Fusion BioLabs Short Protocol for Mouse scFv Phage Display Library Construction Kit

pAPD-m-scFv: Mouse scFv phage display library construction kit

SKU#: APD-05

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-m-scFv are the phagemid vectors for construction of **mouse** single-chain variable fragment (scFv) library. Here are the key steps involved in constructing such a library:

- Amplify V genes from cDNA reverse transcript from RNA isolated from peripheral blood lymphocytes (PBL) or lymphoid tissue of non-immunized or immunized donors using PCR primers corresponding to known V_H, V_κ, and V_λ gene sequences.
- Overlap assembly V_κ and V_H, and V_λ and V_H to make scFv repertoires respectively.
- Restriction enzyme digestion **pAPD-m-scFv** vector and scFv repertoires with SfiI.
- Ligation of digested and purified repertoires into digested and purified **pAPD-m-scFv** vector to make mouse scFv libraries.

Key Features

High expression efficiency: Engineered for efficient expression and display of antibody fragment scFv 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 5'-end SacI and 3'-end SpeI

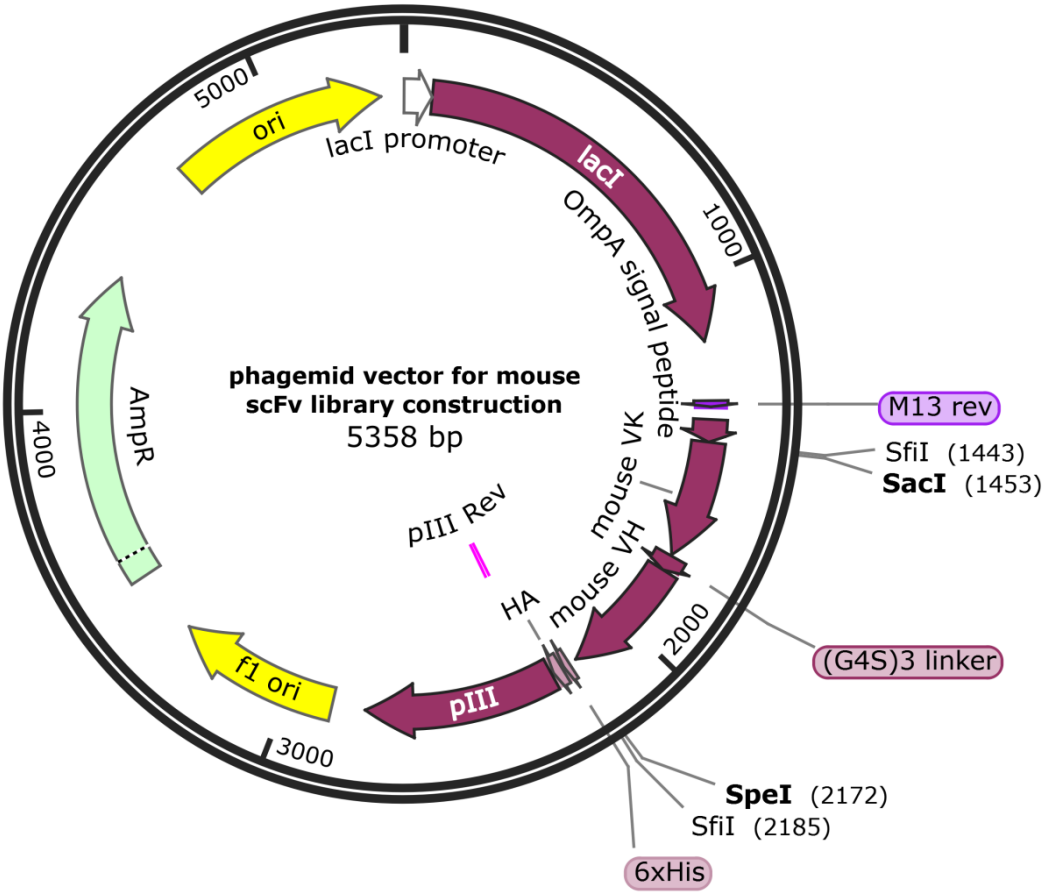
Contents & Storage

scFv Repertoires Construction Primer Set

Vial 1	200 µl, 10 µM	Primer mix (F+R) for V_k repertoires amplification
Vial 2	200 µl, 10 µM	Primer mix (F+R) for V_λ repertoires amplification
Vial 3	200 µl, 10 µM	Primer mix (F+R) for V_H repertoires amplification
Vial 4	200 µl, 10 µM	Assembly primers (F+R) for scFv repertoires construction
pAPD-m-scFv cloning vectors for mouse scFv library construction		
Vial 5	10.0 µg in Tris-EDTA buffer	
Sequencing Primer Set		
Via 6	100 µl, 10 µM	Forward sequencing primer for mouse scFv insert (M13 Reverse)
Via 7	100 µl, 10 µM	Reverse sequencing primer for mouse scFv insert (pIII Reverse)

- 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_H repertoires, V_k repertoires and V_λ repertoires using following condition.

   - Primer mix set and total PCR reaction volume

PCR amplification	Primer mix set	Volume
V_k repertoires	Vial 1	1.0 ml (100 µl /rxn x 10 rxns)
V_λ repertoires	Vial 2	1.0 ml (100 µl /rxn x 10 rxns)
V_H repertoires	Vial 3	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	56°C for 30 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 each repertoires reaction in 2.0 ml nuclease-free tube, respectively.
   - Add gel loading buffer to each tube, mix, and briefly centrifuge the contents.
   - Run the sample on a 2% agarose gel and an appropriate molecular weight marker. *Expect results: 350-400 bp products for each type of repertoire.*
   - 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 3-5 µg of each repertoire. If yields are too low, repeat the PCR and combine the end product for each type of repertoire.

2. Overlapping Extension PCR to generate mouse scFv repertoires according to the below template combination and assembly primers.

- Assembly primer set and total OE-PCR reaction volume

Overlapping extension PCR	Template combination	Assembly primers	Volume
Mouse scFv repertoires (k-scFv repertoires)	V_H repertoires & V_k repertoires	Vial 4	1.0 ml (100 µl /rxn x 10 rxns)
Mouse scFv repertoires (λ-scFv repertoires)	V_H repertoires & $V_λ$ repertoires	Vial 4	1.0 ml (100 µl /rxn x 10 rxns)

- OE-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	20 PCR cycles	Denature	94°C for 30 sec
10 mM dNTPs	2 µl		Anneal	56°C for 30 sec
10 µM Assembly primers	4 µl		Extend	72°C for 2 min
Template combination (**100 ng each)**	x µl	Final Extension		72°C for 10 min
Taq DNA Polymerase (10 units/µl)	0.4 µl	Hold		4°C, indefinitely
Water, nuclease-free	to 100 µl	Note: Recommended for our PCR condition. Optimization maybe needed.		

- Combine each repertoires reaction in 2.0 ml nuclease-free tube, respectively.
- Add gel loading buffer to each tube, mix, and briefly centrifuge the contents.
- Run the sample on a 2% agarose gel and an appropriate molecular weight marker. *Expect results: ~750-800 bp for mouse scFv 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: At least 10-15 µg of mouse scFv repertoire. If yields are too low, repeat the overlapping extension PCR and combine the end product for mouse scFv repertoire.

3. Restriction digestion of scFv repertoires and pAPD-m-scFv cloning vectors by SfiI at 50°C for overnight or 6 h.

Components	scFv repertoires (k-scFv or λ-scFv)	pAPD-m-scFv vectors
DNA	10 µg	10 µg
10X digestion buffer	20 µl	20 µl
SacI (20 units/µl)	10 µl	10 µl
SpeI (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: ~750 bp for digested scFv repertoires, and ~4600 bp for digested pAPD-m-scFv 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 scFv repertoire or digested pAPD-m-scFv vector backbone.*

4. Library ligation using T4 DNA ligase

Components	200 µl reaction for k-scFv library	200 µl reaction for λ-scFv library
T4 DNA Ligase Buffer (10X)*	20 µl	20 µl
Digested pAPD-m-scFv	1.9 µg	1.9 µg
Digested ScFv repertoires	1.5 µg	1.5 µg
T4 DNA Ligase (20 units/µl)	5.0 µl	5.0 µl
Water, nuclease-free	to 200 µl	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.
- Chill on ice and heart transform 1-5 µl of the reaction into 50 µl competent cells.
- 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.

5. Proceed with the phage library preparation and panning.