

Supplementary information

Effective high-throughput isolation of fully human antibodies targeting infectious pathogens

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Supplementary Tables

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| Supplementary Table 1 | Composition of primer mixes used for multiplex single cell PCR |
| Supplementary Table 2 | Composition of primer mixes used for multiplex cloning PCR |
| Supplementary Table 3 | Overview of primers and primer mixes used for single cell PCR |
| Supplementary Table 4 | Overview of primers and primer mixes used for cloning PCR |
| Supplementary Table 5 | Overview of primers used for colony PCR |
| Supplementary Table 6 | Sequences of optimized forward primers (openPrimeR) for multiplex single cell PCR |
| Supplementary Table 7 | Sequences of primers used for heavy and light chain amplification in single cell and colony PCRs |
| Supplementary Table 8 | Sequences of optimized forward primers for multiplex cloning PCR |
| Supplementary Table 9 | Sequences of reverse primers for multiplex cloning PCR |

Supplementary Table 1: Composition of primer mixes used for multiplex single cell PCR

Name of mix	oPR-IGHV	oPR-IGKV	oPR-IGLV	oPR_1st_fwd	oPR_1st_IgG_rev	oPR_1st_IgM_rev
Composition	oPR-IGHV-1_fw	oPR-IGKV-1_fw	oPR-IGLV-1_fw	oPR-IGHV	Cg RT	Cm-RT
	oPR-IGHV-2_fw	oPR-IGKV-2_fw	oPR-IGLV-2_fw	oPR-IGKV	3' Ck 543	3' Ck 543
	oPR-IGHV-3_fw	oPR-IGKV-3_fw	oPR-IGLV-3_fw	oPR-IGLV	3' Cλ	3' Cλ
	oPR-IGHV-4_fw	oPR-IGKV-4_fw	oPR-IGLV-4_fw			
	oPR-IGHV-5_fw	oPR-IGKV-5_fw	oPR-IGLV-5_fw			
	oPR-IGHV-6_fw	oPR-IGKV-6_fw	oPR-IGLV-6_fw			
	oPR-IGHV-7_fw	oPR-IGKV-7_fw	oPR-IGLV-7_fw			
	oPR-IGHV-8_fw	oPR-IGKV-8_fw	oPR-IGLV-8_fw			
	oPR-IGHV-9_fw		oPR-IGLV-9_fw			
	oPR-IGHV-10_fw		oPR-IGLV-10_fw			
	oPR-IGHV-11_fw		oPR-IGLV-11_fw			
	oPR-IGHV-12_fw		oPR-IGLV-12_fw			
	oPR-IGHV-13_fw		oPR-IGLV-13_fw			
	oPR-IGHV-14_fw		oPR-IGLV-14_fw			
	oPR-IGHV-15_fw		oPR-IGLV-15_fw			

Supplementary Table 2: Composition of primer mixes used for multiplex cloning PCR

Name of mix	SLIC_oPR_IGHV	SLIC_oPR_IGKV	SLIC_oPR_IGLV	SLIC_oPR_IgM_HC_rev
Composition	SLIC_oPR_IGHV_1_fw	SLIC_oPR_IGKV_1_fw	SLIC_oPR_IGLV_1_fw	SLIC_oPR_IGMH_1_rev
	SLIC_oPR_IGHV_2_fw	SLIC_oPR_IGKV_2_fw	SLIC_oPR_IGLV_2_fw	SLIC_oPR_IGMH_2_rev
	SLIC_oPR_IGHV_3_fw	SLIC_oPR_IGKV_3_fw	SLIC_oPR_IGLV_3_fw	SLIC_oPR_IGMH_3_rev
	SLIC_oPR_IGHV_4_fw	SLIC_oPR_IGKV_4_fw	SLIC_oPR_IGLV_4_fw	
	SLIC_oPR_IGHV_5_fw	SLIC_oPR_IGKV_5_fw	SLIC_oPR_IGLV_5_fw	
	SLIC_oPR_IGHV_6_fw	SLIC_oPR_IGKV_6_fw	SLIC_oPR_IGLV_6_fw	
	SLIC_oPR_IGHV_7_fw	SLIC_oPR_IGKV_7_fw	SLIC_oPR_IGLV_7_fw	
	SLIC_oPR_IGHV_8_fw	SLIC_oPR_IGKV_8_fw	SLIC_oPR_IGLV_8_fw	
	SLIC_oPR_IGHV_9_fw		SLIC_oPR_IGLV_9_fw	
	SLIC_oPR_IGHV_10_fw		SLIC_oPR_IGLV_10_fw	
	SLIC_oPR_IGHV_11_fw		SLIC_oPR_IGLV_11_fw	
	SLIC_oPR_IGHV_12_fw		SLIC_oPR_IGLV_12_fw	
	SLIC_oPR_IGHV_13_fw		SLIC_oPR_IGLV_13_fw	
	SLIC_oPR_IGHV_14_fw		SLIC_oPR_IGLV_14_fw	
	SLIC_oPR_IGHV_15_fw		SLIC_oPR_IGLV_15_fw	

Supplementary Table 3: Overview of primers and primer mixes used for single cell PCR

PCR Step	1 st single cell PCR		2 nd single cell PCR			
Amplified Chain	IgG heavy/kappa/lambda chain	IgM heavy/kappa/lambda chain	IgG heavy chain	IgM heavy chain	Kappa chain	Lambda chain
Forward primer(s)	oPR_1st_fwd	oPR_1st_fwd	oPR-IGHV	oPR-IGHV	oPR-IGKV	oPR-IGLV
Reverse primer(s)	oPR_1st_IgG_rev	oPR_1st_IgM_rev	3' IgG (internal)	Cm-1st	3' Ck 494	3' Xhol Cλ

Supplementary Table 4: Overview of primers and primer mixes used for cloning PCR

PCR	Cloning PCR			
Amplified Chain	IgG Heavy chain	IgM Heavy chain	Kappa chain	Lambda chain
Forward primer(s)	SLIC_oPR_IGHV	SLIC_oPR_IGHV	SLIC_oPR_IGKV	SLIC_oPR_IGLV
Reverse primer(s)	SLIC_oPR_IgG_HC_rev	SLIC_oPR_IgM_HC_rev	SLIC_oPR_IGKV_rev	SLIC_oPR_IGLV_rev

Supplementary Table 5: Overview of primers used for colony PCR

PCR	Colony PCR		
Amplified Chain	Heavy chain	Kappa chain	Lambda chain
Forward primer	5' Ab-sense		
Reverse primer(s)	3' IgG (internal)	3' Ck 543	3' CA

Supplementary Table 6: Sequences of optimized forward primers (openPrimeR) for multiplex single cell PCR

openPrimeR SLIC IGHV set (oPR-IGHV)		
Name	Sequence	Reference
oPR-IGHV-1_fw	ATGGACTGGACCTGGAGCATCC	openPrimeR ⁴⁷
oPR-IGHV-2_fw	ATGGACTGGACCTGGAGGATCCTC	openPrimeR ⁴⁷
oPR-IGHV-3_fw	ATGGACTGGACCTGGAGGGTCTTC	openPrimeR ⁴⁷
oPR-IGHV-4_fw	ATGGACTGGATTGGAGGGTCTCTTC	openPrimeR ⁴⁷
oPR-IGHV-5_fw	ATGGACACACTTGCTACACACTCCTGC	openPrimeR ⁴⁷
oPR-IGHV-6_fw	ACTTGCTCCACGCTCCTGC	openPrimeR ⁴⁷
oPR-IGHV-7_fw	GGCTGAGCTGGGTTTCCTTGTGTTG	openPrimeR ⁴⁷
oPR-IGHV-8_fw	GGCTCCGCTGGGTTTCCTTGTGTTG	openPrimeR ⁴⁷
oPR-IGHV-9_fw	CACCTGTGGTTCTCCTCTGCTG	openPrimeR ⁴⁷
oPR-IGHV-10_fw	ATGAAACACCTGTGGTTCTCCTCCTCC	openPrimeR ⁴⁷
oPR-IGHV-11_fw	ACATCTGGTTCTCCTCTGGTG	openPrimeR ⁴⁷
oPR-IGHV-12_fw	GCCTCTCCACTTAAACCCAGGCTC	openPrimeR ⁴⁷
oPR-IGHV-13_fw	ATGTCTGTCTCTTCTCATCTTCTGC	openPrimeR ⁴⁷
oPR-IGHV-14_fw	ATGGAGTTGGGGCTGAGCTGG	openPrimeR ⁴⁷
oPR-IGHV-15_fw	ATGGGGTCAACCGCCATCCTC	openPrimeR ⁴⁷
openPrimeR IGKV set (oPR-IGKV)		
Name	Sequence	Reference
oPR-IGKV-1_fw	ATGAGGCTCCTGCTCAGCTCTGG	openPrimeR ⁴⁷
oPR-IGKV-2_fw	ATGGAAGCCCCAGCTCAGCTTC	openPrimeR ⁴⁷
oPR-IGKV-3_fw	CCCAGCTCAGCTCTTCTCCTCG	openPrimeR ⁴⁷
oPR-IGKV-4_fw	TGGTGTTCAGACCCAGGCTTCATTTC	openPrimeR ⁴⁷
oPR-IGKV-5_fw	GTCAGGTTCACCTCCTCAGCTTC	openPrimeR ⁴⁷
oPR-IGKV-6_fw	GCCATCACAACTCATGGGTTCTGCTG	openPrimeR ⁴⁷
oPR-IGKV-7_fw	TCCCTGCTCAGCTCTGGG	openPrimeR ⁴⁷
oPR-IGKV-8_fw	CCTGGGACTCTGCTGCTCTG	openPrimeR ⁴⁷
openPrimeR IGLV set (oPR-IGLV)		
Name	Sequence	Reference
oPR-IGLV-1_fw	CCCTGGGTATGCTCCTCTGAAATC	openPrimeR ⁴⁷
oPR-IGLV-2_fw	CTCTGCTGCTCTCACTCTCTCAC	openPrimeR ⁴⁷
oPR-IGLV-3_fw	ATGGCATGGATCCCTCTCTCG	openPrimeR ⁴⁷
oPR-IGLV-4_fw	CCTCTGGCTCACTCTCTCACTC	openPrimeR ⁴⁷
oPR-IGLV-5_fw	ACACTCTGCTCCACTCTAAC	openPrimeR ⁴⁷
oPR-IGLV-6_fw	ATGGCCTGGATCCCTACTTCTCC	openPrimeR ⁴⁷
oPR-IGLV-7_fw	ATGGCCTGGGCTCTCTTCTACC	openPrimeR ⁴⁷
oPR-IGLV-8_fw	ATGGCCTGGACTCCTCTTCTGTTC	openPrimeR ⁴⁷
oPR-IGLV-9_fw	ATGGCCTGGATGATGCTCTCCTC	openPrimeR ⁴⁷
oPR-IGLV-10_fw	GTCCTCTCTCTCCACCCCTCATC	openPrimeR ⁴⁷
oPR-IGLV-11_fw	CTCTCGCTCACTGCACAGG	openPrimeR ⁴⁷
oPR-IGLV-12_fw	CCTCTCTCTCACCCCTCTC	openPrimeR ⁴⁷
oPR-IGLV-13_fw	CTCTCTCACCCCTCTCACTC	openPrimeR ⁴⁷
oPR-IGLV-14_fw	ATGGCCTGGACCCCTCTCC	openPrimeR ⁴⁷
oPR-IGLV-15_fw	ATGGCCTGGACCCCACTCC	openPrimeR ⁴⁷

Supplementary Table 7: Sequences of primers used for heavy and light chain amplification in single cell and colony PCRs

Primers for Single Cell and Colony PCRs		
Name	Sequence	Reference
5' Ab-sense	GCTTCGTTAGAACGCGGGCTAC	Tiller ⁴²
Cm-RT	ATGGAGTCGGGAAGGAAGTC	Ozawa ⁸⁰
Cm-1st	CCGACGGGGATTCTCACAG	Ozawa ⁸⁰
Cg-RT	AGGTGTGCACGCCGCTGGTC	Ozawa ⁸⁰
3' IgG (internal)	GTTCGGGGAAAGTAGTCCTTGAC	Tiller ⁴²
3' Ck 543	GTTCCTCGTAGCTGCTTGCTCA	Tiller ⁴²
3' Ck 494	GTGCTGTCTTGCTGTCCCTGCT	Tiller ⁴²
3' Cλ	CACCAGTGTGCCCTGTTGGCTTG	Tiller ⁴²
3' Xhol Cλ	CTCCTCACTCGAGGGYGGAACAGAGTG	Tiller ⁴²

⁴²Tiller, T. et al. Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning. *J. Immunol. Methods* 329, 112–124 (2008).

⁸⁰Ozawa, T., Kishi, H. & Muraguchi, A. Amplification and analysis of cDNA generated from a single cell by 5'-RACE: application to isolation of antibody heavy and light chain variable gene sequences from single B cells. *Biotechniques* 40, 469–470, 472, 474 passim (2006).

Supplementary Table 8: Sequences of optimized forward primers for multiplex cloning PCR

openPrimeR SLIC IGHV set (SLIC_oPR_IGHV)	
Name	Sequence
SLIC_oPR_IGHV_1_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGGACTGGACCTGGAGGACATCC
SLIC_oPR_IGHV_2_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGGACTGGACCTGGAGGATCCTC
SLIC_oPR_IGHV_3_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGGACTGGACCTGGAGGGTCTTC
SLIC_oPR_IGHV_4_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGGACTGGGATTTGGAGGGTCTTC
SLIC_oPR_IGHV_5_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGGACACACTTGTACACACTCCTGC
SLIC_oPR_IGHV_6_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGGACACACTTGTCCACGCTCCTGC
SLIC_oPR_IGHV_7_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGGAGTTGGGCTGAGCTGGGTTTCCCTGTTG
SLIC_oPR_IGHV_8_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGGAACTGGGGCTCCGCTGGGTTTCCCTGTTG
SLIC_oPR_IGHV_9_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGAAACACCTGTGGTTCTCCTCCTGCTG
SLIC_oPR_IGHV_10_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGAAACACCTGTGGTTCTCCTCCTCC
SLIC_oPR_IGHV_11_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGAAACATGTGGTTCTCCTCCTG
SLIC_oPR_IGHV_12_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGCAAGTGAGGGCCTCTCACTAAACCCAGGCTC
SLIC_oPR_IGHV_13_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGTCGTCTCCTCATCTCCTGC
SLIC_oPR_IGHV_14_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGGAGTTGGGGCTGAGCTGG
SLIC_oPR_IGHV_15_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGGGGTCAACGCCATCCT
openPrimeR SLIC IGKV set (SLIC_oPR_IGKV)	
Name	Sequence
SLIC_oPR_IGKV_1_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGAGGCTCTTGCTCAGCTCTGG
SLIC_oPR_IGKV_2_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGGAAGCCCCAGCTCAGCTTC
SLIC_oPR_IGKV_3_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGGAAACCCCCAGCTCACCTCTCCCTCCTG
SLIC_oPR_IGKV_4_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGGTGTGAGACCCAGGTCTCATTTC
SLIC_oPR_IGKV_5_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGGGGTCCCAGGTTACCTCCTCAGCTTC
SLIC_oPR_IGKV_6_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGTTGCCATACAACATGGGTTCTGCTG
SLIC_oPR_IGKV_7_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGAGGCTCCCTGCTCAGCTCCTGGG
SLIC_oPR_IGKV_8_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGAGGGTCCCCGTCAGCTCCTGGGACTCCTGCTGCTG
openPrimeR SLIC IGLV set (SLIC_oPR_IGLV)	
Name	Sequence
SLIC_oPR_IGLV_1_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGCCCTGGGTATGCTCCTCCTGAAATC
SLIC_oPR_IGLV_2_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGCCCTGGGTCTGCTCCTCAGCTCCTCAC
SLIC_oPR_IGLV_3_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGGCATGGATCCCTCTCCCTCG
SLIC_oPR_IGLV_4_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGCCCTGGACCCCTCTGGCTCAGCTCCTCACTC
SLIC_oPR_IGLV_5_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGGCATGGCCACACTCCTGCTCCCAGCTCCTAAC
SLIC_oPR_IGLV_6_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGCCCTGGATCCCTACTTCTCC
SLIC_oPR_IGLV_7_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGCCCTGGGTCTCCTTCTACC
SLIC_oPR_IGLV_8_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGCCCTGGACTCCTCTTTCTGTT
SLIC_oPR_IGLV_9_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGCCCTGGATGATGCTTCTCCTC
SLIC_oPR_IGLV_10_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGCCCTGGTCCCCCTCTCCCTCACCCCTCATC
SLIC_oPR_IGLV_11_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGCCCTGGTCTCCCTCCTCCACTCTCCTGCTCAGTCACAGG
SLIC_oPR_IGLV_12_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGCCCGGCTCCCTCTCCCTCACCCCTCCTC
SLIC_oPR_IGLV_13_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGCCAGCTTCCCTCTCCCTCACCCCTCCTCACTC
SLIC_oPR_IGLV_14_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGCCCTGGACCCCTCTCC
SLIC_oPR_IGLV_15_fw	AACTGCACCTCGGTTCTATCGATTGAATTCATGCCCTGGACCCACTCC

Supplementary Table 9: Sequences of reverse primers for multiplex cloning PCR**openPrimeR SLIC reverse primers**

Name	Sequence
SLIC_oPR_IGGH_rev	GGGTGCCAGGGGGAAAGACCGATGGGCCCTTGGTCGAGGC
SLIC_oPR_IGMH_1_rev	GGGGAAGACCGATGGGCCCTTGGTCGAGGCTGAGGAGACGGTGACCAGGGT
SLIC_oPR_IGMH_2_rev	GGGGAAGACCGATGGGCCCTTGGTCGAGGCTGAGGAGACGGTGACCATTGT
SLIC_oPR_IGMH_3_rev	GGGGAAGACCGATGGGCCCTTGGTCGAGGCTGAGGAGACGGTGACCGTGGT
SLIC_oPR_IGKV_rev	CTCATCAGATGGCGGGAAAGATGAAGACAGATGGTGCAGCCACCGTACG
SLIC_oPR_IGLV_rev	GAAGCTCCTCACTCGAGGGYGGAACAGACTG