Nucleic Acid Mini Screen™

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-118 (pg 1)**

The Nucleic Acid Mini Screen™ is an efficient screen formulated to assist in the determination of preliminary crystallization conditions of nucleic acid fragments.

Using 1 to 4 mM oligonucleotide stock concentration the screen requires less than 25 microliters of sample.

The screen evaluates sample concentration, temperature, pH, monovalent cations, divalent cations, and polyamines. To complete the screen, one will need 1) HR2-118 Nucleic Acid Mini Screen, a set of twenty-four, 1 milliliter volumes of reagent and 2) 35% v/v (+/-)-2-Methyl-2,4-pentanediol, the dehydrant for the reagent well / reservoir (available separately as HR2-863 35% v/v (+/-)-2-Methyl-2,4-pentanediol).

The composition of the nucleic acid mini screen allows one to apply the formulation to other nucleic acids such as deoxy- and ribozymes, pseudoknots and tRNAs.

Formulation

All solutions are formulated using ultra-pure chemicals and deionized water and are sterile filtered.

Storage

Recommended long term storage for the HR2-118 Nucleic Acid Mini Screen 24 unique reagents is -20°C. Allow the kit to equilibrate to room temperature prior to use.

Sample Preparation

Nucleic acid solutions should be highly purified and filtered using a 0.2 or 0.45 micron filter prior to crystallization screening. The nucleic acid sample should be suspended in deionized water or buffer of choice to a concentration of approximately 20 to 24 mM mononucleotide concentration. For example, a dodecamer, the single strand concentration should be approximately 2 mM.

No preincubation is recommended for this screen.

Centrifuge the sample to remove amorphous material prior to set up.

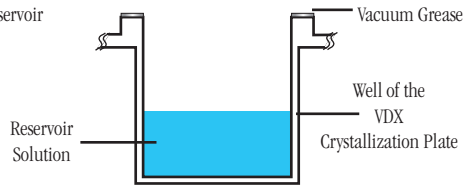
Instructions

The following procedure describes the use of the Nucleic Acid Mini Screen with the Hanging Drop Vapor Diffusion method. The Nucleic Acid Mini Screen is also compatible with the Sitting Drop, Sandwich Drop, Microbatch, and Microdialysis methods. A complete description of crystallization methods is available from the Hampton Research Crystal Growth 101 Library.

Prior to use, remove the kit from -20°C storage and allow the reagents to equilibrate to 4°C or room temperature. The set up may be performed at room temperature and moved to a 4°C incubation or the set up may be performed in a 4°C cold room. To minimize condensation with 4°C incubation of room temperature set ups, place a sealed crystallization plate with water in each reservoir on the top and bottom of the plate stack.

1. Prepare a VDX Plate (HR3-140) for Hanging Drop Vapor Diffusion by applying a thin bead of cover slide sealant to the upper edge of each of the 24 reservoirs. One may also use a VDX Plate with sealant (HR3-170). Twenty-four reservoirs are to be prepared for a complete screen. See Figure 1 below.

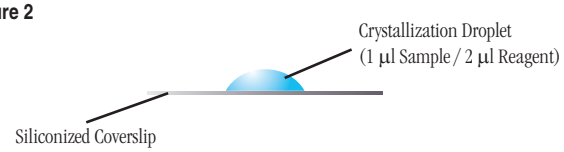
Figure 1
Cross section of a reservoir in the VDX plate.



2. Pipet 1 milliliter of 35 % v/v (+/-)-2-Methyl-2,4-pentanediol dehydrant into the reservoir of the crystallization plate.

3. Pipet 1 µl of sample (1 to 4 mM oligonucleotide stock) onto a clean, siliconized cover slide. Pipet 2 µl of Nucleic Acid Mini Screen reagent 1 to the 1 µl sample drop. It is recommended, but not required to pipet a second drop beside the initial drop. It is recommended the second drop contain 2 µl of sample and 2 µl of reagent. See Figure 2.

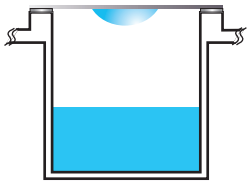
Figure 2



Note: Be sure to pipet the Nucleic Acid Mini Screen reagents / precipitants into sample drops and **NOT** the dehydrant!

3. Seal the reservoir.

Figure 3
Inverted siliconized coverslip placed over the reservoir.



4. Repeat steps 2 through 3 for the remaining reagents 2 through 24.

Nucleic Acid Mini Screen™

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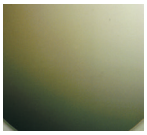
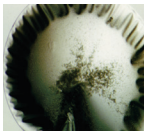
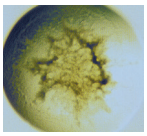
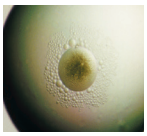
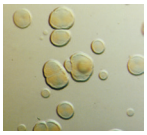
Figure 4
Typical observations in a crystallization experiment

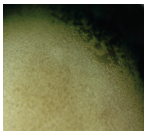

Clear Drop


Skin/Precipitate

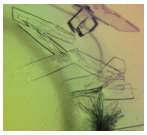

Precipitate


Precipitate/Phase

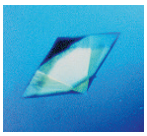

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

Examine the Drop

Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week there after. Records should indicate whether the drop is clear, contains precipitate, and or crystals. It is helpful to describe the drop contents using descriptive terms. Adding magnitude is also helpful. Example: 4+ yellow/brown fine precipitate, 2+ small bipyramid crystals, clear drop, 3+ needle shaped crystals in 1+ white precipitate. One may also employ a standard numerical scoring scheme (Clear = 0, Precipitate = 1, Crystal = 10, etc). Figure 4 (on the left) shows typical examples of what one might observe in a crystallization experiment.

Interpreting the Results

The sparse matrix screen used in Nucleic Acid Mini Screen is designed screen the samples solubility as well as determine preliminary crystallization conditions. When crystals are obtained during the initial screen, conditions may be optimized by varying the concentration of the precipitant (MPD) and/or salt, cation and polyamine, the pH, temperature, as well as other primary crystallization variables.

When crystals are not obtained in the initial screen, review droplets with precipitates for microcrystallinity. Examine the amorphous material under a high power microscope between crossed polarizing lenses to look for birefringence. True amorphous precipitates do not glow. Microcrystalline precipitates may glow under polarization. If the amorphous material is precipitate, consider one of the following:

- Screen an alternate sample or precipitant concentration
- Alter the sample sequence
- Vary the drop ratio
- Change the temperature of the experiment

If the droplet remains clear, continue to observe the screen for several weeks and consider increasing sample concentration, increasing the concentration of the precipitant, varying the salt concentration, or screening an alternate sequence.

Using the unique dehydrant format also allows one simple increase the concentration of the dehydrant by adding concentrated dehydrant (100%) to the 35% v/v dehydrant to increase dehydrant concentration, or one can simply replace the 35% v/v dehydrant with a more concentrated dehydrant such as 50 or 65% v/v (+/-)-2-Methyl-2,4-pentanediol.

If small crystals are grown which are not suitable for X-ray diffraction analysis there are several options to pursue:

- Use the small crystals as seeds to grow larger crystals.
- Set optimization trials, varying the primary crystallization variables to optimize conditions for crystal growth. Review all of the results in the initial screen to obtain information on what affects pH, precipitant type and concentration, as well as the mixing of salts with precipitants have on crystal growth. Design subsequent trials to encompass these variables in a grid.
- Vary polyamine /cation type and concentration.

If the results of the screen performed at 4°C do not appear different from the room temperature screen, pursue varying pH, precipitant type and concentration, salt, cation and polyamine during optimization. If the presence or precipitate or crystals is dependent upon temperature, implement temperature variations into the crystallization strategy.

References and Readings

1. Berger, et al, A Highly Effective 24 Condition Matrix for the Crystallization of Nucleic Acid Fragments. Acta Crystallographica Section D. Vol. D52 Part 3, 465-468, 1996.

Technical Support

Inquiries regarding Nucleic Acid Mini Screen reagent formulation, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 4:30 p.m. USA Pacific Standard Time.

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Tube #	Precipitant	Tube #	Buffer ◊	Tube #	Polyamine	Tube #	Monovalent Ion	Tube #	Divalent Ion
1.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	1.	0.040 M Sodium cacodylate trihydrate pH 5.5	1.	0.020 M Hexamine cobalt(III) chloride	1.	None	1.	0.020 M Magnesium chloride hexahydrate
2.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	2.	0.040 M Sodium cacodylate trihydrate pH 5.5	2.	0.020 M Hexamine cobalt(III) chloride	2.	0.080 M Sodium chloride	2.	0.020 M Magnesium chloride hexahydrate
3.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	3.	0.040 M Sodium cacodylate trihydrate pH 5.5	3.	0.020 M Hexamine cobalt(III) chloride	3.	0.012 M Sodium chloride, 0.080 M Potassium chloride	3.	None
4.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	4.	0.040 M Sodium cacodylate trihydrate pH 5.5	4.	0.020 M Hexamine cobalt(III) chloride	4.	0.040 M Lithium chloride	4.	0.020 M Magnesium chloride hexahydrate
5.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	5.	0.040 M Sodium cacodylate trihydrate pH 6.0	5.	0.012 M Spermine tetrahydrochloride	5.	0.080 M Potassium chloride	5.	0.020 M Magnesium chloride hexahydrate
6.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	6.	0.040 M Sodium cacodylate trihydrate pH 6.0	6.	0.012 M Spermine tetrahydrochloride	6.	0.080 M Potassium chloride	6.	None
7.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	7.	0.040 M Sodium cacodylate trihydrate pH 6.0	7.	0.012 M Spermine tetrahydrochloride	7.	0.080 M Sodium chloride	7.	0.020 M Magnesium chloride hexahydrate
8.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	8.	0.040 M Sodium cacodylate trihydrate pH 6.0	8.	0.012 M Spermine tetrahydrochloride	8.	0.080 M Sodium chloride	8.	None
9.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	9.	0.040 M Sodium cacodylate trihydrate pH 6.0	9.	0.012 M Spermine tetrahydrochloride	9.	0.080 M Sodium chloride, 0.012 M Potassium chloride	9.	0.020 M Magnesium chloride hexahydrate
10.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	10.	0.040 M Sodium cacodylate trihydrate pH 6.0	10.	0.012 M Spermine tetrahydrochloride	10.	0.012 M Sodium chloride, 0.080 M Potassium chloride	10.	None
11.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	11.	0.040 M Sodium cacodylate trihydrate pH 6.0	11.	0.012 M Spermine tetrahydrochloride	11.	0.080 M Sodium chloride	11.	0.020 M Barium chloride
12.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	12.	0.040 M Sodium cacodylate trihydrate pH 6.0	12.	0.012 M Spermine tetrahydrochloride	12.	0.080 M Potassium chloride	12.	0.020 M Barium chloride
13.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	13.	0.040 M Sodium cacodylate trihydrate pH 6.0	13.	0.012 M Spermine tetrahydrochloride	13.	None	13.	0.080 M Strontium chloride hexahydrate
14.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	14.	0.040 M Sodium cacodylate trihydrate pH 7.0	14.	0.012 M Spermine tetrahydrochloride	14.	0.080 M Potassium chloride	14.	0.020 M Magnesium chloride hexahydrate
15.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	15.	0.040 M Sodium cacodylate trihydrate pH 7.0	15.	0.012 M Spermine tetrahydrochloride	15.	0.080 M Potassium chloride	15.	None
16.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	16.	0.040 M Sodium cacodylate trihydrate pH 7.0	16.	0.012 M Spermine tetrahydrochloride	16.	0.080 M Sodium chloride	16.	0.020 M Magnesium chloride hexahydrate
17.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	17.	0.040 M Sodium cacodylate trihydrate pH 7.0	17.	0.012 M Spermine tetrahydrochloride	17.	0.080 M Sodium chloride	17.	None
18.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	18.	0.040 M Sodium cacodylate trihydrate pH 7.0	18.	0.012 M Spermine tetrahydrochloride	18.	0.080 M Sodium chloride, 0.012 M Potassium chloride	18.	0.020 M Magnesium chloride hexahydrate
19.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	19.	0.040 M Sodium cacodylate trihydrate pH 7.0	19.	0.012 M Spermine tetrahydrochloride	19.	0.012 M Sodium chloride, 0.080 M Potassium chloride	19.	None
20.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	20.	0.040 M Sodium cacodylate trihydrate pH 7.0	20.	0.012 M Spermine tetrahydrochloride	20.	0.080 M Sodium chloride	20.	0.020 M Barium chloride
21.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	21.	0.040 M Sodium cacodylate trihydrate pH 7.0	21.	0.012 M Spermine tetrahydrochloride	21.	0.080 M Potassium chloride	21.	0.020 M Barium chloride
22.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	22.	0.040 M Sodium cacodylate trihydrate pH 7.0	22.	0.012 M Spermine tetrahydrochloride	22.	0.040 M Lithium chloride	22.	0.080 M Strontium chloride hexahydrate, 0.020 M Magnesium chloride hexahydrate
23.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	23.	0.040 M Sodium cacodylate trihydrate pH 7.0	23.	0.012 M Spermine tetrahydrochloride	23.	0.040 M Lithium chloride	23.	0.080 M Strontium chloride hexahydrate
24.	10% v/v (+/-)-2-Methyl-2,4-pentanediol	24.	0.040 M Sodium cacodylate trihydrate pH 7.0	24.	0.012 M Spermine tetrahydrochloride	24.	None	24.	0.080 M Strontium chloride hexahydrate, 0.020 M Magnesium chloride hexahydrate

Nucleic Acid Mini Screen contains twenty-four unique reagents.
To determine the formulation of each reagent, simply read across the page.

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Solutions for Crystal Growth

Sample: _____ Sample Concentration: _____

Sample Buffer: _____ Date: _____

Reservoir Volume: _____ Temperature: _____

Drop Volume: Total _____ µl Sample _____ µl Reservoir _____ µl Additive _____ µl

1 Clear Drop
2 Phase Separation
3 Regular Granular Precipitate
4 Birefringent Precipitate or Microcrystals
5 Posettes or Spherulites
6 Needles (1D Growth)
7 Plates (2D Growth)
8 Single Crystals (3D Growth < 0.2 mm)
9 Single Crystals (3D Growth > 0.2 mm)

Nucleic Acid Mini Screen™ - HR2-118 Scoring Sheet	Date:	Date:	Date:
1. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 5.5, 0.020 M Hexamine cobalt(III) chloride, 0.020 M Magnesium chloride hexahydrate			
2. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 5.5, 0.020 M Hexamine cobalt(III) chloride, 0.080 M Sodium chloride, 0.020 M Magnesium chloride hexahydrate			
3. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 5.5, 0.020 M Hexamine cobalt(III) chloride, 0.012 M Sodium chloride, 0.080 M Potassium chloride			
4. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 5.5, 0.020 M Hexamine cobalt(III) chloride, 0.040 M Lithium chloride, 0.020 M Magnesium chloride hexahydrate			
5. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 6.0, 0.012 M Spermine tetrahydrochloride, 0.080 M Potassium chloride, 0.020 M Magnesium chloride hexahydrate			
6. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 6.0, 0.012 M Spermine tetrahydrochloride, 0.080 M Potassium chloride			
7. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 6.0, 0.012 M Spermine tetrahydrochloride, 0.080 M Sodium chloride, 0.020 M Magnesium chloride hexahydrate			
8. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 6.0, 0.012 M Spermine tetrahydrochloride, 0.080 M Sodium chloride			
9. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 6.0, 0.012 M Spermine tetrahydrochloride, 0.080 M Sodium chloride, 0.012 M Potassium chloride, 0.020 M Magnesium chloride hexahydrate			
10. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 6.0, 0.012 M Spermine tetrahydrochloride, 0.012 M Sodium chloride, 0.080 M Potassium chloride			
11. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 6.0, 0.012 M Spermine tetrahydrochloride, 0.080 M Sodium chloride, 0.020 M Barium chloride			
12. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 6.0, 0.012 M Spermine tetrahydrochloride, 0.080 M Potassium chloride, 0.020 M Barium chloride			
13. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 6.0, 0.012 M Spermine tetrahydrochloride, 0.080 M Strontium chloride hexahydrate			
14. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 7.0, 0.012 M Spermine tetrahydrochloride, 0.080 M Potassium chloride, 0.020 M Magnesium chloride hexahydrate			
15. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 7.0, 0.012 M Spermine tetrahydrochloride, 0.080 M Potassium chloride			
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19. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 7.0, 0.012 M Spermine tetrahydrochloride, 0.012 M Sodium chloride, 0.080 M Potassium chloride			
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22. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 7.0, 0.012 M Spermine tetrahydrochloride, 0.040 M Lithium chloride, 0.080 M Strontium chloride hexahydrate, 0.020 M Magnesium chloride hexahydrate			
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24. 10% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.040 M Sodium cacodylate trihydrate pH 7.0, 0.012 M Spermine tetrahydrochloride, 0.080 M Strontium chloride hexahydrate, 0.020 M Magnesium chloride hexahydrate			

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