Fusion BioLabs

Antibody Phage Display Library Construction Kit

pAPD-V_HH: camelid V_HH phage display library construction kit

Catalog#: APD-08

Product Overview

Fusion BioLabs offers a range of library primer sets and phagemid vector combination for antibody phage display and peptide phage display construction. With customizable features and robust performance, our primer sets and phagemid vectors are designed for facilitating phage display library generation as fast as within one week.

pAPD-VHH is a phagemid vector for construction of variable domain of the heavy chain of a heavy chain only antibody (V_HH) library. Here are the key steps involved in constructing such a library:

- Amplify V_HH repertoires from cDNA reverse transcript from RNA isolated from peripheral blood lymphocytes (PBL) or lymphoid tissue of non-immunized or immunized donors using PCR Primer Set.
- Restriction enzyme digestion **pAPD-VHH** vector and VHH repertoires with NcoI/NotI.
- Ligation of digested and purified VHH repertoires into digested and purified **pAPD-VHH** vector to make VHH libraries.

Key Features

High expression efficiency: Engineered for efficient expression and display of camelid VHH on the surface, allowing for easy screening and selection of target molecules.
Flexibility and versatility: One vector for both antibody library construction and downstream antibody fragment expression. No need subcloning into expression vector for downstream application.

Specifications

Antibiotic Resistance	Ampicillin (Amp^R)
Constitutive or Inducible System	Inducible for downstream expression
Delivery Type	Transformation
Product Type	Bacterial Expression Vector
Cloning Method	Restriction Enzyme NcoI/NotI

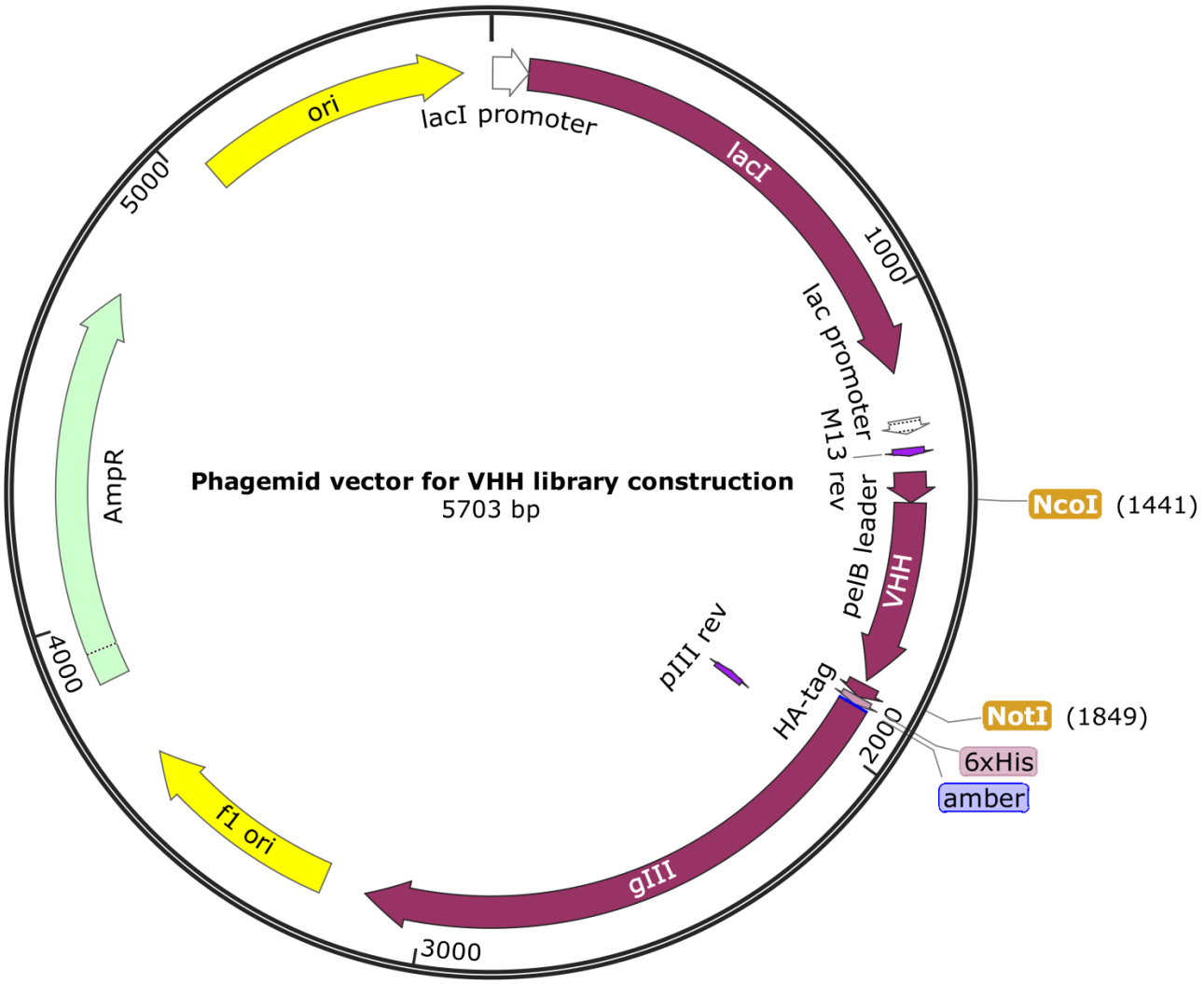
Contents & Storage

Primer set for amplification of camelid heavy chain antibody (V_HH) repertoires		
Vial 1	100 µl, 10 µM	Forward (4 oligos) and reverse primer (2 oligos) mix for V_HH fragment amplification (normalized concentration)
Sequencing primer set		
Vial 2	100 µl, 10 µM	M13 Reverse primer for VHH forward sequencing

Vial 3	100 µl, 10 µM	pIII Reverse primer for VHH reverse sequencing
pAPD-VHH cloning vector for phage display VHH library construction		
Vial 4	10.0 µg in Tris-EDTA buffer	

- Store at -20°C. Primer sets and vectors are guaranteed stable for 12 months when properly stored.

Vector for library Construction



Protocols

1. PCR amplification of the V_HH repertoires using following condition.

- Primer mix set and total PCR reaction volume

PCR amplification	Primer mix set	Volume
V_HH repertoires	Vial 1	1.0 ml (100 µl /rxn x 10 rxns)

- PCR setup and PCR protocol

Component	Amount	PCR Protocol		
10 x PCR buffer, -Mg^{2+}	10 µl	Initial denaturation		94°C for 2 min
50 mM $MgCl^2$	3 µl	30 PCR cycles	Denature	94°C for 30 sec
10 mM dNTPs	2 µl		Anneal	55°C for 45 sec
10 µM Primer mix (F+R)	4 µl		Extend	72°C for 90 sec
cDNA template (500-8000 ng/µl)	1 µl	Final Extension		72°C for 10 min
Taq DNA Polymerase (10 units/µl)	0.4 µl	Hold		4°C, indefinitely
Water, nuclease-free	79.6 µl	Note: Recommended for our PCR condition. Optimization maybe needed.		

- Combine V_HH repertoires reaction in 2.0 ml nuclease-free tube.
- Add gel loading buffer to the tube, mix, and briefly centrifuge the contents.
- Run the sample on a 1.0% agarose gel and an appropriate molecular weight marker. *Expect results: ~400-450 bp for V_HH repertoires.*
- Cut out the correct-size bands, and purify the DNA using any commercial gel DNA extraction kit.
- Quantitate yields using a NanoDrop Spectrophotometer at 260 nm *Expect results: Approximately 10-15 µg V_HH repertoires. If yields are too low, repeat the PCR and combine the end product for the repertoire.*

2. Restriction digestion of V_HH repertoires and **pAPD-VHH** cloning vectors by double enzymes NcoI/NotI at 37°C for overnight or 6 h.

Components	V_HH repertoires	pAPD-VHH vectors
DNA	10 µg	10 µg
10X digestion buffer	20 µl	20 µl
NcoI (20 units/µl)	10 µl	10 µl
NotI (20 units/µl)	10 µl	10 µl
Water, nuclease-free	to 200 µl	to 200 µl

- Add gel loading buffer to each tube, mix, and briefly centrifuge the contents.

- Run the sample on a 1% agarose gel and an appropriate molecular weight marker. *Expect results: ~400-430 bp for digested V_HH repertoires, and ~5300 bp for digested pAPD-VHH vector backbone.*
- Cut out the correct-size bands, and purify the DNA using any commercial gel DNA extraction kit.
- Quantitate yields using a NanoDrop Spectrophotometer at 260 nm *Expect results: At least 1-4 µg of each purified V_HH repertoire or digested pAPD-VHH vector backbone.*

3. Library ligation using T4 DNA ligase

Components	200 µl reaction
T4 DNA Ligase Buffer (10X)*	20 µl
Digested pAPD-VHH	2.5 µg
Digested V_HH repertoires	1.5 µg
T4 DNA Ligase (20 units/µl)	5.0 µl
Water, nuclease-free	to 200 µl

**The T4 DNA Ligase Buffer should be thawed and resuspended at room temperature.*

- Gently mix the reaction by pipetting up and down and microfuge briefly.
- Incubate overnight at room temperature.
- Heat inactivate at 65°C for 10 minutes.
- Purify the ligated samples and dissolve in 50 µl nuclease-free water.
- Transform 2 µl of purified ligation samples into 25 µl tube with TG1 electrocompetent cells (efficiency should be >4 x 10^{10} cfu/µg of plasmid DNA) for 2 min.
- Transfer the mix to electroporation cuvette (0.1 cm gap) and electroporate at 1.8 kV, 10 µF and 600 Ohms. Typical time constants are 3.5 to 4.5 msec for successful electroporation. There are around 25 electroporation in total needed to be done individually.

4. Proceed with the phage library preparation and panning.