

Amino acids that make direct contact with RNA bases in each of the 8 Pum repeats (R1-R8):

○ pos. 13 residue makes stacking interactions with the RNA base

\* pos. 12, 16 residues make hydrogen bond or Van der Waals interactions with RNA base

Protein alignment

|                          |          |           |           |           |           |             |           |           |           |
|--------------------------|----------|-----------|-----------|-----------|-----------|-------------|-----------|-----------|-----------|
| Sp_SPAC4G8               | 19113974 | C R Y L Q | N Y M C Q | T R S M Q | T H V L Q | C C I L Q   | N Y L V Q | S N A I E | N Y V M Q |
| Neurospora crassa        | 16416090 | S I F L Q | N F L V Q | C H V V Q | C H V W Q | S L V V V Q | N W C I Q | S K V V E | S K V V E |
| Sp_SPAC4G9               | 19114599 | S L S L Q | N F L I Q | S H V L Q | C H V W Q | S L V V V Q | N W V I Q | S K V V E | N Y L I Q |
| Encephalitozoon cuniculi | 19074973 | S R C I Q | N Y V I Q | C R V I Q | N H V I Q | C R V I Q   | N Y V I Q | S N V V E | N Y V V Q |
| Sc_Puf5                  | 730058   | C R F L Q | N Y L V Q | T R S L Q | N H V I Q | C C V L Q   | N Y I I Q | S N V V E | N Y A L Q |
| Leishmania major         | 6562659  | C R Y L Q | N F L I Q | T F S V Q | N H V I Q | C C V L Q   | N Y V L Q | S N V M E | N Y V L Q |
| Arabidopsis thaliana     | 15241239 | C R F L Q | N Y L V Q | T R A V Q | N H V I Q | C C V L Q   | N Y V L Q | S N V V E | N Y V I Q |
| Arabidopsis thaliana     | 15219849 | C R F L Q | N Y L M Q | T R V V Q | N H V I Q | C C V L Q   | N Y A V Q | S H M V E | N F V I Q |
| Arabidopsis thaliana     | 15218233 | C R F L Q | N Y L M Q | T R V V Q | N H V I Q | C C V L Q   | N Y A V Q | S H M V E | N F V I Q |
| Sc_Puf4                  | 6321424  | C R F L Q | N Y L I Q | T R A L Q | N H V I Q | C C V L Q   | N Y V V Q | S N V I E | N Y V L Q |
| Neurospora crassa        | 18376126 | C R Y L Q | N Y L C Q | T R A L Q | N H V I Q | C C V L Q   | N Y V V Q | S N V V E | N Y V V Q |
| Sp_SPAC6G9               | 19115035 | C R Y L Q | N Y L C Q | T R A L Q | N H V I Q | C C V V Q   | N Y V L Q | S N V M E | N Y V I Q |
| Ce_Puf8                  | 16259231 | S R F I Q | N Y V V Q | C R V L Q | N H V V Q | C R V V Q   | N Y V V Q | S N V I E | N Y V V Q |
| Sc_Puf3                  | 6323016  | S R F I Q | N Y V I Q | C R V I Q | N H V I Q | C R V I Q   | N Y V I Q | S N V V E | N Y V I Q |
| S. pombe                 | 11282577 | S R F I Q | N Y V V Q | C R V V Q | N H V I Q | C R V I Q   | N Y V V Q | S N V V E | N Y V I Q |
| Saprolegnia parasitica   | 15131678 | S R F I Q | N Y V I Q | C R V I Q | N H V V Q | C R V I Q   | N Y V I Q | S N V I E | N Y V V Q |
| C. elegans               | 17569159 | S R F I Q | N Y V I Q | C R V I Q | N H V I Q | C R V I Q   | N Y V I Q | S N V I E | N Y V V Q |
| dm_Pumilio               | 17737857 | S R F I Q | N Y V I Q | C R V I Q | N H V V Q | C R V I Q   | N Y V I Q | S N V V E | N Y V V Q |
| Anopheles gambiae        | 21301443 | S R F I Q | N Y V I Q | C R V I Q | N H V V Q | C R V I Q   | N Y V I Q | S N V V E | N Y V V Q |
| Xenopus laevis           | 12082247 | S R F I Q | N Y V I Q | C R V I Q | N H V V Q | C R V I Q   | N Y V I Q | S N V V E | N Y V V Q |
| h_Pum1                   | 6681354  | S R F I Q | N Y V I Q | C R V I Q | N H V V Q | C R V I Q   | N Y V I Q | S N V V E | N Y V V Q |
| m_Pum1                   | 20846292 | S R F I Q | N Y V I Q | C R V I Q | N H V V Q | C R V I Q   | N Y V I Q | S N V V E | N Y V V Q |
| h_Pum2                   | 6681354  | S R F I Q | N Y V I Q | C R V I Q | N H V V Q | C R V I Q   | N Y V I Q | S N V V E | N Y V V Q |
| m_Pum2                   | 20846292 | S R F I Q | N Y V I Q | C R V I Q | N H V V Q | C R V I Q   | N Y V I Q | S N V V E | N Y V V Q |
| Arabidopsis thaliana     | 18416639 | S R F I Q | N Y V I Q | C R V I Q | N H V I Q | C R V I Q   | N Y V T Q | S N V I E | N Y V V Q |
| Arabidopsis thaliana     | 9293981  | S R F I Q | N Y V I Q | C R V I Q | N H V I Q | C R V I Q   | N Y V T Q | S N V V E | N Y V V Q |
| Populus tremula          | 8071634  | S R F I Q | N Y V I Q | C R V I Q | N H V I Q | C R V I Q   | N Y V T Q | S N V V E | N Y V V Q |
| Arabidopsis thaliana     | 15228271 | S R F I Q | N Y V I Q | C R V I Q | N H V I Q | C R V I Q   | N Y V I Q | S N V V E | N Y V V Q |
| Arabidopsis thaliana     | 15227038 | S R F I Q | N Y V I Q | C R V I Q | N H V V Q | C R V I Q   | N Y V V Q | S N V V E | N Y V V Q |
| Arabidopsis thaliana     | 20465637 | S R F I Q | N Y V I Q | C R V I Q | N H V V Q | C R V I Q   | N Y V I Q | S N V V E | N Y V V Q |
| Oryza sativa             | 20160612 | S R F I Q | N Y V V Q | C R V I Q | N H V V Q | C R V I Q   | N Y V V Q | S N V V E | N Y V V Q |
| Hordeum vulgare          | 20513851 | S R F I Q | N Y V V Q | C R V I Q | N H V I Q | C R V I Q   | N Y V V Q | S N V I E | N Y V V Q |
| Oryza sativa             | 14719326 | S R F I Q | N Y V V Q | C R V I Q | N H V I Q | C R V I Q   | N Y V V Q | S N V I E | N Y V V Q |

No. of PumHD repeat

Position

Protein {amino acid}

|             |         | R1            | R2  | R3            | R4  | R5            | R6 | R7 | R8 |     |
|-------------|---------|---------------|-----|---------------|-----|---------------|----|----|----|-----|
|             |         | *pos12.       |     | *pos12.       |     | o pos13.      |    |    |    |     |
|             |         | Puf4,5 {C}    |     | Puf4,5 {T}    |     | Puf4,5 {C}    |    |    |    |     |
|             |         | Puf3, Pum {S} |     | Puf3, Pum {C} |     | Puf3, Pum {R} |    |    |    |     |
|             | PUMILIO | A             | U   | A             | Auc | A             | U  | G  | U  | -5' |
| RNA         | Puf3p   | A             | U   | A             | Auc | A             | U  | G  | U  | -5' |
| sequence    | Puf4p   | A             | U   | A             | Uca | A             | U  | G  | U  | -5' |
| preferences | Puf5p   | A U           | Uag | UC            | Au  | A             | U  | G  | U  | -5' |

tetranucleotide core