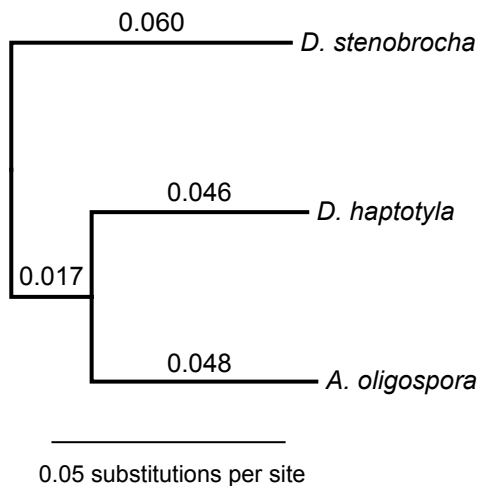**A**

Number of RNAs with motif matches in "ancestral Puf3 targets"
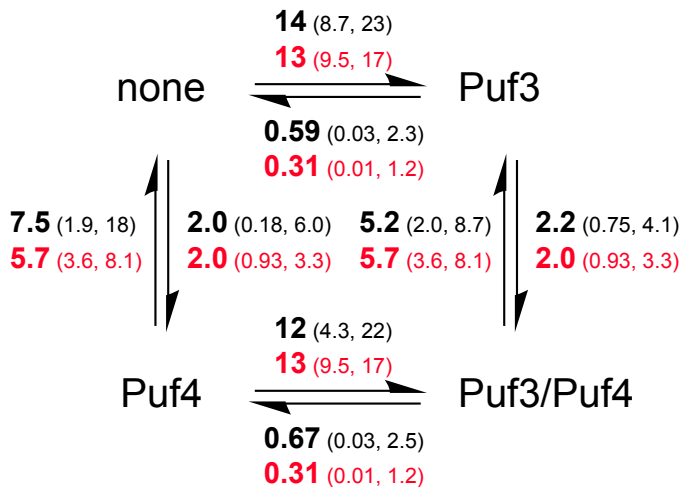
	Puf3 and Puf4*	Puf3 only	Puf4 only*	neither	odds-ratio (95% CI)	p-value
D. stenobrocha	38	71	10	15	0.80 (0.32 - 2.01)	0.65
D. haptotyla	46	63	16	24	1.09 (0.51 - 2.32)	0.85
A. oligospora	49	74	9	19	1.39 (0.56 - 3.58)	0.52

* The Puf4 submotif UGUA[ACU].[AU].UA was removed from this analysis to prevent overlap with the Puf3 motif.

B

0.060 — *D. stenobrocha*

0.046 — *D. haptotyla*

0.017

0.048 — *A. oligospora*

0.05 substitutions per site

C

none → Puf3: **14** (8.7, 23) / **13** (9.5, 17)

Puf3 → none: **0.59** (0.03, 2.3) / **0.31** (0.01, 1.2)

none ↔ Puf4: **7.5** (1.9, 18) / **5.7** (3.6, 8.1)

2.0 (0.18, 6.0) / **2.0** (0.93, 3.3)

Puf3 ↔ Puf3/Puf4: **5.2** (2.0, 8.7) / **5.7** (3.6, 8.1)

2.2 (0.75, 4.1) / **2.0** (0.93, 3.3)

Puf4 → Puf3/Puf4: **12** (4.3, 22) / **13** (9.5, 17)

Puf3/Puf4 → Puf4: **0.67** (0.03, 2.5) / **0.31** (0.01, 1.2)

■ rates in "dependence" model

■ rates in "independence" model

median rate (95% credible interval)

Comparing Model Likelihoods:

log[harmonic mean(**dependence model**)] = -242.2

log[harmonic mean(**independence model**)] = -234.5

log Bayes Factor = 2 * (-242.2 --234.5) = -15

log Bayes Factor < 0, favoring **independence model**

Supp. Figure 16