Electronic Supplementary Material (ESI) for Analyst.

Electronic Supporting Information

PD-L1 aptamer isolation via Modular-SELEX and its applications in cancer cell detection and tumor tissue section imaging

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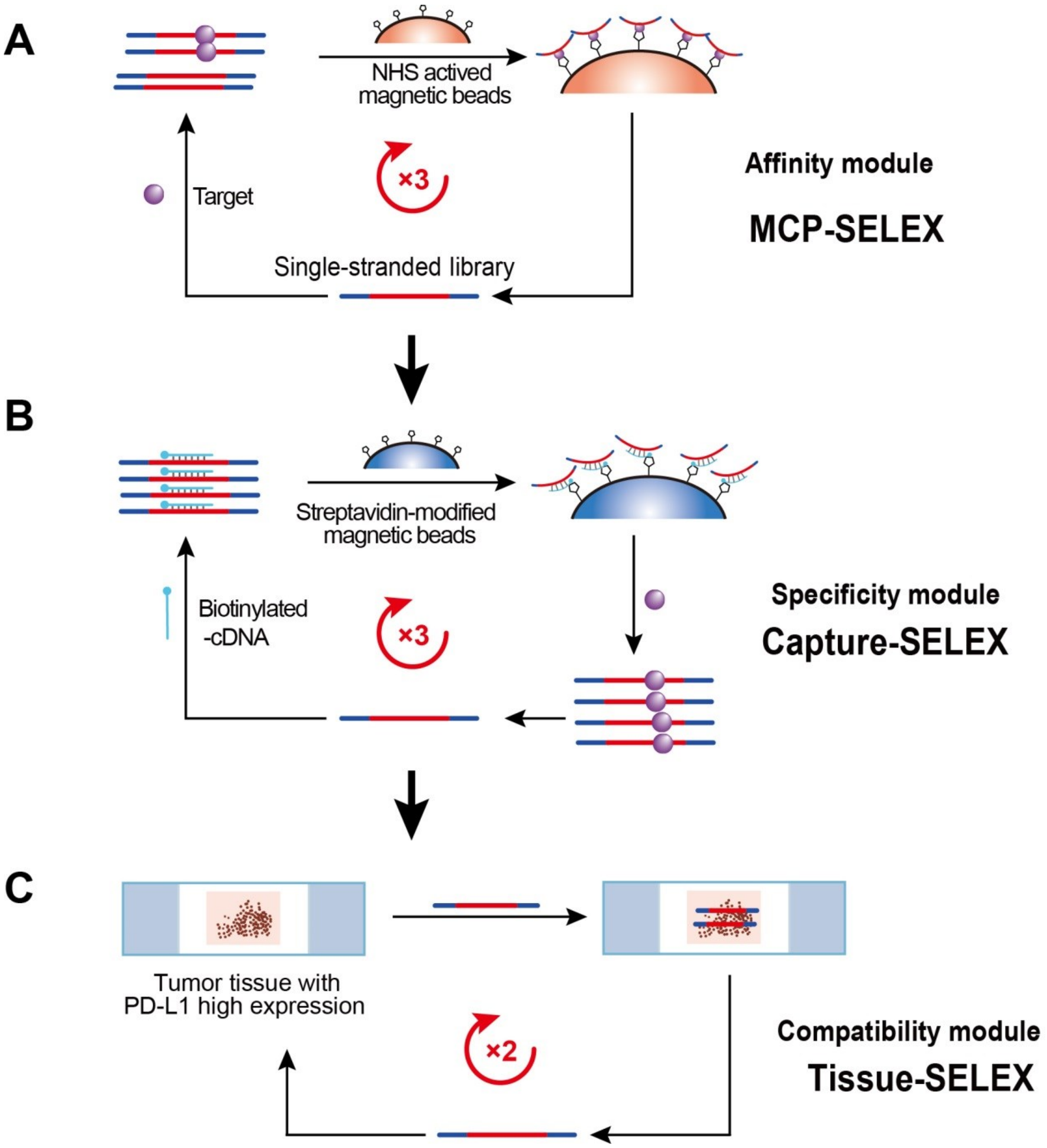

Figure S1. An overview of Modular-SELEX for PD-L1 aptamer isolation, including three sequential modules: (A) affinity module: three rounds of MCP-SELEX; (B) specificity module: three rounds of Capture-SELEX; and compatibility module: two rounds of Tissue-SELEX.

Table S1. DNAs used in this study

Name	Sequences (5’- 3’)	Description
Pool$_0$	TCTCAGCTCTAGTGGTGACG-N$_{20}$-ACGAGACGAGCTTATGCGTA-N$_{20}$-ACTCTGTCCTGAACCTGTTG	Initial random library
FP	TCTCAGCTCTAGTGGTGACG	Forward primer
Biotin-RP	Biotin-CAACAGGTTCAGGACAGAGT	Biotin labeled reverse primer
RP	CAACAGGTTCAGGACAGAGT	Reverse primer used for high-throughput sequencing
Tagged-FP	aaagca-TCTCAGCTCTAGTGGTGACG	Forward primer used for high-throughput sequencing
Biotin-cDNA	Biotin-(CH$_2$CH$_2$O)$_{18}$-GCATAAGCTCGT CTC	Complementary strand used in Capture-SELEX
Clon-3	CCCTCCTCCTAACTGTTCCTACGAAACGAGCTTATGCGTAATGATGACTGTCGTAGTTCG	Clon-3 used in Nano-Affi assay and EMSA assay
Cy5-Clon-3	Cy5-CCCTCCTCCTAACTGTTCCTACGAAACGAGCTTATGCGTAATGATGACTGTCGTAGTTCG	Cy5 labeled Clon-3 used for flow cytometry analysis
Cy5-N-60	Cy5-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	Cy5 labeled N-60 used for flow cytometry analysis as negative control
Cy5.5-Clon-3	Cy5-CCCTCCTCCTAACTGTTCCTACGAAACGAGCTTATGCGTAATGATGACTGTCGTAGTTCG	Cy5.5 labeled Clon-3 used for tissue sections imaging and K$_D$ determination using waveguide
Cy5.5-N-60	Cy5-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	Cy5.5 labeled N-60 used for tissue sections imaging as negative control

Table S2. Experimental parameters used in Modular-SELEX for PD-L1 aptamer isolation

Round	SELEX Method	Library (pmol)	PD-L1(pmol)	Assay (μL)	Temperature	Negative-SELEX	Binding (%)
1	MCP	1000	50	500	Room	Magnetic beads[b]	1.2±0.7 [c]
2	MCP	100	50	500	Room	Magnetic beads[b]	3.6±0.4[c]
3	MCP	100	25	500	37 ℃	Magnetic beads[b]	3.3±0.4[c]
4	Capture	60	12.5	150	Room	His-tag HSA (62.5 pmol)	1.0±0.04[c]
5	Capture	60	12.5	150	Room	His-tag HSA (62.5 pmol)	3.6±0.3[c]
6	Capture	60	12.5	150	Room	His-tag HSA (62.5 pmol)	16.4±3.2[c]
7	Tissue	60	Tissue section [a]	-	Room	None	4.8±0.5[d]
8	Tissue	50	Tissue section [a]	-	Room	None	8.8±0.5[d]
9	Tissue	50	Tissue section [a]	-	Room	None	14.7±0.8[d]

a: PD-L1 highly expressed cancer tissue section (NSCLC)

b: EDC/NHS activated carboxylic acid-magnetic beads

c: the ratio of the amount of eluted library to the amount of input PD-L1

d: the ratio of the amount of eluted library to the amount of input library

Table S3. Top 50 sequences identified by high-throughput sequencing of the final enriched library

Rank	Sequences without primer binding sites (5’-3’)	Quantity
1	GCACGGATCGAGCAATTCCAACGAGACGAGCTTATACGTACCCCCCTCTCGGTCCGCTGC	341
2	CCCCAGGTTCCTACAATCCAAACGAGACGAGCTTATACGTACACCACTCTCTCTGTGAGCT	265
3	CGGCCCAGGATTTCACTCTGACGAGACGAGCTTATACGTACCTTACTCATCCCTTGGGCC	223
4	GCACACTCCAGGACAATCTAACGAGACAAGCTTATGCATACAACCCTCAATTCGTGGGCC	218
5	CCCCCAGACCTTATCCAGTTACGAGACGAGCTTATACATATGTCACCCTAGTATTGTCGC	197
6	CCGGACCAACAATTCTATTCACGAGACGAGCTTATACGTATACTTTACATCGGCTGTCGC	157
7	GGCGGGGGCAAATAAGGGGAACGAGACGAGCTTATACGTATTACTAGGGTGGGGGTTGGC	155
8	GCCCATCCCTTTGTGTTCATACGAGACAAGCTTATGCATACTTTTCACAGGTCTGTGCCC	153
9	CCCGACCCTATATTTGCTTAACGAGACGAGCTTATACGTACCTACCGATCCTCGGGCGCT	149
10	CCCCACGGTTCATTCCCCTCACGAGACGAACTTATGCGTATTCAGTTCTGGTTGGCCCTC	123
11	GGCGCCCTCTGATGTGTTCAACGAGACGAGCTTATACGTACTATTTCTGTCGCCCACCGC	120
12	GCCGCAATGATCTTGTGAAAACGAACGAGCTTATGCATACATCCTACATCTCTGCTGCC	111
13	GCAGGCAACGGTTCCTCTTAACGAGACGAACTTATGCGTAATACTGATCTTTTCCCGGGC	106
14	CAGCACAGCAACCCATATCTACGAGACGAGCTTATACGTATTCCACCTCTGTTCTGCGCT	99
15	CACGAGGGGCCAGCCTATTTACGAGACGAGCTTATACGTACTTTCCTATCGGCCACGTGC	96
16	CACGGGATTCCAGGTTTTTTACGAGACGAACTTATGCATAATCAATGGGGTTCCTCCTGC	90
17	CGCGGGACTCTTCAATCAACACGAGACGAGCTTATACGTATTTACCCACCCCGAGCGCGT	89
18	CCGCAACAGGCAACAGTTCAACGAGACGAGCTTATGCGTAGCCTTTTGTCCCTCTTGGCT	87
19	GGCGGGCATCCTAAAACTTCACGAGACGAGCTTATACATACATGTCCTAGATGTACCCGC	86
20	GCCGGATTGGTTCAGCATCTACGAAACGAGCTTATGCGTACATTTACTTCCTGCTCCCGC	85
21	CGCCCAACTCCCTTTCATTGACGAGACGAACTTATGCGTAACCCTCCTATGGTCCTCTGC	84
22	CGCGGGCAAACTTCCTTCCTACGAGACGAGCTTATACGTACTGCCCCTTCATGTGTGCTC	82
23	CCGGCCAAACAGTATCTTCAACGAACGAGCTTATGCGTACCAATCTTCCGCCAGTGTGT	82
24	GGGGGCCTGGCTAACAATTAACGAGACGAACTTATGCATAAACCTACATTATCCTGCCGC	81
25	CCCGCACAGTATGTAGCCATACGAGACGAGCTTATACGTATATTACTGCCCCCCTGTGCG	81
26	GCGGCAGCGGTCCATTCATAACGAGACGAGCTTATACGTACTACATTCTGCACCGTGCCG	80
27	CCCCCGAAGCTAATGCGATCACGAGACGAACTTATGCGTACTATCACTCACCTGCTCTGC	77
28	CCGACGTGTGTTCCTGTTCTACGAGACGAGCTTATACGTACCTTATTCACTCGGCACCTG	77
29	GCGCCCCCCATGACAATGTTACGAGACAGCTTATGCATATTACCTCTAGCTCCGACCCG	74
30	CCGCACATTTCTCCCTCTGGACGAGACGAACTTATGCGTATTCATGTATCCCTCCTGCCT	72
31	CCGACCGAACCCCAGTTCCTACAAGACGAGCCTTATGCGTATCGGGTGTGCTCCTCTCCGC	71
32	CACCCACCCATTCATTCTCTACACGAGCTTATGCGTAACTGCACCGTCGGACTCTGC	71
33	CGCAACCCTCCCTGTTAAAAACGAGACGAGCTTATACGTATTTAACTGCATGTCTGGCCT	68
34	GCCCAACAGTTCCTCAAGTAACGAGACGAGCTTATACGTACCATGTTGCCAACTCCCTGT	68
35	CCCGGGCCCACAGTATTGCAACGAAACGAGCTTATGCGTACACGGTCTCCTTTTCTGCCG	67

36	CCAGCACAATCTCCCGGATTACGAGACAAGCTTATGCATACCAGGACATCTGCCGTGCGT	66
37	CCCCCCGCCCGACTTCCTCAACGAGACGTTATGCATAGTAAGCCTCTTGGTACGCCC	64
38	CGCAACTGCCTCAATGAAGGACGAAACGAGCTTATGCATACTTTACTCTAGTCTCTGTCC	64
39	GCGGCGGAAGATACCATTCTACGAGACGAACTTATGCATACCCCTACTAGTCATCTGCCG	63
40	CCCAGCCTGTGGTTCCAGATACGAGACAAGCTTATGCATACCTTTCGACTTCCTCATGTG	62
41	GCCCCCGCAACATTCTCTAGACGAAACGAGCTTATGCGTATTCTCCCCGCTCTGTGTGGC	62
42	CCCAAGCAGGTTCCTTTTACACGAGCTTATGCATACAACGGTCTGGCCAACCTCG	60
43	CCCCCAACTTATACCGCTGCACGAGACGAACTTATGCGTATTTAGGTGTCTTGCCTCTGC	60
44	CCGCGCATTCCCAGTATAGGACGAGACGAGCTTATACGTACCTTTTCCTGTCCATCGGCC	60
45	GCACGGTGTACTGTTTTCTTACGAGACGAGCTTATACGTACCTTCTATCGATGCGCCGTG	60
46	CCCACAGCATAGTATACCATACGAGACGAGCTTATACGTACTTTCATTTGCTTGGGCCGT	60
47	GCCACGGTTCCTTCGCGTACACGAAACGAGCTTATGCGTACATTGTCCAACTCCCTGTGC	59
48	GCCCATTTGCACAGAGGAGTACGAGACGAGCTTATATATCCCTTTACCATGCGTGC	59
49	CAACCGGTTGCCAGACTTTTACGAGACGAACTTATGCGTACACTCCCACTTCTTCCTGCC	57
50	GGGGGCTCAAACATCACTCTACGAGACGAGCTTATACATACCATCCGCTGTTTTTGCCC	57

The underlined sequences are the middle constant regions.

Table S4. Sequences identified by cloning sequencing of the final enriched library

Name	**Sequence without primer binding sites (5'-3')**
Clon-1	ACGACAGGAGGATTACAATCACGAGGCGAGCTTATGCGTATGATAATGTGCCCATTTGCT
Clon-2	GACCCGGACTGTAAGTGTGAACGAGACGAGCTTATACGTACTACTACATACCCCATTCAC
Clon-3	CCCTCCTCCTAACTGTTCCTACGAAACGAGCTTATGCGTAATGATGACTGTCGTAGTTCG
Clon-4	CCAGCACTAGTTCTTCACATACGAGACGAGTTTATGCGTACGCTTCTCTGTTTTCTAGTG
Clon-5	GCCCCACCCTCGCTAGTGTTACGAGACGAGCTTATGCGTATTGTAGCGGTTTTCCGTGCG
Clon-6	CGTGATCTGCGATAACCCTAACGAGACGAGCTTATGCGTACATCTACTTGCCCCGCCTTTA
Clon-7	CCCCCCCTGTCCCAGCGTGGACGAGACGAGCTTATACGTACATTTTGACCGGGCGCATAC
Clon-8	TCGTCCTGTGTGTAGTTCGAACGAGACGAGCTTATGCGTAGACTATGAGCCCTTTGGCCT
Clon-9	GCTCACGGGGGTCTTGGCCTACGAGACGAGCTTATGCATATAGGGTTCACGCGATGGGGC
Clon-10	CACCTCACCTCCTTGTGGTGACGAGACGAGCTTATGCGTAATTCGCGAGAGTCCTGTGCG
Clon-11	AACGACGCCCCTGCTAGGTTACGAGACGAGCTTATGCGTAAACGTTTGACCACGGGTGCAA
Clon-12	TCTGGTCGATCTGCTGTATAACGAGACAAGCTTATGCGTACTGGAAATAGCCGTGCATGG
Clon-13	GCTCACGGGGGTCTTGGCCTACGAGACGAGCTTATGCATATAGGGTTCACGCGATGGGGC
Clon-14	CTAGCAGTCCAAATTGAAATACGAGACGAGCTTATGCGTACTGACATATTCGGTTAGCGT
Clon-15	CCCGGGTCCTACCCGAATTCACGAGACGAGCTTATGCGTAGACCACGGATTGGGAAATTG
Clon-16	CGTACAGCGCTAAAAATGCAACAGACGAGCTTATGCGTAGCTTTCACGATCATCTTCGC
Clon-17	GCCCCACCTTAGACATTGGTACGAAACGAGCTTATGCGTAACCTCTTCCTCCGTGTGTGT
Clon-18	CCCGATCATAACTGTCGAGACGAGTTTATGCGTAACATATACGTGTCATTTGTC
Clon-19	CCCGTTTGACCCCGCCGAGTACGAGACGAGCTTATGCGTAACCGCGGGGTGTGCCATCTG
Clon-20	GCCCCAACTGACCCTCGTATGACGAAACGAGCTTATGCGTAACTGTGGATCGTGTCTGATT
Clon-21	GATTACTGCTTGCACTGCTTACGAGACGAGCTTATGTGTAACCTCCCATGTAGGTCTTTG
Clon-22	GCAGCTGGCCCGGAAATATTACGAGACGAGCTTATGCGTATTTGCCCCCAGCCTGGGTAT
Clon-23	CCGCACTCTGGAGGTTGTACGACGAGACGAGCTTATGCAGCTGAGACTCGCGGCGATG

The underlined sequences are the middle constant regions.