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Monitoring intracellular concentrations of small molecules

Lothar Eggeling
Jülich

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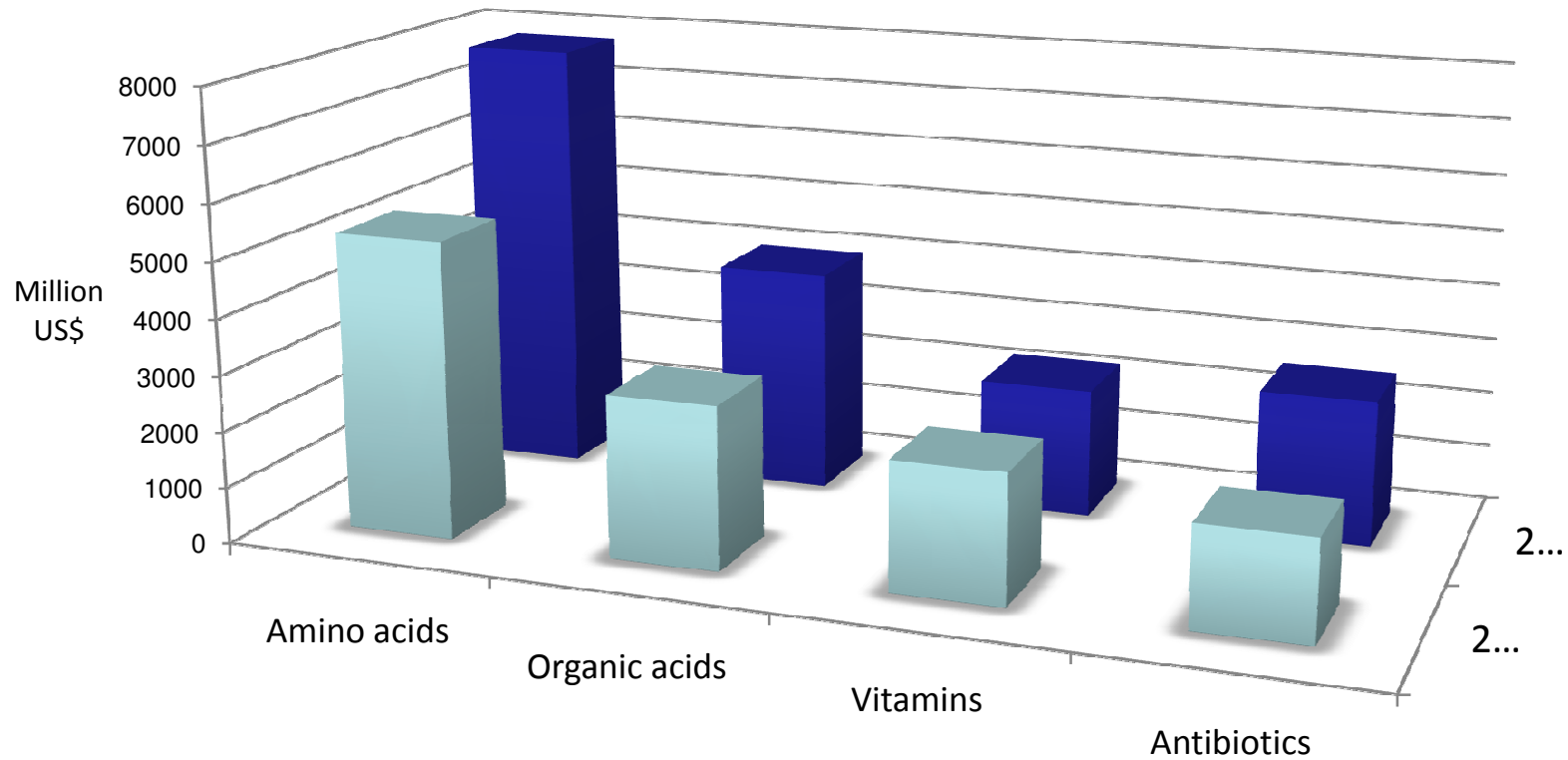
Lothar Eggeling, "Monitoring intracellular concentrations of small molecules" in "Metabolic Engineering IX", E. Heinzle, Saarland Univ.; P. Soucaille, INSA; G. Whited, Danisco Eds, ECI Symposium Series, (2013). http://dc.engconfintl.org/metabolic_ix/10

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Monitoring intracellular concentrations of small molecules

Lothar Eggeling, Amino acids and cell wall

Global market of fermentatively made small-molecules



Small-molecules: Amino acids



Food
L-glutamate
2 300 000 t/a



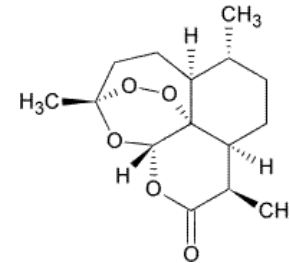
Feed
L-lysine
1 500 000 t/a

Pharma
L-serine
400 t/a

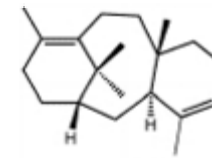


Egging, Amino acids and Cell wall

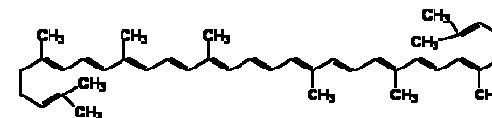
Small-molecules: Desire for new substances



Antimalaria drug
Artemisinin
Amorpha-4,11-diene



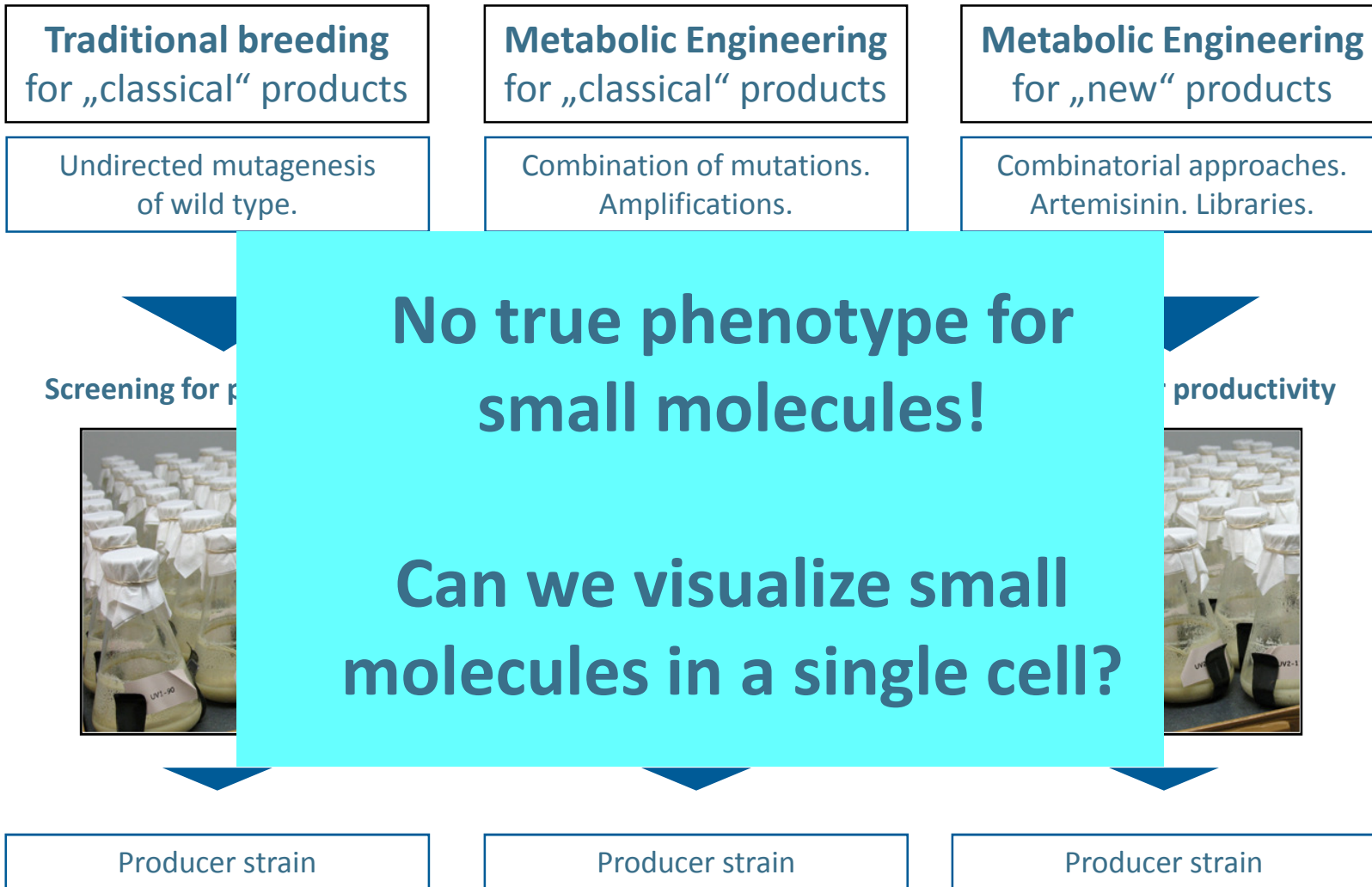
Anticancer drug
Taxol
Taxadiene



Lycopon

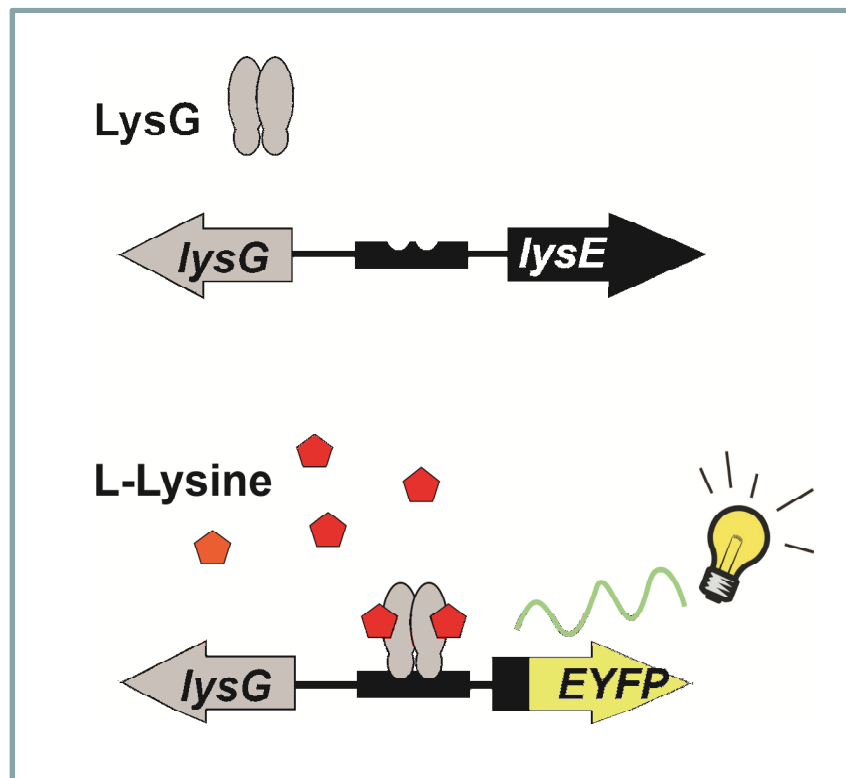
Antioxidant
Lycopene

Strain development: *from wild-type to producer*

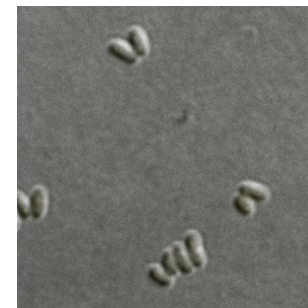


Use of transcriptional regulators to monitor small-molecules in single cells

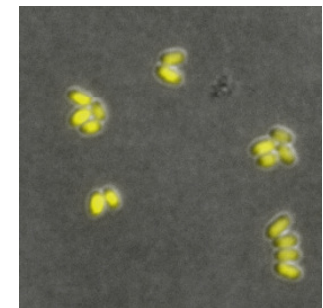
LysG of *Corynebacterium glutamicum* senses L-lysine



Wild type
pSenLys



Lysine producer
pSenLys

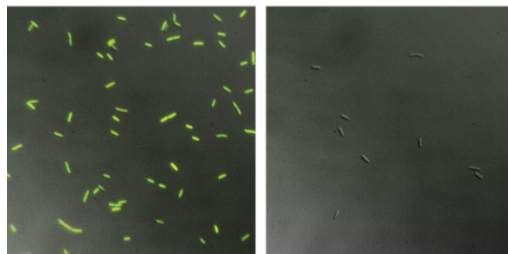


Visualization of
L-lysine at the
single cell level

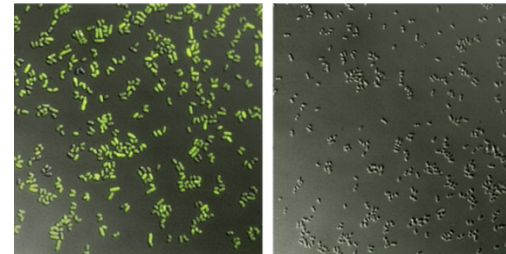
General use of transcription factors for small-molecule monitoring in single cells

„Fluorescent“ Molecule	Sensor	Regulator	Organism	Laboratory
L-Lysine	pSenLys	LysG (LTTR)	<i>C. glutamicum</i>	Eggeling, Jülich
L-Arginine	pSenArg	ArgP (LTTR)	<i>E. coli</i>	Eggeling, Jülich
O-Acetyl-serine	pSenOAS	CysR (LTTR)	<i>C. glutamicum</i>	Eggeling, Jülich
L-Serine	pSenSer	ROK-type	<i>C. glutamicum</i>	Eggeling, Jülich
L-Methionine	Lrp-Sensor	Lrp-type	<i>C. glutamicum</i>	Frunzke, Jülich

E. coli pSenArg



C. glutamicum Ser4 pSenSer



General use of transcription factors for small-molecule monitoring in single cells

Natural transcriptional regulators sensing

Amino acids. Sugars. Sugar phosphates. Vitamins.
Oxoacids. Ions. Antibiotics. Acyl-CoA derivatives

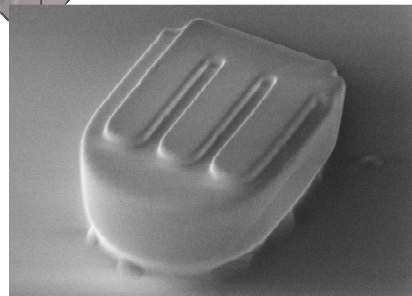
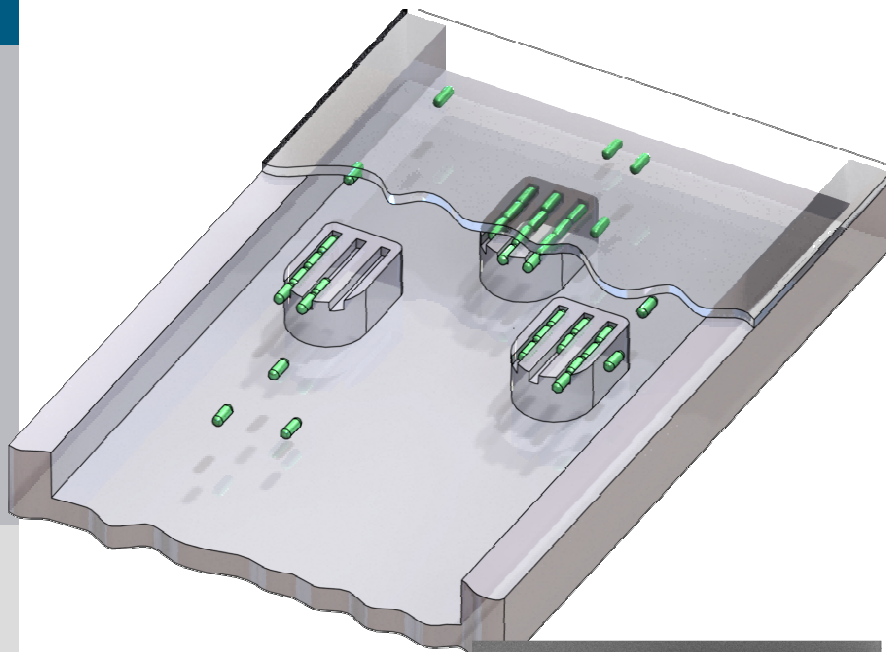
Developed transcriptional regulators sensing

Mevalonate (AraC sensing Arabinose)
Peptides (TetR sensing Tetracycline)

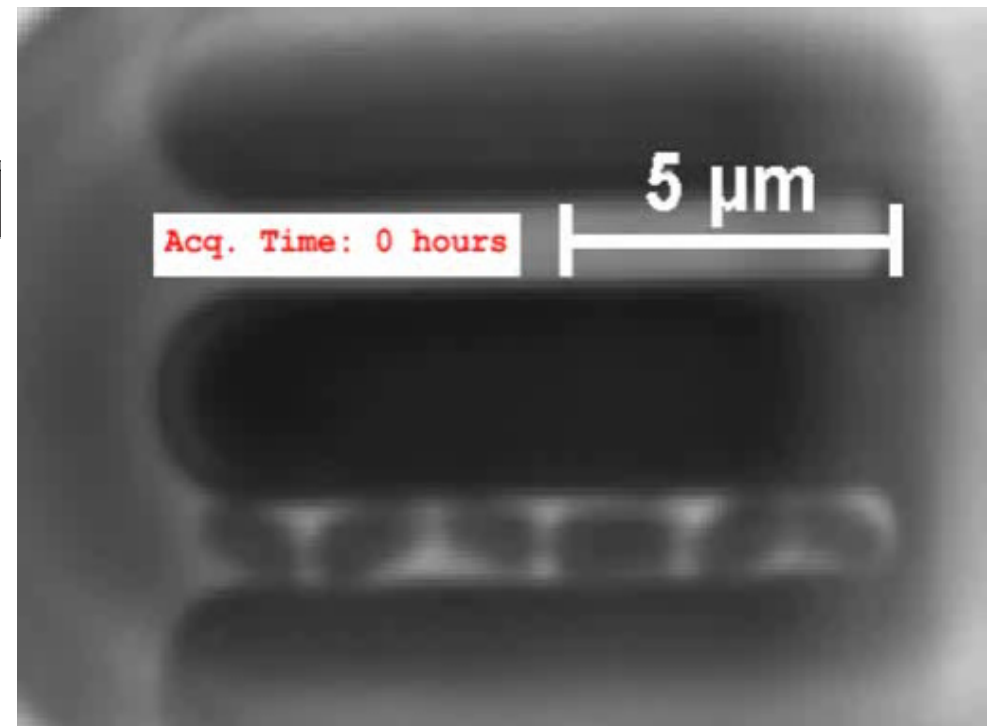
A broad number of new Applications in Single-Cell Analysis

New Applications in Single-cell analysis

Single cell growth and production performance
in Microfluidic Chips

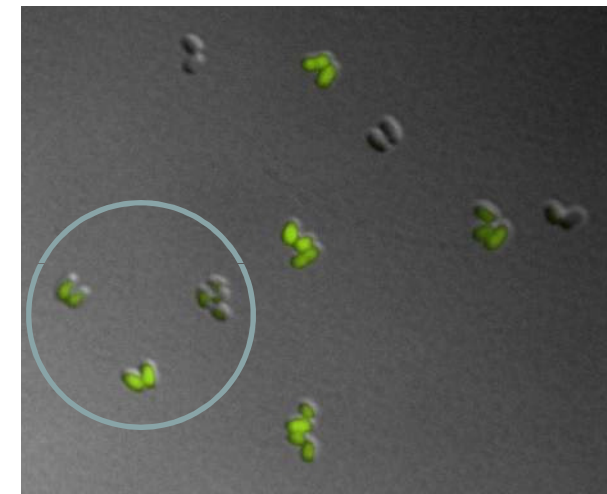
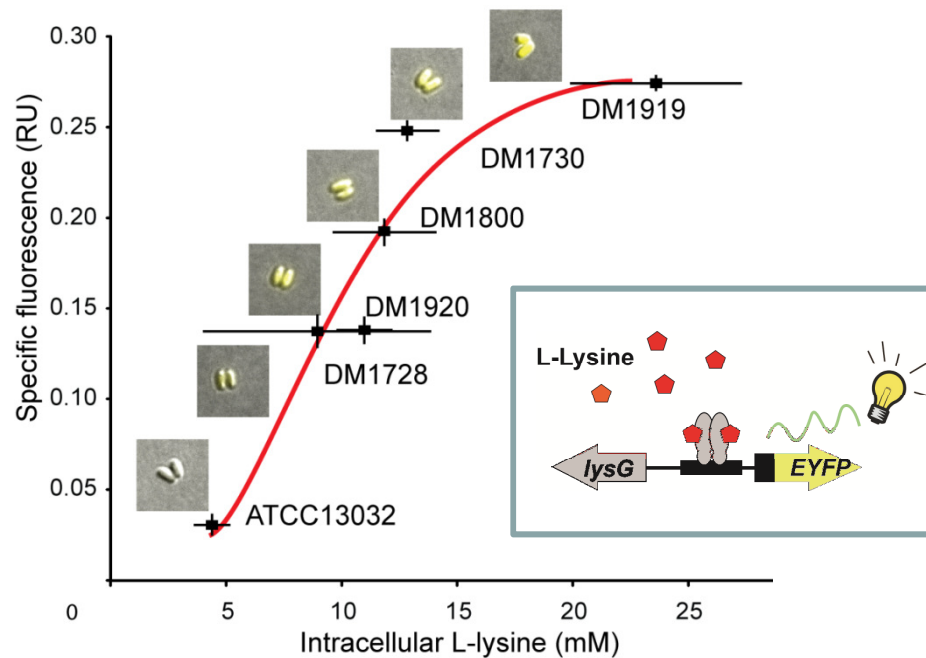


Egeling, Amino acids and Cell wall



New Applications in Single-cell analysis

HT-screening of mutant libraries



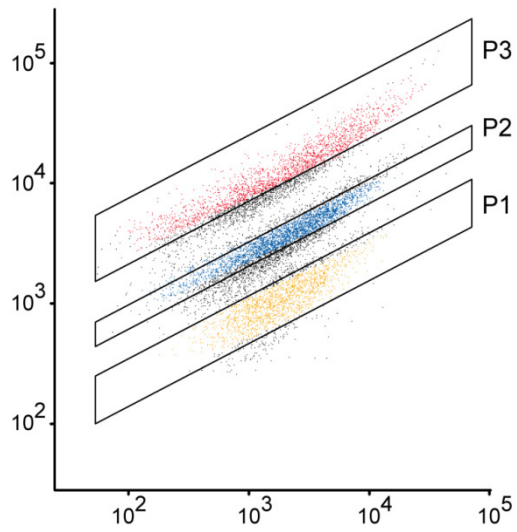
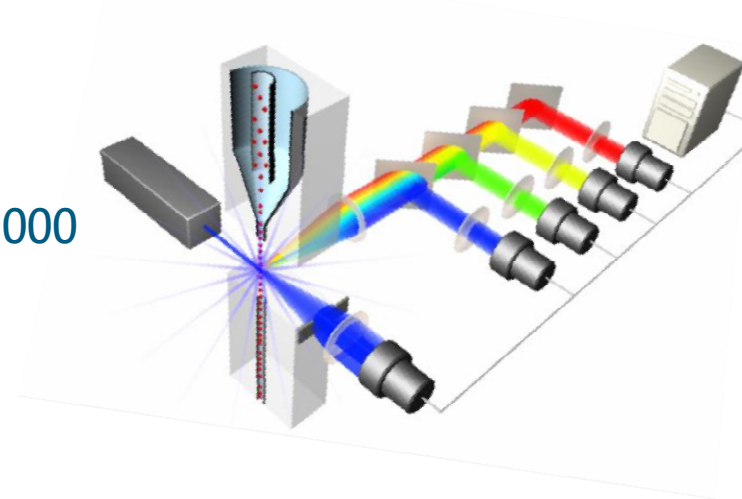
Mixture of WT,
DM1728, DM1919

Correlation between intracellular lysine concentration and fluorescence

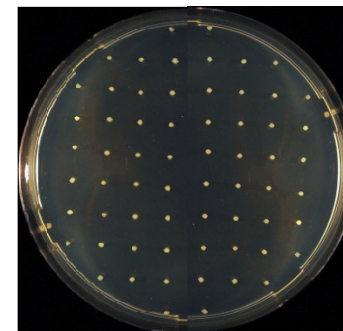
Fluorescence Activated Cell Sorting (FACS) enables detection and isolation of fluorescent cells



HT-analysis of 70.000 cells per second



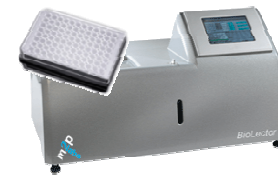
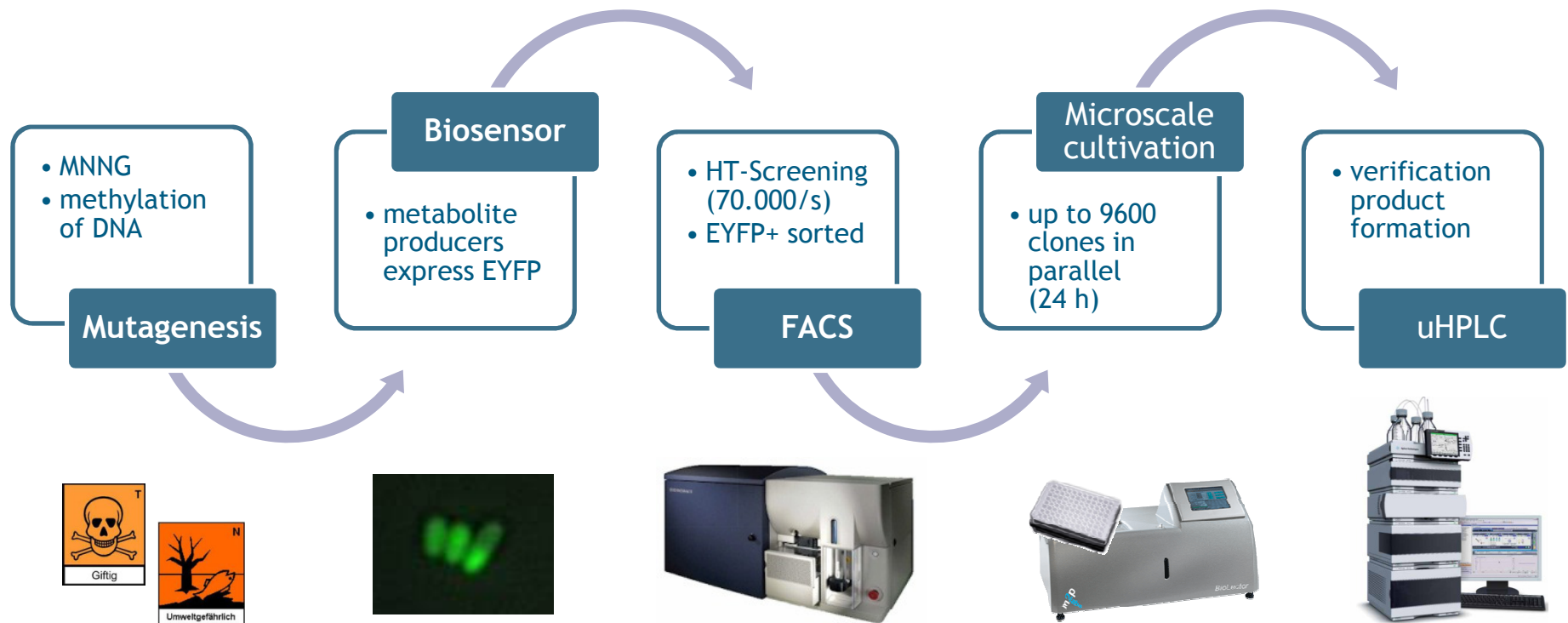
Sorting efficiency for gates $\geq 91\%$



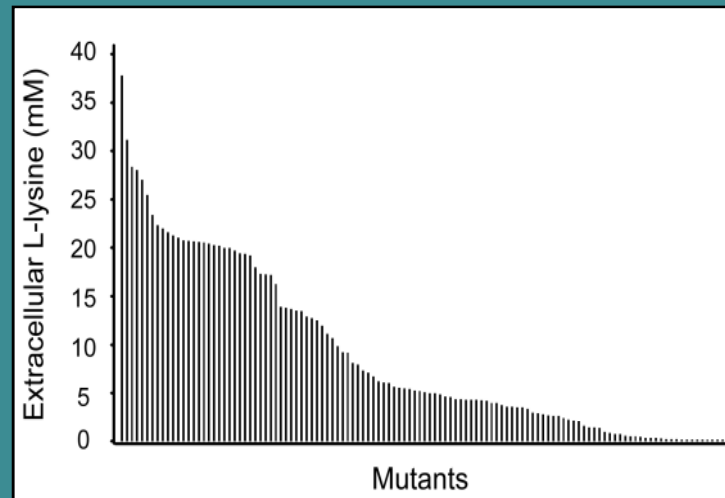
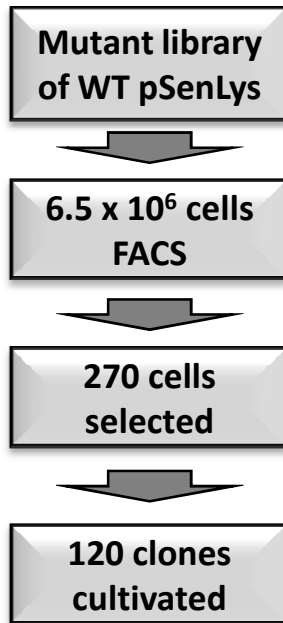
Sorting of single cells on petri dishes

30 °C, 24 h

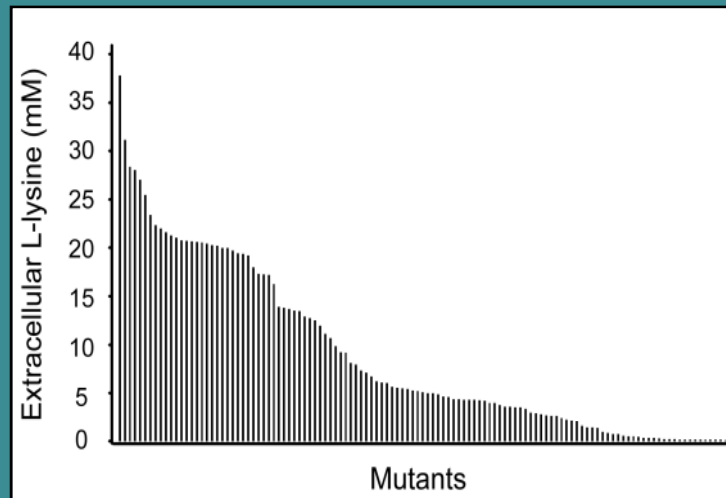
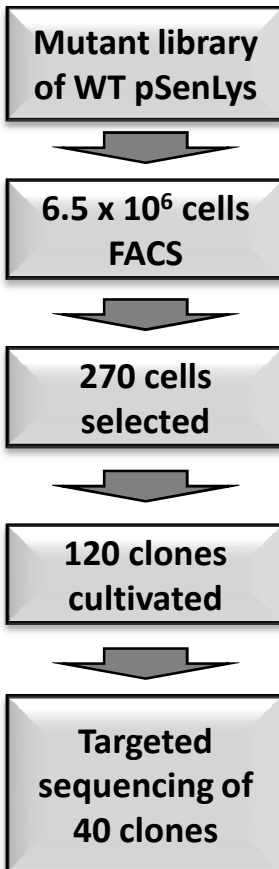
Isolation of metabolite producing bacteria from libraries



Isolation of new L-lysine producers from *C. glutamicum* WT



Isolation of new L-lysine producers and targeted sequencing

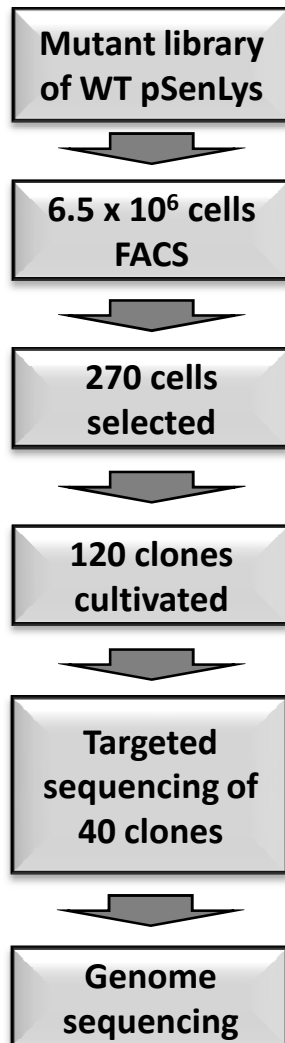


Sequencing of keyplayers:

- 11 known mutations
- 13 new mutations
- 16 with unknown targets

	Lys (mM)	μ (h ⁻¹)	lysC	hom	dapA	thrC	thrB	metX	murE	Mutation
K015	36.9	0.34	x	x	x	x	x	x	x	lysC A279T
K037	27.4	0.33	x	x	x	x	x	x	x	lysC A279T
K053	19.8	0.39	x	x	x	x	x	x	x	lysC A279V
K008	7.2	0.36	x	x						lysC G277D
K106	19.6	0.39	x	x						lysC G277S
K096	0.9	0.25	x	x	x	x	x			lysC H357Y
K035	21.5	0.35	x	x						lysC T308I
K100	20.6	0.29	x	x	x	x	x	x	x	lysC T308I
K047	20.3	0.27	x	x	x	x	x	x	x	lysC T308I
K065	20.2	0.30	x	x	x	x	x	x	x	lysC T308I
K019	20.1	0.23	x	x						lysC T308I
K090	19.8	0.34	x	x	x	x	x	x	x	lysC T308I
K078	0.3	0.18	x	x	x	x	x			lysC T308I
K101	26.4	0.23	x	x	x	x	x			lysC T311I
K115	1.5	0.25	x	x	x	x	x			lysC T313I
K002	27.7	0.30	x	x	x	x	x	x	x	hom A328V
K049	9.6	0.15	x	x	x	x	x			hom A364V
K074	2.9	0.14	x	x	x	x	x			hom G241S thrC A372V
K052	0.2	0.18	x	x	x	x	x			hom G241S thrC A372V
K032	22.9	0.26	x	x	x	x	x	x	x	hom R158C hom T351I
K039	10.9	0.24	x	x	x	x	x			hom V211F
K055	2.7	0.18	x	x	x	x	x	x	x	murE L121F
K016	0.1	0.11	x	x	x	x	x			thrB S102F
K023	16.9	0.17	x	x						
K051	15.9	0.23	x	x						
K118	10.5	0.36	x	x	x	x	x	x	x	
K063	9.0	0.37	x	x	x	x	x	x	x	
K112	7.9	0.38	x	x	x	x	x	x	x	
K117	7.0	0.34	x	x	x	x	x	x	x	
K005	6.6	0.38	x	x	x	x	x	x	x	
K021	5.9	0.37	x	x						
K079	5.2	0.30	x	x	x	x	x	x	x	
K093	5.0	0.39	x	x	x	x	x	x	x	
K013	4.9	0.38	x	x	x	x	x	x	x	
K048	4.5	0.38	x	x						
K062	3.7	0.26	x	x						
K107	3.6	0.36	x	x						
K120	2.6	0.37	x	x	x	x	x	x	x	
K046	0.1	0.39	x	x	x	x	x	x	x	

Isolation of new L-lysine producers and genome sequencing, WGS



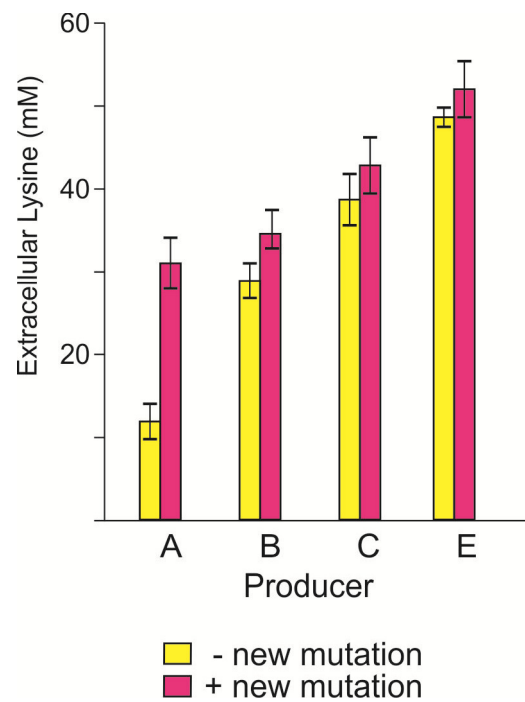
Sequence data of strain K051:

Description	value
Number of sequenced reads	20,156,524
Avg. length of reads after trimming [bp]	48.1
Number of reads mapped to reference	17,877,215
Coverage (# mapped reads * avg. length/3301500)	260.5
SNPs in total	268
Transitions	268
SNPs leading to amino acid exchange	171
Silent mutations	65
Intergenic SNPs	28
Introduced stop-codons	4

One mutation in strain K051:

MurE-G81E
UDP-N-acetylmuramoyl-L-alanyl-D-glutamate:meso-diaminopimelate ligase

Use of new mutation in known L-lysine producers



500 m³ fermenters for lysine production

Monitoring intracellular concentrations of small molecules

M. Bott

W. Wiechert

L. Eggeling

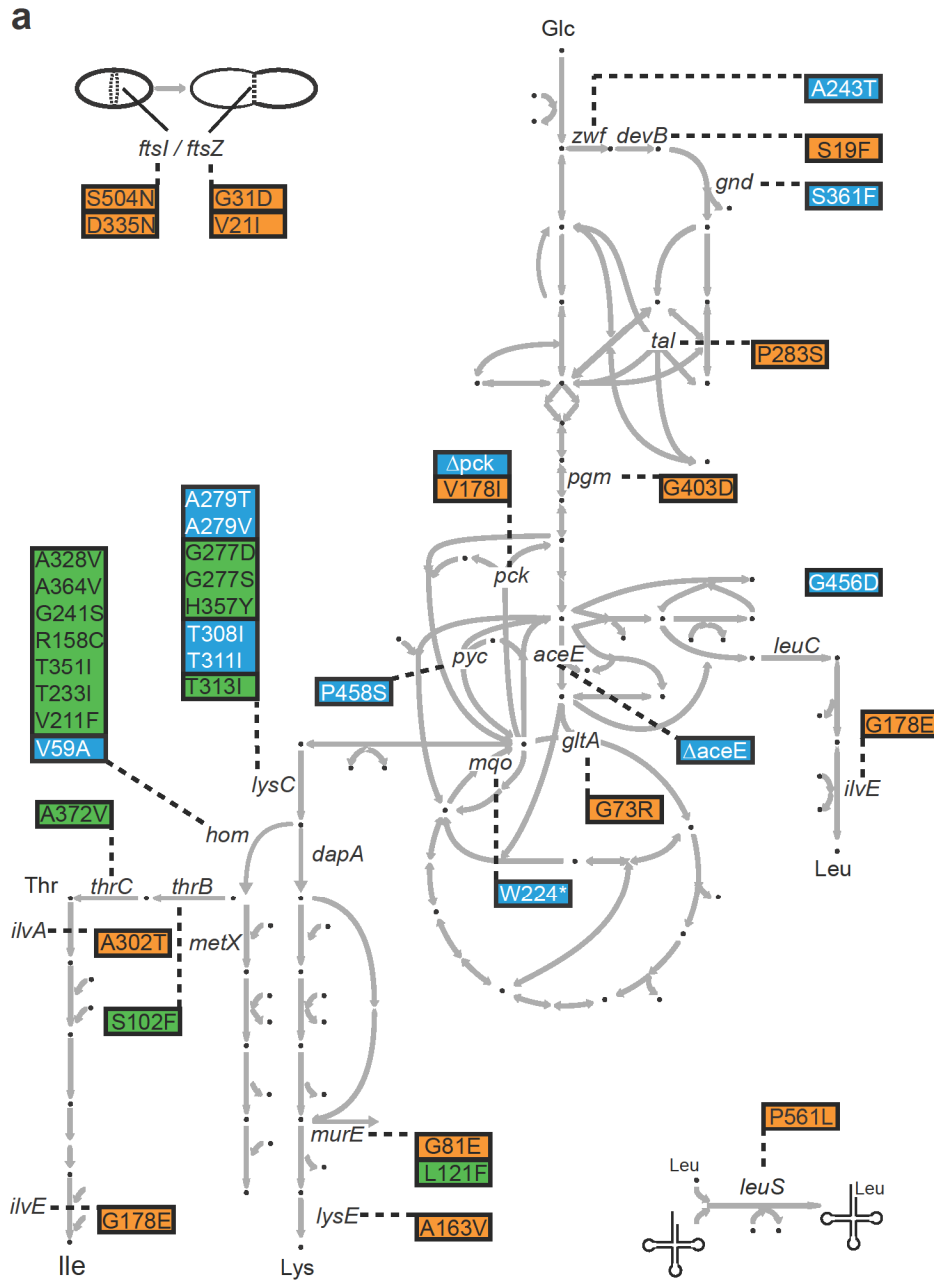
D. Kohlheyer

S. Binder 177

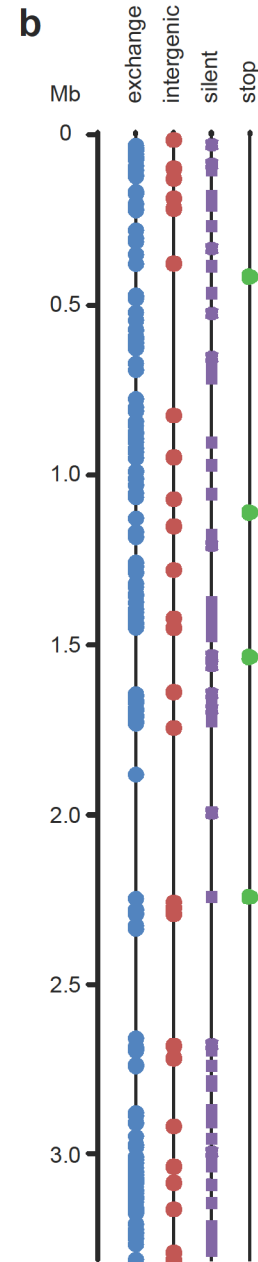
A. Grünberger 31

G. Schendzielorz 215

S. Siedler 182

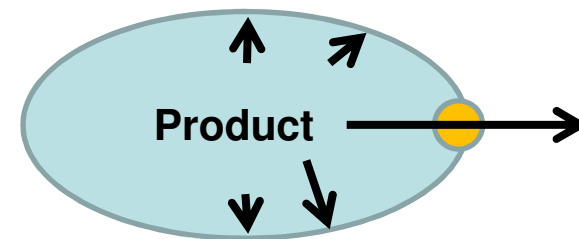
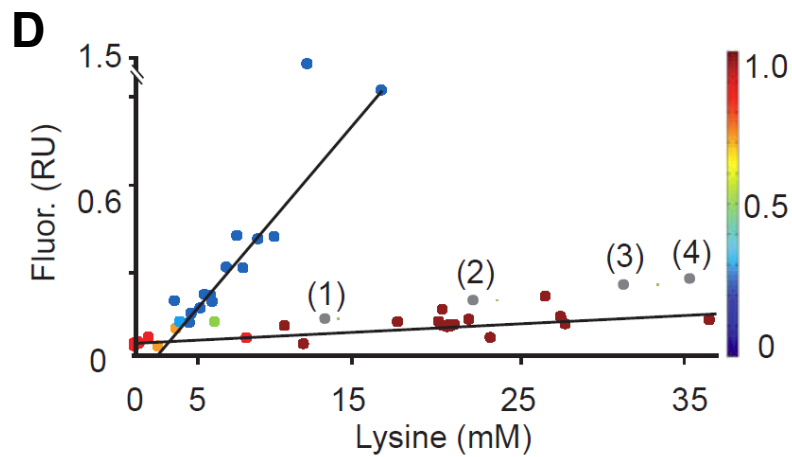
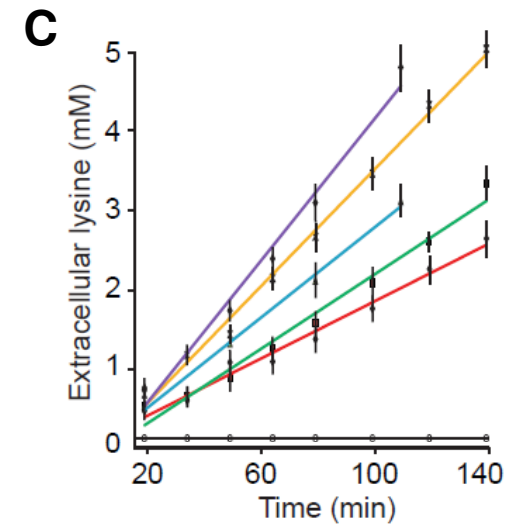
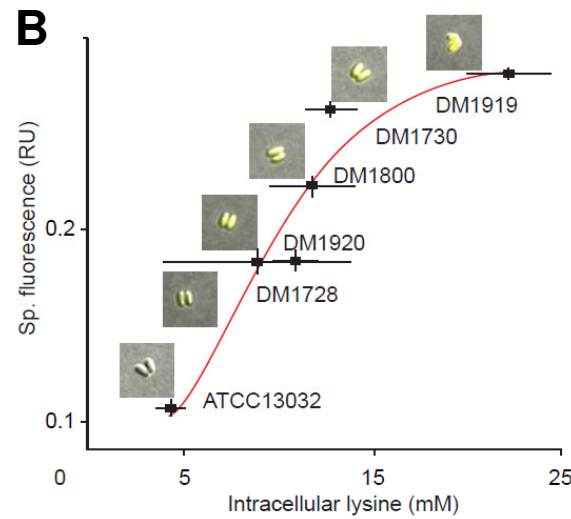
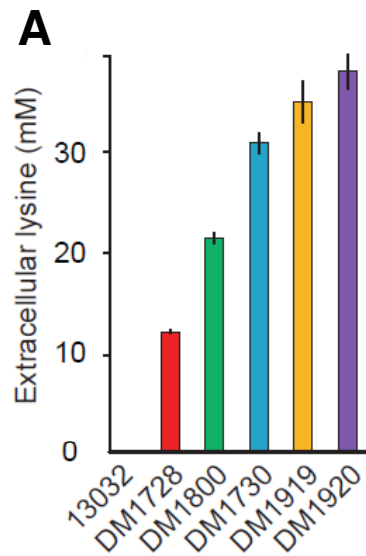


Eggeling Fig6.eps



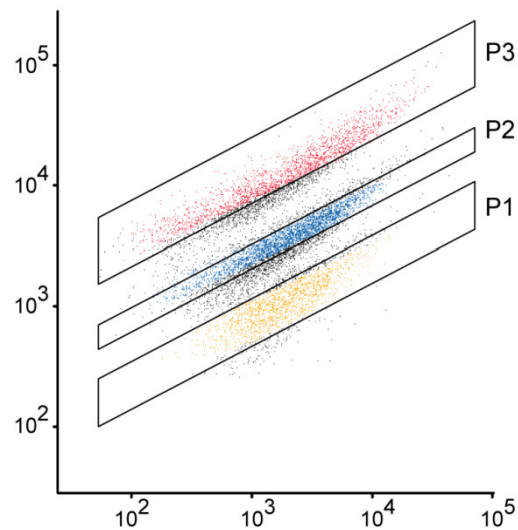
Mutations from targeted sequencing and genome sequencing

Vital in microbial small molecule production: Export



FACS Aria II characterization, and sorting specificity of defined mixtures

A



B

Gate	Viability		Verified strain			Sorting specificity
	Sorted in total	Grown	WT	Interm	High	
P3	288	84.7 (%)	3	6	90	90 (%)
P2	288	89.2 (%)	2	95	2	95 (%)
P1	432	89.4 (%)	98	1	0	98 (%)

FACS selection step works at single cell level