Application Note

BRAND

Characterisation of antibodies with BRAND PCR plates

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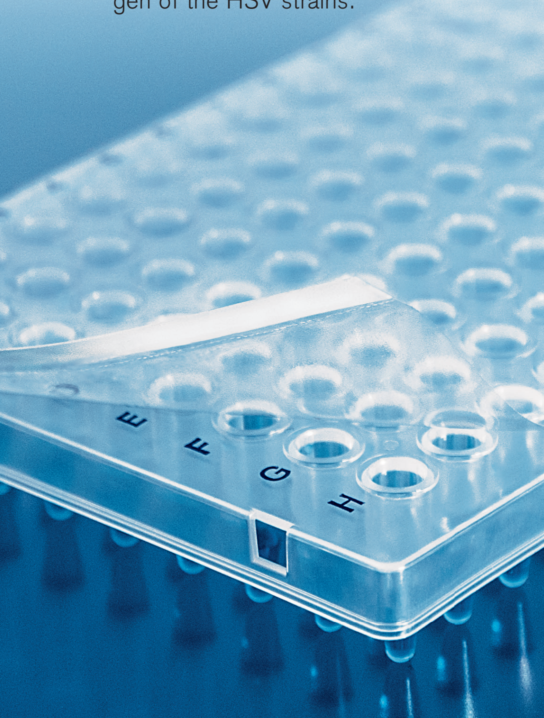
Introduction

The generation of fully human antibodies with unique functional properties that are exploitable for tailored therapeutic interventions remains a major challenge in the antibody technology field. However, it is possible to isolate these antibodies from human B-cellls in contact with specific antigens.

In this study, variable antibody genes of igG were cloned from B-cell repertoires in lymph nodes of head and neck cancer patients. The sequence analysis of the antibodies of lymph nodes revealed a naturally occurring distribution pattern of rearranged antibody sequences, representing all known variable gene families and most functional germ line sequences.
To demonstrate the feasibility for selecting antibodies with therapeutic potential from these repertoires, these antibodies were tested for their affinity to the target antigen of HSV-1 (Herpes simplex viruses) infected cells.
The majority of the scFv (single chain variable fragment)-antibody fragments bound with nanomolar affinity to the target antigen of the HSV strains.



Material and Methods[1]

For antibody isolation IgG donor repertoires from lymph nodes of head and neck cancer patients were used. Thereby independent gene pools of variable domains of light and heavy chains (VH/ VL-kappa and VH/ VL-lambda) could be generated.
Bacterial cells transformed with these genes were grown on agar plates and cultivated overnight. These transformed and cultivated *E.coli* cells were in the next step stored in glycerol aliquots at -80 °C.

Exemplary transformed samples of aliquots were selected and cultivated in shaking flasks, before a tenfold dilution of these *E.coli* cells were transferred on agar plates. Eight clones of each plate were picked and further analysed with a colony PCR to clarify whether the genes of both the heavy chain and the light chain were integrated (fragment size ~ 1 kb).

Table 1: PCR Program characteristics

PCR-Program		Time	
Initial Denaturation	95 °C	5 min	
Denaturation	95 °C	15 sec	35 Cycles
Annealing	51 °C	15 sec	35 Cycles
Elongation	72 °C	15 sec	35 Cycles
Final Elongation	72 °C	10 min	
Cooling	4 °C	unlimited	

Table 2: PCR mixture

	Capacity
KAPA 2 G Fast Ready Mix (buffer, polymerase, dNtPs, gel loading dye)	12,5 µl
Primer A	0,5 µl
Primer B	0,5 µl
Template	picked single colony
H_2O	11,5 µl
Total capacity	25,0 µl

To visualize expression of scFVs in the selected colonies, the colony PCR was subjected to agarose gel electrophoresis.

Result

All eight clones (in triplicates) showed a band at a fragment size of ~ 1 kb. For this reason they include the genes of the heavy chain as well as the genes of the light chain.

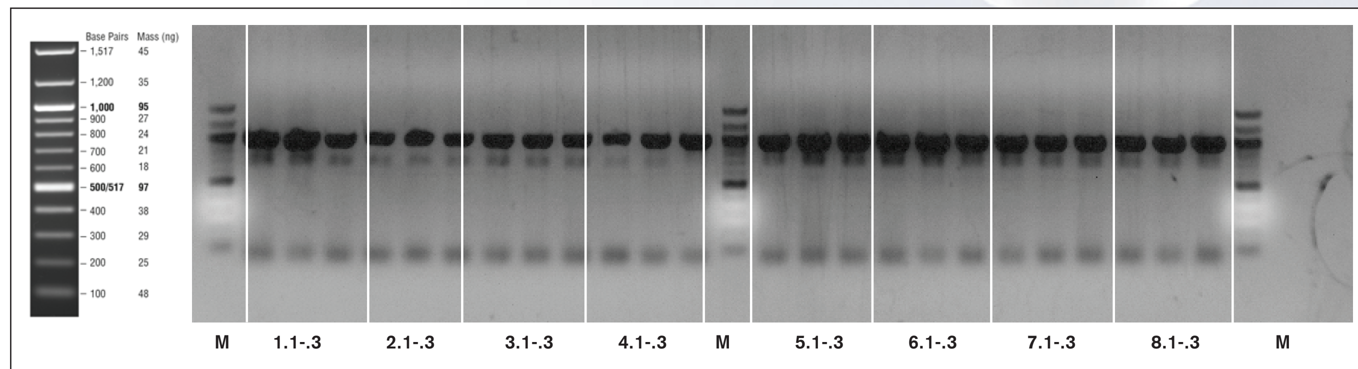


Figure 1: [right]: DNA marker (100bp DNA Ladder (N3231) of NEB at position 1;14 and 27. The 1,5% agarose gel ran at 140 V for 40 minutes) with marker description; [left]: agarose gel (triplicates each)

Subsequently, the isolated B-cell fragments were incubated with HSV-1 infected cells. The equilibrium constant of binding (K_D) of the scFvs with the target antigen of HSV-1 infected cells was determined on the basis of a flow cytometry affinity measurement. The K_D value was in the nanomolar range indicating a high binding affinity.

Discussion

BRAND PCR plates (Cat. No. 7813 75) are perfectly suitable for a colony PCR. The smooth well surfaces minimize attaching of reactants to the well surface, so that the PCR reaction can process completely. Further the high-quality polypropylene of this PCR plate is free of PCR inhibitors that could reduce the yield. In addition these PCR plates were sealed with the optimal fitting sealing mat (Cat. No. 7814 05) to reduce evaporation losses to a minimum.

In the light of the above the antibody fragments of all transformed clones could be reproduced with a high yield and verified with an agarose gel.

1 Philipp Diebolder, Armin Keller, Stephanie Haase, Anne Schlegelmilch, Jonathan D Kiefer, Tamana Karimi, Tobias Weber, Gerhard Moldenhauer, Roland Kehm, Anna M Eis-Hübinger, Dirk Jäger, Phillippe A Ferderspil, Christel Herold-Mende, Gerhard Dyckhoff, Roland E Kontermann, Michaela AE Arndt, and Jürgen Krauss. Generation of „LYmph Node Derived Antibody Libraries“ (LYNDAL) for selecting fully human antibody fragments with therapeutic potential.
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