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

pAPD--ck-scFv: Chicken scFv phage display library construction kit

SKU#: APD-06

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-ck-scFv are the phagemid vectors for construction of **chicken** single-chain variable fragment (scFv) library. Here are the key steps involved in construcckting 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 and V_L gene sequences.
- Overlap assembly V_H and V_L to make scFv repertoires.
- Restriction enzyme digestion **pAPD-ck-scFv** vector and scFv repertoires with either SfiI only or SacI and SpeI.
- Ligation of digested and purified repertoires into digested and purified **pAPD-ck-scFv** vector to make chicken 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

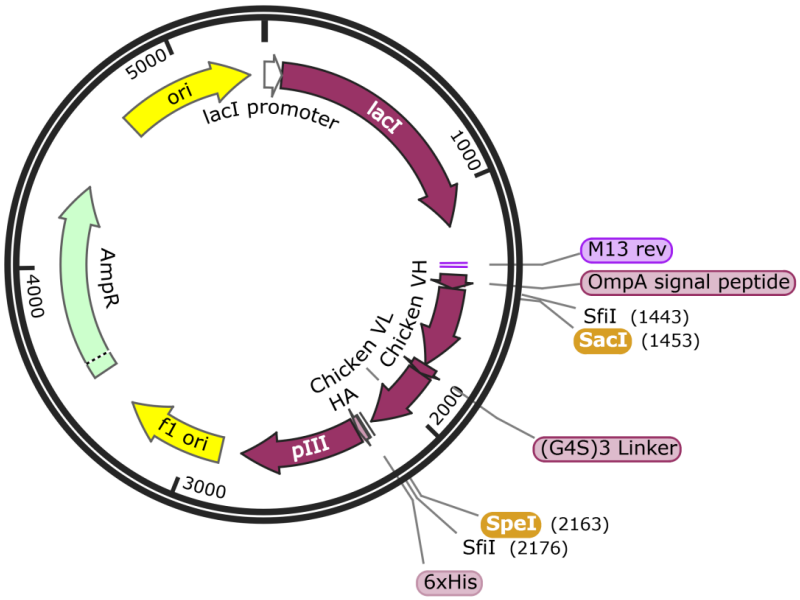
Antibiotic Resistance	Ampicillin (Amp^R)
Constitutive or Inducible System	Inducible for downstream expression
Delivery Type	Transformation
Product Type	Bacterial Expression Vector
Cloning Method	Restriction Enzymes (5'-SfiI and 3'-SfiI or 5'-SacI and 3'-SpeI)

Contents & Storage

Primer for amplification of chicken variable domain of heavy chain (V_H) and light chain (V_L)		
Vial 1	100 µl, 10 µM	Primers (normalized forward and reverse primer mix) for V_H repertoires amplification
Vial 2	100 µl, 10 µM	Primers (normalized forward and reverse primer mix) for V_L repertoires amplification
Primer Set for Cloning/assembly chicken scFv (Format: V_H-$(G_4S)_3$ linker-V_L)		
Vial 3	100 µl, 10 µM	Primers (Forward primer and reverse mix) for chicken scFv repertoires assembly
Sequencing Primer set		
Vial 4	100 µl, 10 µM	M13 Reverse primer for scFv forward sequencing
Vial 5	100 µl, 10 µM	pIII Reverse primer for scFv reverse sequencing
pAPD-ck-scFv cloning vector for phage display chicken scFv library construction		
Vial 6	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



Phagemid vector for chicken scFv library construction
5349 bp

Protocols

1. PCR amplification of the V_H repertoires and V_L repertoires using following condition.

    - Primer mix set and total PCR reaction volume

PCR amplification	Primer mix set	Volume
V_H repertoires	Vial 1	1.0 ml (100 µl /rxn x 10 rxns)
V_L repertoires	Vial 2	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: ~400 bp for VH repertoire and -350 bp for VL 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 chicken 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
Chicken scFv repertoires	V_H repertoires & V_L repertoires	Vial 3	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: ~760-800 bp for chicken 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 chicken scFv repertoire. If yields are too low, repeat the overlapping extension PCR and combine the end product for chicken scFv repertoire.

3. Restriction digestion of scFv repertoires and pAPD-ck-scFv cloning vectors by single enzyme SfiI at 50°C for overnight or 6 h or double enzymes SacI/SpeI at 37°C for overnight or 6 h.

Components	scFv repertoires	pAPD-ck-scFv vectors
DNA	10 µg	10 µg
10X digestion buffer	20 µl	20 µl
SfiI or SacI (20 units/µl)	10 µl	10 µl
SfiI or 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: ~750-780 bp for digested scFv repertoires, and ~4600 bp for digested pAPD-ck-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-ck-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-ck-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.
- 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.

5. Proceed with the phage library preparation and panning.