Supplementary Material (Team 8)

Krushanu Patki (BE23B007), K.Varunkumar (BE23B016)

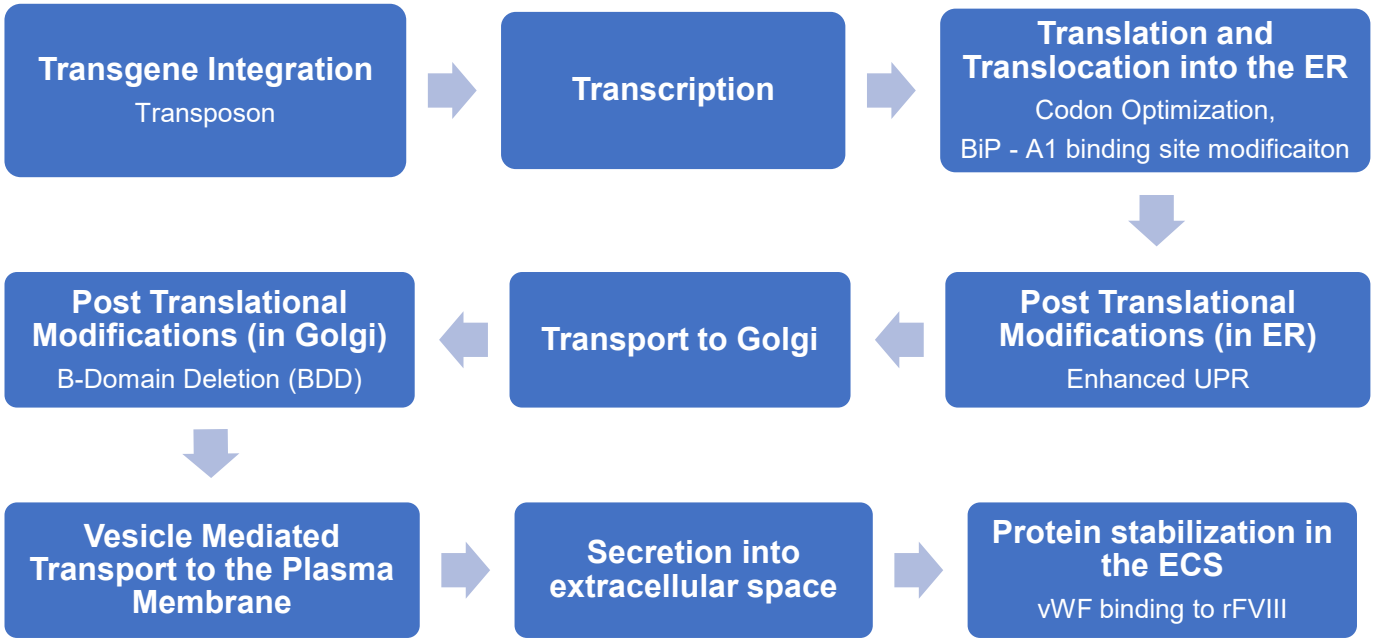

Figure 1: Flowchart of the Secretory Pathway

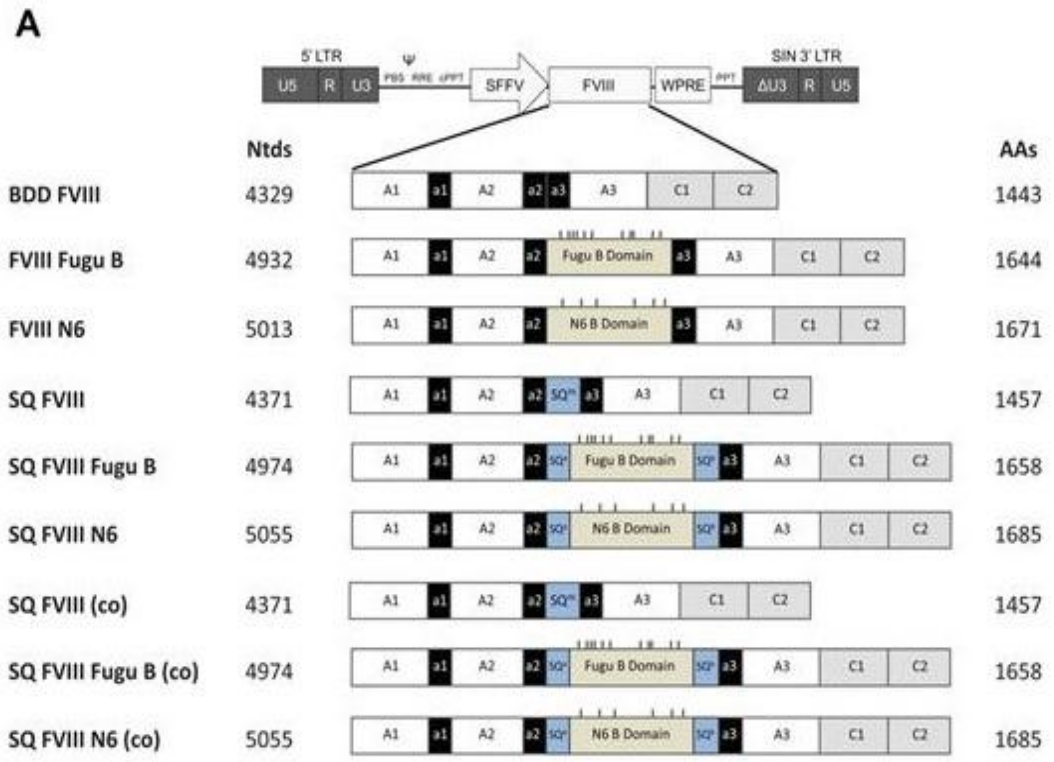

Figure 2: Codon Optimization sequences for BDD-rFVIII transgenes

Various codon optimized BDD-rFVIII sequences were evaluated for their expression

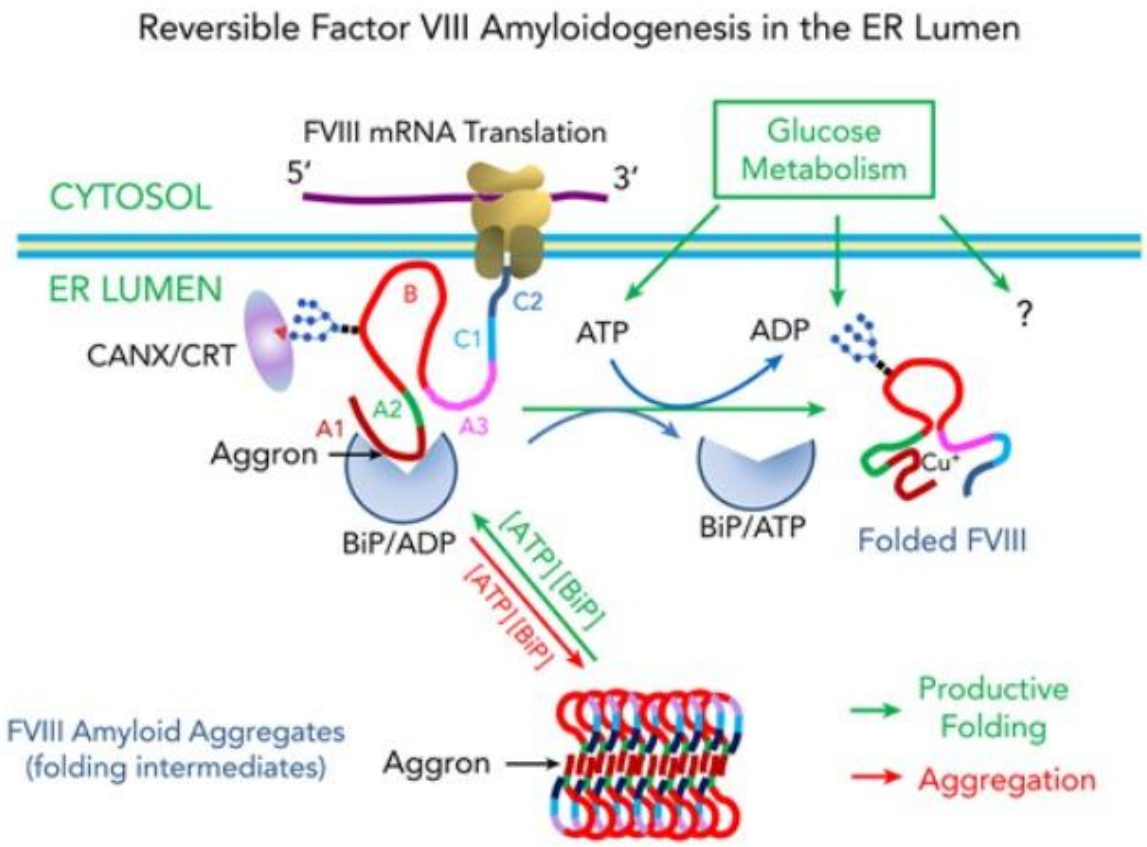

Figure 3: rFVIII binding to BiP through its A1 domain

rFVIII binds to the ER chaperone BiP through a short motif in its A1 domain. BiP binds to nascent, unfolded polypeptides in the ER and aids in proper folding of the protein product.

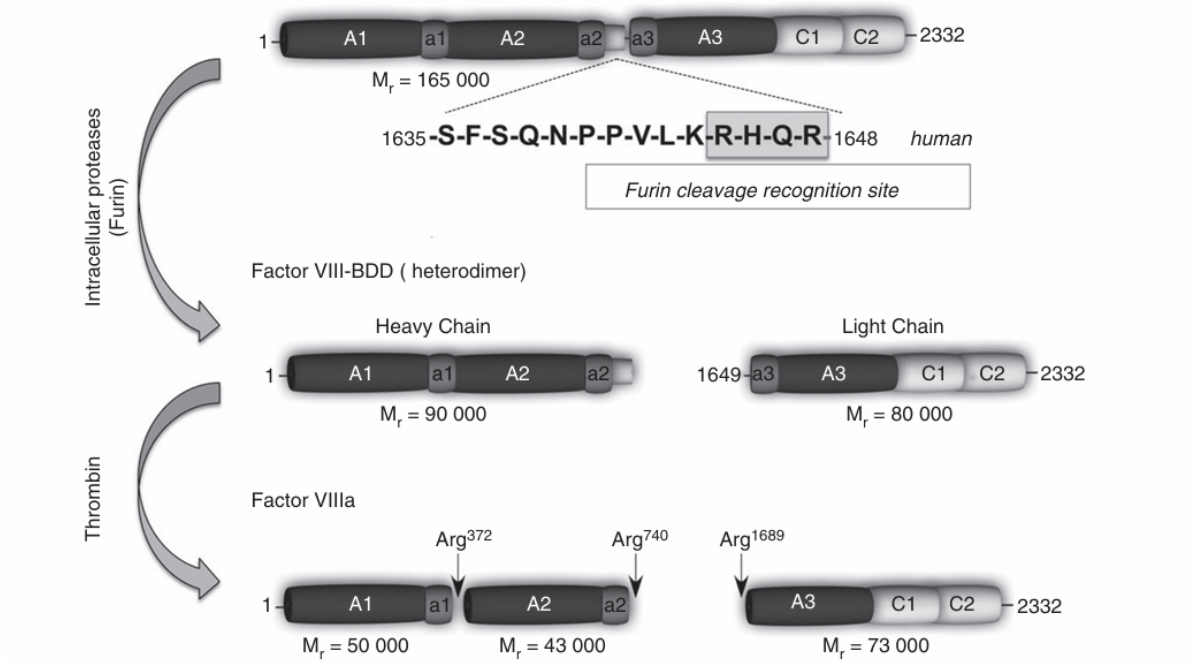

Figure 4: Furin Recognition Motif in FVIII-BDD

The B domain is not required for procoagulant activity. In the B-domain-deleted form of FVIII there are 14 residual amino acids and within that region is the furin recognition motif.

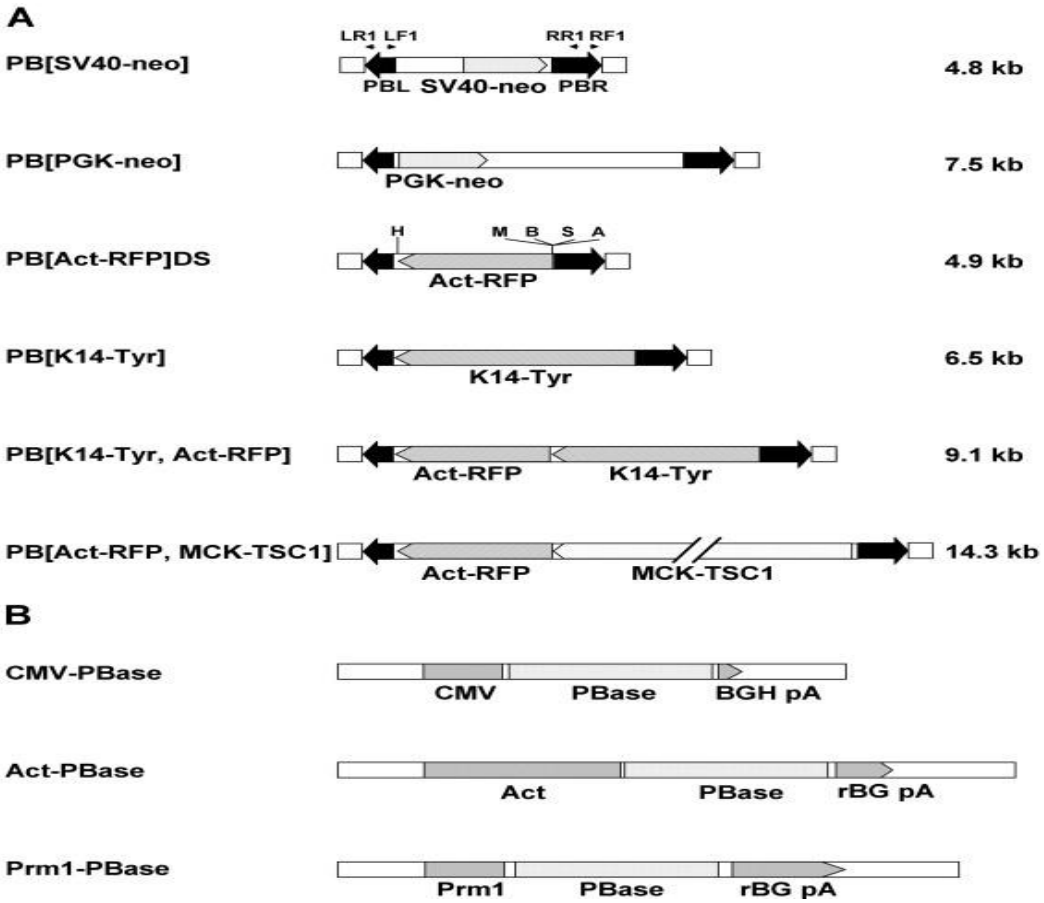

Figure 5 : *PiggyBac* transposon based transgene constructs

Plasmid Construction

[PB 5' ITR] → pPB[Exp] → Cassette A (EF1α → hFVIII-F395S → 6xHis:BGHpA) → Cassette B (CMV → vWF → SV40pA) → Cassette C (PGK → PuroR → BGHpA) → bacterial backbone (ColE1 + KanR/AmpR) → [PB 3' ITR]

1. 5’ and 3’ ITR (Inverse Terminal Repeats) allow the PiggyBac transposase to carry out the transposition reaction.
2. pPB[Exp]: PiggyBac expression backbone
3. Casette A: hVIII gene (with a point mutation at the 395th Amino Acid) and a polyA signal placed under a strong EF1α promoter; 6xHis provides 6 Histidine residues that can be utilized during the separation of the r-protein
4. Casette B: vWF gene and a polyA signal placed under a strong CMV promoter
5. Casette C: Puromycin resistance gene (for selection of successfully transfected mammalian cells) and a poly A signal placed under a strong promoter
6. ColE1: Bacterial Origin of Replication, from the ColE1 plasmid
7. KanR/AmpR: Kanamycin/ Ampicillin resistance gene (for selection among successfully engineered plasmids)

PiggyBac is a Class II (non-retroviral) transposable element that consists of a transposase-encoding gene flanked by Inverse Terminal Repeats (ITRs). The transposase recognizes specific sequences on the ITRs, excises the intervening transposable element, and inserts it at a specific location on another DNA molecule by recognition of TTAA sites at the target location.

This transposition reaction is utilized here to integrate the fVIII-A1*-vWF construct at specific locations in the CHO cell genome to ensure stable integration at constitutively active sites, thereby eliminating issues related to poor efficiency of integration, varying copy number, lack of control over the genomic and epigenetic site of integration, and laborious work required to isolate high-yielding clones.

References:

1) Figure 2 is reproduced from *Ward NJ, Buckley SM, Waddington SN, Vandendriessche T, Chuah MK, Nathwani AC, McIntosh J, Tuddenham EG, Kinnon C, Thrasher AJ, McVey JH. Codon optimization of human factor VIII cDNAs leads to high-level expression. Blood. 2011 Jan 20;117(3):798-807. doi: 10.1182/blood-2010-05-282707. Epub 2010 Nov 1. PMID: 21041718.*

2) Figure 3 is reproduced from *Poothong J, Pottekat A, Siirin M, Campos AR, Paton AW, Paton JC, Lagunas-Acosta J, Chen Z, Swift M, Volkmann N, Hanein D, Yong J, Kaufman RJ. Factor VIII exhibits chaperone-dependent and glucose-regulated reversible amyloid formation in the endoplasmic reticulum. Blood. 2020 May 21;135(21):1899-1911. doi: 10.1182/blood.2019002867. PMID: 32128578; PMCID: PMC7243144.*

3) Figure 4 is reproduced from *Nguyen GN, George LA, Siner JI, Davidson RJ, Zander CB, Zheng XL, Arruda VR, Camire RM, Sabatino DE. Novel factor VIII variants with a modified furin cleavage site improve the efficacy of gene therapy for hemophilia A. J Thromb Haemost. 2017 Jan;15(1):110-121. doi: 10.1111/jth.13543. Epub 2016 Nov 25. PMID: 27749002; PMCID: PMC5280213.*

4) Figure 5 is reproduced from *Matasci M, Baldi L, Hacker DL, Wurm FM. The PiggyBac transposon enhances the frequency of CHO stable cell line generation and yields recombinant lines with superior productivity and stability. Biotechnol Bioeng. 2011 Sep;108(9):2141-50. doi: 10.1002/bit.23167. Epub 2011 Apr 25. PMID: 21495018.*