

Supplementary Information

**Ecobody technology: rapid monoclonal antibody screening method
from single B cells using cell-free protein synthesis for
antigen-binding fragment formation**

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Supplementary Table S1. Recovery ratio of *E. coli* expressed proteins.

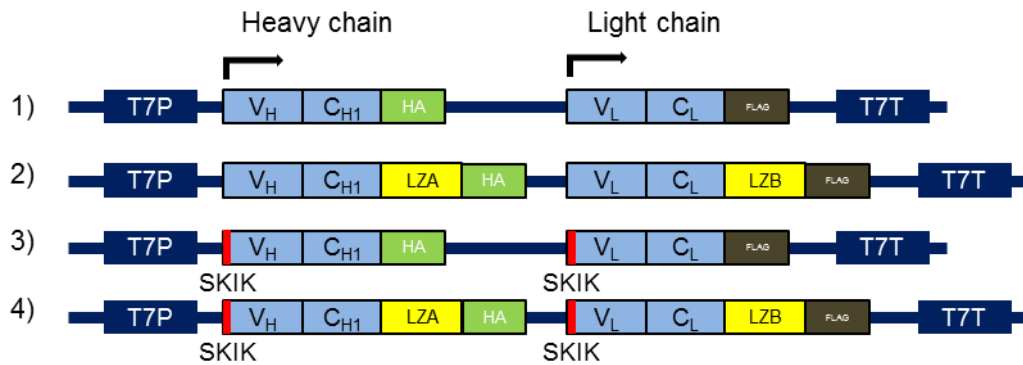
Step	Protein concentration (mg/mL)	Total volume (mL)	Amount of protein (mg)	Recovery ratio (%)
Solubilization by 6 M				
GuHCl	1.62	0.95	1.54	
Refolding	0.98	1.3	1.28	82.8
His tag purification	0.094	4.5	0.423	27.4

Inclusion bodies produced in *E. coli* SHuffle T7 Express in 50 mL of LB medium supplemented with ampicillin were used. The solubilized amount of protein is regarded as 100% in recovery ratio.

Supplementary Table 2. Primers used in this study.

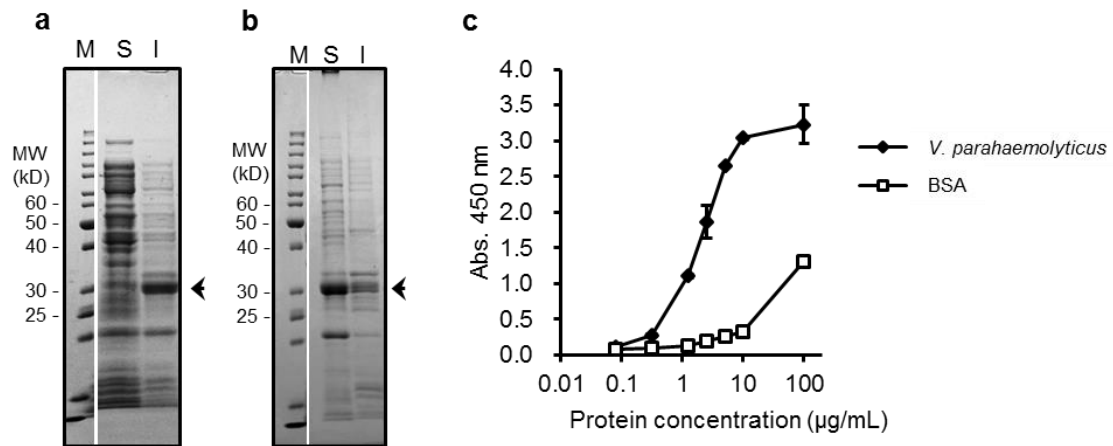
Description	Target gene	Sequence (5'-3')
Reverse transcription	Lc	ACAGTCACCCCTATTGAAGCT
		GCAGTCACCCCTGTTGAAGCT
		ACAGTCTCCCCTATTGAAGCT
	Hc (IgG)	CCATGTGAACTGCACCTCGG
Hc (IgM)	GGGAAAGGACACTCGCA	
First PCR	Lc	ATGGACACGAGGGCCCCAC
		ATGGACATGAGGGCCCCAC
		ATGGACACGAGGGCACCCGC
		GTGCCCTGGGTCACCTTGCAG
		GTGTCCTGGGTCACCTTGCAGGT
		CCCTCGTGCCTGACCTGGCA
	Hc (both IgG and IgM)	ATGGAGACTGGGCTGCGCTGG
Hc (IgG)	TGACCTCGGGGTGCGTGAGAT	
Hc (IgM)	GGTACTCCTCTGTGCCCTGGAGGA	
Second PCR	Lc	CATATGTCTAAAATAAAAAGCSCTTGTGATGACCCAGACTC
		CATATGTCTAAAATAAAAAGCAGCCGTGCTGCTGACCCAGA
		CATATGTCTAAAATAAAAAGACCCTRTGCTGACCCAGACTC
		CATATGTCTAAAATAAAAAGCTCAAGTGCTGACCCAGACTC
		CATATGTCTAAAATAAAAAGCTGACATTGTGATGACCCAGA
		CATATGTCTAAAATAAAAAGCATTTCGAATTGACCCAGACTC
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		CATATGTCTAAAATAAAAAGCCCAAGTGCTGACCCAGACTC
		CATATGTCTAAAATAAAAAGCCGAAGTAGTGATGACCCAGA
		CATATGTCTAAAATAAAAAGCCAACATCGTGCTGACCCGTA
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Hc (both IgG and IgM)	CATATGTCTAAAATAAAAACAGCAGCTGGAGCAGTCCG	
CATATGTCTAAAATAAAAACAGTCGGTGAAGGAGTCCG		
CATATGTCTAAAATAAAAACAGTCGTTGGAGGAGTCCG		

		CATATGTCTAAAATAAAAAACAGTCGCTGGAGGAGTCCG CATATGTCTAAAATAAAAAACAGGAGCAGCTGAAGGAG CATATGTCTAAAATAAAAAACAGTCGGTGAAGGACTCCG CATATGTCTAAAATAAAAAACAGGAGCAGTTGAAGGAGTC CATATGTCTAAAATAAAAAACAGTCGGTGGAGGAGTCC CATATGTCTAAAATAAAAAACAGTCCTTGGAGGAGTCC CATATGTCTAAAATAAAAAACAGGAGCAGCTGGAGGAG CATATGTCTAAAATAAAAAACAGCCGTTGGAGGAGTCC CATATGTCTAAAATAAAAAACAGTCGTCGGAGGAGTCC CATATGTCTAAAATAAAAAACAGGCGTTGGAGGAGTCC CATATGTCTAAAATAAAAAACAGCAGCAGCTGATGGAG CATATGTCTAAAATAAAAAACAGCAGCAGCTGGTGGAG CATATGTCTAAAATAAAAAACAGGAGCAGCTGGTGCAG ACCACAACACGGTGCCAGTGGGAAGACTGATGGAGCCTT ACCACAACACGGTGCCAGTGGGAAGACTGACGGAGCCTT GCTAACCAGCGGATACAGAGTTGGAGATGACAGGCTCAC
Amplification of pRSET-based vector fragment for Gibson assembly	Hc (IgG)	
	Hc (IgM)	
	Lc	TGTGTTGCCAATAAATACT TTTTATTTTAGACATATGTATATCTCCTTCTTAAAGTTAAACAAAAT
	Hc (IgG)	CTGGCACCGTGTTGTGGTG TTTTATTTTAGACATATGTATATCTCCTTCTTAAAGTTAAACAAAAT
Construction of expression plasmid of clone 22G	Hc (IgM)	CTGTATCCGCTGGTTAGCTGT TTTTATTTTAGACATATGTATATCTCCTTCTTAAAGTTAAACAAAAT
	Hc	GATCCGGCTGCTAACAAAGCCC ATGATATCTCCTTCTAGATTATTAAGCGTAATCTGGAAC
	Lc	TCTAGAAGGAGATATCATATGTCTAAAATAAAAGATGTTG GGGCTTTGTTAGCAGCCGGATC
	pET22b-vector	GATCCGGCTGCTAACAAAGCCC TTTTATTTTAGACATATGTATATCTCCTTCTTAAAG ATCTCGATCCCGCAAATTAATACG TCCGGATATAGTTCCTCCTTCAG
Amplification of templates for CFPS, F1 R1		



Supplementary Fig. S1. DNA templates used for CFPS.

Four variations of rabbit anti-*Listeria monocytogenes* monoclonal antibody genes used to assess the effect of leucine zipper fusion and an N-terminal SKIK peptide tag in CFPS. Arrows indicate start positions for open reading frames of heavy and light chains. T7P: T7 promoter, HA: HA tag, FLAG: FLAG tag, T7T: T7 terminator, LZA: leucine zipper A, LZB: leucine zipper B, SKIK: SKIK tag.



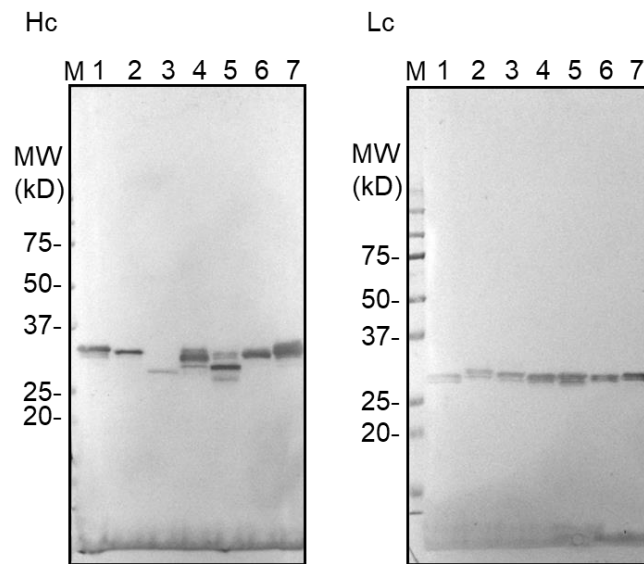
Supplementary Fig. S2. Analyses of the *E. coli*-expressed and refolded proteins.

Anti- *V. parahaemolyticus* clone 22G was expressed in *E. coli* as Zipbody with the SKIK tag. M, size markers; Arrow, expected size of overlapping Hc and Lc bands of Zipbody mAb.

a) CBB staining of the soluble (S) and insoluble (I) fraction expressed in *E. coli* SHuffle T7 Express on SDS-PAGE.

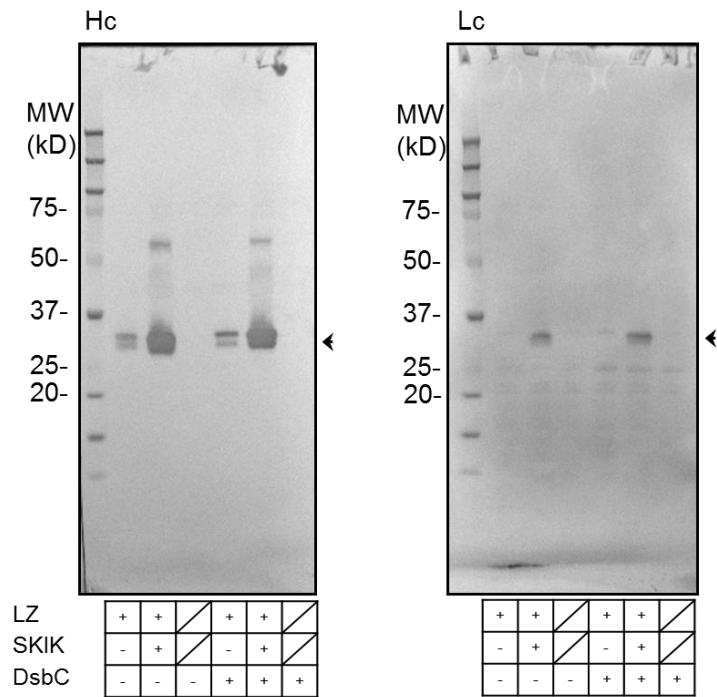
b) CBB staining of the soluble (S) and insoluble (I) fractions after refolding.

c) ELISA results for the refolded Zipbody mAb. The solubilized fraction after refolding was analysed by ELISA at various concentrations with *V. parahaemolyticus* and BSA as the antigens.



Supplementary Fig. S3. Full western blotting images of 1 μ L of anti-*V. parahaemolyticus* Zipbody proteins produced in CFPS.

Hc and Lc were visualised with anti-HA tag-HRP and anti-His tag-HRP conjugated antibodies, respectively. Sample numbers 1-7 correspond to clones 3G, 5M, 7M, 20G, 22G, 30M, and 36G. The left-hand lane in each image indicates molecular size markers.



Supplementary Fig. S4. Full western blotting images of Figure 2b.

Arrows indicate the target antibody fragments used for Image J analysis. The left-hand lane in each image indicates molecular size markers.

Supplementary Information for DNA sequences.

End points in the linearised vector are indicated with red slant lines.

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