Creation and Expansion of Recombinant Adenovirus by Direct Ligation with Vector Core Turnaround Time

Clone Transgene in pSHUTTLE (It has CMV Promoter, RBG PolyA)

Restrict pSHUTTLE Transgene with I-Ceu1 & PI-Sce1

Set Ligation with I-Ceu1 & PI-Sce1 digested pAdeno X pkGFP

Transform bacteria

Screen white vs green colonies under UV light

Pick white colonies

Make minipreps and isolate DNAs

Set appropriate restrictions to check for presence of transgene and intactness of the adenovirus backbone so as to select positive recombinants

Inoculate MidiPrep with the selected bacterial culture

Isolate DNA and recheck with restrictions

Digest with PAC1

Transfect 293 cells

Harvest when shows more than 60% CPE

Freeze thaw and infect 5 plates

Expand to 50 plates and purify the virus

Check infectious titer & presence of any Replication Competent Adenovirus

3-6 weeks

4-6 weeks