Instructions for HETMAT imino experiment

1. Select imino 15N-1H peaks from 2D HSQC/HMQC spectra using peak picking – and save it – automatic peak picking also works great but mind the threshold
2. Create new HETMAT experiment (use parameter set, not prosol compatible)
3. Based on the 15N and 1H chemical shifts, you would need to update frequency list for F1 and F3
4. Roughly divide the peaks based on the broadness and available spectral resolution – choose appropriate nutation field accordingly – rule of thumb 50 Hz for sharper and 75 Hz for broaded imino resonance – you would need to update pldb8 / pldb9 and pldb10 / pldb11 according to chosen nutation field.
5. Choose duration of heteronuclear encoding according to choose CP power: p7=10400 for 75 Hz and p8=14500 for 50 Hz.
6. Choose d8 and d9 – standard options are d8= 30 – 50 ms and d9= 80 – 125 ms
7. Update vclist – number of loops used – by default use 6-8 loops for slower exchanging and 8-14 loops for faster exchanging peaks. Order loops according to the peak list (assign value for loops in the order of peak list according to your division into sharp and broad)
8. Once acquired, extract the data and use MATBAL processing scripts to obtain the pseudo 3D spectra

Here we show a table of acquisition parameters we used in our study:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| RNA | 5\_SL5b+c | | 5\_SL8b+c | | 5\_SL8b+c | |
| Parameters | HMQC  NOESY | HETMAT  NOESY | HMQC  NOESY | HETMAT  NOESY | HMQC  NOESY | HETMAT  NOESY |
| Temperature (K) | 283 | 283 | 283 | 283 | 298 | 298 |
| SW (ppm) | 23.8 | 23.8 | 23.8 | 23.8 | 23.8 | 23.8 |
| TD | 3072 | 3072 | 3072 | 3072 | 3072 | 3072 |
| SW1 (ppm) | 24 | / | 24 | / | 24 | / |
| TD1 | 384 | 13 | 384 | 21 | 384 | 17 |
| NS | 28 | 512 | 32 | 480 | 32 | 512 |
| DS | 32 | 32 | 32 | 32 | 32 | 32 |
| d1 (s) | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| RG | 101 | 101 | 101 | 101 | 101 | 101 |
| O1 (F2, ppm) | 4.697 | 4.697 | 4.694 | 4.694 | 4.698 | 4.698 |
| O1 (F1, ppm) | 116 | 116 | 116 | 116 | 116 | 116 |
| d8 (NOE, ms)\* | 175 | a: 125  b: 30 | 150 | a: 80  b: 50 | 200 | a: 125  b: 50 |
| n\*  (number of loops) | / | a: 7  b: 20 | / | a: 10  b: 17 | / | a: 7  b: 17 |
| RF CP field\*  (Hz) | / | a: 50  b: 75 | / | a: 50  b: 75 | / | a: 50  b: 75 |

\*Choice for NOE mixing time, number of loops and CP RF field according to the broadness of the peak