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	ATGGACACGAGGGCCCCCAC
		ATGGACATGAGGGCCCCCAC
		ATGGACACGAGGGCACCCGC
		GTGCCCTGGGTCACCTTGCAG
		GTGTCCTGGGTCACCTTGCAGGT
		CCCTCGTGCGTGACCTGGCA
	Hc (both IgG and IgM)	ATGGAGACTGGGCTGCGCTGG
	Hc (IgG)	TGACCTCGGGGGTGCGTGAGAT
	Hc (IgM)	GGTACTCCTCTGTGCCCTGGAGGA
Second PCR	Lc	CATATGTCTAAAATAAAAGCSCTTGTGATGACCCAGACTC
		CATATGTCTAAAATAAAAGCAGCCGTGCTGCTGACCCAGA
		CATATGTCTAAAATAAAAGACCCTRTGCTGACCCAGACTC
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	Hc (both IgG and IgM)	CATATGTCTAAAATAAAACAGCAGCTGGAGCAGTCCG
		CATATGTCTAAAATAAAACAGTCGGTGAAGGAGTCCG
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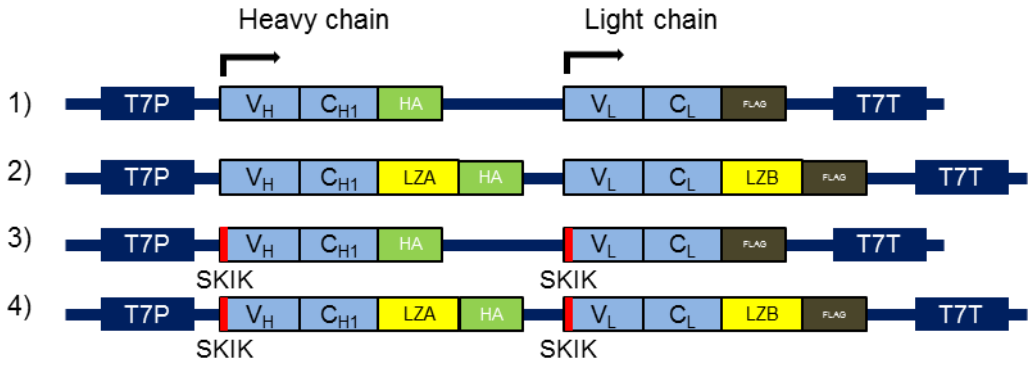
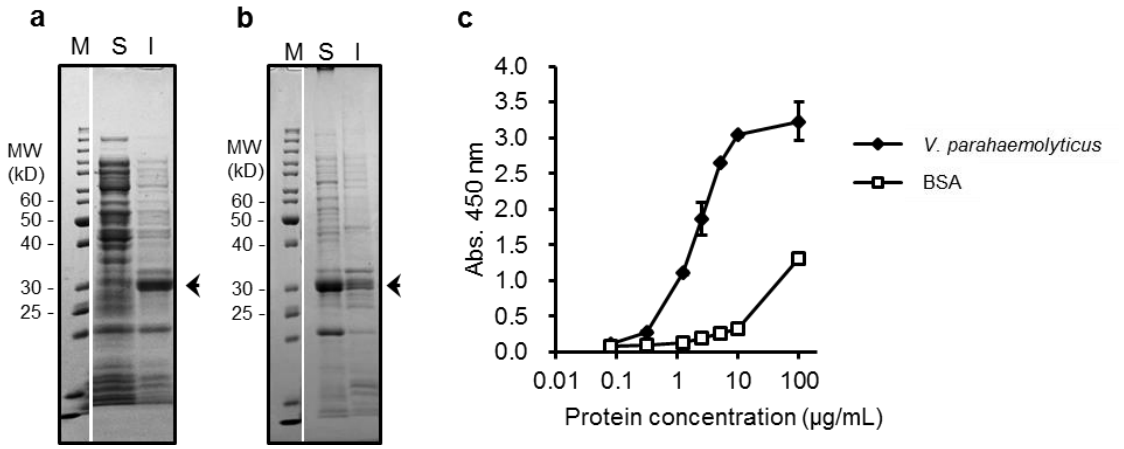
	Hc (IgG)	ACCACAACACGGTGCCAGTGGGAAGACTGATGGAGCCTT
		ACCACAACACGGTGCCAGTGGGAAGACTGACGGAGCCTT
	Hc (IgM)	GCTAACCAGCGGATACAGAGTTGGAGATGACAGGCTCAC
Amplification of pRSET-based vector fragment for Gibson assembly	Lc	TGTGTTGCCAATAAATACT
		TTTTATTTTAGACATATGTATATCTCCTTCTTAAAGTTAAACAAAAT
	Hc (IgG)	CTGGCACCGTGTTGTGGTG
		TTTTATTTTAGACATATGTATATCTCCTTCTTAAAGTTAAACAAAAT
	Hc (IgM)	CTGTATCCGCTGGTTAGCTGT
		TTTTATTTTAGACATATGTATATCTCCTTCTTAAAGTTAAACAAAAT
Construction of expression plasmid of clone 22G	Hc	GATCCGGCTGCTAACAAAGCCC
		ATGATATCTCCTTCTAGATTATTAAGCGTAATCTGGAAC
	Lc	TCTAGAAGGAGATATCATATGTCTAAAATAAAAGATGTTG
		GGGCTTTGTTAGCAGCCGGATC
	pET22b-vector	GATCCGGCTGCTAACAAAGCCC
		TTTTATTTTAGACATATGTATATCTCCTTCTTAAAG
Amplification of templates for CFPS, F1		ATCTCGATCCCGCGAAATTAATACG
R1		TCCGGATATAGTTCCTCCTTTCAG



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.



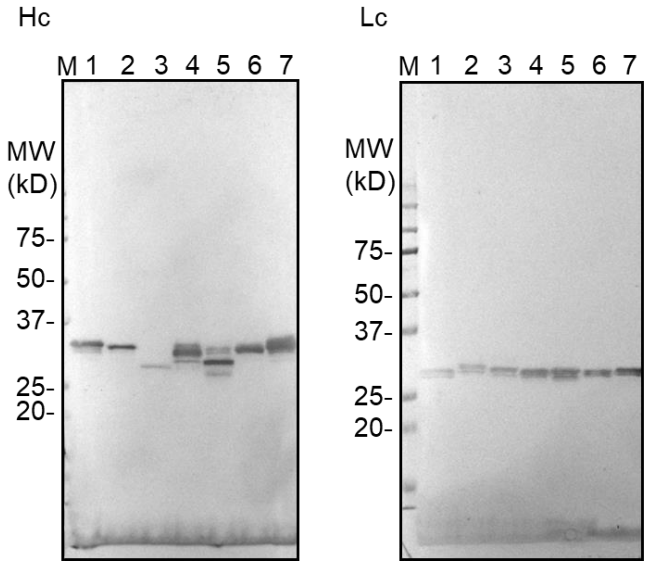
Supplementary Fig. S2. Analyses of the *E. coli*-expressed and refolded proteins.

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.

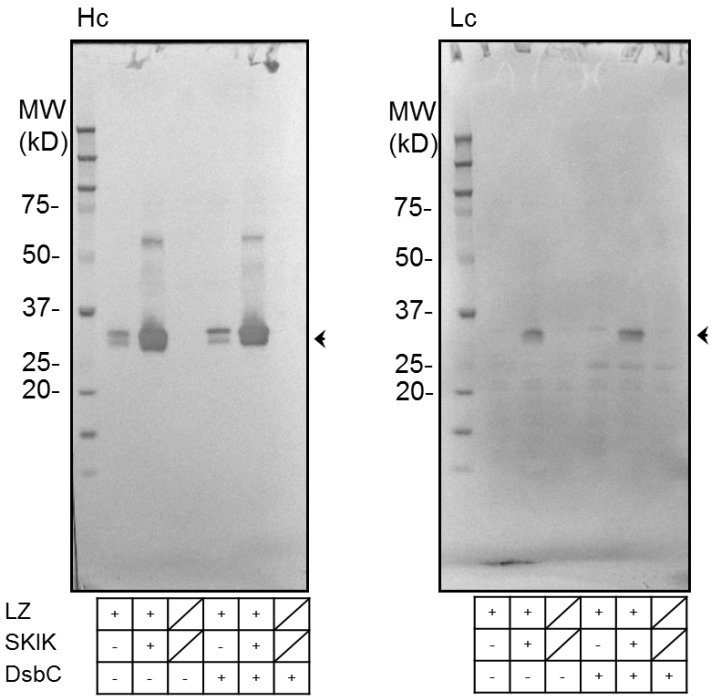
b) CBB staining of the soluble (S) and insoluble (I) fractions after refolding.

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 μL 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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