**Table S1.** Buffers and additives used in the thermofluor protein stability screen

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| No. | Buffer | Additive | No. | Buffer | Additive |
| 1 | 0.2 M MES (pH 5.5) | - | 41 | 0.2 M HEPES (pH 7.5) | - |
| 2 | 0.2 M MES (pH 5.5) | 0.05 M NaCl | 42 | 0.2 M HEPES (pH 7.5) | 0.05 M NaCl |
| 3 | 0.2 M MES (pH 5.5) | 0.1 M NaCl | 43 | 0.2 M HEPES (pH 7.5) | 0.1 M NaCl |
| 4 | 0.2 M MES (pH 5.5) | 0.2 M NaCl | 44 | 0.2 M HEPES (pH 7.5) | 0.2 M NaCl |
| 5 | 0.2 M MES (pH 5.5) | 0.05 M KCl | 45 | 0.2 M HEPES (pH 7.5) | 0.05 M KCl |
| 6 | 0.2 M MES (pH 5.5) | 0.1 M KCl | 46 | 0.2 M HEPES (pH 7.5) | 0.1 M KCl |
| 7 | 0.2 M MES (pH 5.5) | 0.2 M KCl | 47 | 0.2 M HEPES (pH 7.5) | 0.2 M KCl |
| 8 | 0.2 M MES (pH 5.5) | 0.05 M MgCl2+ | 48 | 0.2 M HEPES (pH 7.5) | 0.05 M MgCl2+ |
| 9 | 0.2 M MES (pH 5.5) | 0.1 M MgCl2+ | 49 | 0.2 M HEPES (pH 7.5) | 0.1 M MgCl2+ |
| 10 | 0.2 M MES (pH 5.5) | 0.2 M MgCl2+ | 50 | 0.2 M HEPES (pH 7.5) | 0.2 M MgCl2+ |
| 11 | 0.2 M Bis-Tris (pH 6.0) | - | 51 | 0.2 M Tris (pH 8.0) | - |
| 12 | 0.2 M Bis-Tris (pH 6.0) | 0.05 M NaCl | 52 | 0.2 M Tris (pH 8.0) | 0.05 M NaCl |
| 13 | 0.2 M Bis-Tris (pH 6.0) | 0.1 M NaCl | 53 | 0.2 M Tris (pH 8.0) | 0.1 M NaCl |
| 14 | 0.2 M Bis-Tris (pH 6.0) | 0.2 M NaCl | 54 | 0.2 M Tris (pH 8.0) | 0.2 M NaCl |
| 15 | 0.2 M Bis-Tris (pH 6.0) | 0.05 M KCl | 55 | 0.2 M Tris (pH 8.0) | 0.05 M KCl |
| 16 | 0.2 M Bis-Tris (pH 6.0) | 0.1 M KCl | 56 | 0.2 M Tris (pH 8.0) | 0.1 M KCl |
| 17 | 0.2 M Bis-Tris (pH 6.0) | 0.2 M KCl | 57 | 0.2 M Tris (pH 8.0) | 0.2 M KCl |
| 18 | 0.2 M Bis-Tris (pH 6.0) | 0.05 M MgCl2+ | 58 | 0.2 M Tris (pH 8.0) | 0.05 M MgCl2+ |
| 19 | 0.2 M Bis-Tris (pH 6.0) | 0.1 M MgCl2+ | 59 | 0.2 M Tris (pH 8.0) | 0.1 M MgCl2+ |
| 20 | 0.2 M Bis-Tris (pH 6.0) | 0.2 M MgCl2+ | 60 | 0.2 M Tris (pH 8.0) | 0.2 M MgCl2+ |
| 21 | 0.2 M Bis-Tris (pH 6.5) | - | 61 | 0.2 M Tris (pH 8.5) | - |
| 22 | 0.2 M Bis-Tris (pH 6.5) | 0.05 M NaCl | 62 | 0.2 M Tris (pH 8.5) | 0.05 M NaCl |
| 23 | 0.2 M Bis-Tris (pH 6.5) | 0.1 M NaCl | 63 | 0.2 M Tris (pH 8.5) | 0.1 M NaCl |
| 24 | 0.2 M Bis-Tris (pH 6.5) | 0.2 M NaCl | 64 | 0.2 M Tris (pH 8.5) | 0.2 M NaCl |
| 25 | 0.2 M Bis-Tris (pH 6.5) | 0.05 M KCl | 65 | 0.2 M Tris (pH 8.5) | 0.05 M KCl |
| 26 | 0.2 M Bis-Tris (pH 6.5) | 0.1 M KCl | 66 | 0.2 M Tris (pH 8.5) | 0.1 M KCl |
| 27 | 0.2 M Bis-Tris (pH 6.5) | 0.2 M KCl | 67 | 0.2 M Tris (pH 8.5) | 0.2 M KCl |
| 28 | 0.2 M Bis-Tris (pH 6.5) | 0.05 M MgCl2+ | 68 | 0.2 M Tris (pH 8.5) | 0.05 M MgCl2+ |
| 29 | 0.2 M Bis-Tris (pH 6.5) | 0.1 M MgCl2+ | 69 | 0.2 M Tris (pH 8.5) | 0.1 M MgCl2+ |
| 30 | 0.2 M Bis-Tris (pH 6.5) | 0.2 M MgCl2+ | 70 | 0.2 M Tris (pH 8.5) | 0.2 M MgCl2+ |
| 31 | 0.2 M HEPES (pH 7.0) | - | 71 | 0.2 M CAPS (pH 9.0) | - |
| 32 | 0.2 M HEPES (pH 7.0) | 0.05 M NaCl | 72 | 0.2 M CAPS (pH 9.0) | 0.05 M NaCl |
| 33 | 0.2 M HEPES (pH 7.0) | 0.1 M NaCl | 73 | 0.2 M CAPS (pH 9.0) | 0.1 M NaCl |
| 34 | 0.2 M HEPES (pH 7.0) | 0.2 M NaCl | 74 | 0.2 M CAPS (pH 9.0) | 0.2 M NaCl |
| 35 | 0.2 M HEPES (pH 7.0) | 0.05 M KCl | 75 | 0.2 M CAPS (pH 9.0) | 0.05 M KCl |
| 36 | 0.2 M HEPES (pH 7.0) | 0.1 M KCl | 76 | 0.2 M CAPS (pH 9.0) | 0.1 M KCl |
| 37 | 0.2 M HEPES (pH 7.0) | 0.2 M KCl | 77 | 0.2 M CAPS (pH 9.0) | 0.2 M KCl |
| 38 | 0.2 M HEPES (pH 7.0) | 0.05 M MgCl2+ | 78 | 0.2 M CAPS (pH 9.0) | 0.05 M MgCl2+ |
| 39 | 0.2 M HEPES (pH 7.0) | 0.1 M MgCl2+ | 79 | 0.2 M CAPS (pH 9.0) | 0.1 M MgCl2+ |
| 40 | 0.2 M HEPES (pH 7.0) | 0.2 M MgCl2+ | 80 | 0.2 M CAPS (pH 9.0) | 0.2 M MgCl2+ |

**Table S2**. Directed crystallisation screen for terpene synthase proteins.

|  |  |  |  |
| --- | --- | --- | --- |
| No. | Precipitant | Buffer | Salt |
| 1 | 0.1 M Bis-Tris (pH 6.0) | 5% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 2 | 0.1 M Bis-Tris (pH 6.5) | 5% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 3 | 0.1 M Bis-Tris (pH 7.0) | 5% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 4 | 0.1 M Tris (pH 7.5) | 5% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 5 | 0.1 M Tris (pH 8.0) | 5% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 6 | 0.1 M Tris (pH 8.5) | 5% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 7 | 0.1 M Bis-Tris (pH 6.0) | 15% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 9 | 0.1 M Bis-Tris (pH 6.5) | 15% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 10 | 0.1 M Bis-Tris (pH 7.0) | 15% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 11 | 0.1 M Tris (pH 7.5) | 15% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 12 | 0.1 M Tris (pH 8.0) | 15% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 13 | 0.1 M Tris (pH 8.5) | 15% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 14 | 0.1 M Bis-Tris (pH 6.0) | 25% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 15 | 0.1 M Bis-Tris (pH 6.5) | 25% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 16 | 0.1 M Bis-Tris (pH 7.0) | 25% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 17 | 0.1 M Tris (pH 7.5) | 25% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 18 | 0.1 M Tris (pH 8.0) | 25% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 19 | 0.1 M Tris (pH 8.5) | 25% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 20 | 0.1 M Bis-Tris (pH 6.0) | 35% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 21 | 0.1 M Bis-Tris (pH 6.5) | 35% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 22 | 0.1 M Bis-Tris (pH 7.0) | 35% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 23 | 0.1 M Tris (pH 7.5) | 35% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 24 | 0.1 M Tris (pH 8.0) | 35% PEG-3350 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 25 | 0.1 M Bis-Tris (pH 6.0) | 5% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 26 | 0.1 M Bis-Tris (pH 6.5) | 5% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 27 | 0.1 M Bis-Tris (pH 7.0) | 5% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 28 | 0.1 M Tris (pH 7.5) | 5% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 29 | 0.1 M Tris (pH 8.0) | 5% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 30 | 0.1 M Tris (pH 8.5) | 5% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 31 | 0.1 M Bis-Tris (pH 6.0) | 15% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 32 | 0.1 M Bis-Tris (pH 6.5) | 15% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 33 | 0.1 M Bis-Tris (pH 7.0) | 15% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 34 | 0.1 M Tris (pH 7.5) | 15% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 35 | 0.1 M Tris (pH 8.0) | 15% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 36 | 0.1 M Tris (pH 8.5) | 15% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 37 | 0.1 M Bis-Tris (pH 6.0) | 25% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 38 | 0.1 M Bis-Tris (pH 6.5) | 25% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 39 | 0.1 M Bis-Tris (pH 7.0) | 25% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 40 | 0.1 M Tris (pH 7.5) | 25% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 41 | 0.1 M Tris (pH 8.0) | 25% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 42 | 0.1 M Tris (pH 8.5) | 25% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 43 | 0.1 M Bis-Tris (pH 6.0) | 35% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 44 | 0.1 M Bis-Tris (pH 6.5) | 35% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 45 | 0.1 M Bis-Tris (pH 7.0) | 35% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 46 | 0.1 M Tris (pH 7.5) | 35% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 47 | 0.1 M Tris (pH 8.0) | 35% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2+ |
| 48 | 0.1 M Tris (pH 8.5) | 35% PEG-8000 | 0.2 M NaCl, 0.2 M MgCl2 |

A screenshot of a computer screen

Description automatically generated

**Figure S1.** Purification of CsTPS proteins via Ni-NTA chromatography, visualised by 12% SDS-PAGE. The cell pellet (P) was collected after protein extraction for analysis, while the clarified cell lysate (L) was loaded onto a 2 mL Ni-NTA resin column. Proteins with intact hexahistidine tags were retained on the resin, and non-binding proteins were collected in the flow-through (F). The resin was washed with 10 mM imidazole (W1-2), and the bound protein was eluted in four 1 mL fractions (E1-4) using 200 mM imidazole.

A screenshot of a computer screen

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**Figure S2.** Size-exclusion chromatogram and correlating 12% SDS-PAGE of all CsTPS proteins. Purification was conducted via Superdex S200 16/60 chromatography column (GE Healthcare). Molecular weight markers are indicated in kDa.

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**Figure S3.** A. Thermal shift assay result for the initial screen of 10 CsTPS proteins. B. Changes in the unfolding transition temperature (∆T m) were calculated for measurement on 10 proteins in 56 buffer conditions. The bars represent the median ∆T m values. A negative ∆T m value signifies that the buffer destabilises the proteins, and a positive ∆T m value indicates that the buffer has a stabilising effect.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **CsTPS** | **Retention Time (min)** | | **Identified Terpene Compound** | **NIST Compound** | | **Head to Tail Comparison** | | |
| CsTPS3FN | | 24.05 | β-myrcene | | A graph of a graph  AI-generated content may be incorrect. | | A white background with red and blue text  AI-generated content may be incorrect. |
| CsTPS9FN | | 38.75 | β-caryophyllene | | A graph of a chemical formula  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| 39.7 | humulene | | A graph of a chemical formula  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| 39.87 | epi-β-caryophyllene\* | | A graph of a chemical formula  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| 42.71 | germacrene D\* | | A graph of a chemical structure  AI-generated content may be incorrect. | | A screen shot of numbers  AI-generated content may be incorrect. |
| 43.64 | globulol\* | | A graph with numbers and lines  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| CsTPS16CC | | 35.89 | δ-Eiemene\* | | A graph of a chemical reaction  AI-generated content may be incorrect. | | A graph with numbers and symbols  AI-generated content may be incorrect. |
| 37.48 | β-Elemene\* | | A graph of a chemical reaction  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| 38.55 | y-elemene\* | | A graph of a chemical structure  AI-generated content may be incorrect. | | A line with numbers and lines  AI-generated content may be incorrect. |
| 40.3 | alloaromadendrene\* | | A graph of a chemical structure  AI-generated content may be incorrect. | | A white background with numbers and red and blue text  AI-generated content may be incorrect. |
| CsTPS20CT | | 24.06 | β-myrcene | | A graph of a molecule  AI-generated content may be incorrect. | | A screenshot of a computer  AI-generated content may be incorrect. |
| 25.91 | limonene | | A graph of a molecule  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| 26.11 | (Z)-β-ocimene | |  | |  |
| 27.9 | terpinolene | | A graph of a chemical structure  AI-generated content may be incorrect. | | A screen shot of a computer  AI-generated content may be incorrect. |
| 31.6 | α-terpineol | | A graph of a chemical reaction  AI-generated content may be incorrect. | | A white background with numbers and lines  AI-generated content may be incorrect. |
| 32.8 | geraniol | | A graph of a chemical structure  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| 41.7 | elemol\* | | A graph of a chemical reaction  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| 43.03 | guaiol | | A diagram of a graph  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| 44.03 | γ-eudesmol\* | | A graph of a chemical reaction  AI-generated content may be incorrect. | | A graph of numbers and lines  AI-generated content may be incorrect. |
| 44.79 | α-eudesmol\* | | A graph of a chemical formula  AI-generated content may be incorrect. | | A diagram of a number  AI-generated content may be incorrect. |
| CsTPS1SK | | 22.41 | α-pinene | | A graph of a graph  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| 23.18 | camphene | | A graph of a chemical structure  AI-generated content may be incorrect. | | A screen shot of a graph  AI-generated content may be incorrect. |
| 24.05 | β-myrcene | | A graph with numbers and lines  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| 24.24 | β-pinene | | A diagram of a chemical structure  AI-generated content may be incorrect. | | A graph with numbers and a line  AI-generated content may be incorrect. |
| 25.91 | limonene | | A graph with numbers and lines  AI-generated content may be incorrect. | | A screenshot of a computer  AI-generated content may be incorrect. |
| 27.9 | terpinolene | | A graph of a chemical structure  AI-generated content may be incorrect. | | A white background with red and blue text  AI-generated content may be incorrect. |
| 29.25 | fenchol\* | | A graph of a chemical reaction  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| 29.57 | β-terpineol\* | | A graph of a molecule  AI-generated content may be incorrect. | | A graph with numbers and a white background  AI-generated content may be incorrect. |
| 31.58 | α-terpineol | | A graph of a chemical reaction  AI-generated content may be incorrect. | | A graph with numbers and a line  AI-generated content may be incorrect. |
| 32.8 | geraniol | | A graph of a chemical structure  AI-generated content may be incorrect. | | A graph with numbers and a number on it  AI-generated content may be incorrect. |
| CsTPS13PK | | 25.66 | (E)-β-ocimene | | A graph of a chemical reaction  AI-generated content may be incorrect. | | A graph with numbers and a line  AI-generated content may be incorrect. |
| 26.1 | (z)-β-ocimene | | A graph of a chemical formula  AI-generated content may be incorrect. | | A screen shot of a computer  AI-generated content may be incorrect. |
| 28.94 | allo-ocimene\* | | A graph of a chemical reaction  AI-generated content may be incorrect. | | A screen shot of a graph  AI-generated content may be incorrect. |
| CsTPS37FN | | 22.42 | α-pinene | | A graph of a graph with numbers and lines  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| 23.82 | β-phellandrene\* | | A graph of a chemical structure  AI-generated content may be incorrect. | | A screen shot of a number  AI-generated content may be incorrect. |
| 24.04 | β-myrcene | | A graph with numbers and lines  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| 24.23 | β-pinene | | A graph of a chemical structure  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| 25.25 | ∆-carene | | A graph of a chemical structure  AI-generated content may be incorrect. | | A screen shot of a number  AI-generated content may be incorrect. |
| 25.45 | α-terpinene | | A graph of a molecule  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| 25.92 | limonene | | A graph of a chemical formula  AI-generated content may be incorrect. | | A white background with red and black text  AI-generated content may be incorrect. |
| 26.86 | y-terpinene | | A graph of a chemical structure  AI-generated content may be incorrect. | | A screen shot of a computer  AI-generated content may be incorrect. |
| 27.89 | terpinolene | | A graph of a chemical structure  AI-generated content may be incorrect. | | A screen shot of a computer  AI-generated content may be incorrect. |
| 27.97 | linalool | | A graph of a chemical structure  AI-generated content may be incorrect. | | A screen shot of a computer  AI-generated content may be incorrect. |
| 32.8 | geraniol | | A graph of a chemical structure  AI-generated content may be incorrect. | | A white background with red and blue text  AI-generated content may be incorrect. |
| CsTPS19BL | | 27.98 | linalool | | A graph of a chemical structure  AI-generated content may be incorrect. | | A screenshot of a computer  AI-generated content may be incorrect. |
| CsTPS12PK | | 24.07 | β-myrcene | | NI | | NI |
| 25.44 | α-terpinene | | A graph of a chemical structure  AI-generated content may be incorrect. | | A screenshot of a computer  AI-generated content may be incorrect. |
| 25.91 | limonene | | A graph of a molecule  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |
| 26.87 | γ-terpinene | | A graph of a chemical structure  AI-generated content may be incorrect. | | A graph with numbers and lines  AI-generated content may be incorrect. |

**Figure S4.** Terpene products identified in recombinant *Cannabis sativa* terpene synthase enzyme assays using Gas-chromatography mass-spectrometry (GC-MS).The figure shows retention times, identified terpene compounds, and their corresponding National Institute of Standards and Technology (NIST) library matches. For each compound, the most likely hit is provided, along with head-to-tail plots comparing the search spectrum with the reference spectrum from the NIST library. \*No reference standard available, NI; Not identified