Yeast dispaly based Naïve VHH Library from Camel

Introduction

Yeast display antibody library technology is one of the most prominent research advances in the field of antibody engineering in recent years. This technology mainly displays antibody molecules on the surface of yeast cells, uses target antigen molecules to screen yeast cells expressing specific antibody molecules, and uses genetic engineering methods to express and subsequently identify the functions of antibodies, thereby obtaining functional antibody molecules. According to whether the B cells used for constructing yeast display antibody libraries have been immunized, the constructed yeast antibody libraries can be divided into immune libraries and non-immune libraries. The B cells used to construct the immune bank are mainly derived from memory B cells that have been actively immunized or infected by pathogens in vivo; If the yeast antibody library is constructed from non-sensitized B cells, it is called a non-immune library. Since the diversity of non-sensitized B cells depends on the diversity of B cell clones used to construct antibody libraries, the larger the capacity of non-immune libraries, the more high affinity antibodies can be screened.

The natural Yeast dispaly based Naïve VHH Library from Camel sold by our company was constructed by collecting 20 non-immunized camel lymphocytes. 48 clones were randomly selected for PCR verification with a positive rate of 100%. The sequencing results showed no sequence duplication and good diversity, with a library capacity of approximately 1 × 10^9.

The reagent kit contains

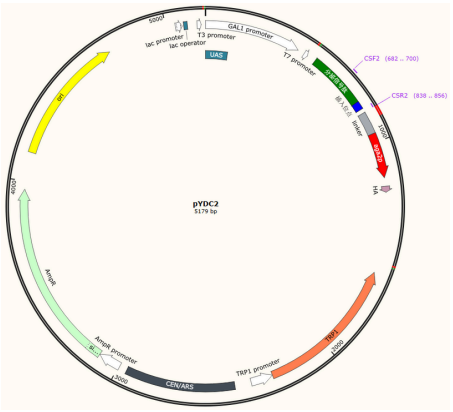
1mL of yeast library cells (containing 25% glycerol).
Please read the instructions carefully before use and prepare the reagents for screening.

Yeast display antibody library vector instructions

This reagent kit is constructed using a modified plasmid pYD1. This yeast vector is designed to display alpaca and camel VHH fragments, which are fused and expressed with yeast Aga1-Aga2 protein and have HA tags for convenient detection of display signals.

Vector type:	Phagemid vector
Vector length:	5300 bp
Tag:	HA tag
Promoter:	GAL1
Resistance:	Amp+
Vector backbone:	pYD1

Backbone size w/o insert (bp) 5100



Medium formulation

Amplification medium SD-CAA

Name	Final Concentration
$Na_2HPO_4 \cdot 12H_2O$	13.62 g/L
$NaH_2PO_4 \cdot 2H_2O$	9.62 g/L
Yeast Nitrogen Base without Amino Acids(No amino acids, containing ammonium sulfate)	6.7 g/L
casamino acids	5 g/L
Glucose	20 g/L
Ampicillin	100 μg/mL
streptomycin	100 μg/mL

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Galactose	20 g/L
Glucose	2 g/L
Ampicillin	100 μg/mL
Streptomycin	100 μg/mL

Recovery and cryopreservation of host bacteri

1. Resuscitation: Take a frozen bacterium and place it in a 30 ℃ water bath for about 3 minutes until completely melted. Inoculate it into 1L SD-CAA medium and shake it overnight at 30 ℃ and 220rpm.
2. Cryopreservation: Prepare cells freshly cultured in SD-CAA medium until logarithmic growth stage, add 25% glycerol to the final volume and mix well, and store at -80 ℃.

Host bacterial culture and induction

1. Proliferation culture: Inoculate in SD-CAA liquid medium, shake and culture at 30 ℃, 220rpm, doubling time of about 3-4 hours; inoculate in SD-CAA solid medium plate, culture box at 30 ℃ for about 2-3 days to produce single colonies.
2. Induced expression: Cultivate in shaking table at 30 ℃, 220rpm, SD-CAA until logarithmic growth stage, replace SG-CAA medium, shake table culture at 20-25 ℃, 220rpm, 24h.

Cell staining for flow sorting and identification

Induced yeast cells were centrifuged, washed, and incubated with biotinylated target proteins for binding. After washing, the target proteins were labeled with avidin fluorescent dye, and anti HA antibody fluorescent dye was added to label the HA tag. After incubation and washing, the yeast cells were sorted or identified using a flow cytometer.

Monoclonal PCR

Amplification Primers:
CSF2: 5'-TTGCTGCCTTGCCATTATC-3'
CSR2: 5'-GTACGTGCTGTTGAACTGT-3'

Monoclonal sequencing and analysis

Sequencing of monoclonal PCR products using sequencing primer CSF2.
Analysis: -- GTACAATTAGATAAAGAGAGGCCGAGCT (upstream vector sequence) - VHH (target sequence)--

Backbone size w/o insert (bp) 5100