Designing a CoDA Zinc Finger Nuclease for your *Glycine max* gene of choice

Pearl Program for ZFA creator (just download and open)

1. Find your gene
    a) Go to www.arabidopsis.org/
    b) Search your gene of interest in the top right corner i.e. RNA Polymerase
    c) Click on a locus (AT#G#####)
    d) Scroll down to the "Protein Data" section and click on the name (AT#G#####)
    e) Scroll down to the "Sequence" section and copy the entire amino acid sequence of your gene (including the numbers is fine)
2. Use BLAST to find your gene's Soybean version
    a) Go to www.phytozome.net/
    b) Click on the large box with the list of species
    c) Click on the little box next to *Glycine max*
    d) Under "2. Choose a tool:" expand "BLAST search"
    e) Select "proteome" instead of "genome: masked"
        a. The bar under proteome should say BLASTP- protein query to protein db
    f) Paste your amino acid sequence from part one into the Query sequence box (the numbers can be included and will not affect the BLAST)
    g) Click "submit" to begin the BLASTp search
3. Pick a target gene from the BLAST results
    a) Browse the list of BLAST results for a corresponding gene
    b) Click the box "Gene Page" to open the details about this gene
    c) Confirm the description, domain, or annotations fit your original interests
    d) Open the "Sequences" tab near the top
    e) Expand "Genomic sequence" to view the genomic context
    f) The color key is in the upper right, Blue indicates CDS
    g) Copy the entire genomic sequence
4. You have a Glyma sequence, now how do you get a Zinc Finger Nuclease?
    a) Open a new tab and go to zifit.partners.org/
    b) Select "ZiFit" and then "Proceed to ZiFit"
    c) Under CoDA (Context Dependent Assembly) click "Design Zinc Finger Nucleases"
    d) Change spacer to "5,6" for our plasmid and uncheck Exon/Intron Case Sensitivity
    e) Paste entire sequence and "Submit"
    f) A ZiFit Graphic Summary window will appear with small color bars above a large red one. These indicate individual ZFN target sites.
    g) On the main page under "Sort By" will be a list of these ZFN target sites
    h) The colored codons indicate where the individual zinc fingers will bind
    i) The lower strand of colored bases is where the Left array will bind and the upper strand is where the Right array will bind
5. Quality check Zinc Finger Nuclease Target sites
    a) Does the ZFN target an exon region?

i. Copy the 25-26 base pair target from the upper strand
ii. Return to your gene's genomic sequence at phytozome.net
iii. Click Edit –>Find and paste your 25-26 base pair target to find the target region ((targets may be spread over two lines)
iv. Determine if your ZFN target site falls in an exon region

b) Does the ZFN target consist primarily of GNN codons?
i. This starts at the spacer in the center and goes out.

```
cCCTTTCTCCCCAGCTGAGGTCGCCa     3 GNN Right array
gGGAAAAGAGGGGTCGACTCCAGCGGt    2 GNN Left array
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ii. GNN fingers appear to work best

c) Is there a limited number of "T"s in my fingers?
i. "T"s appear to have weaker binding, less than 4 is recommended

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cCCTTTCTCCCCAGCTGAGGTCGCCa     1 T Right array
gGGAAAGAGGGGTCGACTCCAGCGGt     0 T Left array
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6. Consider future screening method
a) We screen using a restriction enzyme for enrichment PCR and therefore need the ZFN target to span a restriction enzyme site.
I. Copy the 25-26 base pair target from the upper strand
i. Open two new tabs of tools.neb.com/NEBcutter2/
ii. Paste your 25-26 base pair target into the large white box and click submit
iii. Take note of reliable restriction enzymes that target larger areas
iv. Return to your gene's genomic sequence at phytozome.net and copy a 500-600 base pair region surrounding your ZFN target
v. Paste the 500-600 base pairs in tab two of tools.neb.com/NEBcutter2/ and click submit
vi. Under "Main Options" in the lower left select "Custom Digest"
vii. Check the boxes next to your noted restriction enzymes and click Digest
viii. Choose the restriction enzyme that cuts in the middle of your ZFN target but not in the surrounding 100 base pair region for enrichment PCR.

7. BLAST ZFN target against *Glycine max* genome
a) Return to ZiFit and your list of ZFN targets
b) Click the "+" next to your ZFN target to expand your ZFN design parameters
c) Above the Blast button scroll from Cow down to *Glycine max*
d) Now click the Blast button (this might take some time)
e) Click "View report"
f) Confirm your ZFN target only occurs once in the genome or in places (such as introns or intergenic regions) that won't be devastated by mutation.

8. Collect the sequence for your left and right fingers
a) Click on "ZF DNA Sequence" to reveal the sequence for your left and right finger arrays
b) Order these sequences to be synthesized.

9. Begin assembling your Zinc Finger Nuclease