



**Figure 2. Layout of the of RUBIC Additive Screen**

|   | 1   | 2                      | 3                            | 4                       | 5                                     | 6                                       | 7                       | 8                       | 9                        | 10                                    | 11                            | 12                             |
|---|---|------------------------|------------------------------|-------------------------|---------------------------------------|---|-------------------------|-------------------------|--------------------------|---------------------------------------|-------------------------------|--------------------------------|
| A | water                                       | 100 mM Na Acetate      | 100 mM Ca Acetate            | 100 mM K Acetate        | 100 mM Ammonium Acetate               | 100 mM Na Sulfate                       | 100 mM Mg Sulfate       | 100 mM K Sulfate        | 100 mM Ammonium Sulfate  | 100 mM Na Phosphate (monobasic)       | 100 mM Na Phosphate (dibasic) | 100 mM K Phosphate (monobasic) |
| B | 100 mM K Phosphate (dibasic)                | 100 mM Na Tartrate     | 100 mM Na Citrate (tribasic) | 100 mM Na Malonate      | 100 mM Na Nitrate                     | 100 mM Na Formate                       | 100 mM K Formate        | 100 mM NaF              | 100 mM KF                | 100 mM NH4F                           | 100 mM LiCl                   | 100 mM NaCl                    |
| C | 100 mM KCl                                  | 100 mM NH4Cl           | 100 mM NaI                   | 100 mM KI               | 100 mM NaBr                           | 1 mM MgCl2                              | 1 mM CaCl2              | 1 mM MnCl2              | 1 mM NiCl2               | 1 mM FeCl2                            | 1 mM ZnCl2                    | 1 mM CoCl2                     |
| D | 5 mM EDTA                                   | 5 mM EGTA              | 0.1 M Urea                   | 0.5 M Urea              | 1 M Urea                              | 2 M Urea                                | 4 M Urea                | 150 mM Guanidine-HCl    | 500 mM Guanidin-HCl      | 1 mM NDSB-195                         | 1 mM NDSB-201                 | 1 mM Fos Choline 12            |
| E | 1 mM CHAPS                                  | 1mM CHAPSO             | 1 mM OG                      | 1mM DM                  | 1 mM DDM                              | 25 mM Monosaccharides mix MD2-100-75    | 25 mM Glucose           | 25 mM Sucrose           | 25 mM Maltose            | 50 mM Carboxylic acids mix MD2-100-76 | 50 mM Proline                 | 50 mM Glycine                  |
| F | 50 mM Glutamic acid                         | 500 mM Glutamic acid   | 50 mM Arginine               | 500 mM Arginine         | 50 mM Arginine<br>50 mM Glutamic acid | 500 mM Arginine<br>500 mM Glutamic acid | 50mM Gly-Gly-Gly        | 5 mM Oxaloacetic acid   | 5%(v/v) DMSO             | 5% (v/v) Ethylene glycol              | 5% (v/v) Glycerol             | 20% (v/v) Glycerol             |
| G | 5%(v/v) PEG 400                             | 5% (w/v) PEG 1000      | 5% (w/v) PEG 3350            | 5 mM DTT                | 5 mM TCEP                             | 5 mM Biotin                             | 5 mM Betaine            | 5 mM Coenzyme A         | 5 mM Nicotinic acid      | 1 mM Spermidine                       | 1 mM Spermine                 | 1 mM Sarcosine                 |
| H | ~20 uM Deoxyribonucleic acid library <50 bp | 1 mM ATP<br>1 mM MgCl2 | 1 mM ATPyS<br>1 mM MgCl2     | 1 mM cAMP<br>1 mM MgCl2 | 1 mM GTP<br>1 mM MgCl2                | 1 mM GTPyS<br>1 mM MgCl2                | 1 mM cGMP<br>1 mM MgCl2 | 1 mM NADH<br>1 mM MgCl2 | 1 mM NADPH<br>1 mM MgCl2 | 5 mM Polyethylenimine                 | 200 mM Imidazole              | 400 mM Imidazole               |

**Salts**

**Monovalent ions**

**Multivalent ions, chelating reagents**

**Chaotropic reagents**

**Non detergent , detergents,**

**Carbohydrates**

**Carboxylic acids, amino acids (racemic)**

**Reducing reagents**

**Polyols**

**Co-factor, polyamines**

**Nucleotides**

**Imidazole**

Concentrations shown above are final concentrations based on 25  $\mu$ l assay (16  $\mu$ l RUBIC Additive Screen + 5  $\mu$ l 5X Buffer\* + 2  $\mu$ l Protein sample + 2  $\mu$ l SYPRO Orange dye diluted stock solution).

\*5X Buffer can be the buffers provided or your own stock.