Use of antibody gene library for the isolation of specific single chain antibodies by ampicillin-antigen conjugates

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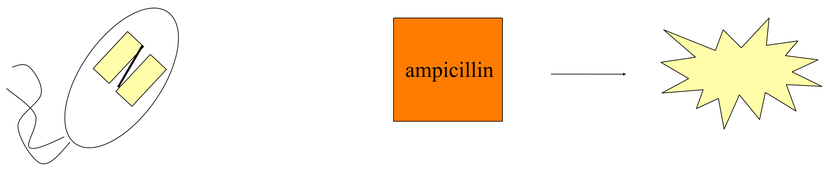
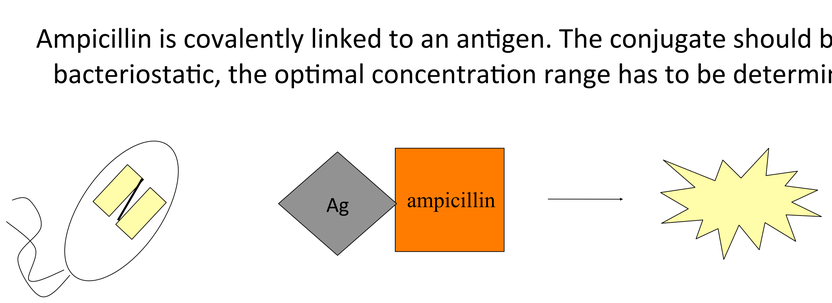
Introduction

Isolation of recombinant antibodies from antibody libraries are commonly performed by different molecular display formats including phage display and ribosome display or different cell-surface display formats. We describe a new plate assay method which allows the selection of *Escherichia coli* cells producing the required single chain antibody by cultivation in presence of ampicillin conjugated to the antigen of interest. The method utilises the neutralization of the conjugate by the produced single chain antibody which is secreted to the periplasm.

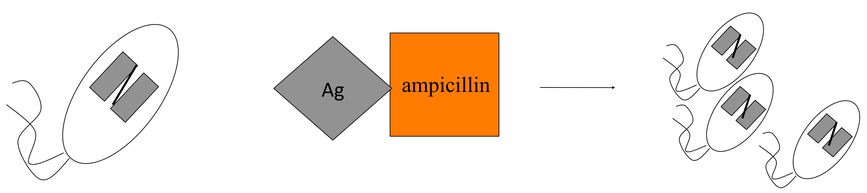
Ampicillin prevents bacterial growth



Ampicillin is covalently linked to an antigen. The conjugate should be still bacteriostatic, the optimal concentration range has to be determined.



Ampicillin-antigen conjugate is no longer bacteriostatic if the antigen-specific scFv is bound to the conjugate.



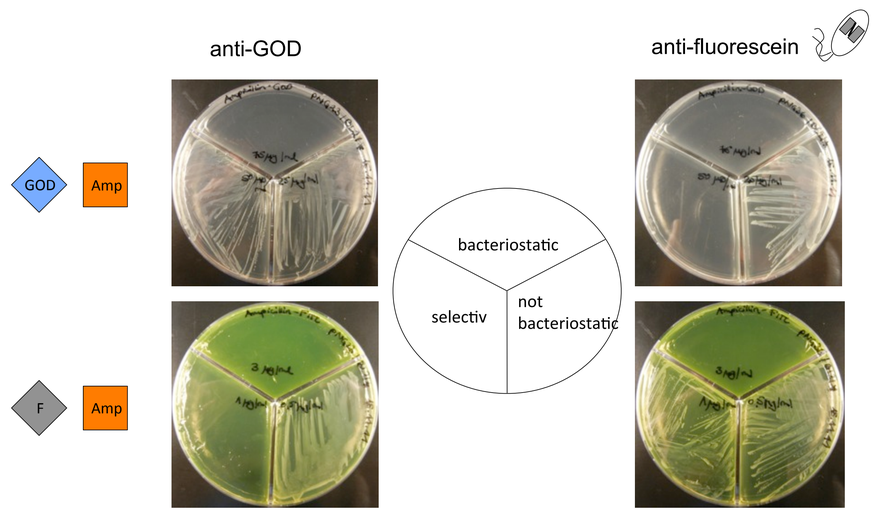
Conclusions

- simple plate assay
- requires only methods and materials available in standard molecular biology labs
- conjugation of the antigen required, ampicillin may loose activity
- optimal conjugate concentration range may vary
- applies the soluble single-chain antibody molecule
- avoids undesired effects e.g. by the phage particle or the yeast fusion protein
- selecting directly in an expression strain
- production and characterization without any further cloning or transformation

1 Neumann-Schaal, M., Messerschmidt, K., Grenz, N., Heilmann, K., 2013. *Immunol. Lett.* 151(1-2), 39-43
2 Sellrie, F., Micheel, B., 2008. *Biochimical Engineering Journal* 38(3), 309-313.

Proof of principle with model antigens

Experimental set-up: preculture, induced with 100 µM IPTG, cells were harvested and plated on LB-agar containing ampicillin-conjugates and 100 µM IPTG. Plates were incubated overnight at 30˚C.



Recovery of cells expressing the plasmid encoding the anti-fluorescein scFv:

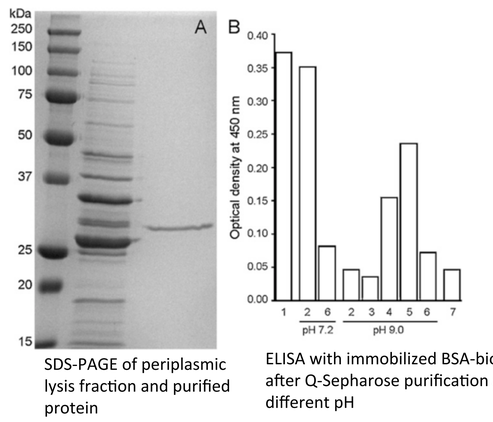
- until a dilution of 1:32,000 after mixing with bacterial cells producing unspecific scFv
- until a dilution 1:5,000 after mixing with plasmids encoding unspecific scFv prior to transformation.

Selection of a biotin binding scFv

sequence of isolated anti-biotin scFV

V_H	FRI	MAQVQLQQSGAELVKPGASVKISCKASGYAFS
	CDRI	NYWMN
	FRII	WVKQRPGKGLEWIG
	CDRII	QIYPGNGDAKYSGKSRD
	FRIII	KATLTADKSSSTAYMQLSSLTSEDSAVYFCSR
	CDRIII	SYGYDEAWFAY
	FRIV	WGQGTLVTVSA
	Linker	GSSGGGGSGGRASGGGGSKL
V_L	FRI	DLLLTQSPASLAVSLGQRATISC
	CDRI	RASESVDNYGISYMH
	FRII	WYQQRPGQPPKLLIY
	CDRII	LAANLDS
	FRIII	GVPARFSGSGSGTDFTLNIHPVEEEDAATYYC
	CDRIII	QHSREVPWT
	FRIV	FGGGTKLEIKRAA
	Tags	A EQKLISEEDL S HHHHHH

purification of anti-biotin scFv



SDS-PAGE of periplasmic lysis fraction and purified protein

ELISA with immobilized BSA-biotin after Q-Sepharose purification at different pH

IC_{50} values of anti-biotin scFv, E11-AE11, streptavidin and E11-AE11 based scFv

competitive ELISA with BSA-Biotin bound to the solid phase and varying free biotin concentrations

Binder	free Biotin [µg/ml]	IC_{50}	R^2 value	Detection antibody
anti-biotin scFv	$5x10^{-4}$ – 1000	5.03 ± 0.11 µM	0.99041	mouse α-c-myc ab (9E10) and POD-labeled goat α-mouse Ig ab
E11-AE11	$5x10^{-7}$ – 1	35.5 ± 1.4 nM	0.98874	POD-labeled goat α-mouse Ig ab
streptavidin	$5x10^{-8}$ – 0.1	28.7 ± 4.5 pM	0.99682	E11-HE11 (mouse α-streptavidin ab) and POD-labeled goat α-mouse Ig ab
E11-AE11-based scFv (10 µg/ml)	$5x10^{-4}$ – 1000	not detectable		mouse α-c-myc ab (9E10) and POD-labeled goat α-mouse Ig ab