Table S1. Single-Factor Optimization Results of the Self-Developed High-Salinity Lysate Method (Concentration)

		C.albicans																	
	(CFU/ml)	10^3						10^5						10^7					
		Concentration (ng/μL)			Mean	SD	CV	Concentration (ng/μL)			Mean	SD	CV	Concentration (ng/μL)			Mean	SD	CV
Concentration of Guanidine Hydrochloride (mol/L)	1	5.33	5.18	5.29	5.27	0.08	1.47%	12.35	12.82	12.93	12.70	0.31	2.43%	71.41	72.53	69.81	71.25	1.37	1.92%
	2	5.24	5.37	5.12	5.24	0.13	2.38%	13.25	13.13	13.33	13.24	0.10	0.76%	76.64	73.24	77.33	75.74	2.19	2.89%
	3	6.05	5.92	6.08	6.02	0.09	1.41%	16.55	16.45	16.63	16.54	0.09	0.55%	112.15	116.46	112.82	113.81	2.32	2.04%
	4	5.22	5.25	5.38	5.28	0.09	1.61%	14.05	13.92	14.13	14.03	0.11	0.76%	91.15	88.43	86.45	88.68	2.36	2.66%
	5	4.82	4.93	4.97	4.91	0.08	1.58%	13.55	13.42	13.63	13.53	0.11	0.78%	63.23	65.52	64.17	64.31	1.15	1.79%
Volume fraction of isopropanol (%)	30	4.85	4.76	4.89	4.83	0.07	1.38%	11.25	11.12	11.33	11.23	0.11	0.94%	69.32	68.31	71.94	69.86	1.87	2.68%
	40	5.52	5.43	5.58	5.51	0.08	1.37%	13.85	13.72	13.98	13.85	0.13	0.94%	100.16	98.63	102.16	100.32	1.77	1.76%
	50	6.23	6.15	6.29	6.22	0.07	1.13%	17.35	17.22	17.33	17.30	0.07	0.40%	131.21	128.96	130.43	130.20	1.14	0.88%
	60	5.42	5.35	5.48	5.42	0.07	1.20%	14.55	14.42	14.63	14.53	0.11	0.73%	113.85	111.23	111.52	112.20	1.44	1.28%
	70	4.72	4.63	4.78	4.71	0.08	1.60%	12.05	11.92	12.42	12.13	0.26	2.14%	74.24	76.96	72.37	74.52	2.31	3.10%
Volume fraction of Triton X-100 (%)	0	4.71	4.53	4.58	4.61	0.09	2.02%	10.53	10.41	10.63	10.52	0.11	1.05%	51.23	51.51	49.87	50.87	0.88	1.72%
	2	5.34	5.28	5.35	5.32	0.04	0.71%	13.13	12.95	13.18	13.09	0.12	0.92%	86.42	83.53	85.24	85.06	1.45	1.71%
	4	6.53	6.42	6.59	6.51	0.09	1.32%	18.26	17.92	18.15	18.11	0.17	0.96%	153.02	154.29	150.85	152.72	1.74	1.14%
	6	5.67	5.57	5.62	5.62	0.05	0.89%	14.70	14.74	14.96	14.80	0.14	0.95%	122.78	121.90	122.36	122.35	0.44	0.36%
	8	5.02	4.93	5.08	5.01	0.08	1.51%	12.24	12.16	12.24	12.21	0.05	0.38%	84.66	82.87	83.23	83.59	0.95	1.13%
	10	4.62	4.53	4.62	4.59	0.05	1.13%	11.46	10.98	11.26	11.23	0.24	2.15%	61.25	58.92	62.65	60.94	1.88	3.09%
pH	6	4.34	4.15	4.25	4.25	0.10	2.24%	9.82	9.61	9.94	9.79	0.17	1.71%	60.83	59.21	61.21	60.42	1.06	1.76%
	7	5.02	4.98	5.03	5.01	0.03	0.53%	12.53	12.42	12.69	12.55	0.14	1.08%	82.56	78.54	82.50	81.20	2.30	2.84%
	8	5.63	5.53	5.68	5.61	0.08	1.36%	15.28	15.12	15.38	15.26	0.13	0.86%	133.57	130.32	133.25	132.38	1.79	1.35%
	9	6.12	6.09	6.17	6.13	0.04	0.66%	17.05	16.96	17.13	17.05	0.09	0.50%	160.62	163.67	164.31	162.87	1.97	1.21%
	10	8.87	8.70	8.89	8.82	0.10	1.18%	19.36	19.41	19.67	19.48	0.17	0.85%	219.13	216.26	218.54	217.98	1.52	0.70%
	11	6.23	6.15	6.24	6.21	0.05	0.79%	16.85	16.75	16.93	16.84	0.09	0.54%	161.28	161.51	166.32	163.04	2.85	1.75%
	12	5.31	5.23	5.32	5.29	0.05	0.93%	13.42	13.02	13.21	13.22	0.20	1.51%	90.17	93.62	94.94	92.91	2.46	2.65%

Table S1. Single-Factor Optimization Results of the Self-Developed High-Salinity Lysate Method (Concentration)

	C.neoformans		
(CFU/ml)	10^3	10^5	10^7

		Concentration（ng/μL）			Mean	SD	CV	Concentration（ng/μL）			Mean	SD	CV	Concentration（ng/μL）			Mean	SD	CV
Concentration of Guanidine Hydrochloride（mol/L）	1	4.75	4.93	4.81	4.83	0.09	1.90%	14.30	14.72	14.45	14.49	0.21	1.47%	32.26	31.53	31.12	31.64	0.58	1.83%
	2	5.12	4.92	5.05	5.03	0.10	2.02%	15.35	14.93	15.12	15.13	0.21	1.39%	46.23	44.16	45.92	45.44	1.12	2.46%
	3	5.95	6.02	6.25	6.07	0.16	2.58%	17.98	17.63	17.85	17.82	0.18	0.99%	62.67	59.47	62.41	61.52	1.78	2.89%
	4	5.32	5.15	5.28	5.25	0.09	1.69%	15.50	15.11	15.31	15.31	0.20	1.27%	47.39	48.85	48.17	48.14	0.73	1.52%
	5	4.58	4.40	4.65	4.54	0.13	2.84%	14.40	14.08	14.25	14.24	0.16	1.12%	26.62	25.28	25.73	25.88	0.68	2.64%
Volume fraction of isopropanol（%）	30	4.30	4.08	4.22	4.20	0.11	2.65%	12.35	11.92	12.14	12.14	0.22	1.77%	49.25	48.89	50.84	49.66	1.04	2.09%
	40	4.88	4.96	4.79	4.88	0.09	1.74%	14.95	14.55	14.73	14.74	0.20	1.36%	57.62	56.47	54.90	56.33	1.37	2.42%
	50	6.20	6.01	6.15	6.12	0.10	1.61%	18.75	18.39	18.57	18.57	0.18	0.97%	83.59	79.54	81.92	81.68	2.04	2.49%
	60	5.35	5.42	5.27	5.35	0.08	1.40%	15.45	15.73	15.26	15.48	0.24	1.53%	61.43	61.18	63.35	61.99	1.19	1.92%
	70	4.32	4.41	4.25	4.33	0.08	1.85%	13.25	12.81	13.25	13.10	0.25	1.94%	42.19	42.37	43.17	42.58	0.52	1.23%
Volume fraction of Triton X-100（%）	0	4.01	4.15	3.95	4.04	0.10	2.54%	11.25	10.81	11.25	11.10	0.25	2.29%	62.24	59.64	60.41	60.76	1.34	2.20%
	2	5.32	5.24	5.33	5.30	0.05	0.93%	14.55	14.17	14.32	14.35	0.19	1.33%	77.75	78.45	75.49	77.23	1.55	2.00%
	4	6.42	6.21	6.35	6.33	0.11	1.69%	19.45	19.32	19.25	19.34	0.10	0.52%	93.41	97.35	99.14	96.63	2.93	3.03%
	6	5.62	5.40	5.55	5.52	0.11	2.03%	15.75	15.34	15.57	15.55	0.21	1.32%	74.54	76.92	73.36	74.94	1.81	2.42%
	8	4.75	4.82	4.65	4.74	0.09	1.80%	13.45	13.21	13.26	13.31	0.13	0.95%	63.42	61.51	60.24	61.72	1.60	2.59%
	10	4.08	4.21	3.98	4.09	0.12	2.82%	12.35	11.95	12.16	12.15	0.20	1.65%	46.72	44.68	45.47	45.62	1.03	2.25%
pH	6	3.88	4.01	3.90	3.93	0.07	1.78%	10.45	10.32	10.29	10.35	0.09	0.82%	65.14	63.14	65.16	64.48	1.16	1.80%
	7	4.82	4.63	4.75	4.73	0.10	2.03%	14.13	13.63	13.86	13.87	0.25	1.80%	83.63	80.54	79.92	81.36	1.99	2.44%
	8	5.54	5.32	5.45	5.44	0.11	2.03%	16.75	16.37	16.54	16.55	0.19	1.15%	126.82	126.51	128.45	127.26	1.04	0.82%
	9	6.12	5.93	6.05	6.03	0.10	1.59%	18.35	17.91	18.14	18.13	0.22	1.21%	158.27	159.73	155.24	157.75	2.29	1.45%
	10	6.72	6.50	6.65	6.62	0.11	1.70%	20.25	19.82	20.12	20.06	0.22	1.10%	214.71	213.90	216.09	214.90	1.11	0.52%
	11	6.22	6.21	6.15	6.19	0.04	0.61%	18.28	19.21	19.15	18.88	0.52	2.76%	145.42	145.25	148.52	146.40	1.84	1.26%
	12	5.35	5.18	5.25	5.26	0.09	1.62%	15.34	15.13	15.25	15.24	0.11	0.69%	90.93	88.34	88.83	89.37	1.38	1.54%

Table S1. Single-Factor Optimization Results of the Self-Developed High-Salinity Lysate Method (Concentration)

		A.fumigatus																	
	（CFU/ml）	10^2						10^4						10^6					
		Concentration（ng/μL）			Mean	SD	CV	Concentration（ng/μL）			Mean	SD	CV	Concentration（ng/μL）			Mean	SD	CV
Concentration of Guanidine	1	4.29	4.38	4.42	4.36	0.07	1.53%	15.98	15.86	16.31	16.05	0.23	1.45%	57.46	56.39	56.53	56.79	0.58	1.02%
	2	8.18	8.36	8.11	8.22	0.13	1.57%	19.46	19.25	19.53	19.41	0.15	0.75%	78.93	78.86	79.99	79.26	0.63	0.80%

Hydrochloride (mol/L)	3	13.95	13.88	14.14	13.99	0.13	0.96%	27.53	27.46	27.69	27.56	0.12	0.43%	83.26	85.19	83.33	83.93	1.09	1.30%
	4	9.72	9.53	9.65	9.63	0.10	1.00%	23.25	23.15	23.32	23.24	0.09	0.37%	66.82	66.75	63.89	65.82	1.67	2.54%
	5	5.86	5.79	5.93	5.86	0.07	1.19%	18.68	18.65	18.75	18.69	0.05	0.27%	40.46	40.39	41.53	40.79	0.64	1.57%
Volume fraction of isopropanol (%)	30	5.84	5.87	5.91	5.87	0.04	0.60%	16.39	16.32	16.56	16.42	0.12	0.75%	64.32	63.75	64.89	64.32	0.57	0.89%
	40	11.52	11.35	11.59	11.49	0.12	1.07%	20.93	20.86	20.99	20.93	0.07	0.31%	86.39	85.82	86.46	86.22	0.35	0.41%
	50	15.88	15.81	15.95	15.88	0.07	0.44%	26.86	26.79	25.93	26.53	0.52	1.95%	123.09	120.02	119.18	120.76	2.06	1.70%
	60	9.15	9.18	9.22	9.18	0.04	0.38%	22.59	22.42	22.66	22.56	0.12	0.55%	109.66	105.49	109.73	108.29	2.43	2.24%
	70	4.93	4.86	5.12	4.97	0.13	2.71%	17.66	17.89	18.03	17.86	0.19	1.05%	73.96	75.85	76.03	75.28	1.15	1.52%
Volume fraction of Triton X-100 (%)	0	6.67	6.68	6.54	6.63	0.08	1.18%	15.46	15.29	15.53	15.43	0.12	0.80%	63.89	65.92	64.06	64.62	1.13	1.74%
	2	10.19	10.32	10.26	10.26	0.07	0.63%	19.93	19.92	20.06	19.97	0.08	0.39%	85.26	86.29	83.53	85.03	1.39	1.64%
	4	13.42	13.15	13.49	13.35	0.18	1.34%	28.16	27.99	28.13	28.09	0.09	0.32%	127.46	122.55	124.73	124.91	2.46	1.97%
	6	10.32	10.84	9.94	8.01	0.12	1.54%	24.56	24.55	24.74	24.62	0.11	0.43%	98.91	97.23	95.16	97.10	1.88	1.93%
	8	7.98	7.91	8.15	4.49	0.04	0.78%	23.82	23.75	23.89	23.82	0.07	0.29%	83.13	88.16	86.20	85.83	2.54	2.95%
	10	4.46	4.49	4.53	4.49	0.04	0.78%	19.09	19.12	19.16	19.12	0.04	0.18%	75.59	77.62	79.66	77.62	2.04	2.62%
pH	6	6.35	6.18	6.32	6.28	0.09	1.44%	16.73	16.46	16.80	16.66	0.18	1.08%	72.39	74.89	75.46	74.25	1.63	2.20%
	7	8.89	8.82	8.96	8.89	0.07	0.79%	20.39	20.32	20.47	20.39	0.08	0.37%	99.93	96.42	95.99	97.45	2.16	2.22%
	8	9.53	9.46	9.67	9.55	0.11	1.12%	24.16	24.49	24.23	24.29	0.17	0.72%	110.66	107.59	112.73	110.33	2.59	2.34%
	9	11.86	12.39	12.43	12.23	0.32	2.60%	26.53	26.46	26.69	26.56	0.12	0.44%	131.69	136.26	135.40	134.45	2.43	1.81%
	10	14.89	14.82	15.16	14.96	0.18	1.20%	27.26	27.19	28.33	27.59	0.64	2.32%	160.82	164.75	162.69	162.75	1.97	1.21%
	11	10.75	10.68	10.72	10.72	0.04	0.33%	23.09	23.32	23.16	23.19	0.12	0.51%	113.46	113.39	116.83	114.56	1.97	1.72%
	12	6.39	6.39	6.46	6.41	0.04	0.63%	18.46	17.89	18.53	18.29	0.35	1.92%	94.09	94.62	96.16	94.96	1.08	1.13%

Table S2. Single-Factor Optimization Results of the Self-Developed High-Salt Lysis Method (Purity：$OD_{260/280}$)

		C.albicans										C.neoformans				
	(CFU/ml)	10^3			10^5			10^7				10^3			10^5	
Concentration of Guanidine Hydrochloride (mol/L)	1	1.94	1.86	1.83	1.85	1.89	1.86	1.81	1.84	1.87	1.82	1.85	1.80	1.82	1.92	1.83
	2	1.88	1.89	1.97	1.81	1.87	1.90	1.88	1.87	1.95	1.87	1.84	1.86	1.88	1.85	1.86
	3	1.92	1.91	1.94	1.92	1.91	1.94	1.92	1.91	1.90	1.91	1.93	1.88	1.92	1.89	1.90
	4	1.77	1.68	1.85	1.87	1.96	1.89	1.87	1.87	1.82	1.86	1.84	1.97	1.87	1.85	1.86
	5	1.73	1.76	1.65	1.83	1.82	1.95	1.83	1.82	1.81	1.82	1.79	1.83	1.83	1.79	1.81
Volume fraction of isopropanol (%)	30	1.84	1.83	1.95	1.84	1.87	1.84	1.84	1.83	1.93	1.85	1.81	1.89	1.85	1.81	1.93
	40	1.89	1.81	1.87	1.89	1.83	1.91	1.89	1.86	1.96	1.89	1.94	1.88	1.79	1.86	1.87
	50	1.93	1.92	1.92	1.93	1.92	1.95	1.83	1.92	1.91	1.94	1.90	1.82	1.94	1.91	1.82
	60	1.86	1.89	1.88	1.86	1.85	1.95	1.86	1.81	1.88	1.86	1.97	1.84	1.86	1.88	1.84
	70	1.82	1.81	1.82	1.82	1.87	1.81	1.86	1.81	1.83	1.82	1.79	1.88	1.82	1.79	1.88
Volume fraction of Triton X-100 (%)	0	1.86	1.84	1.94	1.84	1.80	1.83	1.81	1.85	1.83	1.81	1.87	1.80	1.81	1.78	1.85
	2	1.87	1.82	1.85	1.84	1.81	1.89	1.82	1.86	1.94	1.82	1.85	1.89	1.87	1.81	1.85
	4	1.95	1.92	1.87	1.95	1.83	1.97	1.97	1.83	1.97	1.95	1.82	1.94	1.96	1.93	1.85
	6	1.81	1.87	1.93	1.88	1.81	1.92	1.88	1.94	1.90	1.88	1.85	1.86	1.88	1.83	1.86
	8	1.83	1.88	1.83	1.85	1.82	1.85	1.83	1.81	1.81	1.88	1.84	1.89	1.83	1.84	1.81
	10	1.83	1.79	1.82	1.80	1.80	1.82	1.80	1.86	1.82	1.80	1.84	1.77	1.80	1.77	1.82
pH	6	1.80	1.86	1.83	1.88	1.79	1.84	1.80	1.79	1.82	1.80	1.81	1.87	1.80	1.87	1.78
	7	1.85	1.81	1.92	1.93	1.84	1.87	1.95	1.94	1.87	1.85	1.96	1.84	1.85	1.82	1.86
	8	1.89	1.84	1.91	1.89	1.88	1.85	1.83	1.79	1.91	1.89	1.97	1.82	1.90	1.97	1.88
	9	1.87	1.83	1.94	1.92	1.81	1.84	1.92	1.92	1.84	1.92	1.90	1.91	1.93	1.90	1.81
	10	1.96	1.89	1.82	1.84	1.95	1.88	1.96	1.84	1.91	1.97	1.85	1.83	1.83	1.85	1.96
	11	1.91	1.96	1.83	1.91	1.81	1.83	1.94	1.95	1.82	1.91	1.89	1.89	1.91	1.85	1.89
	12	1.86	1.85	1.92	1.86	1.93	1.88	1.86	1.83	1.87	1.86	1.82	1.87	1.86	1.82	1.85

Table S2. Single-Factor Optimization Results of the Self-Developed High-Salt Lysis Method (Purity：$OD_{260/280}$)

	C.neoformans			A.fumigatus								
(CFU/ml)	10^7			10^2			10^4			10^6		
1	1.84	1.82	1.86	1.82	1.81	1.84	1.81	1.83	1.88	1.84	1.83	1.87

Concentration of Guanidine Hydrochloride (mol/L)	2	1.86	1.84	1.88	1.85	1.94	1.82	1.87	1.80	1.83	1.88	1.93	1.89
	3	1.90	1.88	1.92	1.94	1.84	1.95	1.95	1.94	1.83	1.96	1.95	1.83
	4	1.86	1.85	1.87	1.90	1.80	1.97	1.91	1.84	1.97	1.80	1.81	1.92
	5	1.81	1.87	1.93	1.85	1.84	1.83	1.85	1.88	1.83	1.85	1.84	1.93
Volume fraction of isopropanol (%)	30	1.83	1.82	1.86	1.81	1.90	1.82	1.83	1.94	1.84	1.83	1.86	1.84
	40	1.82	1.89	1.88	1.86	1.83	1.87	1.89	1.88	1.89	1.84	1.82	1.90
	50	1.92	1.83	1.93	1.85	1.95	1.84	1.96	1.83	1.82	1.97	1.84	1.86
	60	1.85	1.94	1.86	1.88	1.97	1.86	1.88	1.85	1.89	1.88	1.83	1.89
	70	1.81	1.86	1.82	1.83	1.88	1.84	1.84	1.83	1.86	1.84	1.83	1.82
Volume fraction of Triton X-100 (%)	0	1.80	1.85	1.81	1.81	1.81	1.79	1.92	1.81	1.84	1.87	1.81	1.83
	2	1.86	1.85	1.92	1.87	1.84	1.88	1.88	1.85	1.89	1.88	1.87	1.84
	4	1.94	1.83	1.89	1.95	1.98	1.96	1.97	1.94	1.78	1.98	1.84	1.82
	6	1.84	1.93	1.87	1.87	1.86	1.88	1.90	1.83	1.91	1.83	1.85	1.91
	8	1.81	1.88	1.82	1.82	1.81	1.83	1.85	1.82	1.86	1.85	1.84	1.83
	10	1.79	1.98	1.85	1.82	1.80	1.97	1.95	1.80	1.86	1.78	1.98	1.85
pH	6	1.79	1.83	1.86	1.79	1.78	1.84	1.84	1.81	1.83	1.82	1.81	1.83
	7	1.84	1.83	1.85	1.84	1.83	1.80	1.87	1.88	1.88	1.87	1.79	1.88
	8	1.88	1.83	1.81	1.89	1.88	1.93	1.98	1.91	1.91	1.92	1.96	1.93
	9	1.91	1.84	1.83	1.92	1.91	1.93	1.94	1.96	1.95	1.95	1.94	1.91
	10	1.86	1.95	1.87	1.97	1.92	1.98	1.96	1.95	1.97	1.97	1.96	1.98
	11	1.90	1.88	1.92	1.91	1.95	1.92	1.78	1.90	1.83	1.91	1.95	1.92
	12	1.85	1.89	1.86	1.85	1.84	1.81	1.85	1.82	1.86	1.85	1.84	1.86

Table S3. ANOVA Results of the Effects of Different Optimization Factors on Fungal Nucleic Acid Extraction Concentration(medium and high concentration)

Fungal species	Optimization factor	Medium concentration				High concentration			
		Number of levels	F-value	P-value	optimum level	Number of levels	F-value	P-value	optimum level
C. albicans	Concentration of Guanidine Hydrochloride (mol/L)	5	247.7	<0.0001	3	5	301.3	<0.0001	3
	Volume fraction of isopropanol (%)	5	745.1	<0.0001	50	5	623.0	<0.0001	50
	Volume fraction of Triton X-100 (%)	6	1016	<0.0001	4	6	2519	<0.0001	4
	System pH	7	1527	<0.0001	10	7	2161	<0.0001	10
C. neoformans	Concentration of Guanidine Hydrochloride (mol/L)	5	164.7	<0.0001	3L	5	520.5	<0.0001	3
	Volume fraction of isopropanol (%)	5	386.4	<0.0001	50	5	379.1	<0.0001	50
	Volume fraction of Triton X-100 (%)	6	734.3	<0.0001	4	6	280.3	<0.0001	4
	System pH	7	479.6	<0.0001	10	7	3174	<0.0001	10

	Concentration of Guanidine Hydrochloride (mol/L)	5	3034	<0.0001	3	5	886.4	<0.0001	3
A. fumigatus	Volume fraction of isopropanol (%)	5	706.8	<0.0001	50	5	683.1	<0.0001	50
	Volume fraction of Triton X-100 (%)	6	7847	<0.0001	4	6	324.3	<0.0001	4
	System pH	7	570.6	<0.0001	10	7	609.1	<0.0001	10

Table S4. Extraction Results of Two Different Fungal Nucleic Acid Extraction Methods (Concentration)

	Methods	CFU/ml	Concentration（ng/μL）			Mean（ng/μL）	SD	CV
C.albicans	High-salt	10^3	9.58	9.17	9.43	9.39	0.21	2.21%
		10^5	20.35	20.30	20.31	20.32	0.03	0.13%
		10^7	222.47	217.46	220.62	220.18	2.53	1.15%
	Kit-based	10^3	10.30	10.33	10.33	10.32	0.02	0.17%
		10^5	18.80	18.75	18.73	18.76	0.04	0.19%
		10^7	204.26	201.78	202.52	202.85	1.27	0.63%
C.neoformans	High-salt	10^3	6.52	6.41	6.59	6.51	0.09	1.39%
		10^5	22.05	21.22	21.31	21.53	0.46	2.12%
		10^7	214.58	208.36	210.32	211.09	3.18	1.51%
	Kit-based	10^3	5.64	5.62	5.63	5.63	0.01	0.18%
		10^5	19.75	19.70	19.77	19.74	0.04	0.18%
		10^7	213.66	208.37	210.43	210.82	2.67	1.26%
A.fumigatus	High-salt	10^2	15.34	15.42	15.53	15.43	0.10	0.62%
		10^4	28.45	28.38	28.40	28.41	0.04	0.13%
		10^6	161.47	160.42	160.95	160.95	0.53	0.33%
	Kit-based	10^2	13.70	13.60	13.62	13.64	0.05	0.39%
		10^4	25.55	25.50	25.51	25.52	0.03	0.10%
		10^6	154.84	152.66	153.96	153.82	1.10	0.71%

Table S5. Extraction results of two different fungal nucleic acid extraction methods(purity)

	Methods	CFU/ml	Concentration		
C.albicans	High-salt	10^3	1.81	1.89	1.82
		10^5	1.92	1.92	1.99
		10^7	1.85	1.81	1.85
	Kit-based	10^3	1.97	1.97	1.94
		10^5	1.88	1.84	1.88
		10^7	1.94	1.93	1.91
C.neoformans	High-salt	10^3	1.83	1.86	1.83
		10^5	1.99	1.98	1.96
		10^7	1.87	1.8	1.87
	Kit-based	10^3	1.91	1.95	1.94
		10^5	1.84	1.83	1.89
		10^7	1.96	1.96	1.97
A.fumigatus	High-salt	10^2	1.89	1.88	1.81

Table S5. Extraction results of two different fungal nucleic acid extraction methods(purity)

	Methods	CFU/ml	Concentration		
		10^4	1.93	1.91	1.92
		10^6	1.82	1.85	1.84
		10^2	1.98	1.99	1.93
	Kit-based	10^4	1.86	1.82	1.86
		10^6	1.9	1.94	1.98

Table S6. Optimization Results of Multiple qPCR Reaction Conditions

		C.albicans						C.neoformans					
			CT		Mean	SD	CV		CT		Mean	SD	CV
Primer concentration (pmol/system)	2	30.98	31.02	30.81	30.94	0.11	0.36%	24.93	24.95	24.99	24.96	0.03	0.12%
	4	30.35	30.35	30.44	30.38	0.05	0.17%	24.43	24.45	24.49	24.46	0.03	0.12%
	6	29.56	29.52	29.54	29.54	0.02	0.07%	24.44	24.47	24.60	24.50	0.09	0.35%
	8	29.3	29.19	29.23	29.24	0.06	0.19%	24.15	24.16	24.06	24.12	0.06	0.23%
	10	29.82	29.83	29.89	29.85	0.04	0.13%	24.97	24.96	24.96	24.96	0.01	0.02%
Probe concentration (pmol/system)	1	29.03	29.12	29.07	29.07	0.05	0.16%	24.46	24.43	24.42	24.44	0.02	0.09%
	2	28.18	28.27	28.35	28.27	0.09	0.30%	24.05	24.12	24.13	24.10	0.04	0.18%
	3	28.64	28.35	28.73	28.57	0.20	0.69%	24.19	24.32	24.36	24.29	0.09	0.37%
	4	28.97	29.13	29.18	29.09	0.11	0.38%	24.14	24.16	24.22	24.17	0.04	0.17%
	5	29.07	29.15	29.22	29.15	0.08	0.26%	24.35	24.48	24.42	24.42	0.07	0.27%
	6	29.35	29.49	29.5	29.45	0.08	0.28%	24.64	24.65	24.64	24.64	0.01	0.02%
MgCl2 concentration (mmol/system)	0	30.64	30.42	30.72	30.59	0.16	0.51%	25.72	24.90	24.81	25.14	0.50	1.99%
	2	30.41	30.30	30.11	30.27	0.15	0.50%	24.72	24.61	24.62	24.65	0.06	0.25%
	4	29.24	29.17	29.4	29.27	0.12	0.40%	24.21	24.24	24.27	24.24	0.03	0.12%
	6	29.56	29.33	29.49	29.46	0.12	0.40%	23.88	23.86	23.95	23.90	0.05	0.20%
	8	29.69	29.23	29.84	29.59	0.32	1.07%	24.65	23.88	23.99	24.17	0.42	1.72%
Annealing Temperature (℃)	54	30.38	30.25	30.28	30.30	0.07	0.22%	24.40	24.38	24.43	24.40	0.03	0.10%
	56	29.88	29.85	30.01	29.91	0.09	0.28%	23.69	23.68	23.73	23.70	0.03	0.11%
	58	28.61	28.67	28.54	28.61	0.07	0.23%	23.43	23.31	23.44	23.39	0.07	0.31%
	60	28.23	28.70	28.88	28.60	0.34	1.17%	23.95	23.94	23.95	23.95	0.01	0.02%
	62	28.91	28.77	28.99	28.89	0.11	0.39%	24.55	24.58	24.58	24.57	0.02	0.07%

Table S6. Optimization Results of Multiple qPCR Reaction Conditions

		A.fumigatus					
			CT		Mean	SD	CV
	2	30.98	31.02	30.81	30.94	0.11	0.36%

Primer concentration (pmol/system)	4	30.35	30.35	30.44	30.38	0.05	0.17%
	6	29.56	29.52	29.54	29.54	0.02	0.07%
	8	29.3	29.19	29.23	29.24	0.06	0.19%
	10	29.82	29.83	29.89	29.85	0.04	0.13%
Probe concentration (pmol/system)	1	29.03	29.12	29.07	29.07	0.05	0.16%
	2	28.18	28.27	28.35	28.27	0.09	0.30%
	3	28.64	28.35	28.73	28.57	0.20	0.69%
	4	28.97	29.13	29.18	29.09	0.11	0.38%
	5	29.07	29.15	29.22	29.15	0.08	0.26%
	6	29.35	29.49	29.5	29.45	0.08	0.28%
MgCl2 concentration (mmol/system)	0	30.64	30.42	30.72	30.59	0.16	0.51%
	2	30.41	30.30	30.11	30.27	0.15	0.50%
	4	29.24	29.17	29.4	29.27	0.12	0.40%
	6	29.56	29.33	29.49	29.46	0.12	0.40%
	8	29.69	29.23	29.84	29.59	0.32	1.07%
Annealing Temperature (℃)	54	30.38	30.25	30.28	30.30	0.07	0.22%
	56	29.88	29.85	30.01	29.91	0.09	0.28%
	58	28.61	28.67	28.54	28.61	0.07	0.23%
	60	28.23	28.70	28.88	28.60	0.34	1.17%
	62	28.91	28.77	28.99	28.89	0.11	0.39%

Table S7. Sensitivity Results of Single qPCR Reaction System

	(CFU/ml)	CT			Mean	SD	CV
C.albicans	10^1	43.8	NoCt	42.84	43.32	0.68	1.57%
	10^2	41.59	41.64	41.43	41.55	0.11	0.26%
	10^3	37.8	37.67	38.23	37.90	0.29	0.77%
	10^4	34.76	35.44	34.63	34.94	0.44	1.24%
	10^5	31.04	31.18	31.16	31.13	0.08	0.24%
	10^6	27.89	28.01	27.86	27.92	0.08	0.28%
	10^7	23.65	23.8	23.75	23.73	0.08	0.32%
	10^8	19.92	19.96	20.09	19.99	0.09	0.44%
C.neoformans	10^1	40.14	40.12	NoCt	40.13	0.01	0.04%
	10^2	37.06	37.21	37.13	37.13	0.08	0.20%
	10^3	33.95	34.21	34.39	34.18	0.22	0.65%
	10^4	31.44	31.24	31.37	31.35	0.10	0.32%
	10^5	28.05	28.76	28.11	28.31	0.39	1.39%
	10^6	25.35	25.39	25.16	25.30	0.12	0.49%
	10^7	20.78	20.82	20.69	20.76	0.07	0.32%
	10^8	17.68	17.58	17.76	17.67	0.09	0.51%
A.fumigatus	10^1	35.34	35.12	34.93	35.13	0.21	0.58%
	10^2	30.67	30.46	30.61	30.58	0.11	0.35%
	10^3	26.95	26.91	26.79	26.88	0.08	0.31%
	10^4	24.44	24.94	24.68	24.69	0.25	1.01%
	10^5	21.05	21.11	25.26	22.47	2.41	10.74%
	10^6	16.35	16.39	16.75	16.50	0.22	1.34%

Table S8. Sensitivity and Reproducibility Results of the Multiplex qPCR Reaction System

		First times within the batch						Second time within the batch					
	(CFU/ml)	CT			Mean	SD	CV	CT			Mean	SD	CV
C.albicans	10^1	42.34	NoCt	41.67	42.01	0.47	1.13%	41.96	NoCt	43.15	42.56	0.84	1.98%
	10^2	40.69	40.13	39.97	40.26	0.38	0.94%	40.45	40.36	40.22	40.34	0.12	0.29%
	10^3	36.32	35.80	34.87	35.66	0.73	2.06%	34.95	35.10	34.85	34.97	0.13	0.36%
	10^4	33.54	33.65	33.73	33.64	0.10	0.28%	32.47	32.50	32.60	32.52	0.07	0.21%
	10^5	29.04	27.86	28.86	28.59	0.64	2.22%	28.90	28.35	28.32	28.52	0.33	1.14%
	10^6	26.69	26.80	26.46	26.65	0.17	0.65%	26.80	26.70	26.65	26.72	0.08	0.29%
	10^7	23.15	23.35	23.35	23.28	0.12	0.50%	23.45	23.30	23.50	23.42	0.10	0.44%
	10^8	18.92	18.68	19.19	18.93	0.26	1.35%	18.85	18.80	19.05	18.90	0.13	0.70%
C.neoformans	10^1	41.32	40.26	NoCt	40.79	0.75	1.84%	42.83	41.72	NoCt	42.28	0.78	1.86%
	10^2	37.89	37.26	37.75	37.63	0.33	0.88%	38.21	37.88	37.53	37.87	0.34	0.90%
	10^3	34.95	34.21	34.53	34.56	0.37	1.07%	35.13	34.46	35.33	34.97	0.46	1.30%
	10^4	32.44	32.44	33.37	32.75	0.54	1.64%	32.17	33.95	32.84	32.99	0.90	2.73%
	10^5	28.54	28.14	28.05	28.24	0.26	0.92%	27.56	27.39	27.81	27.59	0.21	0.77%
	10^6	25.95	25.89	26.62	26.15	0.41	1.55%	26.54	25.33	26.36	26.08	0.65	2.50%
	10^7	22.78	22.82	21.66	22.42	0.66	2.94%	22.23	23.36	22.56	22.72	0.58	2.56%
	10^8	18.48	18.52	17.96	18.32	0.31	1.71%	18.17	19.82	18.78	18.92	0.83	4.41%
A.fumigatus	10^1	34.88	34.27	34.75	34.63	0.32	0.93%	34.73	34.29	34.57	34.53	0.22	0.64%
	10^2	31.67	29.89	31.64	31.07	1.02	3.28%	31.92	31.64	32.79	32.12	0.60	1.87%
	10^3	27.05	27.11	26.79	26.98	0.17	0.63%	27.27	27.26	27.04	27.19	0.13	0.48%
	10^4	23.44	23.94	23.68	23.69	0.25	1.06%	23.59	23.79	23.68	23.69	0.10	0.42%
	10^5	20.26	20.11	20.12	20.16	0.08	0.42%	20.51	19.96	20.17	20.21	0.28	1.37%
	10^6	17.85	17.41	17.75	17.67	0.23	1.31%	17.97	17.66	17.87	17.83	0.16	0.89%

Table S8. Sensitivity and Reproducibility Results of the Multiplex qPCR Reaction System

		Three times within the batch						Between batches					
	(CFU/ml)	CT			Mean	SD	CV	CT			Mean	SD	CV
C.albicans	10^1	41.74	41.33	42.61	41.89	0.65	1.56%	42.01	42.56	41.89	42.15	0.35	0.84%
	10^2	39.18	38.39	40.18	39.25	0.90	2.29%	40.26	40.34	39.25	39.95	0.61	1.53%
	10^3	34.59	35.13	34.54	34.75	0.33	0.94%	35.66	34.97	34.75	35.13	0.48	1.35%
	10^4	32.61	32.84	31.92	32.46	0.48	1.48%	33.64	32.52	32.46	32.87	0.66	2.02%
	10^5	28.95	29.18	28.69	28.94	0.25	0.85%	28.59	28.52	28.94	28.68	0.22	0.78%
	10^6	26.73	26.96	27.68	27.12	0.50	1.83%	26.65	26.72	27.12	26.83	0.26	0.95%
	10^7	23.18	23.68	22.97	23.28	0.36	1.57%	23.28	23.42	23.28	23.33	0.08	0.34%
	10^8	19.18	18.79	18.58	18.85	0.30	1.62%	18.93	18.90	18.85	18.89	0.04	0.21%
C.neoformans	10^1	42.63	41.52	41.97	42.04	0.56	1.33%	40.79	42.28	42.04	41.70	0.80	1.91%
	10^2	37.65	37.53	38.12	37.77	0.31	0.83%	37.63	37.87	37.77	37.76	0.12	0.32%
	10^3	34.67	34.86	34.81	34.78	0.10	0.28%	34.56	34.97	34.78	34.77	0.21	0.59%
	10^4	33.70	32.97	32.63	33.10	0.55	1.65%	32.75	32.99	33.10	32.95	0.18	0.54%
	10^5	27.47	28.26	28.34	28.02	0.48	1.72%	28.24	27.59	28.02	27.95	0.33	1.20%
	10^6	25.38	25.35	26.34	25.69	0.56	2.19%	26.15	26.08	25.69	25.97	0.25	0.96%
	10^7	21.46	22.57	22.58	22.20	0.64	2.90%	22.42	22.72	22.20	22.45	0.26	1.15%
	10^8	19.36	19.45	18.73	19.18	0.39	2.05%	18.32	18.92	19.18	18.81	0.44	2.35%
A.fumigatus	10^1	35.28	34.64	33.95	34.62	0.67	1.92%	34.63	34.53	34.62	34.60	0.06	0.16%
	10^2	32.18	31.58	32.86	32.21	0.64	1.99%	31.07	32.12	32.21	31.80	0.63	1.99%
	10^3	28.36	28.49	27.49	28.11	0.54	1.93%	26.98	27.19	28.11	27.43	0.60	2.19%
	10^4	22.49	23.18	22.62	22.76	0.37	1.61%	23.69	23.69	22.76	23.38	0.53	2.28%
	10^5	20.38	19.94	20.74	20.35	0.40	1.97%	20.16	20.21	20.35	20.24	0.10	0.49%
	10^6	17.46	18.31	17.28	17.68	0.55	3.11%	17.67	17.83	17.68	17.73	0.09	0.51%

Table S9. Sensitivity and Reproducibility Results of the Multiplex qPCR Reaction System

Strain	CFU/mL	Within the batch		Between batches	
		CT（mean±standard deviation）	Coefficient of Variation（CV/%）	CT（mean±standard deviation）	Coefficient of Variation（CV/%）
C. albicans	1×10^1	42.01±0.47	1.13%	42.15±0.35	0.84%
	1×10^2	40.26±0.38	0.94%	39.95±0.61	1.53%
	1×10^3	35.66±0.73	2.06%	35.13±0.48	1.35%
	1×10^4	33.64±0.10	0.28%	32.87±0.66	2.02%
	1×10^5	28.59±0.64	2.22%	28.68±0.22	0.78%
	1×10^6	26.65±0.17	0.65%	26.83±0.26	0.95%
	1×10^7	23.28±0.12	0.50%	23.33±0.08	0.34%
	1×10^8	18.93±0.26	1.35%	18.89±0.04	0.21%
C. neoformans	1×10^1	40.79±0.75	1.84%	41.70±0.80	1.91%
	1×10^2	37.63±0.33	0.88%	37.76±0.12	0.32%
	1×10^3	34.56±0.37	1.07%	34.77±0.21	0.59%
	1×10^4	32.75±0.54	1.64%	32.95±0.18	0.54%
	1×10^5	28.24±0.26	0.92%	27.95±0.33	1.20%
	1×10^6	26.15±0.41	1.55%	25.97±0.25	0.96%
	1×10^7	22.42±0.66	2.94%	22.45±0.26	1.15%
	1×10^8	18.32±0.31	1.71%	18.81±0.44	2.35%
A. fumigatus	1×10^1	42.01±0.47	1.13%	42.15±0.35	0.84%
	1×10^2	40.26±0.38	0.94%	39.95±0.61	1.53%
	1×10^3	35.66±0.73	2.06%	35.13±0.48	1.35%
	1×10^4	33.64±0.10	0.28%	32.87±0.66	2.02%
	1×10^5	28.59±0.64	2.22%	28.68±0.22	0.78%
	1×10^6	26.65±0.17	0.65%	26.83±0.26	0.95%