1-7-2016

2016 AQ Summit: Innovation Update by Laurie Connell

Laurie B. Connell
Principal Investigator; University of Maine, Orono, laurie.connell@umit.maine.edu

Rapid Onsite Detection of Pathogenic Microbes For Aquaculture

Major Collaborators and funding

NOAA
MBARI
Mbio
NSF
MTI
USDA

Find more information about projects at

http://umaine.edu/connelllab/projects/sensor-development/

Working with various backgrounds

Red Tide

Potato wart fungus

Vibrio

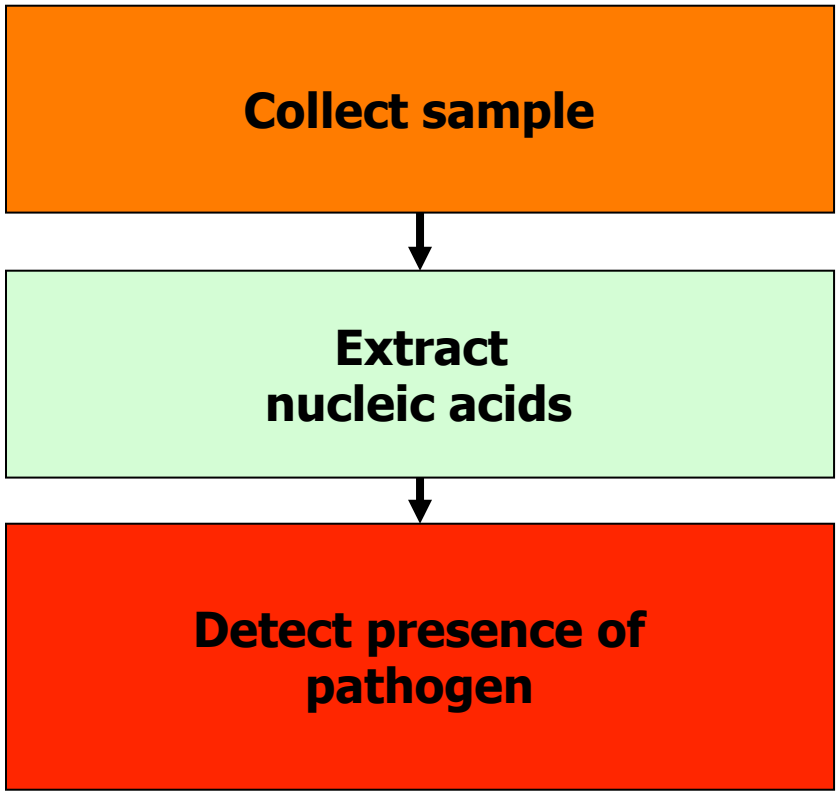
Beverage spoilage yeasts

Variables considered in technique selected

Level of skill for operator
Time involved in assay
Amount of information required
Cost of assay
Location of operation

Tendency to move toward "cheaper, quicker, easier" assays

GOAL: Field-deployable nucleic acid-based detection method



Molecular probe assays

Intact cells

DNA, antibody, lectin probes

Homogenized cells

Toxins, proteins, nucleic acid sequences

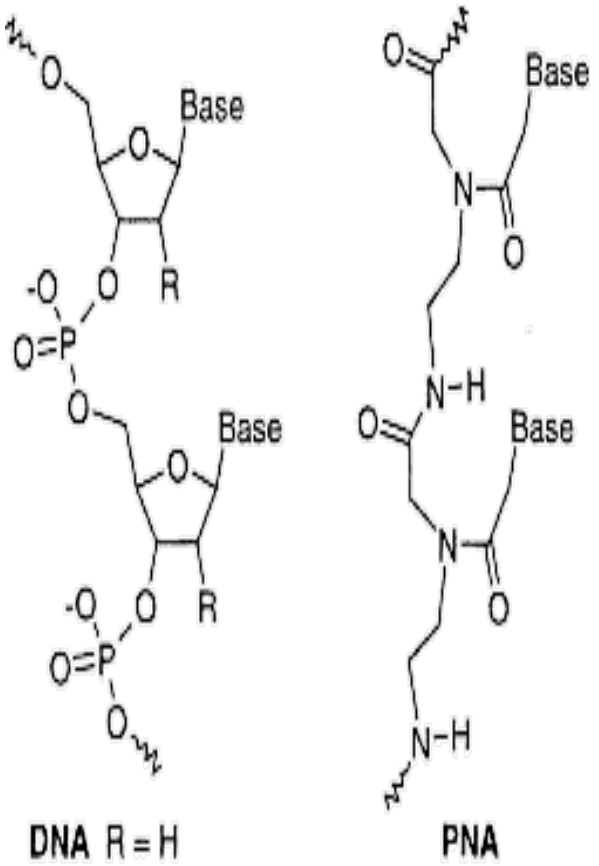
Field-compatible NA detection technologies

Platform	Technology	Time	LOD	Pros	Cons
RAZOR™ EX	Nested PCR	<35 min	100 cells/mL	•Fast •Sensitive •Portable •Minimal training needed	•High cost •Pure DNA sample required
QuickFISH™	FISH	~20 min	N/A	•Fast •Specific •Sensitive •Minimal training needed	•High cost •Slide reader and microscope needed •Requires refrigeration •Not portable •Currently only for blood samples
Bio-Seeq™	PCR	~65 min	100 cells	•Sensitive •Specific •Portable	•High cost •Training needed
IC-NASBA	NASBA + MB	<90 min	1 cell	•Specific •Sensitive •Portable	•High cost •3 enzymes needed •Pure DNA sample required
LFNAB	Lateral flow	~30 min	12.5 aM	•Fast •Sensitive •Portable •Minimal training needed	•Requires refrigeration •Untested robustness •Labor-intensive preparation
SPIRIT	SPR	~20 min	10 cells	•Fast •Sensitive •Minimal sample processing required	• High cost •Training needed •High variability •Needs computer

peptide nucleic acid (PNA)

DNA

PNA

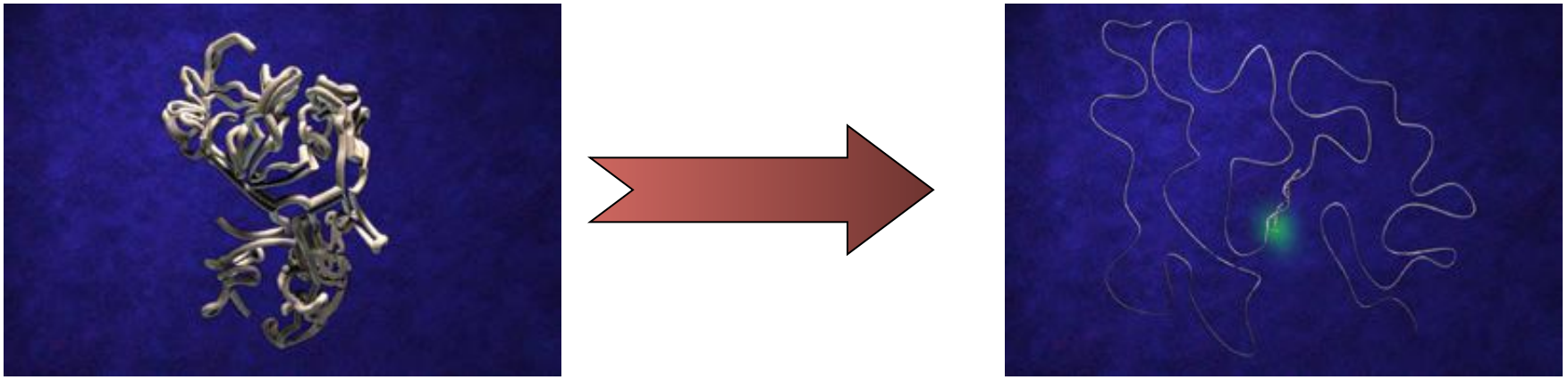


Sugar backbone

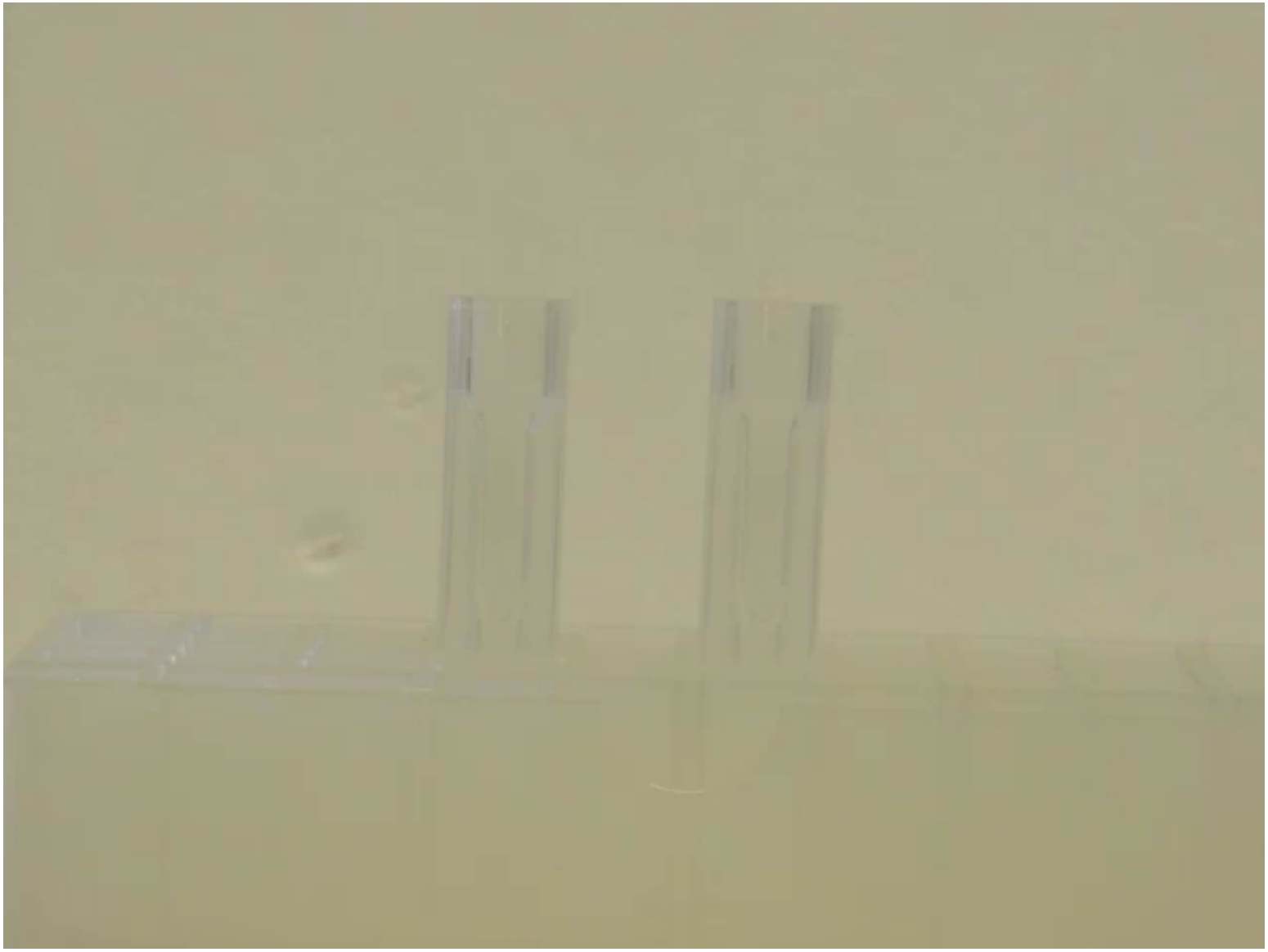
Peptide backbone

PNA for field use

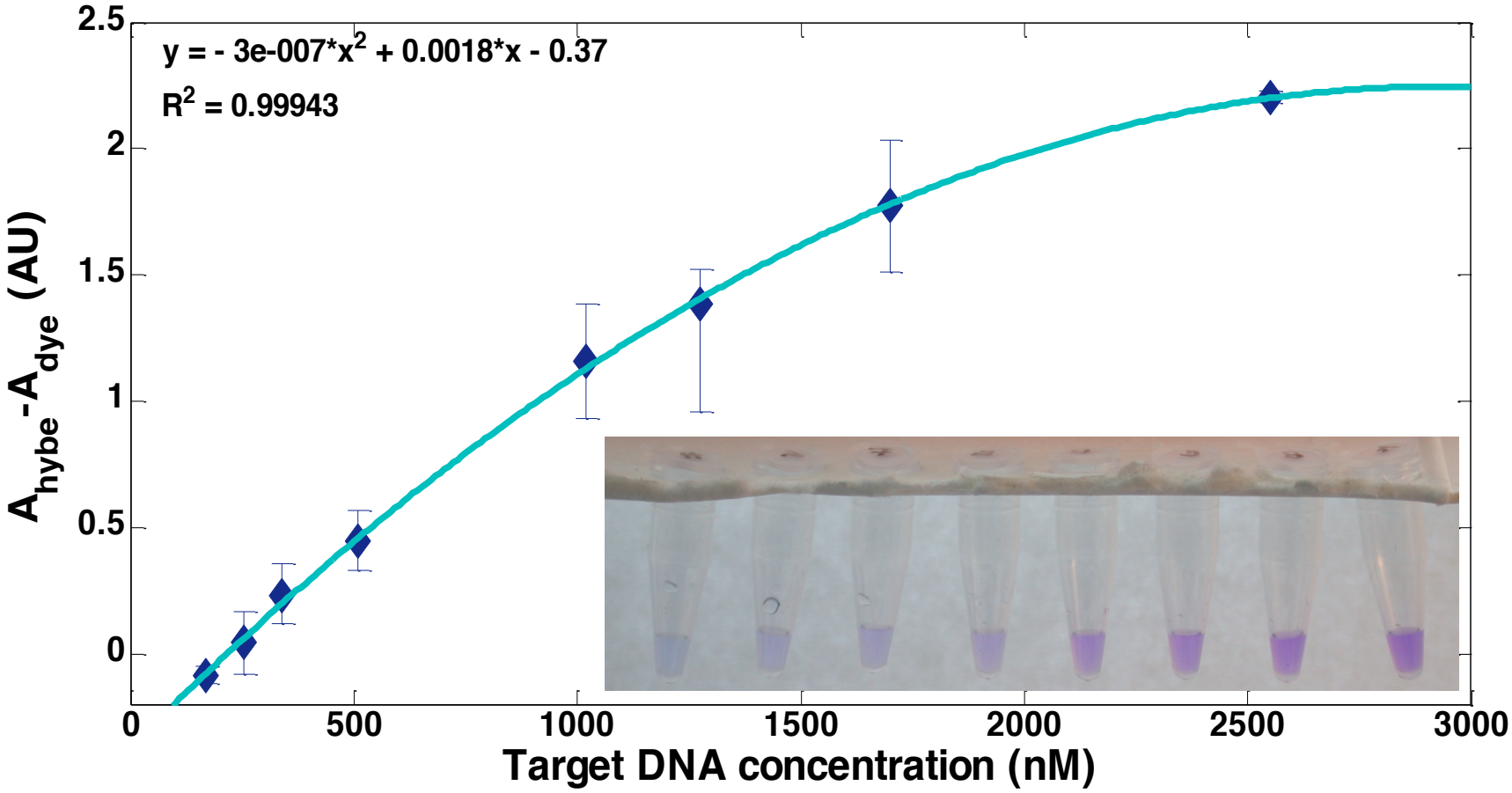
- Tm is independent of ionic strength
- resistant to nucleases and proteases
- bind in low salt conditions

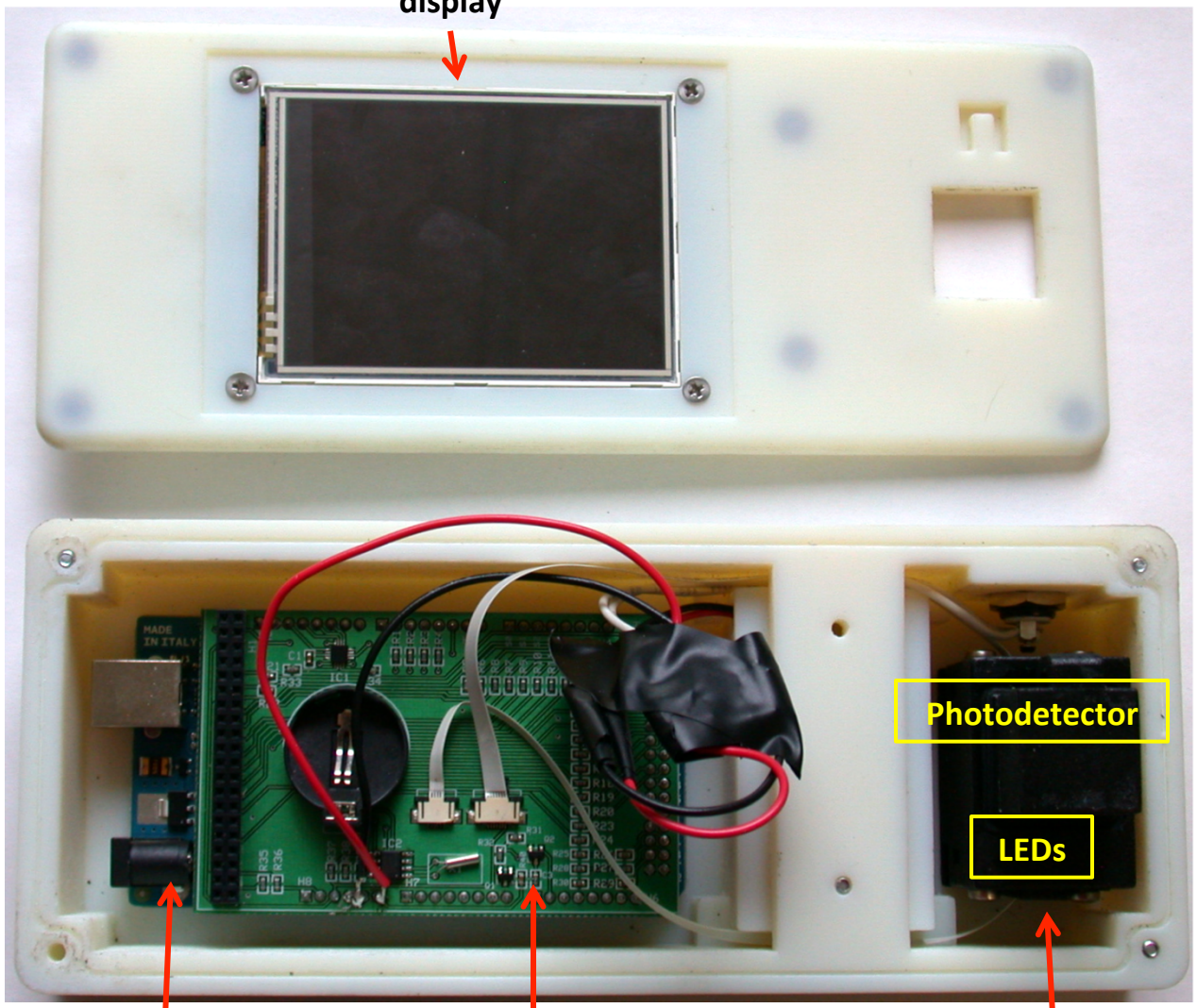


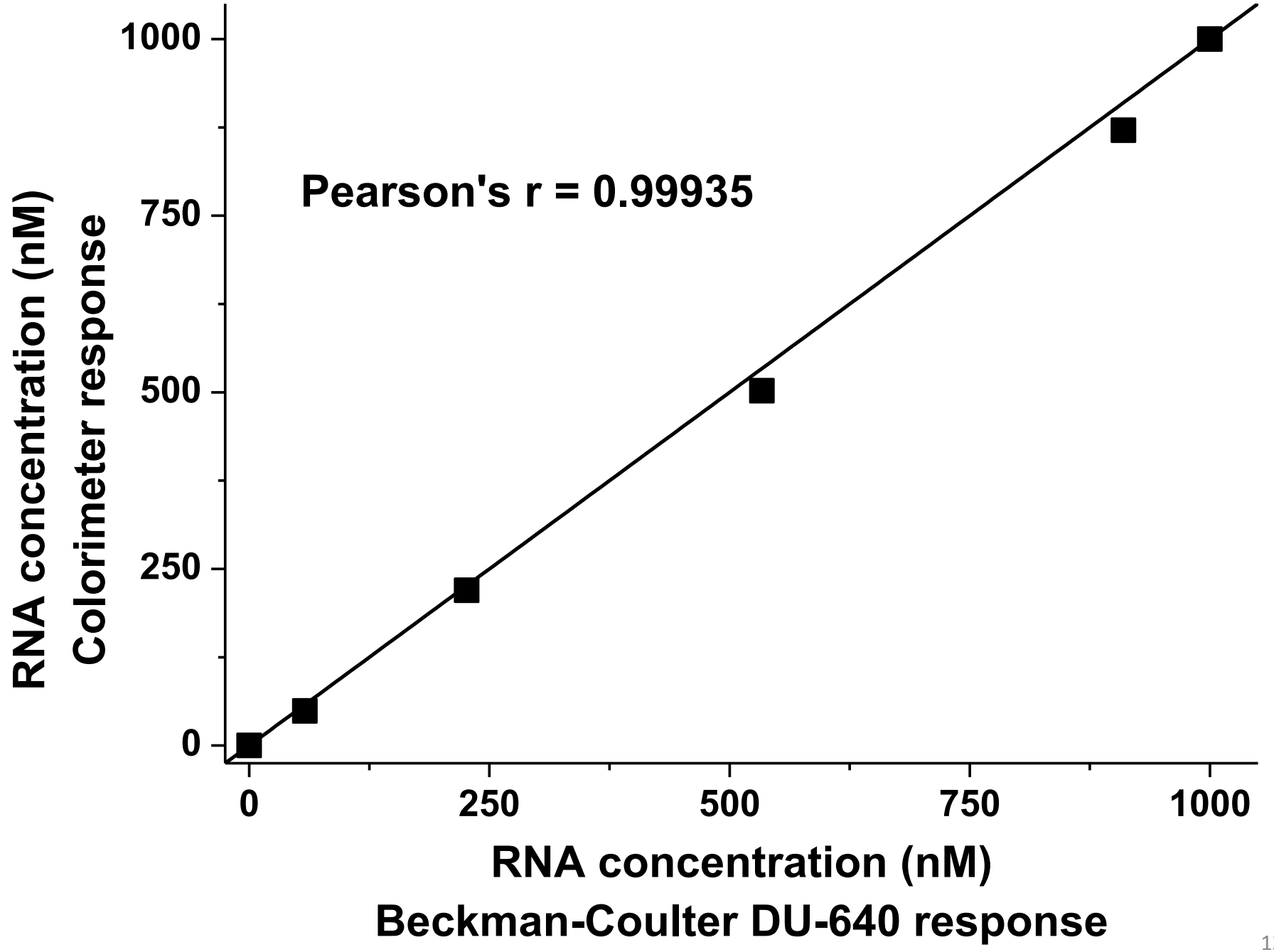
Reaction is near instantaneous



Detection of varying target concentrations




Touchscreen user input/ display
Photodetector
LEDs
Arduino Mega 2560
Custom printed circuit board “shield” for microcontroller
Custom cuvette holder


Pearson's r = 0.99935
RNA concentration (nM)
Colorimeter response
0
250
500
750
1000
RNA concentration (nM)
Beckman-Coulter DU-640 response

Current work Moving to FIT probes for higher sensitivity

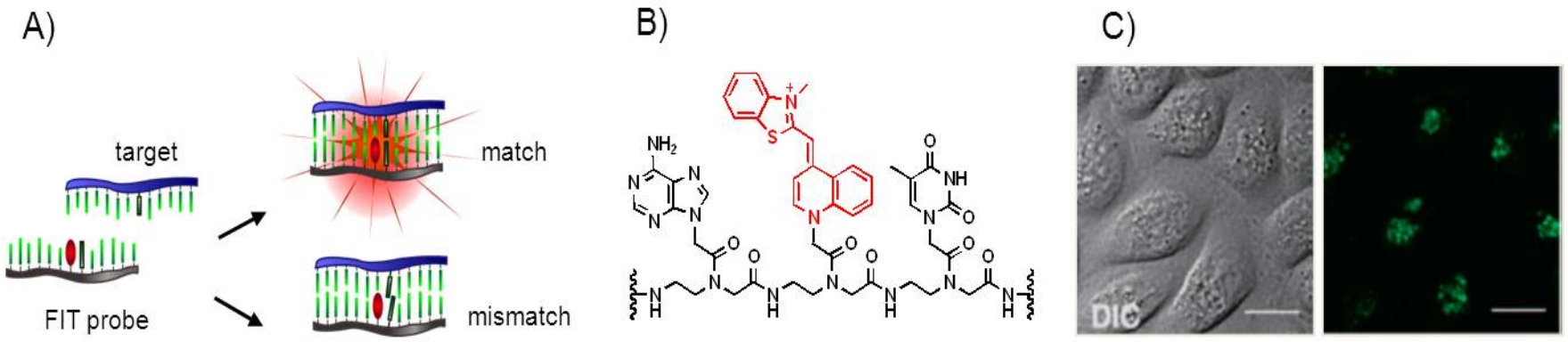


Figure 1. A) Sequence discrimination with Forced Intercalation (FIT) Probes. B) Chemical structure of a PNA FIT-Probe. C) Imaging of mRNA in living cells

https://www.chemie.hu-berlin.de/en/forschung-en/seitz/research/read-detect-nucleic-acid-sequences/figure-01.jpg/image