Multiplex Screening of DNA-Encoded Small Molecule Libraries inside a Living Cell

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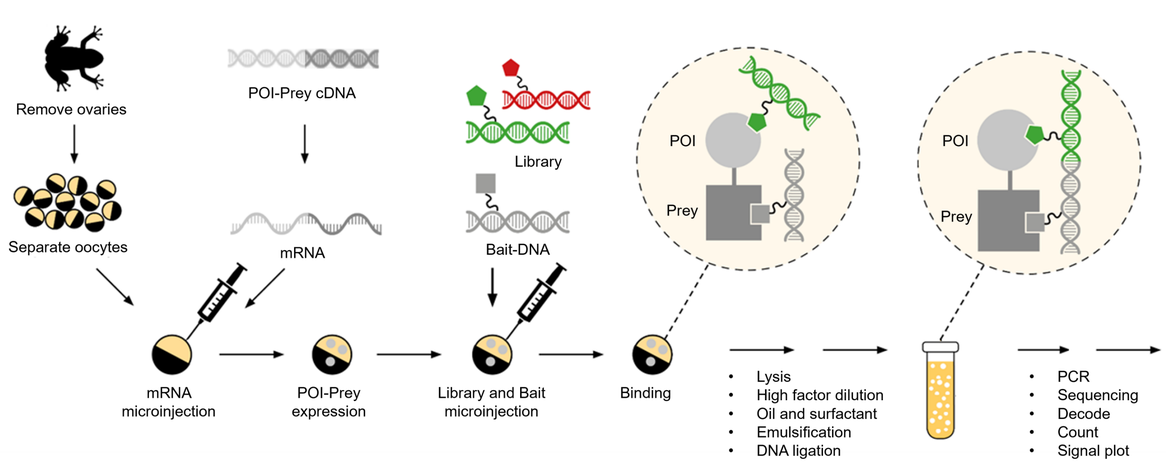
Cellular Binder Trap Enrichment®

The **cellular Binder Trap Enrichment** technology[1] uniquely allows DEL screening inside a living cell. The protein of interest (POI) is expressed as a Prey protein fusion in *Xenopus laevis* oocytes. DEL and Bait-DNA is microinjected into the cell. The affinity of the Prey protein for the small Bait molecule is utilized to label the POI with DNA *in vivo.* DEL binding events are captured by emulsification and DNA ligation. An advantage of the technology is that it allows for **target multiplexing**.



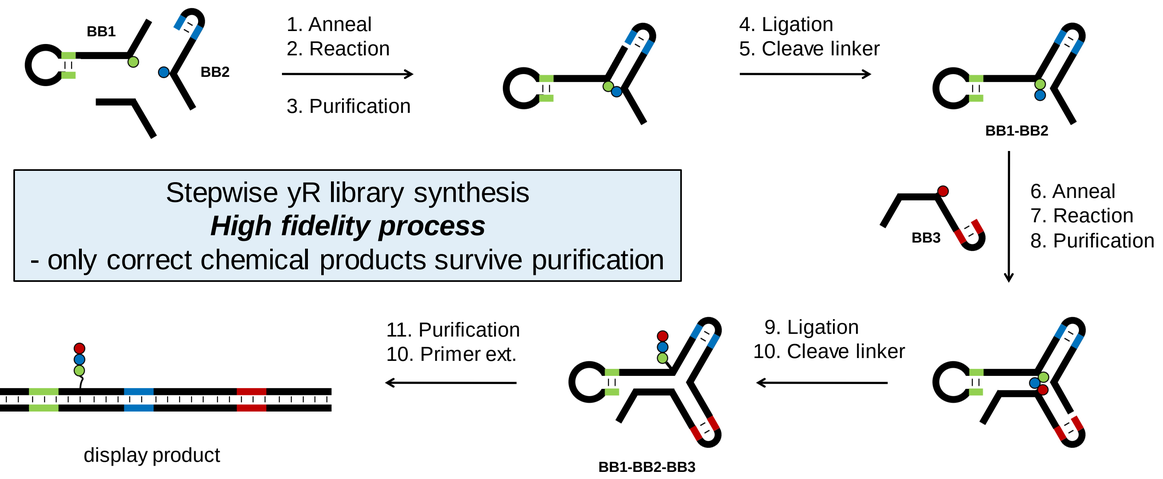
Why screening in cells?

- *Purified protein not needed*
- *Broader target space*
- *Lower attrition rate – physiological screening conditions*
- *Efficient expression – 95% of tested proteins amenable*
- *Higher success rate and faster TAT*

YoctoReactor® and Library Chemistry

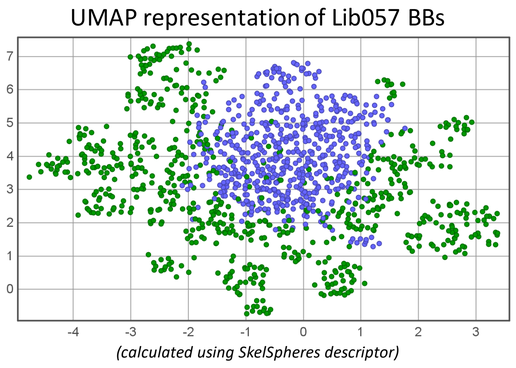
High fidelity libraries are constructed with the **YoctoReactor**[2] which exploits the natural power of DNA to form stable 3D-structures through hybridization. Robust and reliable chemistry (acylation, nucleophilic aromatic substitution, and reductive alkylation) is used to assemble building blocks via simple chemical steps.

Lib081 was designed to allow for rapid off-DNA resynthesis of hits, and is supported by robust logistics, where building blocks are kept in stock to avoid challenges in delivery and synthesis.



Stepwise yR library synthesis
High fidelity process
- only correct chemical products survive purification

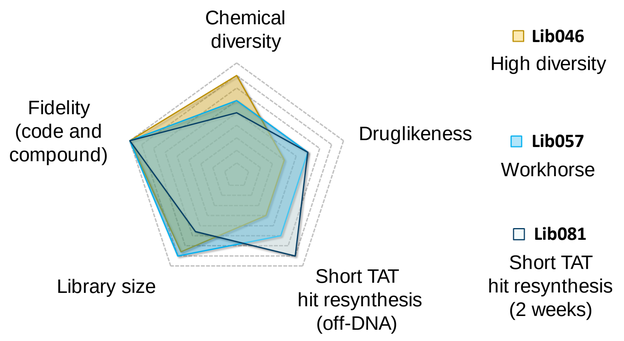
Building blocks



- Commercial BBs (585)
- Vipergen *in-house* BBs (708)

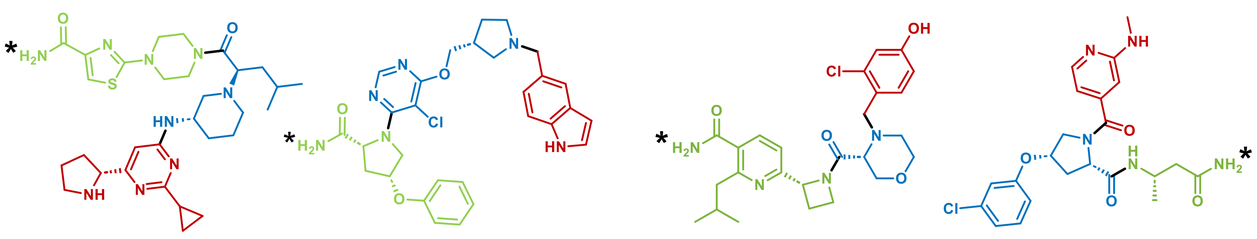
In-house BBs increase chemical space coverage

DEL design criteria



Parameter	Lib046	Lib057	Lib081
Size (million)	445	535	381
Chemistry	Acylation, reductive alkylation and S_NAr	Acylation and reductive alkylation	Acylation and reductive alkylation
Total BBs (#)	1507	1293	1138
Designer BBs (#)	979	708	617
Scaffolds (#)	368	349	295
Molecular weight (avg)	612	525	525
cLogP (avg)	2.8	0.8	0.9
HBA (avg)	8.7	6.2	6.2
HBD (avg)	2.8	2.8	2.9
Rotatable bonds (avg)	10.7	8.7	8.9
TPSA (avg)	153	138	137
Fsp³ (avg)	0.5	0.6	0.6

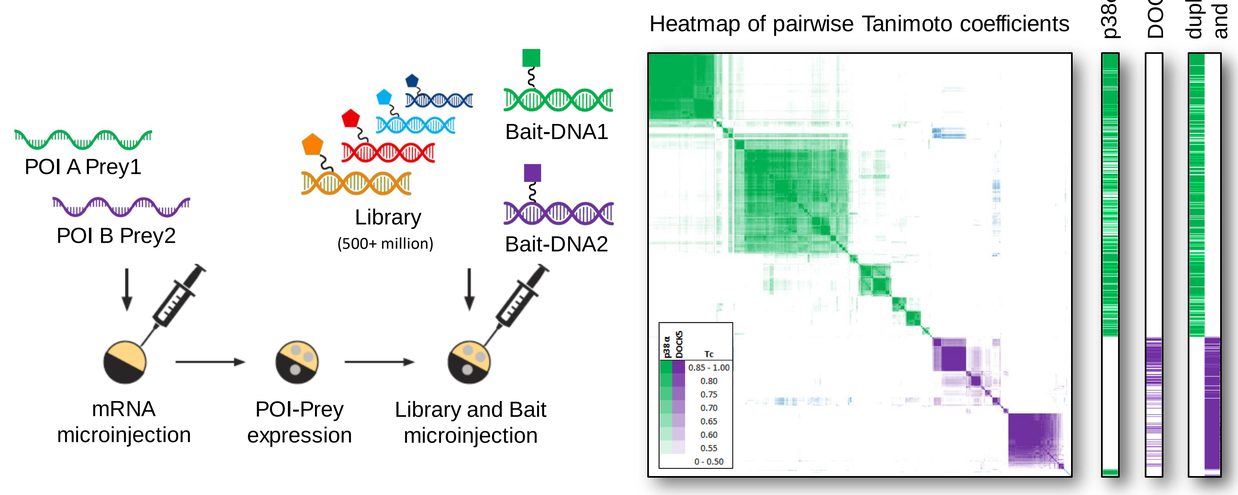
Library compound examples



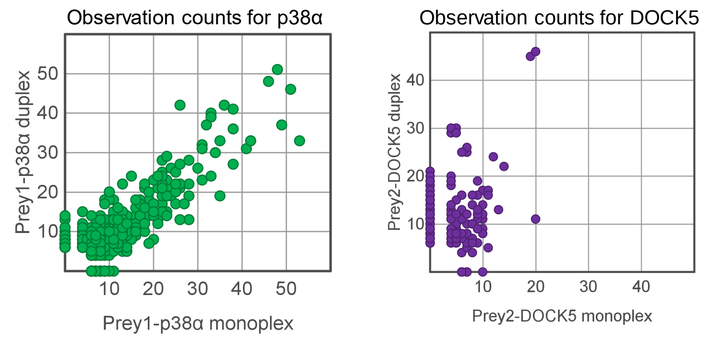
Lib046 * = DNA attachment Lib057 and Lib081

Multiplex screening

In **multiplexing**, a mixture of multiple mRNAs is injected, and POI-Prey fusion proteins are expressed simultaneously. Each carries a distinct Prey. In the second injection, DEL and matching Bait-DNAs are injected, thus specifically labelling each of the fusion proteins. Example of p38α and DOCK5 :



- ✓ High reproducibility between monoplex and duplex
- ✓ No cross-talk for non-interacting POIs



Why multiplexing?

- *Screening of multiple POIs in the same cell*
- *Cost-effective*
- *Enables screening for interacting POIs e.g. molecular glue*

Short Turn-Around-Time

Target amino acid sequence

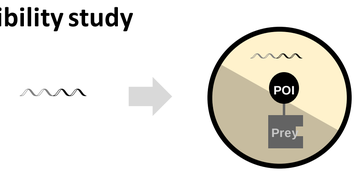
Day 1 — **Feasibility study**

Day 10 — **DEL Screening**

Day 20 — **Resynthesis**

Day 30 — **Hit compounds**

Feasibility study



cDNA design and transcription — Expression in living cells

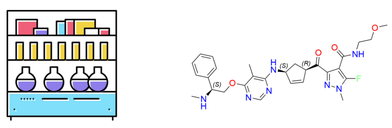
- Target amino acid sequence used for designing cDNA constructs
- mRNA injected into living cells
- Expression validated by Western blotting

DEL screening

Screening in living cells — DNA sequencing, decoding and counting

- Feasibility study results used for the screening design
- DELs optimized for fast resynthesis
- Success rates of DEL screening in cells >75%

Hit resynthesis



Chemical building blocks ready — Rapid resynthesis

- Chemical building blocks kept on stock
- Optimized logistics for rapid off-DNA resynthesis
- >80% of hits can be resynthesized in 2 weeks

Summary

- DEL screening is performed in a living cell, increasing success rates and lowering attrition rates
- The POI is expressed as an intrinsic part of the process → purified protein is not needed
- Multiplex screening can be utilized for to increase throughput, or for screening of interacting proteins
- DELs have been designed with focus on the ability to rapidly resynthesize hit compounds while maintaining good physicochemical properties and diversity
- Logistics and infrastructure of the whole process has been optimized – from gene synthesis to resynthesis of hit compounds enabling short Turn-Around-Time (30 days)

References and Contact Information

1. Petersen, L. K. *et al J. Am. Chem. Soc.* **2021**, *143*, 2751-2756.
2. Hansen, M. H. *et al J. Am. Chem. Soc.* **2009**, *131*, 1322-1327.

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