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| ***Hybridization*** |
| Add 500uL of NF water to new vial of lyophilized 10X Blocking Agent |
| Vortex gently  |
| \*If pellet does not go into solution completely, heat the mix for 4-5 min at 37C |
| Centrifuge for 5-10s |
| \*Can store at -20C for 2 months (vortex and centrifuge before each use) |
| Equilibrate water bath to 60C |

For 8x15k array:

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| Cy3 cRNA (300ng) |
| Cy5 cRNA (300ng) |
| 10X Blocking Agent 5uL |
| NF Water Add until each sample is 24uL |
| 25X Frag. Buffer 1uL |
| \*Mix well but gently on vortex |

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| Incubate at 60C for exactly 30min to fragment RNA |
| Add 25uL 2X hybridization buffer to each cRNA sample |
| Mix well by pipetting and do not introduce bubbles |
| Centrifuge for 1 min at room temp at 13,000rpm |
| Place samples on ice and load onto arrays as soon as possible

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| **Prepare the Individual Assembly** |
| Barcode

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| 1\_1 |  | 1\_2 |  | 1\_3 |  | 1\_4 |
|  |  |  |  |  |  |  |
| 2\_1 |  | 2\_2 |  | 2\_3 |  | 2\_4 |

Load clean gasket slide onto chamber (place unused gasket wells at far end, opposite of bar code |
| Add 40uL of 1X hybridization buffer to each unused well |
| Load each well with 40uL of hybridization samples  |
| Place array slides on top of gaskets |
| \*Ensure mobility of bubbles! |
| Hybridize at 65C for 17 hours at 10 rpm |
| \*Warm Gene Expression Wash Buffer 2 overnight at 37C |

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