Yotta (Y) = 1,000,000,000,000,000,000,000,000
Zetta(Z) = 1,000,000,000,000,000,000,000
Exa(E) = 1,000,000,000,000,000,000
Peta(P) = 1,000,000,000,000,000
Tera(T) = 1,000,000,000,000
Giga(G) = 1,000,000,000
Mega(M) = 1,000,000
Kilo(k) = 1,000
Hecto(h) = 100
Deca(da) = 10

DEL Screening in Living Cells

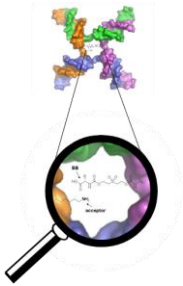
Deci(d) = 0.1
Centi(c) = 0.01
Milli(m) = 0.001
Micro(μ) = 0.000 001
Nano(n) = 0.000 000 001
Pico(p) = 0.000 000 000 001
Femto(f) = 0.000 000 000 000 001
Atto(a) = 0.000 000 000 000 000 001
Zepto(z) = 0.000 000 000 000 000 000 001
Yocto(y) = 0.000 000 000 000 000 000 000 001

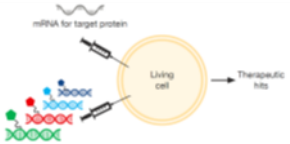
5th Medicinal Chemistry & Protein Degradation Summit
November 16-17, 2020

Dr. Iolanda Micco, Associate Director of Chemistry & Alliances
imi@vipergen.com
www.vipergen.com

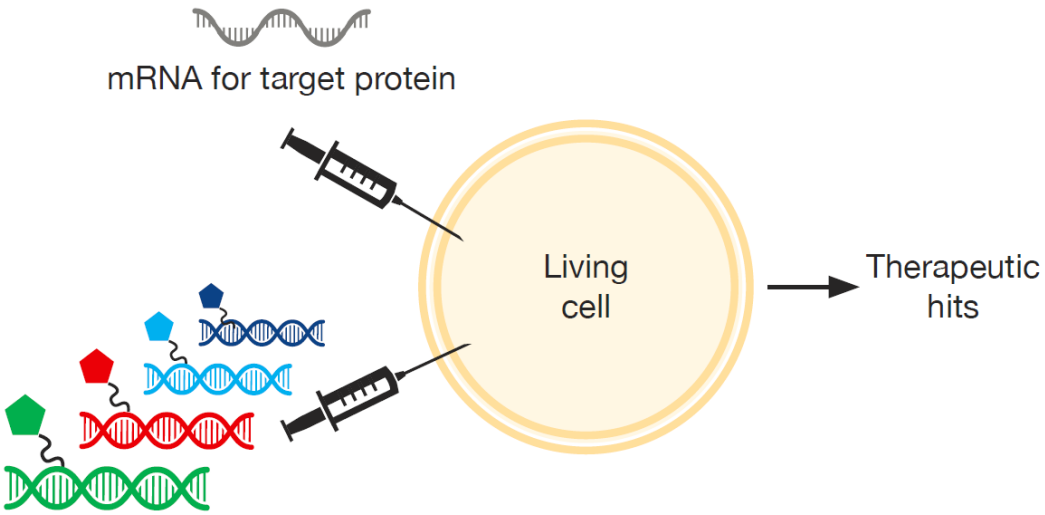
Vipergen in brief

- Vipergen offers to current and future partners to discover hits/leads for their high value targets, including protein-protein interaction targets
- Vipergen is a small molecule drug discovery company – with focus on the hit/lead finding process
- Vipergen introduces the YoctoReactor® (yR) and Binder Trap Enrichment® (BTE) - 2nd generation DNA-encoded small-molecule library technologies
- Vipergen introduces the *in cell* Binder Trap Enrichment® (cBTE) - 3rd generation technology (New)
- The yR, BTE and cBTE technology platforms are exclusively owned by Vipergen and secured by strong patent positions
- The company is privately owned by Gunnar Kjems and Dr. Nils Hansen
- Board members: Gunnar Kjems (chair) and Dr. Nils Hansen
- Founded by CEO, Dr. Nils Hansen
- Founded in 2005
- Incorporated in Copenhagen, Denmark
- Currently 15 employees (14 PhDs) – extensive outsourcing, including 22 chemists
- In 2015-20 >40 deals signed, including with top-5 pharma in both USA, EU and Japan





In Cell Binder Trap Enrichment® (cBTE)



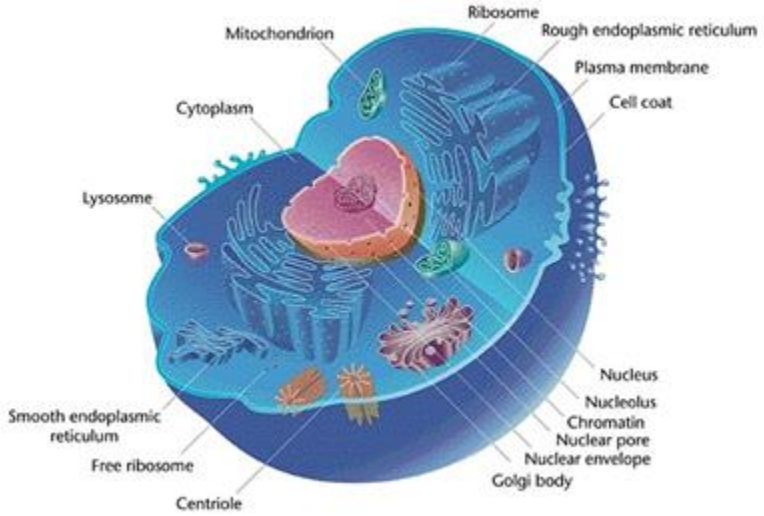
Virtues:

- Screening under physiologically relevant conditions
  - Lower attrition rate
- No need for highly purified target protein
  - Broader protein target space
  - Less work
- Tapping into BTE

Limitations:

- Implemented for soluble protein and protein fragment targets only

Screening in plastic vs in cell



- Physiological relevance low
- Purified active target protein required

- Physiological relevance high
- Purified active target protein not required

- Attrition rates lower
- Target space bigger/complementary
- Less work

Delivery of DEL into the cell an apparent challenge

We simple inject DEL into the cell

- Various methods exist for delivery of DNA into cells, such as electroporation, liposomes, chemical, and by tethered cell-penetrating peptide
- All inefficient
- Limited usefulness for DELs – high cost reagents

Xenopus Laevis Oocytes

- 1 mm diameter
- 1 µL volume
- 100 000 times bigger than somatic cells
- 400 ng heterologous protein per day
- 1 EUR per oocyte, mail order

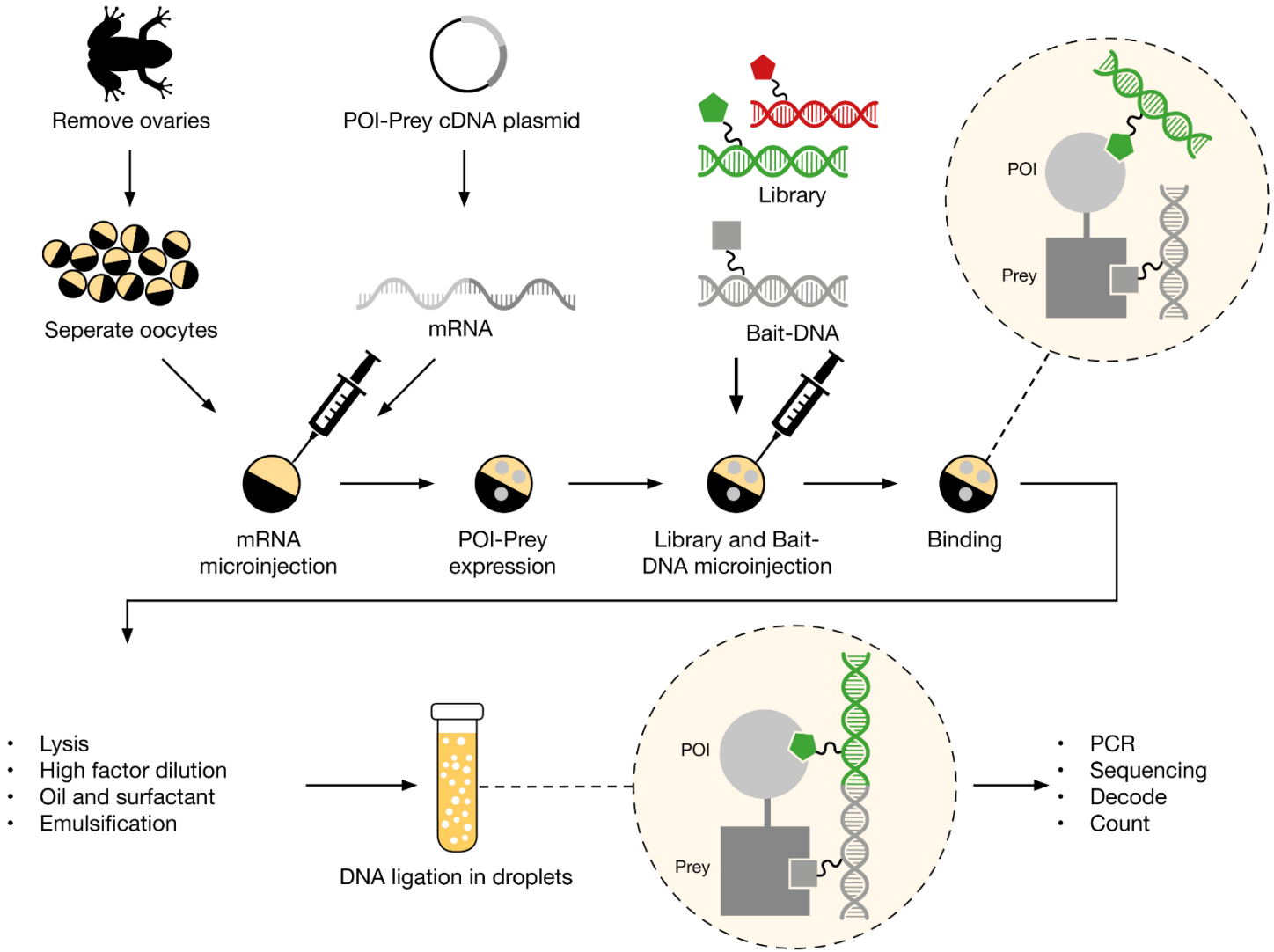
- Microinjection

The oocytes of the South African clawed frog Xenopus Laevis are widely used for the expression of heterologous proteins

DEL members inside a cell can either bind POI or the endogenous cell proteins

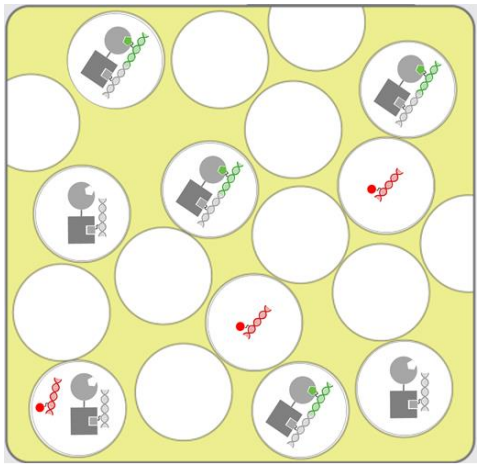
We discriminate between these two binding events by specific labeling POI with DNA, and thereby provide the set-up for Binder Trap Enrichment (BTE)

In cell Binder Trap Enrichment (cBTE)

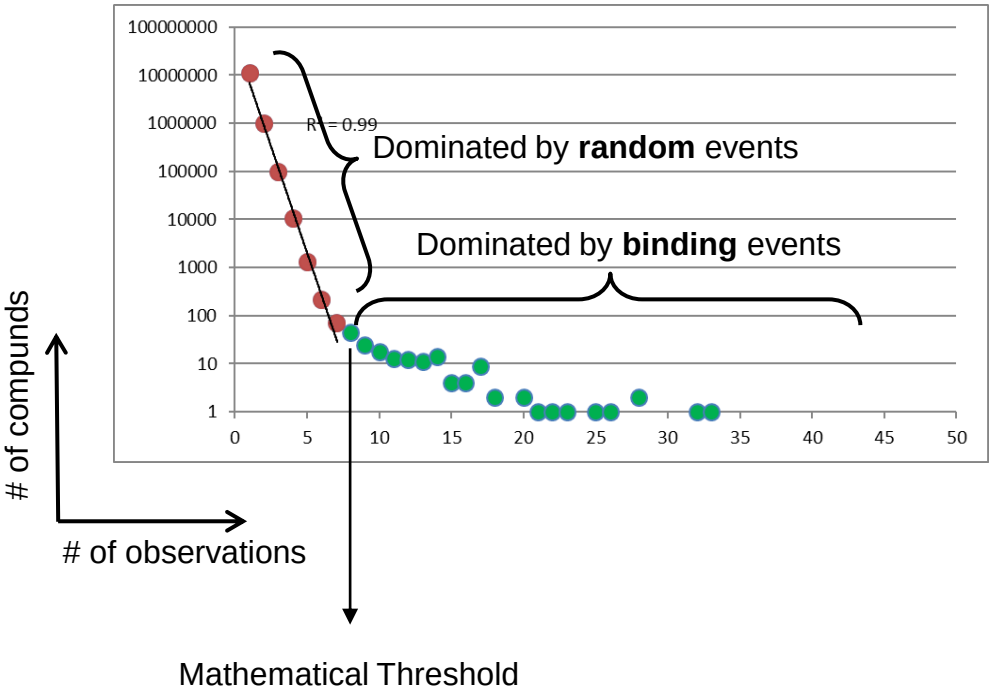


Hits identification

- Decode DNA codes to compounds
- Count
- Generate signal plot
- Apply mathematical threshold



Trapping: due to binding or random events

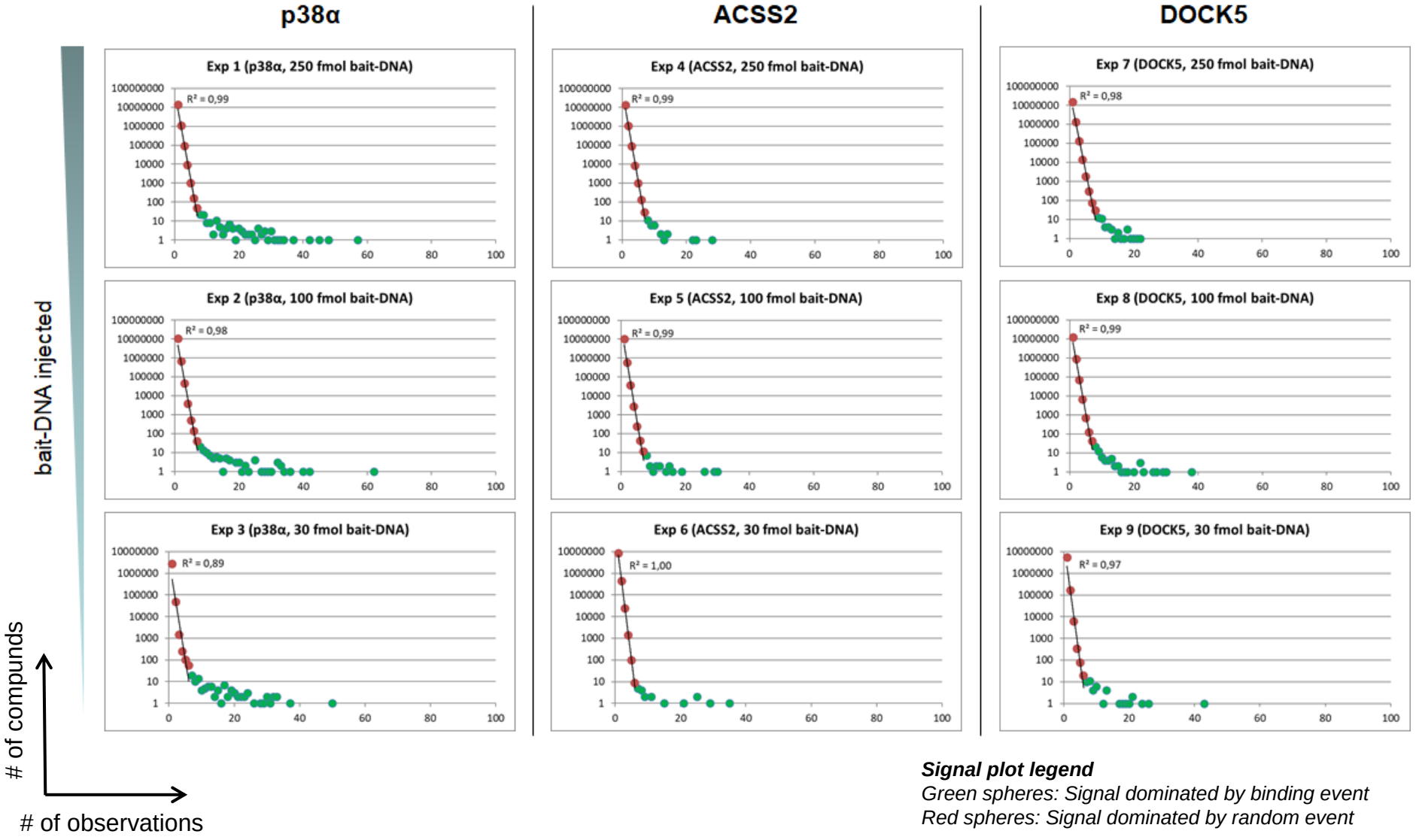


Experimental Overview – VIPS392

- POI-Prey mRNA (3 different POIs) injected into oocytes
- Library (Lib050a, 200 mill members) and Bait-DNA (250, 100, or 30 fmoles) injected into oocytes
- In total 9 experiments all performed in triplicates

- Mitogen-activated protein kinase 14 (**p38α**)
  - Indication: cancer
  - Protein class: kinase
  - Size: MW 41 kDa, 361 aa (full length)
- Acetyl CoA synthetases 2 (**ACSS2**)
  - Indication: cancer
  - Protein class: synthetase
  - Size: MW 78 kDa, 700 aa (full length)
- Dedicator of cytokinesis 5 (**DOCK5**)
  - Indication: cancer
  - Protein class: guanine nucleotide exchange factor
  - Size: MW 51 kDa, 431 aa (domain)

Signal Plots – VIPS392

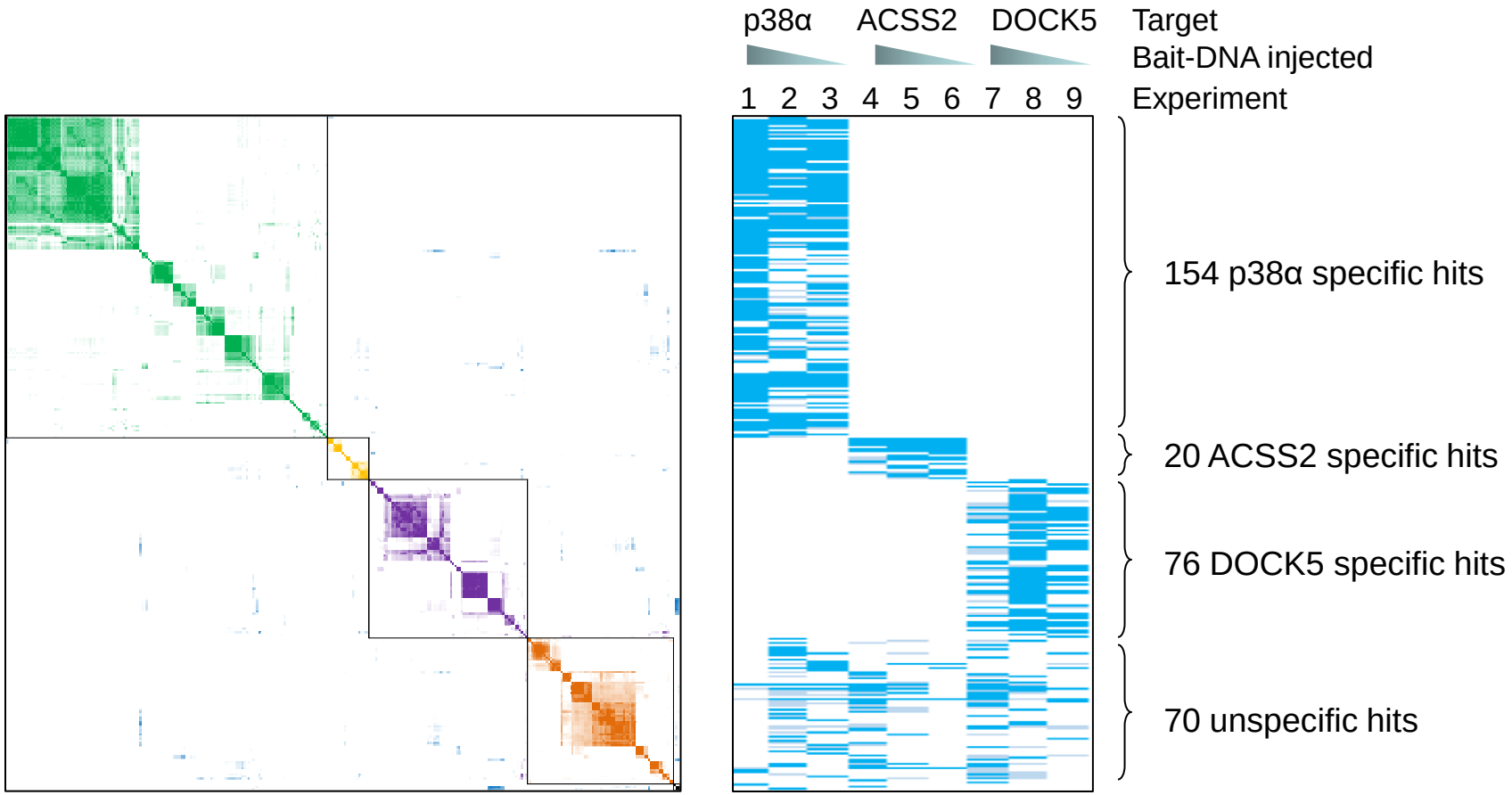


Signal plot legend

Green spheres: Signal dominated by binding event

Red spheres: Signal dominated by random event

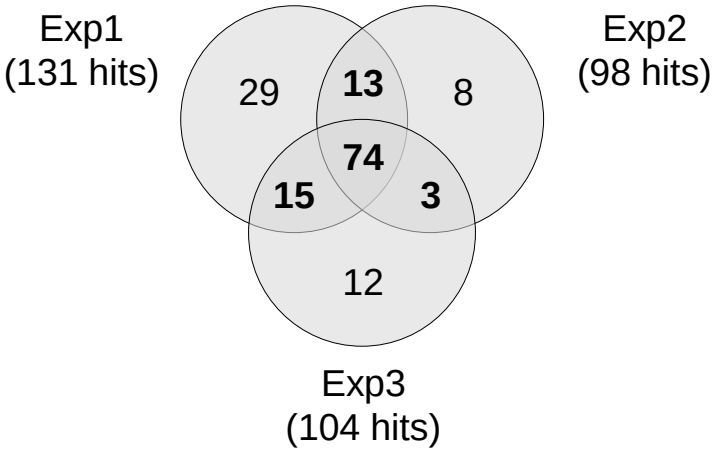
Heat map – VIPS392



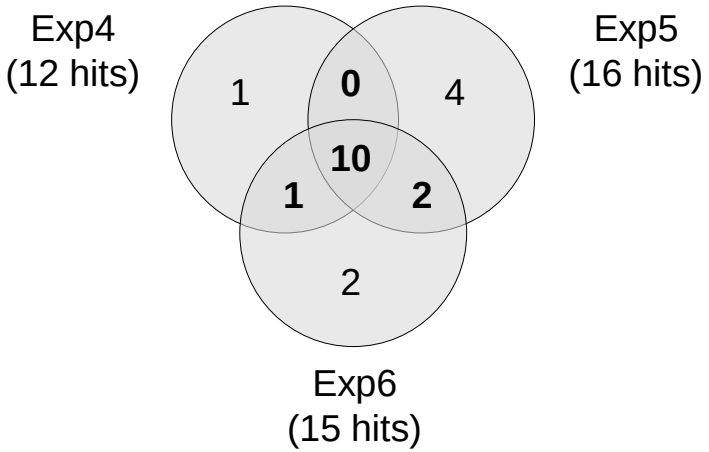
- 323 hits total
- Heat map based on pairwise 2D Tanimoto Similarity Scores
- Best neighbor sorting
- Islands along the diagonal represents chemical clusters
- 3 hits non assignable (lower right corner)

Venn diagrams – VIPS392

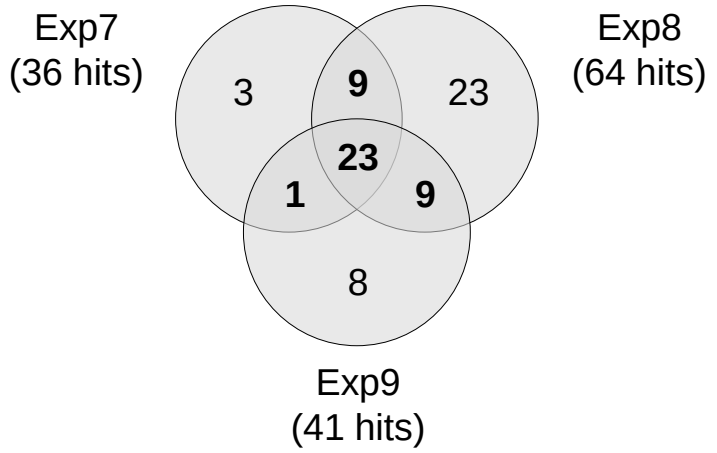
p38α 154 hits



ACSS2 20 hits



DOCK5 76 hits



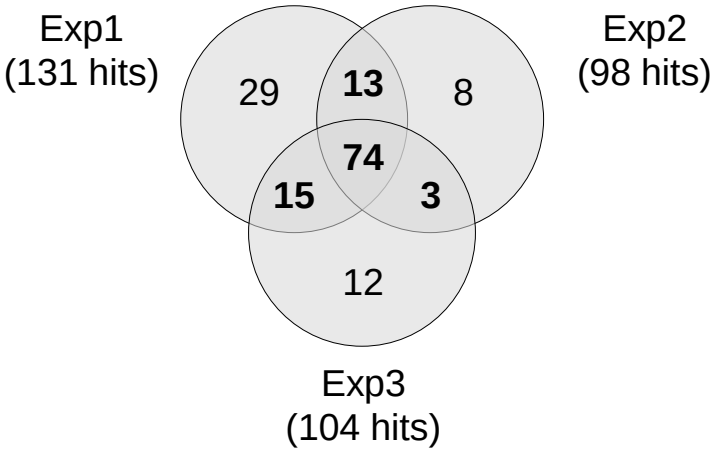
Bait-DNA injected in various amounts

- Exp 1, 4 and 7: 250 fmoles, ~250 nM
- Exp 2, 5 and 8: 100 fmoles ~100 nM
- Exp 3, 6 and 9: 30 fmoles ~30 nM

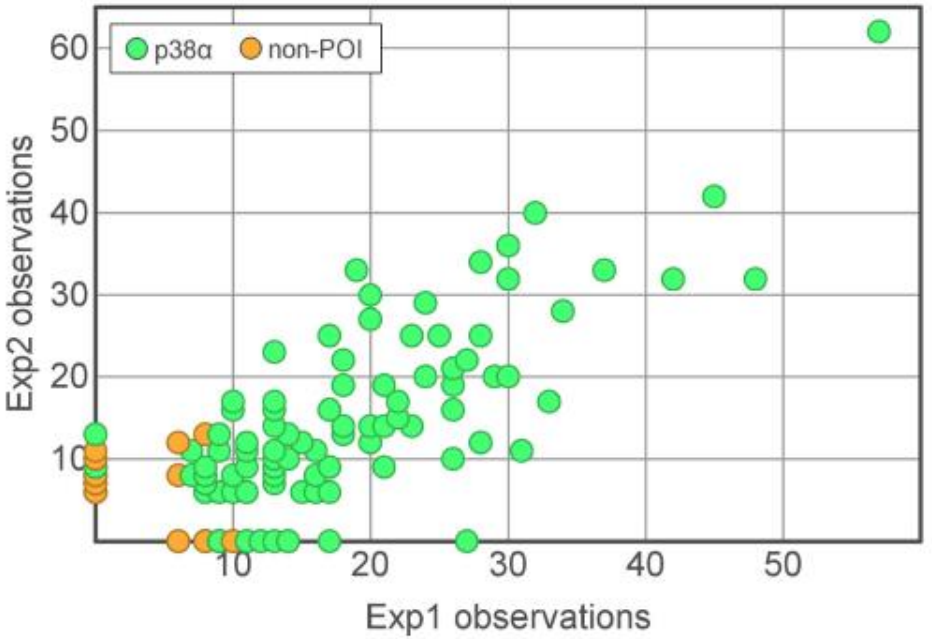
- Similar results across experiments
- A range of bait-DNA concentrations works
- Robust and reproducible

Venn diagrams – VIPS392 – p38α

p38α 154 hits



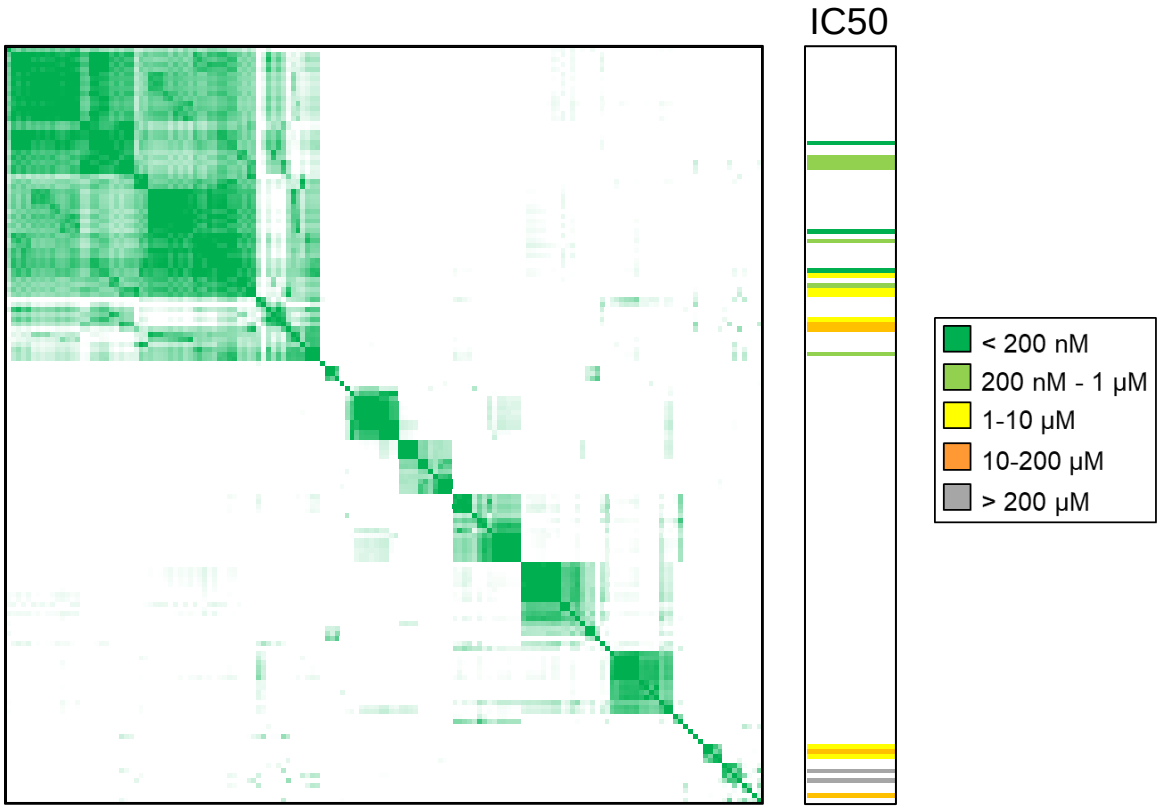
p38α Exp1&Exp2



Direct comparison between Exp1 and Exp2 (250 and 100 fmoles bait-DNA resp) shows:

- that hits with higher numbers of observations are consistently found
- hits with lower number of observations vary
- non-POI hits are observed with lower observations numbers

p38α heat map and IC50 values – VIPS392



154 hits

21 hits resythesized and assayed

- 9 chemical clusters
- 21 hits resynthesized and assayed
- 19 positive in enzymatic ADP quest assay
- IC50 range <25 nM to >200 µM (inactive)

Overlap with finding w conventional screening

Best compound
- Kd 2.5 nM
- Cellular IC50 7 nM

Summary – VIPS392

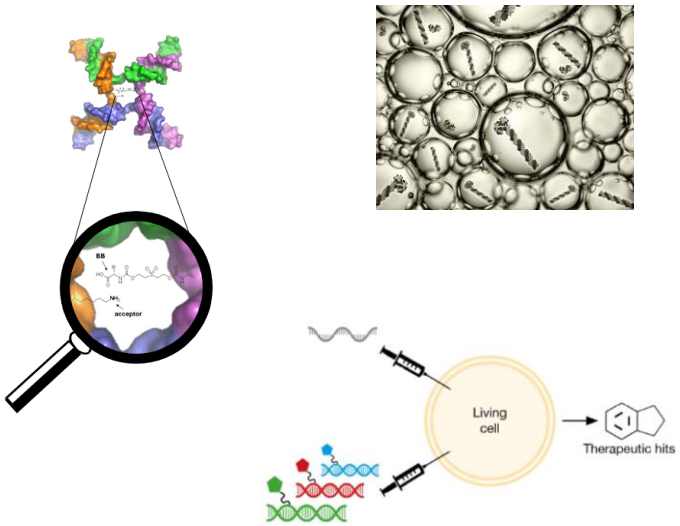
- Target specific hits obtained for all 3 targets
- Chemical clusters obtained for all 3 targets
- Hits confirmed for p38α
- Single digit nM potencies
- Robust and reproducible
- Simple, fast, and scalable analysis

Targets - cBTE

- Human or pathogenic proteins
- Tolerate either N- or C-terminal fusions
- Soluble proteins
  - Full length
  - Fragments
- Membrane proteins
  - Fragments: intra- or extracellular domains
  - Not integral membrane proteins, such as 7TM and ion channels
- Not strong DNA binding proteins (truncated forms with DNA binding domain removed amenable)
- Not too toxic for the cell

Aknowledgement

Vipergen ApS
Charlotte Andersen
Jacob Andersen
Carlos Azevedo
Peter Blakskjaer
Allan B. Christensen
Amaya A. Farinas
Charlotta G. Folkesson
Stine Jørgensen
Ole Kristensen
Daniel Madsen
Lars K. Petersen
Frank A. Sloek
Nils Jakob Vest Hansen



Contact information: Dr Iolanda Micco, imi@vipergen.com