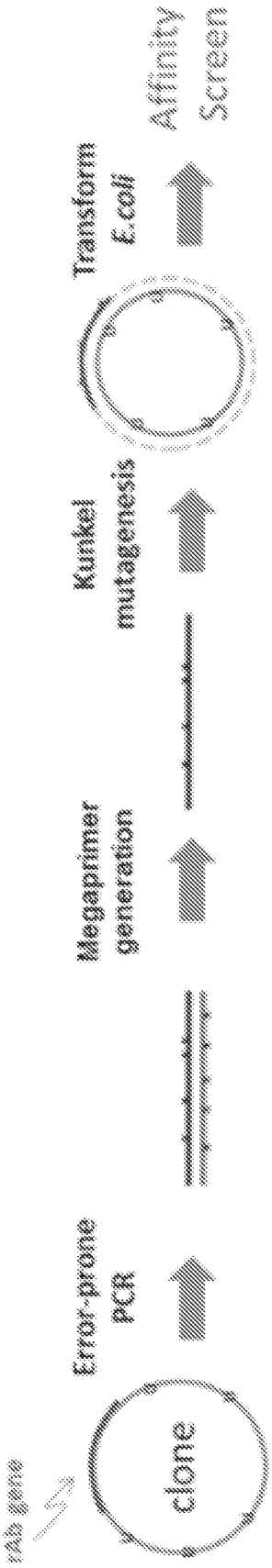
Fig. 1


rAb gene
clone
Error-prone PCR
Megaprimer generation
Kunkel mutagenesis
Transform E.coli
Affinity Screen

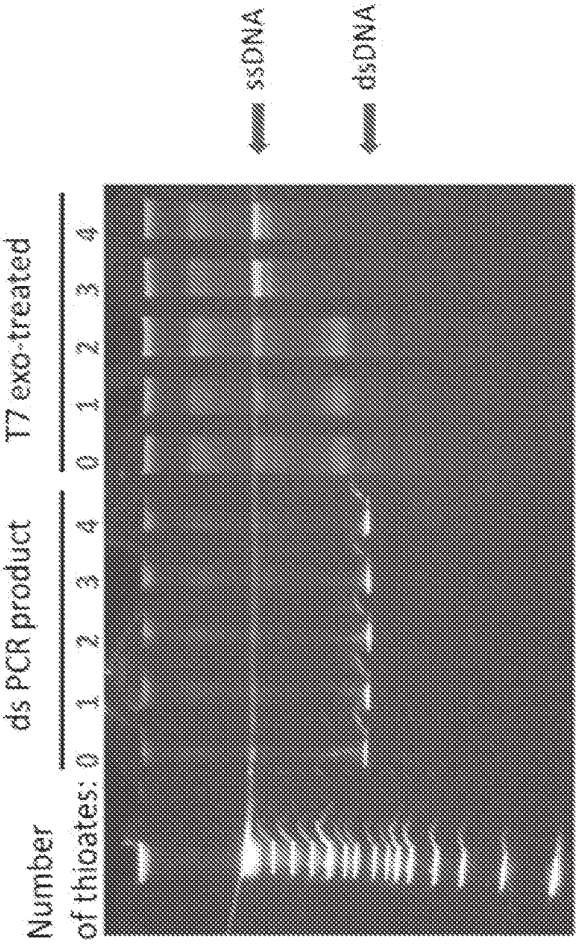

ssDNA
dsDNA
ds PCR product
T7 exo-treated
Number of thioates: 0 1 2 3 4 0 1 2 3 4

Fig. 2

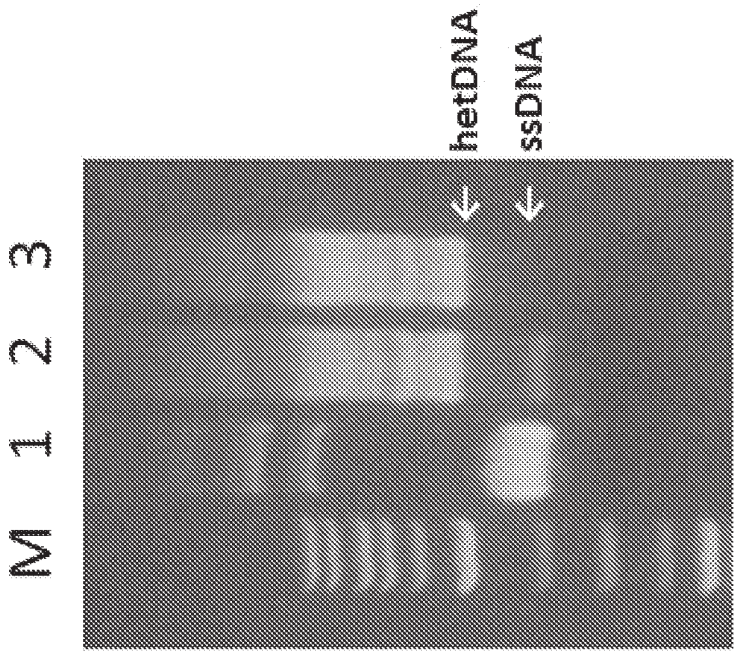


Fig. 3

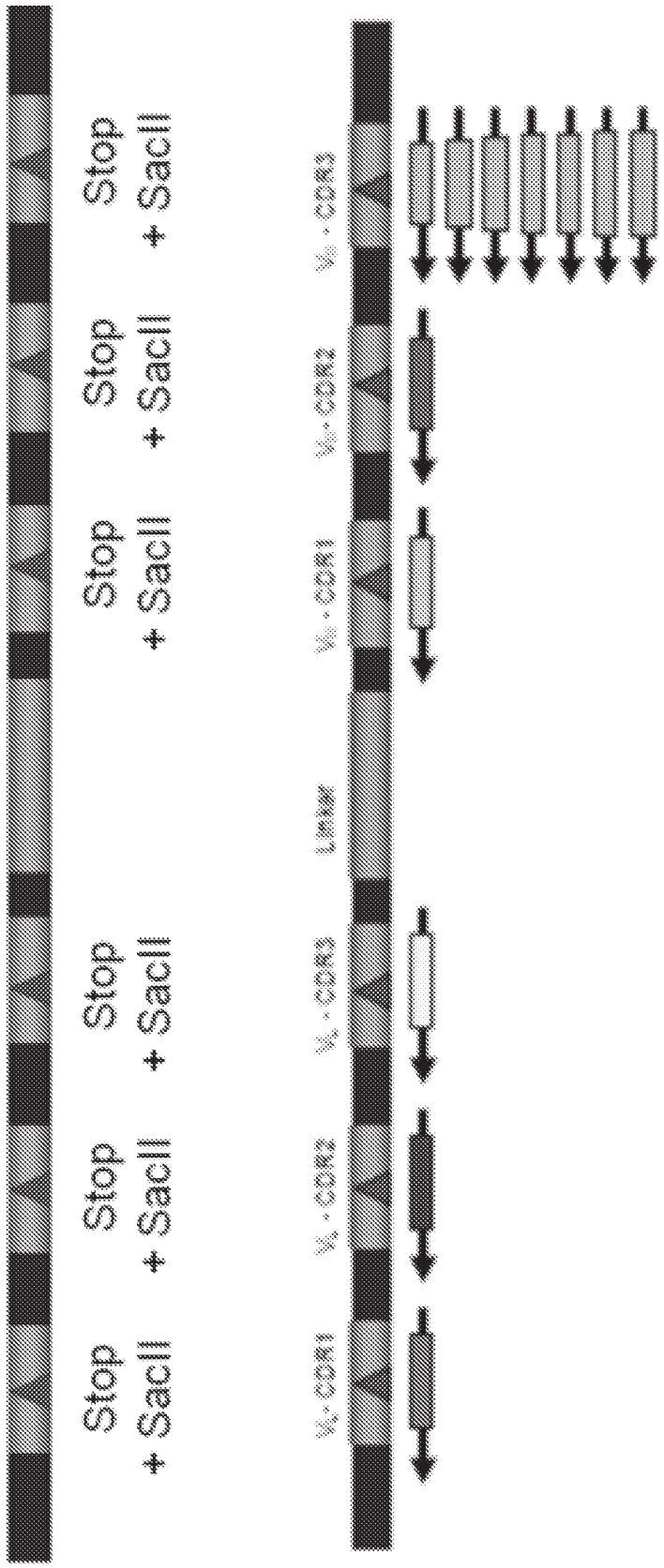
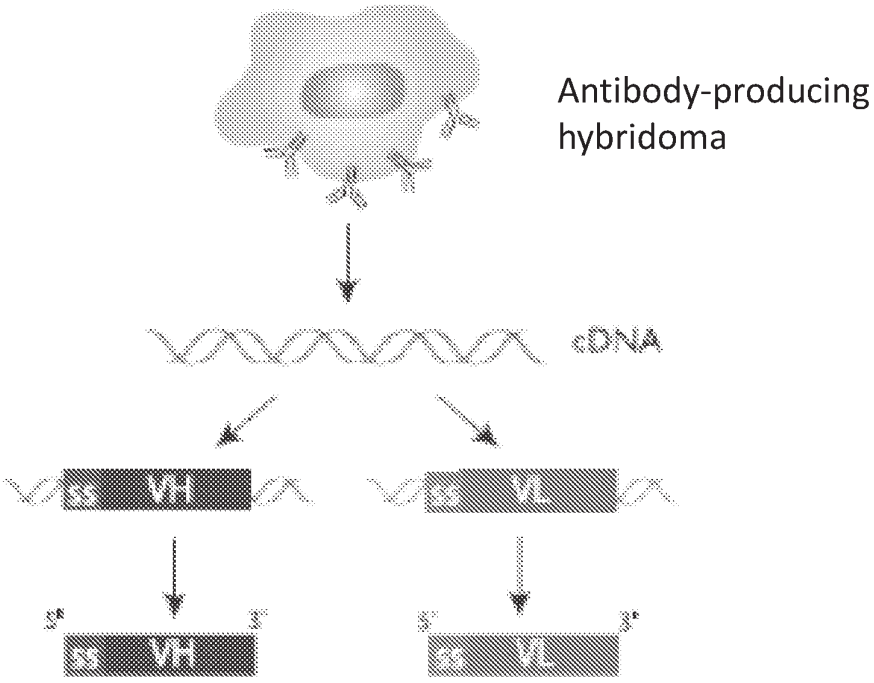
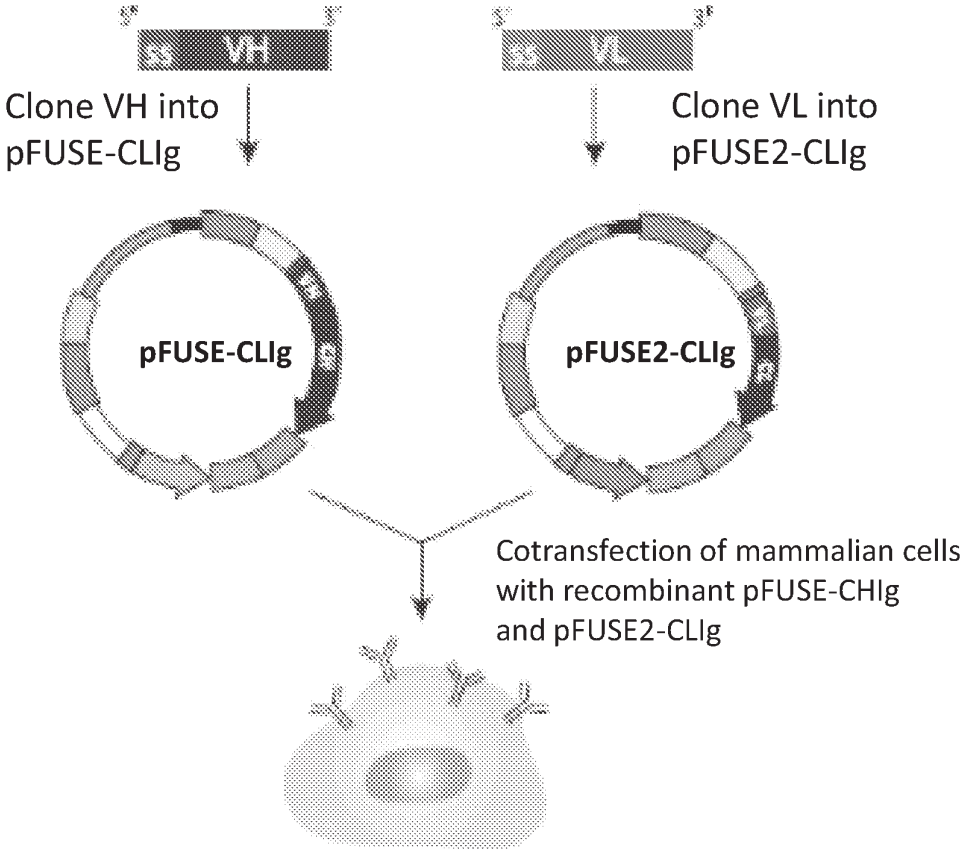

V_L - CDR1
V_L - CDR2
V_L - CDR3
Linker
V_H - CDR1
V_H - CDR2
V_H - CDR3
Stop + SacII
Stop + SacII
Stop + SacII
Stop + SacII
Stop + SacII
Stop + SacII

Fig. 4

1-Obtention of VH and VL sequences



2-Cloning into pFUSE-CHIg and pFUSE2-CLIg



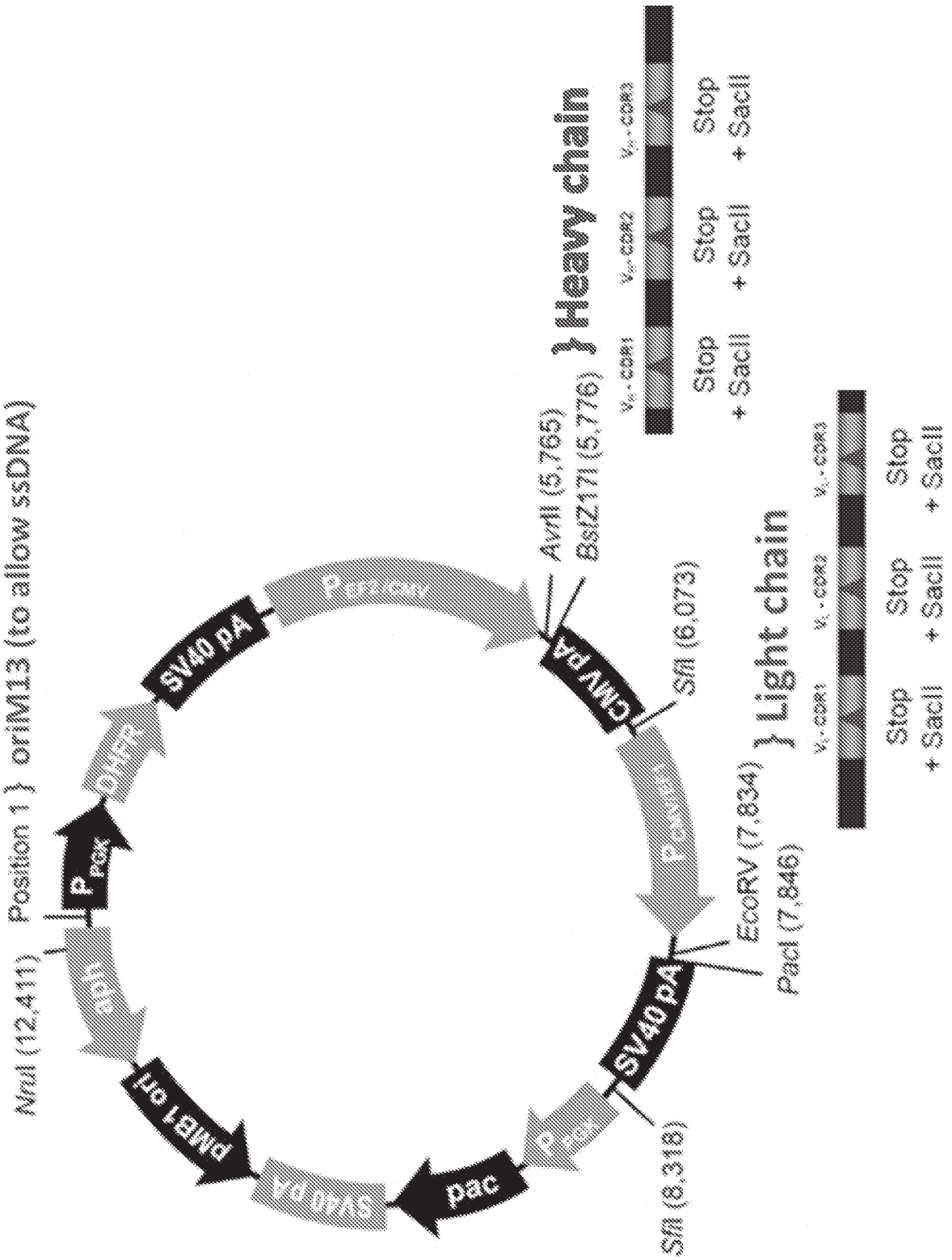

Position 1
oriM13 (to allow ssDNA)
NruI (12,411)
AvrII (5,765)
BstZ17I (5,776)
Heavy chain
Stop + SacII
Stop + SacII
Stop + SacII
SalI (6,073)
EcoRV (7,834)
PacI (7,846)
Light chain
Stop + SacII
Stop + SacII
Stop + SacII
SalI (8,318)
SV40 pA
pac
pMB1 ori
ApoI
DNER
SV40 pA
CMV PA
SV40 pA

Fig. 6

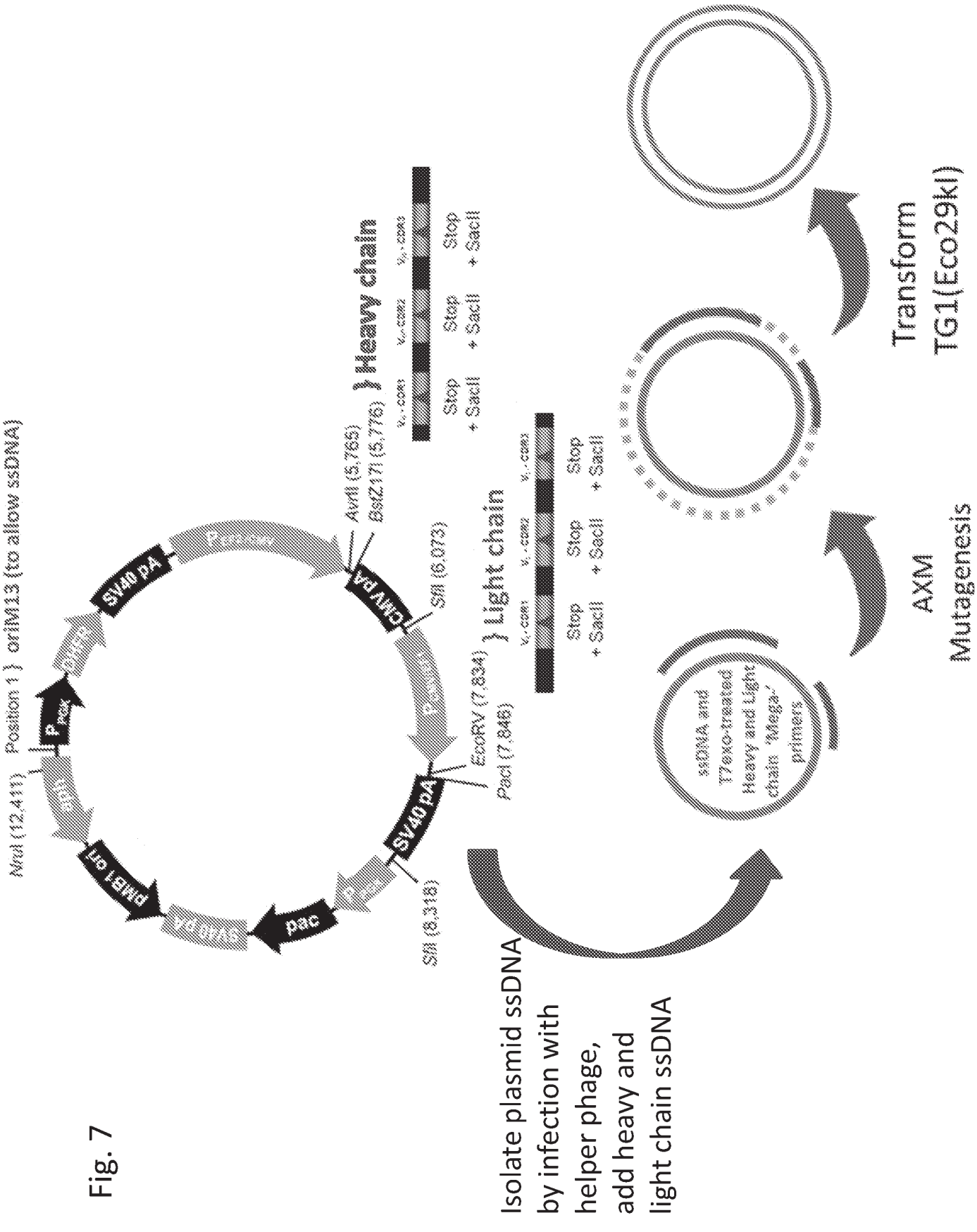
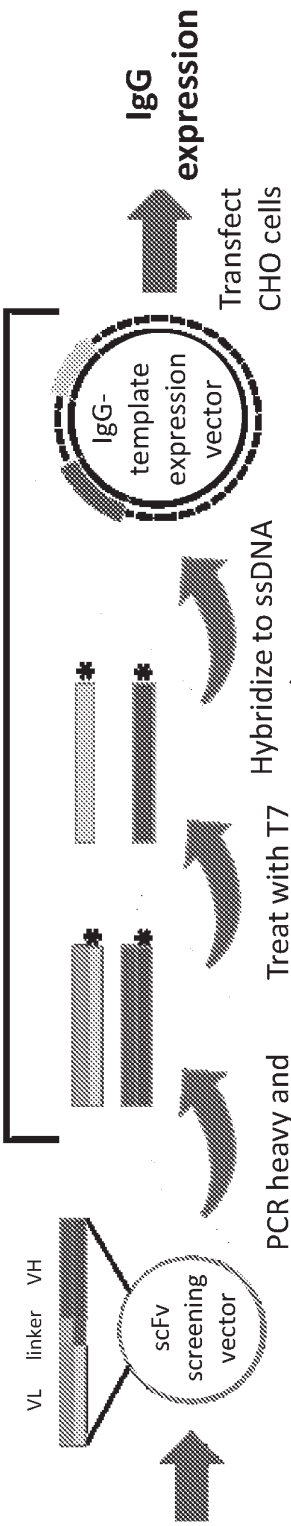

NruI (12,411)
Position 1
oriM13 (to allow ssDNA)
SV40 pA
pIII
pVIII
pIB1 Ori
pac
SfiI (8,318)
SV40 pA
PacI (7,846)
EcoRV (7,834)
SfiI (6,073)
CMV PA
AvrII (5,765)
Bst2171 (5,776)
Heavy chain
Light chain
Stop
+ SacII
+ SacI
Isolate plasmid ssDNA by infection with helper phage, add heavy and light chain ssDNA
ssDNA and T7exo-treated Heavy and Light chain 'Mega' primers
AXM Mutagenesis
Transform TG1(Eco29kI)

Fig. 7

Fig. 8


Recombinant affinity reagent
VL linker VH
scFv screening vector
PCR heavy and light chains with a 5' thiol-protected primer
Treat with T7 exonuclease to remove unprotected strand
Hybridize to ssDNA template vector, polymerase fill-in and ligation
AXM Mutagenesis
IgG-template expression vector
Transfect CHO cells
IgG expression