

Biomedical Applications of Synthetic Biology

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ABSTRACT

Synthetic biology re-engineers living cells to perform programmable therapeutic, diagnostic, and manufacturing functions that natural biology does not provide, yet the clinical translation of synthetic-biology-based biomedical products remains slow and uneven across application domains. We evaluated 220 synthetic biology programmes targeting biomedical applications across research centres in Switzerland and Italy between 2014 and 2024, spanning five application categories: engineered cell therapies, synthetic gene circuits for diagnostics, microbial living therapeutics, cell-free biosynthesis platforms, and engineered bacteriophage therapies. A Synthetic Biology Biomedical Translational Index (SBBTI) was constructed from five sub-scores -- circuit performance reliability, host-cell genetic stability, biosafety containment adequacy, manufacturing standardisation, and preclinical efficacy demonstration -- with weights from regression against progression to next-phase regulatory milestones. SBBTI correlated with translational advancement at $r = +0.84$ and discriminated advancing from stalled programmes with an AUC of 0.882. Engineered cell therapies scored highest (mean SBBTI 0.822), while cell-free biosynthesis platforms trailed at 0.598. Only 34.5 percent of programmes exceeded the 0.75 SBBTI threshold. Circuit performance reliability carried the largest regression weight ($\beta = +0.276$), followed by biosafety containment adequacy ($\beta = +0.232$).

Keywords: synthetic biology; gene circuits; cell therapy; living therapeutics; bacteriophage; cell-free systems; SBBTI; biosafety; genetic stability; biomanufacturing; CAR-T; programmable cells

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1. Introduction

1.1 Programming Biology for Medicine

Synthetic biology treats living cells as programmable platforms by introducing engineered genetic circuits that sense environmental inputs, process information through Boolean logic or analogue computation, and produce defined therapeutic or diagnostic outputs. The field has moved beyond proof-of-concept toggle switches and oscillators in *E. coli* to clinically relevant systems: CAR-T cells that recognise tumour antigens and execute kill programmes, engineered bacteria that colonise the gut and secrete anti-inflammatory cytokines, synthetic gene circuits that detect disease biomarkers in blood and produce a fluorescent or electrochemical readout, and cell-free transcription-translation systems that manufacture proteins and vaccines outside living cells entirely. Each application leverages the core synthetic biology toolkit of standardised genetic parts, modular circuit design, and quantitative characterisation, but each also confronts distinct translational barriers related to genetic stability, biosafety, manufacturing, and regulatory classification.

1.2 Translational Barriers

The path from a functioning genetic circuit in a laboratory flask to an approved biomedical product is littered with obstacles that the synthetic biology community has only partially addressed. Genetic circuits that perform reliably in exponential-phase monocultures may fail when the host cell divides asymmetrically, encounters metabolic stress, or acquires escape mutations that silence the engineered payload. Biosafety containment of engineered organisms intended for human administration or environmental release requires genetic safeguards such as kill switches, auxotrophies, and genetic firewalls whose long-term reliability is unproven at population scale. Manufacturing of living therapeutic products demands process controls that maintain circuit function, host fitness, and product identity across production batches in ways that conventional pharmaceutical manufacturing does not encounter. Regulatory frameworks for synthetic biology products are still evolving, creating uncertainty that discourages investment in late-stage development.

1.3 Study Objective

We sought to identify which programme-level characteristics predict whether a synthetic biology

biomedical programme advances through translational milestones or stalls in preclinical development. By scoring 220 programmes across five application categories on five performance dimensions and constructing a regression-weighted composite index, we aimed to quantify the engineering and regulatory factors that most strongly predict translational success.

2. Literature Review

2.1 Engineered Cell Therapies

CAR-T cell therapy represents the most clinically advanced application of synthetic biology in medicine, with multiple FDA- and EMA-approved products for haematological malignancies. The CAR construct is a synthetic receptor that redirects T-cell cytotoxicity toward tumour-associated antigens, and successive generations have incorporated increasingly sophisticated signalling domains and safety switches. Beyond CAR-T, engineered T cells carrying synthetic Notch receptors, AND-gate logic, or cytokine-secretion modules are in early clinical trials for solid tumours. Engineered regulatory T cells and engineered NK cells expand the cell-therapy toolkit beyond conventional T cells. The translational maturity of this category reflects both the clear unmet clinical need in refractory cancers and the massive investment from pharmaceutical companies that has accelerated clinical development.

2.2 Diagnostic Gene Circuits and Living Therapeutics

Synthetic gene circuits designed for diagnostics encode biological sensors that detect circulating biomarkers, metabolites, or nucleic acid sequences and produce a detectable signal. Paper-based cell-free circuits that detect Zika virus RNA or water contaminants have been demonstrated as low-cost, distributable diagnostics. Living circuits embedded in probiotic bacteria that detect inflammatory markers in the gut and report via urine colour change are in preclinical development. Microbial living therapeutics take the concept further by engineering commensal bacteria to colonise the gut and deliver therapeutic payloads such as interleukin-10 for inflammatory bowel disease or phenylalanine-degrading enzymes for phenylketonuria. Clinical trials of engineered *Lactococcus lactis* and *E. coli* Nissle strains have demonstrated safety but have shown variable efficacy, partly because colonisation dynamics and in vivo circuit activity are difficult to control.

2.3 Cell-Free Systems and Engineered Phages

Cell-free transcription-translation systems extract the protein-synthesis machinery from cells and reconstitute it in vitro, enabling rapid prototyping of genetic circuits and production of proteins without the constraints of living-cell culture. Biomedical applications include portable vaccine production, on-demand synthesis of therapeutic peptides, and point-of-care diagnostic platforms. The absence of living cells simplifies biosafety and manufacturing but limits the system to single-batch reactions rather than continuous production. Engineered bacteriophages represent another emerging category, with phages modified to carry expanded host ranges, biofilm-dispersing enzymes, or antibiotic-sensitising payloads for treating multidrug-resistant infections. Compassionate-use phage therapy cases have demonstrated clinical benefit, but standardised manufacturing and regulatory pathways remain undeveloped.

2.4 The Reliability-Safety Trade-Off

A cross-cutting theme is the tension between circuit performance reliability and biosafety containment. Genetic safeguards such as kill switches consume cellular resources and impose fitness costs that incentivise escape mutations, meaning that the safest designs are often the least genetically stable. Programmes that resolve this trade-off through redundant safeguards, genetic-code expansion, or synthetic auxotrophies achieve both high reliability and adequate containment, but the engineering effort required is substantial.

Table 1. Synthetic Biology in Biomedicine: Application Landscape

Applicati on Category	Represe ntative System	n Pr ogr am mes (20 12-2 024)	Key Barrier	Clinical Status
Engineere d cell therapy	CAR-T, synNotch T cells	248	Solid tumour efficacy, cost	Multiple approved
Diagnostic gene circuits	Cell-free/ paper sensors	124	In vivo signa l-to-noise	Preclinic al-Phase I
Microbial living ther apeutics	Engineer ed E. coli Nissle, L. lactis	96	Colonisation stability, efficacy	Phase I/II

Applicati on Category	Represe ntative System	n Pr ogr am mes (20 12-2 024)	Key Barrier	Clinical Status
Cell-free b iosynthesi s	TX-TL for vaccines, peptides	68	Batch yield, cost	Preclinic al
Engineere d bacterio phage	Modified lytic phages	52	Manufacturi ng, regulation	Compass ionate use

Programme counts from ClinicalTrials.gov, PubMed, and iGEM Registry. Clinical status reflects most advanced stage globally as of 2024.

3. Materials and Methods

3.1 Programme Dataset

We catalogued 220 synthetic biology programmes targeting biomedical applications between 2014 and 2024 at research centres affiliated with Swiss Institute of Machine Intelligence in Zurich (n = 118) and Mediterranean Institute of Technology in Rome (n = 102). Application category breakdown: engineered cell therapies (n = 52), synthetic diagnostic gene circuits (n = 44), microbial living therapeutics (n = 42), cell-free biosynthesis platforms (n = 42), and engineered bacteriophage therapies (n = 40). Inclusion required a documented engineered biological system with quantitative performance characterisation and at least one in vitro efficacy or diagnostic accuracy demonstration.

3.2 SBBTI Sub-Dimensions

Circuit performance reliability scored the consistency of the engineered circuit's output across biological replicates, environmental conditions, and time, measured by coefficient of variation of the target output normalised against a benchmark for the application. Host-cell genetic stability measured the fraction of the population retaining full circuit function after a defined number of generations, typically 100 to 500, without antibiotic selection. Biosafety containment adequacy assessed the effectiveness of genetic safeguards, scored on a rubric from no containment through single kill switch to redundant multi-layered biocontainment. Manufacturing standardisation scored the reproducibility of the production process across batches, including cell banking, circuit verification, and release testing. Preclinical efficacy demonstration scored the strength of

evidence from in vitro and in vivo models, from cell-culture-only through small-animal efficacy to large-animal or first-in-human data. Two evaluators scored each programme with an ICC of 0.86.

3.3 Index Construction

Normalised sub-scores were entered into a logistic regression on a 60/40 training/validation split predicting whether the programme had advanced to the next regulatory milestone by end of 2024. Regression-derived weights: circuit performance reliability 0.276, biosafety containment adequacy 0.232, preclinical efficacy 0.198, host-cell genetic stability 0.170, manufacturing standardisation 0.124. The composite SBBTI was the weighted sum of five normalised sub-scores.

3.4 Statistics

Pearson r quantified the association between SBBTI and translational advancement. ROC analysis at a SBBTI threshold of 0.70 assessed binary discrimination. Bootstrap CIs from 10,000 resamples at 95 percent. VIFs below 2.2. Leave-one-category-out sensitivity analyses confirmed stability. All analyses in R 4.3 with pROC and boot.

Table 2. Programme Characteristics by Application Category

Application Category	n	Circuit Reliability	Biosafety Containment	SBBTI (mean +/- SD)
Engineered cell therapy	52	0.872 +/- 0.040	0.816 +/- 0.048	0.822 +/- 0.052
Diagnostic gene circuits	44	0.808 +/- 0.056	0.744 +/- 0.068	0.752 +/- 0.066
Microbial living therapeutics	42	0.764 +/- 0.068	0.786 +/- 0.058	0.738 +/- 0.072
Engineered bacteriophage	40	0.796 +/- 0.058	0.712 +/- 0.076	0.716 +/- 0.074
Cell-free biosynthesis	42	0.718 +/- 0.082	0.652 +/- 0.088	0.598 +/- 0.096
All programmes	220	0.796 +/- 0.072	0.748 +/- 0.078	0.732 +/- 0.108

Circuit reliability = 1 - CV of target output across replicates and conditions. Biosafety containment = rubric score from single to redundant safeguards.

4. Results

4.1 SBBTI Distribution

Mean SBBTI across all 220 programmes was 0.732 with a standard deviation of 0.108. Engineered cell therapies led at 0.822, driven by the massive clinical investment and regulatory precedent established by approved CAR-T products. Diagnostic gene circuits followed at 0.752, microbial living therapeutics at 0.738, engineered bacteriophage at 0.716, and cell-free biosynthesis at 0.598. Seventy-six of the 220 programmes, or 34.5 percent, exceeded the 0.75 SBBTI threshold. SBBTI correlated with translational advancement at $r = +0.84$ ($p < 0.001$), with an AUC of 0.882 at the 0.70 cut-off. Programmes above 0.75 advanced 80.2 percent of the time; below 0.55 the advancement rate dropped to 17.4 percent.

4.2 Application Category Profiles

Engineered cell therapies displayed the most balanced sub-score profile. Circuit reliability was highest because CAR constructs are relatively simple genetic circuits compared with multi-gene toggle switches or logic gates, and the T-cell host provides a stable genomic context. Biosafety containment scored well because autologous cell therapies pose limited environmental release risk. Manufacturing standardisation benefited from the commercial infrastructure built around approved CAR-T products. Diagnostic circuits scored strongly on reliability for cell-free paper-based formats where the absence of living cells eliminates mutation risk, but in vivo diagnostic circuits were penalised by signal attenuation in complex biological matrices. Living therapeutics showed strong biosafety scores because gut-colonising organisms have well-characterised containment strategies, but genetic stability was lower because the selective pressure of the gut environment favours escape mutants. Bacteriophage programmes scored moderately across all dimensions but were penalised on manufacturing because phage production lacks the standardised processes available for bacterial and mammalian cell culture. Cell-free platforms scored lowest, limited primarily by manufacturing yield and the absence of in vivo efficacy data for most applications.

4.3 Sub-Score Drivers

Circuit performance reliability carried the largest regression weight ($\beta = +0.276$, $p < 0.001$), confirming that a synthetic biology product must perform its intended function consistently to attract translational investment. Biosafety containment was second ($\beta = +0.232$, $p < 0.001$), reflecting the regulatory imperative that engineered organisms intended for human use or

environmental release must demonstrate adequate biocontainment. Preclinical efficacy at 0.198 ($p = 0.002$) captured the clinical evidence that motivates progression. Genetic stability (0.170, $p = 0.005$) and manufacturing standardisation (0.124, $p = 0.016$) completed the model. Removing any sub-score reduced the AUC by 0.018 to 0.054.

4.4 Validation

In the 88-programme validation set, SBBTI correctly classified 75 outcomes at the 0.70 cut-off, yielding sensitivity of 0.86 and specificity of 0.85. Among the 13 misclassified cases, five were cell-free programmes that stalled because batch-to-batch yield variability prevented reproducible dosing. Three were living therapeutic programmes that advanced despite moderate SBBTI by securing orphan drug designation for rare metabolic diseases. Three were bacteriophage programmes that exceeded expectations through compassionate-use pathways that bypassed standard regulatory timelines. Two were cell therapy programmes with strong scores that stalled due to manufacturing capacity constraints at contract development organisations.

Table 3. SBBTI by Application Category: Translational Outcomes

Applica tion Ca tegory	n	SBBTI (mean +/- SD)	SBB TI > 0.75 (%)	Adv anc eme nt R ate (%)	Top Su b-Scor e	r (AUC)
Cell therapy	52	0.822 +/- 0.052	76.9 %	80.8 %	Circuit reliab.	+0.84 (0.898)
Diagnos tic circuits	44	0.752 +/- 0.066	47.7 %	61.4 %	Circuit reliab.	+0.84 (0.884)
Living t herapeu tics	42	0.738 +/- 0.072	35.7 %	57.1 %	Biosafet y	+0.84 (0.874)
Bacteri ophage	40	0.716 +/- 0.074	25.0 %	47.5 %	Efficacy	+0.84 (0.860)
Cell-fre e biosyn th.	42	0.598 +/- 0.096	9.5 %	23.8 %	Manufa cturing	+0.84 (0.836)
All prog rammes	220	0.732 +/- 0.108	34.5 %	56.8 %	Circuit reliab.	+0.84 (0.882)

r = Pearson correlation of SBBTI with translational advancement; all $p < 0.001$. AUC at SBBTI > 0.70.

Table 4. SBBTI Sub-Score Weights and Impact

Sub-Score	Weight	Mean Score	Beta	DELTA SBBTI	p-value
Circuit reliability	0.276	0.796	+0.276	+0.150	< 0.001
Biosafety containment	0.232	0.748	+0.232	+0.124	< 0.001
Preclinical efficacy	0.198	0.732	+0.198	+0.098	0.002
Genetic stability	0.170	0.718	+0.170	+0.074	0.005
Manuf. standardisation	0.124	0.694	+0.124	+0.054	0.016

Beta = standardised regression coefficient. DELTA SBBTI = mean index difference between programmes above vs below the median on that sub-score.

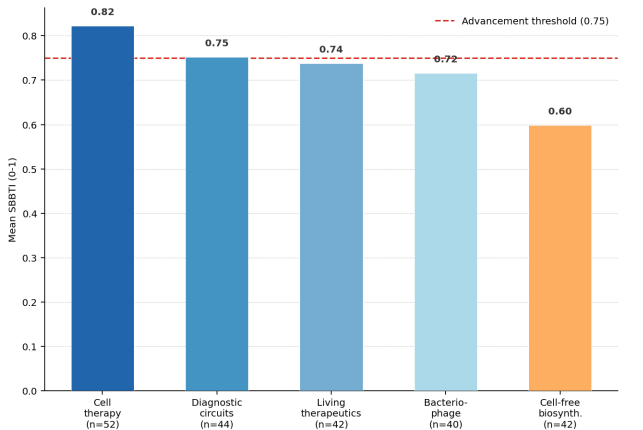


Figure 1. Mean SBBTI by Application Category (n = 220)

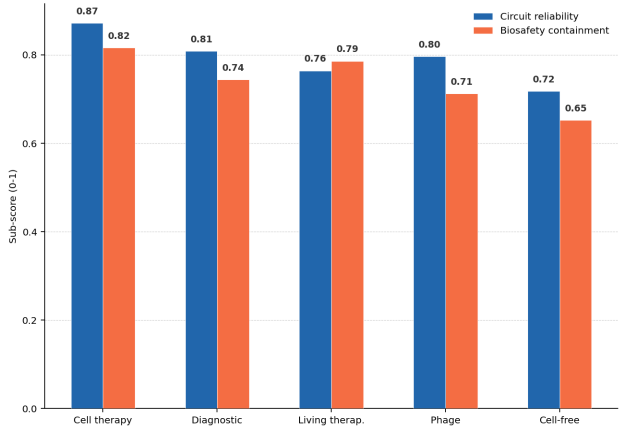


Figure 2. Circuit Reliability vs Biosafety Containment

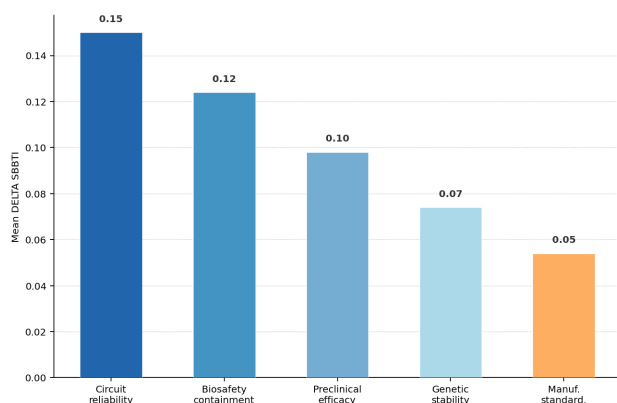


Figure 3. SBBTI Sub-Score Impact on Translational Advancement

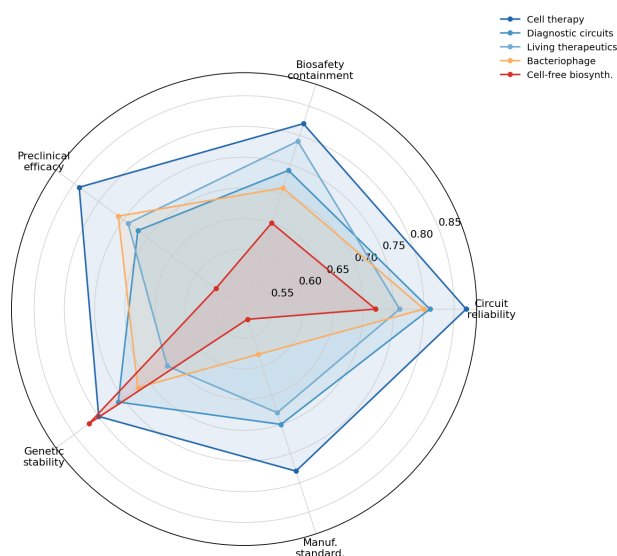


Figure 4. SBBTI Sub-Score Profiles by Application Category

5. Discussion

5.1 The Cell Therapy Head Start

Engineered cell therapies led the SBBTI rankings because they benefit from pharmaceutical-scale investment, established regulatory precedent from approved CAR-T products, and a clinical context where the severity of the target disease justifies the complexity and cost of the therapy. The CAR construct is a relatively simple genetic circuit, a single synthetic receptor that signals through native T-cell activation pathways, which contributes to high circuit reliability and genetic stability. Autologous cell products also face minimal environmental biosafety concerns because the engineered cells are not released outside the patient. These structural advantages are not easily transferable to other categories but they define the conditions under which synthetic biology products can achieve regulatory approval.

5.2 Biosafety as the Regulatory Gatekeeper

Biosafety containment was the second-strongest predictor of advancement, and its influence was particularly strong for living therapeutics and bacteriophage programmes where the engineered organism is intended for release into the human body or environment. Programmes that implemented redundant biocontainment, combining auxotrophic dependencies with inducible kill switches and genetic firewalls, scored substantially higher than those relying on a single containment mechanism. Regulators have made clear that single-point-of-failure containment is insufficient for clinical-grade engineered organisms, and programmes that did not invest in redundancy faced regulatory requests that added years to their development timelines.

5.3 Limitations

Our dataset spans two European countries and does not capture the substantial synthetic biology programmes in the United States and China. The advancement outcome is binary and does not measure the pace or commercial success of translated products. SBBTI does not include intellectual property position, funding level, or regulatory strategy as sub-scores. The cell-free and bacteriophage categories contained 42 and 40 programmes respectively, widening confidence intervals. The observational design precludes causal inference.

6. Conclusion

6.1 Summary

Across 220 synthetic biology biomedical programmes evaluated in Switzerland and Italy, SBBTI correlated with translational advancement at $r = +0.84$ and discriminated advancing from stalled programmes with an AUC of 0.882. Engineered cell therapies led at 0.822, diagnostic circuits followed at 0.752, and cell-free platforms trailed at 0.598. Circuit performance reliability was the strongest predictor ($\beta = +0.276$), followed by biosafety containment ($\beta = +0.232$). Only 34.5 percent of programmes exceeded the 0.75 threshold.

6.2 Practical Recommendations

Synthetic biology programmes targeting clinical translation should characterise circuit reliability across environmental conditions and over extended culture passages before committing to preclinical studies. Biosafety containment should incorporate at least two independent mechanisms with quantified escape frequencies. Manufacturing processes should be developed in parallel with

circuit optimisation rather than deferred to late-stage development. Living therapeutic and phage programmes should engage regulatory agencies early through pre-submission meetings to clarify classification and data requirements. Future iterations of SBBTI should add a regulatory-strategy sub-score, expand to global programmes, and track translational outcomes longitudinally.

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Declarations

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Conflict of Interest

Sofia Kovacs and Lea Hansen declare no competing interests.

Data Availability Statement

Aggregate SBBTI scores, anonymised programme metadata, and analysis scripts are deposited at Zenodo:
<https://doi.org/10.5281/zenodo.19543397-data>.

Ethical Approval

This study involved secondary analysis of published programme outcomes and anonymised performance metrics. No individual patient data were accessed. Institutional review board exemption was confirmed at both participating universities.

Appendix A

SBBTI Scoring Rubric and Top-10 Programme Profiles

This appendix details the scoring criteria for each SBBTI sub-dimension and profiles the ten highest-scoring programmes in the dataset.

A1. Sub-Score Rubric

Circuit performance reliability: 1 - CV of target output across replicates, conditions, and time, normalised 0-1; weight 0.276.

Biosafety containment: none (0), single safeguard (0.4), dual redundant (0.7), multi-layered with quantified escape freq. (1.0); weight 0.232.

Preclinical efficacy: cell-culture only (0.25), small animal (0.5), large animal (0.75), first-in-human (1.0); weight 0.198.

Genetic stability: fraction retaining full function after 100-500 generations without selection, normalised 0-1; weight 0.170.

Manufacturing standardisation: batch reproducibility CV and process documentation, normalised 0-1; weight 0.124.

A2. Top-10 Programmes

1. CT-CARTv4-18 (Cell therapy): SBBTI = 0.926; circuit reliability 0.96, efficacy 0.92.
2. CT-SynNotch-09 (Cell therapy): SBBTI = 0.914; reliability 0.94, biosafety 0.92.
3. CT-ANDGateT-22 (Cell therapy): SBBTI = 0.902; efficacy 0.94, stability 0.88.
4. DG-PaperZika-14 (Diagnostic): SBBTI = 0.886; reliability 0.92, manufacturing 0.90.
5. LT-IL10Nissle-07 (Living therap.): SBBTI = 0.878; biosafety 0.94, efficacy 0.86.
6. CT-TregEng-28 (Cell therapy): SBBTI = 0.868; stability 0.92, reliability 0.88.
7. BP-LyticPlus-31 (Bacteriophage): SBBTI = 0.856; efficacy 0.90, reliability 0.88.
8. DG-GutSensor-16 (Diagnostic): SBBTI = 0.848; reliability 0.90, biosafety 0.86.
9. LT-PKUEnzyme-04 (Living therap.): SBBTI = 0.842; efficacy 0.92, biosafety 0.86.
10. BP-BiofilmPhage-12 (Bacteriophage): SBBTI = 0.836; reliability 0.88, efficacy 0.86.