

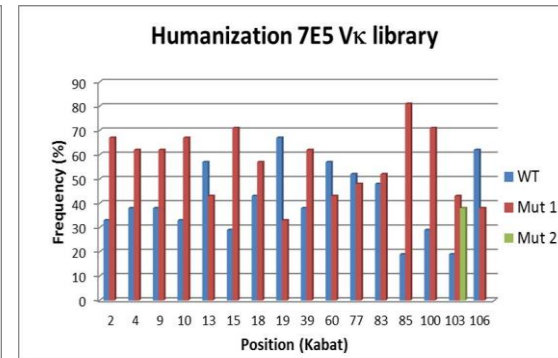
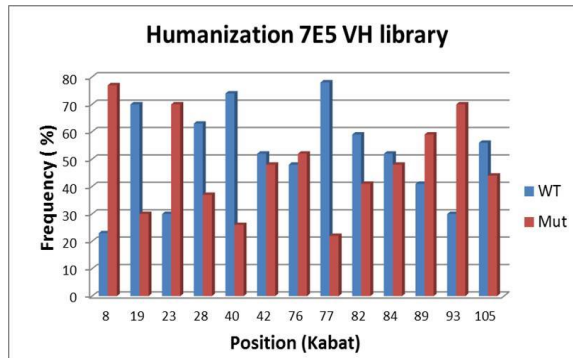
# Case study IX

## Humanization of a rat mAb with no loss of affinity

- Aim: To humanize a rat antibody
- Technology: Library of mutations + Affinity driven selections

### Library design & construction:

- Identification of closest human germline based on CDRs canonical folds
- Assembly of synthetic genes using primers that introduce mutations
- Cloning in phagemid (VH and VL sub-libraries)
- Sub-libraries selection on antigen
- Final humanized Fab library construction



Distribution of frequency of WT and mutated amino acid residue per position in humanized 7E5 VH and V<sub>k</sub> sub-libraries clones

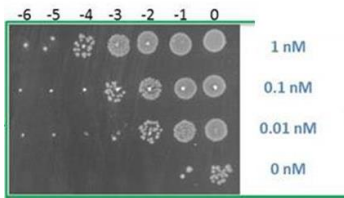
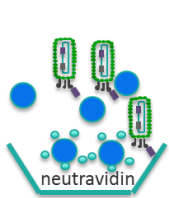
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### Affinity driven selection:

- ↓ biotinylated antigen concentration
- ↓ Phage
- Washing with ↑ non-biotinylated antigen



Titration of *E. coli* infected with phage from humanization Fab library selected after three rounds on llama IgG in the presence or absence of counter antigens

Clone	Binding ELISA (soluble Fab in P.E.)	Biacore			Vκ			VH		
	OD450nm	Kd (s <sup>-1</sup> )	SE(kd)	RO (>10)	%Identity	%Homology	Sequence #	%Identity	%Homology	Sequence #
WT	4.149	4.92E-04	3.09E-05	14.2	83%	84%	WT	86%	89%	WT
8G09	3.859	3.91E-04	1.55E-05	39	94%	94%	18	95%	97%	4
7E12	4.255	5.90E-04	1.37E-05	51.6	94%	94%	57	94%	97%	49
7G09	4.540	1.74E-04	9.93E-06	43.5	94%	94%	49	94%	95%	59
8F07	3.972	2.44E-04	1.21E-05	44.2	94%	94%	14	93%	94%	29
7F06	4.549	2.66E-04	9.10E-06	45.7	94%	94%	17	94%	95%	67
7F11	4.128	2.27E-04	1.40E-05	31	93%	94%	25	98%	99%	8
7E11	3.371	1.42E-03	6.88E-05	7.29	93%	94%	4	97%	98%	3
7F02	4.615	1.96E-04	1.22E-05	36.6	90%	90%	44	95%	97%	66

Correlation of human identity and homology percentage with ELISA binding data and off-rate values of selected humanized Fab variants. Data for WT Fab is shown for comparison

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## Humanization of a rat mAb with no loss of affinity

- Summary

Combination of a randomized FR library with monovalent phage display affinity-based selection allowed the isolation of a very diverse panel of Fabs

- Human identity and homology percentage values varying from 88-99%

Note: Avastin (humanized anti VEGF mAb): 87% Human identity and 95% human homology

- Better or equal off-rates than WT clone