

# Supplementary Material for: “Exploring genomic dark matter: homology search for non-coding RNA”

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## Supplementary methods

**Phylogenetic Sequence Reconstruction** Ancestral sequences were stochastically sampled for each internal node of a phylogeny relating the search sequences (see Figure 1). An empirical Bayesian approach was adopted in which calculation of the posterior probabilities was conditioned on point estimates for the phylogeny, branch lengths, and model parameters <sup>1</sup>. MrBayes v3.1.1<sup>2</sup> was used to obtain a Bayesian consensus tree ( $\tau$ ), posterior expectation of branch lengths ( $v$ ), and substitution model parameters ( $\theta$ ). MrBayes was run for 1 million cycles with a single Markov chain using an RNA doublet model derived from the Shöniger and von Haussler model <sup>3</sup> for paired sites (stems) and the general-time-reversible model <sup>4</sup> for unpaired sites (loops). The all-compatibility consensus tree and posterior expectations ( $v$  and  $\theta$ ) were obtained after excluding the first 500,000 cycles as burn-in.

Ancestral states are stochastically sampled from the posterior distribution at each internal node of the consensus tree. Specifically, the probability of the ancestral state  $i$  for site  $k$  is calculated as,

$$Pr(y_k = i \mid \mathbf{x}_k, \hat{\tau}, \hat{v}, \hat{\theta}) = \frac{l_{k,i} \pi_i I(y_k = i)}{\sum_{j \in \psi_k} l_{k,j} \pi_j I(y_k = j)} \quad (1)$$

where  $l_{k,i}$  is the conditional likelihood,  $\pi_i$  is the frequency of  $i$ ,  $\psi_k$  is the possible set of states at site  $k$ , and  $\mathbf{x}_k$  is a column vector of the observations at site  $k$ . If site  $k$  is a doublet then  $\psi_k$  contains 16 possible states ( $A \cdot A, A \cdot C, A \cdot G, \dots, U \cdot G, U \cdot U$ ), otherwise,  $\psi_k$  is the set of 4 possible nucleotides. A prior is used to constrain sampled ancestral states using the indicator function  $I(y_k = i)$ ,

$$I(y_k = i) = \begin{cases} q & \text{site } k \text{ is a doublet, } i = \text{WC or GU} \\ 1 - q & \text{site } k \text{ is a doublet, } i \neq \text{WC or GU} \\ 1 & \text{site } k \text{ is a singleton.} \end{cases} \quad (2)$$

where,  $q$  is the observed frequency of Watson-Crick (WC) or wobble (GU) pairs at site  $k$ .

The purpose of using reconstructed sequences to enhance homology searches rests on expanding the search sequence diversity in a way that captures the diversity in unidentified orthologs. Unfortunately, when the diversity of search sequences is low ancestral reconstructions alone are unlikely to capture divergent homologs. In order to expand search sequence diversity a posterior predictive method was developed to simulate more divergent search sequences (see Supplementary Figure 1).

Using samples of the model parameters from the posterior (as described above) a predictive sequence is sampled by simulating an imaginary lineage originating from each internal node of the search sequence phylogeny. In the absence of an estimate of the evolutionary distance from the internal node to the tip of the imaginary lineage each branch is assigned an *a priori* length,  $\lambda$ , that has been optimized for the level of divergence among a larger subset of orthologs. The probability of observing a state at the tip of the new lineage is the product of the probability of sampling a state at an internal node and the probability of changing from one state to another along the lineage. Formally, sequences are simulated by stochastically sampling from the following distribution,

$$Pr(z_k = j \mid y_k = i, \mathbf{x}_k, \hat{\tau}, \hat{v}, \hat{\theta}) = Pr(y_k = i \mid \mathbf{x}_k, \hat{\tau}, \hat{v}, \hat{\theta}) p_{ij}(\lambda) \quad (3)$$

where the simulated observation for site  $k$  at the imaginary tip node is  $z_k$ ,  $y_k$  is the state at the parent node,  $\lambda$  is the branch length in terms of the expected number of substitutions, and  $p_{ij}$  is the transition probability of changing from  $i$  to  $j$ . Transition probabilities are derived from the same models used in reconstructing ancestral states. It should be noted that when  $\lambda = 0$  equation 3 gives the same results as equation 1.

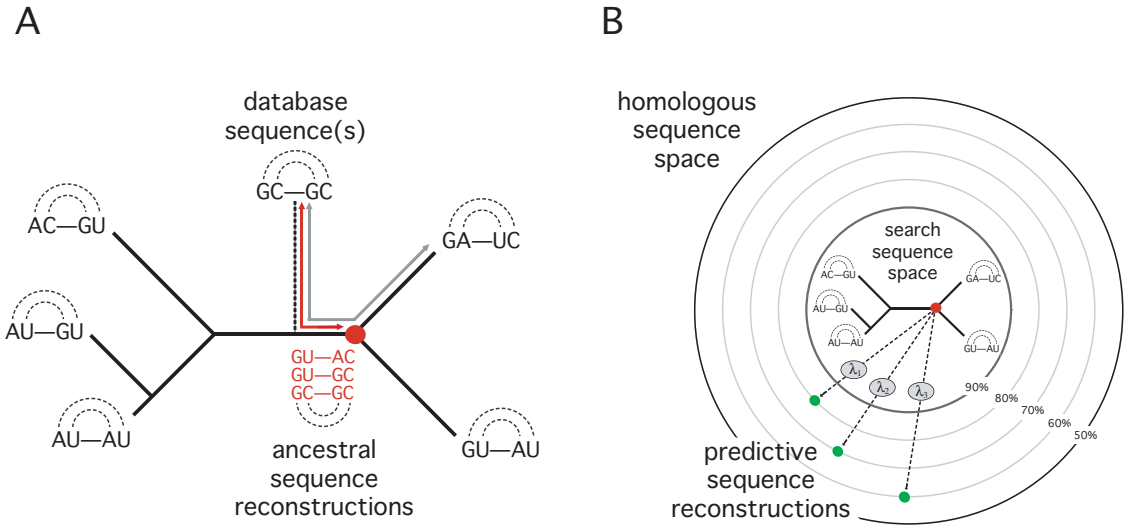


Figure 1: Schematic justification for the use of ancestral state reconstructions (A: ASR) and predictive sequence reconstructions (B: PSR) to enhance homology search. (A) Ancestral state reconstruction of a stem pair demonstrating their use to augment homology discovery. The red and grey arrowed lines represent the distance between the nearest search sequence and an unknown homolog (sampling described in the text). (B) Predictive sequence reconstructions augment homology discovery by sampling highly probable sequences at varying divergences from the backbone nodes of the phylogeny relating the search sequences; divergence, ( $\lambda_i$ ), of the predictive lineages is optimised on training sets to balance gains in sensitivity and loss in specificity.

### Supplementary Figure 1 - Phylogenetic sequence reconstruction

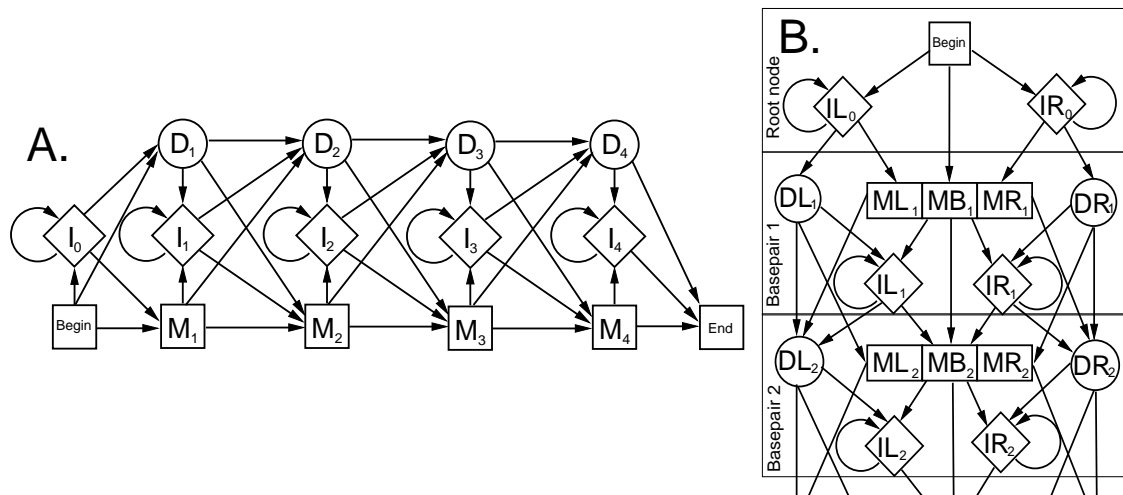


Figure 2: **A.** An illustration of the profile hidden Markov model (HMM) architecture proposed by Krogh *et al.* (1994). States labelled “M” capture matches between database sequences and the query profile, states labelled “I” allow for insertions in the database sequences relative to the profile and states labelled “D” allow for deletions in the database sequences relative to the profile. **B.** An illustration of 2 basepairs of a covariance model (CM) as proposed by Eddy & Durbin<sup>5</sup>. A CM is a generalization of a profile HMM that allows basepairs to be modeled. In the above representation of a CM: “MB” states match both the 5’ & 3’ bases in a basepair, “ML” and “MR” states match only the 5’ or 3’ bases respectively. “DL” and “DR” states model deletions of either a 5’ or a 3’ base respectively. “ML” states are tied to “DR” states and “MR” states are tied to “DL” states. “IL” and “IR” states allow for insertions between bases in either the 5’ or 3’ side of a basepair. Loops in the secondary structure degenerate to a profile HMM.

### Supplementary Figure 2 - A profile HMM architecture

## **Supplementary results**

### **Tables**

Program	Sensitivity	Specificity	MCC	Ave. MCC Rank	
				Median	Mean
Sequence based methods					
NCBI-BLAST	(0.23, <b>0.59</b> ,0.88)	(0.995, <b>0.997</b> ,0.999)	(0.44, <b>0.73</b> ,0.90)	31.0	29.33
NCBI-BLAST (W7)	(0.41, <b>0.70</b> ,0.93)	(0.981, <b>0.987</b> ,0.994)	(0.57, <b>0.74</b> ,0.86)	30.0	27.72
NCBI-BLAST (W7,65%)	(0.40, <b>0.88</b> ,0.96)	(0.998, <b>0.999</b> ,1.000)	(0.61, <b>0.93</b> ,0.96)	17.0	15.84
WU-BLAST	(0.30, <b>0.63</b> ,0.89)	(0.996, <b>0.998</b> ,0.999)	(0.52, <b>0.76</b> ,0.91)	28.0	26.75
WU-BLAST (W7)	(0.37, <b>0.92</b> ,0.97)	(0.999, <b>0.999</b> ,1.000)	(0.59, <b>0.95</b> ,0.97)	15.0	14.51
WU-BLAST (W3)	(0.36, <b>0.93</b> ,0.96)	(0.999, <b>1.000</b> ,1.000)	(0.59, <b>0.95</b> ,0.97)	11.0	13.45
WU-BLAST (W7,65%)	(0.39, <b>0.88</b> ,0.96)	(0.999, <b>1.000</b> ,1.000)	(0.60, <b>0.93</b> ,0.96)	17.0	15.68
WU-BLAST (W7,PUPY)	(0.41, <b>0.84</b> ,0.97)	(0.994, <b>0.995</b> ,0.999)	(0.62, <b>0.88</b> ,0.94)	23.0	21.36
FASTA	(0.43, <b>0.90</b> ,0.96)	(0.998, <b>0.999</b> ,1.000)	(0.64, <b>0.94</b> ,0.97)	14.0	13.04
FASTA [ASR]	(0.52, <b>0.94</b> ,0.98)	(0.994, <b>0.997</b> ,0.999)	(0.70, <b>0.92</b> ,0.95)	12.0	14.86
FASTA (ktup6,65%)	(0.40, <b>0.89</b> ,0.96)	(0.999, <b>0.999</b> ,1.000)	(0.61, <b>0.93</b> ,0.97)	16.0	14.45
FASTA (U)	(0.31, <b>0.89</b> ,0.97)	(0.997, <b>0.998</b> ,1.000)	(0.54, <b>0.92</b> ,0.96)	19.0	19.05
FASTA (RIBOSUM)	(0.06, <b>0.25</b> ,0.58)	(0.993, <b>0.999</b> ,1.000)	(0.15, <b>0.47</b> ,0.64)	34.0	33.84
FASTA (FOLDALIGN)	(0.11, <b>0.74</b> ,0.97)	(0.989, <b>1.000</b> ,1.000)	(0.32, <b>0.84</b> ,0.91)	27.0	27.53
ParAlign	(0.27, <b>0.64</b> ,0.77)	(0.996, <b>0.998</b> ,0.999)	(0.49, <b>0.76</b> ,0.84)	30.0	29.32
ParAlign (65%)	(0.38, <b>0.82</b> ,0.89)	(0.999, <b>1.000</b> ,1.000)	(0.60, <b>0.89</b> ,0.93)	21.0	19.65
SSEARCH	(0.38, <b>0.90</b> ,0.95)	(0.999, <b>1.000</b> ,1.000)	(0.60, <b>0.94</b> ,0.97)	12.0	13.49
SSEARCH [ASR]	(0.46, <b>0.93</b> ,0.97)	(0.997, <b>0.999</b> ,1.000)	(0.66, <b>0.94</b> ,0.96)	13.0	12.77
SSEARCH (65%)	(0.38, <b>0.88</b> ,0.95)	(0.999, <b>1.000</b> ,1.000)	(0.60, <b>0.93</b> ,0.97)	14.0	14.30
SSEARCH (U)	(0.27, <b>0.89</b> ,0.96)	(0.995, <b>0.997</b> ,1.000)	(0.50, <b>0.91</b> ,0.95)	22.0	21.79
Profile HMM methods					
HMMer (1.8.4,local)	(0.50, <b>0.86</b> ,0.95)	(1.000, <b>1.000</b> ,1.000)	(0.69, <b>0.92</b> ,0.97)	13.0	13.77
HMMer (1.8.4,global)	(0.70, <b>0.85</b> ,0.95)	(0.992, <b>0.997</b> ,1.000)	(0.76, <b>0.90</b> ,0.95)	19.0	16.14
HMMer (2.3.2,local)	(0.59, <b>0.89</b> ,0.96)	(0.998, <b>0.999</b> ,0.999)	(0.75, <b>0.93</b> ,0.96)	11.0	12.40
HMMer (2.3.2,global)	(0.74, <b>0.88</b> ,0.97)	(0.991, <b>0.996</b> ,0.999)	(0.77, <b>0.92</b> ,0.96)	12.0	13.37
SAM (local)	(0.48, <b>0.91</b> ,0.96)	(1.000, <b>1.000</b> ,1.000)	(0.67, <b>0.95</b> ,0.97)	<b>10.0</b>	10.61
SAM (global)	(0.40, <b>0.88</b> ,0.94)	(0.999, <b>1.000</b> ,1.000)	(0.61, <b>0.93</b> ,0.96)	14.0	15.68
SAM (model) + HMMer (2.3.2,search)	(0.68, <b>0.90</b> ,0.97)	(0.982, <b>0.995</b> ,0.999)	(0.67, <b>0.91</b> ,0.95)	17.0	17.11
Structure enhanced methods					
ERPIN	(0.01, <b>0.01</b> ,0.03)	(1.000, <b>1.000</b> ,1.000)	(0.07, <b>0.10</b> ,0.15)	36.0	35.93
ERPIN [PSR]	(0.09, <b>0.17</b> ,0.29)	(1.000, <b>1.000</b> ,1.000)	(0.28, <b>0.39</b> ,0.51)	35.0	33.02
Infernal (0.7)	(0.94, <b>0.98</b> ,1.00)	(0.996, <b>1.000</b> ,1.000)	(0.95, <b>0.97</b> ,1.00)	<b>2.0</b>	5.79
Infernal (0.7,local)	(0.91, <b>0.98</b> ,1.00)	(0.999, <b>1.000</b> ,1.000)	(0.95, <b>0.98</b> ,0.99)	<b>3.0</b>	5.19
Infernal (0.55)	(0.32, <b>0.38</b> ,0.52)	(1.000, <b>1.000</b> ,1.000)	(0.55, <b>0.60</b> ,0.71)	32.0	30.64
RAVENNA (ML)	(0.61, <b>0.82</b> ,0.94)	(1.000, <b>1.000</b> ,1.000)	(0.77, <b>0.90</b> ,0.97)	<b>8.0</b>	11.74
RAVENNA (ML,local)	(0.64, <b>0.89</b> ,0.96)	(0.997, <b>0.998</b> ,0.999)	(0.78, <b>0.92</b> ,0.96)	13.0	13.38
RSEARCH	(0.93, <b>0.99</b> ,1.00)	(0.999, <b>1.000</b> ,1.000)	(0.96, <b>0.99</b> ,1.00)	<b>2.0</b>	4.57
RSmatch	(0.31, <b>0.42</b> ,0.51)	(0.997, <b>0.999</b> ,1.000)	(0.53, <b>0.62</b> ,0.68)	32.0	26.60

Table 1: Tabulated results of searches with **five** sequences. From left to right column one contains program names and settings, column two sensitivity, column three specificity, column four Matthew's correlation coefficient (MCC) and column five contains both a median and mean ranking determined by the MCC (see Box 2 for definitions). Sensitivity, specificity and MCC are summarised as 3-tuples displaying the lower quartiles, medians and upper quartiles. Median MCC rankings below ten (one being the best) are indicated with bold font. See Methods section and Supplementary Tables 5&6 for algorithm settings.

Program	Sensitivity	Specificity	MCC	Ave. MCC Rank	
				Median	Mean
Sequence based methods					
NCBI-BLAST	(0.53, <b>0.71</b> ,0.94)	(0.978, <b>0.987</b> ,0.993)	(0.67, <b>0.76</b> ,0.85)	29.0	29.10
NCBI-BLAST (W7)	(0.69, <b>0.83</b> ,1.00)	(0.924, <b>0.951</b> ,0.974)	(0.67, <b>0.70</b> ,0.72)	31.0	30.40
NCBI-BLAST (W7,65%)	(0.83, <b>0.96</b> ,1.00)	(0.991, <b>0.997</b> ,0.998)	(0.89, <b>0.94</b> ,0.96)	16.0	15.61
WU-BLAST	(0.58, <b>0.76</b> ,0.98)	(0.977, <b>0.991</b> ,0.994)	(0.72, <b>0.80</b> ,0.88)	27.0	26.48
WU-BLAST (W7)	(0.88, <b>0.97</b> ,0.98)	(0.989, <b>0.997</b> ,0.998)	(0.90, <b>0.93</b> ,0.97)	20.0	16.28
WU-BLAST (W3)	(0.89, <b>0.97</b> ,0.98)	(0.990, <b>0.997</b> ,0.999)	(0.91, <b>0.93</b> ,0.97)	18.0	15.73
WU-BLAST (W7,65%)	(0.83, <b>0.96</b> ,1.00)	(0.991, <b>0.998</b> ,0.999)	(0.90, <b>0.94</b> ,0.96)	17.0	15.72
WU-BLAST (W7,PUPY)	(0.77, <b>0.97</b> ,1.00)	(0.973, <b>0.981</b> ,0.985)	(0.81, <b>0.87</b> ,0.89)	26.0	23.10
FASTA	(0.85, <b>0.97</b> ,1.00)	(0.988, <b>0.996</b> ,0.998)	(0.88, <b>0.93</b> ,0.96)	15.0	15.78
FASTA (ktup6,65%)	(0.83, <b>0.96</b> ,1.00)	(0.991, <b>0.997</b> ,0.998)	(0.89, <b>0.94</b> ,0.96)	16.0	14.55
FASTA (U)	(0.84, <b>0.97</b> ,0.98)	(0.989, <b>0.993</b> ,0.996)	(0.89, <b>0.93</b> ,0.94)	21.0	20.18
FASTA (RIBOSUM)	(0.25, <b>0.57</b> ,0.79)	(0.936, <b>0.993</b> ,0.998)	(0.46, <b>0.54</b> ,0.69)	32.0	32.08
FASTA (FOLDALIGN)	(0.69, <b>0.85</b> ,0.98)	(0.988, <b>1.000</b> ,1.000)	(0.82, <b>0.89</b> ,0.93)	23.0	21.43
ParAlign	(0.57, <b>0.76</b> ,0.93)	(0.979, <b>0.988</b> ,0.992)	(0.70, <b>0.78</b> ,0.85)	28.0	28.04
ParAlign (65%)	(0.81, <b>0.93</b> ,0.96)	(0.994, <b>0.999</b> ,1.000)	(0.89, <b>0.93</b> ,0.96)	16.0	15.30
SSEARCH	(0.83, <b>0.96</b> ,0.98)	(0.994, <b>0.999</b> ,0.999)	(0.90, <b>0.95</b> ,0.97)	12.0	12.59
SSEARCH (65%)	(0.81, <b>0.96</b> ,0.97)	(0.994, <b>0.999</b> ,0.999)	(0.89, <b>0.95</b> ,0.97)	12.0	13.26
SSEARCH (U)	(0.84, <b>0.96</b> ,0.98)	(0.984, <b>0.989</b> ,0.995)	(0.86, <b>0.91</b> ,0.93)	25.0	23.34
Profile HMM methods					
HMMer (1.8.4,local)	(0.71, <b>0.90</b> ,0.95)	(0.999, <b>1.000</b> ,1.000)	(0.83, <b>0.95</b> ,0.97)	11.0	12.06
HMMer (1.8.4,global)	(0.74, <b>0.84</b> ,0.90)	(1.000, <b>1.000</b> ,1.000)	(0.85, <b>0.91</b> ,0.94)	16.0	15.16
HMMer (2.3.2,local)	(0.74, <b>0.94</b> ,0.97)	(0.996, <b>0.997</b> ,0.998)	(0.84, <b>0.93</b> ,0.97)	12.0	14.36
HMMer (2.3.2,global)	(0.74, <b>0.86</b> ,0.94)	(1.000, <b>1.000</b> ,1.000)	(0.85, <b>0.92</b> ,0.96)	<b>9.5</b>	12.87
SAM (local)	(0.74, <b>0.94</b> ,0.97)	(0.999, <b>1.000</b> ,1.000)	(0.85, <b>0.96</b> ,0.98)	<b>10.0</b>	10.42
SAM (global)	(0.84, <b>0.94</b> ,0.98)	(0.997, <b>0.998</b> ,0.999)	(0.88, <b>0.95</b> ,0.97)	11.0	11.19
SAM (model) + HMMer (2.3.2,search)	(0.78, <b>0.90</b> ,0.95)	(0.998, <b>1.000</b> ,1.000)	(0.83, <b>0.94</b> ,0.97)	12.0	13.17
Structure enhanced methods					
ERPIN	(0.10, <b>0.23</b> ,0.42)	(1.000, <b>1.000</b> ,1.000)	(0.30, <b>0.47</b> ,0.63)	33.0	32.51
Infernal (0.7)	(0.97, <b>0.99</b> ,1.00)	(0.997, <b>1.000</b> ,1.000)	(0.97, <b>0.98</b> ,1.00)	<b>2.0</b>	4.28
Infernal (0.7,local)	(0.96, <b>0.99</b> ,1.00)	(0.999, <b>1.000</b> ,1.000)	(0.97, <b>0.99</b> ,1.00)	<b>2.0</b>	3.11
Infernal (0.55)	(0.88, <b>0.93</b> ,0.98)	(1.000, <b>1.000</b> ,1.000)	(0.93, <b>0.96</b> ,0.99)	<b>4.0</b>	8.37
RAVENNA (ML)	(0.79, <b>0.94</b> ,0.97)	(1.000, <b>1.000</b> ,1.000)	(0.88, <b>0.96</b> ,0.98)	<b>7.0</b>	7.89
RAVENNA (ML,local)	(0.80, <b>0.94</b> ,0.97)	(0.995, <b>0.998</b> ,0.999)	(0.88, <b>0.94</b> ,0.96)	14.0	13.22
RSEARCH	(0.84, <b>0.94</b> ,0.99)	(1.000, <b>1.000</b> ,1.000)	(0.91, <b>0.97</b> ,0.99)	<b>4.0</b>	6.61
RSmatch	(0.57, <b>0.62</b> ,0.91)	(0.989, <b>0.997</b> ,0.998)	(0.72, <b>0.76</b> ,0.89)	26.0	25.80

Table 2: Tabulated results of searches with **twenty** sequences. From left to right column one contains program names and settings, column two sensitivity, column three specificity, column four Matthew’s correlation coefficient (MCC) and column five contains both a median and mean ranking determined by the MCC (see Box 2 for definitions). Sensitivity, specificity and MCC are summarised as 3-tuples displaying the lower quartiles, medians and upper quartiles. Median MCC rankings below ten (one being the best) are indicated with bold font. See Methods section and Supplementary Tables 5&6 for algorithm settings.

Program	Sensitivity	Specificity	MCC	Ave. MCC Rank	
				Median	Mean
Five input sequences					
NCBI-BLAST (W7,65%)	(0.40, <b>0.88</b> ,0.96)	(0.998, <b>0.999</b> ,1.000)	(0.61, <b>0.93</b> ,0.96)	17.0	15.84
WU-BLAST (W7,65%)	(0.39, <b>0.88</b> ,0.96)	(0.999, <b>1.000</b> ,1.000)	(0.60, <b>0.93</b> ,0.96)	17.0	15.68
FASTA (ktup6,65%)	(0.40, <b>0.89</b> ,0.96)	(0.999, <b>0.999</b> ,1.000)	(0.61, <b>0.93</b> ,0.97)	16.0	14.45
ParAlign (65%)	(0.38, <b>0.82</b> ,0.89)	(0.999, <b>1.000</b> ,1.000)	(0.60, <b>0.89</b> ,0.93)	21.0	19.65
SSEARCH (65%)	(0.38, <b>0.88</b> ,0.95)	(0.999, <b>1.000</b> ,1.000)	(0.60, <b>0.93</b> ,0.97)	14.0	14.30
Twenty input sequences					
NCBI-BLAST (W7,65%)	(0.83, <b>0.96</b> ,1.00)	(0.991, <b>0.997</b> ,0.998)	(0.89, <b>0.94</b> ,0.96)	16.00	15.61
WU-BLAST (W7,65%)	(0.83, <b>0.96</b> ,1.00)	(0.991, <b>0.998</b> ,0.999)	(0.90, <b>0.94</b> ,0.96)	17.00	15.72
FASTA (ktup6,65%)	(0.83, <b>0.96</b> ,1.00)	(0.991, <b>0.997</b> ,0.998)	(0.89, <b>0.94</b> ,0.96)	16.00	14.55
ParAlign (65%)	(0.81, <b>0.93</b> ,0.96)	(0.994, <b>0.999</b> ,1.000)	(0.89, <b>0.93</b> ,0.96)	16.00	15.30
SSEARCH (65%)	(0.81, <b>0.96</b> ,0.97)	(0.994, <b>0.999</b> ,0.999)	(0.89, <b>0.95</b> ,0.97)	12.00	13.26

Table 3: Tabulated results of the sequence based methods with identical scoring parameters. From left to right column one contains program names, column two sensitivity, column three specificity, column four Matthew’s correlation coefficient (MCC) and column five a median and mean ranking determined by the MCC. Sensitivity, specificity and MCC are summarised as 3-tuples displaying the lower quartiles, medians and upper quartiles.



Program	Sensitivity	Specificity	MCC	Ave. MCC Rank	
				Median	Mean
Five input sequences					
WU-BLAST	(0.30, <b>0.63</b> ,0.89)	(0.996, <b>0.998</b> ,0.999)	(0.52, <b>0.76</b> ,0.91)	28.0	26.75
WU-BLAST (W7,PUPY)	(0.41, <b>0.84</b> ,0.97)	(0.994, <b>0.995</b> ,0.999)	(0.62, <b>0.88</b> ,0.94)	23.0	21.36
FASTA	(0.43, <b>0.90</b> ,0.96)	(0.998, <b>0.999</b> ,1.000)	(0.64, <b>0.94</b> ,0.97)	14.0	13.04
FASTA (U)	(0.31, <b>0.89</b> ,0.97)	(0.997, <b>0.998</b> ,1.000)	(0.54, <b>0.92</b> ,0.96)	19.0	19.05
FASTA (RIBOSUM)	(0.06, <b>0.25</b> ,0.58)	(0.993, <b>0.999</b> ,1.000)	(0.15, <b>0.47</b> ,0.64)	34.0	33.84
FASTA (FOLDALIGN)	(0.11, <b>0.74</b> ,0.97)	(0.989, <b>1.000</b> ,1.000)	(0.32, <b>0.84</b> ,0.91)	27.0	27.53
SSEARCH	(0.38, <b>0.90</b> ,0.95)	(0.999, <b>1.000</b> ,1.000)	(0.60, <b>0.94</b> ,0.97)	12.0	13.49
SSEARCH (U)	(0.27, <b>0.89</b> ,0.96)	(0.995, <b>0.997</b> ,1.000)	(0.50, <b>0.91</b> ,0.95)	22.0	21.79
Twenty input sequences					
WU-BLAST	(0.58, <b>0.76</b> ,0.98)	(0.977, <b>0.991</b> ,0.994)	(0.72, <b>0.80</b> ,0.88)	27.0	26.48
WU-BLAST (W7,PUPY)	(0.77, <b>0.97</b> ,1.00)	(0.973, <b>0.981</b> ,0.985)	(0.81, <b>0.87</b> ,0.89)	26.0	23.10
FASTA	(0.85, <b>0.97</b> ,1.00)	(0.988, <b>0.996</b> ,0.998)	(0.88, <b>0.93</b> ,0.96)	15.0	15.78
FASTA (U)	(0.84, <b>0.97</b> ,0.98)	(0.989, <b>0.993</b> ,0.996)	(0.89, <b>0.93</b> ,0.94)	21.0	20.18
FASTA (RIBOSUM)	(0.25, <b>0.57</b> ,0.79)	(0.936, <b>0.993</b> ,0.998)	(0.46, <b>0.54</b> ,0.69)	32.0	32.08
FASTA (FOLDALIGN)	(0.69, <b>0.85</b> ,0.98)	(0.988, <b>1.000</b> ,1.000)	(0.82, <b>0.89</b> ,0.93)	23.0	21.43
SSEARCH	(0.83, <b>0.96</b> ,0.98)	(0.994, <b>0.999</b> ,0.999)	(0.90, <b>0.95</b> ,0.97)	12.0	12.59
SSEARCH (U)	(0.84, <b>0.96</b> ,0.98)	(0.984, <b>0.989</b> ,0.995)	(0.86, <b>0.91</b> ,0.93)	25.0	23.34

Table 4: Tabulated results comparing sequence based methods with and without RNA specific scoring parameters. From left to right column one contains program names and settings, column two sensitivity, column three specificity, column four Matthew’s correlation coefficient (MCC) and column five a median and mean ranking determined by the MCC. Sensitivity, specificity and MCC are summarised as 3-tuples displaying the lower quartiles, medians and upper quartiles.

**Figures**

**Thresholds**

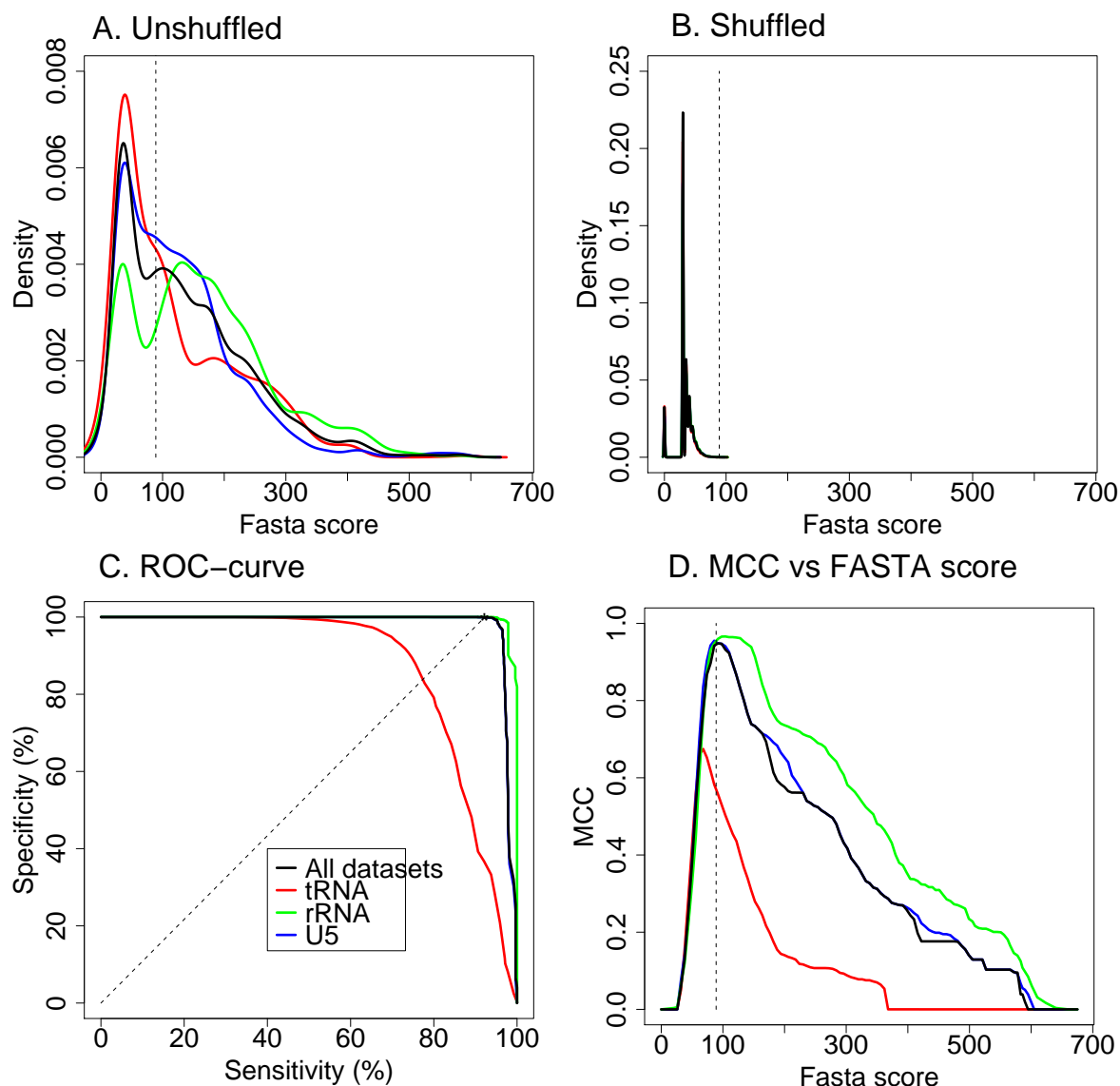


Figure 3:

A FASTA case study for optimal threshold determination. Subplot **A** displays density plots of FASTA score distributions for a selection of inputs from each RNA family (rRNA,tRNA,U5) and identity range (40-60%, 50-70%, 60-80%, 70-85% and 80-95%) when screening the relevant unshuffled database. Subplot **B** similar to subplot **A** except the relevant shuffled database is screened instead. Subplot **C** displays a ROC (receiver operator characteristic) plot showing sensitivity and specificity values over the entire range of possible thresholds. Subplot **D** displays curves of the Matthews correlation coefficient ( $MCC$ ) as a function of score threshold. The red curves indicate rRNA data, green is tRNA and blue is U5. Black curves show data for all 3 families combined. The dashed black vertical line in each subplot indicates the  $MCC$  optimal score threshold (89) over all datasets.

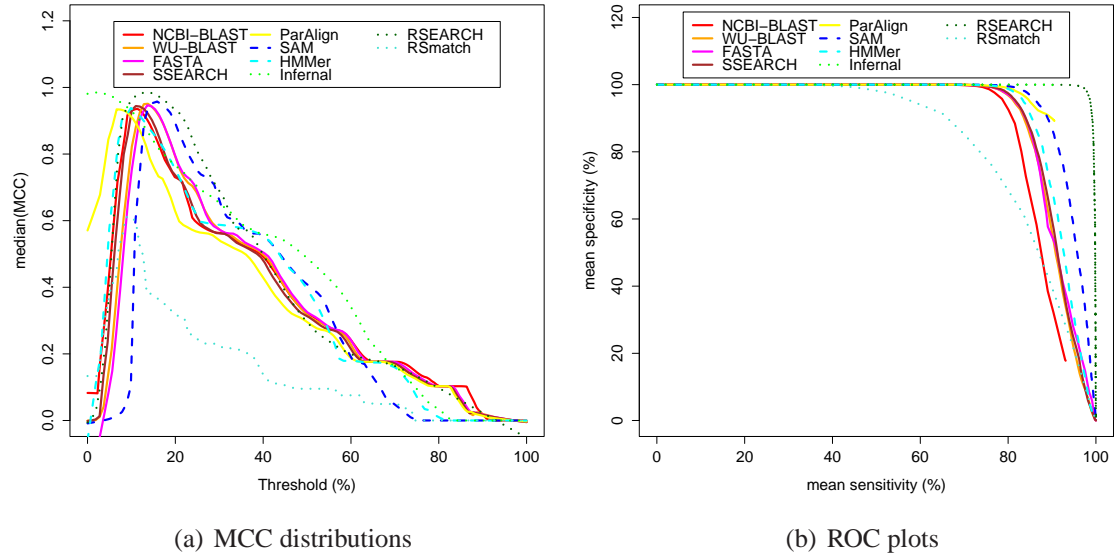


Figure 4: For each algorithm a score threshold is required. We have optimised these for small structured ncRNAs. For the sake of brevity only the highest ranking parameter settings for each algorithm is shown. (a) Distributions of median MCC scores as a function of algorithm score ranging from 0% filtered to 100% filtered fraction. (b) Receiver Operator Characteristic (ROC) plots for each algorithm.

## **Algorithm accuracy and computational efficiency**

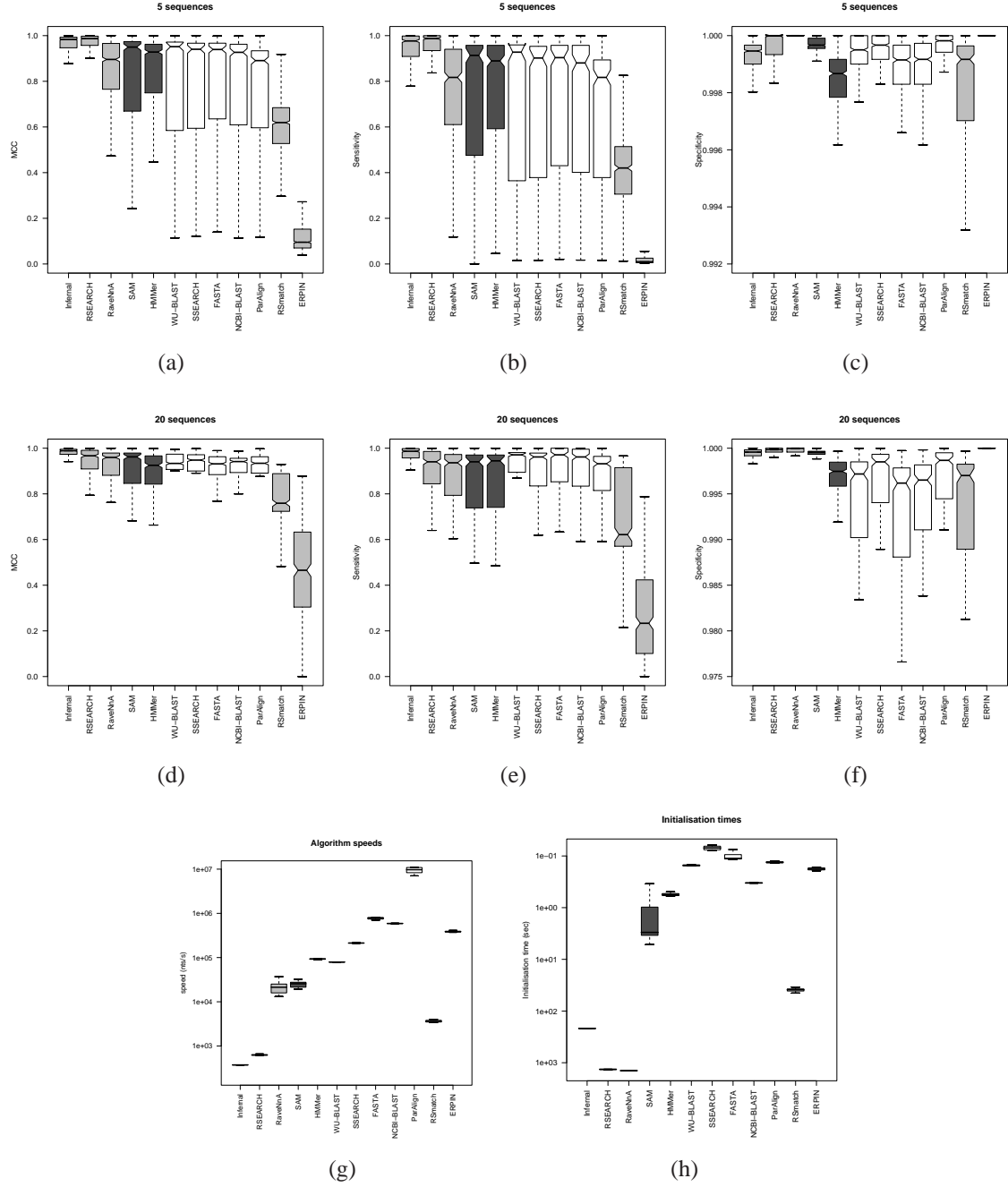


Figure 5: A comparison of the accuracy and efficiencies of homology search methods where only the highest ranking parameter settings for each algorithm from Supplementary Table 1 are shown. These were NCBI-BLAST (W7,65%), WU-BLAST (W3), FASTA, ParAlign (65%), SSEARCH, HMMer (2.3.2,local), SAM (3.5,local), ERPIN, Infernal (0.7,local), RAVENNA, RSEARCH, and RSmatch. (a-c) Boxplots of algorithm accuracies (MCC, sensitivity and specificity respectively) for the 5 sequence datasets. (d-f) Boxplots of algorithm accuracies (MCC, sensitivity and specificity respectively) for the 20 sequence datasets. (g) Boxplots of algorithm speeds in nucleotides per second. (h) Boxplots of initialization times for each algorithm, in seconds.

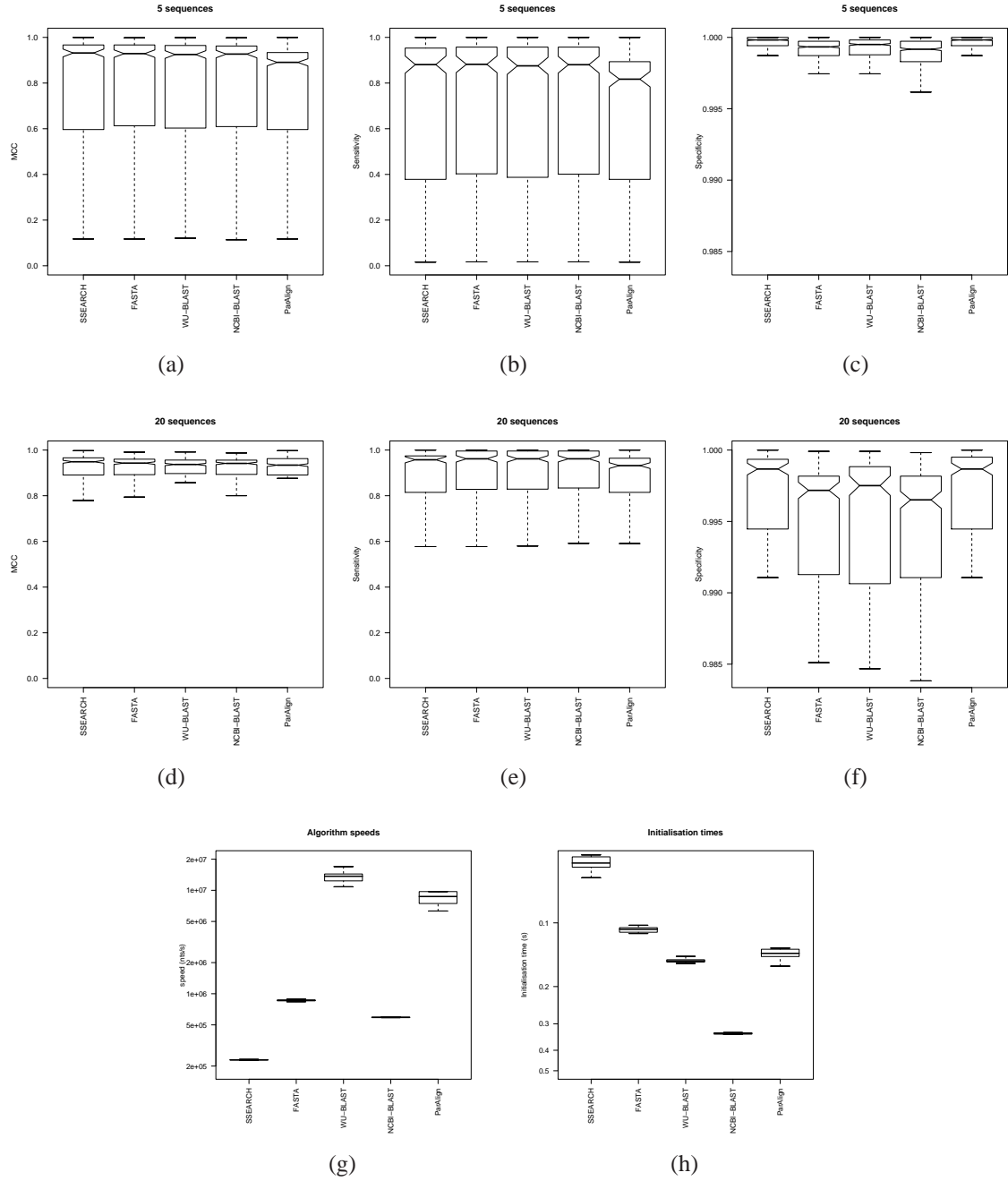


Figure 6: A comparison of the accuracy of sequence based methods with identical scoring parameters. These boxplots show the distributions of the MCC (a&d), sensitivity (b&e) and specificity (c&f) for each of the homology search methods when using a scoring scheme optimised for nucleotide sequences with 65% identity (match=+5, mismatch=-4, gapopen=10,gapextension=10). (g&h) Display algorithm speeds and initialisation times respectively.

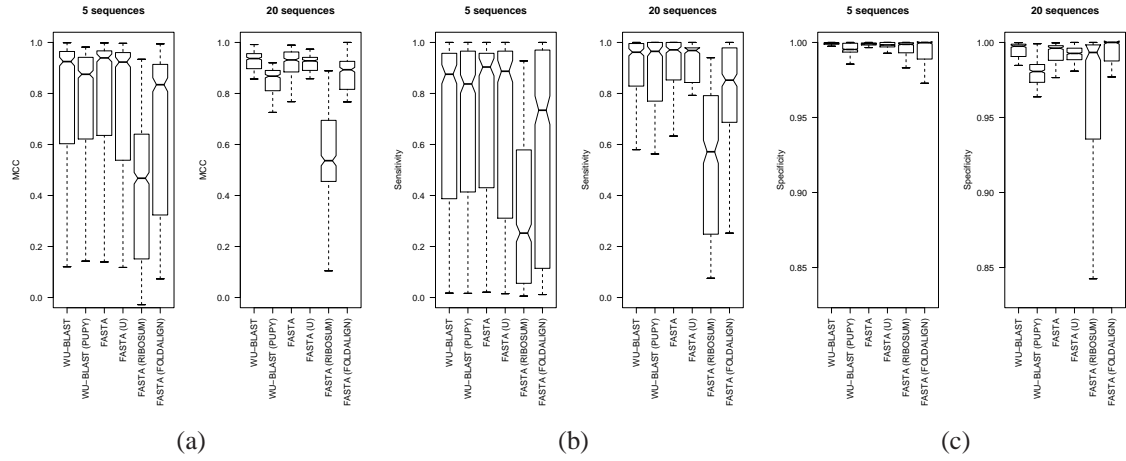


Figure 7: A comparison of the accuracy of sequence based methods with score matrices optimised for ncRNA. These boxplots show the distributions of the MCC (a), sensitivity (b) and specificity (c) for each of the homology search methods when using one of WU-BLAST (W7), WU-BLAST (W7,PUPY), FASTA, FASTA (U), FASTA (RIBOSUM) or FASTA (FOLDALIGN). These matrices are discussed in more detail in the text.



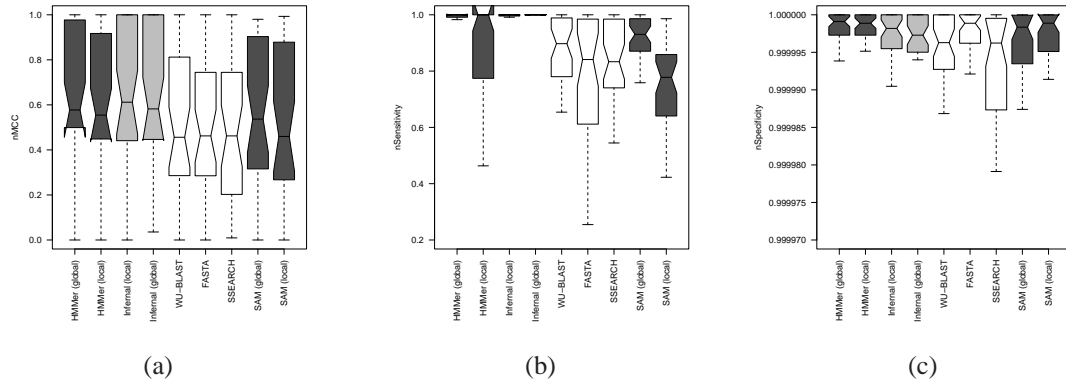


Figure 8: These boxplots indicate the distributions of (a) nMCC, (b) nSensitivity and (c) nSpecificity for a subset of representative programs from each category. Each program was run on a section of human chromosome 12 (coordinates 90,000,000-130,000,000; ver NCBI35).

## Algorithm parameters

Program	Version	Command	Reference
Sequence based methods			
NCBI-BLAST	2.2.19	blastall -p blastn -d database.fa -i query.fa -S 1 -b 0 -v 1000000	6
NCBI-BLAST (W7)		blastall -p blastn -d database.fa -i query.fa -W 7 -S 1 -b 0 -v 1000000	
NCBI-BLAST (W7,65%)		blastall -p blastn -d database.fa -i query.fa -W 7 -r 5 -q -4 -G 10 -E 10 -S 1 -b 0 -v 1000000	
WU-BLAST	2.0	blastn database.fa query.fa -noseqs -top B=9999999 V=9999999	7
WU-BLAST (W7)		blastn database.fa query.fa -noseqs W=7 -top B=9999999 V=9999999	
WU-BLAST (W3)		blastn database.fa query.fa -noseqs W=3 -top B=9999999 V=9999999	
WU-BLAST (W7,65%)		blastn database.fa query.fa -noseqs W=7 M=5 N=-4 Q=20 R=10 -top B=9999999 V=9999999	
WU-BLAST (W7,99%)		blastn database.fa query.fa -noseqs W=7 M=1 N=-3 Q=5 R=2 -top B=9999999 V=9999999	
WU-BLAST (W7,PUPY)		blastn database.fa query.fa -noseqs -matrix=pupy -top W=7 B=9999999 V=9999999	
FASTA	3.4	fasta34 -H -n -d 0 -q -3 query.fa database.fa	8
FASTA (ktup6, 65%)		fasta34 -H -b 9999999 -n -d 0 -q -3 -f 10 -g 10 -r "+5/-4" query.fa database.fa	
FASTA (-U)		fasta34 -H -n -d 0 -q -3 -U query.fa database.fa	
ParAlign	3.4.3	paralign -g sence1.lic -b 1000 -s 1 -n database.fa query.fa	9
ParAlign (65%)		paralign -g sence1.lic -m NT2 -q 10 -r 10 -b 1000 -s 1 -n database.fa query.fa	
SSEARCH		The same options are used as FASTA.	10, 11

Table 5: This table summarises the parameters and provides references for the sequence based methods abbreviated in the body of this manuscript.

Program	Version	Command	Reference
Profile HMM methods			
SAM (build)	3.5	buildmodel name -a RNA -train query.fa	12, 13
SAM (local)		hmmscore name -sw 2 -i name.mod -db database.fa	
SAM (global)		hmmscore name -sw 0 -i name.mod -db database.fa	
SAM (model) + HMMer (search)	3.5, 2.3.2	buildmodel name -a RNA -train query.fa  sam2hmmer name -i name.mod hmsearch name.con.hmm database.fa	
HMMer (global)	2.3.2	hmmbuild -F -n name name.hmm query.aln	14
HMMer (local)		hmmbuild -f -F -n name name.hmm query.aln	
HMMer (search)		hmsearch name.hmm database.fa	
HMMer	1.8.4	hmmt name.hmm query.fa	
HMMer (search)		hmmls name.hmm database.fa (global)	
HMMer (search)		hmmlfs name.hmm database.fa (local)	
Structure enhanced methods			
ERPIN	4.2.5	erpin query.epn database.fa -begin,end -nomask -fwd -sum SUM.dat -pcw 0.1	15
Inferral	0.7	cmbuild -F query.cm query.stk; cmsearch --toponly query.cm database.fa	16
Inferral (local)	0.7	cmbuild -F query.cm query.stk; cmsearch --toponly --local query.cm database.fa	
RAVENNA (ML)		ravenna.pl -filter ML -onlyForwardStrand -global -scoreThreshold 0.00001 -database database.fa -cmFileName query.cm 200	17
RAVENNA (ML,local)		ravenna.pl -filter ML -onlyForwardStrand -local -scoreThreshold 0.00001 -database database.fa -cmFileName query.cm 200	
RSEARCH	1.1	rsearch -n 1000 -E 100000 -m RIBOSUM85-60.mat query.stk database.fa	18
RSmatch	1.0	RSmatch -p dsearch -D database.fa -Q query.fa -n 30000	19

Table 6: This table summarises the parameters and provides references for the profile HMM and structure enhanced methods abbreviated in the body of this manuscript.

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