



Fig. 2: cloning steps to generate the carboxy-terminal backbone fully flexible pTcGW 1.1 vector. Arrows indicate the restrictions sites flanking each element of the vectors. A: pTc6HisP-NH 1.1 was the backbone; B: cassette containing the RP, first TcUIR and hexahistidine tag was removed and a new cassette containing only RP and *Trypanosoma cruzi* ubiquitin intergenic region (TcUIR) was inserted; C: phleomycin resistance marker replaced by neomycin; D: green fluorescent protein (GFP) tag added for carboxy-terminal fusions, generating pTcGFPN-CO 1.1, subsequently used as backbone for the construction of all C-terminal 1.1 vectors; att: attachment; attR1 and attR2: attachment sites required for Gateway[®] recombination; ccdB: gene for negative selection; Cm[®]: chloramphenicol selectable marker.