

Low-temperature and High-efficiency Solid-Phase Amplification Based on Formamide

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1. Image results of the data

The image results of different annealing temperatures for the FA group and the high-temperature group are shown in Figure S1. Notably, a significant increase in cluster density was observed in the formamide group when annealed at 35°C. For the high-temperature group, similar densities were achieved at 45°C and 50°C. Statistical analysis indicated that the density was slightly higher at 45°C, supporting the choice of 45°C as the annealing temperature.

Based on the optimized annealing temperature, we conducted a test of library concentration, as illustrated in Figure S2. In the formamide group, the cluster density increased with concentration, with a notably denser cluster observed at 4 pM and 6 pM. In contrast, the cluster density in the high-temperature group was significantly sparser compared to the formamide group, peaking at approximately 40 pM.

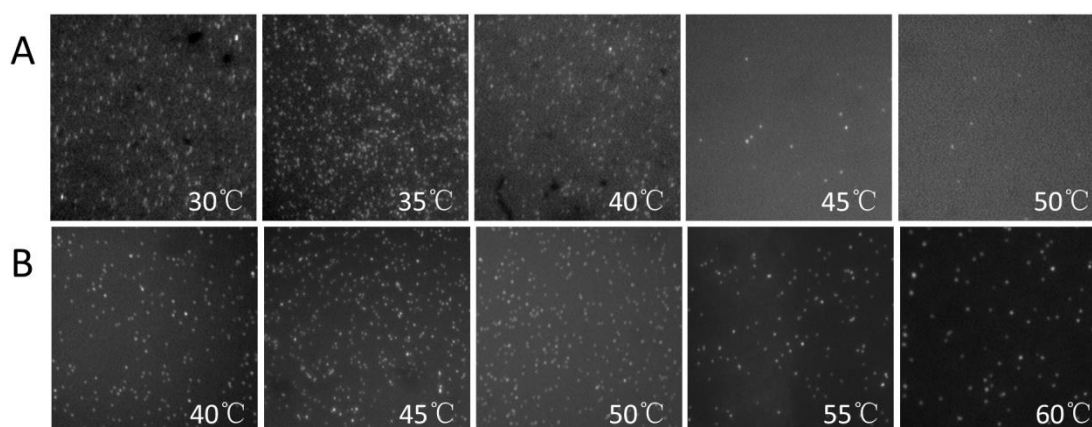


Figure S1. The image results corresponding to two sets of annealing temperatures. (A) Formamide group with a library concentration of 1 pM. (B) High-temperature group with a library concentration of 25 pM.

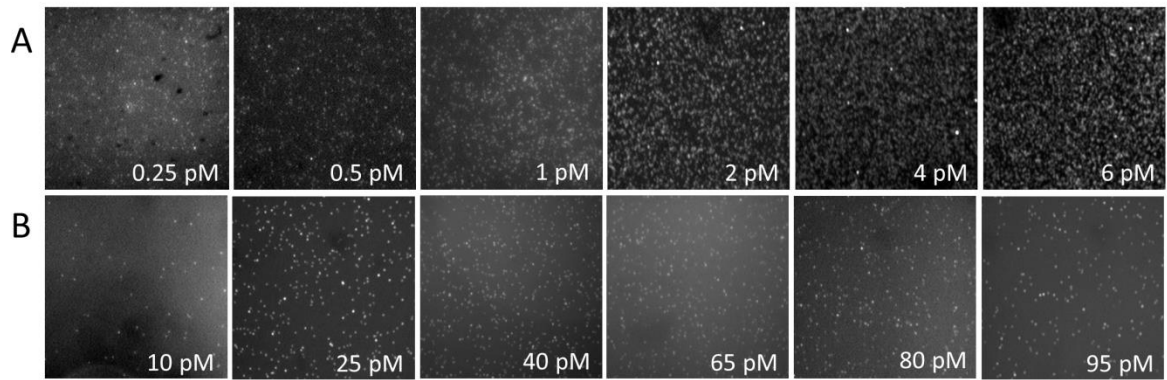


Figure S2. The image results corresponding to two sets of library concentrations. (A) Formamide group with an annealing temperature of 35°C. (B) High-temperature group with an annealing temperature of 45°C.

2. Statistical data of the thermal stability of immobilized primers

Table S1 presents the data on the thermal stability of immobilized primers. We analyzed the statistical differences between the formamide group and the high-temperature group using the Wilcoxon rank-sum test. With a P-value less than 0.01, we concluded that there was a significant statistical difference between the two groups of data.

Table S1. The statistical results of the thermal stability of immobilized primers.

Treatment	Fluorescence Intensity	N	Mean	SD	P
Before PCR	4023.636	9	4219.394889	187.9933737	
	4311.756				
	3839.757				
	4470.987				
	4260.286				
	4115.364				
	4438.568				
	4242.553				
	4271.647				
After PCR (Formamide)	1916.021	9	2311.388444	280.7638659	0.00391**
	1917.064				
	1986.828				
	2471.579				
	2576.642				
	2576.268				
	2249.746				
	2507.071				
	2601.277				
After PCR (High temperature)	1896.402	9	1689.71356	143.30335	
	1849.699				
	1830.197				
	1743.373				

1675.136

1602.176

1592.184

1434.02

1584.235

** $0.001 < p \leq 0.01$