**Miranda et al., 2017**

**Fig 3 Effects of single-nucleotide changes in the atpH site on PPR10 binding affinity.**

(A) Alignment between PPR10 and the atpH binding site.

* Aa position indicated as modules of the protein (boxes of modules)
* Underneath RNA sequence that is important for binding and (N) is not
* asterisks mark nucleotides that are important but outside modules (nonmodular)

(B) RNAs used for gel mobility shift assays. **Summary of C**

* Nucleotide mutation is red
* binding affinities based on REMSA (rel. to wt)
* thermodynamic stability of RNA secondary structure (Mfold for each sequence)

(C) Gel mobility shift assays for PPR10 to assess impact on binding

* **Mutations at nucleotides 2–4, 8–11, and 14–15**
* Position 1 seems to cover the motif so mutating it improved the binding.
* same preparation of PPR10 for the rest.
* What can we see in each position – go through

Answer Q &A:

**Why was this experiment done?**  
To test how **single-nucleotide changes** affect **PPR10 binding**.

**What hypothesis or research question does this address?**  
Which **nucleotides are critical** for PPR10 binding, and how do mutations affect affinity?

**How was it done?**  
**EMSA** tested **wild-type and mutant RNAs** with **different PPR10 concentrations**.

**What experimental setup, controls, and conditions were used?**  
Same **PPR10 prep** for all RNAs (except 1U, 1C), tested across **protein dilutions**.

**What can we conclude?**  
Some positions **are essential**, while others **increase or reduce binding**.

**What do the results tell us about RNA-RBP interactions?**  
PPR10 follows a **binding code**, but **RNA structure also affects affinity**.

**Figure S3 motif assessment using Bind-n-Seq**

**EMSA (Gel Shift Assay) Tests These Predictions**

* To confirm the **Bind-n-Seq results**, **synthesized RNA oligonucleotides** containing motifs.
* tested **how well PPR10 binds to them in a controlled EMSA experiment**.
* **Different PPR10 concentrations** were used to measure **binding affinity**.
* **Combinations that seemed enriched in the Bind-n-Seq were tested**
* **The bigger the jump in Kd the more impact has the mutations**

**Bind-n-Seq**

* Bind and seq can assess multiple mutations and combinations simultaneously and calculate the Kd

**Q&A**

**Why was this experiment done?**  
To confirm whether **PPR10 binds the RNA motifs predicted by Bind-n-Seq**.

**What hypothesis or research question does this address?**  
Do **specific mutations (e.g., 13/14UG, 12/13/14GUG)** alter **PPR10 binding affinity**?

**How was it done?**  
**Bind-n-Seq** identified enriched RNA motifs bound by PPR10

**EMSA tested wild-type and mutant RNAs** with **varying PPR10 concentrations**.

**What experimental setup, controls, and conditions were used?**  
Different **RNA variants** and **PPR10 concentrations**; each assay was **repeated for consistency**.

**What can we conclude?**  
Some mutations **weaken or enhance binding**, confirming **Bind-n-Seq predictions**.

**What do the results tell us about RNA-RBP interactions?**  
PPR10 binding is **sequence-dependent**, but **mutations can shift affinity**.