Reliable sample preparation technology for Point-of-Need Applications

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Vision

To take complex molecular diagnostics out of the lab and place into the hands of non-experts to facilitate rapid, accurate and cost-effective responses to developing situations in real time.
Amplification: PCR/LAMP

- Using amplification chemistry, target sequences unique to an organism.
- Make enough copies and the diagnostic sequence is easy to detect!
- Detection can be by colour change, electrophoresis or fluorescence
DNA sequencing:

- Technology revolution going on in this space since the late 1990s
- Cheaper and faster
- Computationally demanding! Genomes are very big
- New technologies addressing these issues
- Point-of-Need Sample Preparation
- Extraction must be fully self-contained and deliver NA ready for qPCR/Sequencing.
- Robust with few moving parts and low power requirements
- No pipetting steps to avoid sample cross contamination.
- Reliable reaction chemistry capable of dealing with a range of sample types.
PDQeX

- Pretty Damn Quick eXtraction
- Three components: (a) Temperature control unit (b) Plastic disposable (c) Reaction chemistry
**PDQeX DNA extraction process:**

1. **Load** PDQeX Extraction Tube with sample and reagents.
2. Place PDQeX Extraction Tube into top reaction port of the PDQeX device and position a sample collection tube in the bottom collection draw.
3. Select extraction program from either a tethered laptop or mobile phone.
4. Purified DNA expelled into sample collection tube at completion of run.

Sample:
- Plant material
- Card punch
- Swab
- Cells
- Blood
- Etc.
PDQeX Performance – dilution series:

A ten-fold serial dilution of Plasmid.

2μl dried onto a FLOQ swab (Copan). ‘No DNA’ Swabs were included.

Swabs were placed in PDQeX extractor tubes with reagent. Extraction performed using ‘Buccal Program’.

qPCR on all extracted samples using Promega GoTaq Probe qPCR Master Mix with Abbott HPV Probe and Primer set 1.

Cq values and PDQeX loading order in Table. Cq for NTC = - ; HPV18 Positive qPCR control = 16.83

qPCR analysis reflected the amount of input HPV18 DNA added to the FLOQ swab
**PDQeX Performance - repeatability and cross contamination:**

1µl plasmid added to 8 PDQeX extraction tubes. 8 PDQeX extraction tubes received no DNA.

Extraction tubes were loaded in a checker-board pattern of DNA(+) and DNA(-) samples.

The experiment was repeated 3 times, in forward and reverse tube orientation. The device was not decontaminated between each run.

qPCR performed using Promega GoTaq Probe qPCR Master Mix with Abbott HPV Probe and Primer set 1.

Cq values and PDQeX loading order given in Tables A, B and C for each run.

No cross contamination. qPCR was highly reproducible from identical DNA input.

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Average Cq: 18.94  Std Dev: 0.42  CV: 2.23%

No inter-sample cross contamination
**PDQeX Performance - limit of detection:**

*Target DNA:* Plasmid DNA at 9.6 x10^6 molecules/µl

*Method:* 1µl plasmid solution spotted onto different collection material – either Cotton swab or Whatman No. 4

*DNA extraction and qPCR:* prepGEM reagent in PDQeX extraction tube. LC480 using 2 SYBR-based chemistries and Abbott HPV Probe and Primer Set 1.

*Results:* Cq, qPCR amplification chemistry and molecule number in 7µl of extracted DNA sample are given in the Table.

*Conclusion:* PDQeX reliably detected approx. 700 input molecules.
Sample types

• Swabs
• Bacteria
• Blood
• Vaginal Swab
• Plant
• Bone (almost)
• Forensic samples
Two PDQeX Case Studies

Cassava Virus Action Project
Laura Boykin, UWA

Tb DNA direct from Sputum
Htin Lin Aung, Greg Cook, Philip Hill
TB Project:

• Sequencing Tb from an infected patient would confirm both diagnosis and antibiotic resistance status of the disease.
• Currently, some culturing of patient samples are required before sequencing can be performed.
• Can enough DNA be extracted directly from sputum samples for sequencing using PDQeX?
• To date, we have used cultured material to show the PDQeX system removes viable organisms following extraction, delivers DNA ready for downstream detection of Tb in qPCR and MinION.
Cassava Mosaic Virus: East Africa

• Diagnostic sequencing is not readily available in East Africa.
• Samples sent to international facilities are not given priority, can degrade on route and testing is expensive.
• Time delays (+6 months) mean that once a diagnosis is made the opportunity to replant with appropriately resistant cultivars is missed.
• The outcome: Families go hungry, debts are incurred to cover living expenses, children do not go to school.
• A quick, accurate decision changes lives
Tanzania: Asha’s Story

• In 2017, in lab MinION sequencing of Asha’s cassava with three day turnaround made replanting with virus resistant crop possible.
• Harvest in July 2018. Yield from 0 ton/hectare to over 35 ton/hectare.
• Now Asha’s main problem: where do I sell my excess Cassava?
• Asha heads a collective of about 8 women subsistence farmers. We estimate that this work positively impacted the lives of around 3000 people through direct and indirect economic benefit.
Learning from Asha

• Quick accurate diagnosis will positively impact individuals and communities.
• But Asha’s farm is in Dar Es Salaam, close to laboratory facilities.
• What about the farmers that are not close to lab facilities? Can diagnostic sequencing be done on farm?

Morogoro, Tanzania
Sample to sequence diagnostic: In field, no mains power, 3-4 hours.

**PDQeX + MinION with MinIT**

Samples = Cassava Leaf, Stem and Whiteflies

Three in-field sequencing runs, one each in Tanzania, Uganda and Kenya.

All samples generated sequence data.

Cassava Mosaic Virus (CMV) detected in plants with symptoms. Healthy plant showed no virus sequences. Asymptomatic plants grown with virus infected plants carried low viral titers.

CMV detected in single whitefly.
So, can diagnostic sequencing be done on farm?

Almost!

• With a team of experts, PDQeX, MinIT and MinION, on farm sequencing completely off-grid is possible.
• Our current challenge is to take our proof-of-concept and repackage the technology so more people have access to it.
• Bringing diagnostic screening to the farm gives possibilities for immediate action that prevents hunger and loss of income. Food security!
Acknowledgements

Mikocheni Agricultural Research Institute, Tanzania
Prof Joseph Ndunguru
Dr Peter Sseruwagi
Charles Kayuki
Deogratius Mark

University of Western Australia, Australia
Dr Laura Boykin

National Crops Resources Research Institute, Uganda
Prof Titus Alicai
Geoffrey Okao-Okia
Phillip Abidrabo
Apio Hellen Beatrice
Jancinta Akol

JUKAT, Kenya
Prof. Elijah Ateka
Brenda Muga

Mamainana, Kisaxiwene Village, Sarah and Naomi who kindly let us sequence their Cassava and shared the warm hospitality of their farms.
ZyGEM NZ Ltd
David Saul
Orion Scott
Abishek Muralidhar
Grant Broomfield
Kirsty Mayall
Sharon Fisher

AD Instruments
Michael Macknight

University of Otago
Chris Rawle
Christy Rand
Peter Whigham
Htin Lin Aung
Mat McNeil
Merilyn Hibma
Allison Tschirley

CM Programming
Chris Mason

Acknowledgements continued

Funding: Smart Ideas MBIE UOOX1507/ Research Program UOOX1703