotato and yam in-vitro accessions have been selected and multiplied for meristem and small RNA Sequencing and Assembly (sRSA) work. Experiments investigating in-vitro meristem growth in sweetpotato and yam are ongoing after a delay in tissue multiplication to generate sufficient material to initiate the experiments. Standardization of sRSA protocols have been finished and the testing of sRSA against standard indexing techniques is ongoing. Experimentation to determine the sensitivity of the sRSA assays has also been initiated. The VirusDetect software has been integrated with the Chipster platform. And although the enhanced functionality is delivering the desired results, the Chipster platform contains many components that are not needed for VirusDetect; hence modification of the Chipster platform in year 2 is warranted. The catalytic

site for the RNase3 from (sweetpotato chlorotic stunt virus) has been characterized, and high throughput screening of inhibitors for this enzyme is underway, with a preliminary screen yielding 106 compounds for further testing. The simultaneous development of a high throughput screen in plant cells for novel antiviral compounds has been tested and is currently being used for proof of efficacy with a limited number of compounds. The QuickChip prototype is operational and ready to be tested with targeted viruses from sweetpotato, yam, and cassava. This phase of the project was held up due to intellectual property (IP) associated with the QuickChip technology. Capacity-building workshops for the project were given at the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria, and the International Society For Tropical Root Crops (ISTRC), Dar es Salaam, Tanzania, in March 2017. Unused funds will be carried over into year 2 (principally from the VirusDetect and QuickChip components of the project). Any shortfalls in deliverables from year 1 are expected to be 100% caught up in year 2.

INDIVIDUAL PROJECT PROGRESS REPORTS

1. In vitro: sweetpotato (CIP)

   Thirty sweetpotato accessions were initially identified as having inventories (clones) that were both virus infected as well as cleaned of viruses and were bulked up as candidates for both in-vitro and the sRSA experiments. Virus infection of the non-clean inventories were reconfirmed via polymerase chain reaction (PCR), and 5 accessions were selected for in-vitro experiments (a total of 10 clonal inventories from 5 accessions) to enable us to determine whether the results from the experiments are influenced by the virus status of the starting material. The standard protocol for multiplication of sweetpotato in vitro proved too slow and hampered the initiation of the experiments on in-vitro meristem growth. Thus, initial efforts focused on the in-vitro multiplication of shoots to ensure enough meristems could be routinely produced for the experiments. Multiplication rates have been significantly enhanced by the inclusion of a 2–4-week liquid media step as well as a 3-week shoot elongation phase at 28^{o}C. Experimentation on the growth of meristems has been initiated, looking at meristem growth on varying macronutrient levels and the effect of NH_4.

2. In vitro: yam (IITA)

   Ten yam accessions (6 *D. alata* and 4 *D. rotundata*) were selected, meristems excised (15 meristems per accession), and the meristems were cultured under 3 light conditions (continuous light as a control, 7 and 14 first days in the dark before continuous light). Observation after 4 weeks showed that the meristems subjected to dark were chlorotic and gave shorter shoots, compared with those cultured under continuous light. The strength of the macronutrients in mass spectrometry (MS) culture medium was tested with four treatments: full-strength MS (control), ½, ¼ and ⅛ MS; on which 15 meristems per accessions were cultured. Preliminary observations at 4 weeks show no difference between the treatments; however, shoots on full-strength MS appear more vigorous. The effect of liquid (with shaking at 70 rpm) and semi-solid culture media is also tested on yam meristem regrowth (ongoing experiments).

   Twelve cassava accessions have been selected and have been bulked up to get enough material. For cassava, the same experimental design will be used to estimate the effect on meristem growth of light/dark conditions (ongoing), liquid/semi-solid, auxin/cytokinin hormone balance, antioxidants, and meristem location (apical vs. axillary).

3. sRSA: sweetpotato (CIP) (Annex: sRSA presentation Helsinki meeting)

   Of the 30 virus-infected accessions initially selected (see 1. above), 16 had virus infection status fully or at least partially confirmed. A subset of these was selected for sensitivity and specificity analysis by sRNA sequencing before the end of 2017. It is interesting that only ~50% of the virus infection status could be reconfirmed, and it is intriguing to speculate if this was due to low virus titers in in-vitro material or the fact that decades of in-vitro culture could select for tissue free of viruses. We are submitting these accessions to standard virus indexing to confirm that they are free of viruses.

   Separately, 12 different RNA and library preparation protocols were compared on a composite sample containing six different sweetpotato viruses in triplicate to evaluate effects of protocol simplification. It should be noted that all protocols enabled detection of viruses. Whereas the use of cheaper reverse transcriptase and Taq polymerase reduced sensitivity, omitting gel extraction of siRNA, had little effect and the use of sRNA isolation columns showed the best results for simplifying the procedure and reducing costs. Thus, the current protocol will use sRNA columns (or Trizol without sRNA isolation from gel) with standard enzymes, as the reduced sensitivity can be compensated by increased sequencing depth.

A total of 219 sweetpotato accessions that have/are going through virus indexing and cleaning have been selected and will be tested by sRSA before and after virus cleaning, to initiate the comparison and validation of results between sRSA and the standard procedure.

4. sRSA, chemotherapy: yam and cassava (IITA)

   Thirty-five yam and 13 cassava accessions are being multiplied and screened for the project. A sRSA workshop at IITA early in the project cycle ensured alignment of sRSA protocols between IITA and CIP and ensured that sRSA and VirusDetect were set up at IITA. The standard methodology used at CIP was adopted for extracting small dsRNAs (21–24 nt) from 100 to 250 mg tissue samples using either CTAB or Trizol extraction buffer. The small RNAs were precipitated using 2M LiCl, ligated to barcoded oligonucleotide adaptors, and used to construct cDNA libraries. The concentration of the enriched cDNA libraries was estimated using QBIT sample analyzer; samples carrying different adopters were pooled (up to 48 samples) to prepare a mix of SRS libraries for sequencing using HiSeq analyzer. At present, the protocol has been streamlined to analyze 48 samples every 2 weeks. Samples prepared with Trizol extraction buffer resulted in better quality preparations than those prepared in CTAB. Recently, EZNA miRNA kit (Cat. No. R7034, Omega Bio-Tek, USA) was adopted for extraction and precipitation of small RNAs, replacing manual and LiCl precipitation steps in the SRS isolation protocol. Recent work at CIP showed that sRSA library construction direct from the EZNA mRNA kit was equivalent to or even superior to the libraries generated using gel elution of 21–24 nt size SRNAs. This modification will be adopted at IITA to reduce by 50% the time taken to generate an SRS library. Libraries generated at IITA are being pooled and will be submitted for sequencing in October 2017.

   Initial work with antivirals, based on lactoalbumin and salicylic acid, is promising and will serve to develop a protocol to be used at both CIP and IITA.

5. VirusDetect (University of Helsinki)

   The objective is to make VirusDetect more user-friendly, capable of running on laptops and with greater capacity and functionality. To achieve this, VirusDetect has been integrated into the Chipster platform. This move has enabled the incorporation of more user-friendly elements (simplified algorithms, less guess work in calling sequence matches), but it is a relatively heavy program with many elements not needed for virus detection. Hence, the program can be complicated to download, particularly in areas where bandwidth is low (such as most of Africa). That said, it has been a huge advance for the capabilities of VirusDetect, but moving forward in years 2–3, the combining of these two pre-made platforms with much wider scope than needed for this project, needs to be streamlined, keeping the best components but eliminating unneeded elements. As well, certain functions need to be developed and added, to enable characterization of whole genomes from partial sequences or new viruses. Suggested changes include establishing parameters for minimum contig and kmer lengths to balance sensitivity and reliability and improve functions for reliability/stringency by setting parameters for overlap of reads for contig formation. The goal remains that by the end of 2018, VirusDetect will be a simplified, easier-to-use program without the need for huge computing or internet power to run it. All said, Chipster has been a huge improvement and a step in the right direction.

6. High-throughput screening of RNase3 inhibitors (University of Helsinki)

   An experimental system was set up for proof of efficacy and to work out parameters for a screen, which allows screening compounds for inhibition of viral RNase3 activity. The three-dimensional structure of the catalytic site of RNase3 from sweetpotato chlorotic stunt virus was modeled. The structural model was used to test 140,000 chemical compounds *in silico* for their ability to dock to the catalytic site of RNase3. From this, 6,400 compounds were tested in vitro for their ability to dock to the catalytic site of RNase3. Initially, 220 chemical compounds were screened in the Finnish Institute for Molecular Medicine by fluorescence resonance energy transfer to develop the experimental system. This enabled screening of the 6,400 preselected compounds, resulting in 106 compounds having inhibitory activity on the catalytic activity of RNase3, 24 of which have high activity and 12 have 100% inhibition in these initial screens. Next steps are to confirm the results and look closer at the mode of action of the inhibitors (actual binding to the catalytic site or simply blocking the site). These steps will be followed by looking at plant toxicity of the compounds, activity against non-target RNase3s (native plant RNase3), solubility, and biosafety. If these steps are satisfactory, resynthesis and confirmation of the structure of the compound(s) shall follow.

7. High-throughput screening of novel antiviral compounds (UW-Madison)

   The project was delayed until April 1 by the negotiation an IP agreement between CIP and UW-Madison (as mentioned above). A Nicotiana/tobacco mosaic virus (TMV)-based assay system was set up that included the following:

acquiring and characterizing four GFP-labeled TMV strains, setting up the methodology for in-vitro reverse transcription to obtain RNA transcripts, infecting Nicotiana plants with the GFP-labeled TMV strains, collecting fluorescently labeled viral particles, and working out a protocol for creating protoplasts from Nicotiana. Current emphasis is on creating and collecting more fluorescently labeled viral particles and performing pilot-screening assays to feed 1,000 small molecules (including known antivirals as positive hits) to determine a Z-score. In year 1, pilot libraries of small molecules at UW-Madison were selected to initially screen, results of which will be used in year 2 to establish access to services for chemical synthesis of positive hits with potent antiviral activity.

8. QuickChip (UW-Madison)

   The QuickChip phase of the project was also delayed in starting even though the QuickChip patent USPOT 9,050,593 had already been disclosed to the Foundation. A target list of 11 recombinase polymerase amplification and loop mediated isothermal amplification-based assays against yam, sweetpotato, and cassava (6 in sweetpotato, 2 in yam, and 3 in cassava) were identified and primers and probes were developed for all 11 assays. Obtaining plant material with known viruses is ongoing, but efforts to date include the negotiation of an APHIS permit to move viruses around the use, the collection and isolation of viral nucleic acids, the collection of infected and healthy plant tissues (cuttings and leaves), the establishment of plants in the greenhouse, and performing PCR tube-based assays for all viruses. Roughly 100 chips have been fabricated and used to initiate training of QuickChip readers. Assays for the 11 viruses are being optimized and characterized (e.g., performing Receiver Operating Characteristic curves) to enable the movement of the QuickChip into field-testing as well as the fabrication of another 400 chips for assays and capacity building.

YEAR 1 (2017) CAPACITY-BUILDING ACTIVITIES

1. February 20–March 3; IITA (Ibadan, Nigeria): sRSA, VirusDetect, and virus therapy training (photos from training appear in Appendix 1).
   A training course was organized for 20 February–3 March 2017, at IITA. Five staff members from IITA's Virology and Molecular Diagnostics Unit participated in analyzing a diverse set of yam and cassava tissues (fresh samples for established plants grown under screenhouse and field conditions; samples from in-vitro plantlets, dehydrated specimens, and samples from yam tubers). The trained IITA team is currently working actively on SRS library preparation.

2. March 2; IITA (Ibadan, Nigeria): Project Awareness Workshop.
   A 3-hour mini-workshop was held for staff from IITA and the Nigerian Agricultural Quarantine Services, in addition to a few students from the University of Ibadan to present an overview of the science of the proposed research, as well as the expected impact and potential outcome of the project. The presentations were followed by a Q&A session where the principal investigators (PIs) from the two CGIAR centers engaged actively with IITA staff.

3. March 5–10; ISTRC Meeting (Dar es Salaam, Tanzania): Poster presentation promoting the NextGen Phytosanitary Cleaning Project presented at the ISTRC meeting (Appendix 2).
   Ellis, D., Gueye, B., Kreuze, J., Kumar, L., Valkonen, J., and Weibel, D. 2017. NextGen Phytosanitation: Rapid Elimination of Viruses from RTB Plants for Crop Improvement and Seed System. Poster presented at the International Tropical Root Crop Meeting in Dar es Salaam, Tanzania. March 5–10, 2017.

4. March 7; ISTRC Meeting: NextGen Phytosanitary Cleaning Project Initiation Workshop (photos from Workshop Appendix 1).
   A special, open evening session on the NextGen Phytosanitation was organized from 19:30 to 21:30 pm and attended by more than 50 people. In the workshop, all project PIs of the NextGen project participated and introduced the lead themes and opportunities for collaboration and capacity development. Topics covered in this event were as follows:
   - Advances in in-vitro production of virus-free plants (cassava, sweetpotato, and yam)
   - Chemotherapy using antiviral agents
   - Small RNA sequencing for virus detection and VirusDetect software
   - Quick Diagnostics

   A full-page flyer was designed and distributed to the participants.

5. June 16; ASM Annual Meeting (Boston, MA): Demonstration of QuickChip

Provided an invited talk on the QuickChip technology at the American Society for Microbiology General Meeting and solicited interest from several potential users/testers.

6. March 13; MARI training on use of VirusDetect (Appendix 3).
   Twenty-one participants, most from MARI but a few from IITA, attended this workshop on the use of the VirusDetect software.

YEAR 1 PI MEETINGS

1. Face-to-face
   A. Dar es Salaam, Sunday, 5 March
   B. Helsinki, Monday and Tuesday, 18–19 September
2. Quarterly Skype calls (Nov., Feb., June, and Sept.; minutes available upon request).

2. Project Adjustments

For each outcome or output that is behind schedule or under target, explain what adjustments you are making to get back on track.

1. Multiplication of in-vitro material caused an initial delay in setting up meristem growth experiments, testing existing chemotherapy agents, and supplying for sRSA experimentation. Progress in enhancing multiplication rates has been made that will enable all experiments dependent on plant material to be on schedule by the end of year 2.
2. Development of the VirusDetect platform is making excellent progress toward its deliverables. Yet in year 1 the integration with the ChipSter platform was supported by the Center for Super Computing (CSC) at the University of Helsinki. Hence, funds originally budgeted for this integration were not used in year 1. *We are requesting a carry-over of the funds into year 2 to allow further development of the integrated platforms and to make the needed software changes to meet the years 2–3 milestones.* Specifically, the carry-over funds will be used to further develop user-defined parameters for minimum contig and kmer lengths, which should help balance sensitivity and reliability for individual users and needs, improve functions for reliability/stringency in setting parameters for overlap of reads for contig formation, streamline both the VirusDetect and Chipsters platform by removing unneeded components, and move toward increased clarity when using VirusDetect. These activities will be in addition to the planned activities of enhancing the virus detection library function of VirusDetect.
3. As mentioned above, the QuickChip phase of the project was delayed in starting due to the need to negotiate an IP agreement between CIP and UW-Madison for the QuickChip technology (Patent 9,050,593). The realization of patent 9,050,593 delayed the signing of the final project papers to allow UW-Madison to initiate work on the QuickChip portion of the project. Although significant progress has been made in year 1 on the QuickChip, another 8–12 months are needed to fully meet the deliverables of the QuickChip. *Therefore, we are requesting a no-cost extension and carry-over of the funds budgeted for the QuickChip to allow the field testing of the QuickChip against sweetpotato, yam, and cassava viruses as well as capacity building and training of the QuickChip in the African continent.* The no-cost extension is being requested for the QuickChip as that portion of the project was only slated for year 1. We are fully confident that all deliverables for the QuickChip can be met with a one-year extension.
4. The delay in signing the final project papers also delayed start of the antiviral screening project. To offset the delay in starting, we have reorganized the team at UW-Madison working on this project to accelerate it in year 2. *Consequently, we are requesting that some of the budget for screening in year 1 be moved forward into year 2; we do not anticipate any delays in delivering the year 2 deliverables for this project.*

3. Geographic Areas to Be Served

Provide the most updated list of countries and sub-regions/states that have benefitted or will benefit from this work and associated dollar amounts. If areas to be served include the United States, indicate city and state. Reflect both spent and unspent funds. Add more rows as needed. More information about Geographic Areas to Be Served can be found here.

Location	Foundation Funding (U.S.$) – Total Project
Nigeria	$700,000
Ghana	$50,000
Benin	$50,000
Uganda	$100,000

Tanzania	$100,000
Kenya	$100,000
Mozambique	$ 50,000
Other countries W. Africa	$200,000
Other countries in sub-Saharan Africa	$200,000
Global	$836,000

4. Geographic Location of Work

Provide the most updated list of countries and sub-regions/states where this work has been or will be performed and associated dollar amounts. If location of work includes the United States, indicate city and state. Reflect both spent and unspent funds. Add more rows as needed. More information about Geographic Location of Work can be found here.

Location	Foundation Funding (U.S.$) (Year 1)
CIP, Peru – Lead organization	$313,558
IITA, Nigeria	$206,883
U of Helsinki, Finland	$99,475
UW-Madison, Madison, WI	$194,761

5. Feedback for the Foundation

Provide one to three ways the foundation has successfully enabled your work so far. Provide one to three ways the foundation can improve.

ENABLING

1. Funding to enable technology to be developed, transferred, and operationalized
2. Technical support—project partnership
3. Resources for capacity building

IMPROVE

1. Provide more start-up time for project initiation

6. Global Access and Intellectual Property

If your funding agreement is subject to Intellectual Property Reporting, please click the following link to complete an Intellectual Property (IP) Report.

If not, please acknowledge by typing "N/A": N/A

To delegate permissions to another member of your project team or for any questions regarding the Intellectual Property Report, please contact GlobalAccess@gatesfoundation.org.

7. Regulated Activities

Do you represent that all Regulated Activities[1] related to your project are in compliance with all applicable safety, regulatory, ethical and legal requirements? Please mark with an "X":

N/A **N/A (no Regulated Activities in project)**

_____ **Yes**

_____ **No (if no, please explain below)**

Are any new Regulated Activities[1] planned which were not described in any documents previously submitted to the foundation? Please mark with an "X":

_____ **No**

_____ **Yes (if yes, please explain below)**

1 Regulated Activities include but are not limited to: clinical trials; research involving human subjects; provision of diagnostic, prophylactic, medical or health services; experimental medicine; the use of human tissue, animals, radioactive isotopes, pathogenic organisms, genetically modified organisms, recombinant nucleic acids, Select Agents or Toxins (www.selectagents.gov), Dual Use technology (http://export.gov/regulation/eg_main_018229.asp), or any substance, organism, or material that is toxic or hazardous; as well as the approvals, records, data, specimens, and materials related to any of the forgoing.

Financial Update

The purpose of the Financial Update section is to supplement the information provided in the "Financial Summary & Reporting" sheet in the foundation budget template, which reports actual expenditures and projections for the remaining periods of the grant. This section is a tool to help foundation staff fully understand the financial expenditures across the life of the project. Together, the Financial Update section and budget template ("Financial Summary & Reporting" sheet) should provide a complete quantitative and qualitative explanation of variances to approved budget.

Note: If you are using an older version of the budget template, this information could be in a different location in your template.

1. Summary

Briefly describe how total project spending to date compares against the budget and how your assumptions may have changed as the project progressed.

CIP

Project initiation was delayed by the slow multiplication and growth rate in vitro for sweetpotato and the need for larger numbers of shoots in a shorter period than ever needed in the past. Also delayed was the hiring of technical staff for several months; thus the initiation of experiments was delayed for all phases of the sweetpotato project (in vitro and sRSA) as all were dependent on in-vitro material. We expect to be fully caught up by the end of year 2 with all experiments. We will hire another technician to assist the multiplication and maintenance of in-vitro material for the project.

In addition to the hiring of extra personnel, a recent publication suggested that contigs derived from small RNAs may not always be long enough to identify very divergent novel viruses. Thus, we will perform additional sequencing of a select number of libraries using ribosomal RNA depleted total RNA with 150 bp pair end reads as a quality assurance method for sRSA.

IITA

As intended, the project focused initially on yam, which like sweetpotato required extra effort and research to meet the demand for plant material for subsequent experiments. Cassava, as per the work plan, will be included in year 2; issues with multiplication are not anticipated. The sRSA technology has been transferred from CIP and submission of libraries for sequencing will proceed early in year 2. Hiring of another technician and additional sequencing costs are budgeted as no-cost extensions in year 2.

UW–Madison

The 6-month delay in starting the project greatly postponed all experimentation, manufacturing of parts, and initiation of hiring the needed help. Continual discussions with the project lead ensured smooth project coordination during this time, but this delay greatly upset plans for year 1. Despite this, we foresee no major changes in deliverables other than everything will be compressed into a 2.5-year time frame.

- **QuickChip.** Owing to the later-than-expected start date, this effort was pushed back about 6 months into year 2 deliverables. In year 1, plant and nucleic acid samples for all three plants and 12 viruses were obtained. All plant diagnostic assays were developed and established in the lab. These were subsequently converted to assays on the QuickChip platform. We expect to complete the delivery of this milestone and portion of the project in year 2.
- **Antivirals.** The discovery of new antivirals was also delayed. In year 1, the acquisition of key materials (plant strains and viral constructs) was completed and expertise with techniques of propagating and packaging fluorescently tagged viral particles and formation of plant protoplasts was developed. In year 2, we will close the gap to accomplish both the unmet year 1 deliverable as well as the proposed year 2 deliverables. To accomplish this, we are expanding our project team to include Will Gross, a former undergraduate student at UW-Madison who previously worked in our lab and who has since joined our lab as a full-time research assistant.

Helsinki

Research costs for the subcontract with the University of Helsinki have been lower in year 1 than expected, due to the following:

- Institute for Molecular Medicine Finland, Helsinki, did not charge for their services (working time of their technicians or use of the equipment); only the costs of consumables were charged to the project.
- The doctoral candidate Linping Wang obtained a stipend from the China Scholarship Council (CSC), which covered her salary.
- The Information Technology Center for Science in Finland (CSC) has been interested in developing VirusDetect and, thus, has not charged for their time to add needed applications and functions to VirusDetect.
- The remaining funds from year 1 will to transferred to year 2, as the next steps of the research will be much more expensive than anticipated. Therefore, the original budget for year 2 would be insufficient.
- **RNase III inhibition assays.** The initial results must be reconfirmed, requiring that the promising inhibitory compounds be resynthesized to sufficient volumes for experiments, which is very expensive. We must also look closer at the mode of action of the inhibitors (actual binding to the catalytic site). Dose-response validation of the hits (five different concentrations for each compound) will be done, and affinity of compounds to RNase3 will be tested. Although preliminary results suggest that inhibitors bind to the catalytic site, this needs to be confirmed by MicroScal Thermophoresis or Ni-NTA biosensors. Furthermore, the aforementioned steps will be followed by testing the compounds for toxicity to sweetpotatoes in vitro.
- **Further development of VirusDetect.** CSC has developed VirusDetect significantly, but modifications to make VirusDetect genuinely user-friendly remains to be done. This will be done by omitting those functions of Chipster that are not needed and just make the system work slowly in areas where bandwidth is low. This will require a specialist from the Department of Computer Science, University of Helsinki.

2. Latest Period Variance

Provide explanation for any cost category variances outside the allowable range. Explain causes, consequences for the project, and mitigation plans if relevant. Report whether or not approval for the variance has been obtained from your Program Officer.

Note: "Latest period variance" compares actuals to previous projections for the period. See "Financial Summary & Reporting" sheet in the foundation budget template for calculated variance. If you are using an older version of the budget template, this information could be in a different location in your template. Allowable variance is defined in your grant agreement.

Variances are negative due a slow ramp up of the research and thus a slower implementation particularly from the partners which is reflected in the sub-awards:

- For Wisconsin and ITTA, the principal cause of the variance was a delay in implementation.
- For Helsinki, the budgeted year 1 funding was only used for services and supplies since the staff salaries and some services were covered by external (stipend from China) and in-kind funds.
- For CIP, direct costs and personnel funds not utilized in year 1 will be used to ensure deliverables for the second year of the project.

In all cases, the balance from year 1 will be carried over into year 2 and will be used to ensure deliverables for the project.

3. Total Grant Variance

Provide explanation for any cost category variances outside the allowable range. Explain causes, consequences for the project, and mitigation plans if relevant. Report whether or not approval for the variance has been obtained from your Program Officer.

Note: "Total grant variance" compares actuals plus current projections to the budget. See "Financial Summary & Reporting" sheet in the foundation budget template for calculated variance. If you are using an older version of the budget template, this information could be in a different location in your template. Allowable variance is defined in your grant agreement.

N/A

4. Sub-awards (if applicable)

Use the chart to provide the name(s) of the sub-grantee(s) or subcontractor(s), actual disbursement for this reporting period, total disbursement to date from the primary grantee to sub-awardee, total spend to date by the sub-awardee and total contracted amount.

Note: The total of actual disbursements for this reporting period should equal the actual Sub-awards expenses reported on the "Financial Summary & Reporting" sheet in the foundation template for this reporting period. If you are using an older version of the budget template, this information could be in a different location in your template.

Organization Name	Actual Disbursement for this Reporting Period (U.S.$)	Total Disbursed from Primary Awardee to Sub to Date (U.S.$)	Total Sub-Awardee Spent to Date (U.S.$)	Total Contracted Amount (U.S.$)
IITA	$166,546	$166,546	$133,930	$648,702
U of Helsinki	$101,076	$101,076	$10,891	$310,538
UW-Madison	$194,762	$194,762	$6,542	$487,890
	$462,384	$462,384	$151,363	$1,447,130

5. Other Sources of Support (if applicable)

List and describe any sources of *in-kind* project support or resources received in the reporting period.

Note: Names of the other sources of funding and their contributions (U.S.$) should be included in the budget template on the "Financial Summary & Reporting" sheet in the foundation budget template in the Funding Plan table. If you are using an older version of the budget template, this information could be in a different location in your template.

N/A

Describe how interest earned and/or currency gains were used to support the project.

The interest is being accrued until the end of the project. With donor's authorization, these additional fund will be used for the benefit of the project.

Privacy and Non-Confidentiality Notice

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For Foundation Staff to Complete

Analysis (required if contingent payment or PO assessment differs from grantee/vendor assessment)

Progress Analysis

Include analysis of significant project variances and key learnings that may inform portfolio discussions for progress against the strategic goals.

Budget and Financial Analysis

Include analysis of unexpended funds or over expenditures. Refer to the Unexpended Grant Funds Policy for options available when recommending how to handle unexpended grant funds, or reach out to your primary contact in GCM.

Scheduled Payment Amount	$
Carryover Amount	$
Recommended Payment Amount	$

Approver Comments (if applicable)

Name	Title	Date Approved

Comments