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Department of Bioengineering

Amplification-free sensing of circulating nucleic acid biomarkers in blood:
A minimally invasive tool for early diagnosis of cancer

Precision Medicines 2016 Engineering solution for Cancer
London, 14th July 2016

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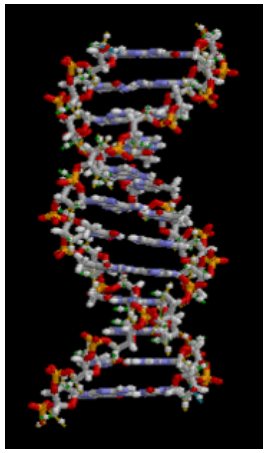
Molecular Bioengineering

How an academically trained chemist (or molecular bioengineer)



working in a Bioengineering department

can address public health problems and engineer solutions for cancer

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Our goal: Diagnose more cancers better and earlier

Increasing survival rate by treating early, only if necessary and with the best suited drugs
requires…

- Highly specific biomarkers (or combination of biomarkers)
- Non-invasive tests for widespread public screening (affordable, robust and easy-to-use)

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Prostate cancer: A case study

Prostate cancer is currently diagnosed too late

In the UK only, around 35,000 new cases diagnosed per year, around a quarter would be already metastatic - but that's only in the UK

And non-invasive diagnostic blood tests lack specificity

Current diagnosis results from the presentation of clinical symptoms such as urine hesitancy, erectile dysfunction and decreased urination flow, whereupon a plasma or serum sample's prostate specific antigen (PSA) concentration will be assessed. Typically, results of 4ng/ml or above result in a prostate biopsy to confirm a neoplasm. However, it is reported that over **75% of positive PSA tests are in fact false positives**

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Public screening

from NHS website

"There is currently no screening programme for prostate cancer in the UK [...] Instead of a national screening programme, there is an informed choice programme on prostate cancer risk management. It aims to give men good information on the pros and cons of a PSA test"

Private healthcare offers patients a prostate cancer screening service.

ProstateCheck: £360
Follow-up appointments range from £200 to £250
Could eliminate 30-50% of all unnecessary biopsies

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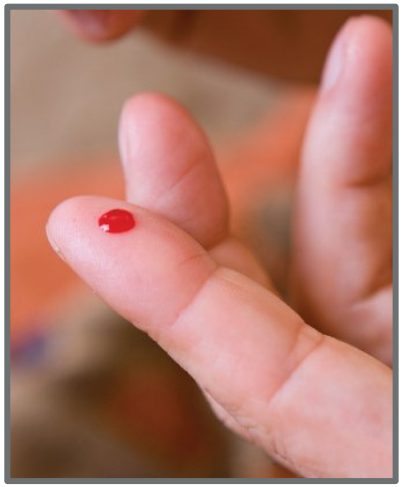
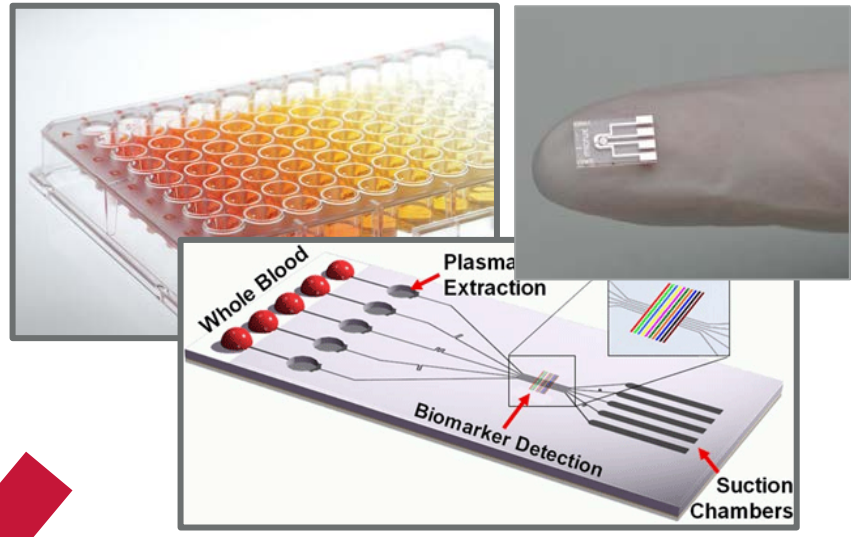
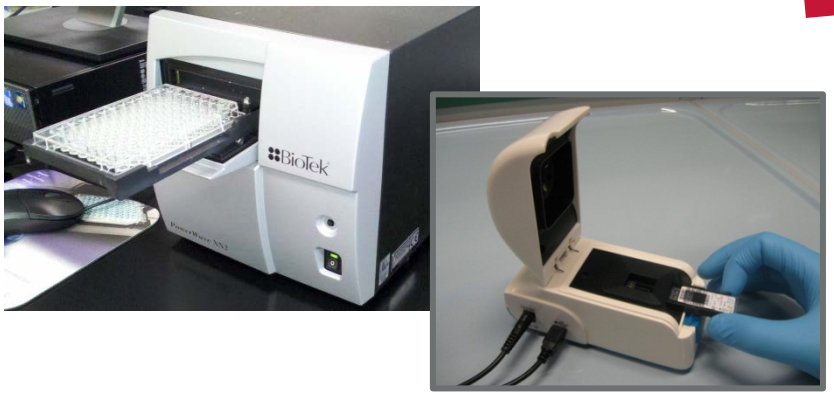
Our answers to an unsolved problem







- Minimal sample volume
- Automated sample processing
- Benchtop analysis
- High specificity
- Low cost

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Our Target: Circulating micro RNAs

Need for reliable, highly sensitive, specific and quantitative sensing technology

- Secretion in body fluids (saliva, serum, plasma…)

Ideal for minimally invasive diagnosis

BUT

- Low concentration in these cell-free fluids
- miRNAs have very similar sequences

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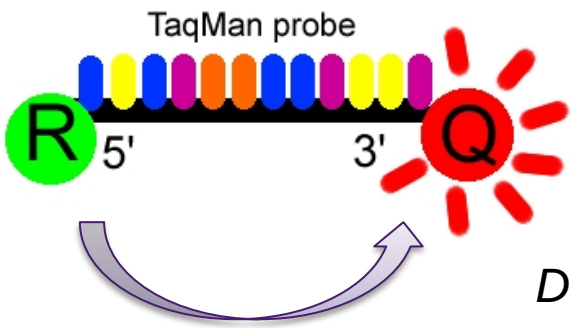
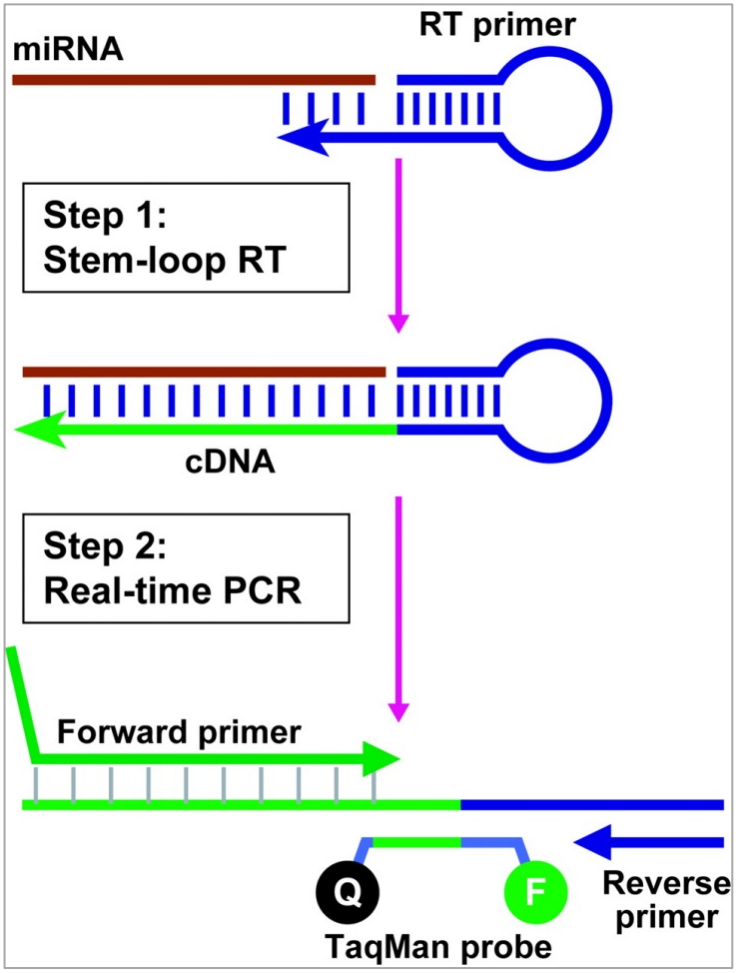
The gold standard: RT-qPCR and Taqman probes



Dual-labelled DNA

- Taqman probes expensive and often unreliable (lack of specificity, reproducibility and strong background fluorescence)
- Double amplification (prone to contamination and source of errors)



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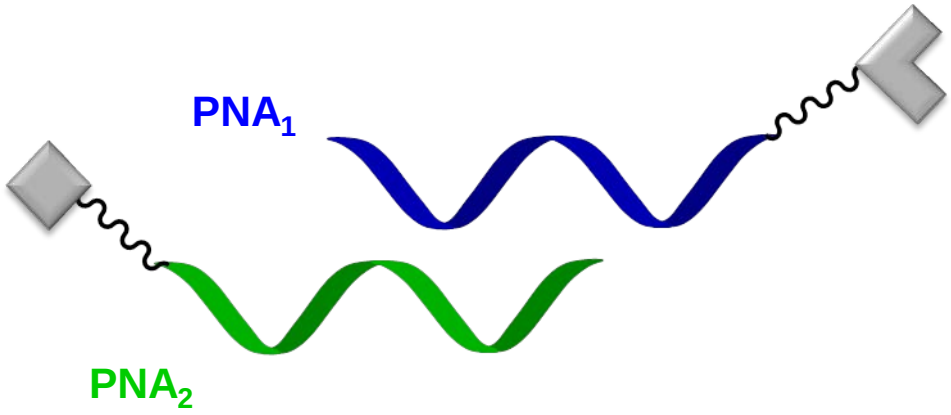
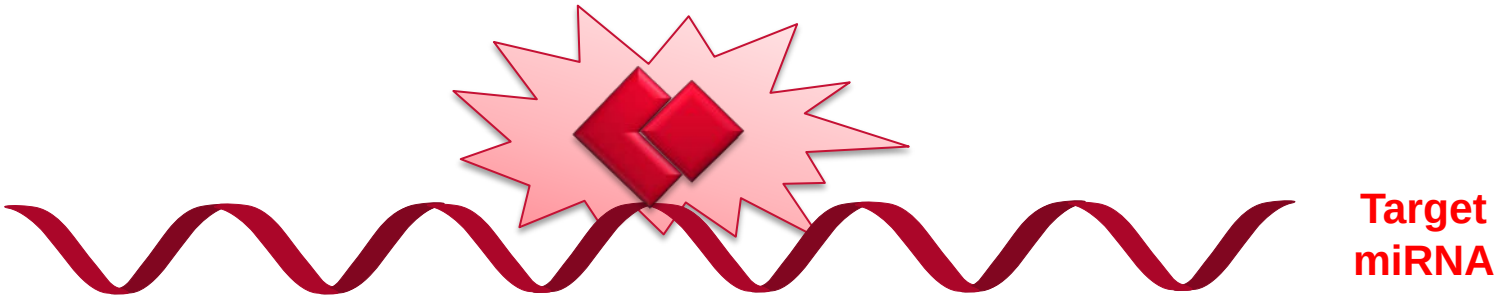
Our Strategy: Oligonucleotide-templated reactions



Optical Readout



Using a DNA or RNA strand as a template to catalyse an otherwise unfavourable reaction of formation of a fluorescent dye

Y. Choi, G. Metcalf, M. Sleiman, D. Vair-Turnbull, S. Ladame *Bioorg. Med. Chem.* **2014**, *22*, 4395.
C. Percivalle, J.F. Bartolo, S. Ladame, *Org. Biomol. Chem.* **2013**, *11,* 16.

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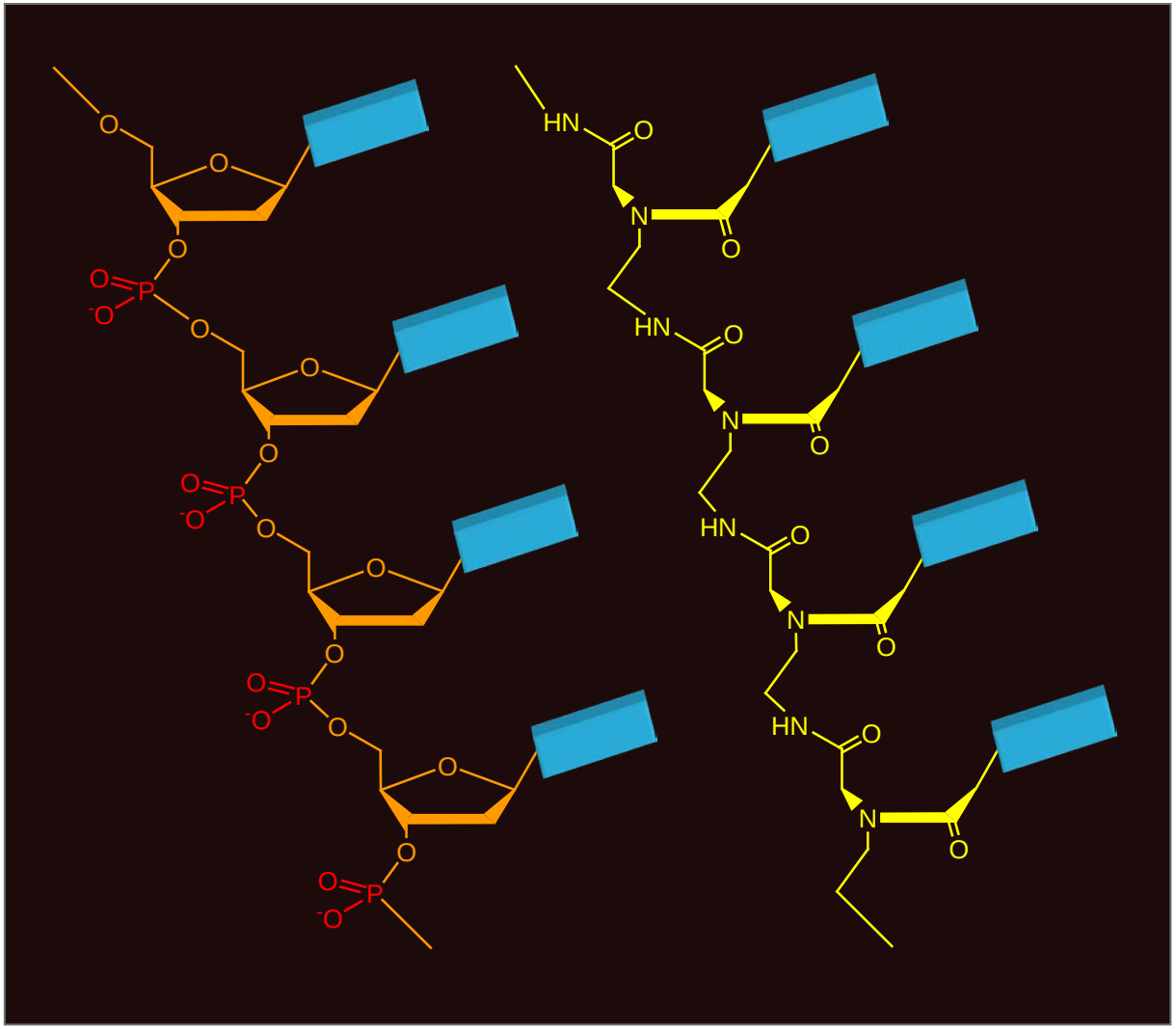
Our scaffold: Peptide Nucleic Acids (PNAs)



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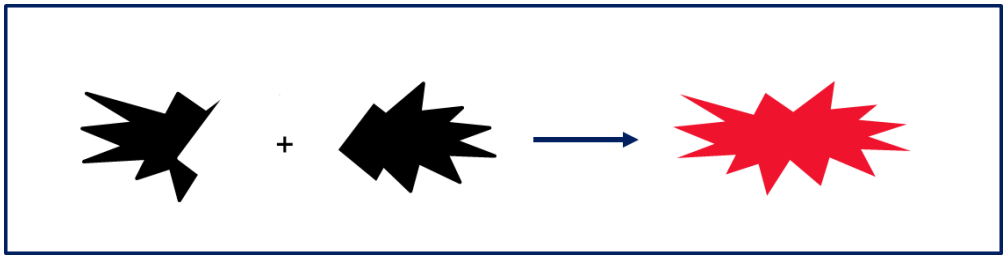
Our probe-heads: A toolbox of multicolour dyes



Emission λ_{max} = 520-530 nm
Coumarin unquenching

Emission λ_{max} = 550-555 nm
Cy3 synthesis

Possibility of multiplexed (multicolour) analysis

Emission λ_{max} = 600-605 nm
C3 synthesis

Emission λ_{max} = 645-650 nm
Squaraine dye synthesis

M. Sleiman, S. Ladame, *Chem. Commun.* **2014**, *50*, 5288.
K. Meguellati, G. Koripelly, S. Ladame, *Angew. Chem. Int. Ed.* **2010**, *49*, 2738.
G. Koripelly, K. Meguellati, S. Ladame, Bioconjug. Chem. **2010**, *21*, 2103.

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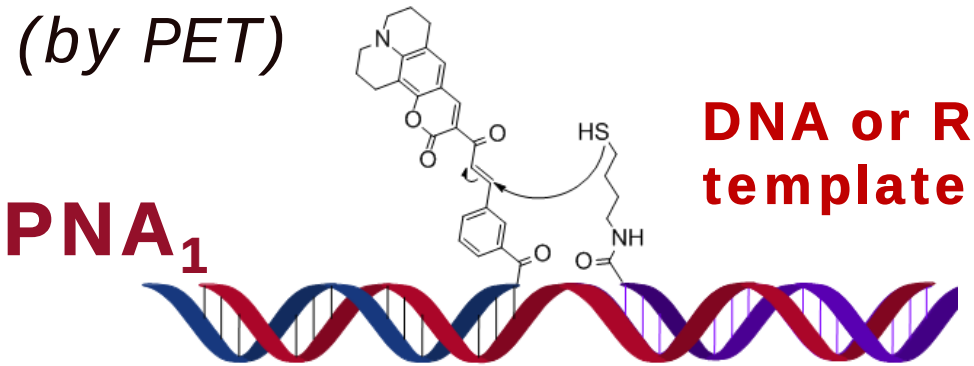
Our Chemistry: Reaction of fluorescence unquenching

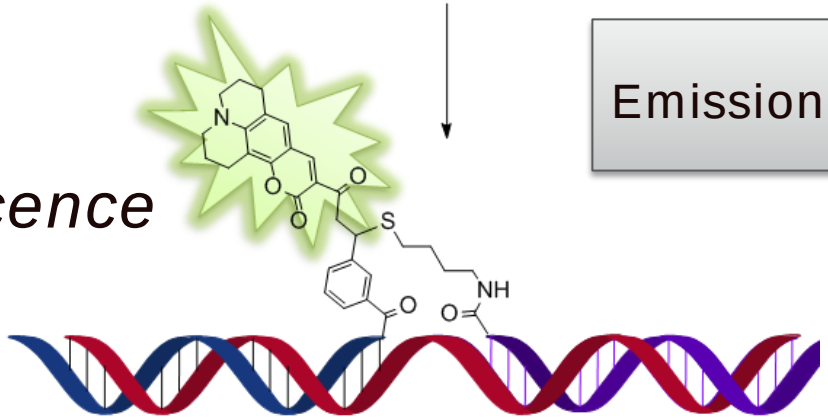
Quenched fluorescence (by PET)



DNA or RNA template

PNA_1

PNA_2



Strong Fluorescence

Emission λ_{max} = 520-530 nm

G. Metcalf, A. Shibakawa, H. Patel, A. Sita-Lumsden, A. Zivi, N. Rama, C.L. Bevan, S. Ladame *Anal. Chem.*, **2016**, accepted

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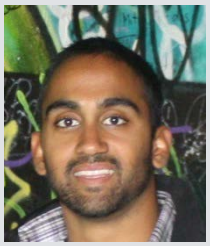
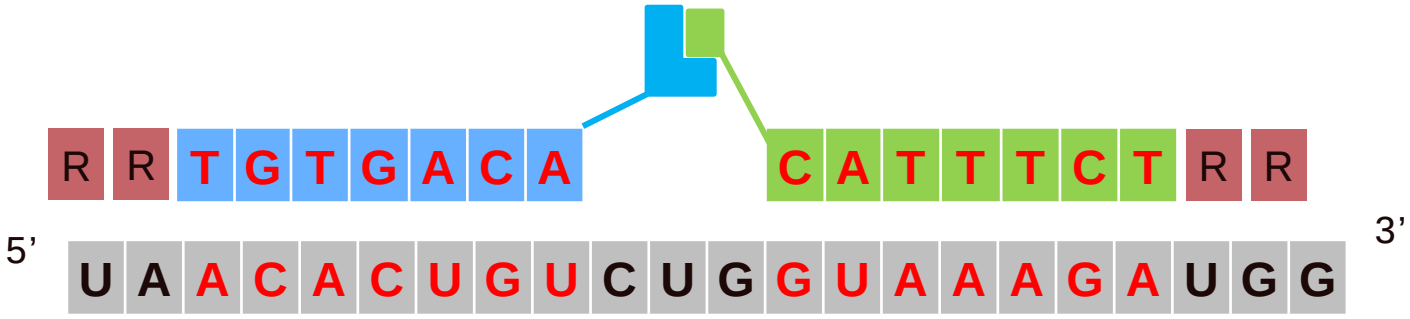
Proof-of-concept study



MiR-141 and miR-375: biomarkers for prostate cancer (high levels compared to healthy controls)

MiR-132: biomarker for ovarian cancer (low levels compared to healthy controls)



miR-141

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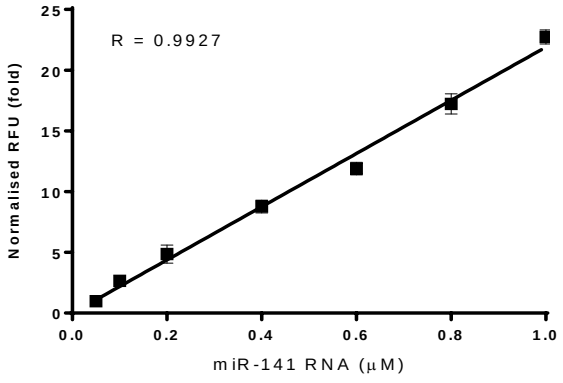
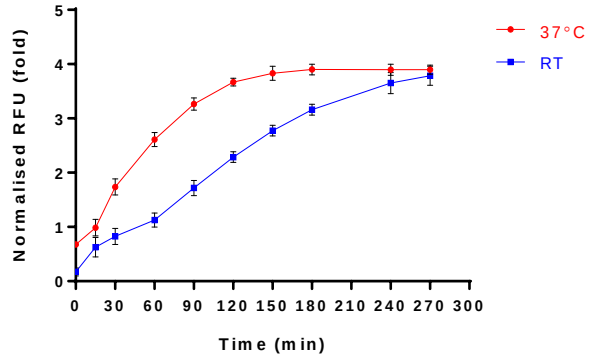
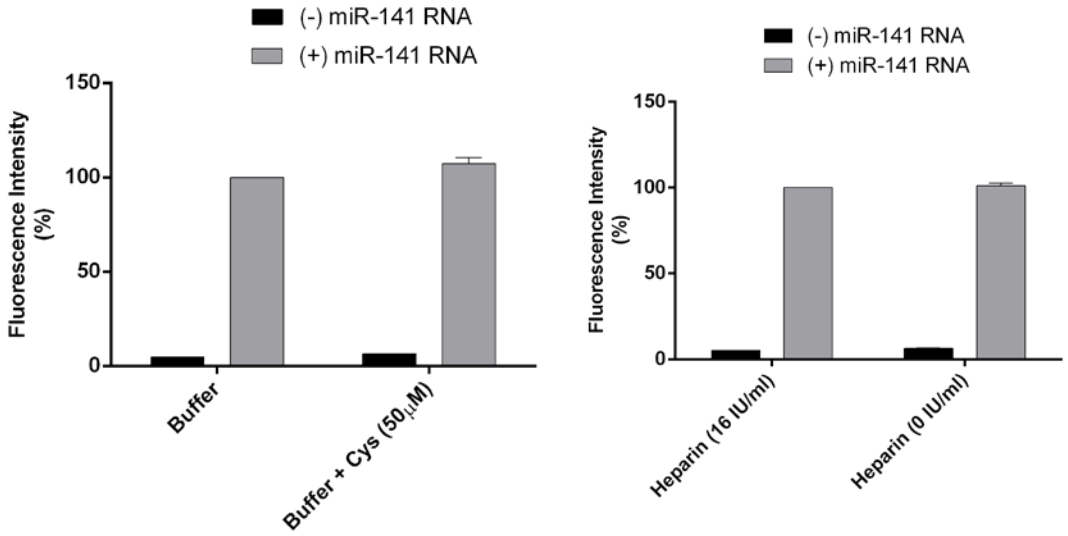
In vitro validation





Quantitative detection
Relatively fast



Specific detection

G. Metcalf, A. Shibakawa, H. Patel, A. Sita-Lumsden, A. Zivi, N. Rama, C.L. Bevan, S. Ladame *Anal. Chem.*, **2016**, accepted

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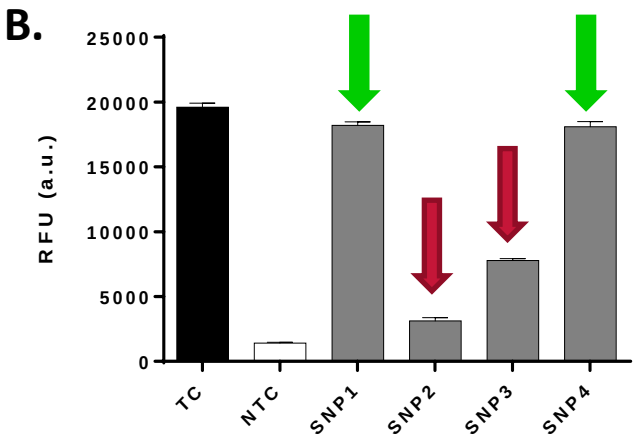
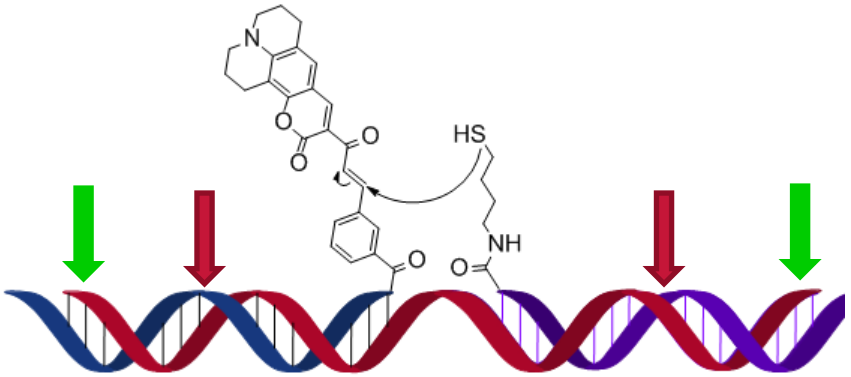
Sequence specificity





Specificity at the single nucleotide level
(but position-dependent)

Implications for miR141 and miR375 sensing?

G. Metcalf, A. Shibakawa, H. Patel, A. Sita-Lumsden, A. Zivi, N. Rama, C.L. Bevan, S. Ladame *Anal. Chem.*, **2016**, accepted

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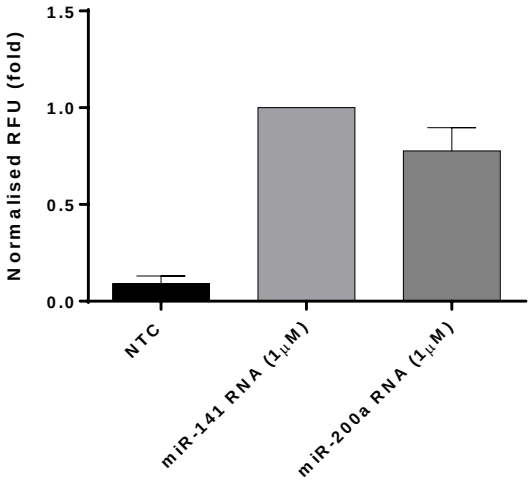
Sequence specificity

miR375: No other miR with sequence homology >75%

miR141: 90.90% homology (20/22bp) with miR-200a

miR-141 sequence (5' to 3'): UAA CAC UGU CUG GUA A**A**G AUG **G**
miR-200a sequence (5' to 3'): UAA CAC UGU CUG GUA A**C**G AUG **U**



Likely to detect both miR141 and miR200a simultaneously

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The issue of Sensitivity

LITTLE* and *COI ***formation on***
endogenous mi ***ons available***

highly depende ***e sample has***
been proces ***analysed***

PRACT **CH:**

Is our techno **PURPOSE?**

Human serum — **To ex** — **NA tion** → **PNA probes**
RT-qPCR

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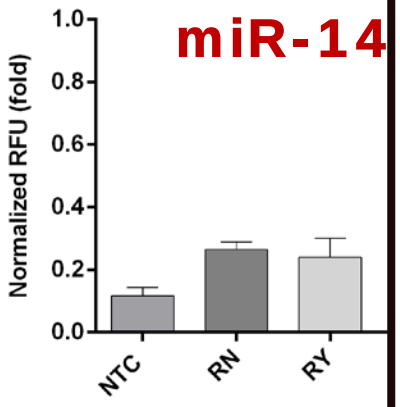
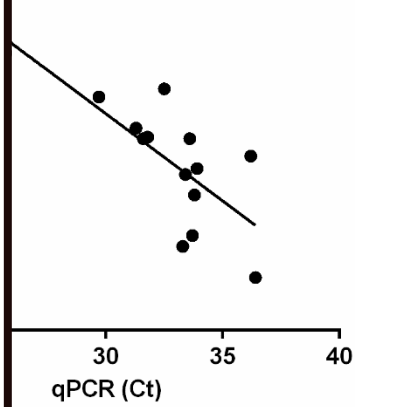
Validation on Pca samples

RN: Remission, post-prostate removal
RY: Remission, prostate gland present
AL: Localised advanced tumour
AM: Metastatic advanced tumour



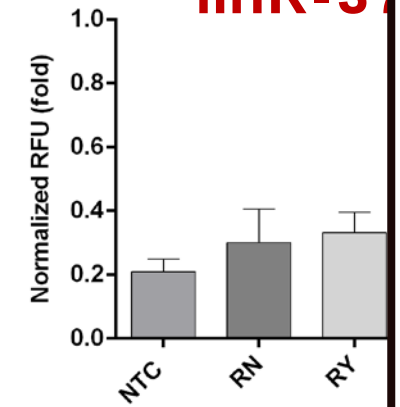


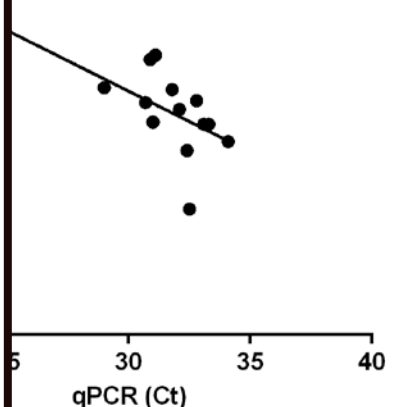
Technology
fit-for-purpose

Amplification-free
Detection

Good correlation with
RT-qPCR data

PCa diagnosis and grading





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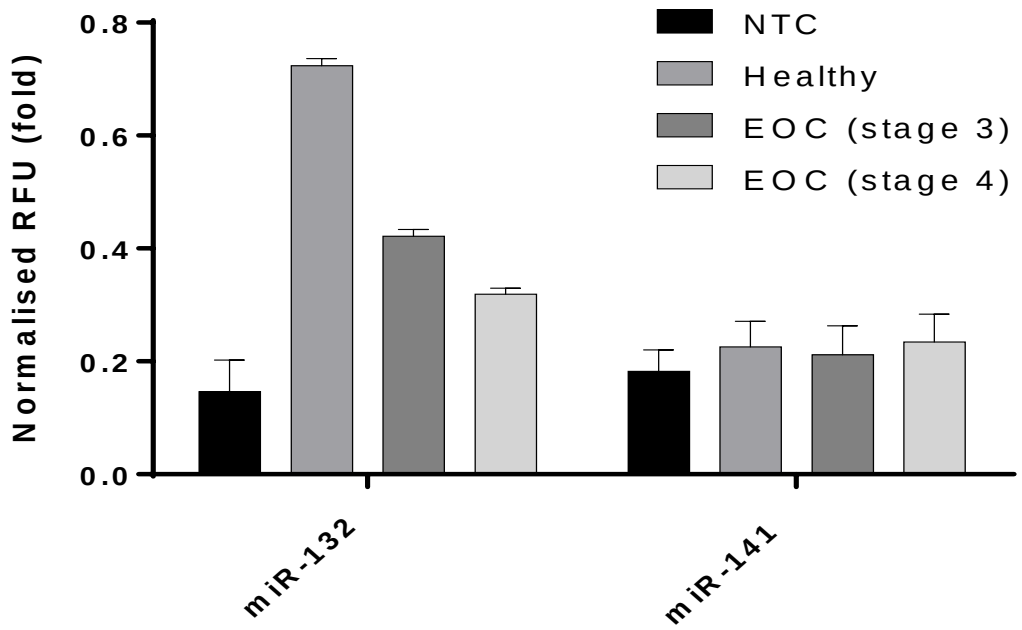
Cross-validation: ovarian vs prostate cancer



- Can detect both increasing and decreasing levels of miRNAs
- miR141 behaves as reference miRNA for ovarian Cancer

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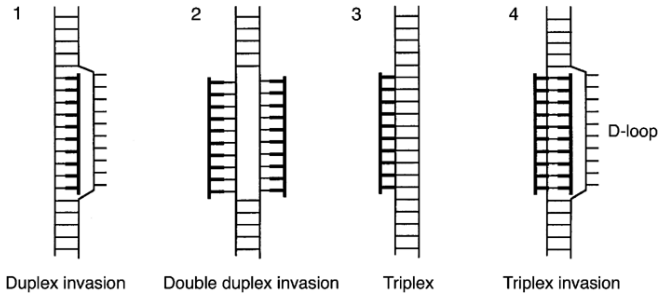
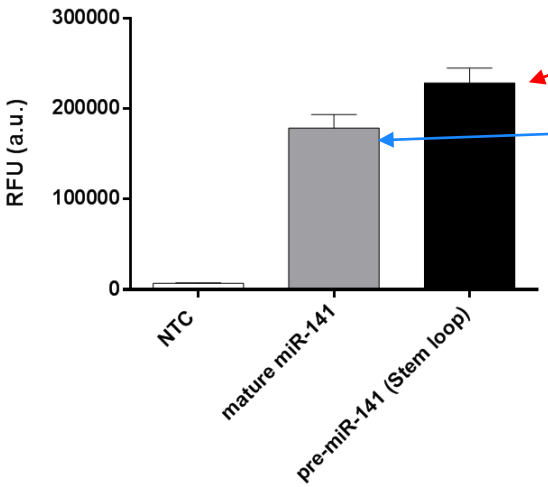
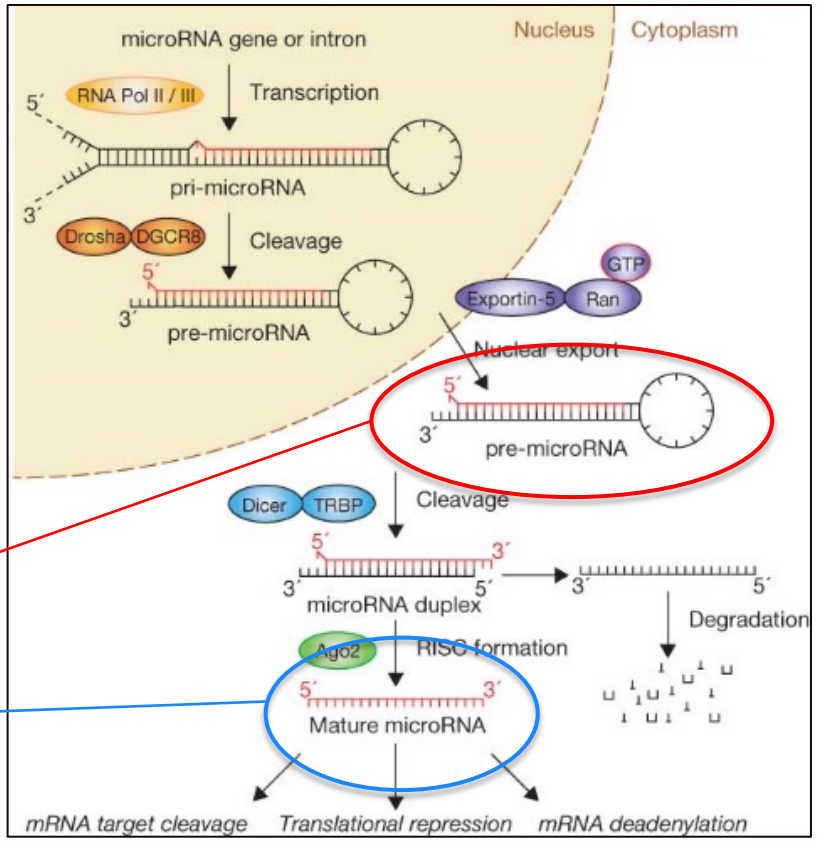
Mature *vs* pre-miR

Why no amplification required?







PNA probes can detect mature miRNAs but also their precursors

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By-passing sample processing?

Blood collection → **Serum separation** → **RNA isolation** → **Detect miRNA**

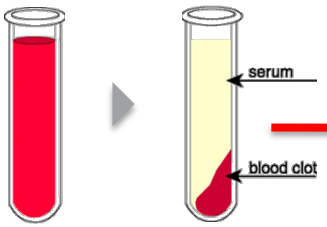
Clot blood – leave upright at RT (0.5-1h)

Spin

Collect supernatant - aspirate

Aliquote (1ml/cryovial)

Freeze at -80°C



Add TRIzol® and chloroform to serum (5min)

Spin

Precipitate RNA from supernatant with isopropanol (1min)

Spin

Wash three times with 75% ethanol

Resuspend RNA in nuclease-free ddH_2O

Heat shock 55-60°C (10min)

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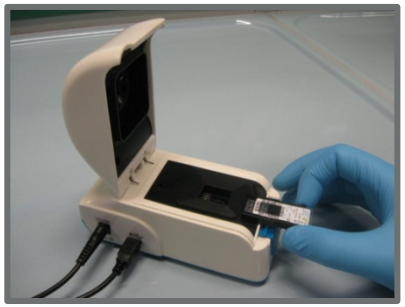
By-passing sample processing?

Can we avoid RNA extraction?



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Vision for the future

- A technology of broad applicability, also to cancers with low survival rates (lung, pancreatic, oesophageal)
- A versatile technology suitable for the detection of any circulating nucleic acid biomarkers (combining miRNA, cell-free DNA and SNP detection in one device for improved specificity).
  - *Minimally invasive* (simple blood test)
  - *Highly automated* (minimal sample processing)
  - *Low production cost* (probes costing <1p per test)
  - *Amenable to incorporation in portable devices* (isothermal, no enzyme, fast)

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Prof. Bob Brown (ICL)
Prof. Charles Coombes (ICL)
Prof. Valerie Taly (France)

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CANCER RESEARCH UK

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