Reliable sample preparation technology for Point-of-Need Applications

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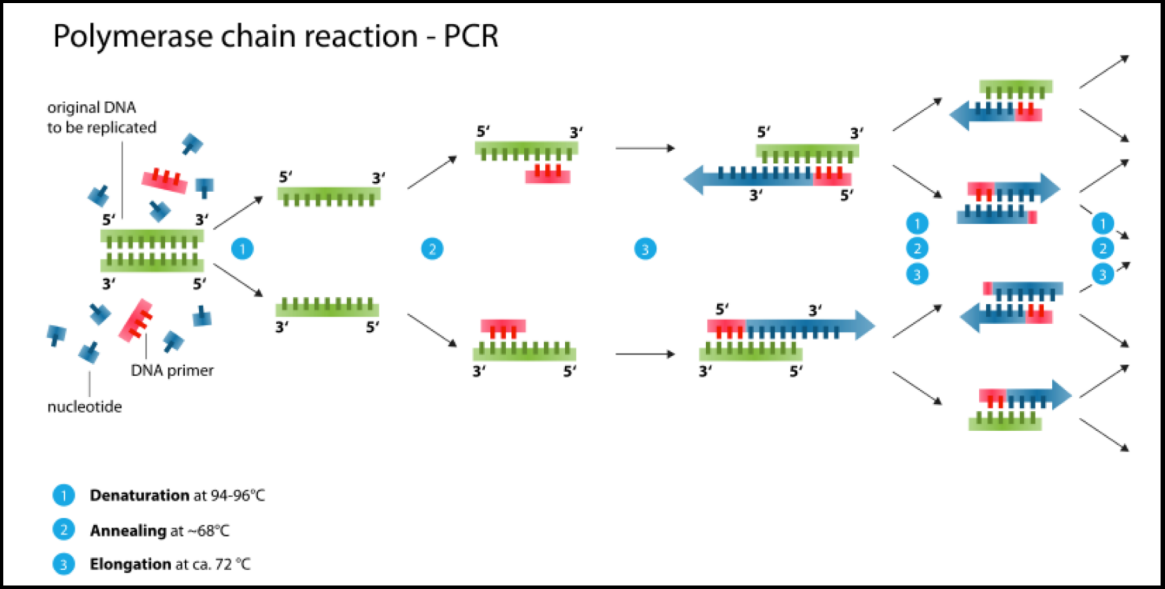
Vision

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

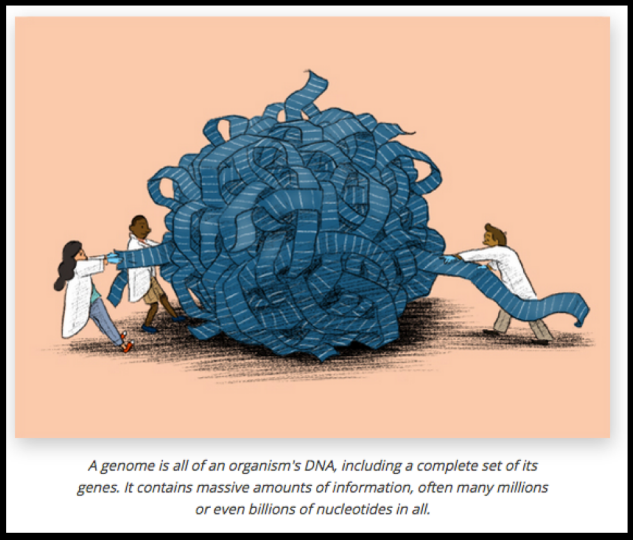
- Using amplification chemistry, target sequences unique to an organism.
- Make enough copies and the diagnostic sequence is easy to detect!
- Detection can be by colour change, electrophoresis or fluorescence




GeneXpert
Xpert® MTB/RIF

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



A genome is all of an organism's DNA, including a complete set of its genes. It contains massive amounts of information, often many millions or even billions of nucleotides in all.

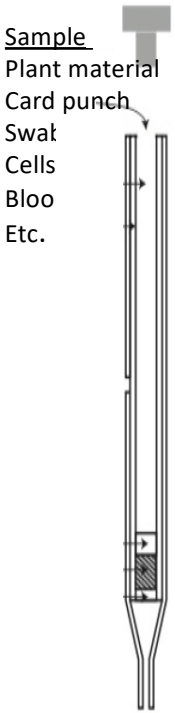
https://tvtropes.org/pmwiki/pmwiki.php/Creator/Banksy

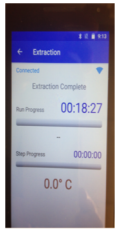
- Point-of-Need Sample Preparation
- Extraction must be fully self-contained and deliver NA ready for qPCR/Sequencing.
- Robust with few moving parts and low power requirements
- No pipetting steps to avoid sample cross contamination.
- Reliable reaction chemistry capable of dealing with a range of sample types.

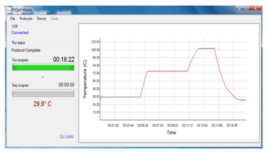
PDQeX

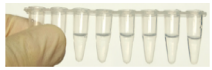
- Pretty Damn Quick eXtraction
- Three components: (a) Temperature control unit (b) Plastic disposable (c) Reaction chemistry

PDQeX DNA extraction process:









Load PDQeX Extraction Tube with sample and reagents

Place PDQeX Extraction Tube into top reaction port of the PDQeX device and position a sample collection tube in the bottom collection draw

Select extraction program from either a tethered laptop or mobile phone

Purified DNA expelled into sample collection tube at completion of run.

PDQeX Performance – dilution series:

A ten-fold serial dilution of Plasmid.

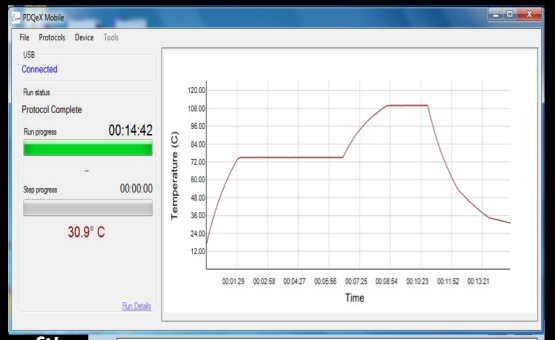
2µl dried onto a FLOQ swab (Copan). ‘No DNA’ Swabs were included.

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

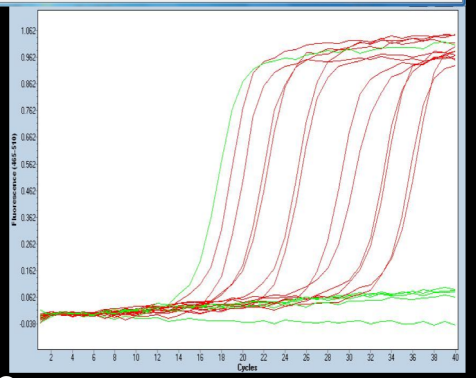
qPCR on all extracted samples using Promega GoTaq Probe qPCR Master Mix with Abbott HPV Probe and Primer set 1.

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 run profile



Load order and Cq

Position 1	Position 2	Position 3	Position 4	Position 5	Position 6	Position 7	Position 8
HPV18 Dilution 1 15.89	HPV18 Dilution 2 19.27	HPV18 Dilution 3 22.41	HPV18 Dilution 4 27.48	HPV18 Dilution 5 30.22	HPV18 Dilution 6 32.48	HPV18 Dilution 7 -	No DNA on Swab -
No DNA on Swab -	HPV18 Dilution 7 -	HPV18 Dilution 6 33.16	HPV18 Dilution 5 30.55	HPV18 Dilution 4 26.05	HPV18 Dilution 3 22.16	HPV18 Dilution 2 18.84	HPV18 Dilution 1 Value not called
Position 9	Position 10	Position 11	Position 12	Position 13	Position 14	Position 15	Position 16

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.

Table A

Position 1	Position 2	Position 3	Position 4	Position 5	Position 6	Position 7	Position 8
HPV16 18.69	No DNA -	HPV16 19.28	No DNA -	HPV16 19.15	No DNA -	HPV16 18.99	No DNA -
No DNA -	HPV16 19.21	No DNA -	HPV16 19.63	No DNA -	HPV16 19.12	No DNA -	HPV16 19.17
Position 9	Position 10	Position 11	Position 12	Position 13	Position 14	Position 15	Position 16

Table B

Position 1	Position 2	Position 3	Position 4	Position 5	Position 6	Position 7	Position 8
HPV16 19.46	No DNA -	HPV16 18.84	No DNA -	HPV16 19.16	No DNA -	HPV16 18.95	No DNA -
No DNA -	HPV16 18.64	No DNA -	HPV16 18.83	No DNA -	HPV16 18.99	No DNA -	HPV16 20.09
Position 9	Position 10	Position 11	Position 12	Position 13	Position 14	Position 15	Position 16

Table C

Position 1	Position 2	Position 3	Position 4	Position 5	Position 6	Position 7	Position 8
No DNA -	HPV16 18.41	No DNA -	HPV16 18.55	No DNA -	HPV16 18.54	No DNA -	HPV16 18.23
HPV16 18.82	No DNA -	HPV16 18.58	No DNA -	HPV16 18.49	No DNA -	HPV16 18.70	No DNA -
Position 9	Position 10	Position 11	Position 12	Position 13	Position 14	Position 15	Position 16

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: *prep*GEM 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.

No. DNA target molecules in 7µl elute*	Whatman No. 4			Cotton Swab	
	A°	B§	C°	qPCR 1§	qPCR 2°
6700000	22	18	21	18	20
670000	26	23	26	22	23
67000	29	25	28	29	30
6700	32	30	32	29	35+
670	35+	35+	35+	32	34
67	0	35+	35+	0	0
0	0	0	0	0	0
qPCR Blank	0	0	0	0	0

*Assumes 100% DNA recovery; °Quanta qPCR Mix; §Promega qPCR reagent.

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 secruity!

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-Okiya
- Phillip Abidrabo
- Apio Hellen Beatrice
- Jancinta Akol

JUKAT, Kenya
- Prof. Elijah Ateka
- Brenda Muga

Mamamaina, Kisaxiwene Village, Sarah and Naomi who kindly let us sequence their Cassava and shared the warm hospitality of their farms

Acknowledgements continued

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

Funding: Smart Ideas MBIE UOOX1507/ Research Program UOOX1703