APD-05 | Mouse scFv Phage Display Library Construction Kit

Fusion BioLabs Short Protocol | pAPD-m-scFv

Product Overview

This Short Protocol describes construction of mouse scFv phage display libraries using the pAPD-m-scFv vector. Two validated restriction-digestion workflows are provided: **Method A (SacI/SpeI)** and **Method B (SfiI)**. All other steps are shared.

Specifications

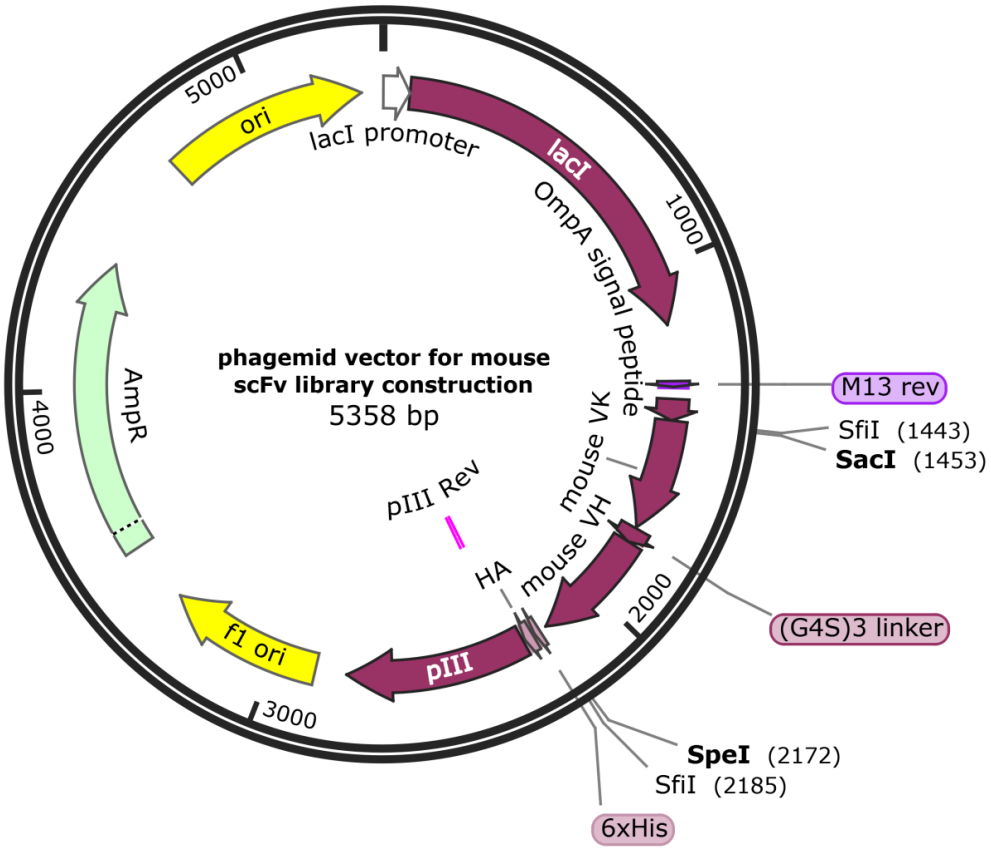
Property	Specification
Catalog number	APD-05
Vector	pAPD-m-scFv (5358 bp)
Cloning	Method A: SacI/SpeI; Method B: SfiI
Antibiotic resistance	Ampicillin (AmpR)
Expression	Inducible for downstream expression

Kit Contents

Vial	Quantity	Component
1	100 µL, 10 µM	Primer mix (F+R) for mouse VL repertoire amplification
2	100 µL, 10 µM	Primer mix (F+R) for mouse VH repertoire amplification
3	100 µL, 10 µM	Assembly primers (F+R) for mouse scFv repertoire construction
4	10.0 µg	pAPD-m-scFv vector in Tris-EDTA buffer
5	100 µL, 10 µM	M13 Reverse forward sequencing primer
6	100 µL, 10 µM	pIII Reverse reverse sequencing primer

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

Vector for Library Construction



Phagemid vector for mouse scFv library construction — 5358 bp

Short Protocol

1. PCR Amplification of Mouse VH and VL Repertoires

Prepare ten 100 µL PCR reactions (1.0 mL total) for each repertoire. Use Vial 1 for VL and Vial 2 for VH.

Component	Per 100 µL reaction
10× PCR buffer, without Mg2+	10 µL
50 mM MgCl2	3 µL
10 mM dNTPs	2 µL
10 µM primer mix (F+R)	4 µL
cDNA template (500–8000 ng/µL)	1 µL
Taq DNA polymerase (10 units/µL)	0.4 µL
Nuclease-free water	79.6 µL

PCR stage	Condition
Initial denaturation	94°C, 2 min
30 cycles	94°C, 30 s; 56°C, 30 s; 72°C, 90 s
Final extension	72°C, 10 min
Hold	4°C

Analyze on a 2% agarose gel and purify the expected 350–400 bp repertoire bands. Typical yield is approximately 3–5 µg per repertoire; repeat PCR and pool products if necessary.

2. Overlap-Extension PCR Assembly

Combine purified VH and VL repertoires (100 ng of each per reaction) using Vial 3 assembly primers. Prepare ten 100 µL reactions (1.0 mL total).

Component	Per 100 µL reaction
10× PCR buffer, without Mg2+	10 µL
50 mM MgCl2	3 µL
10 mM dNTPs	2 µL
10 µM assembly primers	4 µL
VH + VL templates	100 ng each; variable volume
Taq DNA polymerase (10 units/µL)	0.4 µL
Nuclease-free water	To 100 µL

PCR stage	Condition
Initial denaturation	94°C, 2 min
20 cycles	94°C, 30 s; 56°C, 30 s; 72°C, 2 min
Final extension	72°C, 10 min
Hold	4°C

Analyze on a 2% agarose gel and purify the expected 750–800 bp scFv repertoire. It anticipates approximately 10–15 µg of scFv repertoire DNA; repeat overlap-extension PCR if needed.

3. Restriction Digestion — Select One Method

Perform **separate 200 µL reactions** for the purified scFv repertoire and the pAPD-m-scFv vector. Use the same universal 10× digestion buffer for either method.

Method A — SacI/SpeI

Component	scFv repertoire	pAPD-m-scFv vector
DNA	10 µg	10 µg
Universal 10× digestion buffer	20 µL	20 µL
SacI (20 units/µL)	10 µL	10 µL
SpeI (20 units/µL)	10 µL	10 µL
Nuclease-free water	To 200 µL	To 200 µL

Incubate at 37°C for 6 hours or overnight.

Method B — SfiI

Component	scFv repertoire	pAPD-m-scFv vector
DNA	10 µg	10 µg
Universal 10× digestion buffer	20 µL	20 µL
SfiI (20 units/µL)	10 µL	10 µL
Nuclease-free water	To 200 µL	To 200 µL

Incubate at 50°C for 6 hours or overnight. Each digestion contains 200 units of SfiI.

Shared Digestion Cleanup

Following either method, add gel loading buffer and analyze the digested products on a 1% agarose gel. Excise and purify the expected approximately 750 bp scFv insert and approximately 4600 bp vector backbone. Quantify purified DNA. It anticipates approximately 1–4 µg of each purified product.

4. Library Ligation and Transformation

Component	Per 200 µL ligation
10× T4 DNA ligase buffer	20 µL
Digested pAPD-m-scFv vector	1.9 µg
Digested scFv repertoire	1.5 µg
T4 DNA ligase (20 units/µL)	5 µL
Nuclease-free water	To 200 µL

Gently mix and briefly centrifuge. Incubate overnight at room temperature, then heat-inactivate at 65°C for 10 minutes. Purify the ligation products and dissolve in 50 µL nuclease-free water.

Transform 2 µL purified ligation into 25 µL TG1 electrocompetent cells. Use a 0.1 cm cuvette and electroporate at 1.8 kV, 10 µF, and 600 ohms. A typical time constant is 3.5–4.5 ms.

5. Phage Library Preparation and Panning

Proceed with the established phage library preparation and panning workflow.

Documentation Notes

Method A is the SacI/SpeI workflow. Method B is the SfiI workflow. Both use identical DNA inputs, universal digestion buffer, and total reaction volume. All downstream steps are shared.

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