Time: 2024.04.23-2024.04.30

1. **Experiment:** Barcode designing
2. **Time:** 2024.04.23-2024.04.30
3. **Member:** Song Zhang, Yaqi Gao
4. **Result:**

We designed the barcode sequence with Python. There are five parts of the barcode sequence, and they are the forward primer, the reverse primer, a TaqMan Probe and two spacer sequences. We set an algorithm with Python to generate random primer and probe sequence, and then generate the spacer sequences.

Each generated barcode also needs to be verified experimentally to see whether it is practical which can be checked mainly from three dimensions. First, the barcode sequences need to be detected effectively by qPCR, which can be understood as an ideal Ct value under different initial barcode content. Second, it also needs to look at the specificity, including that there can be no cross-reaction between barcodes either detected alone or in a group, and only one barcode can be detected by a pair of primers. Third, the stability of barcode. Whether it is detected alone or mixed with the environment, we need to ensure that it has a stable positive rate.

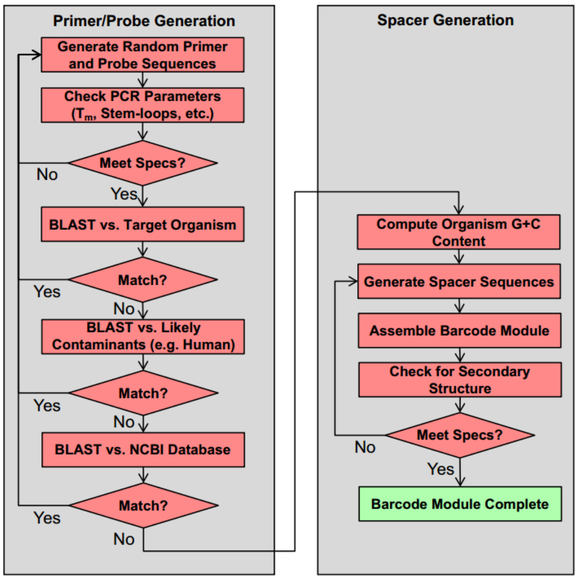

Fig.1 Overview of barcode design and algorithm workflow.

Table 1 Barcode sequences

No.	Squence
1	CCGTAGGCTCGGTAAGTTCGACCAGCGTGGGCCATTGTAAGGCGCTAGGTCA GCCTACCGTGGAACCGCTGCGACCGGAAGTCCGATCGTAGGCCAAGGCCG
2	GTACGTCCATCGGCCGTGAGCTGACCGGTCGACCGGTAAGTCGACCTAGGCG TCAGCTCCGGTAGGCCGCCGTCGACCGATCGGACCTGACGCGTAGGCCG
3	CGAGCTGACCGTCGATGCCGGTAGCTGCGTCCGAGCTAGGCGTCCGTCGACC GTGCGGTCAGCGCTAGGCGGTCAGGCCGTCGACCGGATCGTCCGAGGCT
4	GTCGACCAGCGGTCGACGCTAGGCGTCGACCTAGGCGTCAGCTCCGGTAGCC TGACCGGTCGACTGAGCGTCAGCGGCTGCGAGTAGGCTGACCGGTCGGA
5	CCGGTCGATCGTCCGAGGCTAGGCGTCAGCCCGGTCAGCTCGGATCGTAGGC GTCAGCTCCGGTAGCCTGACCGGGCTAGGCGTCAGCTGACCGATCGGAC
6	GTCGACCGTAGCCTGACCGGTCAGCGCTAGGCGTCAGGCCGTCGACCGGATC GTCCGAGGCTAGGCGTCCGTCGACCGTGCGGTCAGCCCGGTCGACGCTA
7	GACCGGTAGCTGCGTCCGAGCTAGGCGTCCGTCGACCGTGCGGCTGCGAGTA

	GGCTGACCGGTCGACCCGGTAGCCTGACCGGTCGATCGTAGGCGTCGAC
8	GTAGGCGTCAGCCCGGTCAGCTCGGATCGTAGGCGTCAGCTCCGGTAGCCTG ACCGGTCGACTGAGCGTCAGCGGCTGCGAGTAGGCTGACCGGTCGACTG
9	GTCGGATCGTAGGCGTCAGCTCCGGTAGCCTGACCGGTCGACTGAGCGTCAG CGGCTGCGAGTAGGCTGACCGGTCGACCCGGTAGCCTGACCGGTCGATC
10	CGTAGGCGTCAGCTCCGGTAGCCTGACCGGTCGACTGAGCGTCAGCGGCTGC GAGTAGGCTGACCGGTCGACCCGGTAGCCTGACCGGTCGATCGTAGGCG
11	GTCAGCTCCGGTAGCCTGACCGGTCGACTGAGCGTCAGCGGCTGCGAGTAGG CTGACCGGTCGACCCGGTAGCCTGACCGGTCGATCGTAGGCGTCAGCCC
12	GGTAGCCTGACCGGTCGACTGAGCGTCAGCGGCTGCGAGTAGGCTGACCGGT CGACCCGGTAGCCTGACCGGTCGATCGTAGGCGTCAGCTCCGGTAGCCT
13	GACCGGTCGACTGAGCGTCAGCGGCTGCGAGTAGGCTGACCGGTCGACCCG GTAGCCTGACCGGTCGATCGTAGGCGTCAGCTCCGGTAGCCTGACCGGTC
14	CGACTGAGCGTCAGCGGCTGCGAGTAGGCTGACCGGTCGACCCGGTAGCCTG ACCGGTCGATCGTAGGCGTCAGCTCCGGTAGCCTGACCGGTCGACTGAG
15	CGTCAGCGGCTGCGAGTAGGCTGACCGGTCGACCCGGTAGCCTGACCGGTCG ATCGTAGGCGTCAGCTCCGGTAGCCTGACCGGTCGACTGAGCGTCAGCG
16	GCTGCGAGTAGGCTGACCGGTCGACCCGGTAGCCTGACCGGTCGATCGTAGG CGTCAGCTCCGGTAGCCTGACCGGTCGACTGAGCGTCAGCGGCTGCGAG
17	TAGGCTGACCGGTCGACCCGGTAGCCTGACCGGTCGATCGTAGGCGTCAGCT CCGGTAGCCTGACCGGTCGACTGAGCGTCAGCGGCTGCGAGTAGGCTGA
18	CCGGTCGACCCGGTAGCCTGACCGGTCGATCGTAGGCGTCAGCTCCGGTAGC CTGACCGGTCGACTGAGCGTCAGCGGCTGCGAGTAGGCTGACCGGTCGA
19	CCCGGTAGCCTGACCGGTCGATCGTAGGCGTCAGCTCCGGTAGCCTGACCGG TCGACTGAGCGTCAGCGGCTGCGAGTAGGCTGACCGGTCGACCCGGTAG
20	CCTGACCGGTCGATCGTAGGCGTCAGCTCCGGTAGCCTGACCGGTCGACTGA GCGTCAGCGGCTGCGAGTAGGCTGACCGGTCGACCCGGTAGCCTGACCG
21	GTCGATCGTAGGCGTCAGCTCCGGTAGCCTGACCGGTCGACTGAGCGTCAGC GGCTGCGAGTAGGCTGACCGGTCGACCCGGTAGCCTGACCGGTCGATCG
22	TAGGCGTCAGCTCCGGTAGCCTGACCGGTCGACTGAGCGTCAGCGGCTGCGA GTAGGCTGACCGGTCGACCCGGTAGCCTGACCGGTCGATCGTAGGCGTC
23	AGCTCCGGTAGCCTGACCGGTCGACTGAGCGTCAGCGGCTGCGAGTAGGCTG ACCGGTCGACCCGGTAGCCTGACCGGTCGATCGTAGGCGTCAGCTCCGG
24	TAGCCTGACCGGTCGACTGAGCGTCAGCGGCTGCGAGTAGGCTGACCGGTCG ACCCGGTAGCCTGACCGGTCGATCGTAGGCGTCAGCTCCGGTAGCCTGA
25	CCGGTCGACTGAGCGTCAGCGGCTGCGAGTAGGCTGACCGGTCGACCCGGTA GCCTGACCGGTCGATCGTAGGCGTCAGCTCCGGTAGCCTGACCGGTCGA
26	CTGAGCGTCAGCGGCTGCGAGTAGGCTGACCGGTCGACCCGGTAGCCTGACC GGTCGATCGTAGGCGTCAGCTCCGGTAGCCTGACCGGTCGACTGAGCGT
27	CAGCGGCTGCGAGTAGGCTGACCGGTCGACCCGGTAGCCTGACCGGTCGATC GTAGGCGTCAGCTCCGGTAGCCTGACCGGTCGACTGAGCGTCAGCGGCT
28	GCGAGTAGGCTGACCGGTCGACCCGGTAGCCTGACCGGTCGATCGTAGGCGT CAGCTCCGGTAGCCTGACCGGTCGACTGAGCGTCAGCGGCTGCGAGTAG
29	GCTGACCGGTCGACCCGGTAGCCTGACCGGTCGATCGTAGGCGTCAGCTCCG GTAGCCTGACCGGTCGACTGAGCGTCAGCGGCTGCGAGTAGGCTGACCG

30	GTCGACCCGGTAGCCTGACCGGTCGATCGTAGGCGTCAGCTCCGGTAGCCTG ACCGGTCGACTGAGCGTCAGCGGCTGCGAGTAGGCTGACCGGTCGACCC
31	GGTAGCCTGACCGGTCGATCGTAGGCGTCAGCTCCGGTAGCCTGACCGGTCG ACTGAGCGTCAGCGGCTGCGAGTAGGCTGACCGGTCGACCCGGTAGCCT
32	GACCGGTCGATCGTAGGCGTCAGCTCCGGTAGCCTGACCGGTCGACTGAGCG TCAGCGGCTGCGAGTAGGCTGACCGGTCGACCCGGTAGCCTGACCGGTC
33	GCGTCAGCTCCGGTAGCCTGACCGGTCGACTGAGCGTCAGCGGCTGCGAGTA GGCTGACCGGTCGACCCGGTAGCCTGACCGGTCGATCGTAGGCGTCAGC
34	TCCGGTAGCCTGACCGGTCGACTGAGCGTCAGCGGCTGCGAGTAGGCTGACC GGTCGACCCGGTAGCCTGACCGGTCGATCGTAGGCGTCAGCTCCGGTAG
35	CCTGACCGGTCGACTGAGCGTCAGCGGCTGCGAGTAGGCTGACCGGTCGACC CGGTAGCCTGACCGGTCGATCGTAGGCGTCAGCTCCGGTAGCCTGACCG
36	GTCGACTGAGCGTCAGCGGCTGCGAGTAGGCTGACCGGTCGACCCGGTAGCC TGACCGGTCGATCGTAGGCGTCAGCTCCGGTAGCCTGACCGGTCGACTG
37	AGCGTCAGCGGCTGCGAGTAGGCTGACCGGTCGACCCGGTAGCCTGACCGGT CGATCGTAGGCGTCAGCTCCGGTAGCCTGACCGGTCGACTGAGCGTCAG

Table 2 Primer（primer size：18-27///Tm:55-65///GC%:40-60）

No.	Pair	Primer
1	F	CAGAGCTGTGAATTCCCGTAG
	R	CGGCCTTGGCCTACGATC
2	F	CAGAGCTGTGAATTCGTACGTC
	R	CGGCCTACGCGTCAGGTC
3	F	CAGAGCTGTGAATTCCGAGC
	R	AGCCTCGGACGATCCGGTC
4	F	CAGAGCTGTGAATTCGTCGAC
	R	TCCGACCGGTCAGCCTAC
5	F	CAGAGCTGTGAATTCCCGGT
	R	GTCCGATCGGTCAGCTGAC
6	F	CAGAGCTGTGAATTCGTCGAC
	R	TAGCGTCGACCGGGCTGA
7	F	CAGAGCTGTGAATTCGACCG
	R	GTCGACGCCTACGATCGAC
8	F	CAGAGCTGTGAATTCGTAGGC
	R	CAGTCGACCGGTCAGCCTA
9	F	CAGAGCTGTGAATTCGTCGG
	R	GATCGACCGGTCAGGCTAC
10	F	CAGAGCTGTGAATTCCGTAGG
	R	CGCCTACGATCGACCGGTC
11	F	CAGAGCTGTGAATTCGTCAGC
	R	GGGCTGACGCCTACGATC
12	F	CAGAGCTGTGAATTCGACCG
	R	GACCGGTCAGGCTACCGG
13	F	CAGAGCTGTGAATTCGACCG
	R	GACCGGTCAGGCTACCGG

14	F	CAGAGCTGTGAATTCCGACTG
	R	CTCAGTCGACCGGTCAGG
15	F	CAGAGCTGTGAATTCCGTCAG
	R	CGCTGACGCTCAGTCGAC
17	F	CAGAGCTGTGAATTCGCTGC
	R	CTCGCAGCCGCTGACGCTC
18	F	CAGAGCTGTGAATTCCCGGT
	R	TCGACCGGTCAGCCTACTC
19	F	CAGAGCTGTGAATTCCCCGG
	R	CTACCGGGTCGACCGGTC
20	F	CAGAGCTGTGAATTCCCTGAC
	R	CGGTCAGGCTACCGGGTC
21	F	CAGAGCTGTGAATTCGTCGA
	R	CGATCGACCGGTCAGGCTA
22	F	CAGAGCTGTGAATTCTAGGCG
	R	GACGCCTACGATCGACCG
23	F	CAGAGCTGTGAATTCAGCTCC
	R	CCGGAGCTGACGCCTACGAT
24	F	CAGAGCTGTGAATTCTAGCCTGA
	R	TCAGGCTACCGGAGCTGAC
25	F	CAGAGCTGTGAATTCCCGGT
	R	TCGACCGGTCAGGCTACC
26	F	CAGAGCTGTGAATTCCTGAGC
	R	ACGCTCAGTCGACCGGTC
27	F	CAGAGCTGTGAATTCCAGCG
	R	AGCCGCTGACGCTCAGTC
28	F	CAGAGCTGTGAATTCGCGAG
	R	CTACTCGCAGCCGCTGAC
29	F	CAGAGCTGTGAATTCGCTGAC
	R	CGGTCAGCCTACTCGCAG
30	F	CAGAGCTGTGAATTCGTCGAC
	R	GGGTCGACCGGTCAGCCTA
31	F	CAGAGCTGTGAATTCGGTAGC
	R	AGGCTACCGGGTCGACCG
32	F	CAGAGCTGTGAATTCGACCG
	R	GACCGGTCAGGCTACCGG
33	F	CAGAGCTGTGAATTCGCGTC
	R	GCTGACGCCTACGATCGAC
34	F	CAGAGCTGTGAATTCTCCGGT
	R	CTACCGGAGCTGACGCCTA
35	F	CAGAGCTGTGAATTCCCTGAC
	R	CGGTCAGGCTACCGGAGC
36	F	CAGAGCTGTGAATTCGTCGAC
	R	CAGTCGACCGGTCAGGCTA
37	F	CAGAGCTGTGAATTCAGCGTC

R	CTGACGCTCAGTCGACCG