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Introducing *SmartScreen* for the Crystal Former

- **Custom designed for the Crystal Former chip system**
- **Low cost**
- **Enables the power of microfluidics**
- **Enhanced exploration of the phase diagram**

Introduction

The *SmartScreen*, from Microlytic, applies the concepts of sparse screens to microfluidic devices. We developed the *SmartScreen* to release the power of microfluidics designed into our Crystal Former chips.

Sparse screens^{1,2,3} have evolved from conditions that gave crystals for a specific protein, or class of protein. This concept has merit, but given the restrictions of the “trial and error” vapor diffusion methodologies, results are often less than stellar. Regardless, many groups continue to consume numerous plates and liters of screens hoping for a crystallization event. It strikes us that it is time for a change.

The Crystal Former with the *SmartScreen* system is designed to lower overall cost and increase crystallization events.

SmartScreen

SmartScreen is a custom screen consisting of 48 conditions as 3x16 solution sets, which have been selected from many published sparse screens. Each well contains approximately 75µl of screen, arranged in order of productivity, and only a microliter (µl) of each is required with the Crystal Former.

- *The first 16 conditions should give crystals for over 65% of proteins*
- *The next 16 should raise that number to over 85%*
- *The last 16 should produce more than 94% crystal growth.*

These numbers are based on published data from vapor diffusion experiments.^{4,5,6} Higher yields should be expected based on the feedback from customers currently using the Crystal Former (ask about our case studies).

Crystal Former with the SmartScreen System

Few people dispute the physics behind microfluidics. However, some first generation microfluidic devices fell short of their promises. Crystals produced by these devices could not easily be harvested and conditions could not routinely be transferred to other methods (vapor diffusion).

Crystal Former is different, it:

- **Reduces overall cost**
- **Minimizes screen volumes**
- **Reduces labor**
- **Provides easy harvesting of crystals**
- **Increases crystallization hit rates**

The Crystal Former with the *SmartScreen* system uses a microfluidics approach: protein and precipitant are dispensed into wells at the ends of a closed capillary. Diffusion occurs as molecules move from regions of higher concentration to lower concentration. These molecules diffuse at a rate that is proportional to molecular weight/size. Smaller molecules, ions, move very fast and high molecular weight polymers (i.e., PEGs, etc...) migrate more slowly. As a result, the protein is exposed to gradients of conditions. The protein is gently moved through the many regions of saturated to super-saturated nucleation conditions, *producing beam-quality crystals.*

A CASE STUDY:

A researcher at a large pharmaceutical company had been given a novel protein for crystallization. It had gone through their standard protocols, with several commercial screens, in a vapor phase diffusion mode (sitting drop), but without a successful result. None of the screens produced crystals. He consulted the literature, Kimber et al⁴; he chose the top 16 crystallization conditions from a screen and tested his protein in the **Crystal Former**. One channel produced a crystal! He was then able to transfer the conditions to their legacy vapor diffusion system, made a grid around the condition from the **Crystal Former** results, varying PEG, salt and pH, and was also able to grow beam-ready crystals. In this case, the **Crystal Former** proved to be a quick and easy *pilot screening tool.*

This researcher, using the **Crystal Former** as a pilot screening tool, produced crystals ready for structural analysis by X-ray. The time savings and minimized material consumption, paired with a positive result that could not be obtained any other way, makes the **Crystal Former** system a *value added tool.*

The *SmartScreen* is intended to make this practice, of using minimized screens in a microfluidic device, available to all researchers.

Conclusion

SmartScreen, used with the Crystal Former, provides a cost effective means of producing crystals and is changing the paradigm of commonly used protocols.

Fewer plates!

Less imaging!

Lower screen volumes!

Lower purchase cost of screens!

More beam quality crystals!

Lower waste disposal costs!

SmartScreen contents and well positions

Position	MLS-1 #	Condition
A1	1	50% w/v PEG 400, 0.2 M Lithium sulfate, 0.1 M Sodium acetate pH=5.1
A2	2	30% w/v PEG 4000, 0.2 M Magnesium chloride, 0.1 M TRIS pH=8.5
B1	3	20% w/v PEG 3000, 0.1 M Sodium citrate pH=5.5
B2	4	30% w/v PEG 4000, 0.2 M Ammonium acetate, 0.1 M Sodium acetate pH=4.6,
C1	5	20% w/v PEG 3350, 0.2 M Ammonium citrate pH=5
C2	6	20% w/v PEG 8000, 0.2 M Magnesium acetate, 0.1 M Sodium cacodylate pH=6.5
D1	7	20% w/v PEG 6000, 0.1 M Lithium chloride, 0.1 M Citric acid pH=4
D2	8	1.4 M Sodium citrate, 0.1 M HEPES pH=7.5
E1	9	20% w/v PEG 3350, 0.2 M Magnesium formate pH=5.9
E2	10	2% w/v PEG 400, 2 M Ammonium sulfate, 0.1 M HEPES pH=7.5
F1	11	20% w/v PEG 1000, 0.2 M Lithium sulfate, 0.1 M Citric acid pH=4.2
F2	12	30% w/v PEG 1500
G1	13	20% w/v PEG 8000, 0.1 M CHES pH=9.5
G2	14	2 M Ammonium sulfate, 0.1 M TRIS pH=8.5
H1	15	20% w/v PEG 3350, 0.2 M Ammonium formate pH=6.6
H2	16	30% w/v PEG 4000, 0.2 M Lithium sulfate, 0.1 M TRIS pH=8.5
A6	17	20% w/v PEG 3350, 0.2 M Ammonium chloride pH=6.3
A7	18	30% w/v PEG 8000, 0.2 M Ammonium sulfate
B6	19	20% w/v PEG 3350, 0.2 M Potassium formate pH=7.3
B7	20	8% w/v PEG 8000, 0.1 M TRIS pH=8.5
C6	21	0.2 M Ammonium phosphate, 0.1 M TRIS pH=8.5, 50% v/v MPD
C7	22	20% w/v PEG 4000, 0.1 M HEPES pH=7.5, 10% v/v 2-propanol
D6	23	20% w/v PEG 3350, 0.2 M Potassium nitrate pH=6.9
D7	24	18% w/v PEG 8000, 0.2 M Zinc acetate, 0.1 M Sodium cacodylate pH=6.5
E6	25	0.8 M Ammonium sulfate, 0.1 M Citric acid pH=4
E7	26	0.02 M Calcium chloride, 0.1 M Sodium acetate pH=4.6, 30 % v/v MPD
F6	27	20% w/v PEG 3350, 0.2 M Sodium thiocyanate pH=6.9
F7	28	1 M Ammonium phosphate, 0.1 M Sodium citrate pH=5.6
G6	29	20% w/v PEG 6000, 0.1 M Bicine pH=9
G7	30	30% w/v PEG 400, 0.2 M Sodium citrate, 0.1 M TRIS pH=8.5
H6	31	10% w/v PEG 8000, 0.1 M HEPES pH=7.5, 8% v/v ethylene glycol
H7	32	28% w/v PEG 400, 0.2 M Calcium chloride, 0.1 M HEPES pH=7.5
A11	33	5% w/v PEG 8000, 0.1 M Sodium cacodylate pH=7, 40% v/v MPD
A12	34	1.5 M Lithium sulfate, 0.1 M HEPES pH=7.5,
B11	35	5% w/v PEG 1000, 0.1 M Citric acid pH=5.2, 40% v/v Ethanol
B12	36	25% w/v PEG 4000, 0.2 M Ammonium sulfate, 0.1 M Sodium acetate pH=4.6
C11	37	8% w/v PEG 4000, 0.1 M Sodium acetate pH=4.6
C12	38	0.2 M Magnesium acetate, 0.1 M Sodium cacodylate pH=6.5, 30% v/v MPD
D11	39	10% w/v PEG 8000, 0.2 M Magnesium chloride, 0.1 M TRIS pH=7
D12	40	30% w/v PEG 8000, 0.2 M Sodium acetate, 0.1 M Sodium cacodylate pH=6.5
E11	41	20% w/v PEG 6000, 0.1 M Citric acid pH=5
E12	42	4 M Sodium formate
F11	43	50% w/v PEG 200, 0.2 M Magnesium chloride, 0.1 M Sodium cacodylate pH=6.6
F12	44	2 M Sodium formate, 0.1 M Sodium acetate pH=4.6
G11	45	1.6 M Sodium citrate pH=6.5
G12	46	0.8 M Sodium phosphate monobasic, 0.1 M HEPES pH=7.5, 0.8 M Potassium phosphate monobasic
H11	47	20% w/v PEG 3350, 0.2 M Potassium citrate pH=8.3
H12	48	20% w/v PEG 8000, 0.05 M Potassium phosphate monobasic

References

¹ Emerald BioSystems. Wizard screens™, Cryo™ screens ² Hampton Research. Crystal Screens, PEG/ion, Peg 6000 grid, Ammonium Sulfate grid. ³ Jena Bioscience. JBS Classic, JBS Kinase. ⁴ Kimber, M. et al "Data Mining Crystallization Databases: Knowledge-Based Approaches to Optimize Protein Crystal Screens" (2003) Proteins 51:562-568
⁵ Newman, J. et al "Towards Rationalization of crystallization screening for small to medium sized academic labs the PACT/JCSG+ strategy" (2005); Acta Cryst. D 61, 1426-1431 ⁶Page, R. et al "Shotgun strategy for structural genomics: an optimized two-tiered crystallization screen against the Thermotoga maritime proteome" (2003); Acta Cryst. D 59, 1028-1037 ⁷ Kantardjiev, J. et al "Protein isoelectric point as a predictor for increased crystallization screening efficiency" (2004); Bioinformatics 20, 2162-2168



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