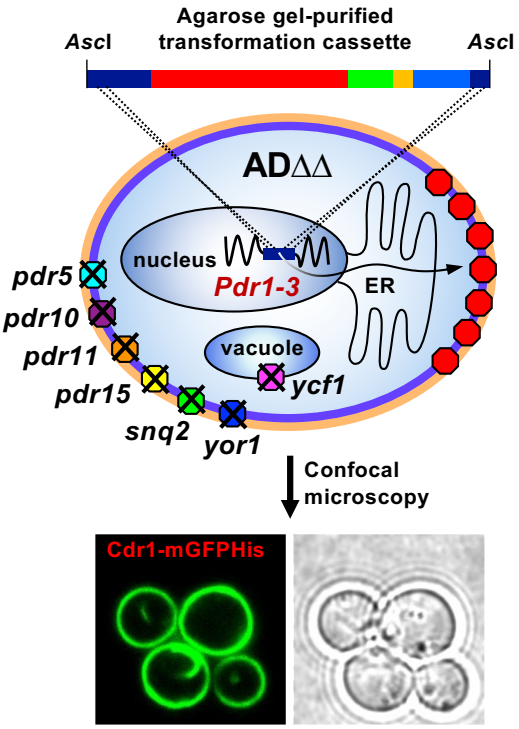
Figure 1

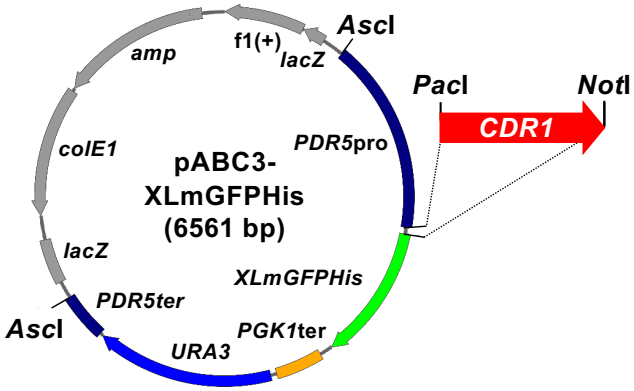
A



B

Traditional cloning strategy

1) Clone *CDR1* into *PacI*/*NotI* of pABC3-XLmGFPHis
2) *AscI* digest pABC3-CDR1-mGFPHis
3) Purify the *CDR1* transformation cassette
4) Transform ADΔΔ with the transformation cassette

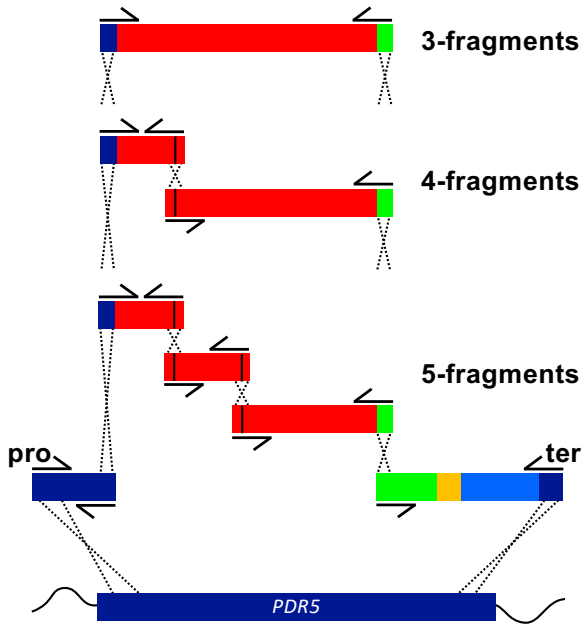


XLmGFPHis double tag (i.e. mGFPHis).	
X:	HRV-3C protease cleavage site (LEVLFQ|GP)
L:	linker (GGSGG)
mGFP:	monomeric yEGFP3 (A206K)
His:	6xHis-tag (GGSHHHHHH)

C

One-step cloning strategy

1) PCR amplify ORF + right + left arm DNA fragments and transform ADΔΔ



D

Whole cell assays of cells overexpressing efflux pump

- Quantification of efflux pump activities with fluorescent substrates (R6G, Nile red).
- Quantification of i) increased resistance to efflux pump substrates [i.e. minimum growth inhibitory concentrations (MICs)] or ii) inhibitor sensitivities of drug efflux (2-dimensional checkboard assays).
- High-throughput screening for novel efflux pump inhibitors.
- Agar disk diffusion assays to determine the substrate specificity or the inhibitor sensitivity of multidrug efflux pumps.