Spring Focus Workshop
Scientific, Regulatory and Technology Challenges in the Development of Oligonucleotide and Peptide Drugs

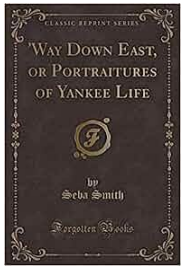
Introduction to Session 3: There's more than one way to skin a cat…

Robert Nelson, on behalf of the EBF

8-9 June 2023 – Malaga, Spain

There’s more than one way to skin a cat…

- There are many ways to do something
- There are many ways to achieve a goal



The oldest known use of the phrase dates back to 1854, in the work “’Way Down East, or Portraitures of Yankee Life” by Seba Smith. However, there’s more than one way to skin a cat has its roots in older, similar phrases such as “*there are more ways to kill a cat than choking it with cream*”, found in the 1830s. It seems that originally the animal in question was a dog, as a 17th proverb is “*there are more ways to kill a dog than hanging”*.

Take home messages from Cecilia's goodiebag

- Oligos ≠ small molecules
- Opportunity to make the undrugable drugable
- Different modes of action
- Limited cellular uptake triggered
  - Chemical evolution and optimisation
  - Targeting strategies

- **Complex bioanalytical requirements**
  - **'Novel' analytical method may have to be explored**
  - **Multiple approaches often required**
  - **Platform strategies may be applied**

Multiple bioanalytical approaches and platforms possible

Liquid Chromatography (LC) / Mass Spectometry (MS) Based Approaches

- The gold standard for oligonucleotide bioanalysis?

- Several different approaches can be applied:
  - LC-MS(MS)
  - TOF-MS/HR-MS
  - Hybridization-based LC-fluorescence assays

LC-MS(MS) and TOF-MS/HR-MS

- A number of case studies throughout this workshop…

Hybridization-based LC-fluorescence assays

- Pairs hybridization with HPLC techniques
  - The target oligonucleotide first hybridizes to a fluorescence-labeled sequence-specific probe oligonucleotide through Watson–Crick base pairs.
  - The resulting fluorescence labeled duplex is then subjected to a anion-exchange HPLC analysis monitored by a fluorescence detector.

- Limitations:
  - Probe design
  - May not detect truncated forms

Hybridization ELISA Approaches

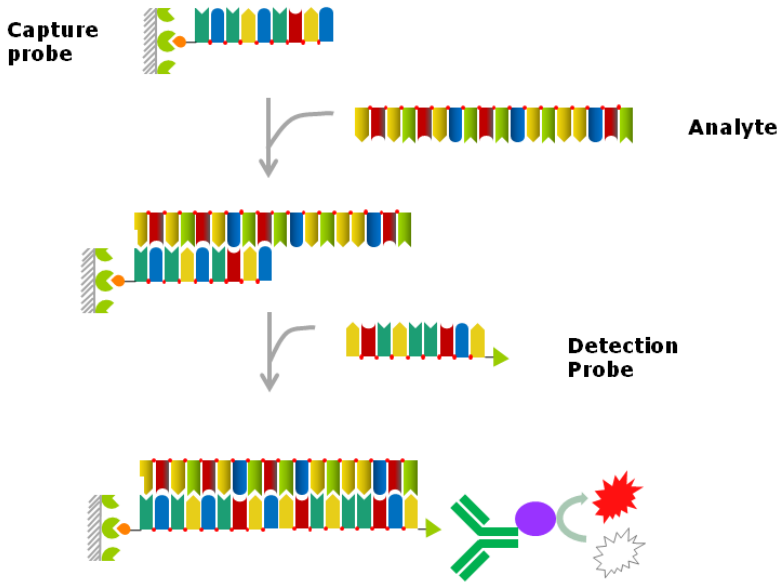
- Several different formats* have been applied
  - Dual probe/sandwich hybridization
  - Nuclease dependent/single probe
  - Ligation
  - Competitive

- May have colorimetric, ECL or fluorescent readout
- Ultra-sensitive assay platforms being evaluated to gain sensitivity

*Note: There are several patents related to assay methodologies that you should familiarise yourself with before taking a final decision on the approach used in your program.

Dual Probe/Sandwich Hybridization ELISA

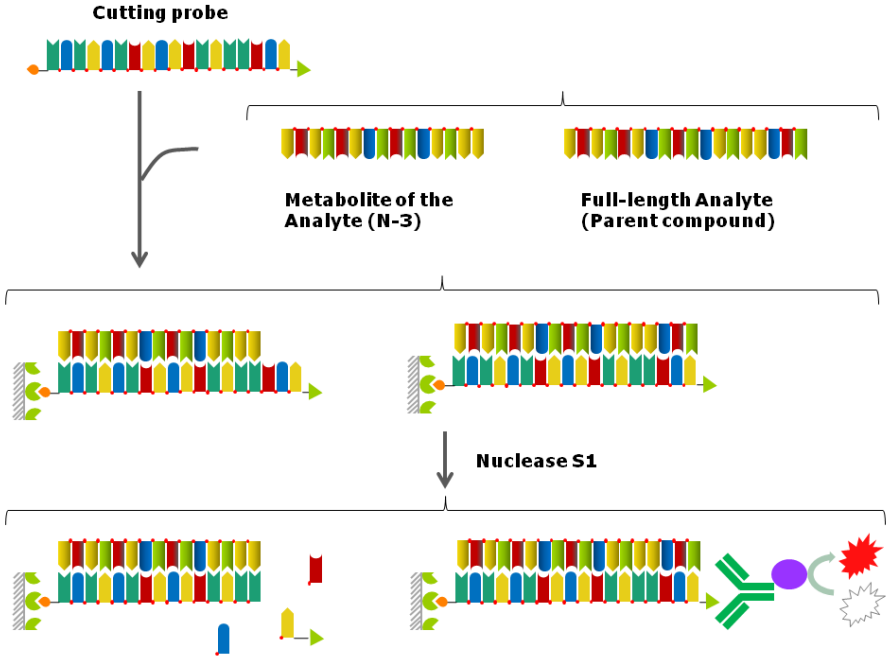
- Advantages:
  - Very sensitive
    - Typical LLOQ low to mid pg/mL range
  - Larger dynamic range than other hELISA formats
- Limitations:
  - Length of analyte
    - Typically >18 nucleotides (min. 9 for each probe)
  - Probe design
  - May not distinguish truncated forms
    - measures total



Source: Bartelmy - Own work, CC BY-SA 4.0
https://commons.wikimedia.org/w/index.php?curid=43156924

Nuclease Dependent/Single Probe Hybridization ELISA

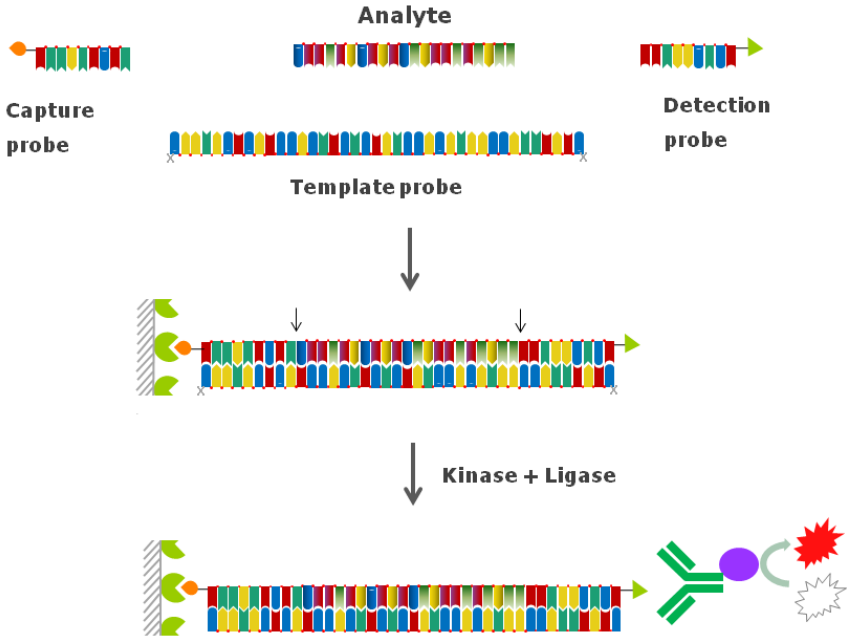
- Advantages:
  - Good sensitivity
    - Typically LLOQ low ng/mL
  - Selective for intact analyte
    - May be able to use design for evaluating metabolites
- Limitations:
  - Probe design
  - Optimisation of nuclease reaction



Source: Bartelmy - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=43201664

Ligation Hybridization ELISA

- Advantages:
  - Good sensitivity
    - Typical LLOQ low ng/mL
  - Selective for intact analyte
    - May be able to use design for evaluating metabolites
- Limitations:
  - Length of analyte
  - Probe design
  - Single ligation assays may also detect 5’ truncations



Dual Ligation Hybridization Assay
Source: Bartelmy - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=38478751

LC-MS vs ELISA approaches

LC-MS	Hybridization ELISA
Internal standard required	Custom probes required
Moderate Sensitivity (typical LLOQ 1-100 ng/mL)	Good sensitivity (typical LLOQ 0.1-10 ng/mL)
Wide dynamic range (3+ log)	Narrower dynamic range (1-2+ log)
Excellent specificity, measure exact species	Moderate specificity, may detect truncated forms
Assays may be more complex than standard MS workflows. Extra instrument cleaning	Assays often more complex than standard ELISAs

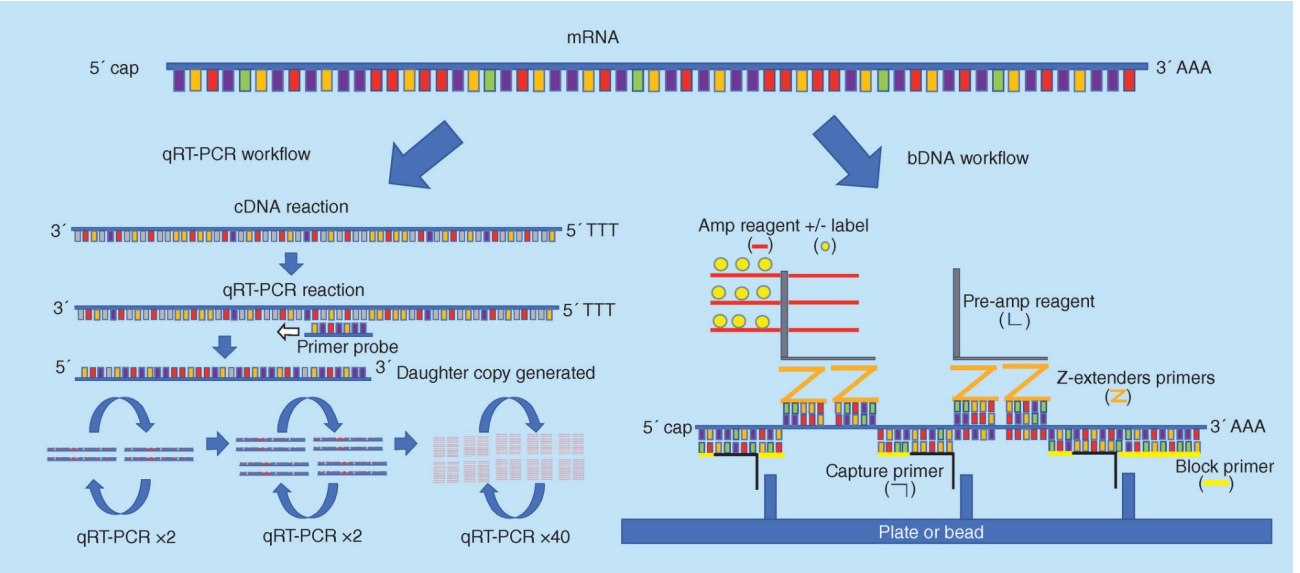
Molecular Assay Approaches

- Several different approaches have been applied
  - (RT)-qPCR
  - Branched DNA
  - Other…?

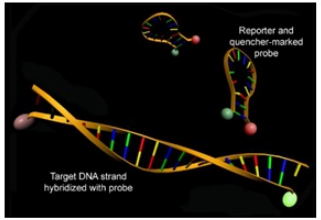
Overview of quantitative reverse transcription PCR and branched DNA modes of action for measuring mRNA

Source: Henderson & Wilson. Bioanalysis 11(21) 2019, 2003-2010

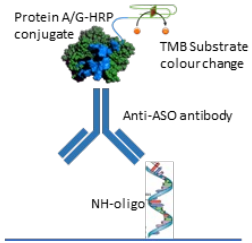
https://doi.org/10.4155/bio-2019-0120



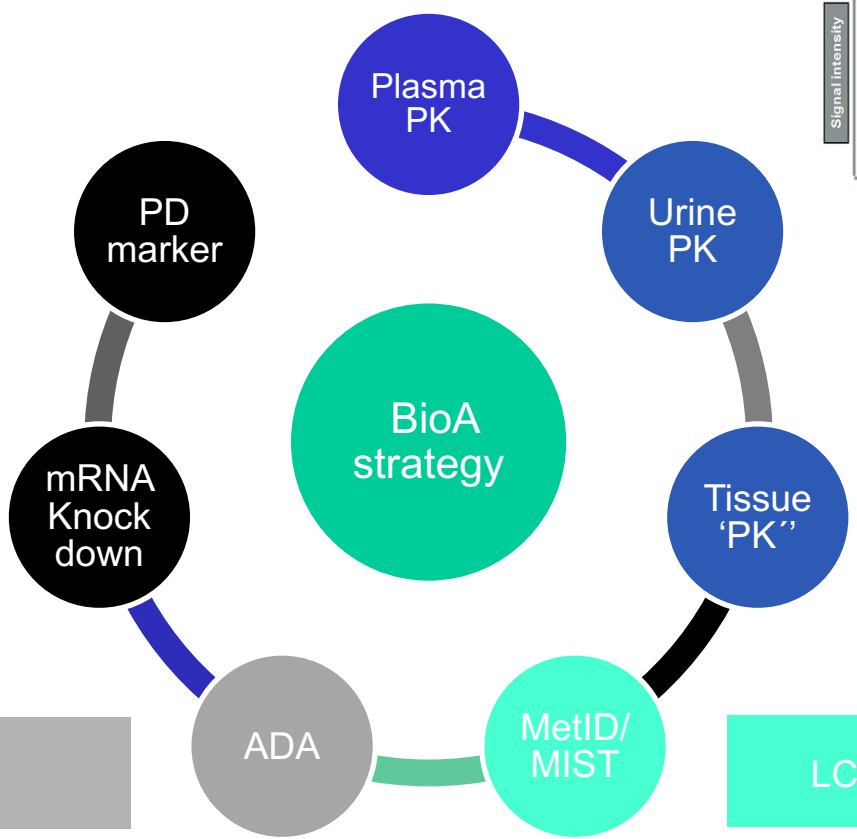
Different assays may be required for different analyses

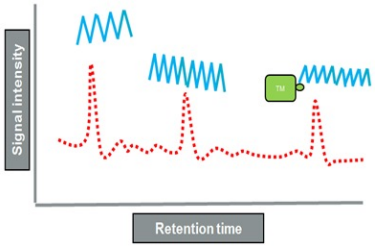


PCR

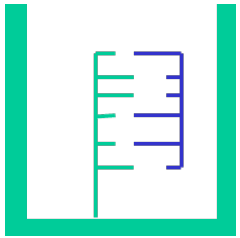


ELISA





LC-MS/MS, Hybridisation Assay (ELISA, ECL LC-FI, Gyros), PCR



LC-MS or LC/UV

In this session...

13:40	**15:00**	**Session 3: More than one way to skin a cat…**
13:40	14:00	*Robert Nelson - on behalf of the EBF* Setting the scene - dealing with tissues and/or non-standard MS approaches
14:00	14:20	*Jill Uhlenkamp - Labcorp Drug Development* The Pursuit of Robust Quantitation of Oligonucleotides by Hybridization Assays
14:20	14:40	*Daniel Schulz-Jander - QPS* Quantitation of siRNA's and Metabolites in Plasma, Excreta and Tissues by LC/ToF-MS in Regulated Studies
14:40	15:00	*Carrie Vyhlidal - KCAS Bio* Quantification of Synthetic DNA Aptamers in Plasma with qPCR

Questions

Acknowledgements

- EBF Focus Workshop Organising Committee
- EBF Community

Contact Information

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