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Glycerol concentrations required for the successful vitrification of cocktail conditions in a high-throughput crystallization screen

The Hauptman-Woodward Medical Research Institute runs a high-throughput crystallization screening service in which macromolecules are screened against 1536 potential crystallization cocktails. Typically, multiple crystallization leads are identified. With a limited amount of sample, the question becomes 'How many leads can be optimized and which leads are most likely to produce X-ray diffraction data?'. In order to prioritize the hits for optimization, the amount of glycerol required to successfully cryocool each cocktail has been determined for the cocktails used in the high-throughput screen. Those hit conditions that require the minimum amount of cryoprotectant for successful vitrification will be closer in chemical make-up to the mother liquor. Hence, if the physical properties of the crystals are similar, one could logically prioritize leads that are more likely to produce diffraction based upon the chemical similarity of the native to the cryopreserved mother liquor.

1. Introduction

High-throughput crystallization is a highly automated process; hundreds of experiments can be conducted with a few milligrams of the macromolecule of interest. A high-throughput screening service is currently offered at the Hauptman-Woodward Medical Research Institute (HWI). Samples solicited from the biological community are screened against 1536 different biochemical cocktails (Luft et al., 2003) using the microbatch-under-oil crystallization method (Chayen et al., 1992). Individual experiments are composed of 200 nl macromolecule solution ($\sim 10 \text{ mg ml}^{-1}$) and 200 nl of a crystallization cocktail. Experiments are incubated at 296 K and the outcomes are imaged for four weeks. The images are archived and are immediately available to the investigator providing the sample. The cocktails used are broken down into three different groups: highly soluble salts, different molecular-weight PEG combinations and commercially available screens that complement the previous groups. Currently, the success rate is \sim 50%, *i.e.* half of the screened samples result in a lead: a likely crystallization condition that can be optimized. Frequently, leads are observed from several chemically distinct cocktails. With a limited supply of macromolecule available for crystallization, can we devise a strategy to rationally prioritize these leads for optimization?

For X-ray structural data collection, the majority of samples are cryocooled in order to reduce radiation damage (Garman & Owen, 2006). Cryoprotective agents (cryoprotectants) are typically required to eliminate crystalline ice formation. One of these cryoprotectants is glycerol. The amounts of glycerol needed to successfully vitrify the Hampton Research Crystal

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Screen (50 different biochemical cocktails) were determined by Garman & Mitchell (1996). A similar study expanded these data with the addition of 48 cocktails (adding Hampton Research Crystal Screen II) using glycerol and also PEG 400, ethylene glycol and 1,2-propanediol as cryoprotectants for all 98 (50 + 48) cocktails (McFerrin & Snell, 2002). In both studies, solutions were tested for successful vitrification using X-ray diffraction. McFerrin and Snell noted that 73% of the glycerol concentrations required to produce a visually clear solution were successfully vitrified as determined by X-ray diffraction. The remaining solutions required a 5% increase (the minimum glycerol concentration step used) to be successfully vitrified. Simple visual observation provided a good guide to the initial cryoprotectant condition within the sampling constraints.

We have expanded on previous studies and visually determined the concentrations of glycerol required to vitrify the first two groups of cocktails used in the HWI high-throughput screening laboratory. The introduction of any non-native component into a crystal, *e.g.* a cryoprotective agent, has the potential to cause damage (Mitchell & Garman, 1994). By determining the minimum concentration of glycerol required for successful vitrification of a lead condition, we can use this information as one of the criteria to prioritize the leads that are subsequently optimized, *i.e.* those where minimal additional of cryoprotection would be needed for data collection.

2. Experimental

The 1536-condition HWI crystallization screen can be divided into three groups. Groups 1 and 2 were constructed using an

incomplete factorial design (Audic et al., 1997) and are buffered with 100 mM concentrations of CAPS (pH 10.0). TAPS (pH 9.0), Tris (pH 8.0), HEPES (pH 7.5), MOPS (pH 7.0), MES (pH 6.0), sodium acetate (pH 5.0) and sodium citrate (pH 4.0). Group 1 cocktails are highly soluble salts (262 cocktails). They include 36 different salts (11 cations and 14 anions) at \sim 30, 60 and 90% saturation, buffered as described. Group 2, PEG/salt (722 cocktails), includes five different molecular-weight PEGs (20, 8, 4, 1 kDa and 400 Da), combined with 35 salts at 100 mM concentration and buffered as described. Group 3 are the commercial screens (552 cocktails). This group is comprised of Hampton Research Natrix, Quick, PEG/Ion, PEG Grid, Ammonium Sulfate Grid, Sodium Chloride Grid, Crystal Screen HT, Index and SaltRx screens. For historical reasons, the first 22 cocktails from Hampton Research Crystal Screen Cryo are distributed within groups 1 and 2. These and other occurrences of Hampton Research cryocondition cocktails serve as a control during the experimental process. The first two groups were studied by the addition of 2.5%(w/v) increments of glycerol concentration to identify cryoprotectant conditions. For the third group, glycerol concentrations for Crystal Screen HT have been described elsewhere (McFerrin & Snell, 2002). Grid Screen Ammonium Sulfate and Grid Screen PEG/LiCl were used to investigate the fine sampling of chemical space, complementing the incomplete factorial sampling of the first two

The instrumentation used consists of an offline goniometer system with an Oxford 700 cryostream positioned to cool the sample from directly above (Fig. 1*a*). Each sample was imaged with a Navitar zoom lens coupled to a Pixelink color firewire-

groups. The remainder were studied in somewhat less detail.

linked CCD camera. Each component could be precisely translated. A Fibre-Lite metal halide machine-vision illuminator was used to illuminate the sample from the front. To bias the experiment towards the worst case, large 0.7–1.0 mm Hampton Research cryoloops mounted on magnetic heads were used to hold the solutions. Multiple loops were used, all of a similar measured size. They were washed and dried between each experiment.

In the high-throughput crystallization screening laboratory, the crystallization cocktail is mixed in a 1:1 ratio with the macromolecule in buffer. For the vitrification studies described here, all the cocktails were studied at full strength and then diluted in a 1:1 ratio with double-distilled water (ddH₂O). At full strength, the data provide an indication of the initial cryoprotectant properties of the cocktail. As solutes lower the vapor pressure of a solvent and decrease the freezing point, the data from the 1:1 dilution with ddH₂O



Figure 1

(a) Photograph of the experimental setup showing the video microscope lens, the fiber-optic illuminator, cryostream and goniometer mount. The instrument focused on the sample to the right-hand side is a thermal imaging camera used for other studies (Snell *et al.*, 2002). Examples of (b) a successful vitrification and (c) a poor flash-cooling result are also shown.

(having no solutes) represent a worst-case scenario. A total of 10 μ l solution was pipetted onto a glass microscope slide and the loop was used to pick up solution and place it on the goniometer with the gas stream blocked. Once on the goniometer, the gas stream was swiftly unblocked to cool the cryoloop and the solution it contained. Magnified images of the loops were examined to determine whether the solution had vitrified successfully (Fig. 1b) or whether crystalline ice was present (Fig. 1c).

The first experiment, with the cocktail at full strength, identified conditions that already had cryoprotectant properties and the second with 50% ddH₂O was used as the starting point to study the glycerol concentrations needed for vitrification. If the 50% cocktail solution did not show successful vitrification, further investigation of the cocktail took place. Glycerol solutions containing 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10 and 5%(v/v) glycerol were prepared by volumetric dilution with ddH₂O. The glycerol solution was warmed in a water bath to reduce its viscosity and increase pipetting accuracy. For the first 984 cocktails, each solution was pipetted in equal volumes onto a glass microscope slide, aspirating and dispensing the mixed drop several times. The effective percentage of cryoprotectant was therefore from 30% to 0% glycerol in 2.5%(v/v) steps. Starting from the highest concentration of cryoprotectant, each solution was loaded in a loop and cooled and then imaged until evidence of crystalline ice was seen. The cryoprotectant concentration that remained clear was then recorded. The initial 984 cocktails (excluding the 22 Crystal Screen Cryo cocktails) provide an incomplete factorial sampling of crystallization space.

A similar procedure was followed for the Hampton Research Grid Screen Ammonium Sulfate and Grid Screen PEG/LiCl cocktails. Sample volume can be an important factor in cryoprotectant concentration and successful vitrification (Chinte et al., 2005). Therefore, a single cocktail that required a larger than average amount of cryoprotectant was selected from the high-salt cocktails group (1.14 M ammonium sulfate pH 6) and from each of five different molecular-weight PEGs in the PEG group (lithium chloride pH 10, calcium acetate pH 6.0, ammonium sulfate pH 7.5, potassium phosphate pH 7.0 and ammonium phosphate dibasic pH 4.0 all at 0.1 M concentration for PEGs 20, 8, 4, 1 kDa and 400 Da, respectively). The experimental procedure was repeated in 5% steps (rather than the previous 2.5% steps) with these cocktails, using a succession of smaller loops ranging from 1.0 to 0.05 mm across. Each loop was independently measured using a light microscope to confirm its size.

The remaining cocktails were studied with 1:1 dilutions of the cocktails with 20, 10 and $5\%(\nu/\nu)$ glycerol solutions. These cocktails (and the Ammonium Sulfate and PEG/LiCl Grid Screens) are used as reference points with the HWI crystallization screen in order to understand the behavior of the macromolecules over fine-sampled chemical shifts, to pinpoint the best category of potential crystallization chemicals and as a means to sample outliers in chemical space not covered by the 962-cocktail incomplete factorial sampled cocktails. Note that the Crystal Screen HT has been studied in detail elsewhere (Garman & Mitchell, 1996; McFerrin & Snell, 2002).

3. Results

Figs. 2-7 list the cocktails and the concentrations of glycerol required to successfully vitrify the solution. The percentage column is divided into three sections, with the first identifying whether or not the cocktail was successfully vitrified at 100% concentration (without added cryoprotectant), the second if it was successful at 50%(v/v) concentration and the third the percentage of glycerol needed needed to vitrify a solution containing a 1:1 dilution of the cocktail with ddH₂O. In Figs. 8 and 9 the Hampton Research Grid Screen Ammonium Sulfate and Grid Screen PEG/LiCl results are displayed in a similar manner. Figs. 10-15 show the effect of loop size on the amount of cryoprotectant needed for successful vitrification. Fig. 16 provides a listing of the remaining screens. For brevity, only those conditions that displayed natural cryoprotectant qualities or that were cryoprotected with 20%(v/v) glycerol or less are displayed. Finally, Tables 1 and 2 summarize the results.

The highly soluble salts (223 cocktails; Fig. 2) required on average the highest concentrations of cryoprotectant [22.5%(v/v)], with the exceptions of lithium chloride, magnesium acetate and magnesium chloride hexahydrate at high concentration and pH. This was also observed in the data for 100% concentration cocktail conditions, *i.e.* no glycerol. In general, a higher initial salt concentration required a lower cryoprotectant concentration, as observed by Garman (1999). High salt concentrations as cryoprotectant agents have been observed elsewhere (Holyoak *et al.*, 2003; Rubinson *et al.*, 2000).

The PEG 20K results are shown in Fig. 3; for all the PEGs the salt concentration was 0.1 M. For 81 cocktails containing 20.0%(v/v) PEG 20K there was little variation in the required cryoprotectant concentration; that for glycerol averaged 23.9%. At 40%(ν/ν) PEG 20K (61 cocktails) the average cryoprotectant concentration was 16.0%(v/v). Two conditions required no cryoprotectant: ammonium bromide pH 7 and magnesium acetate pH 9. In the case of magnesium acetate, as the pH decreased the required concentration of cryoprotectant increased [0%(v/v)] at pH 9, 10%(v/v) at pH 6 and 15%(v/v) at pH 5]. Ammonium bromide was only sampled once at 40%(v/v) PEG, so the extent of any pH trends are unknown. For PEG 8K (Fig. 4) at 20%(v/v) concentration (83 conditions), the average required cryoprotectant was 24.1%(v/v), similar to that for PEG 20K. PEG 8K at 40%(v/v) (70 conditions) reduced the average cryoprotectant to 16.2%. Again, there were a number of samples that needed no cryoprotectant. These were ammonium chloride pH 4, ammonium nitrate pH 7, magnesium acetate pH 7, sodium nitrate pH 4, lithium sulfate monohydrate pH 5 and manganese sulfate monohydrate pH 6. These cocktails included only single occurrences of magnesium, sodium and manganese salts and so no pH trends could be observed. PEG 4K (Fig. 5) at 20%(v/v) (75 conditions) required an average of 24.7% cryoprotectant and for 40%(v/v) (73 conditions) a concen-

No.	Salt (M)		pH		%		No.	Sa	lt (M)	pH		%		No.	Salt (A	1)	pН		%
1		3.56	8	0	0	30.0	81		1.59	9	0	0	22.5	159		4.48	6	0 () 22.5
2	E	3.56	10	0	0	27.5	82	. E .	1.59	8	0	0	22.5	160	fe	4.48	7.5	0 0) 22.5
3	niur ide	2.38	4	0	0	30.0	83	fate	1.06	7	0	0	25.0	161	lorić	4.48	10	0 0	22.5
4	omi	2.38	7.5	0	0	Fail	84	su ag	0.53	5	0	0	27.5	162	- ch	2.99	4	0 () 22.5
5	hu	1.19	5	0	0	Fail	85	- × ·	0.53	7.5	0	0	27.5	163		2.99	5	0 () 22.5
6		1.19	7	0	0	30.0	86		0.53	8	0	0	27.5	164	Sod	1.49	7	0 0	25.0
7		1.19	9	0	0	Fail	87	-	3,82	4	1	1	0	165	-	1,49	8	0 0	27.5
8	U	3./4	4	0	0	30.0	88	- 2	3.82	8	+÷	1	0	100		1.49	9	0 0	30.0
9	orid	3.74	75	0	0	27.5	89	ane	3.82	2	1		125	10/	-	2.05	0	0 0	215
10	cht	3./4	1.5	0	0	30.0	90	ang	2.54	3	0		22.5	108	- 3 0	1.25	7	0 0	22.5
12	E.	2.5	0	0	0	Eail	91	- × ,	1.27	4	0	0	22.5	109	drat drat	1.35	0	0 0	25.0
12	IOL	2.5	10	0	0	Fail	03	-	1.27	4	0	0	22.5	170	Sod	0.68	4	0 0	23.0
13	E .	1.25	6	0	0	30.0	94	-	8.64	8	1	1	0	172	E - E - E - E - E - E - E - E - E -	0.68	8	0 0	250
15	~	1.25	8	0	0	30.0	95	-	8.64	0	ti	+÷	0	172	-	0.68	10	0 0	22.5
16		6.46	4	0	0	27.5	96	state	5.76	4	1	i	0	174	-	3.9	6	0 0	22.5
17	E	6.46	9	0	0	25.0	97	1 30	5.76	5	0	0	22.5	175		3.9	7.5	0 (22.5
18	ie nie	6.46	10	0	0	22.5	98	- in	5.76	7.5	1	1	0	176	itrat	2.6	5	0 0	22.5
19	nitra	3.23	6	0	0	30.0	99	lass	2.88	6	0	0	22.5	177		2.6	8	0 0	0 25.0
20	An	3.23	7	0	0	30.0	100	- ²	2.88	7	0	0	22.5	178	diu	2.6	9	0 () 25.0
21		3.23	8	0	0	30.0	101	1	2.88	10	1	1	0	179	So L	1.3	4	0 () 22.5
22		0.87	8	0	0	30.0	102		4	5	0	0	25.0	180		1.3	7	0 () 22.5
23		1.74	4	0	0	30.0	103	de	4	8	0	0	22.5	181		3.32	4	1 () 17.5
24	asic	1.74	6	0	0	27.5	104	omi	2.66	6	0	0	27.5	182	ie.	3.32	7	1 () 17.5
25	mon nobi	1.74	10	0	0	22.5	105	- Pa	2.66	7	0	0	27.5	183	ic pha	3.32	9	1 1	0
26	Amphe	0.87	5	0	0	30.0	106	sim	2.66	10	0	0	27.5	184	shos	2.21	6	0 () 22.5
27		0.87	7	0	0	Fail	107	otas	1.33	4	0	0	25.0	185	d m	2.21	7.5	0 () 22.5
28		0.87	9	0	0	Fail	108	- a	1.33	7.5	0	0	27.5	186	n	1.11	5	0 0) 27.5
29		2.13	7	0	0	22.5	109	_	1.33	9	0	0	30.0	187	Ň	1.11	8	0 () 22.5
30	sic	3.2	8	1	0	22.5	110		2.54	4	0	0	22.5	188		1.11	10	0 () 22.5
31	lin agi	3.2	9	0	0	22.5	111	nate	2.54	7	0	0	22.5	189	-	2.83	6	1 () 22.5
32	ite, e	2.13	6	0	0	22.5	112	- f	2.54	8	0	0	22.5	190	fate	2.83	8	1 () 17.5
33	um and	2.13	7.5	0	0	22.5	113	5	1.69	5	0	0	25.0	191	la sul	2.83	9	1 () 15.0
34	A set	1.07	4	0	0	Fail	114	siur	1.69	1.5	0	0	27.5	192	- ih di	1.88	1	0 0	25.0
35	-	1.07	5	0	0	30.0	115	otas	1.09	9	0	0	22,5	193	- m T	1,88	9	0 0	22.5
30		2.42	9	0	0	22.5	110		0.85	0	0	0	25.0	194	- by	0.94	10	0 0	25.0
37		3.42	4	0	0	22.5	117		0.85	10	0	0	27.5	195		0.94	7.5	0 0	22.0
30	Ifat	3.42	7	0	0	22.5	110		2.52	7	0	0	22.5	190		1.18	7.5	0 0	25.0
40	nsu	2.28	75	0	0	22.5	120	- in	2.32	7.5	0	0	25.0	108		1.18	8	0 0	225
41	ini	2.28	8	0	0	22.5	120	- Tr	1.55	6	0	0	30.0	190	etat	0.79	5	0 0	225
42	10	1.14	6	0	0	30.0	122	E	1.55	8	0	0	30.0	200	cac	0.79	8	0 0	27.5
43	ЧШ	1.14	9	0	0	25.0	123	assi	1.55	9	0	0	30.0	201	- g	0.39	5	0 0	25.0
44		1.14	10	0	0	30.0	124	Pot	0.77	5	0	0	30.0	202		0.39	6	0 0	25.0
45		1.09	7	0	0	27.5	125	-	0.77	10	0	0	30.0	203		3.46	6	1 (0 15.0
46	CHCHON	0.55	6	0	0	30.0	126		0.88	5	0	0	30.0	204	ite,	3.46	7.5	0 (22.5
47	$Ca(C_2H_3O_2)_2$	0.55	5	0	0	Fail	127	ate	0.88	9	0	0	30.0	205	- ilds	2.3	7	0 (25.0
48	CaCl ₂ .2H ₂ O	3.57	5	0	0	17.5	128	lite –	0.88	8	0	0	30.0	206	sic	2.3	9	0 (25.0
49	HR-0	Cryo-1		1	0	15.0	129	E E	1.77	5	0	0	30.0	207	mi	2.3	5	0 () 22.5
50	HR-0	Cryo-2		0	0	12.5	130	assi	0.88	4	0	0	30.0	208	ISSI	1.15	4	0 () 22.5
51	HR-0	Cryo-3		1	0	17.5	131	Pot	0.88	7.5	0	0	30.0	209	Pott	1.15	5	0 (22.5
52		5.6	5	1	0	17.5	132		0.88	10	0	0	20.0	210		1.15	8	0 0	22.5
53	ide	5.6	4	1	0	12.5	133		1.28	8	0	0	25.0	211		0.53	8	0 0) 25.0
54	EI OL	3.73	6	0	0	22.5	134	1.10	0.85	7	0	0	30.0	212		1.06	9	0 () 22.5
55	É E	3.73	7	1	0	22.5	135	ium tate,	0.85	7.5	0	0	25.0	213	fate	0.53	5	0 0) 25.0
56	iļi	1.87	4	0	0	22.5	136	ospl	0.85	10	0	0	27.5	214	hydi	1.06	4	0 () 22.5
57	E.	1.87	8	0	0	27.5	137	8 H	0.43	4	0	0	30.0	215	ptal	1.06	5	0 (22.5
58		1.87	9	0	0	27.5	138	-	0.43	6	0	0	30.0	216	- Ŭ [#]	1.06	8	0 (22.5
59		6.62	5	1	0	15.0	139	-	0.43	9	0	0	Fail	217	-	0.53	6	0 (25.0
60	de	6.62	7.5	1	0	12.5	140	3	9.5	4	1	0	20.0	218	-	0.53	1	0 0	27.5
61	ilon	0.62	10	1	1	0	141	ana	9.5	0	0	0	22.5	219		2.03	0	0 (22.5
62	n ch	4.42	0	1	0	20.0	142	locy	6.34	0	0	0	22.5	220	- 89	1.35	4	0 0	22.5
64	, in	4.42	0	1	0	20.0	143	- ifi	6.34	8	0	0	27.5	221		1.35	10	0 0	22.0
65	City	9.42	8	1	0	20.0	144	siur	3.17	10	0	0	30.0	222	-	3.05	3	0 0	1 23.0
66		2.21	-	0	0	27.5	145	Atass	3.17	75	0	0	50.0 Fail	223	-	3.05	8		225
67		2.69	7	1	1	0	140	- Å	317	9	0	0	30.0	225	- g	3.05	0	ili	22.5
68	5.0	2.69	9	1	1	0	148		2.85	5	0	0	30.0	226	- R	2.03	5	1 0	25.0
69	5	1.79	4	0	0	12.5	140		1.9	4	0	0	25.0	227	1	2.03	7	1 0	20.0
70	tate	1.79	6	1	1	0	150	RECI	1.9	6	0	0	30.0	228		8.1	4	0 0	20.0
71	lagr	1.79	8	1	1	0	151	1	5.14	4	0	0	22.5	229	1	8.1	5	0 0	22.5
72	N	0.9	5	0	0	12.5	1.52	-	5.14	5	0	0	22.5	230	Ex	5.4	6	0 0	25.0
73		0.9	7	0	0	15	154	nide	5.14	6	0	0	22.5	231	niur	5.4	8	0 0	25.0
74		3.73	5	1	1	0	154	pron	3.43	7	0	0	22.5	232	umo ochi	5.4	9	0 (27.5
75	- 1 A.O	3.73	4	1	1	0	155	Ē	3.43	7.5	0	0	25.0	233	An An	2.7	7	0 0) 25.0
76	de	2.48	7.5	1	0	20.0	156	odiu	3.43	8	0	0	25.0	234		2.7	7.5	0 () 30.0
77	inter lori lori	2.48	8	1	0	15.0	157	S	1.71	9	0	0	25.0	235		2.7	10	0 0) 22.5
78	Mag ch hexa	1.24	4	0	0	27.5	158		1.71	10	0	0	25.0	236	MnSO ₄ .H ₂ O	2.05	4	0 () 22.5
79		1.24	6	0	0	25.0		36		50 C			25 - D	237	HR	Cryo-4		1 1	0
80		1.24	7	0	0	22.5									546				

Figure 2

The first 237 crystallization cocktails representing 46 highly soluble salts, 11 different cations and 14 distinct anions (conditions 49–51 and 237 are from Hampton Research Crystal Screen Cryo). The % column shows 1 in the left column if vitrified with no cryoprotectant and 1 in the middle column if vitrified without cryoprotectant when diluted 1:1 with ddH₂O; the third column shows the percentage (v/v) of glycerol in ddH₂O needed to vitrify a solution cocktail at a 1:1 ratio. Each salt is present at 0.1 *M* concentration with the buffer at 0.01 *M*.

28 Annownine monite 7<	No.	Salt	pH	PEG		9	6	No.	Salt	pH	PEG		%	
207 Amexim bank 9 9 0 23.2 343 Amexim back 6 9 2 3 344 Amexim back 6 9 2 3 344 Amexim back 6 9 2 3 344 Amexim back 6 9 2 3 347 Amexim back 6 9 2 3 348 Amexim back 6 9 2 3 349 Amexim back 6 9 2 3 340 Amexim back 7 9 2 3 351 Amexim back 7 9 2 3 363 Amexim back 7 9 2 3 364 Amexim back 7 9 2 3 373 Amexim back 7 9 2 3 374 Amexim back 7 9 3 3 375 Amexim back 7 9 3 3 374 Amexim back 7 9 3 3 375 Amexim back 7 9 3 3	238		10		0	0	22.5	314	Ammonium bromide	7	~	1	1	0
2 3 3 3 4 4 5 5 4 0	239	Ammonium bromide	9		0	0	27.5	315	Ammonium chloride	6	8	1	0	15.0
1.33 1.34 <t< td=""><td>240</td><td></td><td>7</td><td></td><td>0</td><td>0</td><td>22.5</td><td>310</td><td></td><td>7</td><td>3 20</td><td>1</td><td>0</td><td>15.0</td></t<>	240		7		0	0	22.5	310		7	3 20	1	0	15.0
int Annoxian short int	241		6	000	0	0	22.5	318	Ammonium nitrate	5	PEC	1	0	17.5
344 Amexim ploque, number 6 7 <td>243</td> <td>Ammonium chloride</td> <td>4</td> <td>20</td> <td>0</td> <td>0</td> <td>22.5</td> <td>319</td> <td></td> <td>4</td> <td>%01</td> <td>1</td> <td>0</td> <td>15.0</td>	243	Ammonium chloride	4	20	0	0	22.5	319		4	%01	1	0	15.0
343 Amonium phopple, models 7	244	·	6	L BEG	0	0	22.5	320	Ammonium phosphate, monobasic	8	1	1	0	15.0
346 Amountamplane data P 0 0 2.52 323 Amountam unifale 4 6	245	Ammonium intrate	9	80	0	0	22.5	321	Ammonium phosphate, dibasic	7	20%	0	0	25.0
317 Ammanina matrix 37 0 0 235 3 Ammanina matrix 40 1 0 1 0 1 500 Ammanina matrix 0 0 225 3 Calama 0 1	246	Ammonium phosphate, monobasic	10	6	0	0	22.5	322		4		1	0	15.0
1 1 0 0 2 2 1 0 1	247	Ammonium aboorbota, dibacia	7.5	-	0	0	22.5	323	Ammonium sulfate	8	40%	1	0	17.5
1 1 1 1 0 7.3 Calmin scatte 7.3 1 0 1.53 32 IRCyo 5 7 0 0 2.53 1.1 0 1.53 </td <td>248</td> <td>Ammonium phosphale, dibasic</td> <td>7</td> <td>1</td> <td>0</td> <td>0</td> <td>22.5</td> <td>324</td> <td>HR Cryo-9</td> <td>5</td> <td></td> <td>1</td> <td>0</td> <td>0</td>	248	Ammonium phosphale, dibasic	7	1	0	0	22.5	324	HR Cryo-9	5		1	0	0
31) 0 0.120 32 32 1 0 0 12 32) 1 1 0 0 1 0 0 1 0 0 0 1 0	250	HR Cryo-5	,		Ť	1	0	326	Calcium acetate	7		1	0	15.0
1 1 0 1 0 1 0 1 0 </td <td>251</td> <td>HR-Cryo-6</td> <td></td> <td></td> <td>1</td> <td>0</td> <td>12.5</td> <td>327</td> <td></td> <td>10</td> <td></td> <td>1</td> <td>0</td> <td>15.0</td>	251	HR-Cryo-6			1	0	12.5	327		10		1	0	15.0
135 Lahian shouk 7 0 0 225 320 Lahian shouk 6 7 1 0 1 0 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 0 1<	252	HR-Cryo-7			1	0	15.0	328	Lithium bromide	7.5	8	1	0	17.5
244	253	Lithium bromide	7		0	0	22.5	329		6	20	1	0	15.0
233 235 4 0 0 2 33 3 Magacian catcate 0 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 0 1 0 0 1 0 <td>254</td> <td></td> <td>9</td> <td></td> <td>0</td> <td>0</td> <td>22.5</td> <td>330</td> <td>Lithium chloride</td> <td>8</td> <td>DEG</td> <td>1</td> <td>0</td> <td>17.5</td>	254		9		0	0	22.5	330	Lithium chloride	8	DEG	1	0	17.5
intro intro <t< td=""><td>255</td><td></td><td>5</td><td>000</td><td>0</td><td>0</td><td>22.5</td><td>332</td><td>Magnesium acetate</td><td>0</td><td>250</td><td>+</td><td>0</td><td>0.0</td></t<>	255		5	000	0	0	22.5	332	Magnesium acetate	0	250	+	0	0.0
259 Magaeian aceta 9 9 Magaeian aceta 9 1 0 150 350 Magaeian aceta 9 0 0 225 33 Magaeian aceta 9 9 1 0 150 7 351 Magaeian aceta 6 0 225 33 Pacasian aceta 6 0 225 350 Magaeian aceta 6 0 225 33 Pacasian aceta 6 0 1 0 150 351 Magaeian aceta 6 0 0 225 33 Pacasian aceta 6 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0 10 0	257	Lithium chloride	10	50	0	0	27.5	333	Phighesten accure	5	4	1	0	15.0
190 Magasian aceta 9 8 20 0 0 22 337 Magasian aceta/aria 0 0 23 338 Namesian inflex perfamilies p	258		6	PEG	0	0	25.0	334	Magnesium chloride hexahydrate	5		1	0	15.0
200 App Part of the section of the se	259	Magnesium acetate	9	8	0	0	22.5	335	121	5		0	0	27.5
261 Magnesium chorde hexalydme 4 0 0 250 337 9 9 225 363 HR-Cyo. 0 0 250 337 Potasium bronide 10 0 150 363 HR-Cyo. 0 0 250 337 Potasium bronide 10 0 150 363 Magnesium chorde 6 0 0 252 337 Potasium bronide 10 0 150 360 Potasium bronide 6 0 0 2525 344 Potasium choride 7 7 700 Potasium bronide 5 0 0 2525 344 Potasium choride 7 10 0 17 775 7 Potasium choride 7	260		8	6	0	0	22.5	336	Magnesium sulfate heptahydrate	6	20%	0	0	27.5
2.4 1 0 0 2.25 338 $76380m$ mbcmk 0	261	Magnesium chloride hexahydrate	4		0	0	25.0	337	n	9		0	0	22.5
1 1 1 0 2 50 Potasiam bromide 10 10 150 556 Mangaces chicrife 4 0 0 2 50 Potasian carbonate 6 150 566 Potasian in actuale 7 0 0 2 2 34 Potasian carbonate 7 700 7<	262	UP Cran 8	1		0	0	12.5	338	Potassium acetate	9		1	0	15.0
$\frac{1}{205}$ Magazes choide $\frac{4}{4}$ $\frac{4}{7}$ $\frac{5}{10}$ 0 0 225 341 Petasium cubotate 6 266 75 0 0 225 341 Petasium cubotate 75 267 75 0 0 225 344 Petasium cuborite 75 772 75 0 0 225 346 Petasium nitrate 75 772 75 0 0 2255 347 Petasium nitrate 75 772 9 75 0 0 2255 350 Petasium thorita 10 0 225 777 76 0 0 2255 552 755 0 0 225 78 76 0 0 2255 356 Sodium thorita 8 78 75 50 0 2255 356 Sodium thorita	263	НК-СТУО-8	6		0	0	22.5	340	Potassium bromide	6		1	0	15.0
267 Petasian actual 7	265	Manganese chloride	4	1	0	0	22.5	341		6		1	0	15.0
267 Ptassiam actate 7 8 0 0 250 343 Petassiam clucic 8 200 75 0 0 250 345 Petassiam clucic 75 270 77 Petassiam clucic 75 346 75 346 75 270 7 Petassiam clucic 75 347 Petassiam clucic 75 770 7 Petassiam clucic 75 348 75 348 75 770 7 Petassiam clucic 75 9 9 9 10 225 770 7	266		7.5	1	0	0	22.5	342	Potassium carbonate	9		1	0	15.0
268 1 0 0 250 344 Potassium chloride 7 270 Potassium bronide 75 0 0 255 346 - - 1 0 150 271 Potassium bronide 75 - - - - 75 272 Potassium chloride 75 - - - - - 1 0 125 276 Potassium chloride 70 0 225 353 Potassium chloride 90 0 225 276 Potassium nitrate 6 0 0 225 353 Robidium chloride 75 0 0 225 354 Robidium chloride 80 0 0 225 355 Scalium hronide 80 0 0 225 356 Scalium hronide 80 0 0 225 356 Scalium hronide 80 0 0 0 225 356 Scalium hronide	267	Potassium acetate	7]	0	0	25.0	343		8		1	0	15.0
260 77 78 75 346 75 74 90 1 0 17 772 Potassium chonide 5 0 0 225 347 Potassium nirate 75 9 774 Potassium chonide 75 0 0 275 347 Potassium phoophate, monobaic 9 776 Potassium chonide 75 0 0 225 353 Potassium chonide 8 777 Potassium phoophate, monobaic 6 351 Potassium chonide 8 778 6 0 0 225 353 Redidium chonide 8 788 Potassium phoophate, monobaic 6 357 Sodium bronide 4 784 0 0 225 353 Sodium chonide 7 788 Potassium phoophate, monobaic 6 357 Sodium minitale 7 7 788 9 Sodium monigbate dingram 7 367 Sodium nininitale <td>268</td> <td>1 outstant accure</td> <td>8</td> <td></td> <td>0</td> <td>0</td> <td>25.0</td> <td>344</td> <td>Potassium chloride</td> <td>7</td> <td></td> <td>1</td> <td>0</td> <td>17.5</td>	268	1 outstant accure	8		0	0	25.0	344	Potassium chloride	7		1	0	17.5
2/10 Potassium shomide 2/3 0 0 2/23 3/47 Potassium nitrate 7/5 1 0 1/15 272 9 9 3/47 Potassium nitrate 9 3/47 Potassium nitrate 9 273 9 7 7 Potassium clarbonate 5 3/48 Potassium tinocyanate 6 7/5 3/48 Potassium tinocyanate 6 7/5 3/48 Potassium tinocyanate 6 0 2/25 3/54 Potassium tinocyanate 6 0 0 2/25 3/54 Potassium tinocyanate 6 0 0 2/25 3/54 Robidium chloride 8 0 0 2/25 3/54 Robidium chloride 6 0 0 2/25 3/54 Robidium chloride 6 0 0 0 2/25 3/54 Robidium chloride 7 0 0 2/25 3/54 Robidium chloride 7 0 0 1 0 1/25 1 0 <td>269</td> <td></td> <td>6</td> <td></td> <td>0</td> <td>0</td> <td>22.5</td> <td>345</td> <td></td> <td>4</td> <td></td> <td>1</td> <td>0</td> <td>15.0</td>	269		6		0	0	22.5	345		4		1	0	15.0
1 0 0 0 2 0 0 2 0 0 2 0 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 1 0 1 1 0	270	Potassium bromide	1.5	-	0	0	22.5	340	Potassium nitrate	15		1	0	17.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	272	i oussium promitie	8		0	0	25.0	348	r oussium maac	9		1	0	17.5
274 Potassium carbonate 5 0 0 275 330 Potassium thiocyanate 6 1 0 225 276 Potassium chloride 10 75 331 Potassium thiocyanate 5 332 333 Potassium thiocyanate 5 0 0 225 276 Potassium nitrate 8 0 0 225 333 Rubidium chloride 75 0 0 225 280 Potassium phosphate, monobasic 6 0 0 225 336 Sodium nolybdate dhlydrate 75 1 0 15 284 Potassium thiocyanate 7 361 Sodium molybdate dhlydrate 75 361 363 Sodium molybdate dhlydrate 75 1 0 15 1 0 15 1 0 15 1 0 15 1 0 15 1 0 15 1 0 15 1 0 15 1 0 15 <td>273</td> <td></td> <td>9</td> <td>1</td> <td>0</td> <td>0</td> <td>27.5</td> <td>349</td> <td>Potassium phosphate, monobasic</td> <td>9</td> <td></td> <td>1</td> <td>0</td> <td>12.5</td>	273		9	1	0	0	27.5	349	Potassium phosphate, monobasic	9		1	0	12.5
275	274	Potassium carbonate	5	1	0	0	27.5	350		6		1	0	22.5
277 Potassium chloride 10 0 0 2.25 352 9 5 0 0 2.25 278 Potassium nirate 6 0 0 2.25 354 Rubidium chloride 8 0 0 2.25 280 - 4 0 0 2.25 355 Sodium thonide 6 8 281 - 6 0 0 2.25 355 Sodium thonide 6 8 284 - 6 0 0 2.25 361 369 Sodium chloride 8 10 0 12.5 284 - 9 - 0 0 2.25 361 Sodium chloride 7.5 1 0 17.5 1 0 17.5 1 0 17.5 1 0 17.5 1 0 17.5 1 0 17.5 1 0 17.5 1 0 17.5 1 <	275		7.5		0	0	22.5	351	Potassium thiocyanate	10		1	0	22.5
277 6 0 0 250 353 Rubidium chloride 8 0 0 225 279 Potassium nitrate 8 0 0 225 355 Rubidium chloride 8 75 0 0 225 281 Potassium phosphate, monobasic 6 0 0 0 225 355 Sodium thomide 8 2 1 0 155 284 Potassium nitrate 7 0 0 225 360 360 75 90 0 0 11 0 155 284 Potassium minocyonate 75 90 0 0 2255 360 360 75 90 0 0 11 0 155 288 Sodium molybdate dihydrate 75 90 0 0 225 366 Sodium nitrate 75 1 0 155 1 0 155 1 0 155 1 0 <td>276</td> <td>Potassium chloride</td> <td>10</td> <td></td> <td>0</td> <td>0</td> <td>22.5</td> <td>352</td> <td></td> <td>5</td> <td>8</td> <td>0</td> <td>0</td> <td>22.5</td>	276	Potassium chloride	10		0	0	22.5	352		5	8	0	0	22.5
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	277		1.5	-	0	0	25.0	353	Rubidium chloride	10	200	0	0	22.5
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	279	Potassium nitrate	8		0	0	27.5	355	Rabianan emorae	7.5	EG	1	0	15.0
281 Potassium phosphate, monobasic 8 9 37 Sodium monule 8 4 283 Potassium phosphate, monobasic 6 0 0 22.5 355 Sodium chloride 8 284 Potassium thiocyanate 7 0 0 22.5 360 Sodium molybdate dihydrate 7 1 0 150 286 0 0 22.5 360 Sodium molybdate dihydrate 7 1 0 17.5 287 Rubidium chloride 4 0 0 22.5 363 Sodium nitrate 7.5 9 288 0 0 22.5 363 Sodium nitrate 7.5 9 290 Sodium chloride 7.5 0 0 22.5 366 Sodium nitrate 7.5 1 0 12.5 291 Sodium mitosulfate pentalydrate 8 0 0 22.5 369 Sodium nitrate pentalydrate 8 0 1 0 15.5	280		4		0	0	22.5	356	6 - 11 14 -	6	2% F	1	0	22.5
282 283 284 Potassium phosphate, monobasic 5 5 6 9 0 0 0 25.0 35.0 Sodium chloride 6 4 8 1 0 0 15.0 1 1 0 1 1 1 0 1 1 1 0 1 <th< td=""><td>281</td><td></td><td>8</td><td>1</td><td>0</td><td>0</td><td>22.5</td><td>357</td><td>Sodium bromide</td><td>8</td><td>4</td><td>1</td><td>0</td><td>17.5</td></th<>	281		8	1	0	0	22.5	357	Sodium bromide	8	4	1	0	17.5
283 9 39 39 8 8 284 0 0 22.5 360 7 10 125 284 Potassium thiceyanate 7 0 0 22.5 360 360 7 286 Aubidium chloride 9 9 360 0 22.5 363 360 7 7 7 1 0 17.5 1 0 17.5 9 289 Sodium chloride 7 0 0 22.5 363 366 Sodium nitrate 7 1 0 17.5 9 291 Sodium chloride 7 0 0 22.5 366 Sodium nitrate 7 1 0 12.5 293 Sodium phosphate, monobasic 6 0 0 22.5 367 Sodium nitrate 8 1 0 15.0 294 Sodium phosphate, monobasic 6 0 0 22.5 371 Zin	282	Potassium phosphate, monobasic	5		0	0	25.0	358	Sodium chloride	4		1	0	17.5
284 Potassium thicoyanate 7 9 0 0 22.5 361 7.5 7 7 7 7 7 9 1 0 17.5 287 Rubidium chloride 9 0 0 25.0 361 7.5 9 9 363 363 363 363 363 364 7.5 9 1 0 17.5 1 0 17.5 1 0 17.5 1 0 15.0 0 0 22.5 366 Sodium nirrate 7.5 0 0 22.5 366 Sodium nirrate 7.5 0 0 22.5 366 Sodium nirrate 7.5 0 0 22.5 366 Sodium nintrate 6 1 0 17.5 1 0 15.0 0 0 22.5 370 Sodium nintrate 6 1 0 15.0 1 0 15.0 1 0 15.0 1 0 <td< td=""><td>283</td><td></td><td>6</td><td></td><td>0</td><td>0</td><td>25.0</td><td>359</td><td>1.40.5.1 1.00.00 1.00.5 20.01 1.00</td><td>8</td><td></td><td>1</td><td>0</td><td>15.0</td></td<>	283		6		0	0	25.0	359	1.40.5.1 1.00.00 1.00.5 20.01 1.00	8		1	0	15.0
126 121 1 0 1 <td>284</td> <td>Potassium thiograpata</td> <td>9</td> <td></td> <td>0</td> <td>0</td> <td>22.5</td> <td>360</td> <td></td> <td>10</td> <td>-</td> <td>1</td> <td>0</td> <td>12.5</td>	284	Potassium thiograpata	9		0	0	22.5	360		10	-	1	0	12.5
287 Rabidium chloride 10 0 0 25.0 288 Sodium bromide 4 0 0 25.0 363 9 289 Sodium bromide 10 0 0 25.0 365 366 366 366 366 366 366 9 1 0 15.0 15.0 10 0 22.5 367 366 367 367 367 367 367 367 367 367 367 377 377 377 377 377 377 377 377 377 377 377 377 377 377 377	286	Totassiani unocyanac	4		0	0	22.5	362	Sodium molybdate dihydrate	7		1	0	17.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	287	Rubidium chloride	10	000	0	0	25.0	363	1	9		1	0	17.5
289 Sodium bromide 4 1 0 250 290 Sodium chloride 7.5 0 0 250 366 366 366 366 366 9 9 291 Sodium chloride 7.5 0 0 225 366	288		9	0.20	0	0	25.0	364		4		1	0	17.5
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	289	Sodium bromide	4	퓐	0	0	25.0	365	Sodium nitrate	7.5		1	0	15.0
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	290		10	20%	0	0	25.0	366		9		0	0	22.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	291	Sodium chloride	1.5		0	0	22.5	367		5		1	0	12.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	292		7	1	0	0	25.0	369	Sodium phosphate, monobasic	10		1	0	17.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	294	Sodium molybdate dihydrate	8	1	0	0	22.5	370	Sodium thiosulfate pentahydrate	8	1	1	0	17.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	295	Sodium nitrate	7]	0	0	27.5	371	Zinc acetate	6	1	1	0	15.0
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	296	Sodium phosphate, monobasic	4	1	0	0	22.5	372	HR-Cryo-10			1	0	15.0
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	297	Cadlum this 10	6		0	0	22.5	373	HR-Cryo-11			1	0	22.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	298	Sodium thiosulfate pentahydrate	8	-	0	0	22.5	374	HR-Cryo-12			0	0	22.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	300	Zinc acetate	5	1	0	0	25.0	375	пт-стуо-13	8		0	0	25.0
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	301	Potassium phosphate, dibasic	10	1	0	0	22.5	377	Cobalt sulfate heptahydrate	5	20%	0	0	22.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	302	Cobalt sulfate heptahydrate	7	1	0	0	25.0	378		4		0	0	25.0
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	303	Lithium sulfate monohydrate	6		0	0	25.0	379	Lithium sulfate monohydrate	7.5	40%	1	0	15.0
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	304		4		0	0	22.5	380		7		1	0	12.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	305	Potassium phosphate, tribasic	9	-	0	0	22.5	381	HR-Cryo14	6		1	0	15.0
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	300	Ammonium thioceanate	75		0	0	25.0	382	Potassium phosphate, tribasic	4	20%	0	0	30.0
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	308	, manomum unocyanate	5	1	0	0	22.5	384	HR-Crw-15			1	0	15.0
310 7 0 0 22.5 386 Annonun nicoganate 9 40% 0 0 150 311 5 0 0 22.5 386 Annonun nicoganate 9 40% 0 0 150 311 5 0 0 22.5 387 HR-Cryo-16 1 0 12.5 313 7 0 0 25.0 389 Magnesium nitrate hexahydrate 8 40% 1 0 15.0 300 7 0 0 25.0 389 Magnesium nitrate hexahydrate 8 40% 1 0 15.0	309	Manganese sulfate monohydrate	6	1	0	0	22.5	385	Ammonium thiographia	8	100	1	0	15.0
311 5 0 0 22.5 387 HR-Cryo-16 1 0 12.5 312 Magnesium nitrate hexahydrate 8 0 0 22.5 387 HR-Cryo-16 1 0 12.5 313 7 0 0 22.5 388 Magnesium nitrate hexahydrate 5 1 0 17.5 300 7 0 0 25.0 389 Magnesium nitrate hexahydrate 8 40% 1 0 15.0	310		7		0	0	22.5	386	Ammonium thiocyanate	9	40%	0	0	15.0
312 Magnesium nitrate hexahydrate 8 0 0 22.5 388 Magnesium nitrate hexahydrate 5 1 0 17.5 313 7 0 0 25.0 389 Magnesium nitrate hexahydrate 8 40% 1 0 15.0	311		5	1	0	0	22.5	387	HR-Cryo-16			1	0	12.5
515 / 0 0 25.0 389 Magnesium nitrate hexahydrate 8 40% 1 0 15.0 300 300 300 300 1 0 23.5	312	Magnesium nitrate hexahydrate	8	-	0	0	22.5	388	Magnationstruct	5	100	1	0	17.5
	313		7		0	0	25.0	389	Magnesium nitrate nexahydrate	8	40%	1	0	15.0

Figure 3

PEG 20 000 cocktail conditions (Nos. 238–390) shown in a similar manner to Fig. 2. Within these conditions several Hampton Research Crystal Screen Cryo condition screens are also included; Nos. 250–252, 263, 325, 372–375, 381, 384 and 387.

N Amount monitoria A N<	No.	Salt	pH	PEG		%		No.	Salt	pH	PEG		%	6
92 mamoin data a 93 Annonian data/e a 93 Annonian data/e a 93 Annonian data/e a 93 Annonian planplat, auncha: a 93 Chican aucca: a 94 0 223 94 0 223 94 0 223 94 0 223 94 0 223 94 0 223 9	391	Ammonium bromide	5		0	0	27.5	468		4		1	0	15.0
100 Amound matrix 7 100 Amound matrix 7 100 2 <th2< th=""> <th2< th=""> <th2< th=""></th2<></th2<></th2<>	392	Annonun oronide	8		0	0	27.5	469	Ammonium bromide	7		0	0	25.0
94 Amonian divide interm 8 9 1 0 2 97 Amonian floring fue, method 8 9 1 0 2 97 Amonian floring fue, method 8 9 1 0 0 97 Amonian floring fue, method 5 1 0 0 2 97 Amonian floring fue, method 5 0 0 2 0 0 2 97 Amonian floring fue, method 5 0 0 2 2 0 0 2 98 Amonian floring fue, fue, fue, fue, fue, fue, fue, fue,	393	Ammonium chloride	7		0	0	25.0	470		5		1	0	17.5
383 Annonian matches 1 1 1 0 384 Annonian pice picta, methods 7 1 0 7 994 Annonian pice picta, methods 7 0 2 3 Annonian mice 7 0 1 0 1 0 904 Annonian pice picta, methods 7 0 0 2 2 Annonian mice 7 0 0 2 0 0 2 0	394		8		0	0	25.0	471		5	8	1	0	17.5
36 4 5	395	Ammonium nitrate	10		0	0	22.5	472	Ammonium chloride	4	800	1	1	0
377 380 390 400 Ammanian phophate, methods 400 4 4 4 4 4 4 4 4 4 4	396		7		0	0	27.5	473		8	BG	1	0	17.5
initial Ammaline plocplate, instancing initial Ammaliane plocplate, datales initial Ammaliane plocplate, datales initial Ammaliane plocplate, datales initial	397		8		0	0	25.0	474		4	E.	1	0	17.5
100 110 1 <td>308</td> <td>Ammonium phosphate, monobasic</td> <td>0</td> <td></td> <td>0</td> <td>0</td> <td>22.5</td> <td>475</td> <td>Ammonium nitrate</td> <td>75</td> <td>£02</td> <td>0</td> <td>0</td> <td>22.5</td>	308	Ammonium phosphate, monobasic	0		0	0	22.5	475	Ammonium nitrate	75	£02	0	0	22.5
initian initian <t< td=""><td>300</td><td></td><td>5</td><td></td><td>0</td><td>0</td><td>25.0</td><td>476</td><td>-</td><td>7</td><td></td><td>1</td><td>1</td><td>0</td></t<>	300		5		0	0	25.0	476	-	7		1	1	0
Annonin modular, discuss in the sector of the sec	400		0		0	0	22.5	470		5		1	0	15.0
add Ammenium suffac 0 0 2.50 ddi Calciam accas 6 0 0 2.50 ddi Calciam decide diplate 3 0 1 0	400	Ammonium phosphate, dibasic	9		0	0	22.5	477	 Ammonium phosphate, monobasic 	3		-		15.0
Annomin Mathe Column Lation Mathe	401		0		0	0	22.5	470		0		1	1	26.0
non-	402	Ammonium sulfate	1.5		0	0	22.5	479	Ammonium phosphate, dibasic	2	20%	0	0	25.0
non- transmip Calcian matrix no- second sec	403		10		0	0	25.0	480		7.5		0	0	22.5
non- non- <th< td=""><td>404</td><td>Calcium acetate</td><td>6</td><td></td><td>0</td><td>0</td><td>27.5</td><td>481</td><td>-</td><td>5</td><td>40%</td><td>1</td><td>0</td><td>15.0</td></th<>	404	Calcium acetate	6		0	0	27.5	481	-	5	40%	1	0	15.0
dot Cklaim dbrick dhydrat 3 43 HRCycl ¹⁵ 1 1 0 203 deg Lihan brenik 7 0 233 0 233 0 233 0 233 0 233 deg Lihan brenik 6 0 233 0 233 0 233 0 233 diff Magacian actac 7 0 0 233 433 Chlinin dbrick 6 0 233 diff Magacian actac 7 0 0 233 0 0 233 diff Passian brenik 0 0 233 0 0 233 0 0 233 diff Passian mona 7 0 0 233 0 0 0 233 diff Passian mona 7 0 0 233 0 233 0 233 0 233 0 233 233	405		7.5		0	0	22.5	482	Ammonium sulfate	6	PEG	1	0	15.0
407 1 1 1 0 1 1 0 1	406	Calcium chloride dihydrate	5		0	0	25.0	483		9	8000	1	0	20.0
488 Calcian darke dalpata 6 7 401 7.5 0 0 2.3 411 Lithian honisk 0 0 2.3 413 Magnesian mateire 7 0 0 2.3 414 Magnesian mateire 7 0 0 2.5 417 Magnesian mateire 7 0 0 2.5 417 Magnesian mateire bepalydrate 7 0 0 2.5 418 Patasian caboant 70 0 0 2.5 417 Magnesian mateire bepalydrate 7 7 40 Magnesian mateire bepalydrate 7 410 0 2.5 7 40 Magnesian mateire bepalydrate 7 410 0 2.5 7 40 Magnesian mateire bepalydrate 7 410 0 2.5 7 7 7 7 7 411 0 2.5 7 7 7 7 </td <td>407</td> <td></td> <td>7</td> <td></td> <td>0</td> <td>0</td> <td>27.5</td> <td>484</td> <td>HR-Cryo-18</td> <td></td> <td></td> <td>1</td> <td>1</td> <td>0</td>	407		7		0	0	27.5	484	HR-Cryo-18			1	1	0
400 1.1dium loondix 10 2.35 486 1.000000000000000000000000000000000000	408		5		0	0	22.5	485	Calcium chloride dihydrate	6		1	0	17.5
410 1 1 1 1 2 4	409	Lithium bromide	10		0	0	22.5	486	cureiun enoride uniyurute	5		1	0	17.5
411 Lihim chooke 9 1 0 72 413 Magnesiam accase 6 0 2.5 40 Lihim choick 7 413 Magnesiam chorkle beachystae 7 0 0 2.5 40 Magnesiam chorkle beachystae 7 0 0 2.5 413 Magnesiam chorkle beachystae 7 0 0 2.5 40 Magnesiam chorkle beachystae 7 0 0 2.5 414 Magnesiam chorkle beachystae 7 0 0 2.5 40 Magnesiam chorkle beachystae 7 0 0 2.5 420 Porasiam morkle 6 0 0 2.5 40 Magnesiam actea 7 1 0 0 2.5 420 Porasiam morkle 7 0 0 2.5 50 Porasiam thorma 7 1 0 0 2.5 420 Porasiam thorma 7 0 0 2.5 50	410		7.5		0		22.5	487	Lithium bromide	4	8	0	0	22.5
112 Limin Monos 9 1 0 2.50 141 Magnesian advise becalytane 6 0 2.50 9 1 0 1.50 1.50 1415 Magnesian advise becalytane 7 0 0 2.50 90 1.60 1.50	411	Lithium chlorida	8		0	0	22.5	488	Linnan oronnac	9	80	1	0	17.5
131 Magnesian extence 7 9 1 0 1 1 0 1 0 1 0 1 1 0 1 0 1 1 0 1 0 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 0 1 0 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 0 1 1 1 1	412	Liunum emoride	9		0	0	25.0	489		6	EG	1	0	15.0
141 Magessian action (brink beaulydate) 7.5 9.0 0 2.23 416 Magessian action (brink beaulydate) 7.5 9.0 0 2.23 417 Magessian action (brink beaulydate) 7.5 9.0 0 2.23 418 Magessian action 0 0 0 2.50 49.0 Magessian action 7.5 0 0 2.23 418 Magessian action 0 0 0 0 0 2.50 49.0 Magessian action 7.5 0 0 0 2.50 49.0 Magessian action 7.5 0<	413	Magnesium acetate	6		0	0	22.5	490	Lithium chloride	5	18	1	0	17.5
	414		7.5		0	0	22.5	491	1	7.5	40.	0	0	22.5
1417 Magnesian sufface hepedaydrate 7 1 0 150 413 Magnesian sufface hepedaydrate 7 5 0 0 2.53 419 Peassium recluite 0 0 2.53 405 Magnesian sufface hepedaydrate 7 5 0 0 2.53 420 Peassium carbonate 10 0 0 2.53 407 7 7 7 0 0 2.50 421 Peassium carbonate 0 0 2.53 407 7 7 7 1 0 0 2.50 423 Peassium chronite 7 7 7 7 7 1 0 10 0 2.55 423 Peassium chronite 7 7 7 7 7 10 0 10 0 10 0 10 0 10 0 2.25 410 0 2.25 0 0 2.25 50<	415	Magnesium chloride hexahydrate	8		0	0	25.0	492	Magnesium acetate	7		1	1	0
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	416		7		0	0	22.5	493	Magnesium chloride hexahydrate	5		1	0	15.0
Magnesian satiae heprahydrate 7 80 400 525 90 10 255 420 Potassium carbonate 10 0 225 497 Magnesian satiae heprahydrate 7 1 0 0 256 422 Potassium carbonate 6 0 0 255 497 Magnesian satiae heprahydrate 7 1 0 0 0 0 1 0 <t< td=""><td>417</td><td></td><td>4</td><td>8</td><td>0</td><td>0</td><td>22.5</td><td>494</td><td>inghesium enternae nexaliyarate</td><td>10</td><td></td><td>0</td><td>0</td><td>22.5</td></t<>	417		4	8	0	0	22.5	494	inghesium enternae nexaliyarate	10		0	0	22.5
110 Preasium acetae 10 225 10 225 10 225 121 Petasium thremide 10 255 0 0 255 10 0 255 122 Petasium carbenat 6 0 255 0 0 255 498 Manganese chorisk 5 1 0 10 10 255 122 Petasium carbenat 6 1 0 225 501 Petasium nicrate 7 1 0 17.5 123 Petasium nitrate 7 1 0 225 505 Petasium carbenate 75 501 Petasium carbenate 75 10 0 125 505 Petasium carbenate 75 10 0 10	419	Magnesium sulfate heptahydrate	7	8	0	0	25.0	494	Magnesium sulfate hertabydrate	7.5	20%	0	0	25.0
130 Potassium contonit etc. 0 0 235 90 30 90 200 231 Potassium carbonate 0 0 255 0 0 255 232 Potassium carbonate 6 0 0 255 0 0 255 241 Potassium carbonate 5 0 0 255 501 Petassium actuae 7 232 Potassium nitrate 7 0 0 225 501 Petassium carbonate 7 232 Potassium flooplate, morobaic 7 0 0 225 501 Petassium carbonate 7 433 Sodium mitrate 7 0 0 225 505 Petassium flooplate, morobaic 7 440 6 0 0 250 0 0 255 443 Sodium mitrate 7 0 0 255 444 Sodium intrate 7 0 0 225 <	418	Potentine exterio	10	B	0	0	23.0	495	- Magnesium sunate nepianyurate	1.5	20%	0	0	25.0
420 Parasiam bronike 9 9 0 0 22.50 400 Marganese chloride 1 0 0 22.50 421 Parasiam chronite 6 0 0 22.50 60 0 22.50 60 0 22.50 60 0 22.50 60 0 22.55 60 0	419	Potassium acetate	10	18	0	0	22.5	490		2		0	0	25.0
421	420	Potassium bromide	9	20	0	0	22.5	497		/		1	0	10.0
423 Potassium carbonane 10 0 0 0 0 225 423 Potassium chloride 3 0 0 225 501 Potassium actize 7 425 Potassium chloride 5 0 0 225 501 Potassium bronide 7 426 Potassium thiceyanate 5 0 0 225 503 Potassium actize 7 420 Potassium thiceyanate 5 0 0 225 503 Potassium carbonate 8 420 Potassium thiceyanate 5 0 0 225 503 Potassium carbonate 8 430 Potassium thiceyanate 5 0 0 225 503 Potassium carbonate 8 431 Sodium thiceyanate 6 507 Potassium thiceyanate 75 10 0 225 433 Sodium thiceyanate 75 513 Potassium thiceyanate 75 10 0 10 0 10 10 10 10 10 10 10 <	421		10		0	0	25.0	498	Manganese chloride	2		1	0	22.5
123 0 <th0< th=""> 0 0 0</th0<>	422	Potassium carbonate	10		0	0	25.0	499		6		1	0	17.5
423 Potassium chleride 5 0 0 22.5 502 Potassium bromide 7.5 425 Potassium ploxplate, monobasic 7.5 503 Potassium bromide 7.5 428 Potassium ploxplate, monobasic 7.5 503 Potassium carbonate 8 430 Potassium chloride 7.5 503 Potassium carbonate 8 433 Sodium molybdate dhydrate 6 0 2.25 503 Potassium nitrate 7.5 433 Sodium molybdate dhydrate 6 0 0 2.25 503 Potassium nitrate 7.5 433 Sodium molybdate dhydrate 6 0 0 2.25 510 Potassium nitrate 7.5 443 Sodium nitrate 7.5 9 9 9 9 9 9 444 Sodium nitrate 7.5 9 9 9 9 9 9 9 9 443 Sodium ninitrate 7.5 9	423		6		0	0	22.5	.500	Potassium acetate	5		1	0	22.5
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d26 427 Potassium nitrate 10 7 0 0 2.25 503 Potassium chomide 7 428 Potassium phosphate, monobaic 439 7,5 0 0 2.25 504 506 5 430 Potassium thiceyanate 5,5 0 0 2.25 506 Potassium carbonate 5 430 Rubidum thioride 7,5 0 0 2.25 506 Potassium carbonate 9 433 Sodium mohydate. dihydrate 6 0 0 2.50 511 Potassium phosphate, monobasic 7 435 Sodium mohydate. dihydrate 7 7 0 0 2.50 511 Potassium phosphate, monobasic 7 443 Sodium nitrate 7 7 0 0 2.55 516 7 7 1 0 1.50 443 Sodium nitrate 7 7 0 0 2.55 516 7 7 1 0 1.50 <tr< td=""><td>425</td><td>r ottostum etnortee</td><td>4</td><td></td><td>0</td><td>0</td><td>22.5</td><td>502</td><td></td><td>7.5</td><td></td><td>1</td><td>0</td><td>12.5</td></tr<>	425	r ottostum etnortee	4		0	0	22.5	502		7.5		1	0	12.5
427 Protessium phosphate, monobasic 5 0 0 22.5 505 90 9 8 428 Protessium phosphate, monobasic 7.5 0 0 22.5 505 90 9 9 10 15.0 1 0 15.0 430 Potassium chorade 6 0 0 22.5 507 90 9 9 10 0 0 22.5 90 0 22.5 507 90 0 22.5 90 0 0 22.5 90 0 0 22.5 90 0 0 22.5 90 9 9 9 1 0 15.0 1 0 15.0 1 0 15.5 1 0 0 22.5 1 1 0 15.5 1 0 1 0 15.5 1 0 0 22.5 1 0 0 0 1 0 15.5 1 0	426	Potassium nitrate	10		0	0	22.5	503	Potassium bromide	7		1	0	17.5
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420 Potassium fixedutais. 7.5 0 0 22.5 906 Potassium carbonate 8 430 Potassium finequate. 7.5 0 0 22.5 907 Potassium carbonate 8 431 Robidium chloride 6 0 0 22.5 907 Potassium finequate. 8 433 Sodium molybdate dihydrate 6 0 0 22.5 907 Potassium finequate. 7.5 907 443 Sodium molybdate dihydrate 7.5 0 0 22.5 510 Potassium finequate. 7.5 1 0 150 443 Sodium molybdate dihydrate 7.5 0 0 22.5 516 Potassium finequate. 7.5 1 0 150 1 0 150 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 <th< td=""><td>428</td><td>Batassium abasakata manahasia</td><td>5</td><td></td><td>0</td><td>0</td><td>22.5</td><td>505</td><td></td><td>5</td><td></td><td>1</td><td>0</td><td>15.0</td></th<>	428	Batassium abasakata manahasia	5		0	0	22.5	505		5		1	0	15.0
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	429	Potassium phospitate, monodasie	7.5		0	0	22.5	506	Patasium and anota	8		1	0	15.0
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$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	433	Sodium bromide	6		0	0	25.0	510	Potassium chloride	10		0	0	22.5
436 Sodium chloride 6 0 0 22.5 512 Potassium nitrate 7.5 0 0 1.6 0 1.50 437 Sodium molybdate dihydrate 7 9 0 0 25.0 513 Potassium nitrate 7.5 7.5 440 6 7 0 0 25.0 515 7.5	434		7.5		0	0	25.0	511	-	6		1	0	15.0
36. 437 438 438 438 Sodium molybdate dihydrate 9 6 9 0 0 25.0 0 513 513 Potassium nitrate 7.5 8 10 0 10 10 10 10 15.0 10 439 Sodium mitrate 7 0 0 25.0 514 7.5 8 440 Sodium mitrate 7 0 0 25.0 516 7.5 8 441 Sodium mitrate 7.5 0 0 22.5 516 7.5 1 0 10 12.5 444 Sodium mitrate 7.5 0 0 22.5 516 7.5 10 0 12.5 1 0 10 12.5 443 Sodium mitrate 50 0 22.5 521 Rubidium chloride 9 0 0 22.5 522 Sodium bronside 9 0 0 22.5 523 Sodium chloride 9 0 0 10 15.0 1 0 15.0 1 0 15.0	435	Sodium chloride	6		0	0	22.5	512		9		1	0	15.0
437 Sodium molybdate dihydrate 7 1 1 2 2 5 10 9 9 10 12 2 5 10 10 12 10 12 10 12 10 12 10 <th12< th=""> 10 12 10<td>436</td><td></td><td>6</td><td></td><td>0</td><td>0</td><td>25.0</td><td>513</td><td>Potassium nitrate</td><td>7.5</td><td></td><td>1</td><td>0</td><td>15.0</td></th12<>	436		6		0	0	25.0	513	Potassium nitrate	7.5		1	0	15.0
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	430		7		0	0	25.0	514		0		1	0	15.0
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$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	441	Sodium nitrate	7		0	0	22.5	518		10	20	1	0	17.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	442		4		0	0	27.5	519	Potassium thiocyanate	5	च	1	0	12.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	443		7.5		0	0	27.5	520		8		1	0	22.5
445 Zinc acetate 4 0 0 250 522 Notional diologe 10 446 5 0 0 30.0 523 Sodium bronide 9 448 7.5 1 1 22.5 524 Sodium bronide 9 449 Potassium phosphate, dibasic 7 0 0 22.5 526 Sodium chloride 9 451 8 0 0 22.5 526 Sodium chloride 9 453 0 0 22.5 526 Sodium chloride 9 453 0 0 22.5 527 Sodium chloride 9 454 Lithium sulfate monohydrate 4 0 0 22.5 530 6 1 0 17.5 456 7 0 0 22.5 533 Sodium thiosulfate pentahydrate 9 1 0 15.0 458 7 0 0 22.5 533	444	Sodium thiosulfate pentahydrate	10		0	0	22.5	521	Rubidium chloride	9		0	0	22.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	445	Zinc scetate	4		0	0	25.0	522	Rubidian chioride	10		0	0	22.5
447 HR-Cryo-17 1 1 22.5 Sodium bromide 9 448 7.5 0 0 22.5 0 0 1 0 17.5 450 7 0 0 22.5 0 0 22.5 526 527 Sodium chloride 9 451 8 0 0 22.5 0 0 22.5 527 Sodium chloride 9 453 0 0 25.0 0 0 25.0 530 6 1 0 15.0 455 8 0 0 25.0 531 Sodium phosphate, monobasic 6 1 0 17.5 456 7 0 0 22.5 0 0 22.5 533 Sodium phosphate, monobasic 6 1 0 17.5 457 Potassium phosphate, tribasic 7 0 0 22.5 533 Sodium thiosulfate pentahydrate 9 1 0 15.0 458 0 0 22.5 0 0	446	Line accure	5		0	0	30.0	523		7.5		0	0	22.5
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450 Protassium prosphate, dibasic 7 451 8 452 Cobalt sulfate heptahydrate 4 453 0 0 22.5 454 Lithium sulfate monohydrate 4 455 8 0 0 25.0 454 Lithium sulfate monohydrate 4 5 529 Sodium nitrate 4 455 7 5 0 0 22.5 531 Sodium thiosulfate pentahydrate 6 457 Potassium phosphate, tribasic 7 0 0 22.5 533 Sodium thiosulfate pentahydrate 9 458 0 0 22.5 533 Sodium thiosulfate pentahydrate 9 460 Ammonium thiocyanate 5 0 0 22.5 536 Zinc acetate 6 463 Magnesium nitrate hexahydrate 9 0 0 22.5 539 Sadium chloride 7 1 0 17.5 464 0 <td< td=""><td>449</td><td>Bata da da da da da da</td><td>4</td><td></td><td>0</td><td>0</td><td>22.5</td><td>526</td><td></td><td>8</td><td></td><td>1</td><td>0</td><td>17.5</td></td<>	449	Bata da da da da da da	4		0	0	22.5	526		8		1	0	17.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	450	Potassium phosphate, dibasic	7		0	0	22.5	527	Sodium chloride	9		1	0	15.0
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455 8 0 0 2.5.5 531 350 and prophate, tribusic 0 1 0 15.0 455 7 Potassium phosphate, tribasic 7 0 0 22.5 533 533 Sodium phosphate, tribusic 1 0 15.0 457 Potassium phosphate, tribasic 7 0 0 22.5 533 Sodium thiosulfate pentahydrate 9 1 0 15.0 458 460 Ammonium thiocyanate 8 0 0 22.5 536 Zinc acetate 8 1 0 15.0 461 10 0 0 22.5 536 Zinc acetate 8 0 0 22.5 463 Manganese sulfate monohydrate 5 0 0 22.5 538 Potassium phosphate, tribasic 5 20% 1 0 22.5 466 Magnesium nitrate hexahydrate 9 0 0 22.5 541 1 0 17.5 <	454	Lithium sulfate monohydrate	4		0	0	25.0	\$21	Sodium phoephate, monobasie	6		1	0	150
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	4,54	Laurum sunate mononyurate	4		0	0	22.0	531	soonan prospirate, monousie	7			0	17.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	433		8	8	0	0	22.5	532		/		1	0	17.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	450	The second state of the se	1.5	800	0	0	22.5	533		10		1	0	15.0
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	457	Potassium phosphate, tribasic	7	EG	0	0	25.0	534	Sodium thiosulfate pentahydrate	9		1	0	15.0
459 Ammonium thiocyanate 8 5 0 0 25.0 536 Zinc acetate 6 1 0 17.5 461 10 0 0 22.5 537 20% 1 0 22.5 461 100 0 22.5 538 Potassium phosphate, dibasic 5 20% 1 0 22.5 463 Magnesium nitrate hexahydrate 4 0 0 22.5 540 Lithium sulfate monohydrate 7 40% 1 0 22.5 466 Magnesium nitrate hexahydrate 9 0 0 22.5 540 Lithium sulfate monohydrate 7 40% 1 0 22.5 466 0 0 22.5 541 9 1 0 22.5 466 0 0 22.5 541 9 1 0 22.5 467 0 0 22.5 543 Potassium phosphate, tribasic 5	458		4	P P	0	0	22.5	535		8		1	0	17.5
460 Ammonium thiceyanate 5 0 0 22.5 461 10 0 0 22.5 537 Line accure 8 0 0 22.5 461 10 0 0 22.5 538 Potassium phosphate, dibasic 5 20% 1 0 22.5 463 Magnesium nitrate hexahydrate 5 0 0 22.5 541 540 Lithium sulfate monohydrate 7 40% 1 0 22.5 466 9 6 0 0 22.5 541 10 0 22.5 466 6 0 0 22.5 541 10 0 22.5 466 6 0 0 22.5 541 10 0 15.0 467 0 0 22.5 543 Potassium phosphate, tribasic 5 20% 0 0 25.0 467 7.5 0 0 22.5 <td>459</td> <td></td> <td>8</td> <td>502</td> <td>0</td> <td>0</td> <td>25.0</td> <td>536</td> <td>Zinc acetate</td> <td>6</td> <td></td> <td>1</td> <td>0</td> <td>17.5</td>	459		8	502	0	0	25.0	536	Zinc acetate	6		1	0	17.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	460	Ammonium thiocyanate	5		0	0	22.5	537	Lane accure	8		0	0	22.5
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	461		10		0	0	22.5	538	Potassium phosphate, dibasic	5	20%	1	0	22.5
463 manganese summe monohydrate 7 0 0 22.5 464 4 0 0 22.5 541 9 1 0 22.5 465 9 0 0 22.5 541 9 1 0 15.0 466 9 6 0 0 22.5 541 9 0 0 17.5 467 7,5 0 0 22.5 541 1 0 17.5 467 7,5 0 0 22.5 542 HR-Cryo-19 0 0 17.5 543<	462	Managanasa sulfeta manahudaata	5		0	0	25.0	539	Shudha and same	5		1	1	0
464 4 0 0 22.5 541 9 1 0 15.0 465 9 6 0 0 25.0 542 HR-Cryo-19 0 0 17.5 466 7.5 0 0 22.5 543 Potassium phosphate, tribasic 5 20% 0 0 25.0 467 544 Ammonium thiocyanate 4 0 0 22.5 544 Ammonium thiocyanate 6 40% 1 0 10 545 546 Mananage sulfate monthurgets 6 1 0 10	463	manganese surrate mononyurate	7		0	0	22.5	540	Lithium sulfate monohydrate	7	40%	1	0	22.5
465 Magnesium nitrate hexahydrate 9 0 0 25.0 466 6 0 0 22.5 542 HR-Cryo-19 0 0 17.5 467 6 7.5 0 0 22.5 543 Potassium phosphate, tribasic 5 20% 0 0 25.0 546 545 Ammonium thicoyanate 4 4 0 0 22.5 545 Magnesium phosphate, tribasic 5 20% 0 0 22.5 546 Magnesa pulfate monthicoyanate 4 4 40% 1 0 15.0	464		4		0	0	22.5	541		9		1	0	15.0
466 Magnesium nitrate hexahydrate 6 0 0 22.5 543 Potassium phosphate, tribasic 5 20% 0 0 25.0 467 7.5 0 0 22.5 543 Potassium phosphate, tribasic 5 20% 0 0 25.0 467 7.5 0 0 22.5 544 Ammonium thiocyanate 4 0 0 22.5 545 Magnese sulfate monchultere 6 40% 1 0 15.0	465		9		0	0	25.0	542	HR-Cryo-19			0	0	17.5
467 7.5 0 0 22.5 544 Ammonium thioxyanate 4 0 0 22.5 545 Ammonium thioxyanate 6 40% 1 0 15.0 546 Managages sulfate monobusteria 6 40% 1 0 15.0	466	Magnesium nitrate hexahydrate	6		0	0	22.5	543	Potassium phosphate, tribasic	5	20%	0	0	25.0
545 Ammonium thiocyanate 6 40% 1 0 54.0 546 Managage sulfate manchulente 6 1 0 15.0	467		7.5		0	0	22.5	544	to the second se	4		0	0	22.5
unu unu <thu< th=""> <thu< th=""> <thu< th=""></thu<></thu<></thu<>			1.10					545	Ammonium thiocyanate	6	40%	1	0	15.0
								546	Manganese sulfate monolustrat-	6	10.10	1	1	0

Figure 4

PEG 8000 cocktail conditions (Nos. 391–546), similar to Fig. 2. Within these conditions several Hampton Research Crystal Screen Cryo condition screens are also included: Nos. 447, 484 and 542.

No.	Salt	pH	PEG	ĺ.	%		[No.	Salt	pH	PEG		%	1
547	Ammonium bromide	10		0	0	25.0		621	Ammonium bromide	4		0	0	22.5
548	, minionium province	8		0	0	25.0		622		6		1	0	12.5
549	Ammonium chloride	10		0	0	25.0		623	Ammonium chloride	10	18	1	0	17.5
550		5		0	0	25.0		624		7	40	1	0	12.5
551	Ammonium nitrate	8		0	0	25.0		625		7.5	000	1	0	12.5
552	Ammonium phoenhata monohasio	9	-	0	0	25.0		620	Ammonium nitrate	0	G 4	1	0	17.5
554	Annionani phosphate, monobasie	7	{	0	0	23.0		628		10	PE	1	0	12.5
555		4	1	0	0	25.0		629	the second second second second second	7.5		1	1	0
556	Ammonium phosphate, dibasic	9	1	0	0	25.0		630	Ammonium phosphate, monobasic	10		0	0	22.5
557	8 A C	7	1	0	0	22.5		631	Ammonium phosphate, dibasic	10	20%	0	0	27.5
558		7.5	1	0	0	27.5		632		10		1	0	17.5
559	Ammonium sultate	9	1	0	0	25.0	1	633	Ammonium sulfate	4		0	0	22.5
560	Calcium acetate	7	1	0	0	25.0		634		6		1	0	15.0
561	Calcium chloride dihydrate	5]	0	0	25.0		635	Calcium chloride dihydrate	7		1	0	17.5
562	culcium emoriae amyarate	7.5		0	0	25.0		636	culcum entoniae emperate	5		1	0	17.5
563	Lithium bromide	5		0	0	25.0		637	Lithium bromide	4		1	0	17.5
564		8		0	0	27.5		638		10		1	0	15.0
565		8		0	0	25.0		639	Magnesium acetate	7.5		0	0	22.5
567	Lithium chloride	10	-	0	0	25.0		640	Magnesium chloride hexahydrate	76		1	0	22.5
568		75		0	0	25.0		642	Manganasa eklorida	6		1	0	22.5
560		6	1	0	0	25.0		643	Wanganese emoride	5		1	0	20.0
570	Magnesium chloride hexahydrate	7.5	1	0	0	22.5		644	Potassium acetate	8		1	1	0
571		7	1	0	0	22.5	1	645	1.24-00048-00018-004-00058-0	7.5		1	i	0
572		8	1	0	0	22.5	1	646		7		1	0	20.0
573	Magnesium sulfate heptahydrate	9	1	0	0	25.0	1	647	Potassium bromide	9		1	0	17.5
574		6]	0	0	25.0		648		6		0	0	22.5
575		4]	0	0	25.0	[649		7.5		1	0	15.0
576		5		0	0	22.5		650	Potassium carbonate	8		0	0	22.5
577	Manganese chloride	7		0	0	27.5		651		10		1	0	20.0
578		6		0	0	25.0		652	Potassium chloride	9		1	0	15.0
579	Potassium acetate	4		0	0	25.0		653		6		1	1	0
580		0		0	0	22.5		655	Potassium nitrata	10		-	0	10.0
582	Potassium bromide		8	0	0	22.5		656	r otassium mirate	4		1	0	17.5
583	r oussium oronnac	7.5	940	0	0	22.5		657		8		1	0	17.5
584		7	PEC	0	0	27.5		658	Potassium phosphate, monobasic	4		1	0	17.5
585	Potassium carbonate	8	20	0	0	25.0		659		6		1	0	12.5
586	Determinen ekleside	10	1 14	0	0	22.5	1	660	Petersium this summtry	7		1	0	17.5
587	Potassium chiorkie	8]	0	0	27.5	[661	Polassium infocyanate	8	8	1	Ι	0
588	Potassium nitrate	7]	0	0	27.5		662		4	140	1	0	17.5
589	Potassium phosphate, monobasic	4		0	0	25.0		663		7.5	PEC	1	0	22.5
590		4		0	0	25.0		664	Rubidium chloride	8	250	1	0	15.0
591	Potassium thiocyanate	9		0	0	22.5		665		5	4	1	0	15.0
592	Dubidium ablasida	7.5	{	0	0	22.5		667				1	0	12.5
593	Rublatum chioride	7	1	0	0	21.5		669	Sodium bromide	75		1	0	12.5
595	Sodium bromide	5		0	0	22.5		669	Sodium chloride	5		1	1	22.5
596		5	1	0	0	25.0		670		10		1	0	15.0
597		7	1	0	0	25.0		671	Sodium molybdate dihydrate	8		1	0	15.0
598	Sodium chloride	9	1	0	0	25.0	1	672	Codium alterta	9		1	0	17.5
599		7.5		0	0	27.5		673	Sourun mittate	5		1	0	12.5
600	Sodium molybdate dihydrate	10	1	0	0	22.5		674		7.5		1	1	0
601		4		0	0	22.5		675	Sodium thiosulfate pentahydrate	9		1	0	10.0
602	Sodium nitrate	8	-	0	0	27.5		676		10		1	1	0
603		0	-	0	0	25.0		670	Zinc acetate	3		1	0	12.5
605	Sodium phosphate, monobasic	0	-	0	0	25.0		670	Potessium phosphate dibasis	0		0	0	12.5
606	Sodium thiosulfate pentabydrate	4	1	0	0	25.0	1	680	Polassium phosphate urbasic	7		1	0	0
607	souriar anosariae permanyariae	4	1	0	0	25.0		581	Lithium sulfate monohydrate	10		i	1	0
608	Potassium phosphate, dibasic	9	1	0	0	22.5		682	No. 1	9		0	0	25.0
609		5	1	0	0	25.0		683	Potassium phosphate, tribasic	8		0	0	22.5
610	Cobalt sulfate heptahydrate	8]	0	0	22.5		684		9		1	0	17.5
611		4]	0	0	27.5		685	Ammonium thiocyanate	4		1	0	12.5
612	Lithium sulfate monohydrate	5		0	0	27.5		686		6		1	0	22.5
613		4		0	0	22.5		687		6		0	0	22.5
614		8		0	0	25.0		688	Manganese sulfate monohydrate	7		1	0	12.5
615	Potassium phosphate, tribasic	10		0	0	22.5		689		1		1	0	15.0
616	1.1.5.1 No. 141.444	4	-	0	0	22.5		690		0		1	0	12.5
618	Ammonium thiocyanate	5		0	0	22.3		602		6		1	0	22.5
619	Manganese sulfate monohydrate	6	1	0	0	25.0		693	Magnesium nitrate hexahydrate	5		1	0	12.5
620	Magnesium nitrate hexahydrate	9	1	0	0	22.5		694		9		1	0	15.0
1 100031377				0.001		000010702	- L	11/02/57875						0.00.0250

Figure 5 PEG 4000 cocktail conditions (Nos. 547–694), similar to Fig. 2.

No.	Salt	pH	PEG		%	6	N	lo.	Salt	pH	PEG		%	
695	Ammonium bromide	7.5		0	0	25.0	76	58	1	8		0	0	22.5
696		4	1	0	0	27.5	76	59	Ammonium bromide	5		0	0	22.5
697		5	1	0	0	22.5	77	70	Ammonium chloride	8		1	0	17.5
698	Ammonium chloride	9	1	0	0	22.5	77	71	Ammonium nitrate	7		1	0	15.0
699		8	1	0	0	25.0	77	72		10		1	0	12.5
700		7.5	1	0	0	22.5	77	73		4		1	0	17.5
701	Ammonium nitrate	5	1	0	0	22.5	77	74	Ammonium phosphate, monobasic	7.5		1	1	0
702		4	1	0	0	25.0	77	75		7		1	0	15.0
703	A STREAM STREAM	7	1	0	0	25.0	77	76	Ammonium phosphate, dibasic	4		1	0	10.0
704	Ammonium phosphate, monobasic	6	1	0	0	25.0	77	77	Sources Incoloured and an	7		1	1	0
705		4	1	0	0	22.5	77	78	Ammonium sulfate	10		1	0	15.0
706	Ammonium phosphate, dibasic	0	1	0	0	22.5	77	70		8		1	0	25.0
707		6	1	0	0	22.5	78	80	Calcium acetate	5		i	0	17.5
708	Ammonium sulfate	7	1	0	0	22.5	75	81	Calcium chloride dihydrate	7		-i-	0	12.5
700	Calcium chloride dibydrate	5	1	0	0	22.5	75	82	Calcium entoride onlyurate	6		1	0	12.5
710	Lithium bromide	7	1	0	0	25.0	75	23	Lithium beamida	4		-	0	17.5
711	Eduluti oronnac	8	1	0	0	25.0	75	24	Liunum bronnde	8		1	0	17.5
712	Lithium chloride	6	{	0	0	25.0	76	25		0		÷	0	12.5
712		0	{	0	0	23.0	70	26		5		1	0	12.5
713	Magnacium chlorida havahudrata	9	{	0	0	27.5	70	07		0		0	0	12.5
714	Wagnesium chorue nexaryurate	6	-	0	0	22.5	70	57	Lithium chloride	9		0	0	12.5
715		0	{	0	0	22.5	78	00		4		1	1	0
710	Mamorium culfute hantshuderte	7.2	-	0	0	25.0	70	59 59	M	10		0	1	17.6
717	Magnesium suitate neptanyurate	9	-	0	0	25.0	79	90	Magnesium acetate	8		-	0	17.5
718		2	-	0	0	25.0	75	22	Magnesium chioride hexanydrate	9	-		0	17.5
719		2	_	0	0	25.0	/5	92	Magnesium suitate neptanydrate	9	000	1	0	17.5
720	manganese chioride	4	00	0	0	25.0	79	23	Manganese chloride	3	01	0	0	22.3
721		6	19	0	0	25.0	79	94		6	PE	1	0	17.5
722	Potassium acetate	4	E	0	0	22.5	79	95	Potassium acetate	7	250	0	0	22.5
723	Potassium bromide	4	250	0	0	22.5	79	96		6	4	1	1	0
724	Potassium carbonate	5	10	0	0	22.5	79	97		5		1	1	0
725		4		0	0	22.5	79	98	Potassium bromide	8		1	0	17.5
726	Potassium chloride	5		0	0	25.0	79	99		4		0	0	25.0
727	2003/01/2002/2007/2005/	7		0	0	25.0	80	00		10		1	0	17.5
728		5		0	0	25.0	80	01	Potassium carbonate	4		1	0	17.5
729	Potassium nitrate	10		0	0	22.5	80	02		5		1	0	15.0
730		6		0	0	22.5	80	03		9		1	0	20.0
731	Potassium phosphate, monobasic	7		0	0	22.5	80	04		10		1	0	15.0
732	i cuiscian prospinite monorant	6		0	0	27.5	80	05	Potassium chloride	9		1	0	17.5
733	Potassium thiocyanate	10		0	0	25.0	80	06		7.5		1	0	17.5
734	r otassiani unocyanate	8		0	0	27.5	80	07	Potassium nitrate	7.5		0	0	22.5
735	Rubidium chloride	6		0	0	25.0	80	08	i oussium muute	4		1	0	17.5
736	Rubidian enoride	9		0	0	25.0	80)9	Potassium phosphata, monohasia	8		1	0	17.5
737	Sodium bromide	7		0	0	22.5	81	10	rotassium phosphate, monooasie	4		1	0	17.5
738	Somm bronnac	4		0	0	25.0	81	11	Potessium thioguanata	7.5		1	0	15.0
739	Sodium chloride	10	1	0	0	25.0	81	12	Potassium unocyanate	6		1	0	17.5
740	Sadium malukdata dikudaata	9	1	0	0	25.0	81	13		4		1	0	10.0
741	Sodium moryodate umyorate	7.5	1	0	0	25.0	81	14	Rubidium chloride	8		1	0	15.0
742	Soutions alterets	7		0	0	27.5	81	15		6		1	0	17.5
743	Soutum Intrate	8		0	0	27.5	81	16	Sodium bromida	10		1	0	17.5
744	Sodium phosphate, monobasic	7.5		0	0	22.5	81	17	Sourum bronnide	9		1	0	10.0
745	Sodium thiosulfate pentahydrate	8		0	0	22.5	81	18	Sodium ablasida	8		1	0	17.5
746	Zinc acetate	5]	0	0	25.0	81	19	sourum entoride	10		1	0	10.0
747		8	1	0	0	25.0	82	20	Sodium moluk-bas dibadaata	7.5		1	0	17.5
748	Potassium phosphate dibasic	4	1	0	0	25.0	82	21	Sodium moryodate dinydrate	8	20%	1	0	22.5
749	3 72. 0474	10	1	0	0	22.5	82	22	Out the second second	4		0	0	12.5
750		8	10%	0	0	27.5	82	23	Sodium nitrate	10		1	0	17.5
751		7		0	0	22.5	82	24	Sodium phosphate, monobasic	6		1	0	15.0
752	Cobait sulfate heptahydrate	6	1	0	0	27.5	82	25		5		1	0	15.0
753		5	1	0	0	25.0	82	26	Zinc acetate	8		1	0	10.0
754		6	1	0	0	25.0	82	27		5		1	0	15.0
755	Lithium sulfate monohydrate	7	1	0	0	25.0	82	28	Potassium phosphate, dibasic	7		0	0	22.5
756		9	1	0	0	27.5	82	29		8	000	1	0	12.5
757		9	0	0	0	22.5	83	30	Lithium sulfate monohydrate	6	3.10	0	0	17.5
758	Potassium phosphate, tribasic	7	100	0	0	27.5	83	31		10	PEC	0	0	17.5
759	Fspinner in some	7.5	0	0	0	25.0	81	32		75	2%	0	0	17.5
760		10	PF	0	0	22.5	81	33	Potassium phosphate-tribasic	6	40	0	0	25.0
761	Ammonium thiocyanate	8	20%	0	0	25.0	83	34		0		1	0	17.5
762	. inanomani unocyanace	7.5		0	0	25.0	97	35	Ammonium thiocyanate	10		1	0	15.0
763		4		0	0	23.0	0.0	36	Annositan unocyanate	7		1	0	17.5
764	Manganese sulfate monohydrate	5	1	0	0	27.5	0.1	37	AND THE REAL PROPERTY OF	6			0	17.5
765		0	1	0	0	25.0	0.3	29	Manganese sulfate monohydrate	7		1	0	17.5
766	Magnesium niteata keyakudasta	6	1	0	0	23.0	63	20	Magnacium nitrata herrelanderte	0		1	0	17.5
767	Magnesium mulate nexanyurate	3	-	0	0	25.0	6.3	19	magnesium nurate nexatiyurate	9		1	V	14.3
/0/		1 /		0	0	4.3.0								

Figure 6 PEG 1000 cocktail conditions (Nos. 695–839), similar to Fig. 2.

#	Salt	pH	PEG		%	l i	
840	A	8.0		1	1	0	1
841	Ammonium bromide	4.0	1	1	0	17.5	1
842		6.0	1	I	0	20.0	t i
843	Ammonium chloride	10.0		0	0	22.5	ł –
045		7.0		0	0	22.5	
844	Ammonium nitrate	7.0		0	0	22.5	
845	Construction of the same construction of the same	5.0	8	1	0	17.5	1
846	Ammonium phosphate, monobasic	5.0	5	1	0	17.5	
847	Annikolitani phosphate, nonobase	7.5) ě	1	0	12.5	
848	Ammonium phosphate, dibasic	8.0	18	1	0	15.0	1
840		7.0		1	0	17.5	1
049	Ammonium sulfate	7.0	1		0	17.5	
850		7.5		1	0	17.5	1
851		5.0		1	0	10.0	
852	Calcium acetate	7.5	1	1	0	17.5	
853		6.0	1	1	1	0	f i
954	Calaium ablacida dibudeata	7.5	1	1	0	17.5	1
0.54	Calcium enionide dinydrate	1.5		1	0	17.5	
855	HR-Cryo-20			1	1	0	
856		6.0		1	0	15.0	
857	2427-24	4.0	1	0	0	22.5	1
858	Lithium bromide	8.0	1	1	0	20.0	1
050		0.0			0	15.0	1
859		9.0		1	0	15.0	4
860	Lithium chloride	7.0		1	0	20.0	
861	Magnesium acetate	5.0		-1	0	17.5	
862	Magnesium chloride hexahydrate	6.0	1	1	0	17.5	
862	- Aguestani enstrac nexanyatate	6.0		1	0	17.5	
803	Magnesium sulfate heptahydrate	0.0		1	0	17.5	
864		7.0		1	0	17.5	
865	Potassium acetate	10.0		-1	0	12.5	
866		7.0	1	1	0	17.5	1
867		7.5		1	1	0	
007	Potassium bromide	1.5		-	1	0	
868		6.0		1	0	12.5	
869		4.0		1	0	20.0	
870		9.0	1	1	0	12.5	1
871	Potassium carbonate	5.0	1	1	0	12.5	1
071	r outstant en pointe	7.0			, i	10.0	
872		7.0		1	1	0	4
873	Potassium chlorida	4.0		1	0	10.0	
874	r oussium entonice	6.0		1	1	0	
875		7.5	1	1	1	0	1
876	Potassium nitrate	9.0	1	1	0	0	1
070	1 outstand induce	6.0		-		0	1
8//		6.0		1.	1	0	4
878		7.0		1	1	0	
879		10.0		1	0	12.5	
880	Potassium phosphate, monobasic	6.0	1	1	1	0	1
001		0.0			i	0	1
001		9.0		1	1	0	
882		10.0		1	1	0	1
883	Potassium thiocyanate	4.0	8	1	1	0	
884		9.0	9	1	1	0	1
885		4.0	8	1	0	20.0	1
00.0		4.0	P		0	20.0	
880	Rubidium chloride	7.0	038	1	0	20.0	1
887		5.0	4	1	0	12.5	
888	e	8.0		1	0	22.5	
889	Sodium bromide	7.0	1	0	0	12.5	
800	Sodium ablasida	72		1	1	0	
890	Sodium chloride	1.5		1	1	0	
891	Sodium molybdate dihydrate	8.0		1	0	25,0	
892	Sodium nitrata	6.0		1	1	0	
893	sourum nitrate	8.0	1	1	0	17.5	
894		50	1	1	1	0	1
805	Sodium phoephata, manahasia	60		0	0	22.6	
693	Socium phosphate, monobasic	0.0		0	U	44.5	
896		8.0		1	0	22.5	
897		8.0		0	0	22.5	
898	Sodium thiosulfate pentahydrate	9.0	1	1	1	0	1
800		10.0		1	0	75	
000		10.0		-		1.0	
900		8.0.		1	1	0	1
901	Zinc acetate	6.0		1	1	0	
902		5.0	1	1	1	0	1
002		50	1	1	1	0	ŧ .
903	Potassium phosphate, dibasic	3.0	1	-	1	0	
904	ana ana amin'ny fanisa dia mampiasa amin'ny fanisa dia mampiasa dia mampiasa dia mampiasa dia mampiasa dia mampi	10.0	1	1	1	0	1
905	Lithium sulfate monohudeate	7.5		1	1	0	
906	Lithium surfate mononyarate	10.0	1	1	0	22.5	1
907		50	1	1	1	0	
000	Potassium phosphate, tribasic	5.0		-	1	0	
908		7.0		1	1	0	1
909		9.0		1	0	12.5	
910		7,5	1	1	1	0	
911	Ammonium thiocyanate	80	1	1	0	10.0	1
012		0.0		-	0	22.5	
912		4.0		1	0	22.5	ł
913	Magnesium nitrata havahudesta	7.5		1	0	12.5	
914	magnesium intrate nexanydrate	9.0	1	1	1	0	1
1000		M		-			

#	Salt	nH	PEG		%	
915		5.0	120	1	1	0
916	Ammonium bromide	7.0	800	1	1	0
917	Ammonium chloride	5.0	80%	1	1	0
918	Annonum entorac	8.0	-	1	1	0
919	Ammonium nitrate	7.5	60%	1	1	0
920	Ammonium phosphate, monobasic	8.0	-	0	0	30.0
921		9.0		0	0	25.0
922		7.0	340	0	0	25.0
923	Ammonium phosphate, dibasic	4.0	- BE	0	0	27.5
925		8.0	80	0	0	25.0
926	Ammonium sulfate	9.0	~	0	0	25.0
927	Calcium acetate	5.0	1	0	0	25.0
928	HR-Cryo-21	-		1	1	0
929	Calcium chloride dihydrate	7.5	80%	1	1	0
930	Lithium bromide	8.0		1	1	0
931		10.0	20%	0	0	25.0
932	Lithium chloride	7.0	4	1	1	0
933	Magnesium acetate	7.0	80%		1	0
934	Magnesium chloride hexahydrate	8.0		1	1	0
955		5.0		0	0	25.0
937	Magnesium sulfate heptahydrate	7.5	20%	0	0	27.5
938	Manganese chloride	7.0		1	1	0
939	Potassium acetate	9.0		1	1	0
940	Manganese chloride	8.0	80%	1	1	0
941		5.0	1	1	1	0
942	Potassium acetate	10.0	20%	0	0	22.5
943	r oussium accure	6.0		1	1	0
944		7.0	80%	1	1	0
945	Potassium bromide	9.0		1	1	0
946	Potassium carbonate	7.0	60%	1	1	0
947	Potassium chloride	8.0	80%	1	1	0
948		10.0	20%	0	0	27.5
950	Potassium nitrate	7.0	80%	0	0	22.5
951	Potassium phosphate, monobasic	5.0	20%	0	0	25.0
952	Beer to difference	7.5	80%	1	1	0
953	Potassium thiocyanate	10.0	20%	1	0	27.5
954	Rubidium chloride	7.5		1	1	0
955	Sodium bromide	5.0		1	1	0
956		7.5	80%	1	1	0
957	e	5.0	1	1	1	0
958	Sodium chloride	9.0		1	1	0
959		4.0	60%	1	0	7.5
961	Sodium molybdate dihydrate	9.0	00%	1	1	0
962		10.0	20%	0	0	25
963	Sodium nitrate	5.0	000	1	1	0
964		7.0	80%	1	1	0
965	Sodium phosphate, monobasic	10.0	20%	0	0	25
966	HR-Cryo-22			-1	0	17.5
967	Sodium thiosulfate pentahydrate	8.0	0.00	1	1	0
968		6.0	80%	1	1	0
969	Zinc acetate	8.0		1	1	0
970	Potassium phosphate, dibasic	5.0	20%	0	0	27.5
972	KAA	5.0		0	0	27.5
973		5.0	40%	1	1	0
974	Cobalt sulfate heptahydrate	8.0	2007	0	0	27.5
975		4.0	20%	0	0	25.0
976	Lithium sulfate monohydrate	8.0	60%	1	1	0
977		4.0		0	0	27.5
978	Potassium phosphate, tribasic	8.0	400	0	0	22.5
979		10.0	EG	0	0	25.0
980	Management of the second second	4.0	4 8	0	0	27.5
981	Manganese suitate monohydrate	6.0	8	0	0	30.0
982		60	60%	1	1	0
984	Magnesium nitrate hexahydrate	7.5	80%	1	1	0
		1			· ·	10.56

Figure 7

PEG 400 cocktail conditions (Nos. 840–984), similar to Fig. 2. Note that within these conditions several Hampton Research Crystal Screen Cryo screens are also included: Nos. 855, 928 and 966.

Table 1

Summary of the cryoprotection needed for the different components of the first two groups of the HWI crystallization cocktails as described in Figs. 1-7.

The data are tabulated excluding results from the Crystal Screen Cryo cocktails distributed through the first 984 cocktails. The cryoprotectant concentrations are final concentrations (ν/ν) .

			Cocktail a	and ddH ₂ O	Cocktail	solution w	ith 1:1 cryo	protectant		
		No. of cocktails	0%	50%	30%	25%	20%	15%	10%	5%
Salts (1-237)	All	233	16.9%	5.5%	94.0%	75.5%	16.3%	10.7%	7.7%	6.9%
PEG 20K (238-390)	All	141	35.3%	1.3%	100%	92.9%	36.2%	24.8%	2.8%	1.4%
× /	20%	81	0%	0%	100%	87.7%	0%	0%	0%	0%
	40%	60	93.3%	3.3%	100%	100%	85.0%	58.3%	6.7%	3.3%
PEG 8K (391-546)	All	153	37.9%	4.6%	100%	92.8%	34.6%	19.6%	5.2%	4.6%
	20%	83	1.2%	0%	100%	86.7%	0%	0%	0%	0%
	40%	70	81.4%	10.0%	100%	100%	74.3%	42.9%	11.4%	10.0%
PEG 4K (547-694)	All	148	42.6%	6.8%	100%	90.5%	37.2%	25.7%	8.1%	6.8%
	20%	75	0%	0%	100%	82.4%	0%	0%	0%	0%
	40%	73	86.3%	13.7%	100%	100%	75.3%	52.1%	16.4%	13.7%
PEG 1K (695-839)	All	145†	39.6%	4.1%	100%	91.7%	42.8%	21.4%	7.6%	4.1%
× ,	20%	72	0%	0%	100%	84.9%	0%	0%	0%	0%
	40%	72	80.3%	8.5%	100%	100%	87.3%	43.7%	15.5%	8.5%
PEG 400 (840-984)	All	142	76.0%	46.5%	100%	90.1%	72.5%	59.2%	50.0%	46.5%
	20%	28	0%	0%	100%	51.9%	0%	0%	0%	0%
	40%	75	90.7%	39.5%	100%	100%	86.7%	61.3%	44.0%	38.7%
	60%	7	100%	85.7%	100%	100%	100%	100%	85.7%	85.7%
	80%	32	100%	100%	100%	100%	100%	100%	100%	100%
1-984 (962 excluding Crys	tal Screen Cryo)	962	39.0%	11.1%	98.8%	85.4%	37.8%	25.4%	12.9%	11.1%

† One condition is 10% PEG 1K.

tration of 14.9% was required. PEG 1K (Fig. 6) was similar; at $20\%(\nu/\nu)$ PEG (72 conditions) the average cryoprotectant concentration was 24.5% and for $40\%(\nu/\nu)$ PEG (72 conditions) it was 15.3%. The reduction in cryoprotectant concentration required for vitrification of 20% and 40% PEG for the 20K, 8K, 4K and 1K PEGs were similar.

The PEG 400 group (Fig. 7) was more complex in composition and sampled 20% (28 conditions), 40% (75 conditions), 60% (seven conditions) and $80\%(\nu/\nu)$ (32 conditions) PEG 400 with glycerol. The concentrations of glycerol required averaged 26.3, 10.43, 1.1 and $0\%(\nu/\nu)$, respectively. Unfortunately, comparison with the other PEGs in the screen is difficult as PEG 400 is sampled at a larger number of concentrations but at a reduced number of chemical conditions. We can say that the 20% PEG 400 conditions required cryoprotectant concentrations comparable to similar conditions in the other PEG screens. It is also noticeable that at 40% PEG 400 the concentration of cryoprotectant required is significantly less than that of the higher molecular-weight PEGs.

In the case of the Ammonium Sulfate Grid Screen (Fig. 8) there was a small decrease in the cryoprotectant required with an increasing concentration of ammonium sulfate and no apparent pH effect. Similarly, as shown in Fig. 9, as the PEG 6000 concentration increased there is a slight decrease in the cryoprotectant needed. The most dramatic effect arises from

#	Chen	nical (M)	Buffer (0.1 M)	pH	1/	%					
1201			Citric acid	4	0	0	30.0				
1202			Citric acid	5	0	0	30.0				
1203		0.0	MES	6	0	0	27.5				
1204		0.8	HEPES	7	0	0	30.0				
1205			Tris	8	0	0	30.0				
1206			Bicine	9	0	0	27.5				
1207			Citric acid	4	0	0	27.5				
1208			Citric acid	5	0	0	25.0				
1209		16	MES	6	0	0	25.0				
1210	ate	1.0	HEPES	7	0	0	27.5				
1211	sult		Tris	8	0	0	25.0				
1212	E		Bicine	9	0	0	27.5				
1213	miu		Citric acid	4	0	0	22.5				
1214	m		Bitric acid	5	0	0	22.5				
1215	Am A	24	MES	6	0	0	22.5				
1216		2.4	2.4	2.4	2.4	2.4	HEPES	7	0	0	22.5
1217				Tris	8	0	0	22.5			
1218			Bicine	9	0	0	22.5				
1219		3.2	Citric acid	4	0	0	25.0				
1220			Citric acid	5	0	0	22.5				
1221			MES	6	0	0	22.5				
1222			HEPES	7	0	0	22.5				
1223			Tris	8	0	0	22.5				
1224			Bicine	9	0	0	22.5				

Figure 8

Hampton Research Grid Screen Ammonium Sulfate, cocktail Nos. 1201–1224.

No.	Chemic	als (0.1 M)	pH	C	hemicals		%	
1105		Citale sold	4			0	0	30.0
1106	1	Citric acid	5	1		0	0	30.0
1107	1	MES	6	1	1	0	0	30.0
1108	1	HEPES	7	1		0	0	30.0
1109	1	Tris	8	1		0	0	27.5
1110	1	Bicine	9	1		0	0	27.5
1111	1	Citric acid	4			0	0	25.0
1112	1	Citric acid	5	1	1	0	0	25.0
1113	2	MES	6	1		0	0	27.5
1114	orie	HEPES	7	1	10%(w/v)	0	0	27.5
1115	공	Tris	8	1		0	0	30.0
1116	E	Bicine	9	1	1	0	0	22.5
1117	hiu	Citric acid	4	1		0	0	25.0
1118	F	Citric acid	5	8		0	0	22.5
1119	W	MES	6	1 3		0	0	22.5
1120	1 2	HEPES	7	0	20%(w/v)	0	0	22.5
1121	1	Tris	8		10 10 10	0	0	22.5
1122	1	Bicine	9	1	1	0	0	22.5
1123	1	Citric acid	4	1		0	0	22.5
1124	1	Citric acid	5	1		0	0	22.5
1125	1	MES	6	1		0	0	22.5
1126	1	HEPES	7	1	30%(w/v)	1	0	20.0
1127		Tris	8	1	25 126 2	0	0	22.5
1128	1	Bicine	9	1		0	0	22.5

Figure 9

Hampton Research Grid Screen PEG/LiCl, cocktail Nos. 1105–1128. With the exception of PEG and LiCl, all chemicals are at 0.1 M concentration.

Table 2

The percentage of cocktails from commercial screens used in the 1536condition HWI high-throughput screening laboratory that show cryoprotectant properties without dilution, diluted 1:1 with ddH_2O and diluted 1:1 with 20, 10 and 5% glycerol solution.

Note that those conditions that did not require cryoprotectant at 1:1 dilution with dH_2O are not counted in the figures for those requiring cryoprotectant. The cryoprotectant numbers are cumulative, *i.e.* the 20% cryoprotectant numbers also encompass those that were successful with 10% and 5% cryoprotectant.

		Conditio	ons success	fully cryo	oprotecte	ed
		Cocktail and ddF	solution I ₂ O	Glycero	ol tration	
Hampton Research Screen name	No. of conditions	0%	50%	20%	10%	5%
Natrix	48	10.4%	4.2%	18.8%	0.0%	0.0%
Quick Screen	24	0.0%	0.0%	0.0%	0.0%	0.0%
Nucleic Acid	24	25.0%	0.0%	0.0%	0.0%	0.0%
Sodium Malonate	24	33.3%	4.2%	33.3%	4.2%	0.0%
PEG/LiCl	24	4.2%	0.0%	4.2%	0.0%	0.0%
PEG/Ion	48	0.0%	0.0%	0.0%	0.0%	0.0%
PEG 6000	24	0.0%	0.0%	20.8%	0.0%	0.0%
Ammonium Sulfate	24	0.0%	0.0%	0.0%	0.0%	0.0%
Sodium chloride	24	0.0%	0.0%	4.2%	0.0%	0.0%
Crystal Screen HT	96	21.8%	6.2%	57.3%	11.0%	5.2%
Index HT	96	20.8%	6.2%	45.8%	5.2%	0.1%
Salt RX	96	19.8%	3.1%	9.4%	0.0%	0.0%
All	552	14.5%	3.2%	23.9%	3.0%	0.9%

the reduction of solution volume (cryoloop size). In each case (Figs. 10–15) there is a clear trend in the reduction of cryoprotectant required as a function of the cryoloop size. All of the cocktails studied still required cryoprotectant, even for the smallest cryoloop size.

Loop size	Gly	cerol	conc	entra	tion [%(v)	(v)]
(mm)	30	25	20	15	10	5	0
0.7-1.0	-	-		-	Х	X	X
0.5-0.7	-	-			Х	Х	X
0.4-0.5	-	-	- 22	14	X	X	X
0.3-0.4		+	-	1.00	-	X	X
0.2-0.3	123	1		14	4	X	X
0.1-0.2		-	(#)	-	-	-	X
0.05-0.1	-	-	-	-	-	-	X

Figure 10

1.14 *M* ammonium sulfate pH 6. 'X' indicates the observation of ice, while '-' indicates that vitrification was visually successful.

Loop size	Glycerol concentration $[\%(v/v)]$									
(mm)	30	25	20	15	10	5	0			
0.7-1.0		-		X	X	X	X			
0.5-0.7	-	-			Х	Х	X			
0.4-0.5	- 4	-	-	12	Х	Х	X			
0.3-0.4	-		-	-	Х	X	X			
0.2-0.3	120	-		-	X	X	X			
0.1-0.2		-	-	-	-	X	X			
0.05-0.1	-	-	-	-	-	-	X			

Figure 11

20% PEG 20 000, 0.1 M lithium chloride pH 10. 'X' indicates the observation of ice, while '-' indicates that vitrification was visually successful.

The results for the 984-condition incomplete factorial screen are summarized in Fig. 16. They are broken down into the salt and PEG groups. Within the PEG group, the results are broken down as a function of the PEG concentration. Cryoprotectant conditions for the remaining cocktails (the Hampton Research Natrix, Quick Screen, Nucleic Acid Mini

Loop size	Glycerol concentration $[\%(v/v)]$									
(mm)	30	25	20	15	10	5	0			
0.7-1.0		Х	X	X	X	X	X			
0.5-0.7	-	-	Х	Х	X	Х	X			
0.4-0.5	-	-	X	Х	X	X	X			
0.3-0.4	-	-		-	-	X	X			
0.2-0.3		-		-	-	X	X			
0.1-0.2		-		-	-	X	X			
0.05-0.1	-	-	-	-		-	X			

Figure 12

20% PEG 8000, 0.1 *M* calcium acetate pH 6. 'X' indicates the observation of ice, while '-' indicates that vitrification was visually successful.

Loop size	Glycerol concentration $[\%(v/v)]$									
(mm)	30	25	20	15	10	5	0			
0.7-1.0		X	X	X	X	X	X			
0.5-0.7	-	-	-	Х	Х	Х	X			
0.4-0.5	141	-		-	X	X	X			
0.3-0.4		+	X	X	X	X	X			
0.2-0.3	123	1	-	Х	X	X	X			
0.1-0.2		-		10	Х	X	X			
0.05-0.1	-	-	-	-	-	-	X			

Figure 13

20% PEG 4000, 0.1 M ammonium sulfate pH 7.5. 'X' indicates the observation of ice, while '-' indicates that vitrification was visually successful.

Loop size	Glycerol concentration $[\%(v/v)]$									
(mm)	30	25	20	15	10	5	0			
0.7-1.0		-	X	X	X	X	X			
0.5-0.7	-	-	Х	Х	Х	Х	Х			
0.4-0.5	141	-		Х	X	X	X			
0.3-0.4		+	X	1.00	Х	X	X			
0.2-0.3	120	-	- 1	-	X	X	Х			
0.1-0.2		-		-	Х	X	X			
0.05-0.1	-	-	-	-	-	-	X			

Figure 14

20% PEG 1000, 0.1 M potassium phosphate tribasic pH 7.0. 'X' indicates the observation of ice, while '-' indicates that vitrification was visually successful.

Loop size	Glycerol concentration $[\%(v/v)]$									
(mm)	30	25	20	15	10	5	0			
0.7-1.0	-	-	X	X	X	X	X			
0.5-0.7		-	Х	X	Х	X	X			
0.4-0.5	-	-	-	X	X	X	X			
0.3-0.4		-	-	-	X	X	X			
0.2-0.3	144	-	-	-	X	X	X			
0.1-0.2		-	-	-	X	X	X			
0.05-0.1	-	-	-	142	-	-	X			

Figure 15

20% PEG 400, 0.1 M ammonium phosphate dibasic pH 4.0. 'X' indicates the observation of ice, while '-' indicates that vitrification was visually successful.

Screen, Grid Screens Sodium Malonate, PEG/Ion Screen, PEG 6000, Sodium Chloride, Index and SaltRx) are shown in Table 1. For the Quick Screen and PEG/Ion screen no cock-tails could be satisfactorily cryoprotected with 20% glycerol or less.

Table 1 breaks down the results into the percentages of cocktail groups 1 and 2 that were successfully vitrified without cryoprotectant, diluted 1:1 with ddH₂O and finally as a func-

tion of the 1:1 dilution with different concentrations (v/v) of glycerol in ddH₂O. The latter represents the final cryoprotectant concentration and is cumulative, *e.g.* a cocktail vitrified with 10% cryoprotectant is also counted as successful with higher concentrations. Of the 962 cocktails in groups 1 and 2 (excluding the Crystal Screen Cryo cocktails), ~40% were natively cryoprotected, ~38% were cryoprotected with 20% glycerol and almost all were cryoprotected with 30%

-				Hampton Resea	arch N	latrix, cockta	il Nos.	985	-103	2						
No.	Salt (M)			Buffer		Precipitan	t		рН	t	Ot	her		100%	50%	%
985	MeCla	0.01	-		Liso, H-0 2.0			M	-					0	0	20
987	Magnesium acetate	0.1	1	MES		MPD	20%(v/v)	5.5					0	0	20
996	Magnesium sulfate	0.01				Li2SO4.H2O	1.8	М	1					0	0	20
1000	Magnesium acetate	0.04	1			MPD	30%((v/v)	0					1	1	0
1011	Ammonium acetate	0.2	Sc	dium cacodylate		PEG 8000	30%(w/v)	6.5	0	.1M magne	sium ac	cetate	0	0	20
1013	Magnesium chloride	0.01				LiCl	4.0	М						1	0	-
1015	Magnesium emoride	0.005			P	EG MME 550	25%((v/v)						1	0	20
1016	Potassium chloride	0.2		Sodium HEPES	1	.6-Hexanediol	20%(w/v)	7		0.01 M	MgCl	i (0	0	20
1017	Ammonium chloride	0.2				LIDD	30%(1	w/v)	-		005 1/11			0	0	20
1018	Potassium chloride	0.1				6 Havanadial	15%((WV)	-	0	.005 M Mg	504 aq	ueous	1	0	20
1030	Ammonium chloride	0.005	Ti	ris hydrochloride	1	PEG 4000	30%(w(v)	8.5	-	0.01 M	CoCl.		0	0	20
1032	Annionan circrac	0.2	Ham	nton Desearch	Ouid	Screen coo	Ltail N	los 1	033	1057	0.01 //	Caciz	2	0	0	20
			man	ipton Research	Quici	C Screen, coc	Ktall IN	105. 1	055-	-1057						
		10000		No condi	itions fou	ind at 20% or less cry	yoprotectar	nt								
		Han	npton 1	Research Nucle	ic Ac	id Mini Scree	en cock	ctail 1	Nos.	1058	-1080			- 10 kg		
			,									i i				
No.	Chemical (mM)		Buffer	pH		Chemica		cal			100%		6 50%		%
1061	Potassium chlorid	e	80					2	20 mM	magnesiu	um chloride		1	(,	2
1066	Sodium chloride		12	12 2		e	-		80 mM	potassiu	m chloride		1	()	2
1071	Potassium chlorid	e	80	yla nM		W	HOH				1		1	()	-
1075	Sodium chloride		12	cod 10 1	7	26	M s etta		80 mM	potassiu	tassium chloride			0		<u> </u>
1078	Lithium chloride		40	Ca s	10	2	0	8	0 mM 9	SrCl ₂ , 20	mM MgCl;	2	1	()	-
1080	Strontium chlorid	e	80			20				magnesiu	um chloride		1	()	-
		Ham	pton R	esearch Grid So	creen	Sodium Male	onate, o	cond	ition	s 108	1-1104					
No	Chemical	M	nH	100%		50%	0%		No	М	nH	100	nez.	50%		CZ.
1000	Chennear	24	pii	100 %		50 %	10	_	1000	2.4	pii	100	0.10	0	-	20.0
1080		3.4	4	1		0	12.5	20.0 11		J98 3.4 0				0	-	20.0
1090	Sodium malanata	2.9		0	-	0	20.0	-	1102	2.4	7			0	-	20.0
1091	Somin matchate	3.4	- 5	1	-	0	12.5	-	1103	3.4		1	1	1	-	0.0
1092		2.9	6	1		0	10.0	-	1104	5.4	5.4		·	1	-	U
1027		L	mator	Desearch Crid	Cana	creen PEG/LiCL cocktail Nos 1105-1128								-		
		Па	imptor	r Kesearch Onu	Scie	ell FEO/LICI,	, COCKI	ann	05. 1	105-	1120					
					Data	available in Fig. 9.										
		Hamp	oton R	esearch Grid sc	reen l	PEG/Ion Scre	en, coo	cktai	l No	s. 112	9–1176	5				
				No condi	itions fou	nd at 20% or less cry	yoprotectar	nt								
		Ha	mpton	Research Grid	Scree	en PEG 6000	, cockt	ail N	los. 1	177-	1200					
No.	Buffer (0.1 M)	1 100 100		рН			Chem	icals		S. C*20224 - 13		100%		50%		%
1195	0	2		4								0	-	0		20.0
1196	Citric acid	C		5								0		0		20.0
1197	MES	7		6		PEG 6000			30%(w/v)		0	-	0		20.0
1198	HEPES	-		7								0		0		20.0
1200	Bicine			9									0		20.0	
		Hampto	on Res	earch Grid Scre	en A	nmonium Su	lfate, c	cockt	ail N	los. 12	201-12	24				
					Data	available in Fig. 8										
		Hamp	ton Re	search Grid So	reen (Sodium Chlor	ide co	rekte	il No	s 12	25_124	8				
N		Charles	ion Ke	search onu sei			140,00	ARIA	T	. 124	1/1/24				1.0	
1242	Sodium obtasi	Chemica		40M	+	Burler (0.	rid (M)		-	рн	100%	0	50	0	90	0
1243	Sodium chlorid	ic.		4.0 M		Citric ad	uid		- Ciana	-+	1 0		2	0	20	.0
		H	lampto	on Research, Cr	ystal	Screen HT, c	ocktail	I Nos	s. 12	49-13	344					
				Data	available	in McFerrin & Snel	1(2002)									

Figure 16

Components of the commercial screens used in the 1536 cocktails that could be successfully cryocooled with 20% glycerol or less or showed cryoprotectant properties alone and after 1:1 dilution with H_2O . For brevity, the cocktails that were not successfully vitrified are omitted.

Hampton Research Index, cocktail Nos. 1345–1440											
No.	Chemical (M)		Buffer (0.1 <i>M</i>)	рН	Chemica	d		100%	50%	%	
1345	Ammonium sulfate	2.0	Citric acid	3.5				0	0	20.0	
1364	Tri-sodium citrate dihydrate	1.4	HEPES	7.5	-			0	0	20.0	
1365	m Malic acid pH 7.0	1.8	-					0	0	20.0	
1368	Sodium acetate trihydrate pH 7.0	2.8	-					0	0	20.0	
1369	Sodium formate pH 7.0	3.5	1					0	0	20.0	
1370	Di-ammonium tartrate pH 7.0	1.1]					1	0	20.0	
1371	Sodium malonate pH 7.0	2.4			A.(1	1	0	
1380	Tacsimate pH 7.0	15%	-		2%(w/v) PEG	3350		0	0	20.0	
1382		1	HEPES	7.0	30%(v/v) leffamine M-6	0 reagen	t nH 7.0	1	0	10.0	
1383		1			30%(v/v) Jeffamine ED-20	001 reage	nt pH 7.0	0	0	20.0	
1384		1	Citric acid	3.5	25%(w/v) PEC	3 3350		0	0	20.0	
1386		1	Bis-tris	5.5	-			0	0	20.0	
1388		1	HEPES	7.5	-			1	0	20.0	
1389		1	Ins	8.5	28%(wh)	a::		0	0	20.0	
1393	Calcium chloride	-	-	6.5	20 // (11)	,		1	1	0	
1394		1	Bis-tris	5.5	1			1	1	0	
1395	Ammonium acetate	0.2		6.5	45%(w/v) N	1PD		1	0	10.0	
1396	- internation accuracy		HEPES	7.5	-			1	1	0	
1397	Coloium ablasida	0.05	Tris	8.5	2001.1.5 0001	ME SEA		1	1	0	
1398	Magnesium chloride	0.05	DIS-UIS	0.5	30%(WV) PEG N	INE 330		1	1	0	
1400	Potassium chloride	0.05	HEPES	7.5	Pentaerythritol propoxyl	ate (5/4 1	PO/OH)	1	0	10.0	
1401	Ammonium sulfate	0.05	Die tein	6.5	Pentaerythritol ethoxyla	EO/OH)	1	0	20.0		
1402			DIS-UIS	6.5	45%(v/v) polypropyler	P 400	1	0	10.0		
1403	Magnesium chloride	0.02	HEPES	7.5	22%(w/v) polyacrylic acid	1	0	10.0			
1404	Cobalt chloride	0.1	Tris	8.5	20%(w/v) polyvinylpy	e K15	1	0	20.0		
1406	A mmonium culfate	0.20	LIEDES	8.5	20%(W/V) PEG N)	0	0	20.0		
1412	Annionium surrate	1	Bis-tris	5.5	25% (WV) FEG	3 3330		0	0	20.0	
1415	6 . P	1	12/13/11/13	6.5	1			0	0	20.0	
1416	Sodium chloride	1	HEPES	7.5	1			0	0	20.0	
1417		1	Tris	8.5				0	0	20.0	
1418	Lithium sulfate	1	Bis-tris	5.5				0	0	20.0	
1419	419		LIEDER	6.5	-			0	0	20.0	
1420		-	Tris	85				0	0	20.0	
1422		1	Bis-tris	5.5	1			0	0	20.0	
1423	Ammonium acetate			6.5	1		0	0	20.0		
1424		1	HEPES	7.5]		0	0	20.0		
1426		1	Bis-tris	5.5	-			0	0	20.0	
1427	Magnesium chloride		LIEDES	6.5	-		0	0	20.0		
1420			Tris	8.5	-			1	0	20.0	
1435	DL-Malic acid pH 7.0	0.15			20////.) DE/	1 2 2 5 0		0	0	20.0	
1438	Tri-sodium citrate	0.20]		20%(W/V) PEC	1 2220		0	0	20.0	
1439	Potassium thiocyanate	0.10			30%(w/v) PEG M	ME 2000)	0	0	20.0	
1440	Potassium bromide	0.15						0	0	20.0	
		H	lampton Research	SaltRX, cocktai	il Nos. 1441–1536.						
No.	Salt (M)			Buffe	er (0.1 M)	pH	100%	50%	0	%	
1442	Sodium acetate		2.8	Bis-tr	is propane	7.0	1	0			
1452	Sodium chloride		3.2	Sodiu	im acetate	4.6	0	0		-	
1453	Tri-ammonium citeste all 7.0		2.0	Die wie		7.0	1	0		20.0	
1458	Tri-ammonium citrate pH 7.0 2.0		1.2	Bis-tris propane			1	0	2	- 20.0	
1467		2.0		Sodiu	im acetate	4.6	1	0		× .	
1468	1468 Sodium formate		2.0	Bis-tr	is propane	7.0	1	0		-	
1470			3.5	Sodiu	im acetate	4.6	1	0		20.0	
1472	State of the state		2.2	Tris		8.5	1	0		10.5	
1474	DL-Malic acid pH 7.0	\rightarrow	2.2	2.2 Ristris morane		7.0	1	0		200	
1478	Ammonium nitrate		2.5	2.5		1.0		0			
1488	Sodium nitrate		4.0	4.0		0.5	1	0			
1512	Lithium sulfate monohydrate		Tris		8.5 0		0	0		20.0	
1513	Magnesium sulfate hydrate		1.0	1.0 Sodiu		im acetate 4.6 1				0	
1514	inglesian surface hydrate			Bis-tr	s propane 7.0 1			0		•	
1523	Di-ammonium tartrate		1.3	creation.	Teic	0.5	0	0		20.0	
1532	rotassium iniocynate		0.5	Sodia	im acetate	0.5	1	0		20.0	
1533	Ammonium acetate		4.0	Bis-tr	is propane	7.0	1	1		0	
1534					Tris	8.5	1	1		0	
1536	Tacsimate		60%(v/v)	Bis-tr	is propane	7.0	1	0		20.0	

Figure 16 (continued)

glycerol. There was a sharp increase in cryoprotection going from 20% to 30% glycerol. The commercial screens (Table 2) were not as well suited to cryoprotection, with only 14.5% natively cryoprotected and 24% protected with 20% glycerol. This should not be construed as a criticism of the commercial screens, since cryoprotection was not a factor in their design.

4. Discussion

Cryocooling for X-ray data collection requires transforming the crystal and any mother liquor surrounding it into an amorphous form, i.e. vitrification. Vitrifying pure water, even for the smallest volumes, requires cooling to below 136 K (Mayer, 1991) in less than 10^{-4} s (Bruggeller & Mayer, 1980; Mayer, 1988). Glycerol is thought to work as a cryoprotectant by causing bulk water depletion and hydrogen-bond linearization and by increasing alkyl backbone interactions within the macromolecule (Dashnau et al., 2006). There are many cryoprotectants available, but in addition to its cryoprotective properties glycerol is an effective enhancer of both macromolecular structural order and stabilizes against noncovalent modification (Gekko & Timasheff, 1981; Priev et al., 1996; Sousa, 1995). Practically, glycerol can be formulated as a component in the storage-buffer component and on crystallization it can be readily incorporated into the crystal lattice, effectively displacing water (Charron et al., 2002). The Heterocompound Information Centre (HIC-Up; Kleywegt, 2007) lists over 2280 macromolecules in the Protein Data Bank (PDB; Berman et al., 2000) in which glycerol is observed within the structure. Ethylene glycol is the next most common crvoprotectant and is observed in over 700 structures. Similarly, a survey of crystallization reports published in Acta Crystallographica Section D in 2000 and 2001 showed that glycerol was used in 50% and ethylene glycol was used in 10% of cases (Garman & Doublié, 2003). This does not necessarily imply that glycerol is the best cryoprotectant to use. For reasons of convenience it is often the first; if it works, no further optimization is carried out (Garman & Doublié, 2003).

McFerrin & Snell (2002) determined the amounts of glycerol, PEG 400, ethylene glycol and 1,2-propanediol needed to successfully vitrify the 98 Hampton Research Crystal Screen I and II conditions. In comparing the concentration of glycerol required for vitrification versus other cryoprotectants, there were differences in a small number of samples, e.g. Crystal Screen I condition No. 44 (0.2 M magnesium formate) required 50% glycerol but only 35%, 30% and 30% PEG 400, ethylene glycol and 1,2-propanediol, respectively. However, the average magnitudes of the difference in cryoprotectant concentration when compared with glycerol were 4.0%, 3.2% and 5.9% for PEG 400, ethylene glycol and 1,2-propanediol, respectively. The data for glycerol can thus be used as a guide for the concentration of these cryoprotectants. McFerrin and Snell also used (2R,3R)-(-)-2,3-butanediol for the nine conditions under study that required the highest concentration of glycerol. On average, 10.6% less butanediol than glycerol was required for vitrification.

The cryoprotective properties of glycerol, methanol, 2-propanol, sucrose, xylitol, dextrose, trehalose, ethylene glycol, PEG 200, PEG 2K, PEG 20K, dimethyl sulfoxide (DMSO), 2-methyl-2,4-pentanediol (MPD) and salt (NaCl) with pure water have been systematically studied as a function of volume from 1 nl to 20 µl. Cryoprotectant conditions were determined for plunge-cooling into liquid nitrogen (Berejnov et al., 2006). The concentration required for vitrification decreased with volume, especially in the range \sim 5–0.1 µl. This range includes the typical volumes held in a sample loop and the observation is similar to previous observations that smaller loops require less cryoprotectant for vitrification (Chinte et al., 2005) and is empirically well known. Berejnov et al. (2006) note the presence of three regimes in the cooling process: large volume and therefore slow cooling rate where the critical concentration is nearly constant, intermediate volumes where the concentration shows a sharp decrease with volume and small volumes where the cooling rate saturates and the critical cryoprotectant concentration levels off. From Figs. 10-15 it is clear that typical crystallographic samples are in the intermediate regime. The results of Berejnov and coworkers also illustrate that there are cryoprotectants, i.e. 2-propanol, MPD and dextrose, that successfully vitrify solutions at significantly lower concentrations than glycerol. Our results are in agreement with Berejnov et al. (2006) and Chinte et al. (2005): smaller volumes require less cryoprotectant. However, the crystal volumes required for X-ray diffraction coupled with currently available cooling technologies make it impossible to rapidly cool pure H_2O in the time required for vitrification, *i.e.* in less than 10^{-4} s, even for the smallest cases (Bruggeller & Mayer, 1980; Mayer, 1988). Unlike Chinte et al. (2005), we do not observe any evidence indicating that the concentration of cryoprotectant needed tends to be zero at the smallest loop size. This may be a consequence of the fact that we chose worst-case cocktails while Chinte et al. (2005) used a random sampling of conditions.

Cryocooling samples requires both a good cryoprotectant and good experimental technique and there are many excellent articles that cover these in detail (Pflugrath, 2004; Garman & Schneider, 1997; Garman & Owen, 2006; Garman, 1999; Rodgers, 1997; Garman & Doublié, 2003). Garman & Owen (2006) make a number of suggestions for the choice of cryoprotectant. For two-thirds of cases they suggest that 15-25% glycerol is appropriate. For conditions with PEGs less than 4K, increasing the PEG concentration or adding other low-molecular-weight PEGs is effective. PEGs greater than 4K can be cryoprotected with lower molecular-weight PEGs and crystallization conditions that already contain MPD can be crvoprotected by increasing the MPD concentration. Finally, those with salt that were not protected with glycerol can be cryoprotected with ethylene glycol, with a mixture of sugars, by increasing the salt concentration or by exchanging the salt for an organic solvent. While there are many cryoprotectants, given the ability of glycerol to form ordered conformations within the crystal structure (Charron et al., 2002) and its

stabilizing effect (Sousa, 1995) it seems prudent to incorporate at least a small amount during the crystallization step or earlier unless there is the potential for competition with a ligand of interest. For penetrating cryoprotectants, adding them before or during the crystallization step prevents possible disruption to the lattice by addition of the cryoprotectant after crystals have formed (Pflugrath, 2004).

5. Conclusion

In terms of high-throughput crystallization-condition screening, the data presented here provide a criterion for prioritizing subsequent optimization of crystallization conditions. However, it is important to note that the data represent a worst-case scenario for vitrification: a dilution of the cocktail with glycerol solution was used rather than replacement of the water with glycerol and larger than typical sample volumes were examined. Replacing water in the cocktail with the cryoprotectant agent maintains the original cocktail composition at the same concentration and thereby minimizes deleterious effects to the crystal (unlike the dilution used here). This is the optimum and recommended method to produce a good cryoprotectant solution (Garman, 1999). In terms of volume, a balance is required between the reduction in cryoprotectant needed owing to sample size and practical considerations for collecting X-ray data. The optimum concentration required for the collection of the best X-ray data may not be the same as that which is just sufficient for vitrification (Mitchell & Garman, 1994). Similarly, annealing techniques that could be used to improve crystal quality (Hanson et al., 2003) have the potential to work well with a higher than required cryoprotectant concentration but will not work so well if the concentration is too low (Juers & Matthews, 2004). The data presented here provide a starting point for the optimization of cryoprotectant concentrations under similar biochemical conditions.

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