

## CRISPR- Guide RNA (gRNA) Design Process:

- <http://www.e-crisp.org/E-CRISP/>
  - **This is the best program I've found so far for designing gRNAs. It's the only one that has Neurospora.**
- Input FASTA sequence of targeted gene
- Select relaxed, medium, or strict parameters
- Decide between single design or double design.
  - **I had been using double for my Al-3 knockout**
- <http://www.e-crisp.org/E-CRISP/aboutpage.html>
  - **This is a link to the help page for the program. It explains the SAE score well, which will aid in picking between the provided gRNAs.**

Name	Nucleotide sequence	SAE-Score	Target	Matchstring	Number of Hits
NC_026505.1:C40329 13-4030155_44_0	GTTGGAGAGAAGGGA AAGCG NGG	S A E	EFNCRG00000004013 ::al-3	Matchstring Info	1
NC_026505.1:C40329 13-4030155_42_0	GGAAATGGAACACGT CACCA NGG	S A E	EFNCRG00000004013 ::al-3	Matchstring Info	1
NC_026505.1:C40329 13-4030155_53_0	GCGATTAGAGGGTAT GGCAA NGG	S A E	EFNCRG00000004013 ::al-3	Matchstring Info	1
NC_026505.1:C40329 13-4030155_30_0	GTTCACCTCAGCAGGC AAGCA NGG	S A E	7.14	Matchstring Info	1
NC_026505.1:C40329 13-4030155_6_0	GGGGCTCGTCAAAAA TGGGT NGG	S A E	7.14	Matchstring Info	1
NC_026505.1:C40329 13-4030155_41_0	GAAACCCGTCGGTAC TGTA NGG	S A E	EFNCRG00000004013 ::al-3	Matchstring Info	1
NC_026505.1:C40329 13-4030155_56_0	GGCGCAAACCCGTAA ACGCA NGG	S A E	EFNCRG00000004013 ::al-3	Matchstring Info	1
NC_026505.1:C40329 13-4030155_87_0	GAGATGGTATCCAAC AAGAC NGG	S A E	EFNCRG00000004013 ::al-3	Matchstring Info	1
NC_026505.1:C40329 13-4030155_4_0	GTGGACCTTGCGCAA ATTAG NGG	S A E	7.14	Matchstring Info	1
NC_026505.1:C40329 13-4030155_29_0	GATGCAATCTCGAGT TTTGC NGG	S A E	7.14	Matchstring Info	1

- The above diagram is what should display your Nucleotide sequences for your gRNAs.