Gateway cloning pipeline
Note: be sure to propagate the pDONR and destination vectors with ccdB cassettes in DB3.1 or equivalent cells

To make an entry vector.
**1) Design gene-specific oligos.**
**F primer Gateway tag (*att*B1)**
5' GGGGACAAGTTTGTACAAAAAAGCAGGCTGG (31bp)+template specific sequence in frame

**R primer Gateway tag (if stop codon is needed) *(att*B2)**
5' GGGGACCACTTTGTACAAGAAAGCTGGGTCCTA+template specific sequence in frame (to make C-terminal fusion omit the stop codon, which is the underlined CTA)

**2) PCR amplify, cut out band and purify DNA.**

**3) BP clone:**
1.5 µl dH20
1 µl (Purified PCR product)
1 µl Entry vector (~50 ng)
0.5 µl BP clonase
Incubate on bench 1 hr- overnight.

Transform entire reaction into DH5a cells. We use homemade ones. Plate 5% µl and 95% of transformation on LB+Kan. Screen 3-4 colonies per entry clone. I usually do colony PCR. Sequence with
M21 (-21) [TTGTAAAACGACGGCCAGT]
T7 [TAATACGACTCACTATAGGG]

Use sequenced plasmid for the next step. Dilute to ~30 ng/µl

**4) LR clone.**
1 µl entry clone (the one you generated)
1 µl dest vector
1.5 µl dH20
0.5 µl LR clonase

Incubate on bench 1 hr- overnight.
Transform entire reaction into DH5a cells. Pellet transformation and plate entire reaction on LB+Amp.

I colony PCR screen with these two oligos. Sequencing not necessary. These are universal oligos that I use for screening all B1-B2 entry vectors
JW3 [ACAAGTTTGTACAAAAAAGCAGGCT]
JW4 [ACCACTTTGTACAAGAAAGCTGGGT]