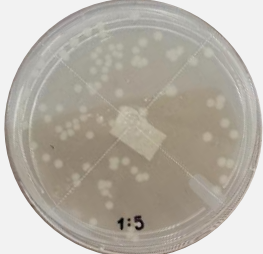
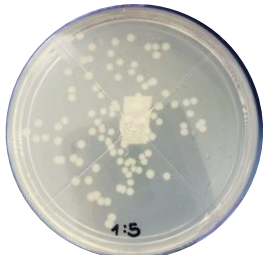
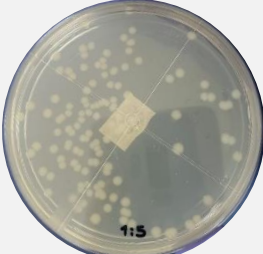


Supplementary data 1. Adjustment of *Salmonella typhimurium* concentration The bacterial concentration in saline solution was adjusted to achieve an absorbance of 0.08 at 625 nm using a microplate reader. After this adjustment, serial dilutions were prepared up to 1:50,000 (final dilution 1:5), were then spread-plated on TSA agar. Table S1 shows the colony recovery from three independent experiments. On average, a concentration of 4.5×10^7 CFU/mL was obtained.

Table S1. Concentration adjustment for the quantification of *Salmonella* cells by Spectrophotometry

Assay	Colony Count	Plate
Experiment 1.	Dilution factor: 50.000 Colonies: 86 UFC 86 \rightarrow 100 μ L X \rightarrow 1000 μ L = 860 850 \times 50.000 = 43000000 4.3 \times 10 ⁷ CFU/mL	
Experiment 2.	Dilution factor: 50.000 Colonies: 92 UFC 92 \rightarrow 100 μ L X \rightarrow 1000 μ L = 920 920 \times 50.000 = 46000000 4.6 \times 10 ⁷ CFU /mL	
Experiment 3.	Dilution factor: 50.000 Colonies: 94 CFU 94 \rightarrow 100 μ L X \rightarrow 1000 μ L = 940 940 \times 50.000 = 47000000 4.7 \times 10 ⁷ CFU mL	

Supplementary data 2. Calculation of final library concentration and number of Sequences

- A stock library concentration of 10 μM (1×10^{-5} mol/L) in nuclease-free water was used.
- A working solution of 100 nM was prepared in a volume of 50 μL . This solution was heated in a thermocycler at 80°C for 2 minutes and then cooled to 25°C until use.
- The total volume of the previously prepared solution was added to 1 mL of the reaction mixture containing *Salmonella typhimurium* cells.
- Based on this procedure, a total of 5 pmol of the library was added, corresponding to 3.011×10^{12} sequences.

The above calculation was verified:

- The molecular weight of the library, as reported by the manufacturer, was 20,266.5 g/mol.
- The amount of library in grams present in the 10 μM stock solution was calculated as follows
 $1 \text{ mol Savory} \rightarrow 20266.5 \text{ g}$
 $1 \times 10^{-5} \text{ mol/L} \rightarrow X$
 $= 0.2026 \text{ g/L}$

Subsequently, the amount present per μL was calculated.

$$0.2026 \text{ g/L} \rightarrow 1 \times 10^6 \mu\text{L}$$

$$X \rightarrow 1 \mu\text{L}$$

$$= 2.026 \times 10^{-7} \text{ g}/\mu\text{L}$$

Since only 0.5 μL was added, the mass added was 1.013×10^{-7} g/ μL or 101.3 ng.

- The number of copies was calculated using the open access tool "ssDNA Copy Number Calculator" (<https://nebiocalculator.neb.com/#!/ssdnaamt>) by entering the number of bases in the sequence (66 bp) along with the mass added (100.7 ng). This calculation estimated 2.99×10^{12} library copies added to the reaction. Subsequent calculations used a value of 3×10^{12} sequences added to the reaction was used.

Supplementary data 3. Composition and properties of buffer systems

The composition of each buffer was used to calculate osmolality and ionic strength. The latter was determined using the formula described below:

$$I = \frac{1}{2} \cdot \sum c_i \cdot Z_i^2$$

The calculations were performed using the LENNTECH website, accessible at:

<https://www.lennotech.com/calculators/activity/activity-coefficient.htm>.

The millimolar (mM) values for each ion were converted to molarity. Since the website does not include the HPO_4^{2-} ion, it was substituted in the SO_4^{2-} field, which has the same charge. Tris, with a pKa of 8.06, was considered ionized since the pH was adjusted to 7.4. The anionic value was added to HCO_3^- , and the cationic value was added to sodium.

Table S2. Composition, Osmolarity, and ionic strength of the evaluated buffer systems

Buffer	Composition	Osmolarity (mOsm/L)	Ionic Strength (mol/L)			
			0 mM MgCl2	1 mM MgCl2	2.5 mM MgCl2	5 mM MgCl2
PBS	137mM NaCl 2.7mM KCl 10mM Na_2HPO_4 2mM KH_2PO_4	313.4	0.161	0.163	0.168	0.176
TBS	10mM Tris-Cl 150mM NaCl 5mM KCl	333	0.160	0.163	0.169	0.175
TK	20 mM Tris-Cl 50 mM KCl	140	0.06	0.063	0.067	0.075

Supplementary data 4. Results of sequence quantification by qPCR: Influence of the buffer system

Table S3. Number of copies recovered from F1 and F2 fractions of the PBS, TBS, and TK buffer systems in two independent experiments.

Experiment	Replicate	Number of copies in 2 μ L of sample					
		PBS		TBS		BI	
		F1	F2	F1	F2	F1	F2
No. 1	1	1.28E+07	3.02E+05	9.38E+06	2.57E+05	6.11E+06	3.26E+05
	2	1.05E+07	2.57E+05	8.39E+06	3.60E+05	1.37E+07	3.8E+05
	3	5.63E+06	1.91E+05	9.67E+06	3.45E+05	8.47E+06	6.44E+05
No. 2	1	5.73E+05	1.99E+04	2.91E+04	3.8E+04	2.06E+05	7.85E+04
	2	2.56E+06	5.02E+02	8.05E+04	3.31E+05	4.26E+05	8.53E+04
	3	5.22E+05	N/A	1.02E+05	9.27E+04	2.5E+04	8.70E+04
	Mean	5.42E+06	1.54E+05	4.61E+06	2.37E+05	4.83E+06	2.67E+05
	SD	5.21E+06	1.37E+05	4.99E+06	1.39E+05	5.62E+06	2.28E+05

Values represent the number of copies found in 2 μ L of each fraction. SD: Standard deviation. Since the methods used to obtain the F1 and F2 fractions were different, the total number of copies in the final volume of each fraction was calculated. This was done by transforming the values as follows:

$$Total\ Sequences = \frac{(Number\ of\ copies * Dilution\ Factor) * Final\ Volume}{2}$$

Number of Copies: Value obtained from qPCR quantification.

Dilution Factor: Dilution applied to the sample for quantification. For all cases, this was 100.

Final Volume: For F1, it was 950 μ L, and for F2, it was 20 μ L.

2: Amount of sample used from each fraction for qPCR.

Table S4. Total number of copies adjusted to the recovered volume of each fraction.

SD: Standard Deviation.

Experiment	Replicate	Number of copies					
		PBS		TBS		BI	
		F1	F2	F1	F2	F1	F2
No.1	1	6.08E+11	3.02E+08	4.45E+11	2.57E+08	2.90E+11	3.26E+08
	2	4.97E+11	2.57E+08	3.98E+11	3.60E+08	6.51E+11	3.80E+08
	3	2.67E+11	1.91E+08	4.59E+11	3.45E+08	4.03E+11	6.44E+08
No. 2	1	2.72E+10	1.99E+07	1.38E+09	3.8E+07	9.79E+09	7.85E+07
	2	1.22E+11	5.02E+05	3.82E+09	3.31E+08	2.02E+10	8.53E+07
	3	2.48E+10		4.84E+09	9.27E+07	1.19E+09	8.7E+07
	Mean	2.58E+11	1.54E+08	2.19E+11	2.37E+08	2.29E+11	2.67E+08
	SD	2.47E+11	1.37E+08	2.37E+11	1.39E+08	2.67E+11	2.28E+08

SD: Standard Deviation.

Supplementary data 5. Results of sequence quantification by qPCR: influence of MgCl₂.

Table S5. Number of copies recovered from F1 and F2 fractions of the TBS buffer system without supplementation or with 1, 2.5, and 5 mM of MgCl₂ in two independent experiments.

Experiment	Replicate	Concentrations MgCl ₂ (mM)							
		0		1		2,5		5	
		F1	F2	F1	F2	F1	F2	F1	F2
1	1	9,20E+05	8,49E+05	6,39E+06	7,39E+05	3,27E+06	9,51E+05	3,22E+06	9,62E+05
	2	4,34E+06	7,88E+05	1,22E+07	9,44E+05	1,06E+07	7,30E+05	7,09E+06	8,81E+05
2	3	4,62E+06	7,30E+05	3,14E+06	2,09E+06	2,20E+06	9,05E+05	6,38E+06	8,62E+05
	4	7,74E+06	2,48E+05	4,24E+06	9,02E+05	6,57E+06	8,70E+05	4,97E+06	8,86E+05
Mean		4.40E+06	6.54E+05	6.49E+06	1.17E+06	4.17E+06	8.64E+05	5.41E+06	8.98E+05
SD		2.79E+06	2.75E+05	4.02E+06	6.21E+05	4.39E+06	9.50E+04	1.71E+06	4.43E+04

The values represent the number of copies found in 2 µL of each fraction. SD: Standard Deviation.

Table S6. Total Number of Copies Adjusted to the Recovered Volume of Each Fraction.

Experiment	Replicate	Concentrations MgCl ₂ (mM)							
		0		1		2,5		5	
		F1	F2	F1	F2	F1	F2	F1	F2
1	1	4.37E+10	8.49E+08	3.01E+11	7.39E+08	1.55E+11	9.51E+08	1.53E+11	9.62E+08
	2	2.06E+11	7.88E+08	5.78E+11	9.44E+08	5.02E+11	7.30E+08	3.37E+11	8.81E+08
2	3	2.19E+11	7.30E+08	1.49E+11	2.09E+09	1.04E+11	9.05E+08	3.03E+11	8.62E+08
	4	3.67E+11	2.48E+08	2.01E+11	9.02E+08	3.12E+10	8.70E+08	2.36E+11	8.86E+08
Mean		2.09E+11	6.54E+08	3.08E+11	1.17E+09	1.98E+11	8.645E+08	2.57E+11	8.98E+08
SD		1.32E+11	2.75E+08	1.91E+11	6.21E+08	2.09E+11	9.50E+07	8.10E+10	4.43E+07

SD: Standard Deviation.

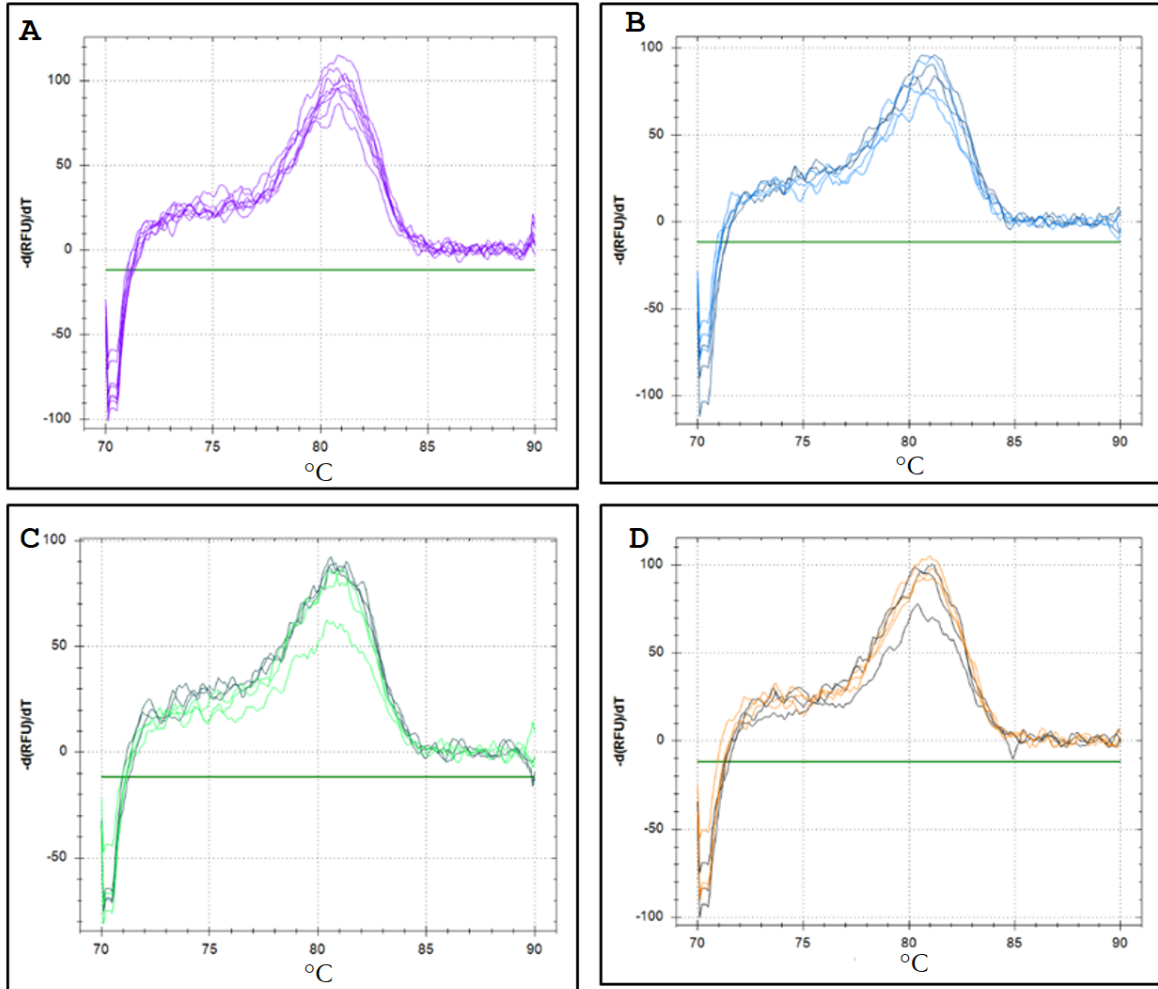


Figure S1. Dissociation peaks obtained during qPCR amplification of F1 and F2 fractions. Results are shown for the quantification curve generated from the library (A) and amplifications recovered from PBS (B), TBS (C), and TK (D). Each line represents an amplified sample. For the buffers (B, C, and D), light lines correspond to F1, while dark lines represent F2. No distinct peaks were observed for any of the buffers analyzed

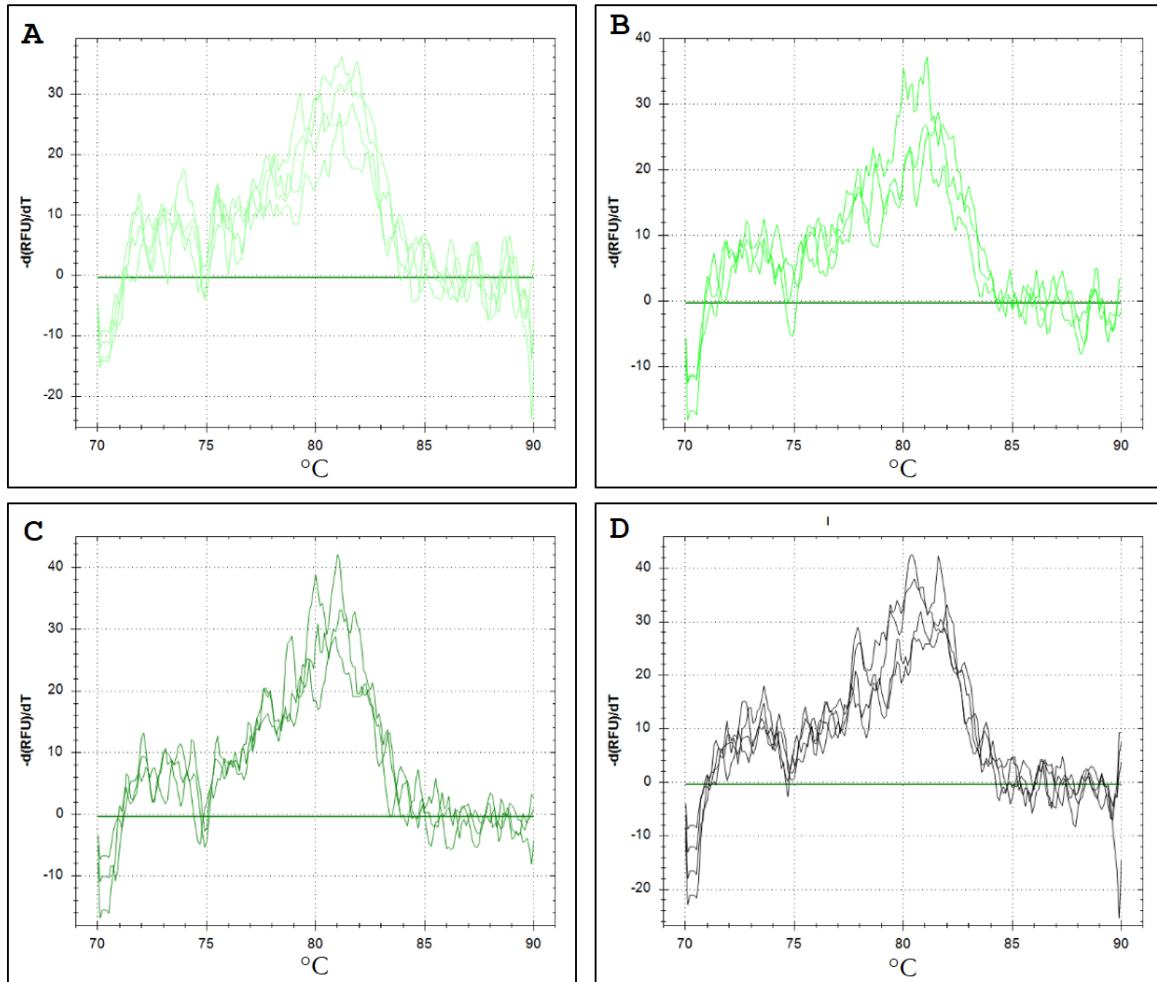


Figure S2. Dissociation Peaks Obtained After qPCR Amplification of F2 Fractions. Dissociation patterns are shown for amplifications recovered at different MgCl_2 concentrations: 0 mM (A), 1 mM (B), 2.5 mM (C), and 5 mM (D). Each line represents an amplified sample.