

Yotta (Y) = 1,000,000,000,000,000,000,000,000

Zetta(Z) = 1,000,000,000,000,000,000,000,000

Exa(E) = 1,000,000,000,000,000,000,000

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

A yoctoliter-scale DNA reactor for small molecule evolution

2nd International Symposium on
"DNA-Encoded Chemical Libraries"

August 20th 2010,
Zurich, Switzerland

Dr Nils Jakob Vest Hansen, CEO
nils_hansen@vipergen.com
www.viper-gen.com

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

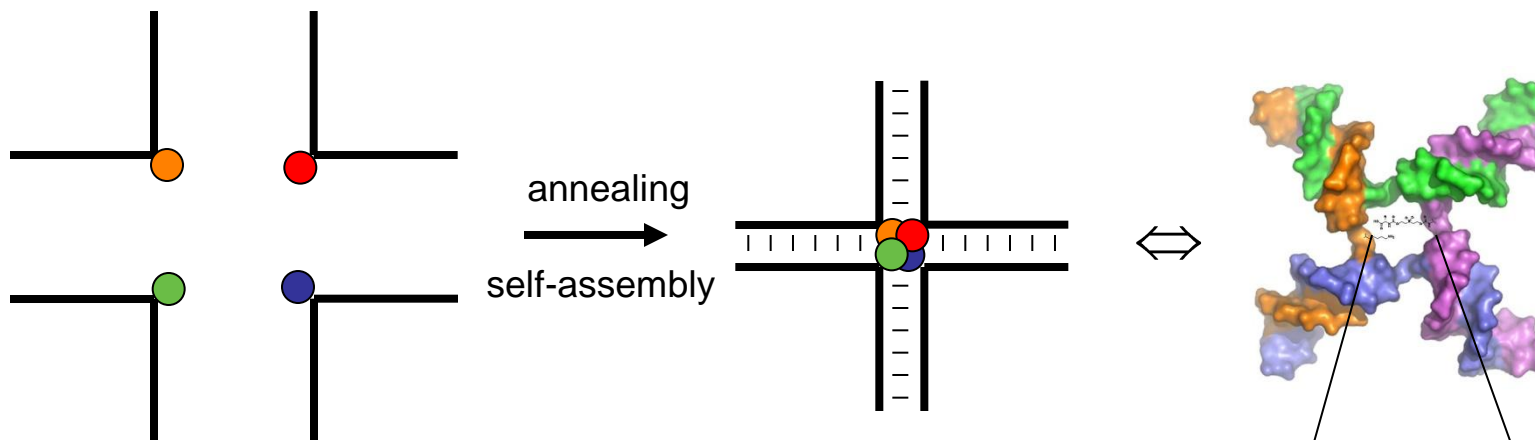
Femto(f) = 0.000 000 000 000 001

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

The YoctoReactor®

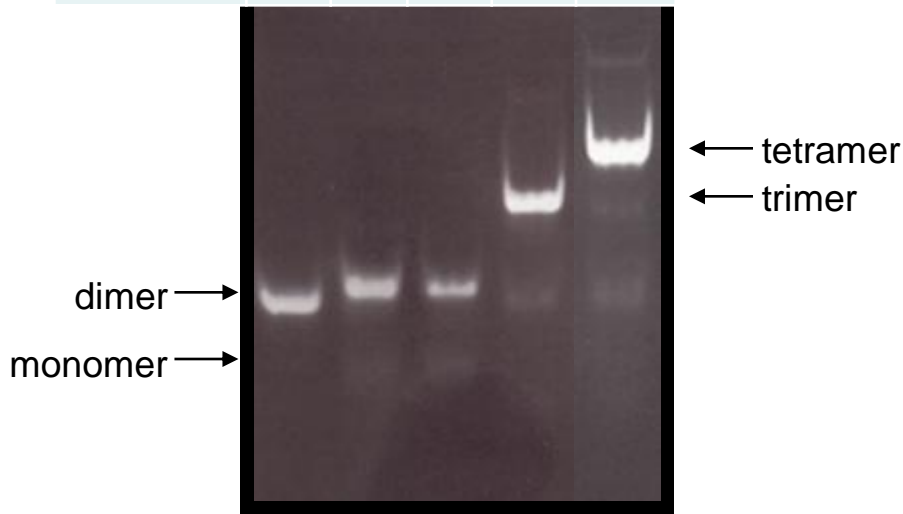
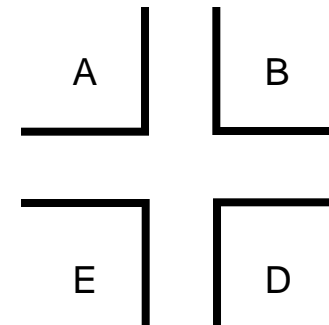
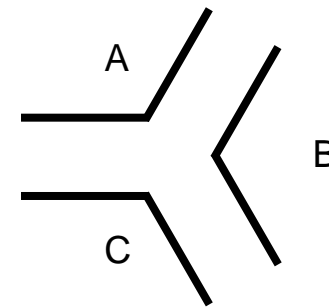


Complementary oligo's with
chemical reactants attached

***The volume of the reactor is on the order of
a yoctoliter (10^{-24} liter), hence the name.***

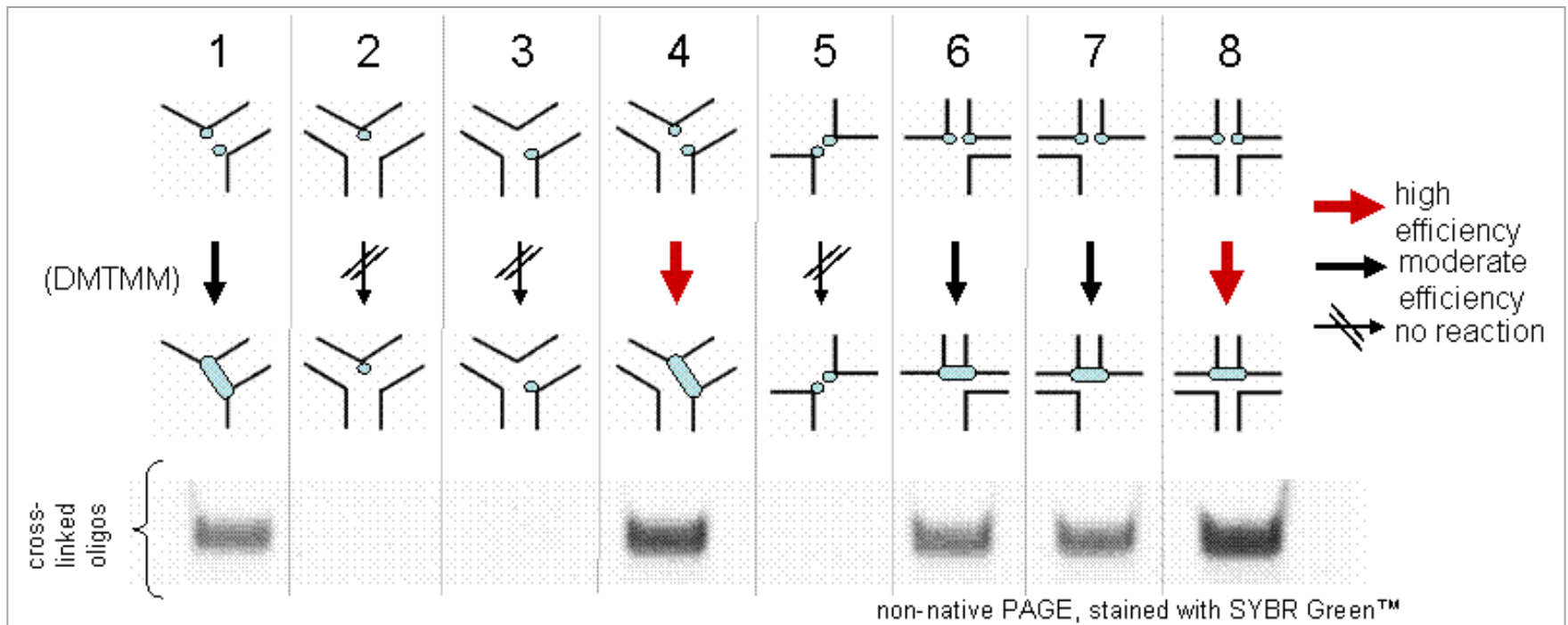
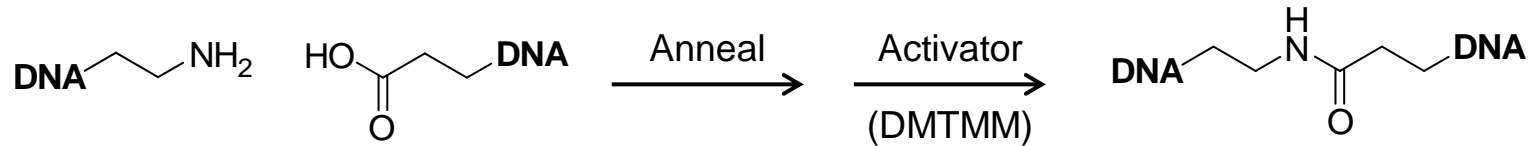
Simple self-assembly of the yR DNA structures

Lane	1	2	3	4	5
A oligo	+	+		+	+
B oligo	+		+	+	+
C oligo		+	+	+	
D oligo					+
E oligo					+

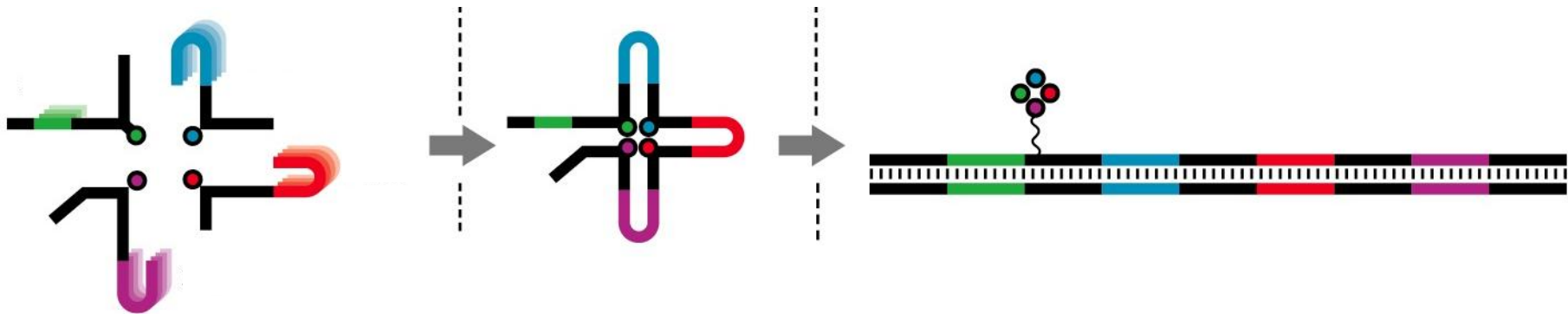


7.5% polyacrylamid gel, stained with EthBr

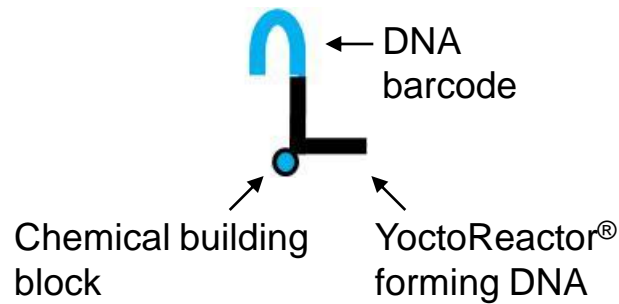
The yR affords strict control over chemical reactions



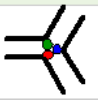
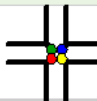
DNA barcoded yR libraries



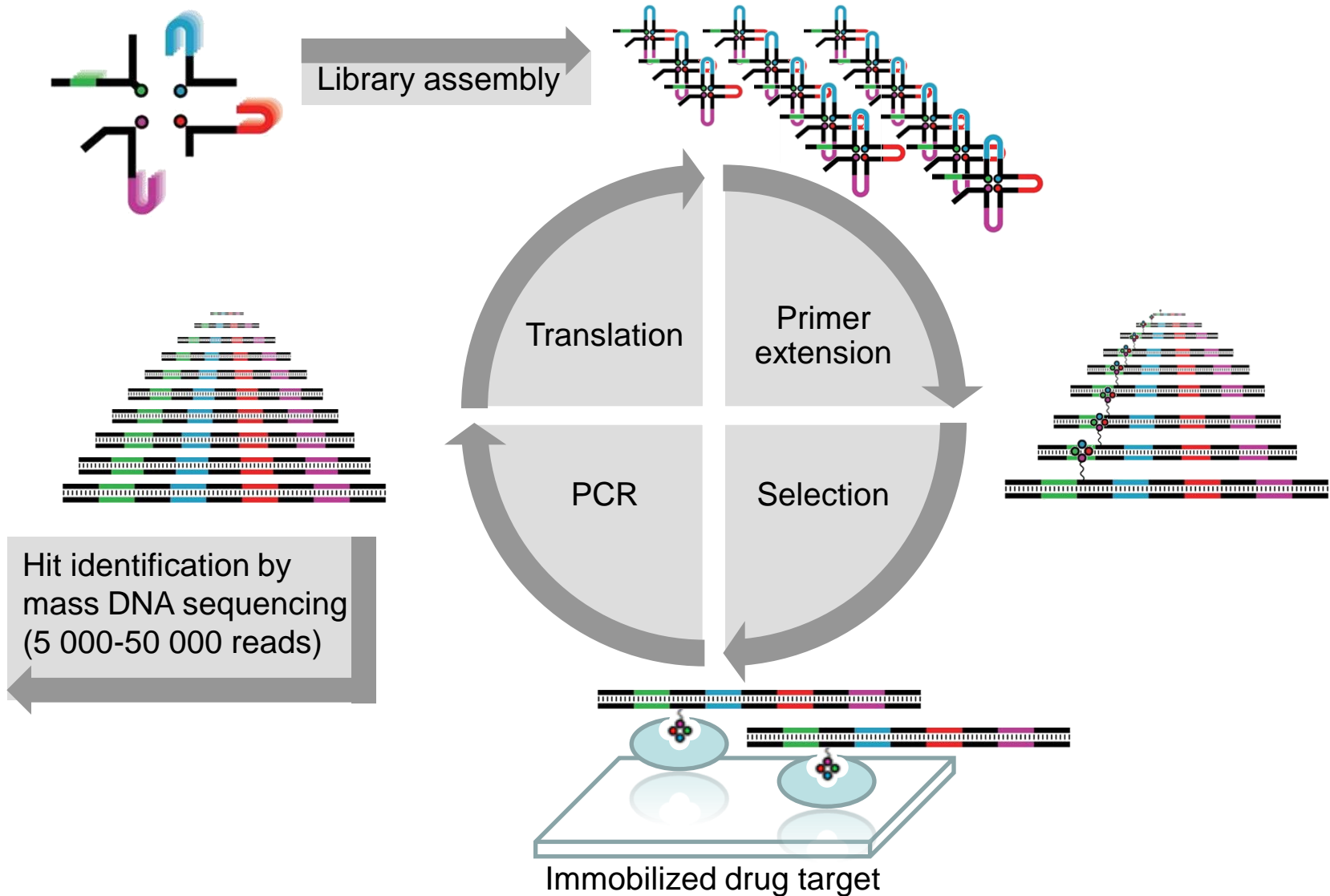
DNA barcoding



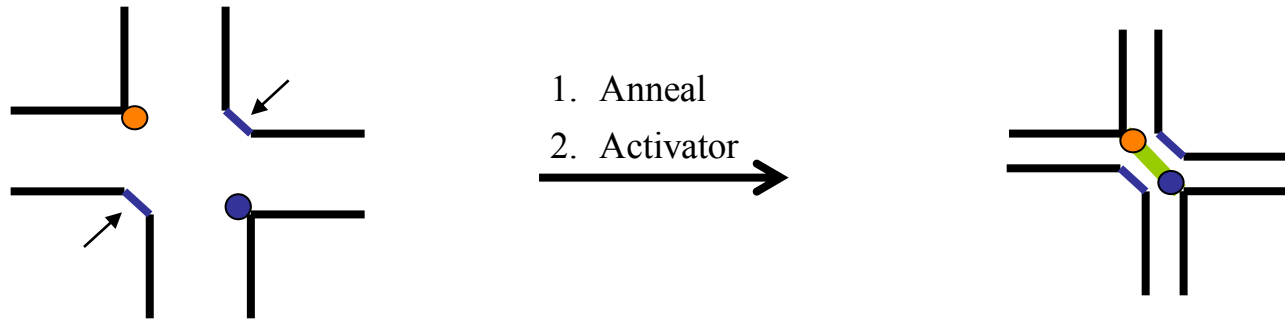
Library sizes

Number of oligos per position	Reactor Geometry	
		
100	1×10^6	1×10^8
400	6×10^7	3×10^{10}
1000	1×10^9	1×10^{12}

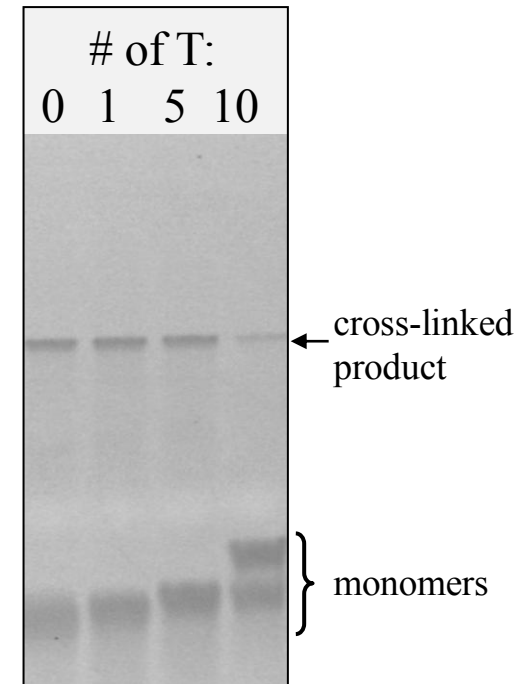
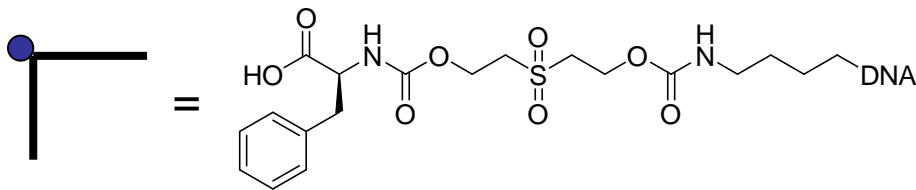
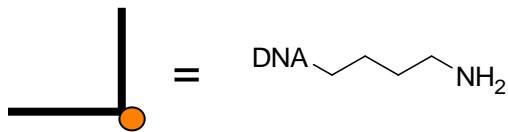
Using nature's solution - Evolution



YoctoReactor Size

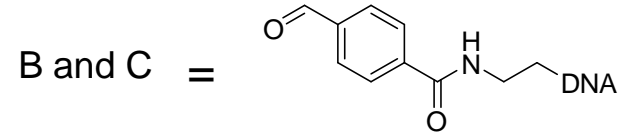
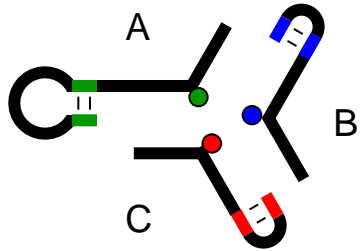


Variable number of nucleotides
(# of T = 0, 1, 5 or 10)

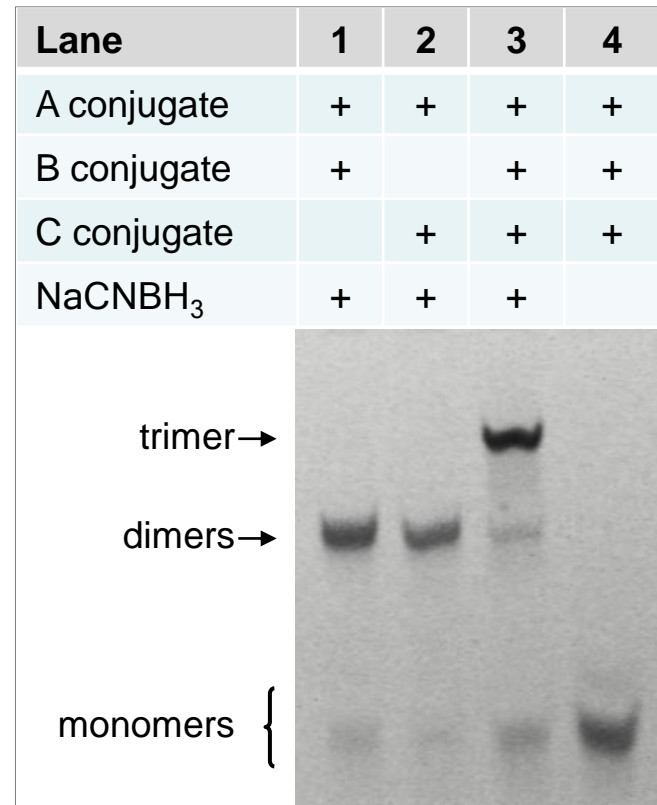
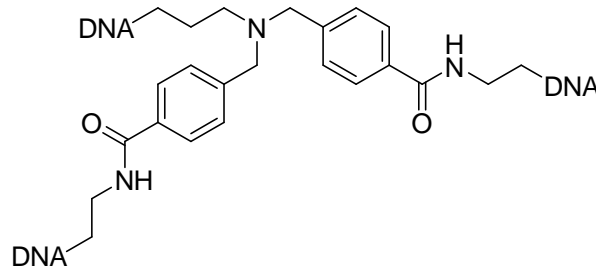
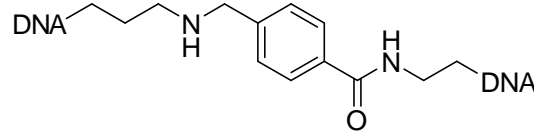
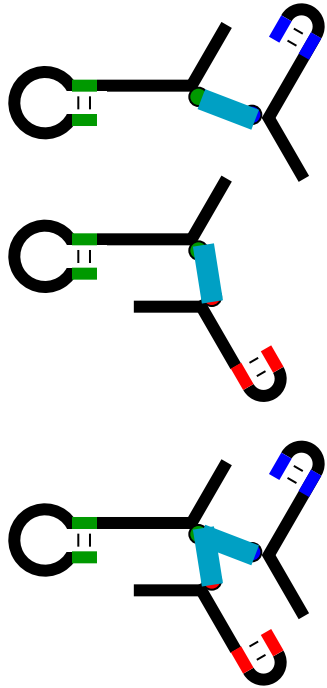


10% non-native PAGE, stained with SYBR Green™

3-way cross linking - double reductive amination

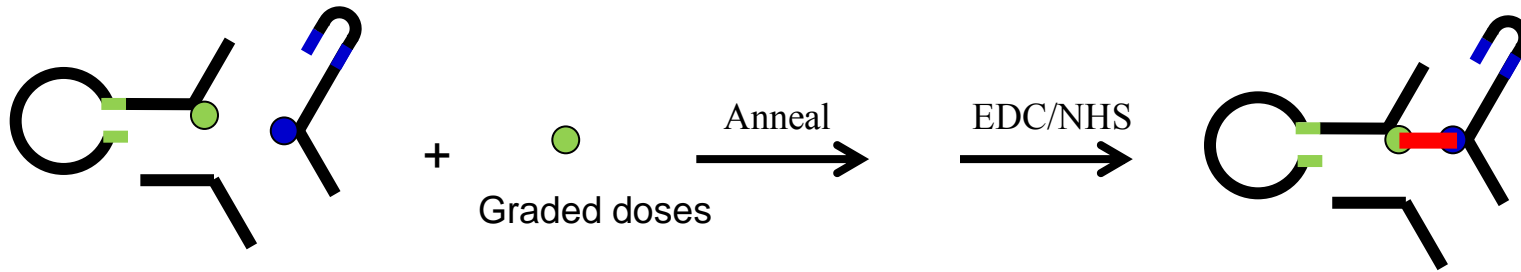


1. Anneal
2. NaCNBH₃

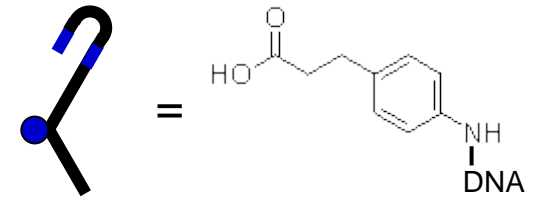
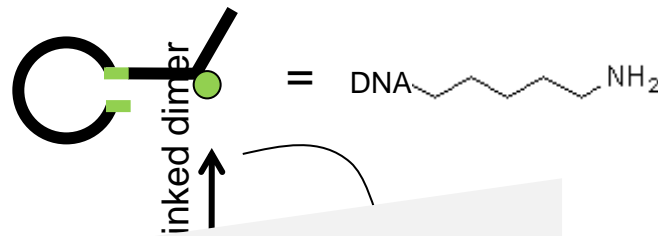


10% non-native PAGE, stained with SYBR

Effective local concentration



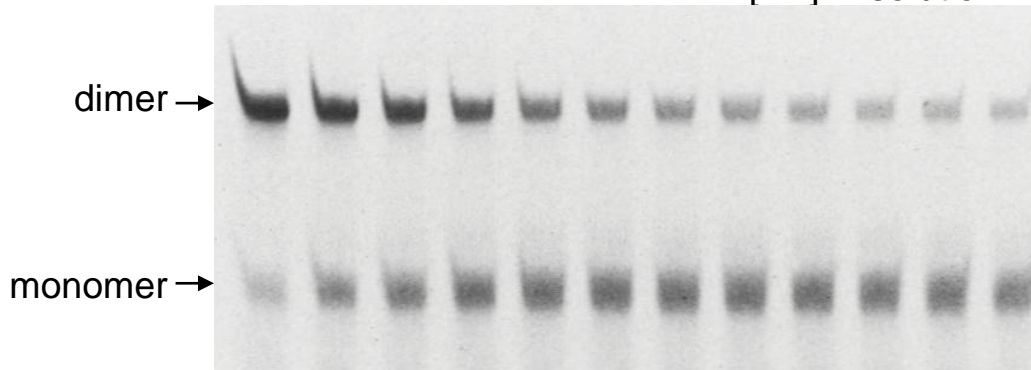
● = MeNH₂-HCl



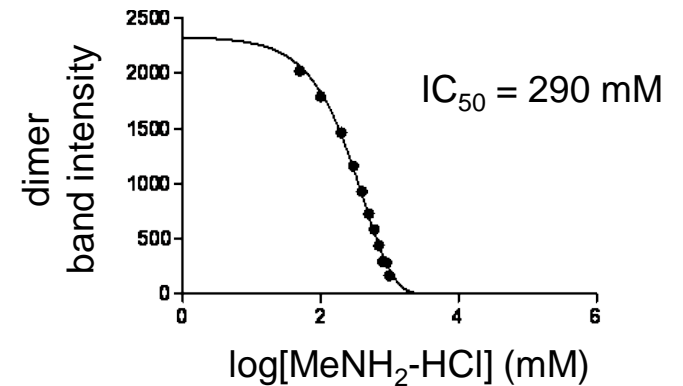
MeNH₂-HCl
(mM)

0 50 100 200 300 400 500 600 700 800 900 1000

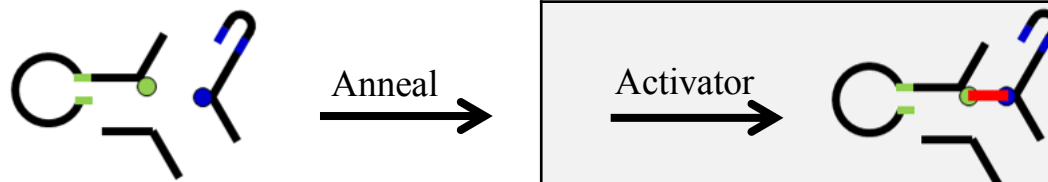
50%



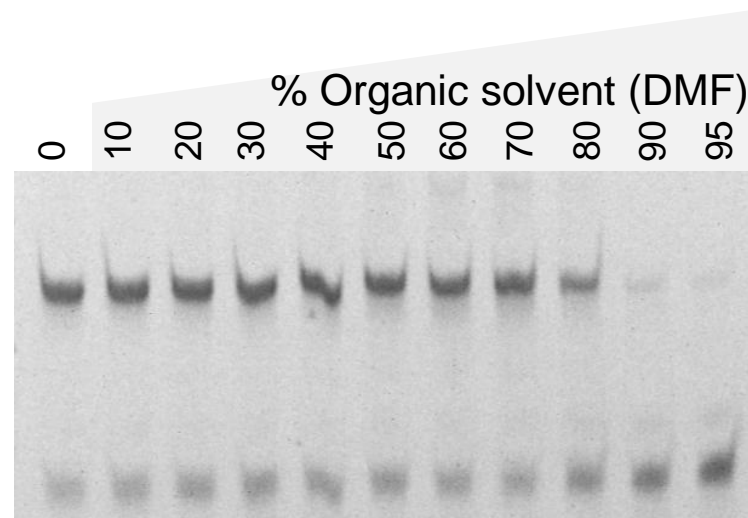
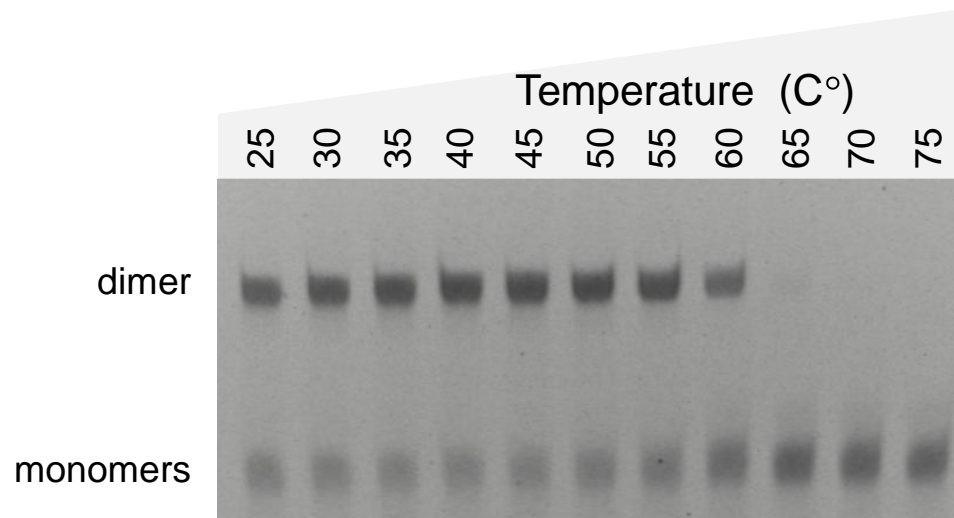
10 % non-native PAGE, stained with SYBR green™



Conditions for γ R chemistry



Temperature	< 60 C
Organic solvent concentration	< 80 %
pH range	4-10
Microwave compatibility	yes

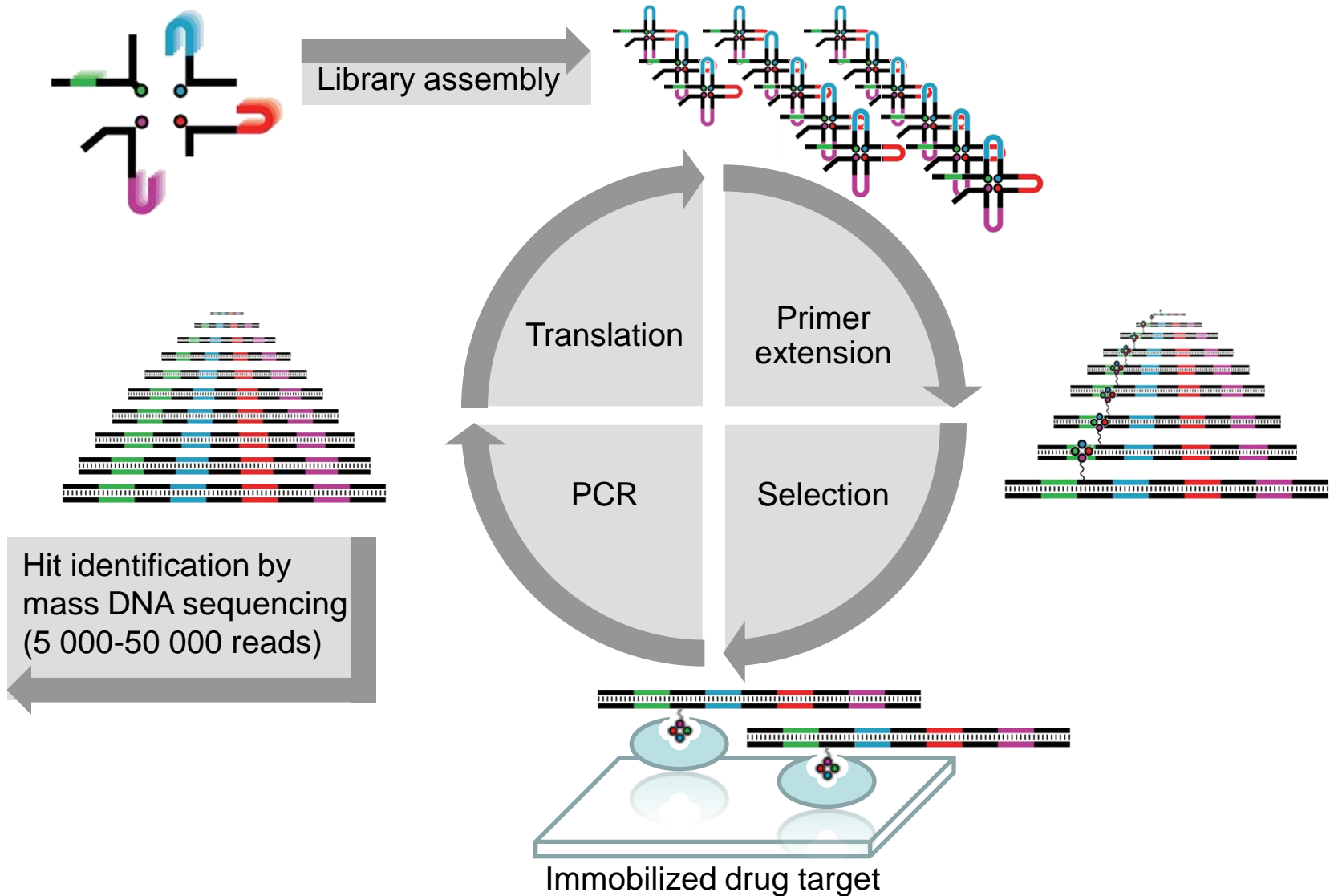


10 % non-native PAGE, stained with SYBR green™

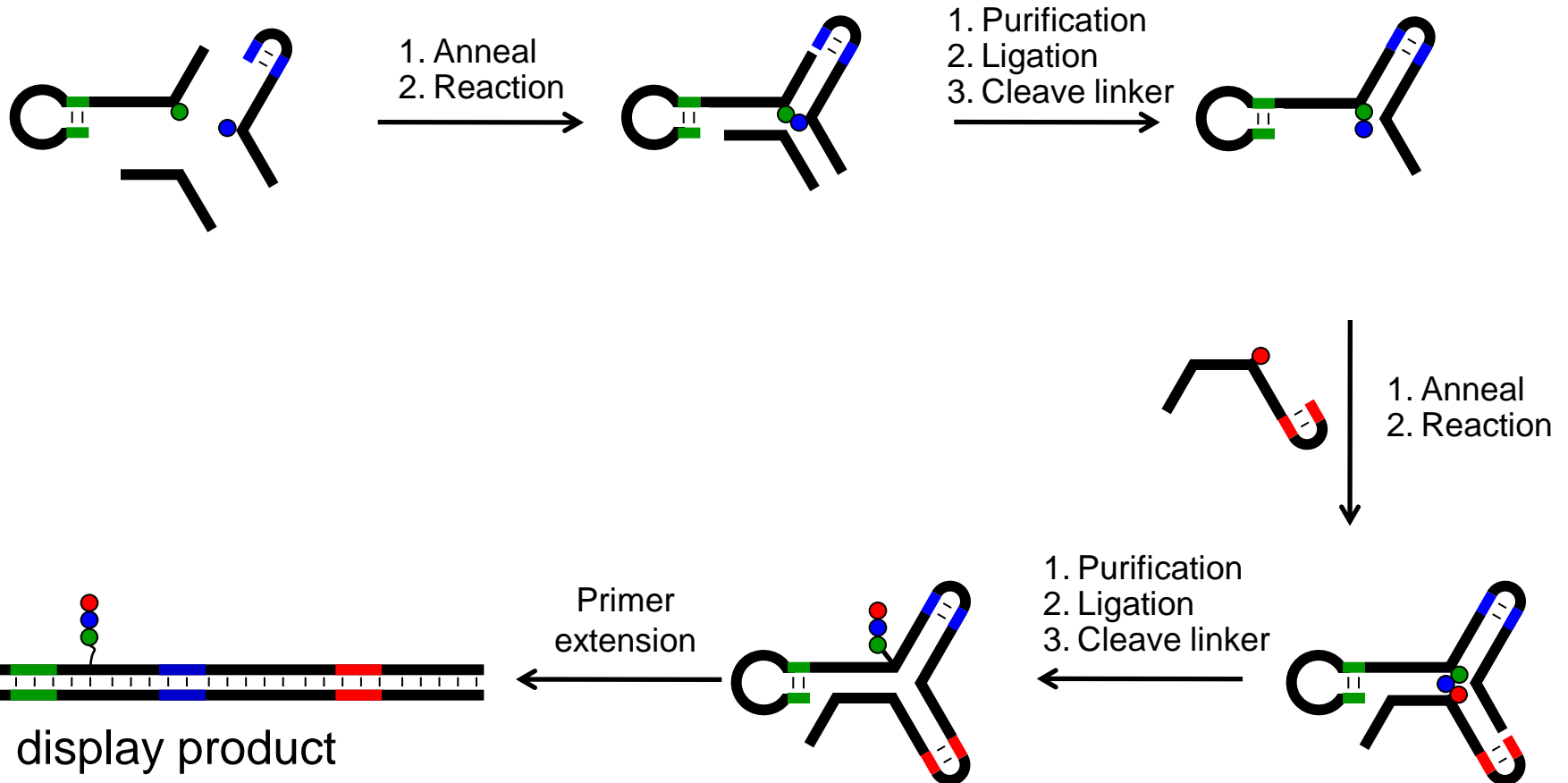
ViperGen Chemistries demonstrated in the yR

Reaction name	Structure	Bond type
Amine acylation		C(O)-N
Urea formation		2 × C(O)-N
Reductive amination		C-N
Nucleophilic aromatic substitution		Ar-N
Conjugate addition		C-Nu (Nu = S or N)
Aldol condensation		C-C
Wittig olefination		C=C
Heck coupling		C-C
Alkyne-alkene coupling		C-C
Sonogashira coupling		C-C

Using nature's solution - Evolution

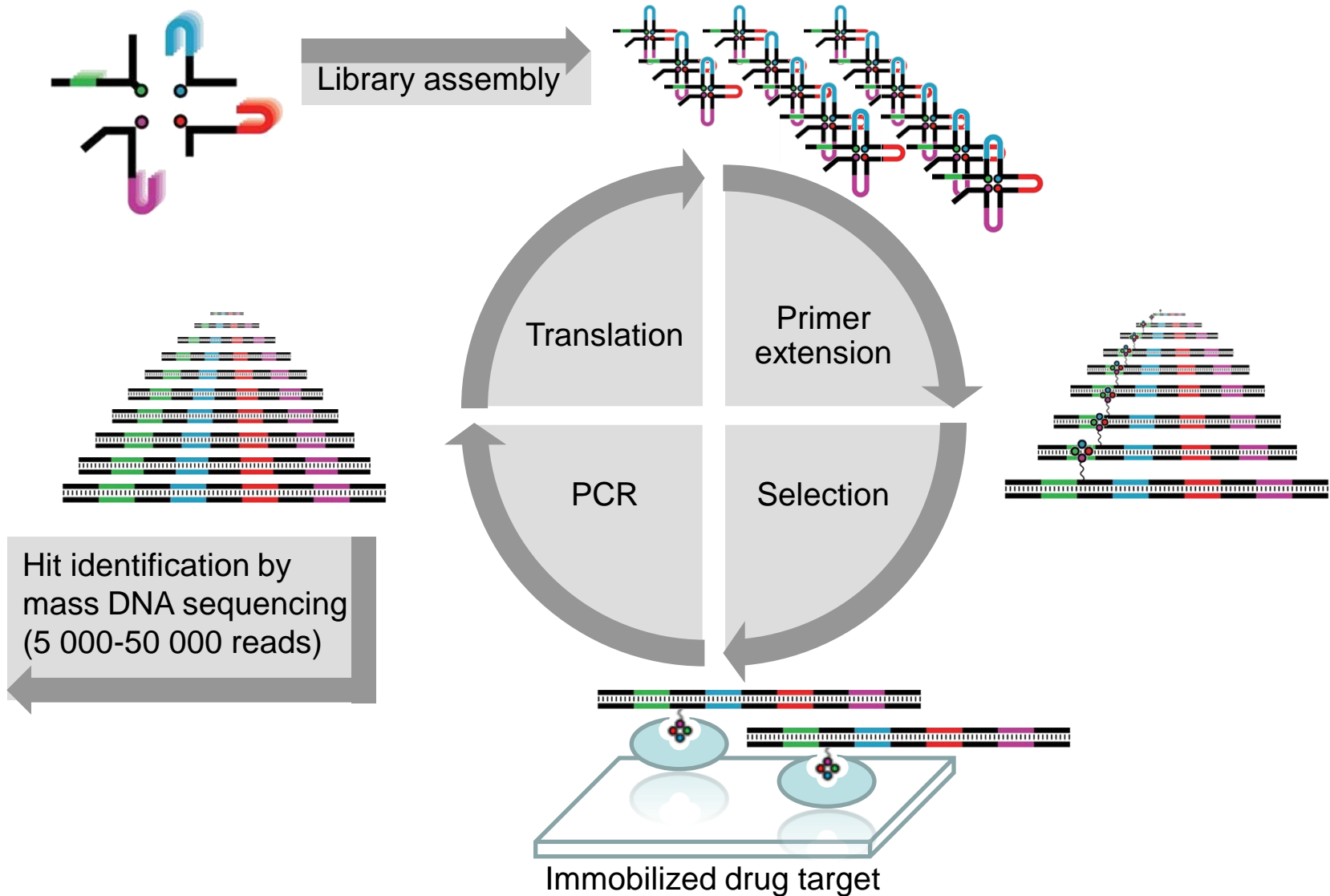


Stepwise yR library synthesis



High fidelity process with built in quality control

Using nature's solution - Evolution

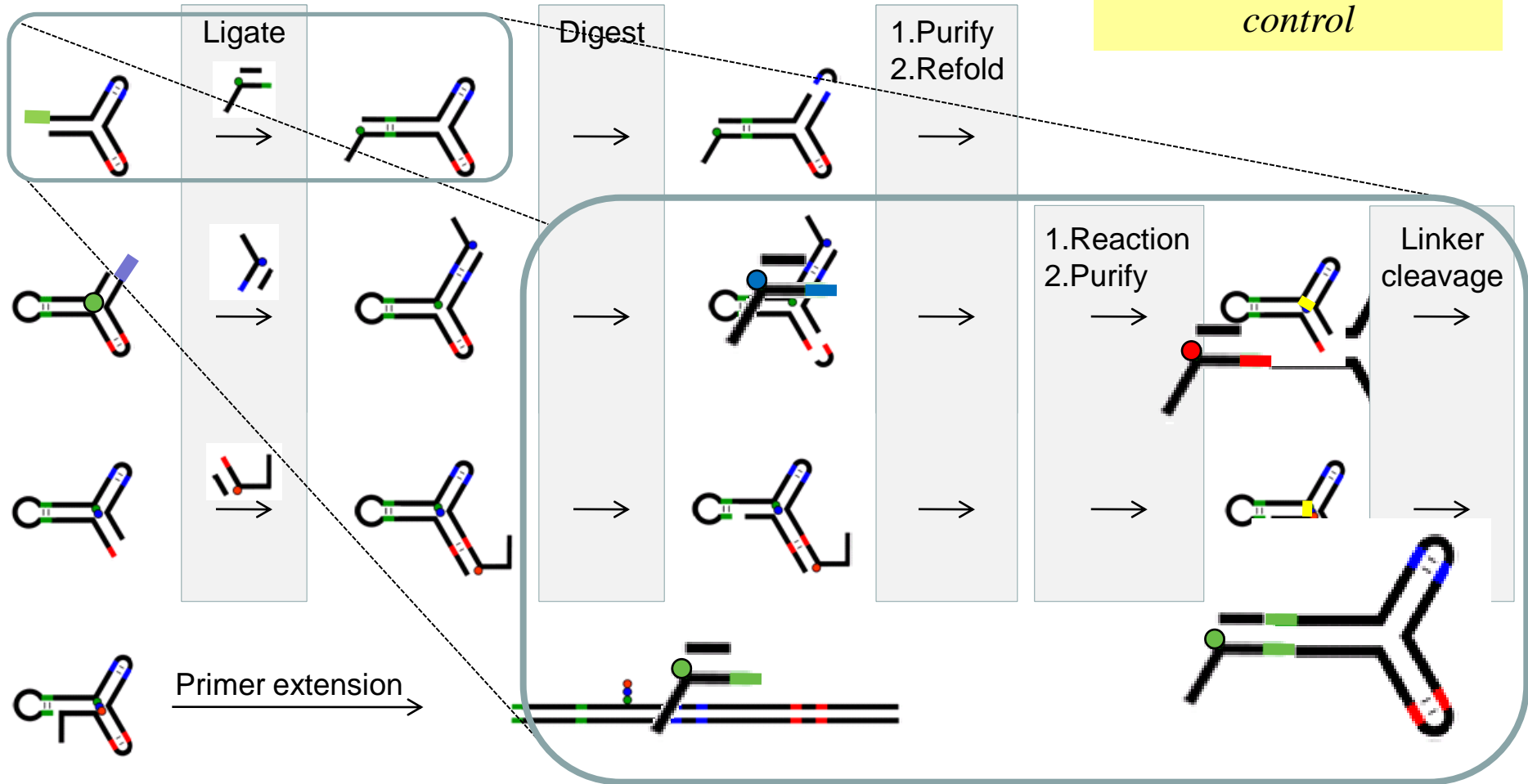


Rolling Translation

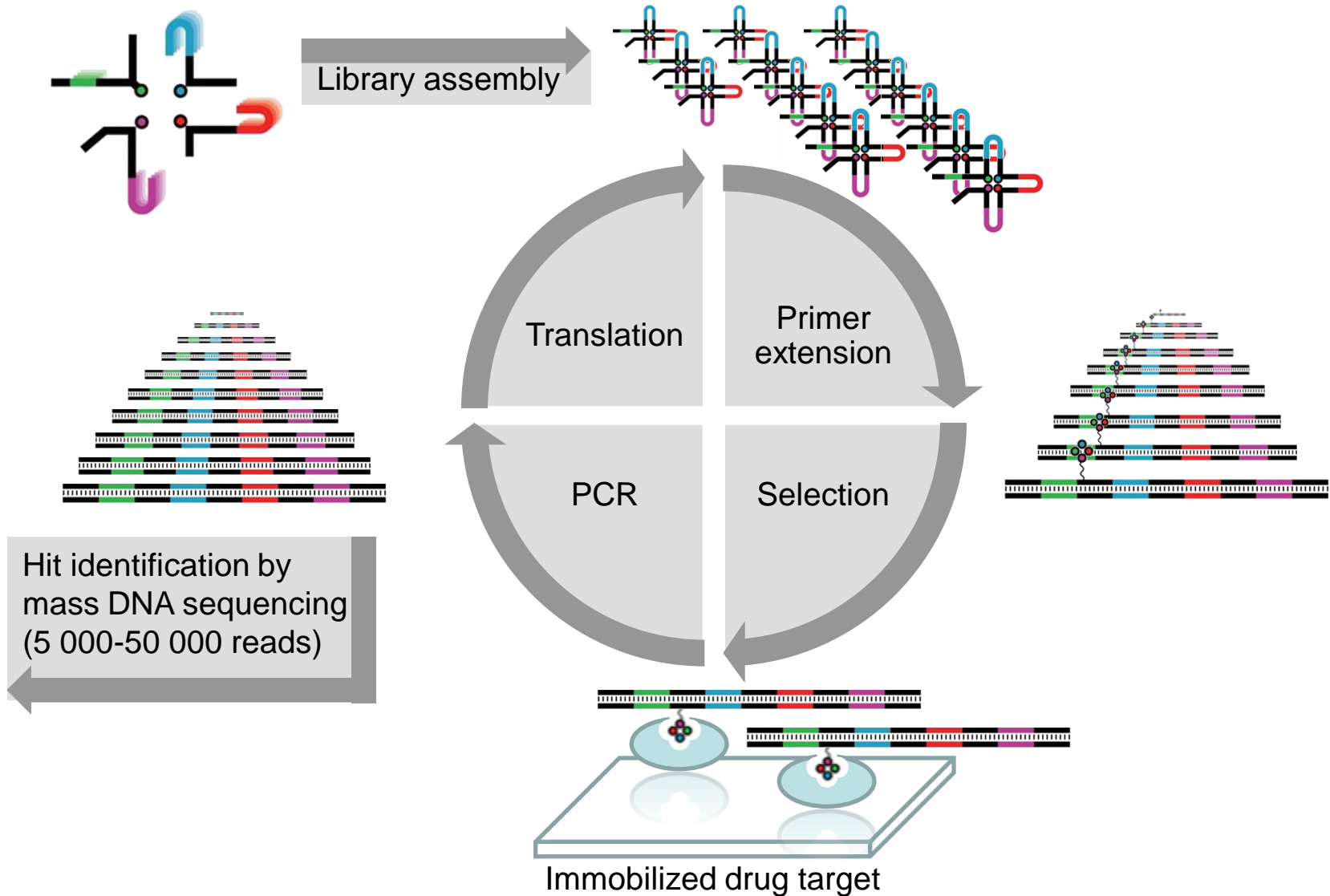
1.Purify
2.Refold



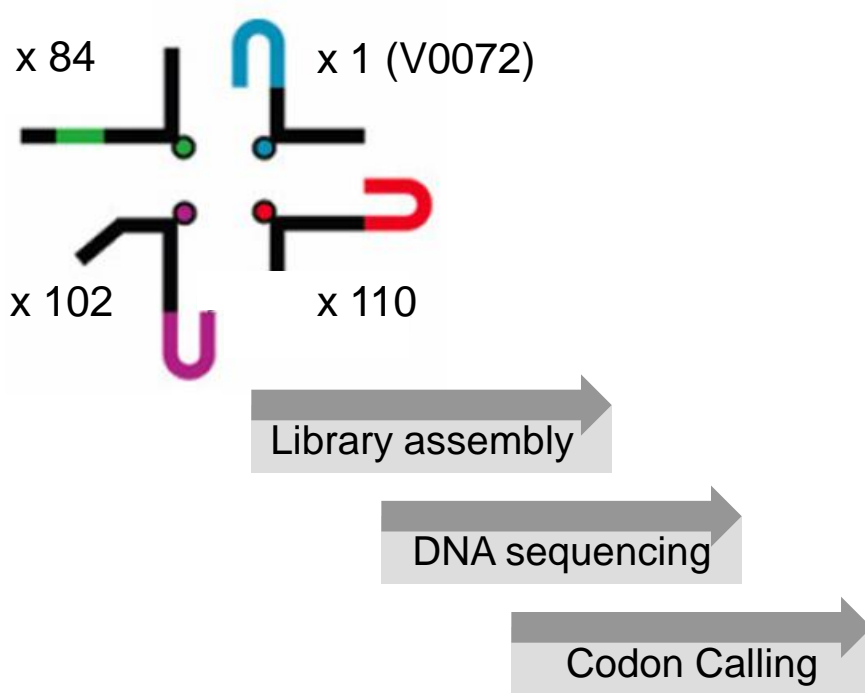
*High fidelity process
with built in quality
control*



Using nature's solution - Evolution

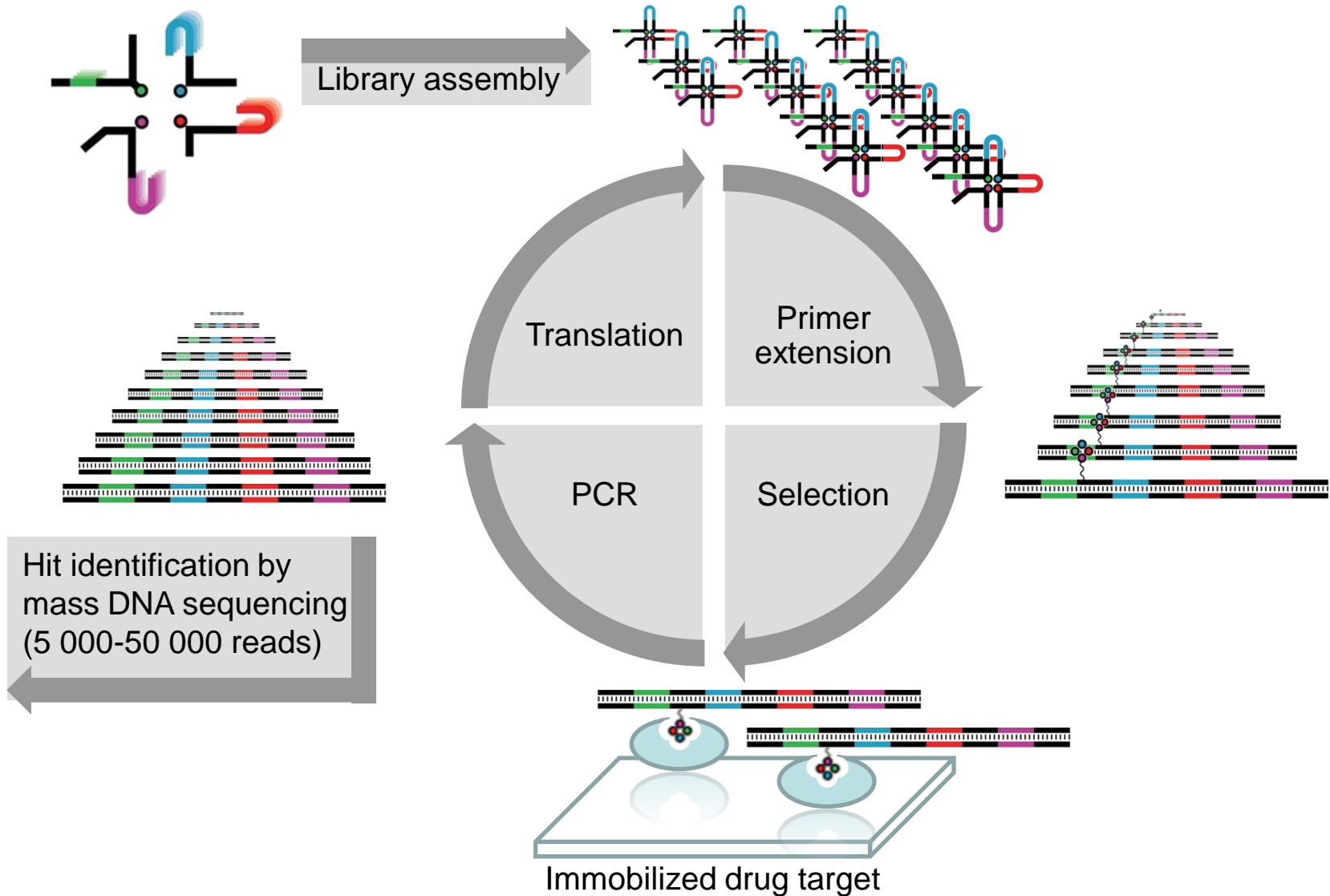


Coding/Decoding Fidelity



BB2	# reads	fraction
V0072	275 250	99.95%
V0350	84	0.03%
V0199	30	0.01%
V0202	8	0.00%
D0015	5	0.00%
V0181	5	0.00%
D0019	3	0.00%
D0014	2	0.00%
D0001	1	0.00%
D0017	1	0.00%
V0166	1	0.00%
V0173	1	0.00%
Total	275391	100.00%

Using nature's solution - Evolution

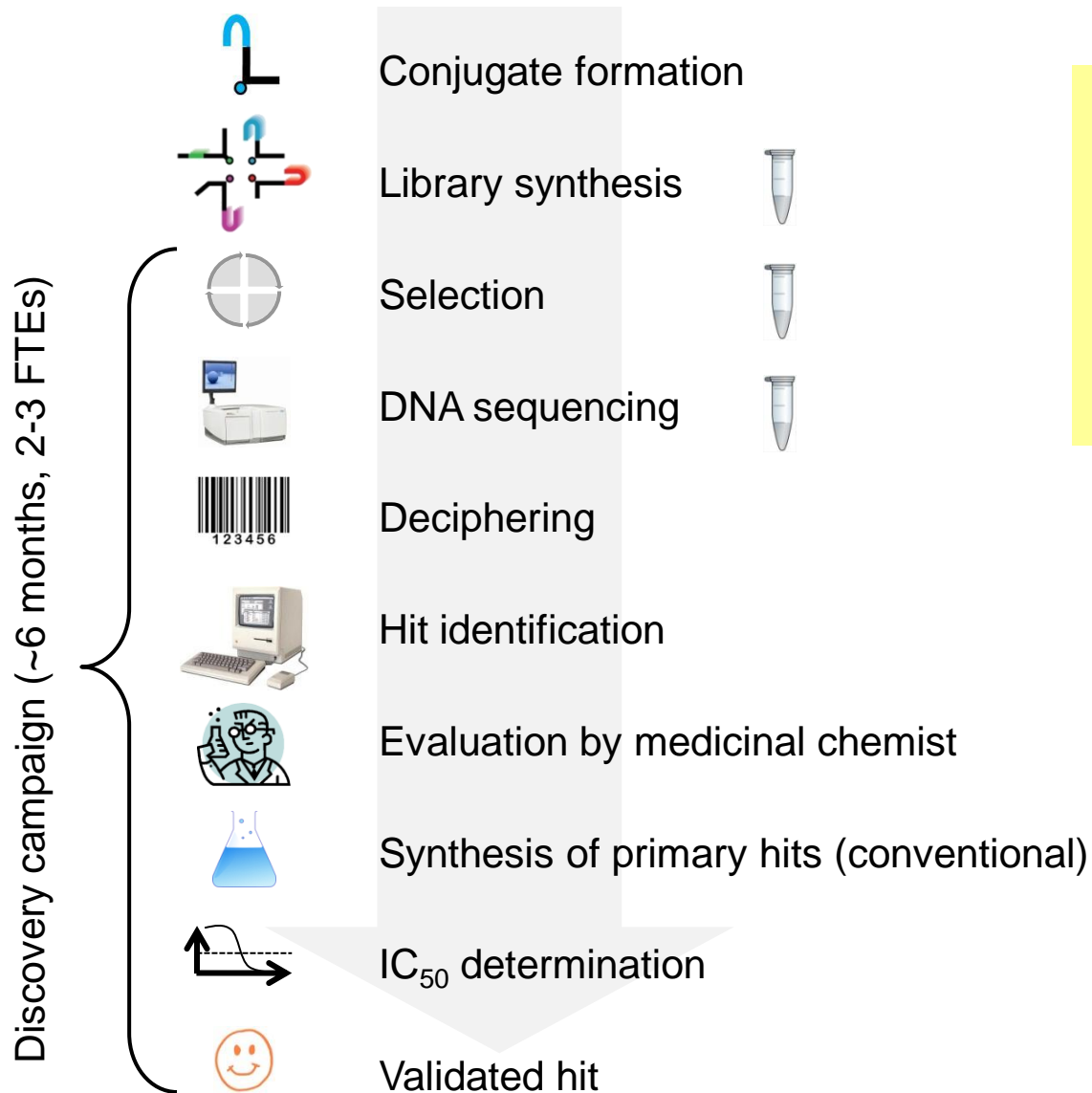


The yR drug discovery technology platform

	HTS	YoctoReactor
Approach	Robotics	single tube
Cost & resources	\$\$\$\$\$	\$
Pharmacophore motifs	any	any
BB functionalities	≥ mono	≥ bi
Chemistries	★★★★	★★
Library size	~10 ⁶	up to 10 ¹²
Molecular evolution	No	Yes

Access to chemical libraries of significant size and diversity along with the technology to handle such diversity will increase the quantity of quality drug hits coming out of hits discovery efforts.

yR Drug Discovery



High fidelity essential

- Cost of synthesis of primary hits potentially significant
⇒ Low false positive rate critical
- Scalability

Example: million compound library (Lib012)

Major considerations in library design

- Drug-likeness
- Library size
- Diversity in structure & pharmacophore motif
 - i. Multiple YoctoReactor[®] chemistries
 - ii. BBs from three groups: flexible, constrained, and planar
 - iii. BBs with a range of pharmacophore motifs
 - iv. BBs with pharmacophore motifs with a range of orientations

	RO5	Lib012
Mw	<500	465
HBA	<10	7.9
HBD	<5	3.8
clogP	<5	1.4
PSA	-	114 Å ²
# rotatable bonds	-	9

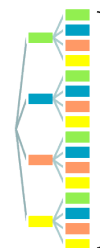
>90% drug-like compounds

	RO5	Lib012
reductive amination	<500	5
acylation		
HBD		
nuc. arom. subst		
urea	-	Å ²
# rotatable bonds	-	

>90% drug-like compounds



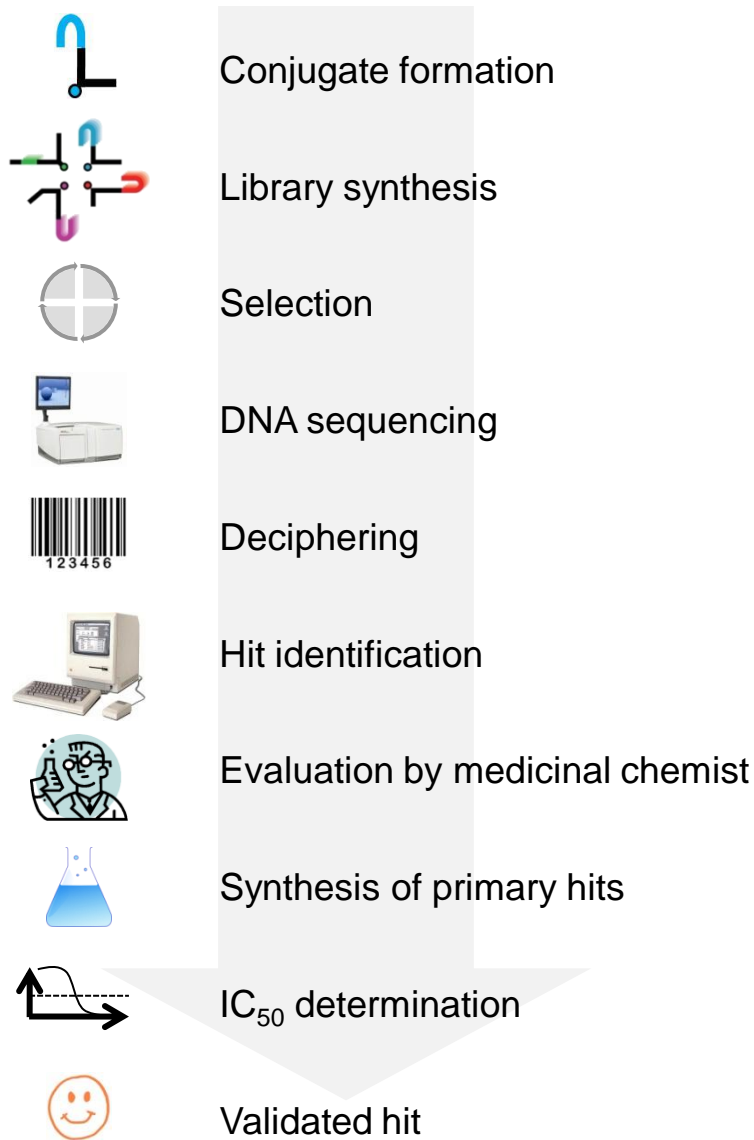
1.2 million compounds



16 different backbone classes

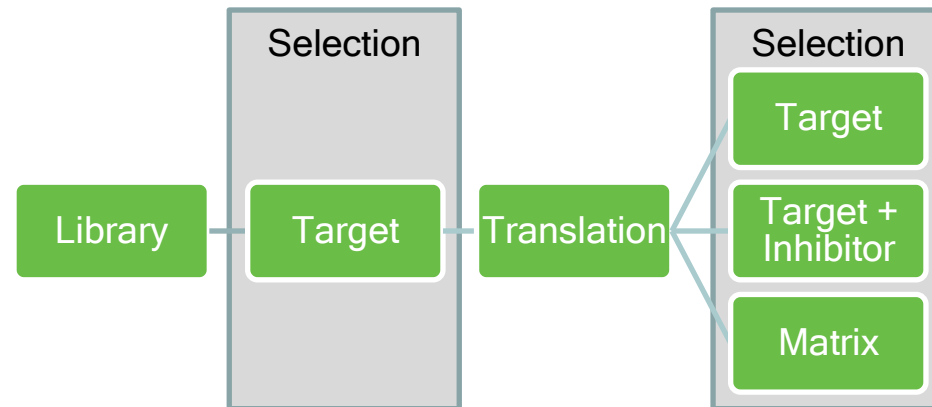
Library synthesis →

yR Drug Discovery



Selection regimen

Parallel selections performed on appropriate controls



Hit ranking (analysis of DNA sequencing data)

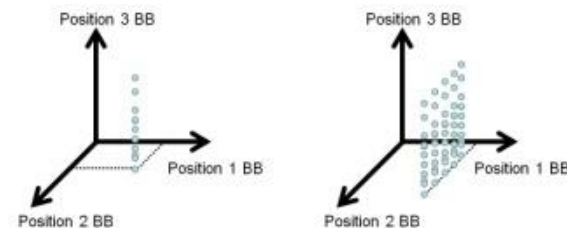
Statistical analysis: Multiple Testing Procedures

- Pairwise comparisons
- Triplewise comparisons

Cube analysis for identifying hit families

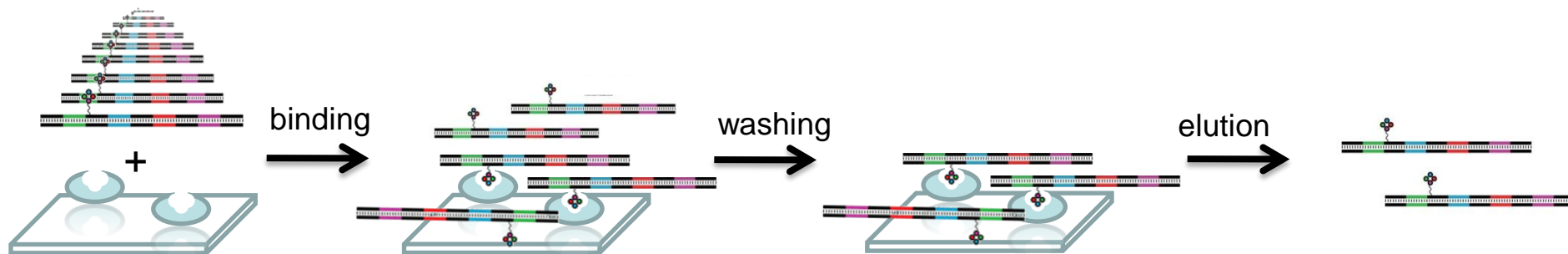
- Shared BB on two positions (line in cube)
- Shared BB on one position (plane in cube)

Chemoinformatics



Affinity Selection

- example: 3E7 antibody, million compound library (Lib012)



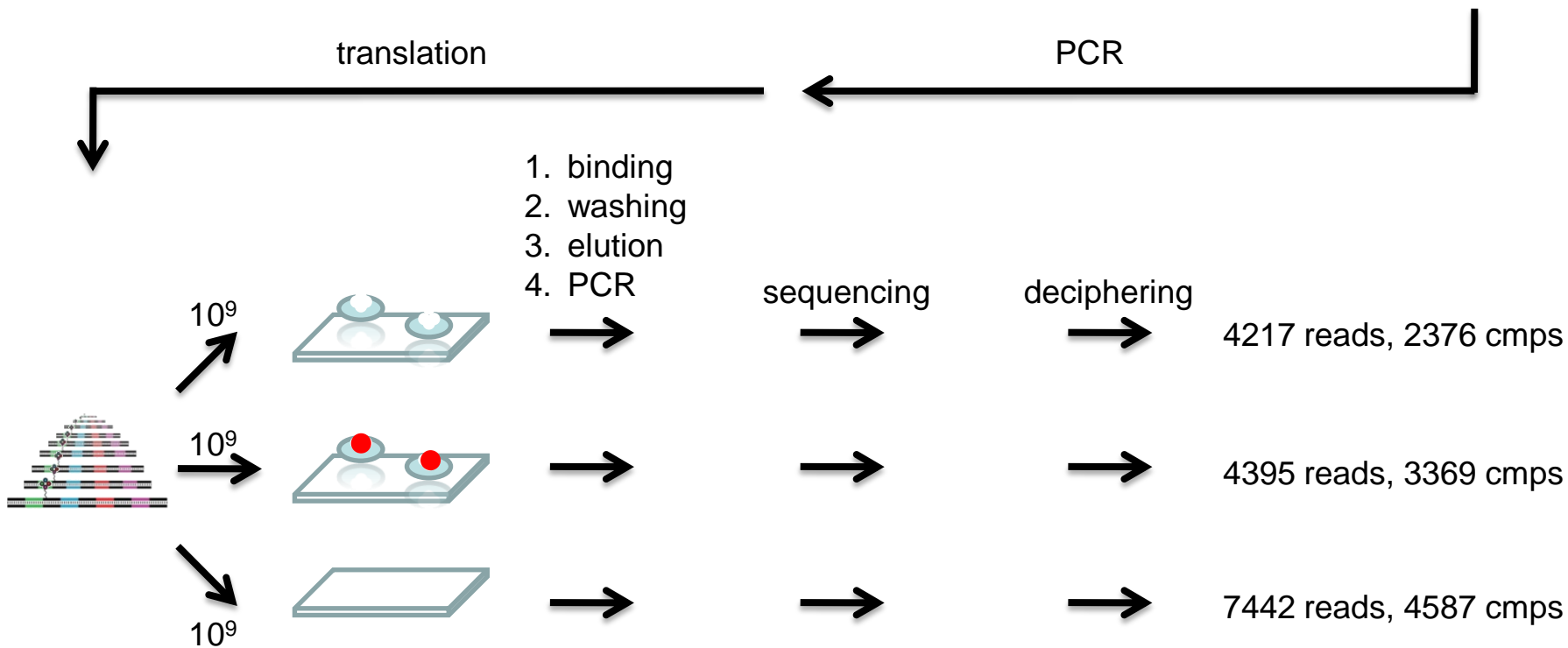
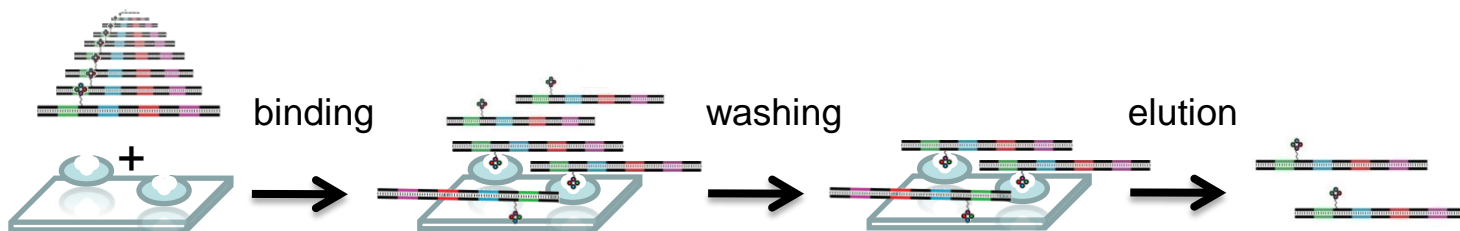
Partition assay

1st Round

- Input: 10^{10} molecules Lib012
- Target: 3E7 antibody (2 μg biotinylated)
- Solid phase: Streptavidin coated paramagnetic beads
- Binding Buffer: PBS, 0.1 % Tween20, 0.01 $\mu\text{g}/\mu\text{L}$ blocking DNA
- Binding volume: 20 μL , ([3E7] = 0.7 μM)
- Binding time: 30 min.
- Washing: 5 x 1 min., 500 μL , PBS, 0.1 % Tween20
- Elution: 500 μL 6 M GuHCl

Affinity Selection

- example: 3E7 antibody, million compound library (Lib012)



Statistical analysis: Multiple Testing Procedures

Primary hit	#Obs(Target)	#Obs(Control)	p-value
a	20	2	2.40E-11
b	5	0	0.0006
c	3	0	0.0122
d	3	1	0.0403
e	3	1	0.0403
f	2	0	0.0529
...			
Totals	2375	7950	NA

Assessment of multiple tests

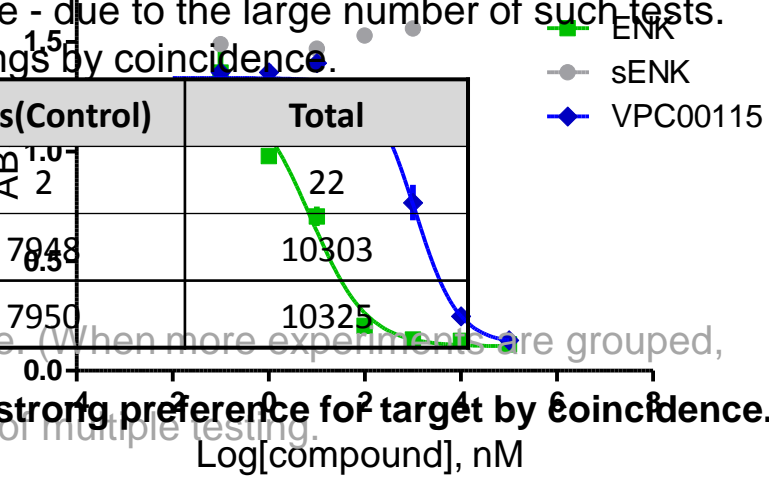
Very rare events (small p-values) DO happen by coincidence - due to the large number of such tests. "Exp" therefore indicates the expected number of such findings by coincidence.

Summary

- A small p-value is not evidence for target.
- A small Exp (expected number of findings) is not by chance.
- The method is not a so-called Mantel-Haenzel test is used.

Hit in question	#Obs(Target)	#Obs(Control)	Total
Hit in question	20	2	22
Another hit	2355	7948	10303
Total	2375	7950	10325

ENK
 $IC_{50} = 9.9 \text{ nM}$
 (published 7.1 nM)

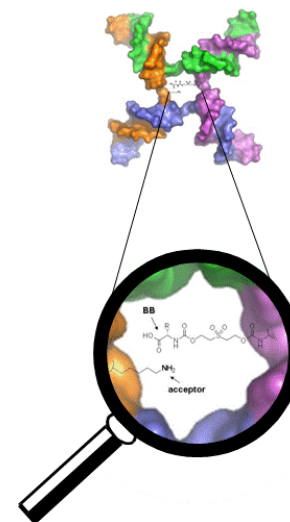


The p-value is the chance, given the totals, of finding such a strong preference for target by coincidence.

The yR drug discovery technology platform

Key features

- General applicable across drug target classes and disease areas
- Large libraries of drug-like small molecules
- Single tube format
- 3D proximity driven chemistry
- High fidelity process
- Molecular evolution
- Low amounts of target protein required (mg)
- Short turn around time
- Instant structure-activity relationship (SAR)
- Hit explosion (optional)
 - secondary libraries
- Instant specificity (optional)
 - parallel selections on family of targets



Acknowledgements

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ViperGen ApS

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Margit H. Hansen, PhD

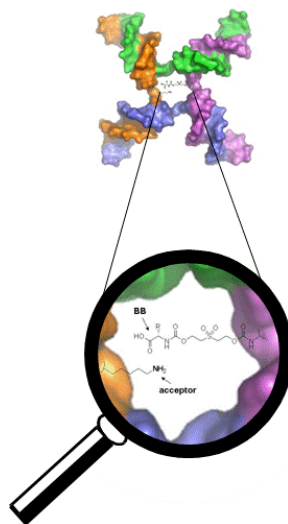
Tara Heitner, PhD

Johan Holmkvist, PhD

Leif K. Larsen, PhD

Lars K. Pedersen, PhD

Frank A. Sloek, PhD



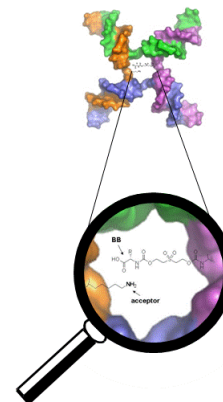
Publications

- J. Am. Chem. Soc., 2009, 131 (3), 1322
- Expert Opinion on Drug Discovery, 2009, 4 (11), 1201

Vipergen in brief

Vipergen is a stream-lined, tightly integrated and adaptive enterprise dedicated to the development and commercialization of the YoctoReactor[®] drug discovery platform.

- The YoctoReactor[®] technology platform is exclusively owned by Vipergen and secured by a strong patent position
- The company is privately owned by Eigil Bjerl Nielsen, Gunnar Kjems and Dr. Nils Jakob Vest Hansen
- Board members: Eigil Bjerl Nielsen (chair), Gunnar Kjems and Dr. Nils Jakob Vest Hansen
- Founded by CEO, Dr. Nils Jakob Vest Hansen
- Founded in 2005
- Incorporated in Copenhagen, Denmark
- Currently 8 employees (8 PhDs) – extensive outsourcing



Dr Nils Jakob Vest Hansen, CEO
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www.vipergen.com