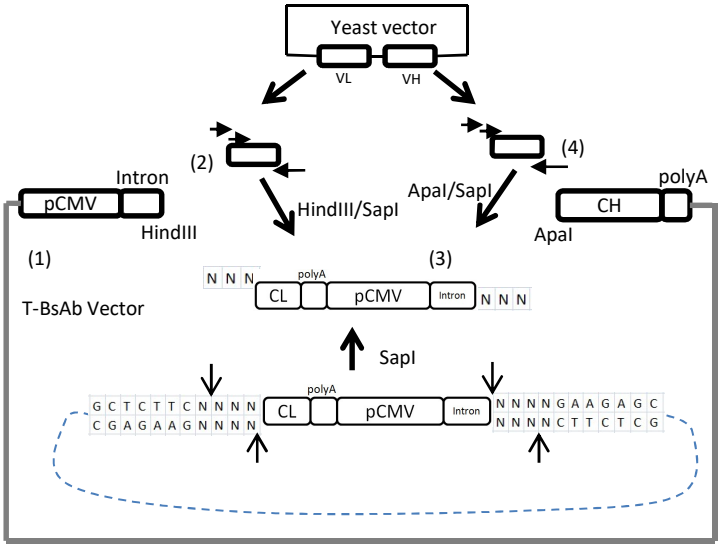
A



B

Clones	Heavy Chain							Light Chain						
			CDR1		CDR2				CDR1		CDR2		CDR3	
	5	13	28	32	52	53	107	18	31	32	53	83	90	91
	V	K	A	Y	S	S	W	R	T	V	N	F	Q	H
31		E	T											
32	E		T											Y
48											D			Y
49			T							L				
53			T											Y
56							R			L				Y
57			T					S				V		Y

C

	K_D	Fold difference/Parental
A33 31	3.87E-11	4.3
A33 32	5.64E-12	29.7
A33 48	3.29E-12	51.0
A33 49	2.20E-11	7.6
A33 53	1.75E-11	9.6
A33 56	2.43E-11	6.9
A33 57	1.56E-11	10.7
A33-BsAb	1.68E-10	1.0

D

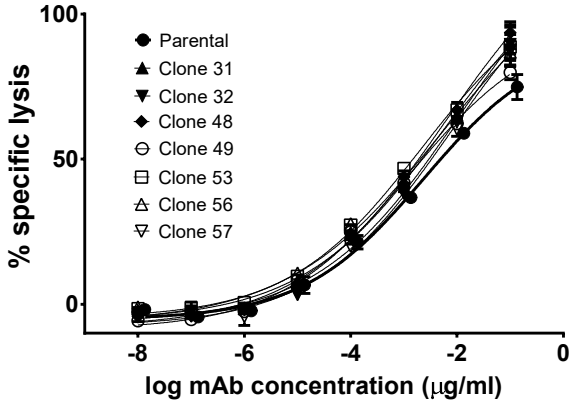


Figure S5. Affinity maturation of huA33-BsAb

A. Strategy for rapid reformation of scFv to T-BsAb format. Vector (1) was linearized with HindIII/ApaI. Promoter fragment (3) was prepared from SapI digested promoter-containing vector. Both vector and promoter fragment could be prepared in large amount for higher throughput cloning. VH (4) and VL (2) were amplified directly from yeast with two 5' primers to add the leader sequences and digested with HindIII/SapI (VL) or ApaI/SapI (VH). The 4 fragments were ligated in a one-step reaction. **B**. Summary of mutations obtained from yeast display. **C**. Summary of Kd values of parental and affinity matured huA33-BsAb. **D**. TDCC assay of parental and 7 clones of affinity matured huA33-BsAb.