siRNA PK hybridization assay

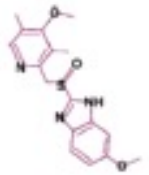
Solvej Lund Lippert, PhD, Senior Scientist

Novo Nordisk A/S

09 June 2023

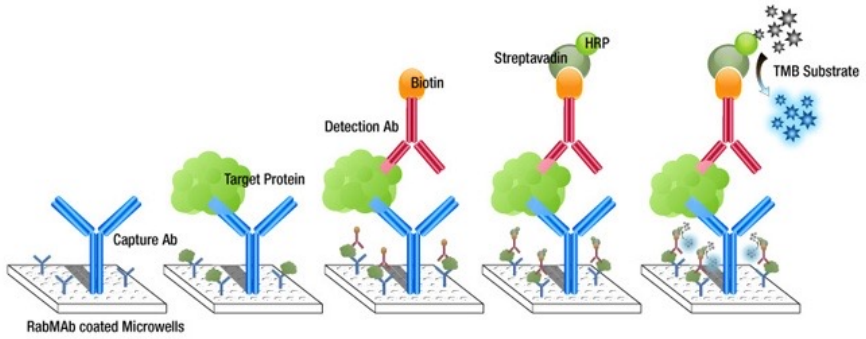
siRNA versus conventional drugs

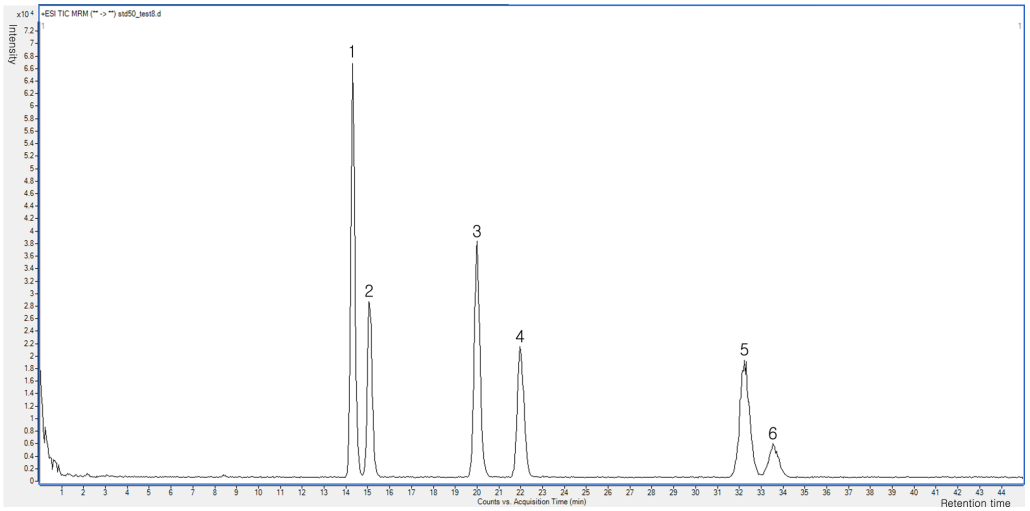
- Structure
- Mechanism of action
- Specificity
- Delivery
- Half-life

	Typical small molecule	Single-stranded DNA (e.g., anti-microRNA, ASO)	Peptides and proteins
			
Size **Physicochemical properties** **Pharmacokinetic characteristics**	200–700 Da Variable solubility Variable charge state • Distribution dependent on chemistry • Hepatic and renal clearance	6–9 kDa High solubility Polyanionic • High distribution to kidney and liver • Hepatic and renal elimination	1.5–70 kDa High solubility Variable charge state • Low tissue distribution • Stability and half-life dependent on plasma-protein binding • Hepatic and renal elimination
Route of administration	Oral	SC or IV	SC or IV
Metabolizing enzymes	Oxidases, e.g., P450s, hydrolases, conjugations	Nucleases	Peptidases

Reference: Andersson et al., Drug metabolism and pharmacokinetic strategies for oligonucleotide- and mRNA-based drug development. Drug Discov Today 2018, 1733-1745.

Traditional PK assays in Novo Nordisk





Ligand binding assays

LC-MS/MS assay

siRNAs and PK assays

LBA hybridization assay

- High sensitivity
- Measure parent and metabolites

LC-MS/MS assay

- Lower sensitivity
- Specific for parent

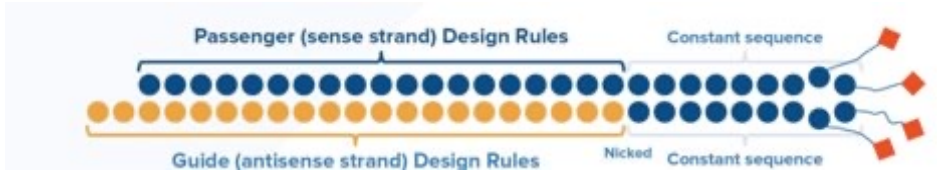
HPLC-PNA assay

- Lower sensitivity
- Specific for parent

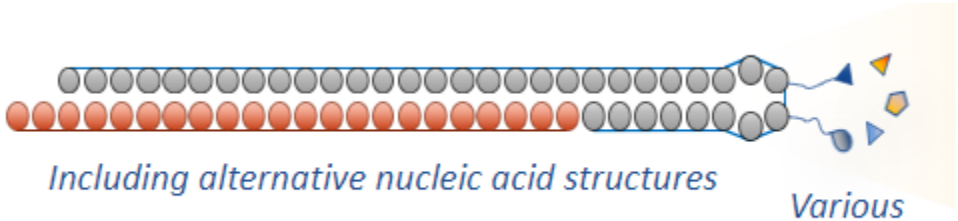
PCR assay

- Sensitivity?
- M10 guidelines not for PCR

Novo Nordisk PK assay strategy



GalXC™



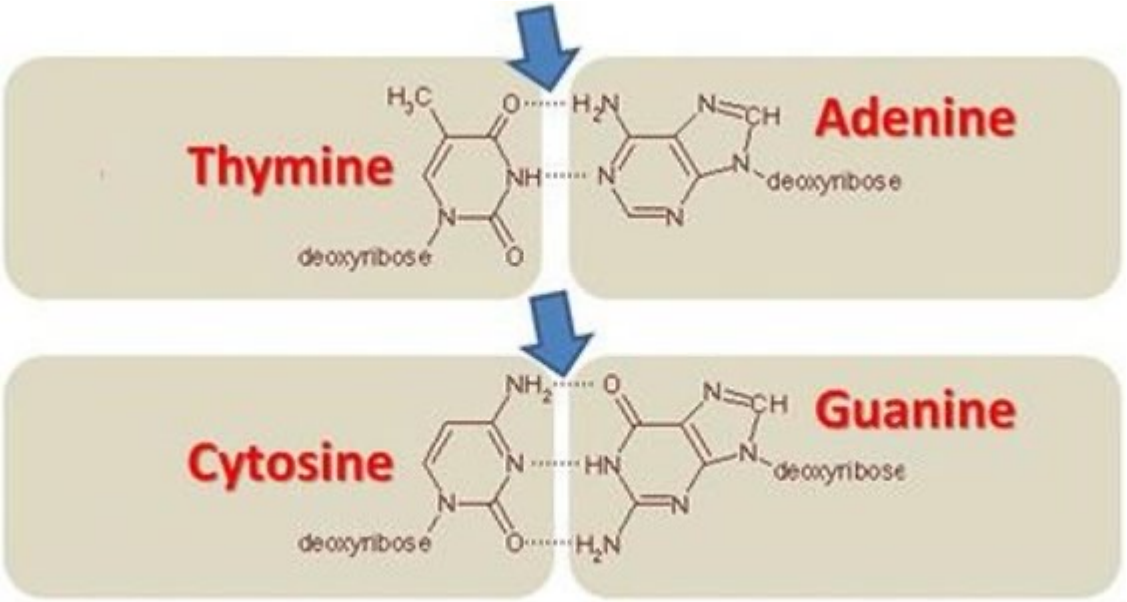
GalXC Plus™

LBA hybridization assay

- High sensitivity
- High throughput

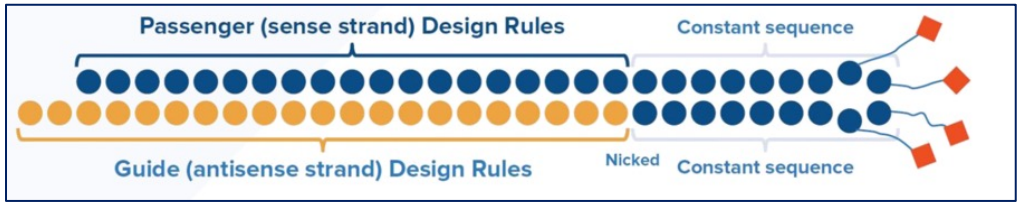
Design of probes

- Adding of locked nucleic acids (LNA) to increase Tm and improve specificity and affinity in basepairing
- Consider length of probe
- Approx. same Tm for both probes
- Digoxigenin- and biotin-labeling of probes



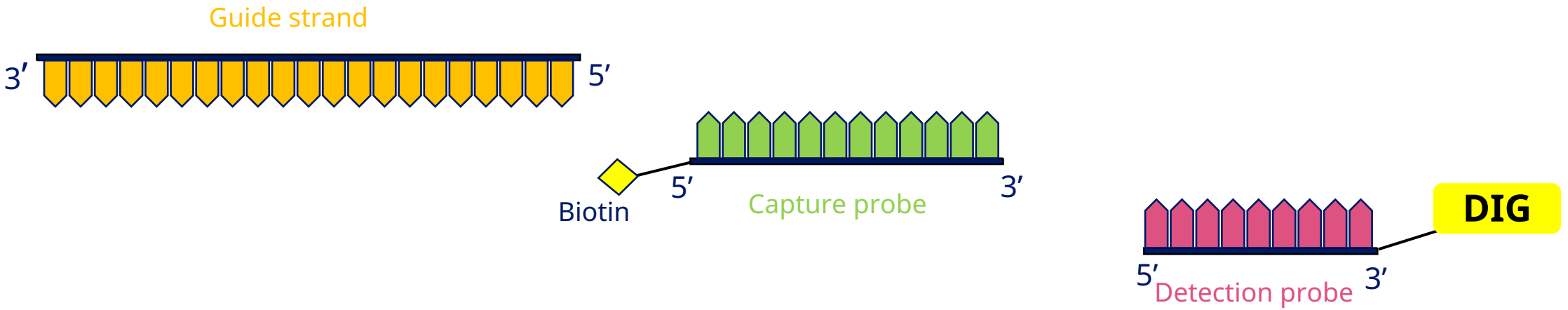
Hybridization assay

In thermocycler



- Biotinylated capture probe
- Guide strand
- DIG labelled detection probe
- Biotin

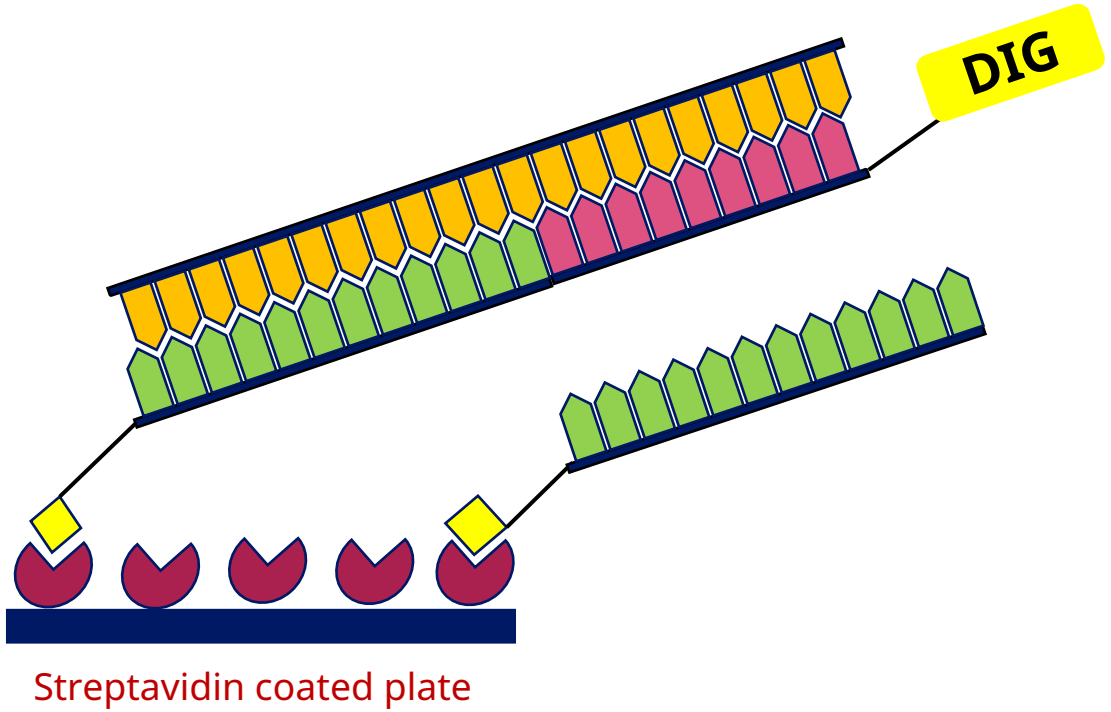
DIG: Digoxigenin



<u>Step 1:</u> Denaturation of siRNA and hybridization of guide strand with sequence specific capture and detection probe

Hybridization assay

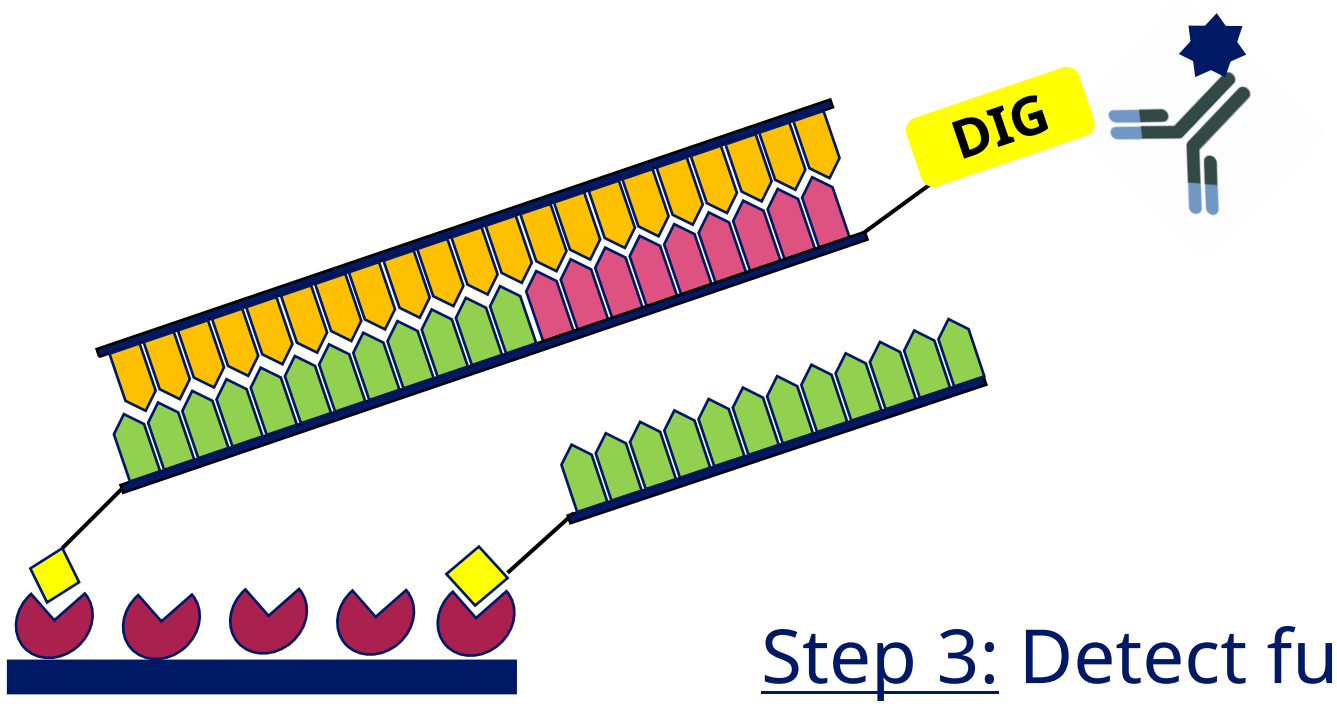
Step 2: Attach hybridized product to streptavidin coated plate



- Biotinylated capture probe
- Guide strand
- DIG labelled detection probe
- Biotin

DIG: Digoxigenin

Hybridization assay



- Biotinylated capture probe
- Guide strand
- DIG labelled detection probe
- Biotin
- Sultfotag labelled Ab

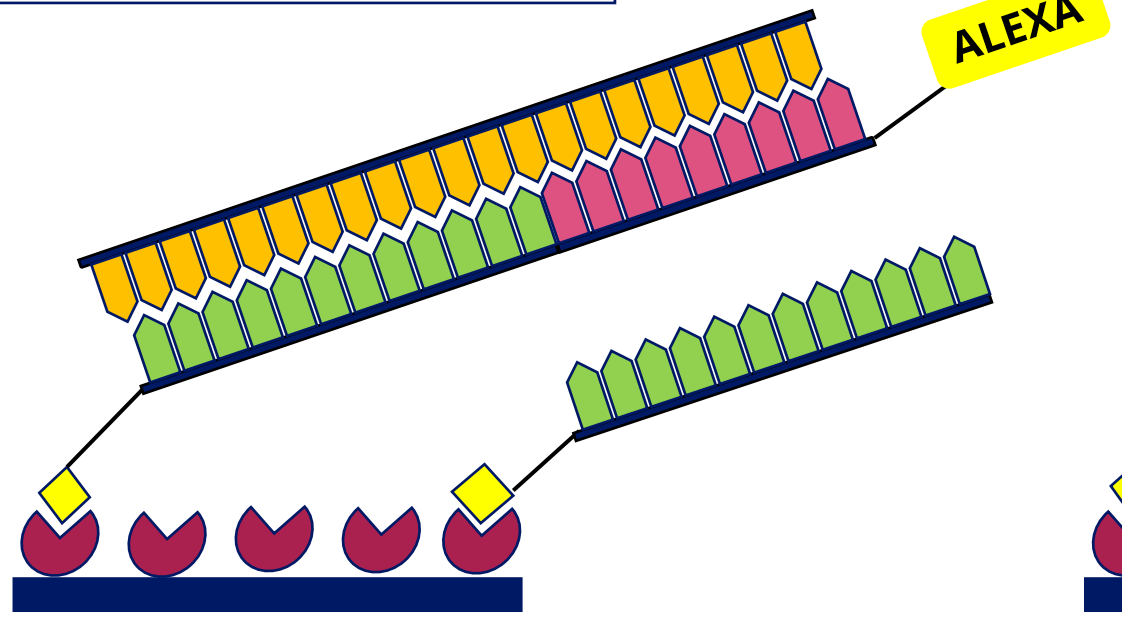
DIG: Digoxigenin

Step 3: Detect full length complement with Sulfotag labelled anti-DIG antibody

Step 4: Read at MSD Sector Imager

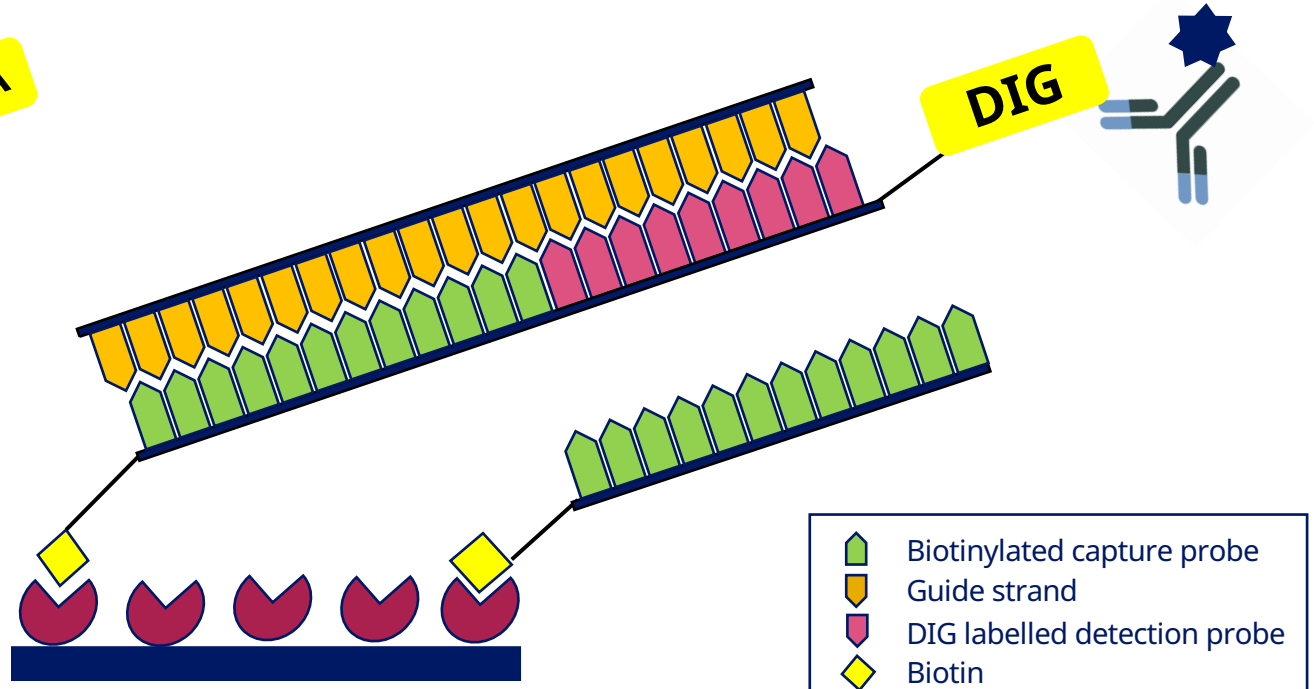
Hybridization assay

- Biotinylated capture probe
- Guide strand
- Alexa flour labelled detection probe
- Biotin



Streptavidin coated disc (Gyrolab)

Gyrolab



Streptavidin coated plate

Mesoscale

- Biotinylated capture probe
- Guide strand
- DIG labelled detection probe
- Biotin
- Sultfotag labelled Ab

DIG: Digoxigenin

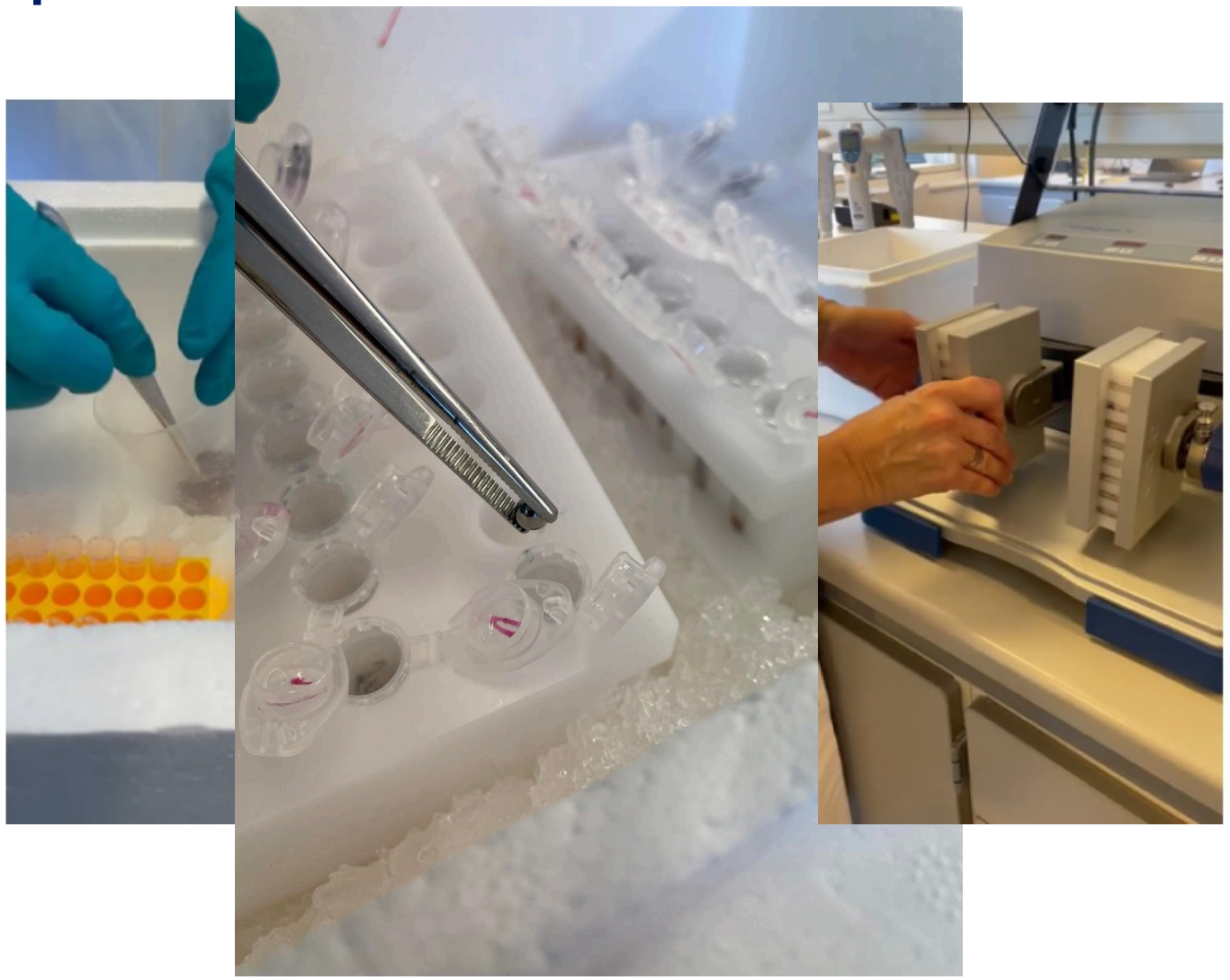
Plasma and tissue preparation

Plasma

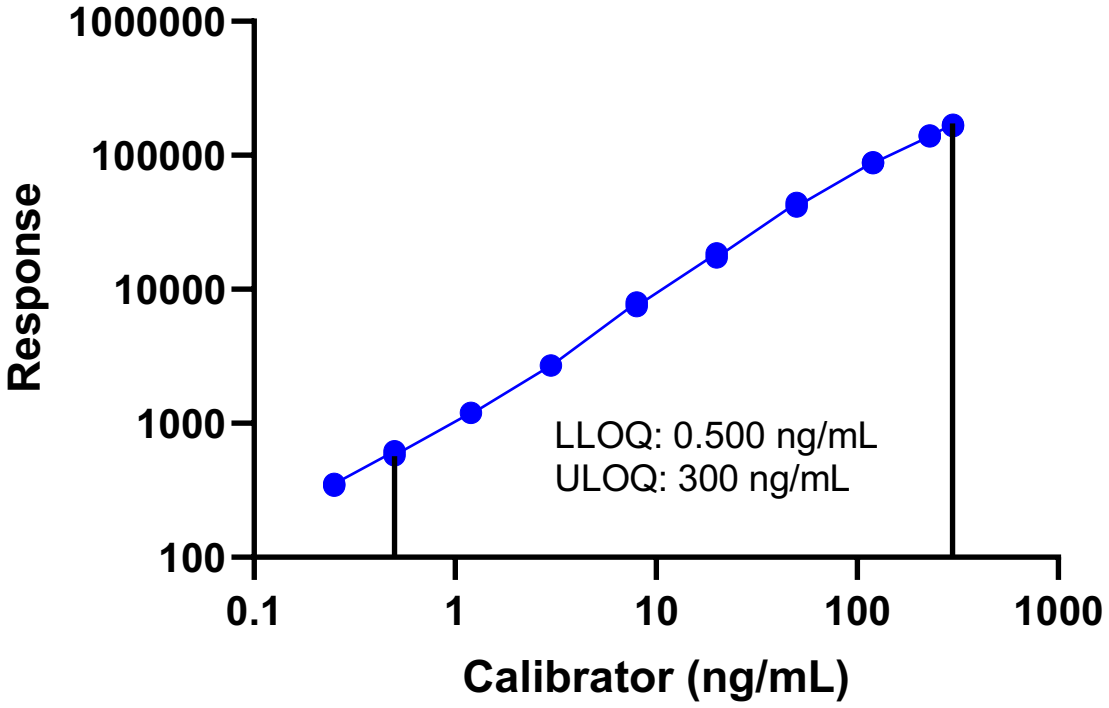
- Plasma will coagulate in thermocycler if not diluted in buffer
- MRD differ between species, e.g. mouse ×2, NHP/human ×5

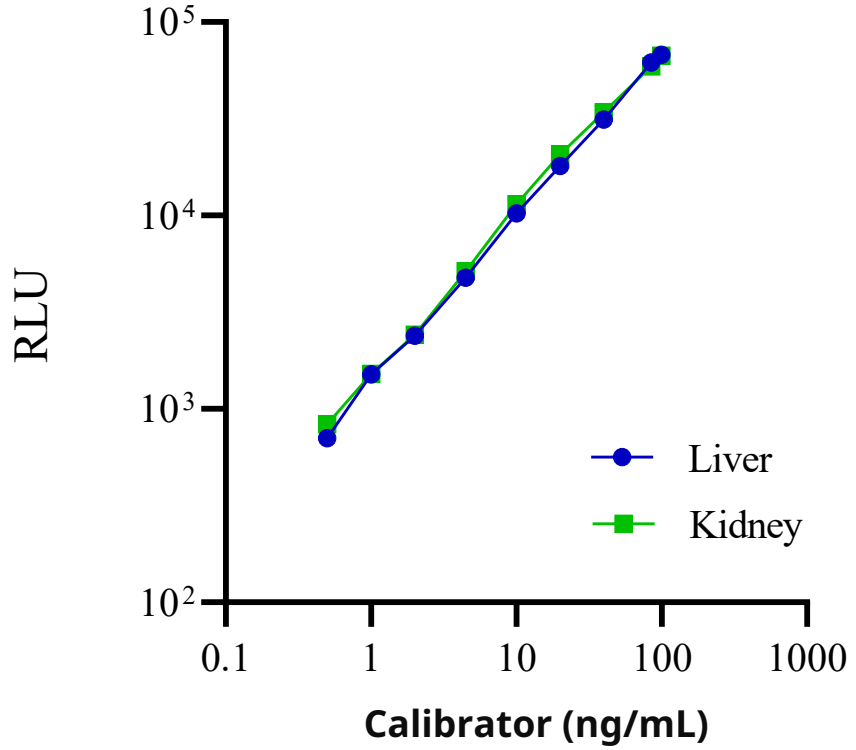
Tissue

- Tissue (approx. 25 µg) needs to be homogenised before hybridisation
- Tissue will coagulate in thermocycler if not diluted in buffer



Hybridization assay in plasma and tissue



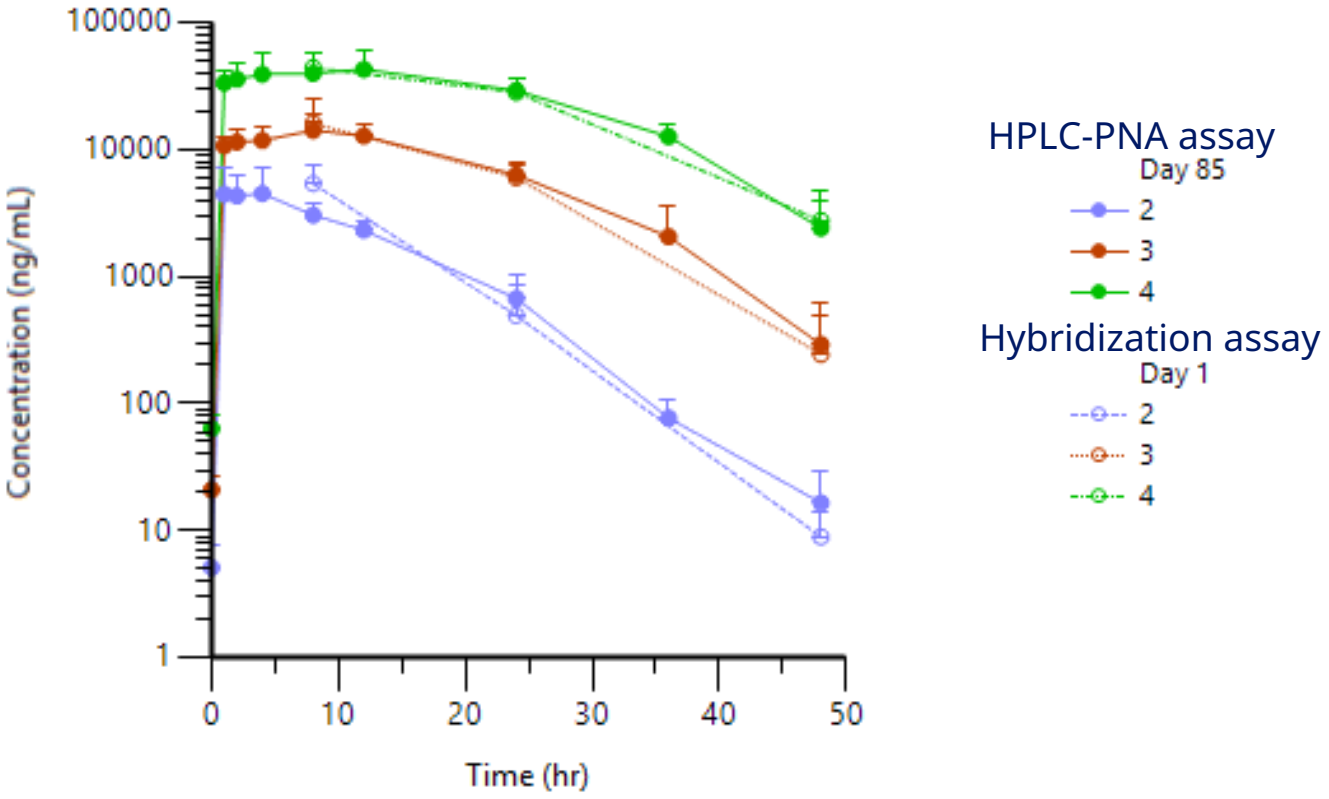


Plasma exposure

- Plasma (primary matrix):
  - Hybridization assay will with a high likelyhood co-measure metabolites

Experience

- Hybridization assay
  - Analysis of Day 1 samples
- HPLC-PNA assay
  - Analysis of Day 85 samples
- Concentrations are comparble on the two platforms hence metabolites not expected in plasma



Tissue exposure

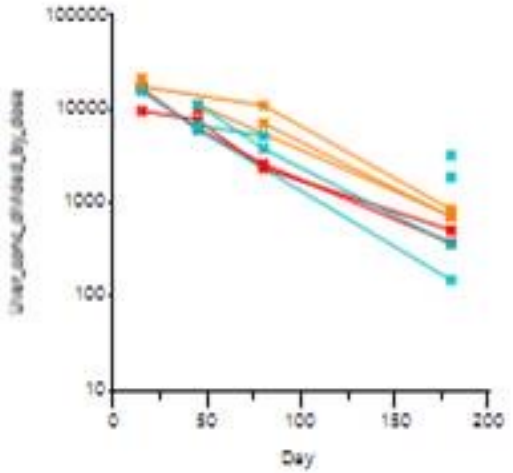
- Tissue (secondary matrix):
  - LC-MS/MS and HPLC-PNA assays can discriminate between the full lengths and metabolites, but only main peak will be reported

Experience

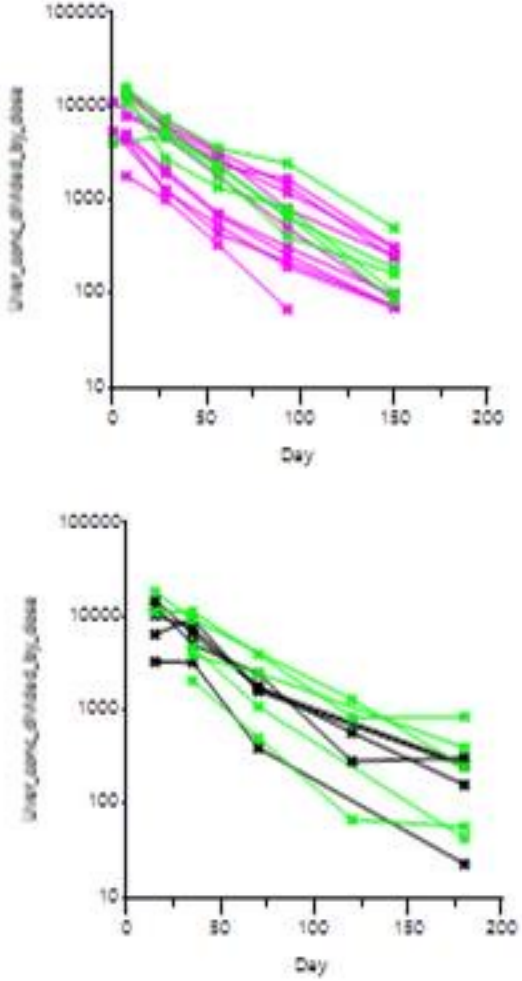
- Hybridization assay
  - Analysis of compound 1

- HPLC-PNA assay
  - Analysis of compound 2 and 3

- Concentrations on the hybridization platform seem higher, maybe due to co-measurement of metabolites

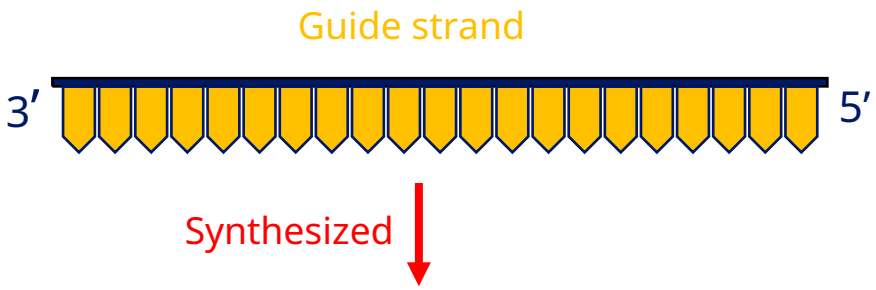


Hybridization assay

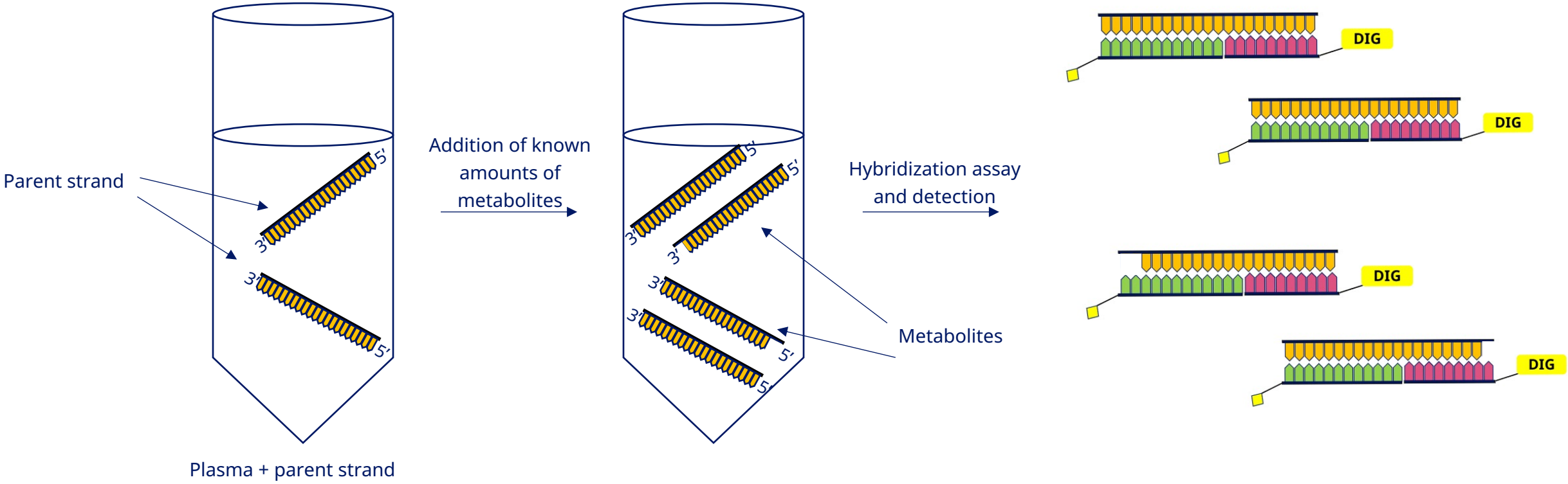


HPLC-PNA assay

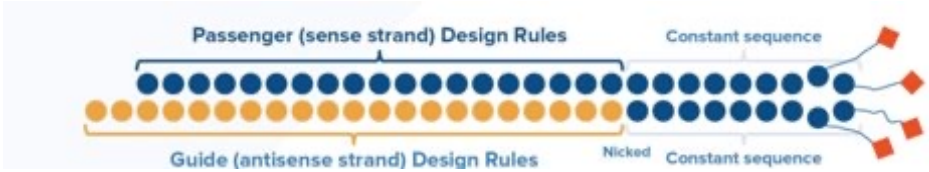
Step wise approach for metabolite testing in PK assays – 1



Step wise approach for metabolite testing in PK assays – 2



Ongoing GalXc studies

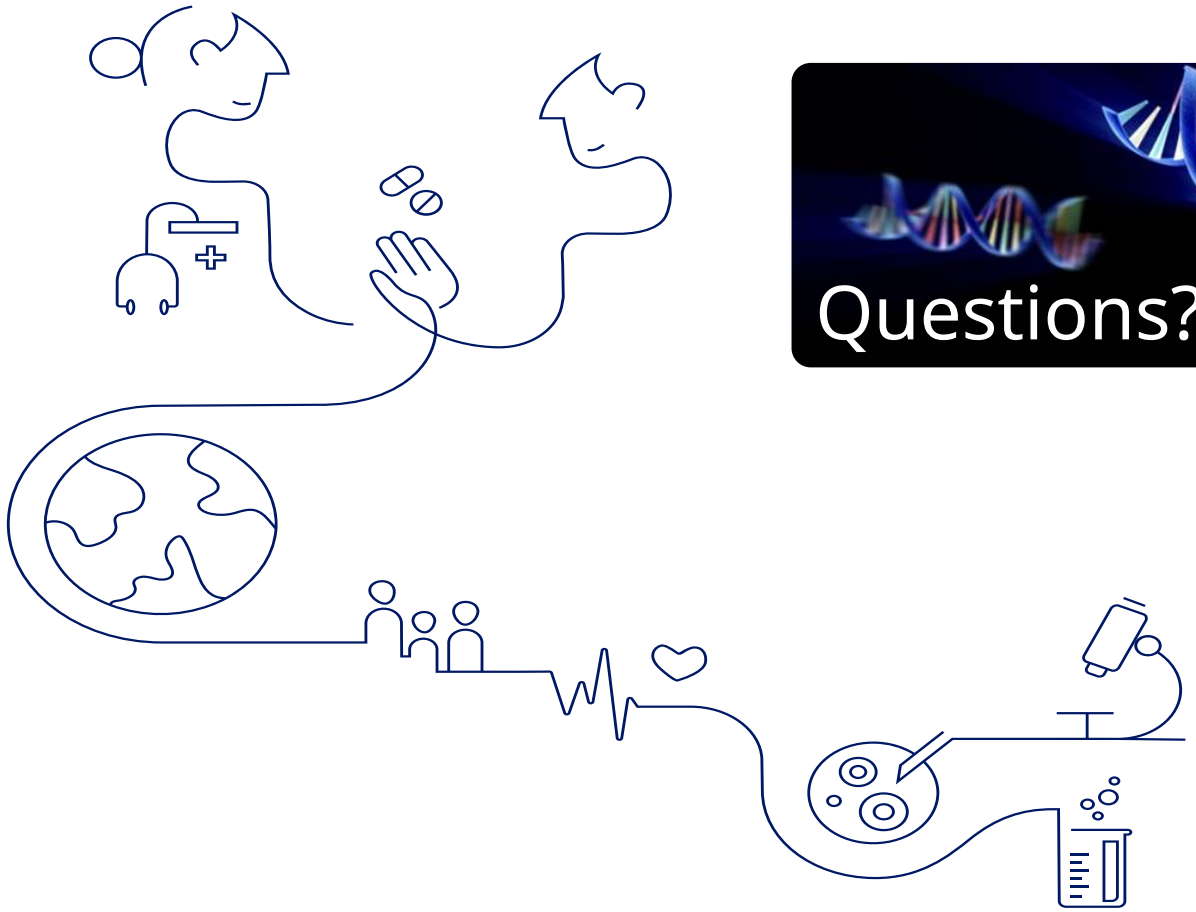


GalXC™

- Two first human dose trials
  - Plasma PK analsyis in-house on hybridization assay
  - Plasma PK analysis by CRO on HPLC-PNA

- NHP and mouse tox studies
  - Plasma PK analysis in-house on hybridization assay
  - Tissue PK analysis (samples are taken but only analysed on request)

Thank you for your attention



Solvej Lund Lippert
Novo Nordisk A/S
Non-clinical & Clinical Assay Sciences
slul@novonordisk.com