Figure 1

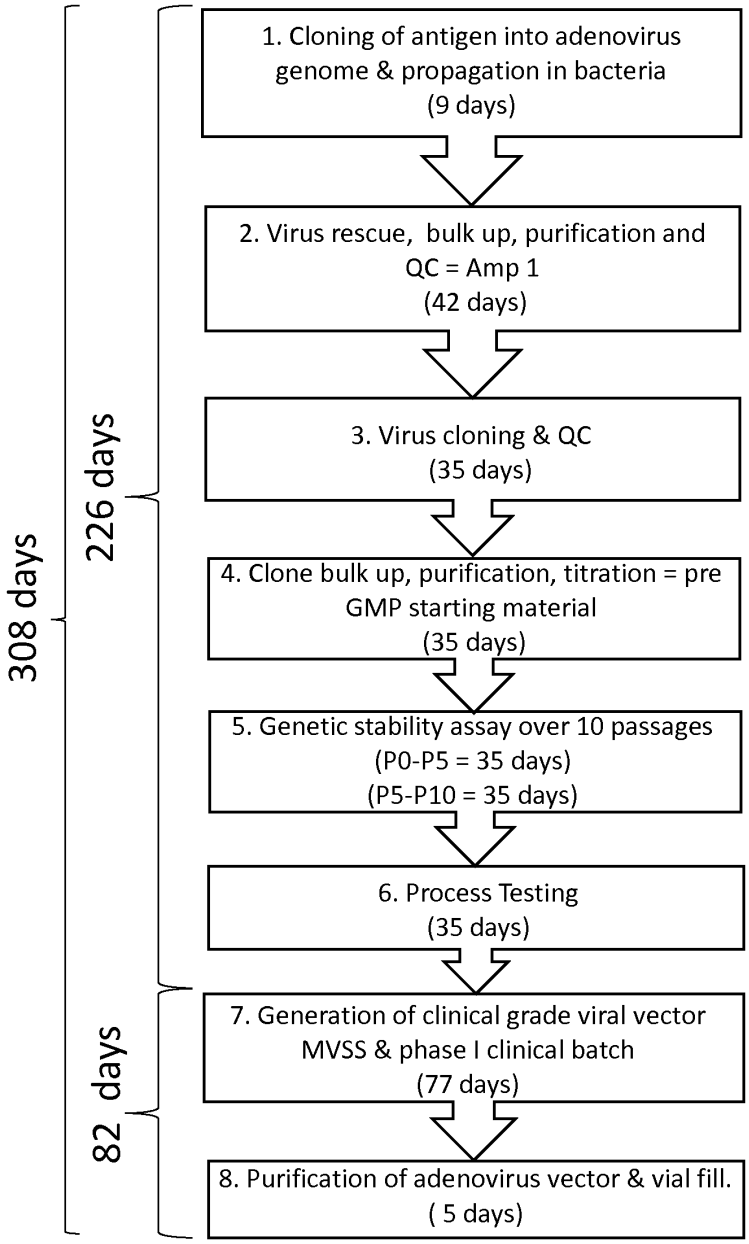
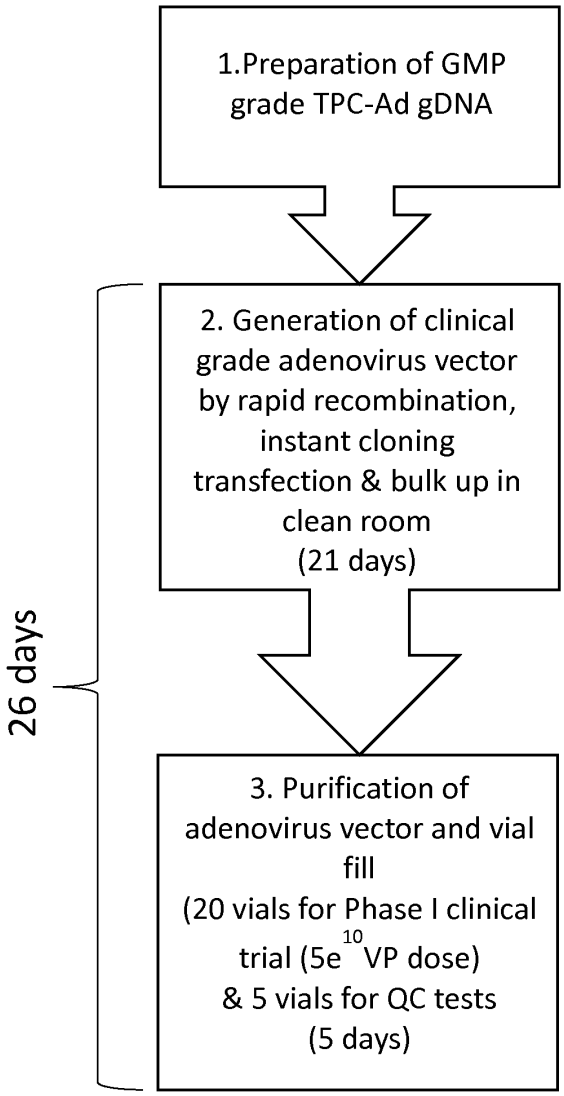

1. Cloning of antigen into adenovirus genome & propagation in bacteria (9 days)
2. Virus rescue, bulk up, purification and QC = Amp 1 (42 days)
3. Virus cloning & QC (35 days)
4. Clone bulk up, purification, titration = pre GMP starting material (35 days)
5. Genetic stability assay over 10 passages (P0-P5 = 35 days) (P5-P10 = 35 days)
6. Process Testing (35 days)
7. Generation of clinical grade viral vector MVSS & phase I clinical batch (77 days)
8. Purification of adenovirus vector & vial fill. (5 days)
226 days
82 days
308 days

Figure 2


1.Preparation of GMP grade TPC-Ad gDNA
2. Generation of clinical grade adenovirus vector by rapid recombination, instant cloning transfection & bulk up in clean room (21 days)
3. Purification of adenovirus vector and vial fill (20 vials for Phase I clinical trial (5e10 VP dose) & 5 vials for QC tests (5 days)
26 days

Methods and Compositions for Producing a Virus

Figure 3

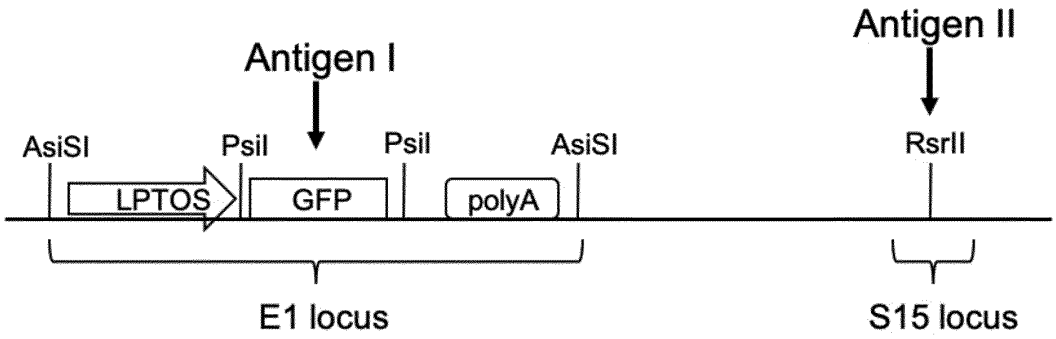


Figure 4

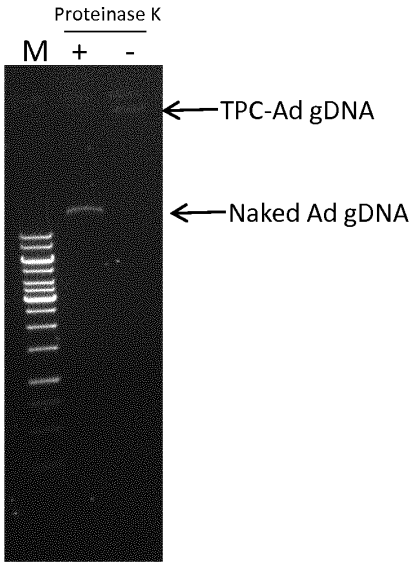
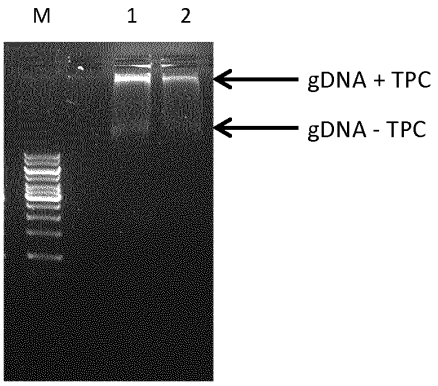
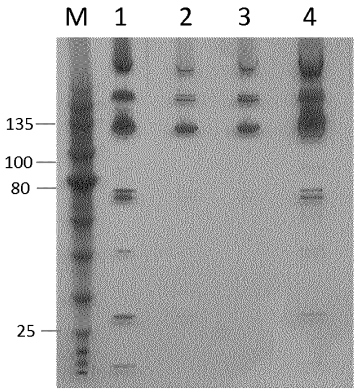


Figure 5



1. PD10 desalted/ unfiltered
2. PD10 desalted/ 0.2uM filtered

Figure 6



M NEB broad colour range
1 ChAdOx1-GFP
2 ChAdOx1-GFP *+ GuHCl 50ng*
3 50ng ChAdOx1-GFP TPC-gDNA concentrate
4 500ng ChAdOx1-GFP TPC-gDNA concentrate

Methods and Compositions for Producing a Virus

Figure 7A

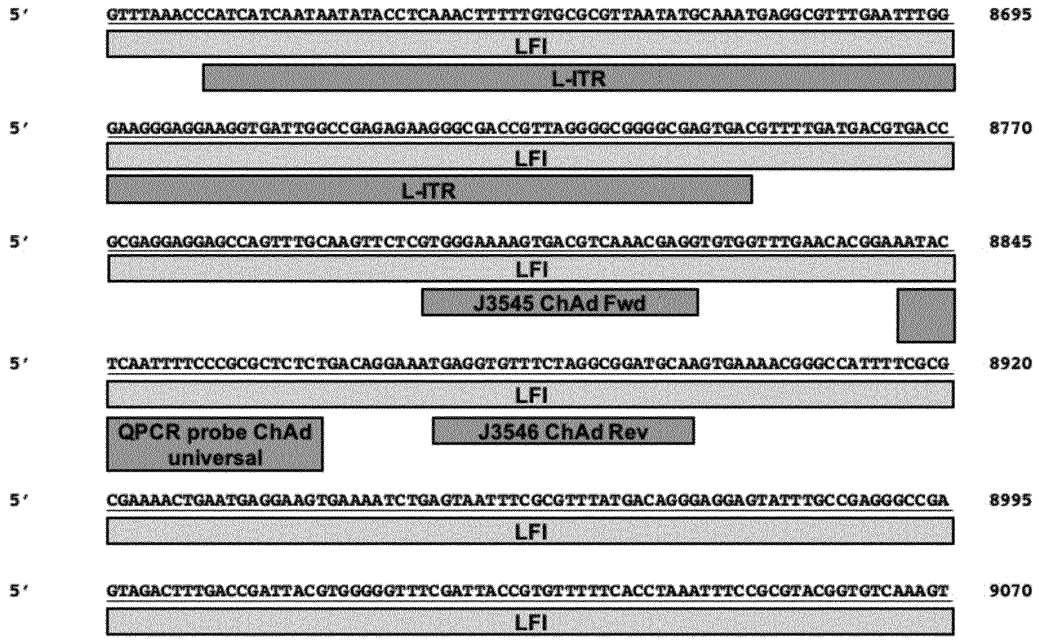


Figure 7B

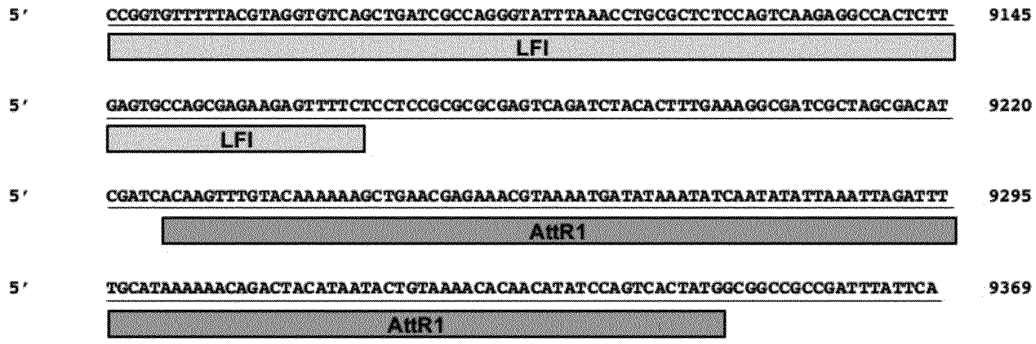

5′
CCGGTGTTTTTACGTAGGTGTCAGCTGATCGCCAGGGTATTTAAACCTGCGCTCTCCAGTCAAGAGGCCACTCTT
9145
LFI
5′
GAGTGCCAGCGAGAAGAGTTTTCTCCTCCGCGCGCGAGTCAGATCTACACTTTGAAAGGCGATCGCTAGCGACAT
9220
LFI
5′
CGATCACAAGTTTGTACAAAAAAGCTGAACGAGAAACGTAAAATGATATAAATATCAATATATTAAATTAGATTT
9295
AttR1
5′
TGCATAAAAAACAGACTACATAATACTGTAAAACACAACATATCCAGTCACTATGGCGGCCGCCGATTTATTCA
9369
AttR1

Figure 8

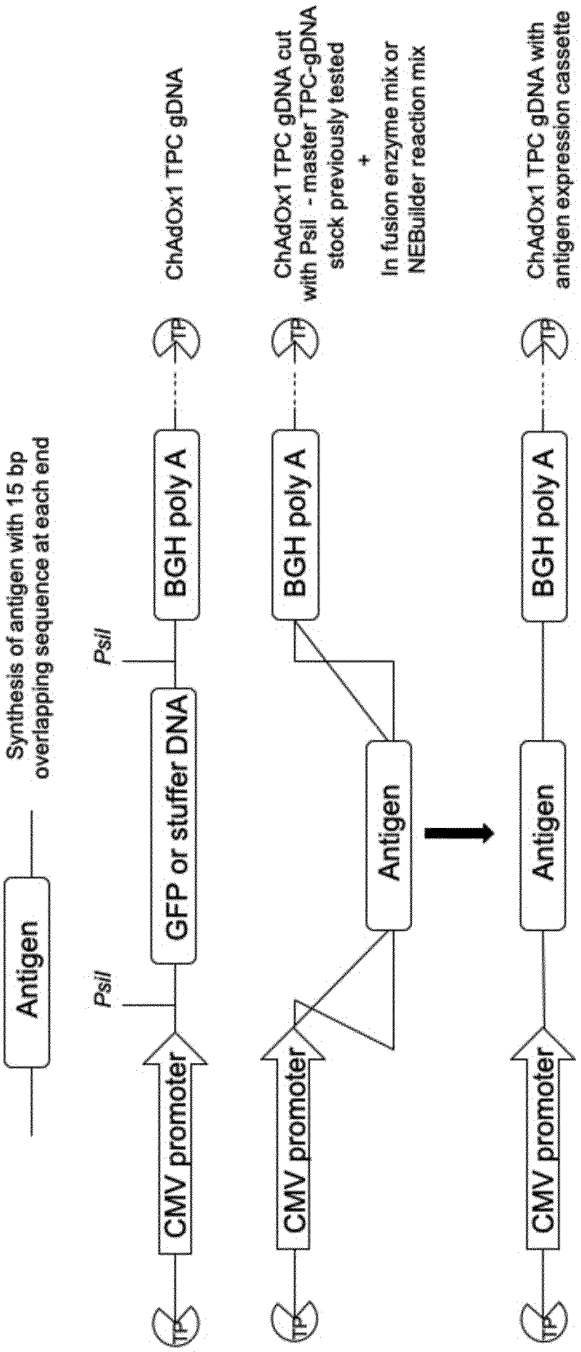

Synthesis of antigen with 15 bp overlapping sequence at each end
Antigen
PsiI
CMV promoter
GFP or stuffer DNA
PsiI
BGH poly A
ITR
ChAdOx1 TPC gDNA
ITR
CMV promoter
BGH poly A
ChAdOx1 TPC gDNA cut with PsiI - master TPC-gDNA stock previously tested
+
In fusion enzyme mix or NEBuilder reaction mix
Antigen
ITR
CMV promoter
BGH poly A
ChAdOx1 TPC gDNA with antigen expression cassette
Antigen
ITR

Figure 9

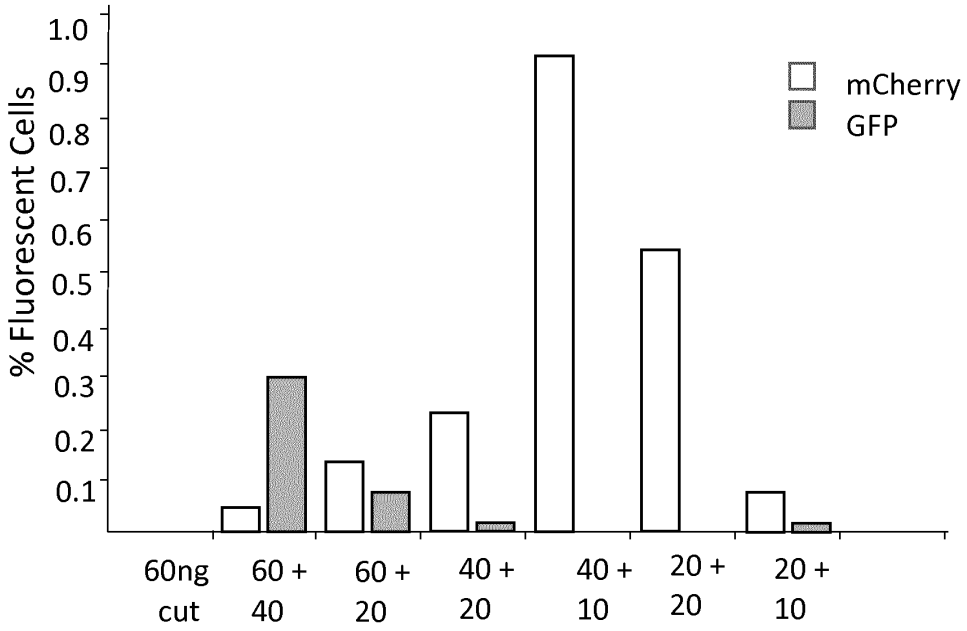
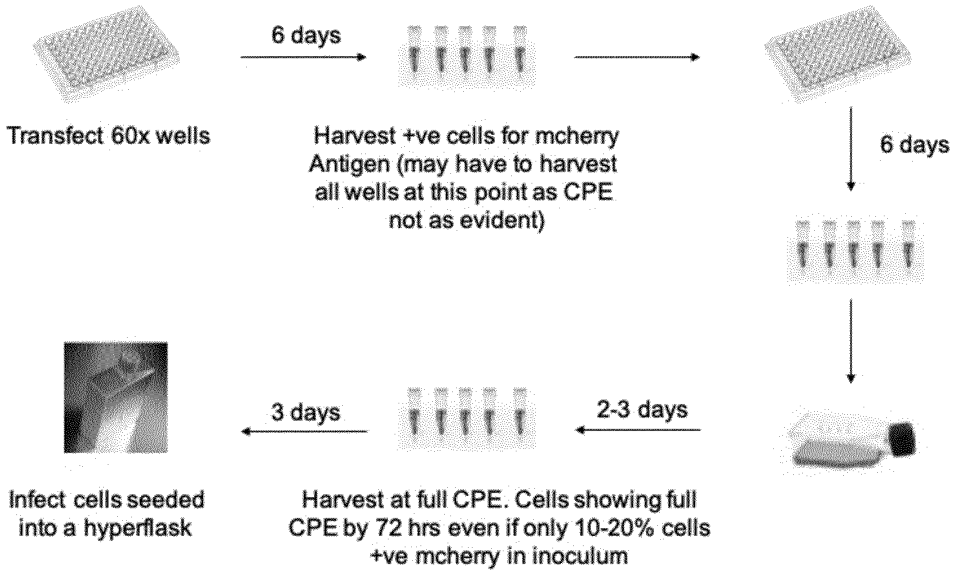

% Fluorescent Cells
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
mCherry
GFP
60ng cut
60 + 40
60 + 20
40 + 20
40 + 10
20 + 20
20 + 10

Figure 10


6 days
Transfect 60x wells
Harvest +ve cells for mcherry Antigen (may have to harvest all wells at this point as CPE not as evident)
6 days
3 days
2-3 days
Infect cells seeded into a hyperflask
Harvest at full CPE. Cells showing full CPE by 72 hrs even if only 10-20% cells +ve mcherry in inoculum