Fig. 1

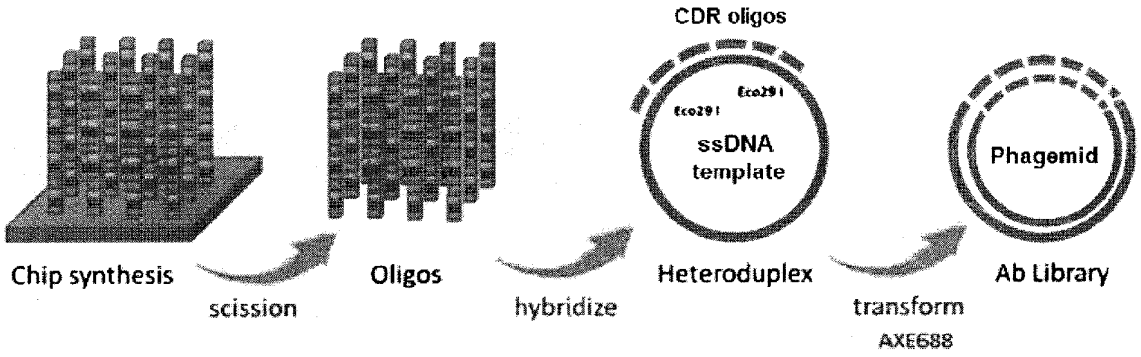

CDR oligos
Eco291
Eco291
ssDNA
template
Phagemid
Chip synthesis
scission
Oligos
hybridize
Heteroduplex
transform
AXE688
Ab Library

Fig. 2

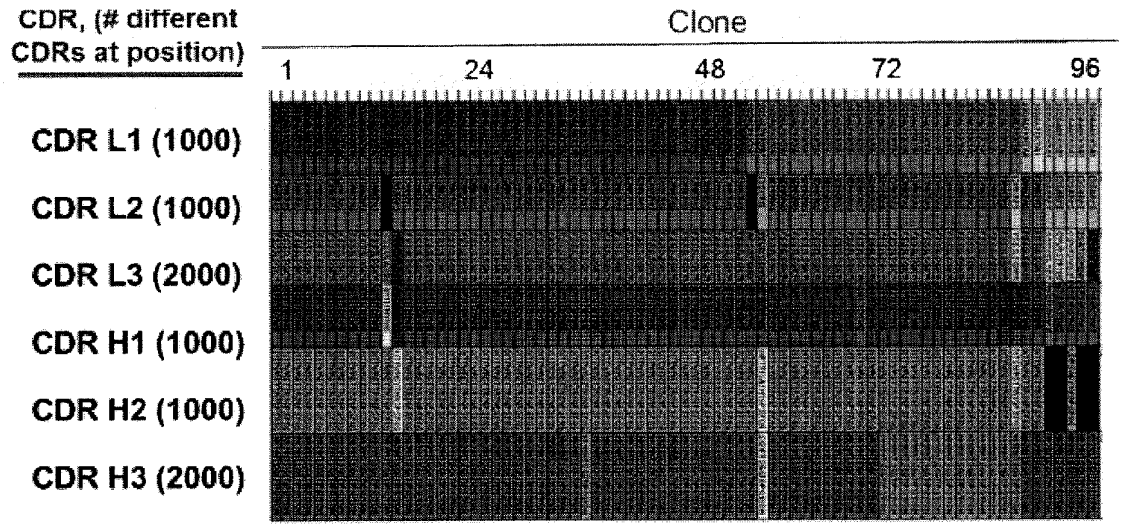

CDR, (# different CDRs at position)
Clone
1
24
48
72
96
CDR L1 (1000)
CDR L2 (1000)
CDR L3 (2000)
CDR H1 (1000)
CDR H2 (1000)
CDR H3 (2000)

Fig. 3

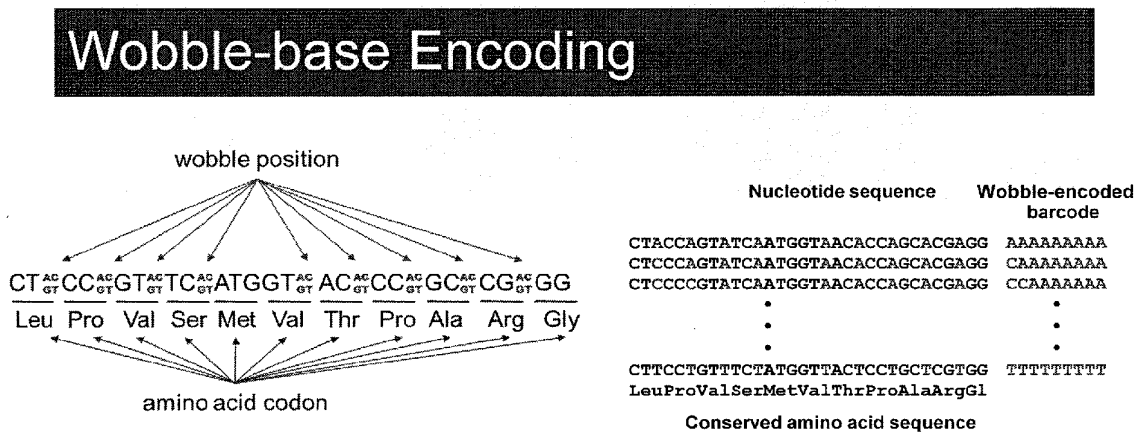
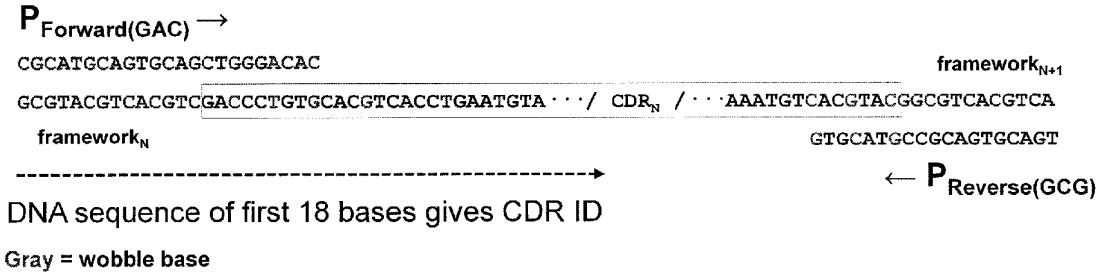

Wobble-base Encoding
wobble position
CT(A/C/G/T)CC(A/C/G/T)GT(A/C/G/T)TC(A/C/G/T)ATGGT(A/C/G/T)AC(A/C/G/T)CC(A/C/G/T)GC(A/C/G/T)CG(A/C/G/T)GG
Leu Pro Val Ser Met Val Thr Pro Ala Arg Gly
amino acid codon
Nucleotide sequence
Wobble-encoded barcode
CTACCAGTATCAATGGTAACACCAGCACGAGG AAAAAAAAA
CTCCCAGTATCAATGGTAACACCAGCACGAGG CAAAAAAAA
CTCCCCGTATCAATGGTAACACCAGCACGAGG CCAAAAAAA
CTTCCTGTTTCTATGGTTACTCCTGCTCGTGG TTTTTTTTT
LeuProValSerMetValThrProAlaArgGl
Conserved amino acid sequence

Fig. 4

Use of amino acid wobble position for encoding and retrieving CDRs



DNA sequence of first 18 bases gives CDR ID

Gray = wobble base

Fig. 5

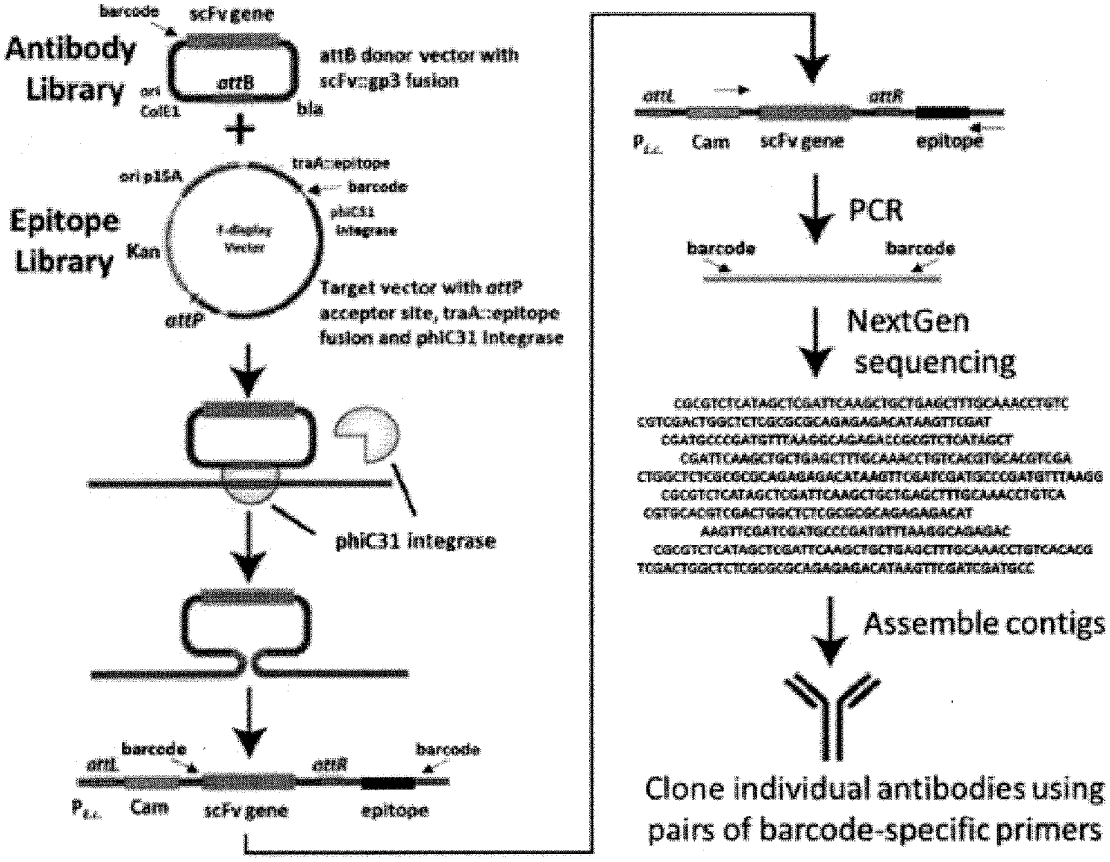

barcode
scFv gene
Antibody Library
attB donor vector with scFv::gp3 fusion
attB
ori ColE1
bla
ori p15A
traA::epitope
barcode
phiC31 integrase
Epitope Library
Kan
f1 display Vector
attP
Target vector with attP accepter site, traA::epitope fusion and phiC31 integrase
phiC31 integrase
attL
attR
barcode
barcode
P
Cam
scFv gene
epitope
PCR
barcode
barcode
NextGen sequencing
CGCGTCTCATAGCTCGATTCAAGCTGCTGAGCTTTGCAAACCTGTC
CGTCGACTGGCTCTCGCGCGCAGAGAGACATAAGTTCGAT
CGATGCCCGATGTTTAAGGCAGAGACCGCGTCTCATAGCT
CGATTCAAGCTGCTGAGCTTTGCAAACCTGTCACGTGCACGTCGA
CTGGCTCTCGCGCGCAGAGAGACATAAGTTCGATCGATGCCCGATGTTTAAGG
CGCGTCTCATAGCTCGATTCAAGCTGCTGAGCTTTGCAAACCTGTCA
CGTGCACGTCGACTGGCTCTCGCGCGCAGAGAGACAT
AAGTTCGATCGATGCCCGATGTTTAAGGCAGAGAC
CGCGTCTCATAGCTCGATTCAAGCTGCTGAGCTTTGCAAACCTGTCACACG
TCGACTGGCTCTCGCGCGCAGAGAGACATAAGTTCGATCGATGCC
Assemble contigs
Clone individual antibodies using pairs of barcode-specific primers

Fig. 6

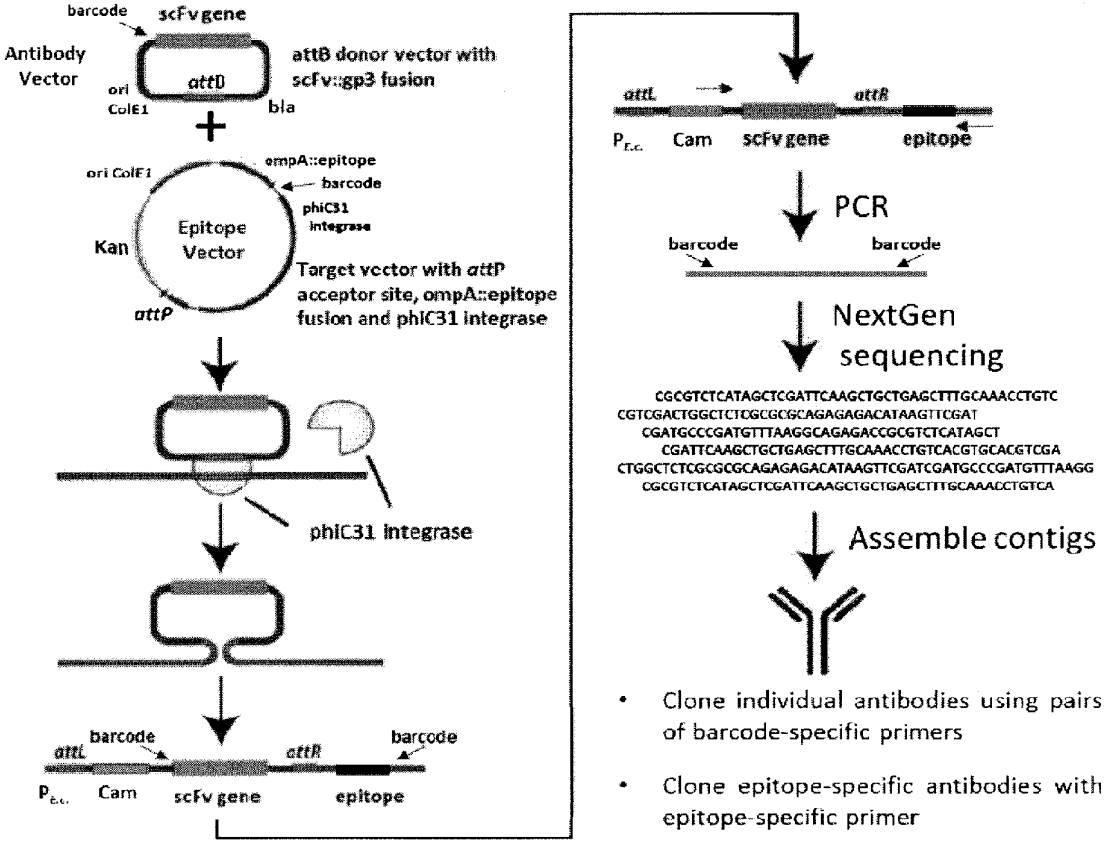

barcode
scFv gene
Antibody Vector
attB donor vector with scFv::gp3 fusion
ori ColE1
attB
bla
ori ColE1
ompA::epitope
barcode
phiC31 integrase
Kan
Epitope Vector
attP
Target vector with attP acceptor site, ompA::epitope fusion and phiC31 integrase
phiC31 integrase
attL
barcode
attR
barcode
Cam
scFv gene
epitope
PCR
NextGen sequencing
CGCGTCTCATAGCTCGATTCAAGCTGCTGAGCTTTGCAAACCTGTC
CGTCGACTGGCTCTCGCGCGCAGAGAGACATAAGTTCGAT
CGATGCCCGATGTTTAAGGCAGAGACCGCGTCTCATAGCT
CGATTCAAGCTGCTGAGCTTTGCAAACCTGTCACGTGCACGTCGA
CTGGCTCTCGCGCGCAGAGAGACATAAGTTCGATCGATGCCCGATGTTTAAGG
CGCGTCTCATAGCTCGATTCAAGCTGCTGAGCTTTGCAAACCTGTCA
Assemble contigs
• Clone individual antibodies using pairs of barcode-specific primers
• Clone epitope-specific antibodies with epitope-specific primer

Fig. 7

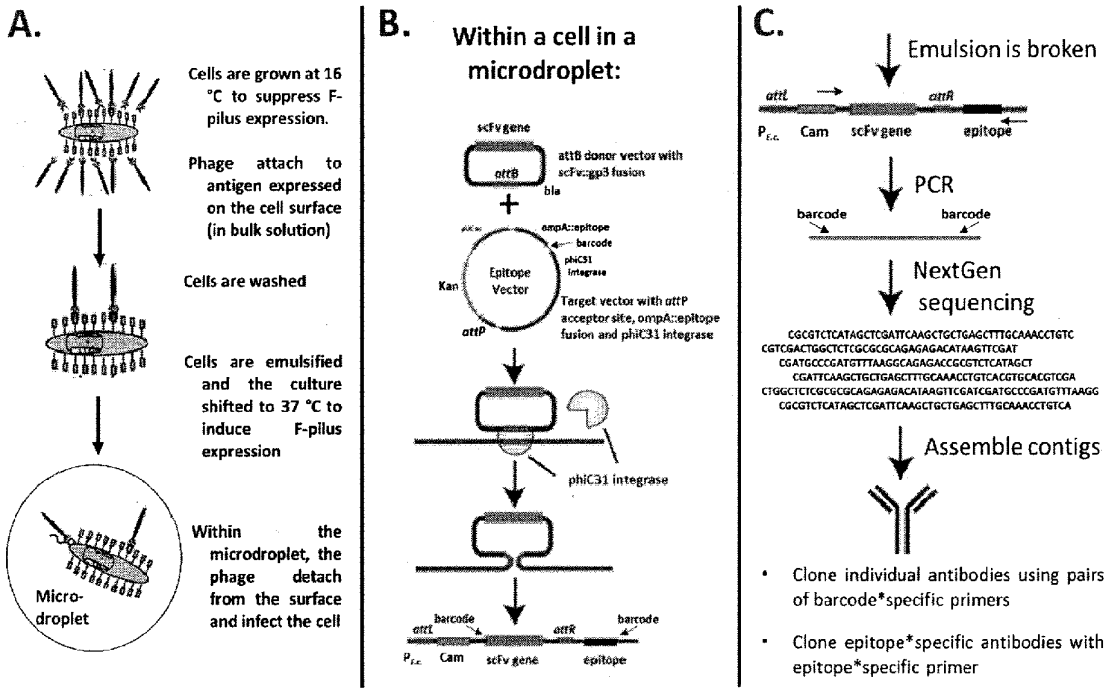

A.
Cells are grown at 16 °C to suppress F-pilus expression.
Phage attach to antigen expressed on the cell surface (in bulk solution)
Cells are washed
Cells are emulsified and the culture shifted to 37 °C to induce F-pilus expression
Within the microdroplet, the phage detach from the surface and infect the cell
Micro-droplet
B.
Within a cell in a microdroplet:
scFv gene
attB
attB donor vector with scFv::gp3 fusion
bla
ompA::epitope
barcode
phiC31 integrase
Epitope Vector
Kan
attP
Target vector with attP acceptor site, ompA::epitope fusion and phiC31 integrase
phiC31 integrase
attL
barcode
attR
barcode
Cam
scFv gene
epitope
C.
Emulsion is broken
attL
attR
Cam
scFv gene
epitope
PCR
barcode
barcode
NextGen sequencing
CGCGTCTCATAGCTCGATTCAAGCTGCTGAGCTTTGCAAACCTGTC
CGTCGACTGGCTCTCGCGCGCAGAGAGACATAAGTTCGAT
CGATGCCCGATGTTTAAGGCAGAGACCGCGTCTCATAGCT
CGATTCAAGCTGCTGAGCTTTGCAAACCTGTCACGTGCACGTCGA
CTGGCTCTCGCGCGCAGAGAGACATAAGTTCGATCGATGCCCGATGTTTAAGG
CGCGTCTCATAGCTCGATTCAAGCTGCTGAGCTTTGCAAACCTGTCA
Assemble contigs
• Clone individual antibodies using pairs of barcode*specific primers
• Clone epitope*specific antibodies with epitope*specific primer

Fig. 8

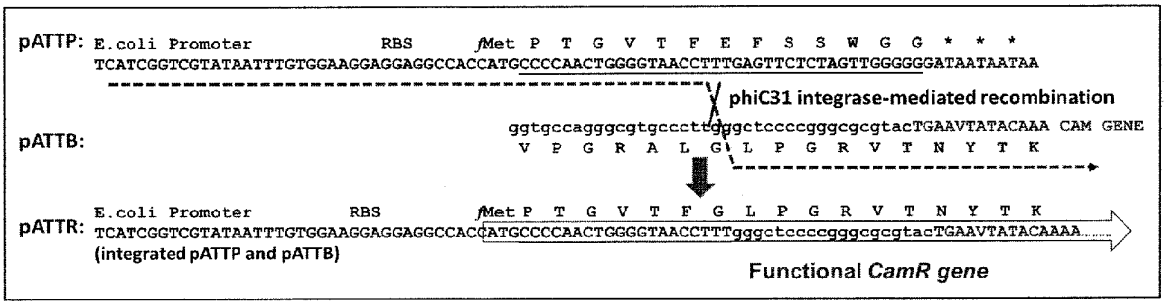
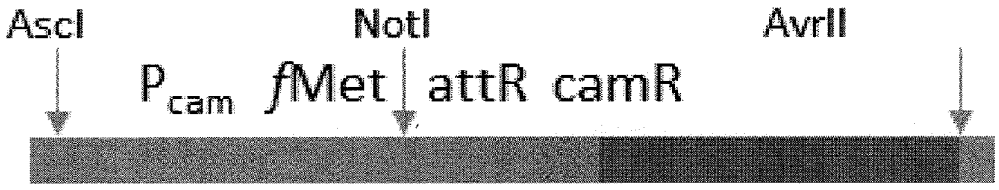

pATTP:
E.coli Promoter
RBS
fMet P T G V T F E F S S W G G * * *
TCATCGGTCGTATAATTTGTGGAAGGAGGAGGCCACCATGCCCCAACTGGGGTAACCTTTGAGTTCTCTAGTTGGGGGGATAATAATAA
phiC31 integrase-mediated recombination
pATTB:
ggtgccagggcgtgcccttgggctccccgggcgcgtacTGAAVTATACAAA CAM GENE
V P G R A L G L P G R V T N Y T K
pATTR:
E.coli Promoter
RBS
fMet P T G V T F G L P G R V T N Y T K
TCATCGGTCGTATAATTTGTGGAAGGAGGAGGCCACCATGCCCCAACTGGGGTAACCTTTgggctccccgggcgcgtacTGAAVTATACAAAA
(integrated pATTP and pATTB)
Functional CamR gene

Fig. 9



attR x 2 (5' to 3' frames 2 and 3)

Fig. 10

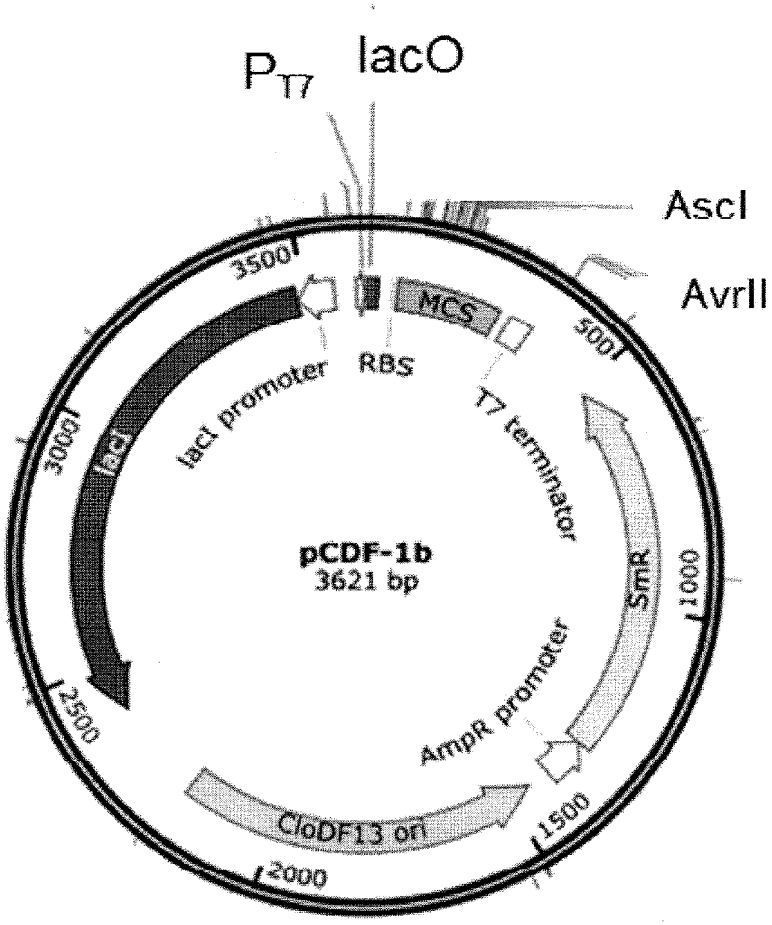
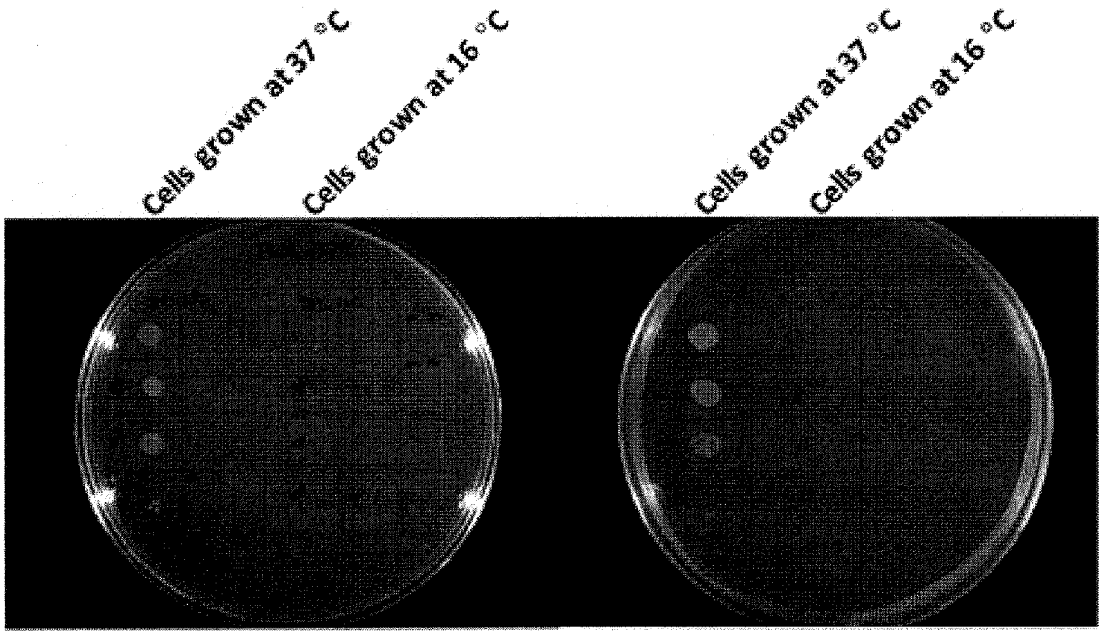

P_{T7}
lacO
AscI
AvrII
3500
500
MCS
RBS
lacI promoter
T7 terminator
3000
lacI
pCDF-1b
3621 bp
SmR
1000
2500
AmpR promoter
CloDF13 ori
1500
2000

Fig. 11


Cells grown at 37 °C
Cells grown at 16 °C
Cells grown at 37 °C
Cells grown at 16 °C
Transduction Plate at 37 °C O/N
Transduction Plate at 16 °C after 4 Days