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Optimised Sparse Matrix

JCSG-*plus*™ Screen MD1-37

JCSG-*plus* is a 96 reagent, optimized sparse-matrix screen of classic and modern conditions,
devised at the Joint Centre for Structural Genomics and developed further by Newman and Perrakis at the Netherlands Cancer Institute.

Features of JCSG-*plus*

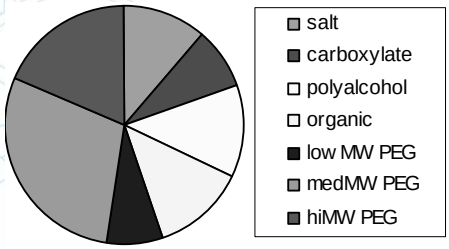
- Optimized sparse-matrix screen.
- Reduced redundancy.
- Screens classic PEG and salt conditions.
- Access more areas of crystallization space.
- Neutralized organic acids: Formate, acetate, citrate, succinate, malate, malonate.
- More organic and polyalcohol conditions.
- Precipitant synergy.
- Wide pH range 4.0 – 10.0.

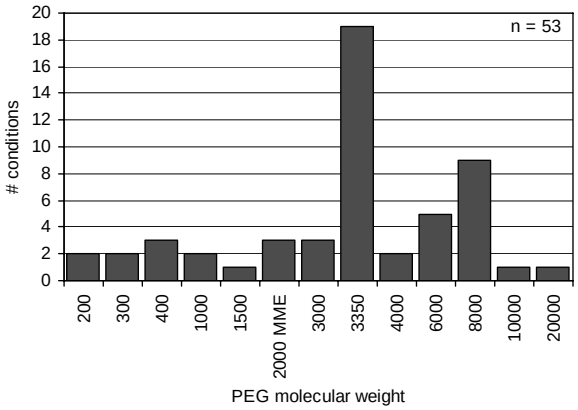
Introduction

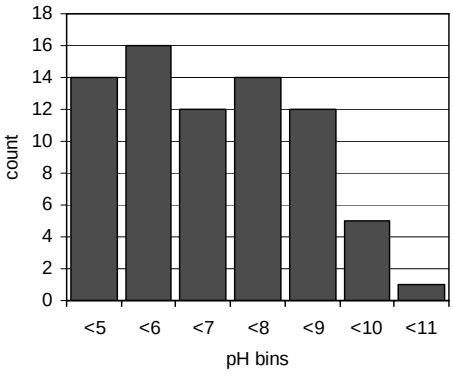
Commercially available sparse matrix screens are devised using conditions based on previously successful crystallization conditions. Since increasing numbers of researchers now use commercially available sparse matrix screens, the same sub-sets of conditions are used repeatedly. A number of structural genomics initiatives have published results of data-mining exercises using internally consistent datasets and analysing negative results as well as positive hits. The results have been startling!

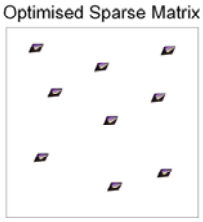
Members of the Joint Centre for Structural Genomics analysed the crystallization of over 500 different proteins against commercially available sparse matrix screens totalling 480 conditions, compiled to sample a wide range of precipitant, buffer, additive and pH. The core screen (JCSG) was developed when data mining revealed massive redundancy between clusters of conditions in commercial screens, particularly where high molecular weight PEGs are used as precipitants (1). Using a novel algorithm, members of the JCSG identified *"conditions most essential for promoting crystal formation for the most diverse set of proteins*

Analysis of precipitants used in JCSG-plus









In-filling the optimized screen

The second issue to come to light was that even extensive suites of sparse matrix screens represent incomplete coverage of crystallisation space – 480 conditions failed to crystallise 15% of the target proteins.

The **JCSG-*plus*** screen is supplemented with additional conditions to provide a more complete coverage of crystallisation space and improved chemical complementarity (2).

i. In-filling the pH profile
ii. introduce conditions using neutralised organic acids as the precipitant (3)
iii. expanded range of organic and polyalcohol conditions
iv. precipitant synergy

Usage

JCSG-*plus* is designed for the rapid, efficient screening for crystallization leads of a new protein or preparation. In the first instance, drops should be set-up using equal volumes of protein solution and reagent. Protein samples should be in a minimal solvent system containing a low concentration of buffer. Starting protein concentrations should be between 5 mg/ml and 40 mg/ml. Protein concentration can be varied in subsequent rounds depending on initial results.

The conditions in JCSG-*plus* are compatible with all commonly used crystallisation methods, sitting drop, hanging drop, sandwich drop, microbatch, vapour microbatch and microdialysis.

The JCSG-*plus* sparse matrix screen is highly effective when used alongside a systematic screen such as PACT-*premier*. The two screens provide a thorough exploration of crystallization conditions and the unique design of PACT-*premier* facilitates rational interpretation of results from both itself and JCSG-*plus* assisting the design of subsequent experiments.

Formulation Notes:

JCSG-*plus* reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 µm filters. No preservatives are added.

50% Stock solutions of Jeffamine are adjusted to pH 7.0 using HCl prior to inclusion in the reagents. Final pH may vary from that specified on the datasheet. Molecular Dimensions will be happy to discuss the precise formulation of individual reagents.

Individual reagents and stock solutions for optimization are available from Molecular Dimensions.

Enquiries regarding JCSG-*plus* formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

Contact and product details can be found at www.moleculardimensions.com

References.

1. Page *et al* (2003). Shotgun crystallization strategy for structural genomics: an optimized two-tiered crystallization screen against the *Thermotoga maritima* proteome. *Acta Cryst*. **D59**, 1028-1037
2. Newman *et al* (2005). Towards rationalization of crystallization screening for small- to medium-sized academic laboratories: the PACT/JCSG+ strategy. *Acta Cryst*. **D61**, 1426-1431
3. McPherson *et al* (2001). A comparison of salts for the crystallisation of macromolecules, *Protein Science* **10**, 418422
4. Crystallization of Nucleic Acids and Proteins, Edited by A. Ducruix and R. Giegé, The Practical Approach Series, Oxford Univ. Press, 1992
5. Protein Crystallization Techniques Strategies & Tips, Edited by Terese Bergfors, IUL 1999
6. Methods and Results in the Crystallization of Membrane Proteins, Edited by So Iwata, IUL 2003.

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Tube No.	Salt	Buffer	pH	Precipitant
1.1	0.2 M lithium sulfate	0.1 M sodium acetate	4.5	50 % v/v PEG 400
1.2	None	0.1 M sodium citrate	5.5	20 % w/v PEG 3000
1.3	0.2 M di-ammonium hydrogen citrate	None	-	20 % w/v PEG 3350
1.4	0.02 M calcium chloride	0.1 M sodium acetate	4.6	30 % v/v MPD
1.5	0.2 M magnesium formate	None	-	20 % w/v PEG 3350
1.6	0.2 M lithium sulfate	0.1 M phosphate/citrate	4.2	20 % w/v PEG 1000
1.7	None	0.1 M CHES	9.5	20 % w/v PEG 8000
1.8	0.2 M ammonium formate	None	-	20 % w/v PEG 3350
1.9	0.2 M ammonium chloride	None	-	20 % w/v PEG 3350
1.10	0.2 M potassium formate	None	-	20 % w/v PEG 3350
1.11	0.2 M ammonium dihydrogen phosphate	0.1 M Tris	8.5	50 % v/v MPD
1.12	0.2 M potassium nitrate	None	-	20 % w/v PEG 3350
1.13	None	0.1 M citrate	4.0	0.8 M ammonium sulfate
1.14	0.2 M sodium thiocyanate	None	-	20 % w/v PEG 3350
1.15	None	0.1 M Bicine	9.0	20 % w/v PEG 6000
1.16	None	0.1 M HEPES	7.5	10 % w/v PEG 8000/ 8 % v/v Ethylene glycol
1.17	None	0.1 M sodium cacodylate	6.5	40 % v/v MPD/ 5 % w/v PEG 8000
1.18	None	0.1 M phosphate/citrate	4.2	40 % v/v Ethanol/ 5 % w/v PEG 1000
1.19	None	0.1 M sodium acetate	4.6	8 % w/v PEG 4000
1.20	0.2 M magnesium chloride	0.1 M Tris	7.0	10 % w/v PEG 8000
1.21	None	0.1 M citrate	5.0	20 % w/v PEG 6000
1.22	0.2 M magnesium chloride	0.1 M sodium cacodylate	6.5	50 % v/v PEG 200
1.23	None	None	6.5	1.6 M tri-sodium citrate
1.24	0.2 M tri-potassium citrate	None	-	20 % w/v PEG 3350
1.25	0.2 M sodium chloride	0.1 M phosphate/citrate	4.2	20 % w/v PEG 8000
1.26	1.0 M lithium chloride	0.1 M Na citrate	4.0	20 % w/v PEG 6000
1.27	0.2 M ammonium nitrate	None	-	20 % w/v PEG 3350
1.28	None	0.1 M Na HEPES	7.0	10 % w/v PEG 6000
1.29	None	0.1 M Na HEPES	7.5	0.8 M sodium dihydrogen phosphate 0.8 M potassium dihydrogen phosphate
1.30	None	0.1 M phosphate/citrate	4.2	40 % v/v PEG 300
1.31	0.2 M zinc acetate	0.1 M sodium acetate	4.5	10 % w/v PEG 3000
1.32	None	0.1 M Tris	8.5	20 % v/v Ethanol
1.33	None	0.1 M Na/K phosphate	6.2	25 % v/v 1,2-propanediol 10 % v/v Glycerol
1.34	None	0.1 M Bicine	9.0	10 % w/v PEG 20,000/ 2% v/v Dioxane
1.35	None	0.1 M sodium acetate	4.6	2.0 M ammonium sulfate
1.36	None	None	-	10 % w/v PEG 1000/ 10 % w/v PEG 8000
1.37	None	None	-	24 % w/v PEG 1500/ 20 % v/v Glycerol
1.38	0.2 M magnesium chloride	0.1 M Na HEPES	7.5	30 % v/v PEG 400
1.39	0.2 M sodium chloride	0.1 M Na/K phosphate	6.2	50 % v/v PEG 200
1.40	0.2 M lithium sulfate	0.1 M sodium acetate	4.5	30 % w/v PEG 8000
1.41	None	0.1 M HEPES	7.5	70 % v/v MPD
1.42	0.2 M magnesium chloride	0.1 M Tris	8.5	20 % w/v PEG 8000
1.43	0.2 M lithium sulfate	0.1 M Tris	8.5	40 % v/v PEG 400
1.44	None	0.1 M Tris	8.0	40 % v/v MPD
1.45	0.17 M ammonium sulfate	None	-	25.5 % w/v PEG 4000/ 15 % v/v Glycerol
1.46	0.2 M calcium acetate	0.1 M sodium cacodylate	6.5	40 % v/v PEG 300
1.47	0.14 M calcium chloride	0.07 M sodium acetate	4.6	14 % v/v 2-propanol/ 30 % v/v Glycerol
1.48	0.04 M potassium dihydrogen phosphate	None	-	16 % w/v PEG 8000/ 20 % v/v Glycerol

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Tube	Salt	Buffer	pH	Precipitant
2.1	None	0.1 M sodium cacodylate	6.5	1.0 M tri-sodium citrate
2.2	0.2 M sodium chloride	0.1 M sodium cacodylate	6.5	2.0 M ammonium sulfate
2.3	0.2 M sodium chloride	0.1 M HEPES	7.5	10 % v/v 2-propanol
2.4	0.2 M lithium sulfate	0.1 M Tris	8.5	1.26 M ammonium sulfate
2.5	None	0.1 M CAPS	10.5	40 % v/v MPD
2.6	0.2 M zinc acetate	0.1 M imidazole	8.0	20 % w/v PEG 3000
2.7	0.2 M zinc acetate	0.1 M sodium cacodylate	6.5	10 % v/v 2-propanol
2.8	None	0.1 M sodium acetate	4.5	1.0 M di-ammonium hydrogen phosphate
2.9	None	0.1 M MES	6.5	1.6 M magnesium sulfate
2.10	None	0.1 M Bicine	9.0	10 % w/v PEG 6000
2.11	0.16 M calcium acetate	0.08 M sodium cacodylate	6.5	14.4 % w/v PEG 8000/ 20 % v/v glycerol
2.12	None	0.1 M imidazole	8.0	10 % w/v PEG 8000
2.13	0.05 M caesium chloride	0.1 M MES	6.5	30 % v/v Jeffamine M-600
2.14	None	0.1 M Na Citrate	5.0	3.2 M ammonium sulfate
2.15	None	0.1 M Tris	8.0	20 % v/v MPD
2.16	None	0.1 M HEPES	7.5	20 % v/v Jeffamine M-600
2.17	0.2 M magnesium chloride	0.1 M Tris	8.5	50 % v/v ethylene glycol
2.18	None	0.1 M Bicine	9.0	10 % v/v MPD
2.19	None	None	7.0	0.8 M succinic acid
2.20	None	None	7.0	2.1 M DL-malic acid
2.21	None	None	7.0	2.4 M sodium malonate
2.22	1.1 M sodium malonate	0.1 M HEPES	7.0	0.5 % v/v Jeffamine ED-2001
2.23	1.0 M succinic acid	0.1 M HEPES	7.0	1 % w/v PEG 2000 MME
2.24	None	0.1 M HEPES	7.0	30 % v/v Jeffamine M-600
2.25	None	0.1 M HEPES	7.0	30 % v/v Jeffamine ED-2001
2.26	0.02 M magnesium chloride	0.1 M HEPES	7.5	22 % w/v polyacrylic acid 5100 sodium salt
2.27	0.01 M cobalt chloride	0.1 M Tris	8.5	20 % w/v polyvinylpyrrolidone K15
2.28	0.2 M tri-methylamine N-oxide	0.1 M Tris	8.5	20 % w/v PEG 2000 MME
2.29	0.005 M cobalt chloride 0.005 M cadmium chloride 0.005 M magnesium chloride 0.005 M nickel chloride	0.1 M HEPES	7.5	12 % w/v PEG 3350
2.30	0.2 M sodium malonate	None	7.0	20 % w/v PEG 3350
2.31	0.1 M succinic acid	None	7.0	15 % w/v PEG 3350
2.32	0.15 M DL - malic acid	None	7.0	20 % w/v PEG 3350
2.33	0.1 M potassium thiocyanate	None	-	30 % w/v PEG 2000 MME
2.34	0.15 M potassium bromide	None	-	30 % w/v PEG 2000 MME
2.35	None	0.1 M Bis Tris	5.5	2.0 M ammonium sulfate
2.36	None	0.1 M Bis Tris	5.5	3.0 M sodium chloride
2.37	None	0.1 M Bis Tris	5.5	0.3 M magnesium formate
2.38	1.0 M ammonium sulfate	0.1 M Bis Tris	5.5	1 % w/v PEG 3350
2.39	None	0.1 M Bis Tris	5.5	25 % w/v PEG 3350
2.40	0.2 M calcium chloride	0.1 M Bis Tris	5.5	45 % v/v MPD
2.41	0.2 M ammonium acetate	0.1 M Bis Tris	5.5	45 % v/v MPD
2.42	0.1 M ammonium acetate	0.1 M Bis Tris	5.5	17 % w/v PEG 10000
2.43	0.2 M ammonium sulfate	0.1 M Bis Tris	5.5	25 % w/v PEG 3350
2.44	0.2 M sodium chloride	0.1 M Bis Tris	5.5	25 % w/v PEG 3350
2.45	0.2 M lithium sulfate	0.1 M Bis Tris	5.5	25 % w/v PEG 3350
2.46	0.2 M ammonium acetate	0.1 M Bis Tris	5.5	25 % w/v PEG 3350
2.47	0.2 M magnesium chloride	0.1 M Bis Tris	5.5	25 % w/v PEG 3350
2.48	0.2 M ammonium acetate	0.1 M HEPES	7.5	45 % v/v MPD

Abbreviations: Bis Tris; Bis-(2-hydroxyethyl)imino-tris(hydroxymethyl)methane, **CAPS**; N-Cyclohexyl-3-aminopropanesulfonic acid**, CHES**; 2-(N-Cyclohexylamino)ethane Sulfonic Acid**, HEPES;** 2-(4-(2-Hydroxyethyl)-1-piperazinyl)ethanesulfonic Acid, **Na HEPES**; 2-(4-(2-Hydroxyethyl)-1-piperazinyl)ethanesulfonic Acid Sodium Salt, **MES**; 2-(N-morpholino)ethanesulfonic acid, **MPD;** 2,4-methyl pentanediol, **PEG**; Polyethylene glycol, **Tris**; 2-Amino-2-(hydroxymethyl)propane-1,3-diol.

Manufacturer's safety data sheets are available upon request.