

Figure 1. Creation of Preliminary Master Cell Bank of Albiglutide producing strain. Steps from KEX2-KanMX Expression cassette PCR product to BXP10KEX2PDIERO1 Production Host are the sequential integrations of expression cassettes to construct Process IV host strain, BXP10_KEX2_PDI_EROI. After transformation of pCID3610 plasmid, the final production clone was selected and was used in making a Preliminary Master Cell Bank (PCB).

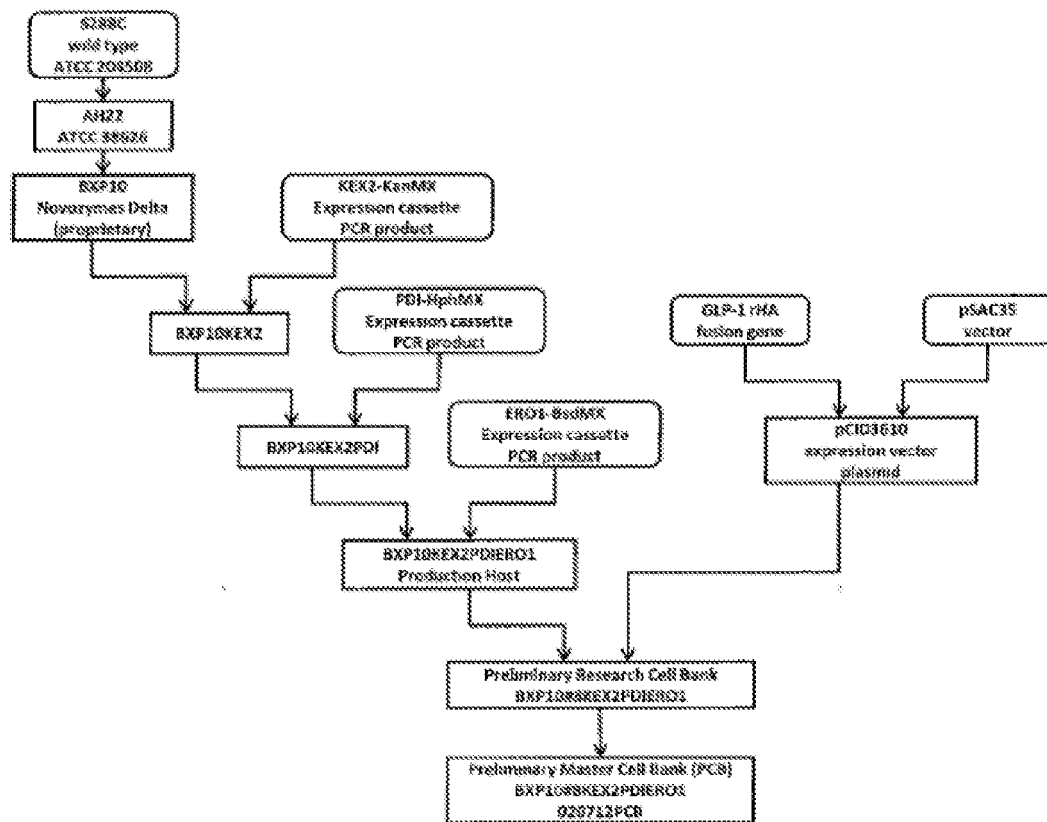


Figure 2: Southern blot analysis of *PDI1* and *KEX2* in the host strains. The endogenous *KEX2* and *PDI1* genes are located in the chromosome XIV and III, respectively, as a single copy (wild type). The target site of integration was shown below the wild type in the chromosome XII. The detecting probes for each gene are shown as solid rectangle.

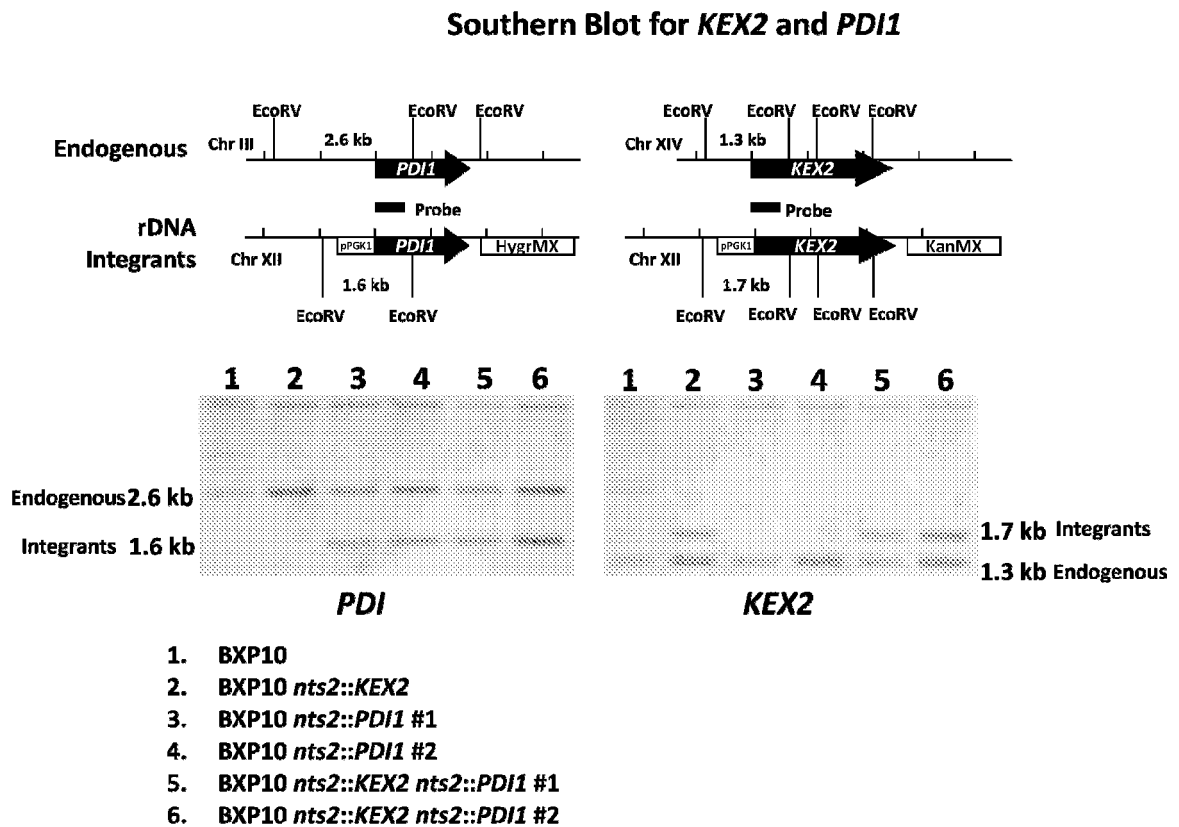


Figure 3: Western blot analysis of *PDII* and *KEX2* from the host strains. Samples in the lane, Lanes 1-5: 5 clones of BXP10-*KEX2*-*PDII* strain; PDI: BXP10 overexpressing *PDII*; *KEX2*: BXP10 overexpressing *KEX2*; and BXP10: host strain as a control. Equivalent amount of proteins were loaded.

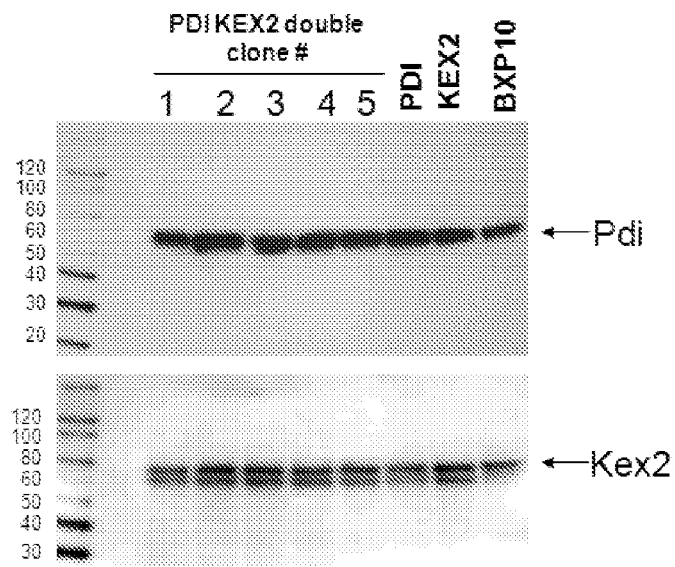


Figure 4. SDS-PAGE of 12 supernatant samples from shaking plate test. Lanes in the gel; L: SeeBlue2 prestained protein ladder (Invitrogen); RS. Reference standard of pCID3610 protein; 1-12: 12 subclones expressing pCID3610

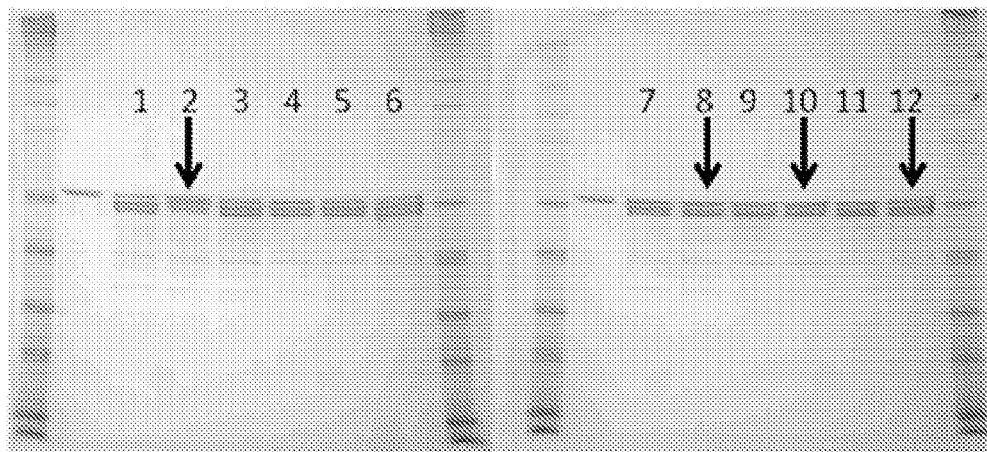


Figure 5: Analysis of titer (A) and quality (B) of pCID3610 protein produced in DasGip fermentation run. The supernatant titer yield and 6-AA levels (%) of pCID3610 protein were compared with BXP10-*KEX2-PDII*, as a control, which is BXP10 overexpressing *KEX2* and *PDII*.

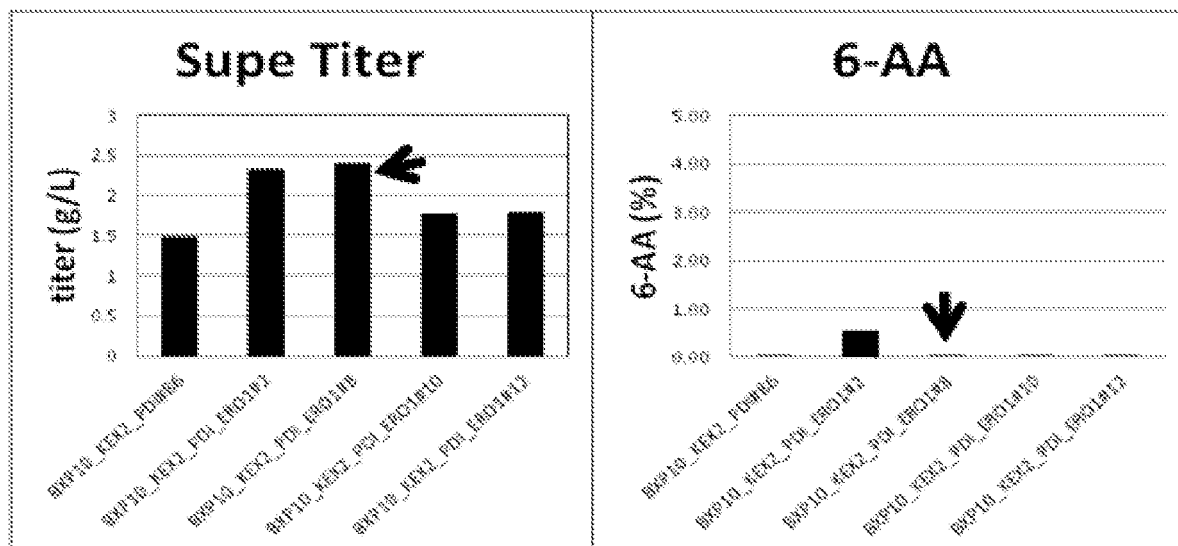


Figure 6: Growth curves generated from Research Cell Bank Vial cells.

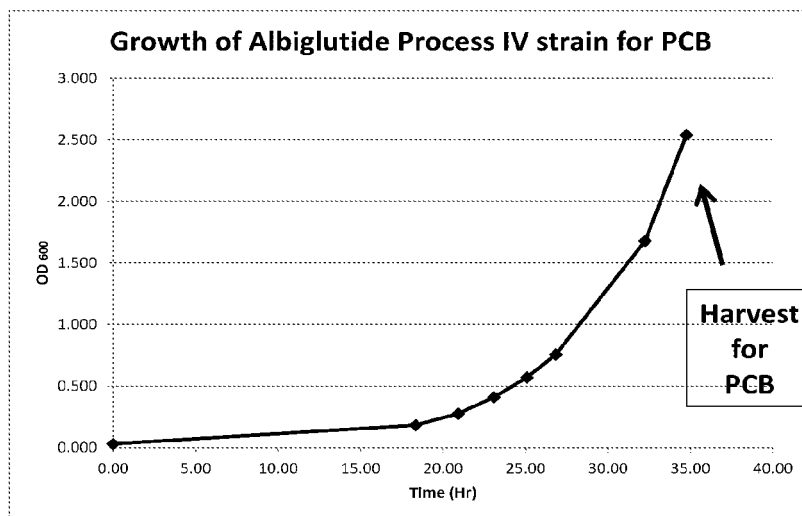


Figure 7: Growth curves generated from Pre-Master Cell Bank cells.

