



Engineering the Medicines  
of Tomorrow

## The Path to Platinum: The Evolution of Human Combinatorial Libraries (HuCAL®)

*Antibody Engineering*  
*December 2008, San Diego*  
*MorphoSys AG, Munich, Germany*

## ■ MorphoSys' Technology

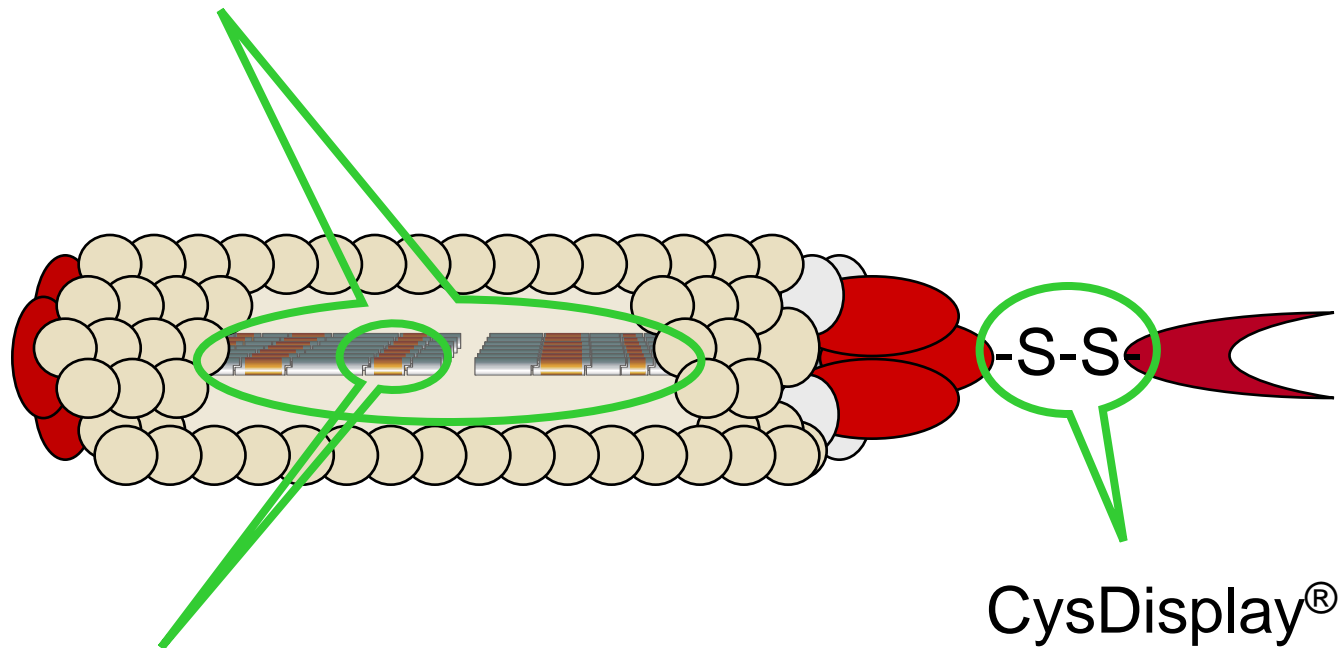
- Synthetic antibody genes
- Cys Phage Display
- Trinucleotide Technology

## ■ History: Evolution of HuCAL Libraries

## ■ Presenting HuCAL Platinum



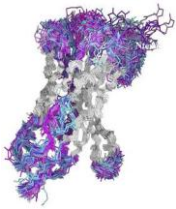
HuCAL<sup>®</sup> : Human Combinatorial Antibody Library<sup>®</sup>



TRIM: Trinucleotide Mutagenesis

# HuCAL is Unique in its Design: Master Genes Cover the Structural Diversity of Human Antibodies

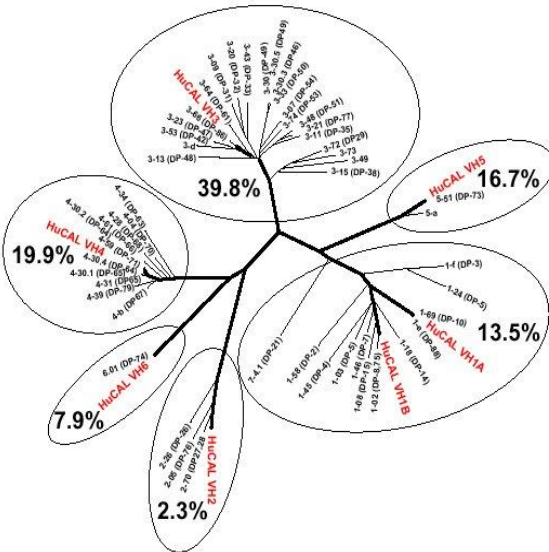
## Structures / Folds



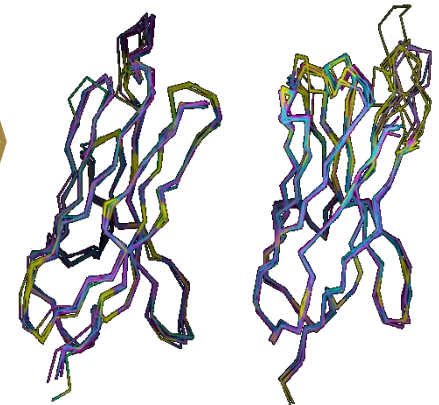
132 Ig Variable Domain Structures

## Germline Genes

- ~ 51 VH Genes
- ~ 40 Vk Genes
- ~ 30 VI Genes



## HuCAL Master Frameworks



## Bioinformatics

Analysis of human antibody germline and rearranged genes

## Analysis

Grouping into VH/VL sub-families according to sequence homology

## Gene synthesis

Combination of 7 VH and 7 VL HuCAL Master Genes covers structural diversity

Source: Knappik et al. J Mol Biol 2000, 296: 57ff

Bioinformatics



TRIM Oligo Synthesis



Library Construction

Rearranged Sequences

G	N	G	N	T	K
N	S	G	N	T	A
G	G	S	N	T	K
N	S	G	N	T	A
N	S	G	N	T	G
N	T	G	G	T	N
S	G	V	T	T	K
N	S	G	G	T	N
Y	N	G	N	T	N
N	S	G	N	T	G
Y	N	G	N	T	N
N	S	G	G	T	N



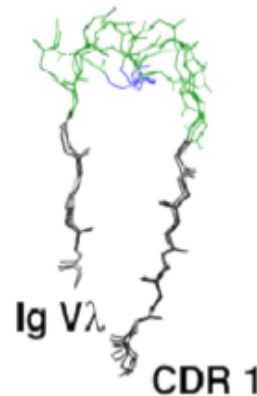
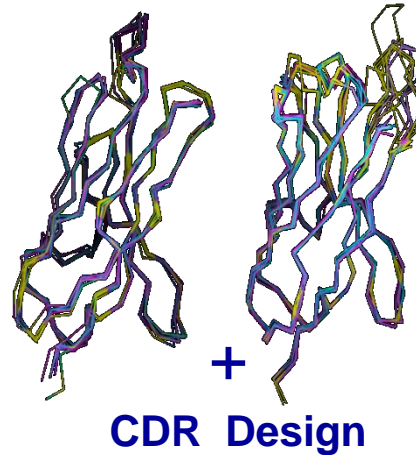
Positional Frequency  
(Statistic)



CDR Design

60N	50S	80G	70N	100T	50N
15G	25N	10S	20G		25K
15Y	15G	10V	10T		15A
5S	15T				10G

HuCAL Frameworks




Taking care of  
structural residues

Using diversity where  
it matters

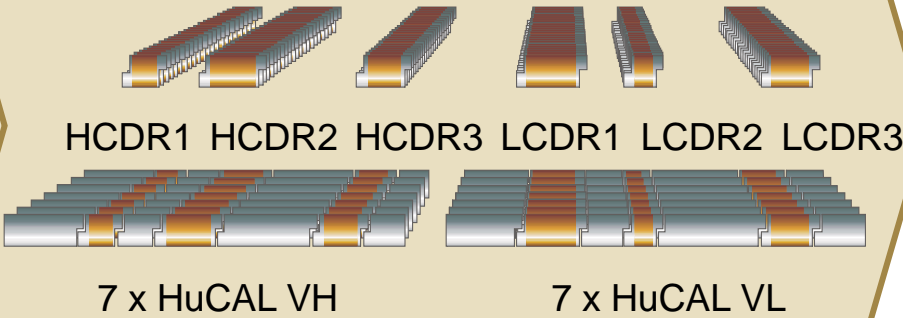
Capturing the natural  
repertoire

Minimizing the risk of  
immunogenicity

# HuCAL – A Modular Fully Human Library with Diversified CDRs

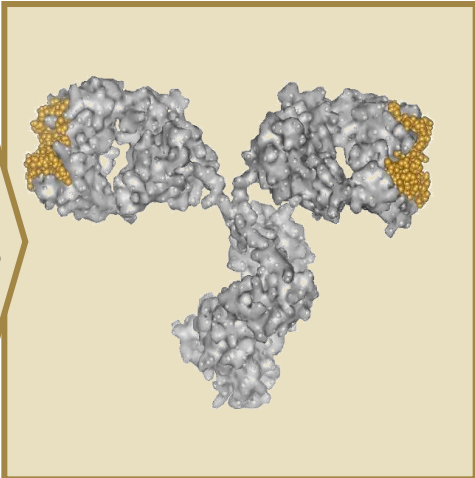


**Pre-Built Trinucleotide Cassettes**



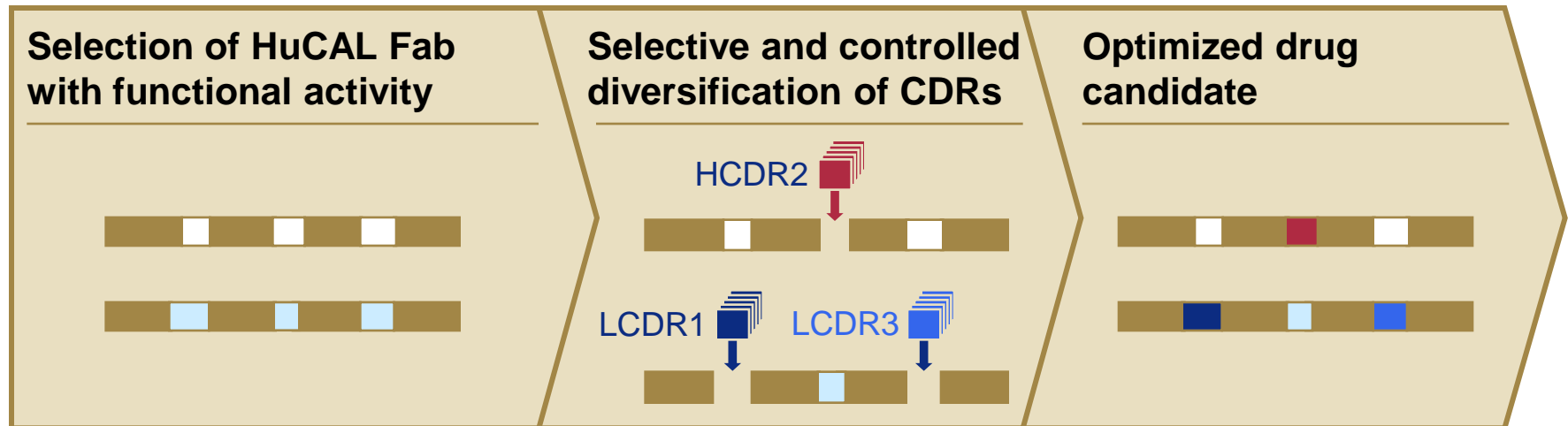
HCDR1 HCDR2 HCDR3 LCDR1 LCDR2 LCDR3

7 x HuCAL VH 7 x HuCAL VL



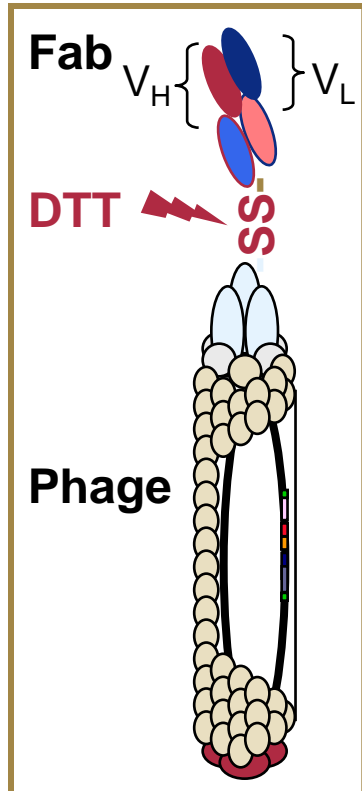
- **Master genes are close to human germline sequences**
  - Cover structural diversity in man
- **Diverse repertoires in all 6 CDRs**
  - Diversity designed according to natural distribution in man
  - Tailor made using TRIM technology
- **Fully modular**
  - Engineering, optimization

# HuCAL's Modularity Facilitates Efficient, Targeted and Controlled Affinity Optimization



- CDR optimization without altering framework regions
- Highly diverse CDRs designed according to natural distribution in man
- Optimization of a single Fab as well as a pool of Fabs
- CDRs can be optimized in sequentially or parallel
- Combination of optimized heavy and light chain possible

# CysDisplay Uniquely Enables Selection of High Affinity Antibodies



- **Display of Fab via disulfide linkage to phage (pIII)**
  - No genetic fusion between Fab and phage protein
  - Cysteines at N-terminus of pIII as well as C-terminus of Fab
  - Disulfide bond formation between Fab and pIII in the periplasm ensures linkage of phenotype and genotype
- **Specific elution of Fabs**
  - Independent of antibody affinity
  - Especially suited for selection of high affinity antibodies
  - Easy and fast procedure, independent of type of antigen
  - Applicable for high throughput

## Trinucleotide-directed mutagenesis (TRIM)

[Exclusive license from Johns Hopkins University in Baltimore, MD, U.S.A.]

5600–5607 *Nucleic Acids Research*, 1994, Vol. 22, No. 25

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### Trinucleotide phosphoramidites: ideal reagents for the synthesis of mixed oligonucleotides for random mutagenesis

Bernhard Virnekäs\*, Liming Ge, Andreas Plückthun<sup>1</sup>, K.Christian Schneider, Günter Wellenhofer and Simon E.Moroney

MorphoSys GmbH, Frankfurter Ring 193a, 80807 Munich, Germany and <sup>1</sup>Biochemisches Institut, University of Zurich, Winterthurerstr. 190, 8057 Zurich, Switzerland

Received September 23, 1994; Revised and Accepted November 1, 1994

- Excellent coupling yields 96%-98.5%
- Controlled relative coupling rates of each individual trinucleotide

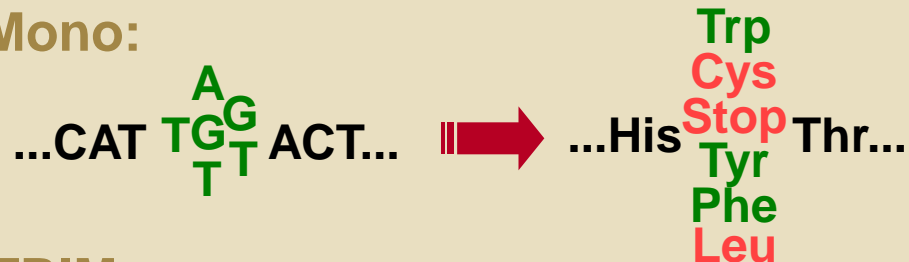
# The TRIM Technology Ensures HuCAL's High Quality and Functionality

## TRIM: Trinucleotide Mutagenesis

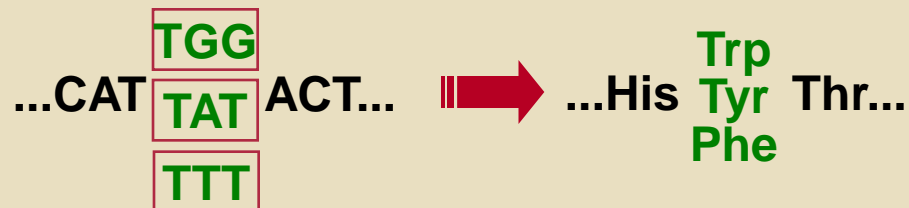
TRIM uses 20 pre-assembled trinucleotides in standard DNA synthesis

Codon based TRIM technology provides solely the desired amino acids

**Mono:**



**TRIM:**



## HuCAL CDRs are highly diverse and human

- No stop codons or frameshifts
- No undesired or rare codons
- Length variation
- Ratio (bias) of amino acids in any subset
- Framework specific CDR subsets

## Diversification of amino acid composition

	T 1	T 2	T 3
D	5%		4.4%
E	5%		4.4%
K	5%		4.4%
R	5%	20%	4.4%
H	5%	20%	4.4%
T	5%		4.4%
S	5%		15%
N	5%		4.4%
Q	5%		4.4%
G	5%		15%
A	5%		4.4%
C			
P	5%		4.4%
V	5%		4.4%
I	5%		4.4%
L	5%	50%	4.4%
M	5%		4.4%
F	5%	5%	4.4%
Y	5%		15%
W	5%	5%	4.4%

T 1:  
Full randomized

T 2:  
Biased

T3:  
Full randomized but weighted

T = TRIM Mixture

## Diversification of CDR Regions

- Single Position

atg-ggc-**T1**-ccg-atc

- Multiple Position (e.g. CDR1/2)

ggc-**T1**-ccg-**T2**-**T3**-ccg-

- Continuous Sequences (e.g. HCDR-3)

**T1**-(**T2**)<sub>4</sub>-(**T3**)<sub>3</sub>-**T4** -**T5** - **T6** -**T7** -

# Limits of Trinucleotide Oligos Synthesis

- High coupling efficiency in trinucleotide cassette synthesis is mandatory
- Working example: HCDR3 cassette with 15 - 23 TRIM coupling steps

Coupling Efficiency	Synthesis Yield	
	15 aa	23 aa
95%	46%	30%
90%	20%	9%
85%	8%	2%
80%	3.5%	0.6%

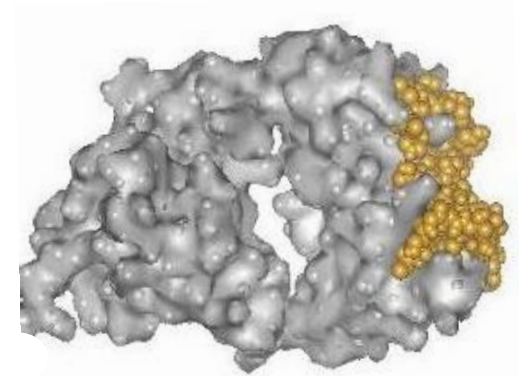
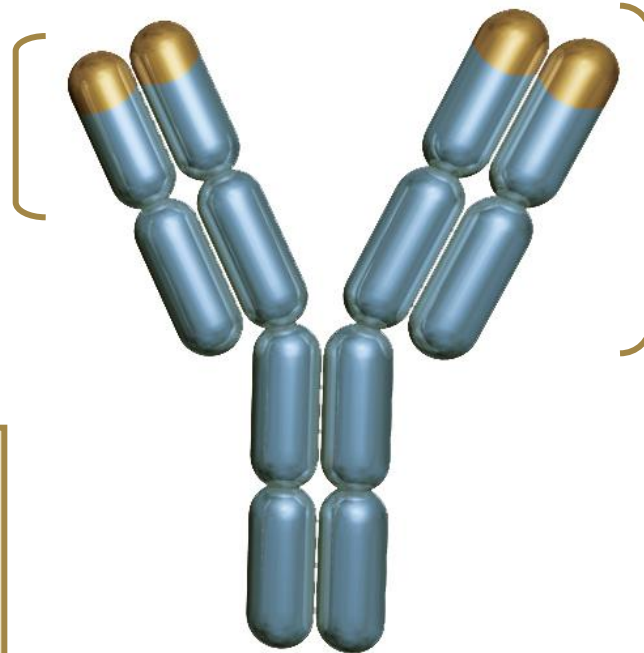
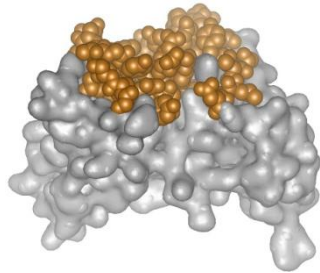
HCDR3 Length Trim Positions	15aa	16aa	17aa	18aa	19aa	20aa	21aa	22aa	23aa
No sequences	33	42	20	35	39	36	36	31	32
No seq without mistake	27	32	14	30	26	21	26	19	19
% without mistake	82	76	70	86	67	58	72	61	59
No seq functional (+/-TRIM)	31	37	20	34	36	30	33	29	26
TRIM Insertion	2	4	6	3	9	8	7	7	7
TRIM Deletion	2	1	0	0	1	1	0	3	0
Nucleotide Insertion/Deletion	2	5	0	2	3	5	3	2	6
% functional (+/-TRIM)	94	88	100	97	92	83	92	94	81



MorphoSys commissioned ELLA Biotech for TRIM services

Kabat Position	97-100 b		100 c-d		100 e-h	
TRIM Mixture	TRIM3		TRIM4		TRIM5	
	F	P	F	P	F	P
D	4	5	4	3	1	1
E	1	1	1	3	2	1
K	0,5	1	1	3	1	1
R	5	5	4	3	8	8
H	1	1	1	3	4	1
T	7	5	6	3	2	1
S	18	15	17	10	11	8
N	1	1	1	3	1	1
Q	1	1	2	3	3	1
G	12	15	12	15	7	8
A	5	5	5	3	2	1
C						
P	7	5	6	3	11	8
V	8	5	7	3	3	1
I	5	5	5	3	2	1
L	6	5	5	3	2	1
M	0,5	1	1	3	2	1
F	1	1	2	3	3	1
Y	16	15	18	20	31	30
W	2	1	2	3	3	1
Occupation	19	19	19	19	19	19
No Seq	3640		1213		1565	

- Positional amino acid frequency planned (P) and found (F) can be precisely adjusted even in complex TRIM mixtures



- HuCAL-scFv 1
- HuCAL-scFv 2
- HuCAL-scFv 3

- HuCAL-Fab 1
- HuCAL-Fab 2

▪ **HuCAL GOLD**

▪ **HuCAL Platinum**

	Antibody Format	Phage Display system	Diversified CDR regions
scFv-1	scFv	genetic fusion	2
scFv-3	scFv	cys display	2
Fab-1	Fab	genetic fusion	2
HuCAL Gold	Fab	cys display	6
HuCAL Platinum	Fab	cys display	6

## Major steps in HuCAL Technology

- Fab antibody format
- CysDisplay
- Increase diversity in CDR-1 and CDR-2

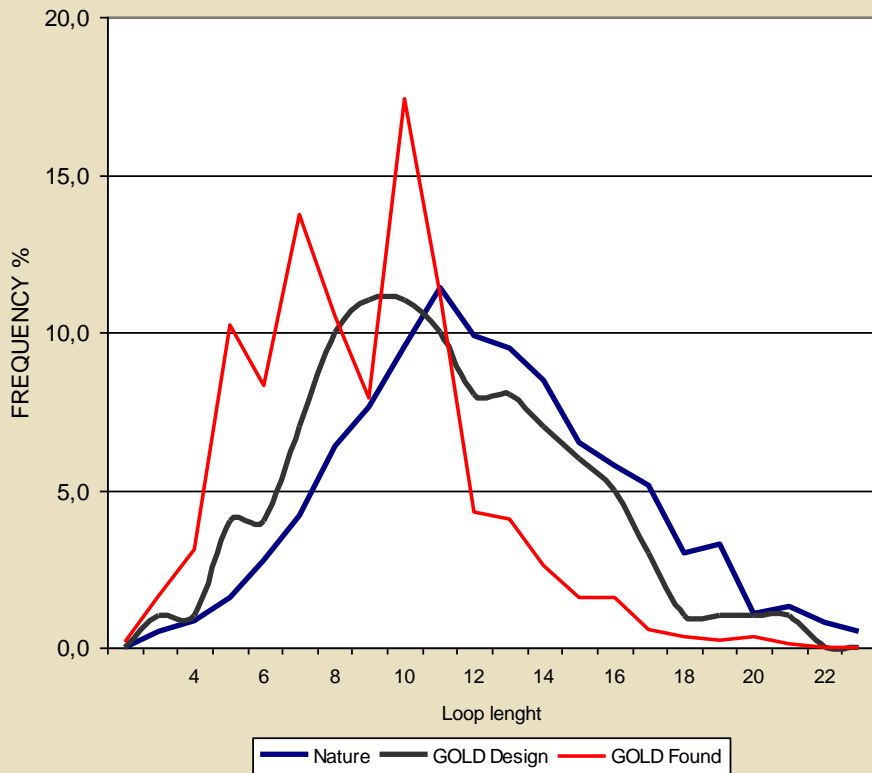
## HuCAL Platinum Concept

### Key Features:

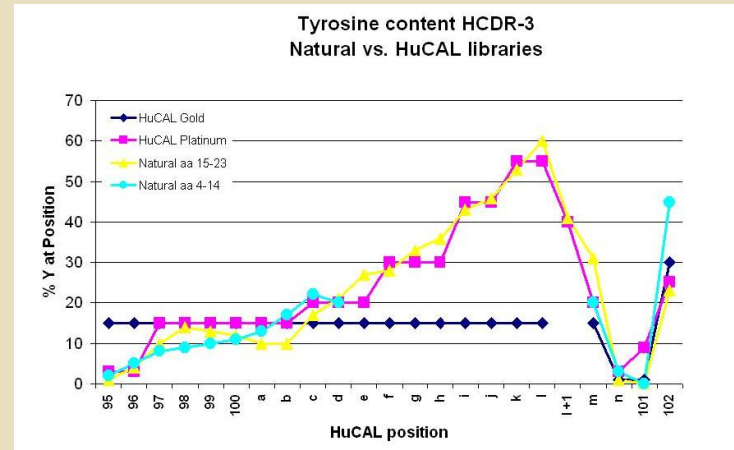
- Increase functional HCDR3 by length-dependent design of HCDR3
- Optimized selection of frameworks
- Minimized numbers of non-germline positions
- Gene optimization of master genes
- Massively reduced number of potential glycosylation sites
- Three times the size of HuCAL GOLD



# A New HCDR3 Design to Cover Human Structure and Functional Diversity



- Zemlin et al. showed a length dependent aa composition of HCDR3
- Increase in long CDR: **Y, S, P, K** and **C**
- Decrease in long CDR: **G, R**



Zemlin et al. , JMB\_2003\_334(4)\_733-479

1. Separate analysis of HCDR3 length 4-23aa (in total 8,900 sequences)
2. Alignment of all lengths according to aa content of HCDR3

	97	98	99	100a	100b	100c	...	100m	100n	100o	101	102	
<b>13aa</b>	15% G 10% S 9% Y	15% G 12% S 11% Y	16% G 16% S 10% Y	19% G 15% S 11% Y	16% G 13% S 11% Y	13% G 11% S 17% Y		14% G 6% S 24% Y	15% G 4% S 26% Y	65% F 13% M	88% D	40% Y 17% I 15% V	
<b>14aa</b>	16% G 11% S 7% Y	14% G 13% S 9% Y	17% G 15% S 13% Y	17% G 15% S 7% Y	16% G 15% S 12% Y	13% G 12% S 17% Y	13% G 10% S 22% Y	14% G 6% S 27% Y	18% G 3% S 24% Y	58% F 17% M	87% D	37% Y 20% V 16% I	
<b>15aa</b>	18% G 9% S 13% Y	11% G 12% S 14% Y	14% G 13% S 11% Y	17% G 17% S 10% Y	18% G 16% S 9% Y	16% G 17% S 16% Y	14% G 11% S 23% Y	11% G 9% S 26% Y	21% G 4% S 23% Y	60% F 21% M	92% D	33% Y 24% V 14% I	
<b>16aa</b>	16% G 8% S 12% Y	14% G 12% S 14% Y	14% G 15% S 9% Y	13% G 18% S 10% Y	15% G 18% S 9% Y	18% G 17% S 11% Y	14% G 8% S 26% Y	9% G 8% S 33% Y	7% G 8% S 33% Y	11% G 4% S 37% Y	26% G 1% S 25% Y	52% F 31% M	92% D 26% Y 12% I

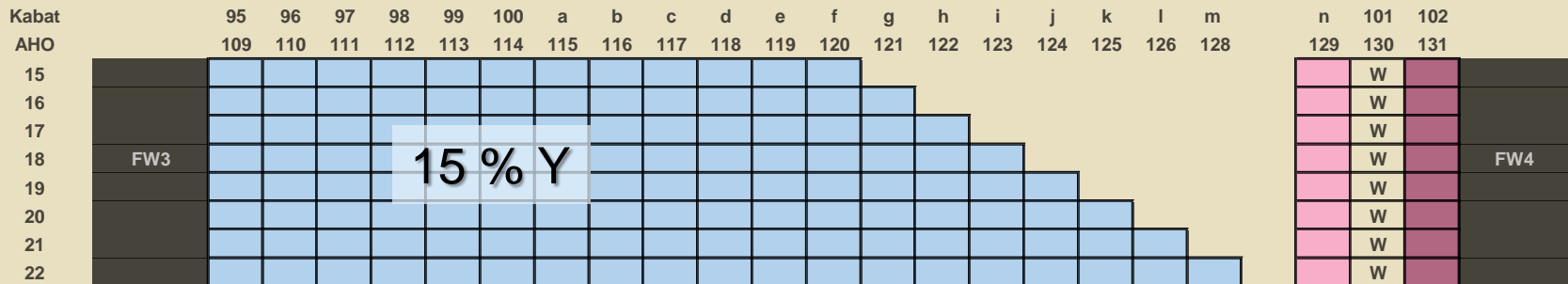
Increasing Y-content

Conserved C-terminus

# HCDR3 Design in HuCAL

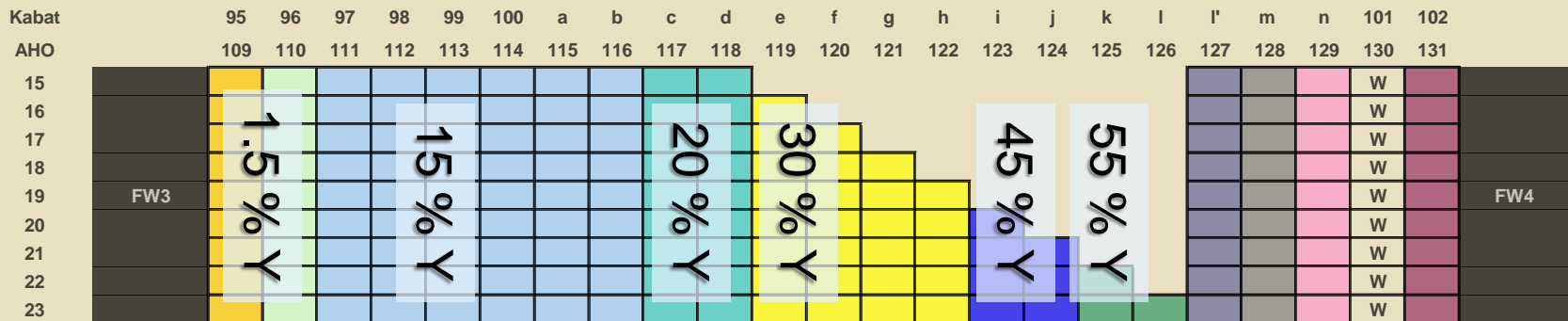
## HuCAL Gold Design

HCDR3 Length aa



## HuCAL Platinum Design

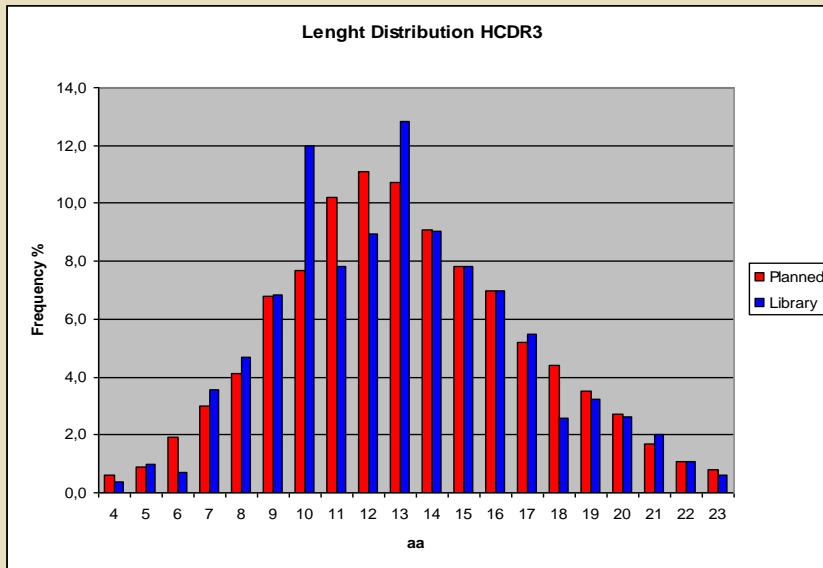
HCDR3 Length aa



- HCDR3 design in HuCAL Platinum reflects the length-dependent aa composition found in nature

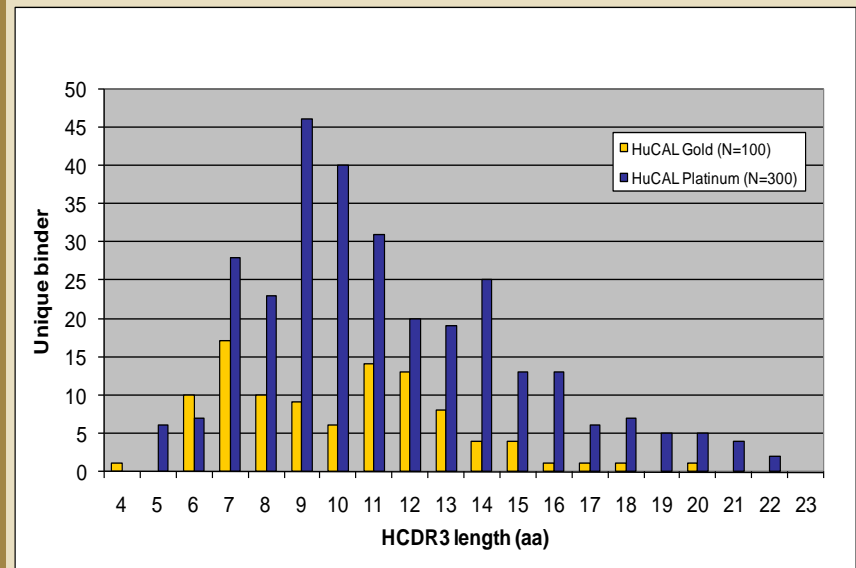
# HuCAL Platinum: HCDR3 Length Distribution

## Design and composition of Library



- Composition Library = Design

## HCDR3 selected from Library



- Increased number of long HCDR3

**HuCAL Platinum delivers higher number of sequences with long HCDR3**

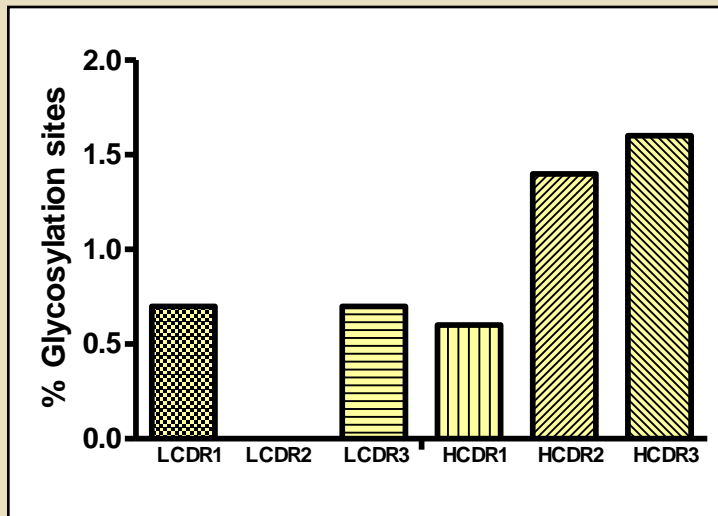
# Controlled Diversity to Avoid .....Undesired Motifs

HCDR2									
	Nat. Seq			Gold			Platinum		
Kabat	50	51	52	50	51	52	50	51	52
25% V		<b>90% I</b>	<b>63% S</b>	20% V	<b>I</b>	<b>66% S</b>	24% V	<b>I</b>	<b>66% S</b>
15% A	...	....	....	12% A		....	14% A		....
11% S	...	....	....	12% S		....	14% S		....
11% Y		....	....	12% Y		....	14% Y		....
	<b>10% N</b>			<b>12% N</b>					
	9 % G			12% G			14% G		
	....			....			....		
	....			....			....		
	....			....			....		
<b>N x S/T</b>	<b>6,3%</b>			<b>7,9%</b>			<b>0%</b>		

■ Potential N-glycosylation site can be avoided by omitting either N or S/T in TRIM Mixtures of known hot spots

# Reduced Number of Potential Glycosylation Sites

- Hot spots of NxS/T site found in Gold were removed or at least dramatically reduced in Platinum
- NxS/T could not be eliminated completely without losing significant sequence diversity



Library Platinum	Number of clones analysed	Glycosylation sites
<b>Lambda</b>		
VH1A	83	4.8%
VH1B	89	2.2%
VH2	87	5.7%
VH3-23	142	4.9%
VH3-15	80	3.7%
VH5	67	4.8%
VH6	88	3.4%
<b>kappa</b>		
VH1A	85	3.5%
VH1B	106	5.4%
VH2	78	5%
VH3-23	141	5.7%
VH3-15	85	3.5%
VH5	94	5%
VH6	86	6%
	<b>1311</b>	<b>5%</b>

**Potential Glycosylation sites: Gold (30%) vs. Platinum (5%)**

# HuCAL Platinum: Size and Correctness

Platinum Library	Library size Gold	Library size Platinum	Number of correct clones, Sequencing (95 clones) [%]
<b>λ-Libraries</b>			
pM30_VL1-3_VH1A	6,1E+08	3,8E+09	72%
pM30_VL1-3_VH1B		1,4E+09	76.4%
pM30_VL1-3_VH2	5,4E+08	7,7E+08	74.7%
pM30_VL1-3_VH3-23	3,4E+09	3,7E+09	70,4%
pM30_VL1-3_VH3-15	-	5,2E+09	64%
pM30_VL1-3_VH5	6,9E+08	4,3E+09	70%
pM30_VL1-3_VH6	1,7E+09	3,8E+09	72%
<b>κ-Libraries</b>			
pM30_Vk1-3_VH1A	5,0E+08	5,1E+09	83%
pM30_Vk1-3_VH1B		9,0E+08	76%
pM30_Vk1-3_VH2	2,9E+09	1,2E+09	79%
pM30_Vk1-3_VH3-23	3,3E+09	1,7E+09	72%
pM30_Vk1-3_VH3-23 II	3,3E+09	5,0E+09	75%
pM30_Vk1-3_VH3-15	-	4,6E+09	76%
pM30_Vk1-3_VH5	1,2E+09	2,0E+09	73%
pM30_Vk1-3_VH6	7,7E+08	1,8E+09	65%
<b>Combined Library</b>	<b>1,6E+10</b>	<b>4,5E+10</b>	<b>74%</b>

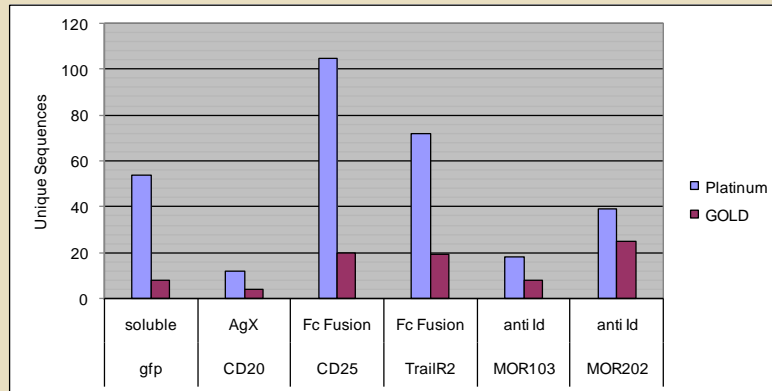
- HuCAL Platinum is around three times as large as HuCAL GOLD
- HuCAL GOLD contains 64% correct clones, Platinum even 74%\*



# HuCAL Platinum: Test Selections



Antigen	Type	Primary Hit rate Platinum > 5 x bgd	Primary Hit rate GOLD > 5 x bgd	Platinum	GOLD
gfp	soluble	352	75	54	8
CD20	AgX	36	10	12	4
CD25	Fc Fusion	702	526	105	20
TrailR2	Fc Fusion	651	468	72	19
MOR103	anti Id	251	346	18	8
MOR202	anti Id	646	662	39	25
<b>Sum</b>				<b>300</b>	<b>84</b>



**HuCAL Platinum shows 3.6 fold increased hit diversity compared to HuCAL GOLD**



- HuCAL Master genes optimized in collaboration with GENEART AG
- Optimized codons for *E.coli* expression and eukaryotic expression system
- mRNA secondary structures removed
- Negative regulatory sequence motifs in prokaryotic and eukaryotic system were avoided



# HuCAL Platinum: Optimized on DNA Level

Genes optimized by GeneOptimizer® Software  
(no aa changes introduced)

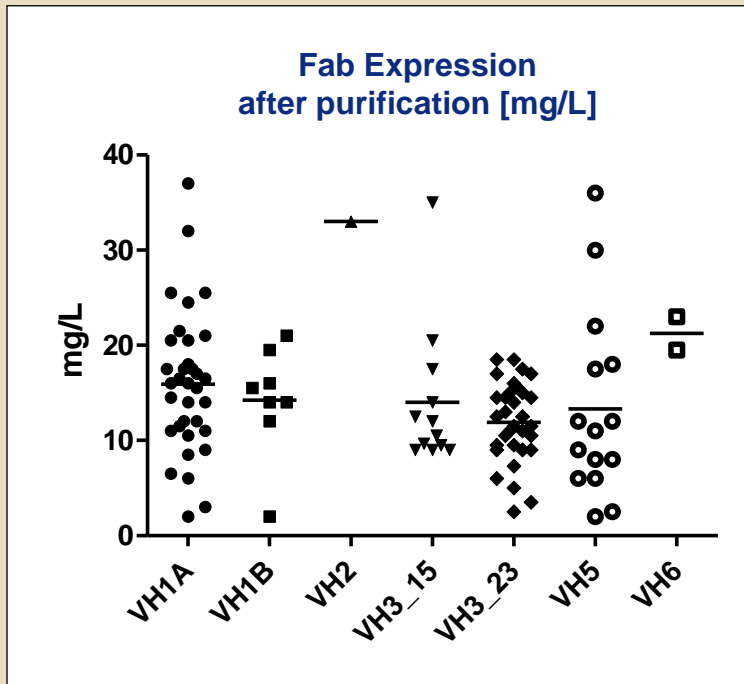


	Removed problematic sites				
	Prokaryotic inhibitory motif	Consensus splice site	Cryptic splice site	RNA instability motif	Alternative start codon
VH1A	1				
VH1B	1		1		
VH2		1			
VH3-23		1			
VH3-15				1	
VH5			1	1	
VH6			1		
VI-1				1	
VI-2		1		1	
VI-3				1	
Vk-1		1		2	
Vk-2				2	
Vk-3				2	
CH1			1		3
CL-k	1	1			
CL-l	1				

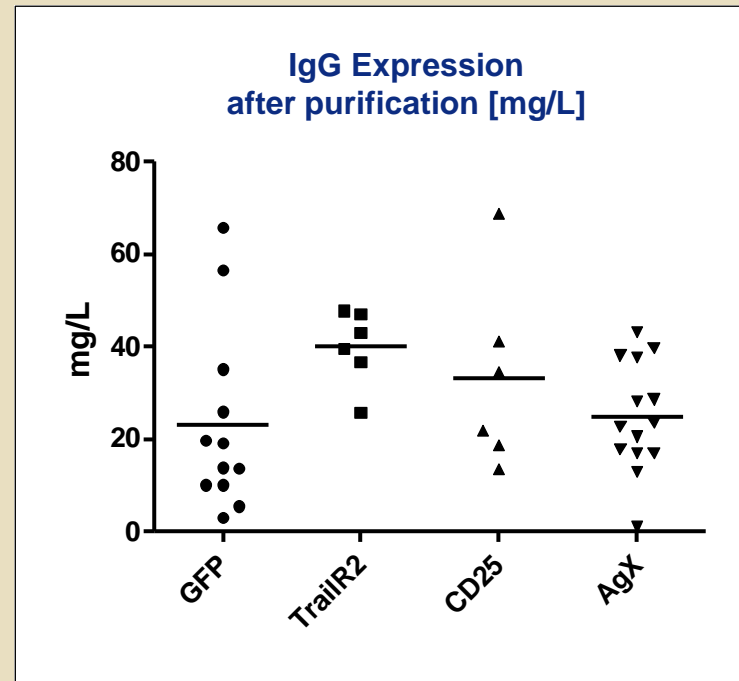
Codon usage optimized for *E. coli*  
Rare mammalian codons avoided



**Number of potential problematic DNA sites significantly reduced**



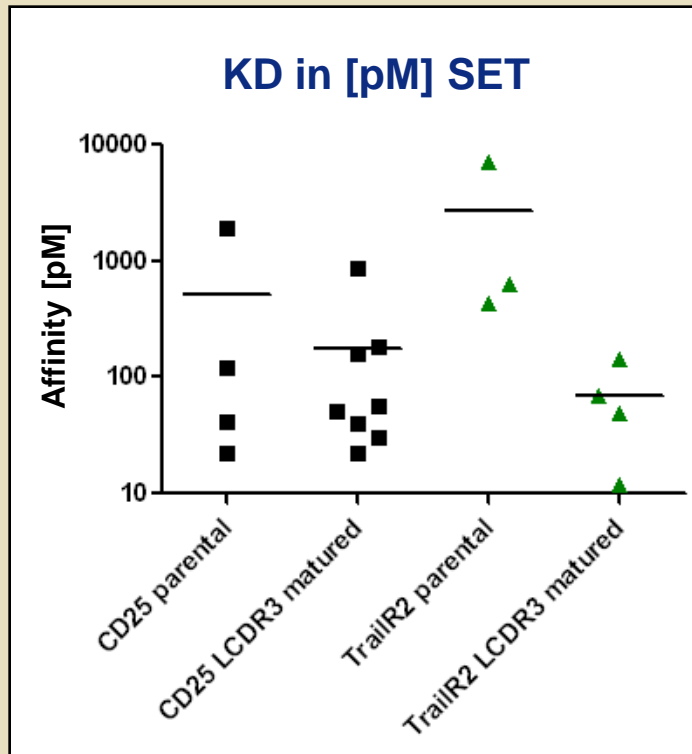
Fab Expression levels from four different projects  
Average 14.2mg/l (n=104)



IgG1f pMorph4 Expression levels from four different projects  
Average 28.1mg/l (n=38); HuCAL Gold 14mg/l

**HuCAL Platinum shows twice the expression rate on IgG level compared to GOLD**

# HuCAL Platinum Delivers Extraordinarily High Affinities: Parental vs. RapMat



Best affinities naïve library: 22pM

Best affinities RapMAT: 12pM

Preselected by affinity screen

**Platinum delivers extraordinarily high affinities from the naïve library which can be quickly improved further by RapMAT**



- TRIM Technology applied to generate a high quality library with over 74% correct clones
- Library size:  $4.5 \times 10^{10}$
- HCDR3 length distribution mimicking nature
- Low number potential glycosylation sites: 5%
- 3.6-fold higher diversity vs. GOLD on average
- Improved expression rate on IgG and Fab level



Thank You.



Engineering the Medicines  
of Tomorrow

[www.morphosys.com](http://www.morphosys.com)