Strategies of CHO Cell Line Development and Toolbox Implementation for Improving Cell Line Productivity and Stability

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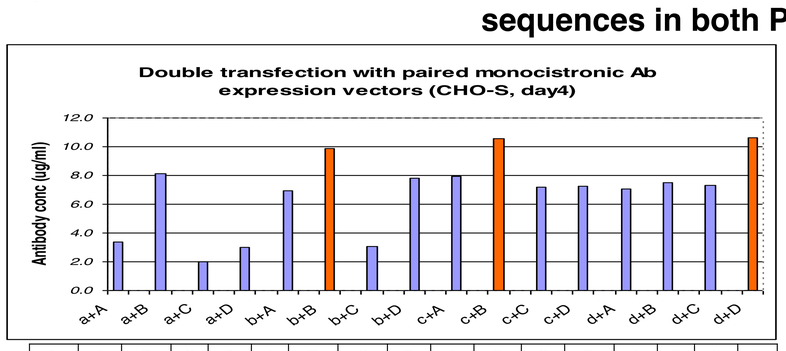
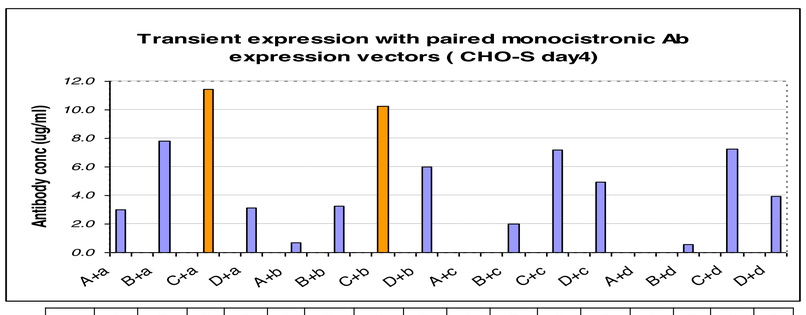
It is well known that for better expressing protein of interest (POI), the codon usage has to be compatible to host cells, but when we ordered synthetic genes optimized for expressing a monoclonal antibody (mAb) in CHO-S cells, that pair of synthetic genes expressed the target Ab very poorly. After thorough investigation, we concluded that current codon optimization cannot guarantee good POI expression. To get good pair of synthetic genes for expressing Ab in CHO cells, multiple pairs of synthetic genes need to be evaluated. The selection of good pair of synthetic genes becomes a process that is not only labor intense, time consuming, but also costly. **To simplify this unappealing process, we use a scaffold that contains both our proven compatible secretory signal peptide and hIgG1 Constant region DNA sequences, where we simply incorporated a single optimization at the variable region. Our data demonstrate that this strategy is successful.** Using commercially available dicistronic mammalian expression vector for antibody cell line development, we encountered two challenges: 1) higher selection stringency cannot guarantee higher productivity, and 2) extremely low recovery rate of stable high producer clones. To overcome these issues, **Cytovance®** built its own **dicistronic mammalian expression vectors**. These vectors link the genes of interest (GOI) and the genes for selection (GFS) together *via* Internal Ribosome Entry Site (IRES). As a result, the GOI and GFS are on a same mRNA where we use a higher selection stringency that leads to a higher GOI expression. Moreover, the vectors also contain CHO genomic DNA targeting elements that direct the transgenes integration into specific sites of CHO genome to enhance the transgene stability. Consequently, the recovery rate of stable high producer clones should be much higher. To date, our preliminary data prove that it is the case.

Cytovance Toolbox uses a mAb scaffold of constant regions to evaluate multiple variable region DNA sequences in both P1/P2 on the vector



p2	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
p1	Native LC seq				*High Prod LC seq				Vendor 1 LC seq				Vendor 2 LC seq			



p1	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
p2	Native LC seq				*High Prod LC seq				Vendor 1 LC seq				Vendor 2 LC seq			

p1 = cloning site 1, p2 = cloning site 2 in pCHO1.0; A = Native HC seq, B = *High Prod HC, C = GrHC, D = BmHC; *High Prod seq used C regions of previously proven good IgG1 DNA sequences

(1) All the LC and HC were optimized for expression in CHO cells, but the outcome varies from nil to >10 ug/ml
(2) 3 out of the 5 good expressers (the red bars) contain previously proven good constant regions, only the variable regions were optimized (optimized variable regions in IgG1 scaffolds) for Ab cell line development, which greatly improved the odds of success

According to these data we come up with a strategy using scaffolds contain both proven good IgG HC and LC constant regions ("hpHC and hpLC") and secretory signal peptide DNA sequences and plug in optimized variable regions for IgG type of mAbs expression vector construction.

Two major Issues encountered using commercially available mammalian expression system for Ab cell line development

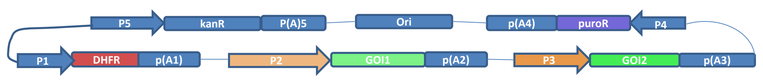
- Productivity is not driven by selection pressure

  Under 1000 nM MTX + 50 ug/ml puromycin selection force only 32 out of 1000+ clones produce higher level of mAb

- Stable high productivity clone recovery rate is very low

  Starting from $3x10^7$ transfectants, only 7 clones achieved >10 pg/c/d.
  (2 were > 20 pg/c/d & can reach > 2 g/L)

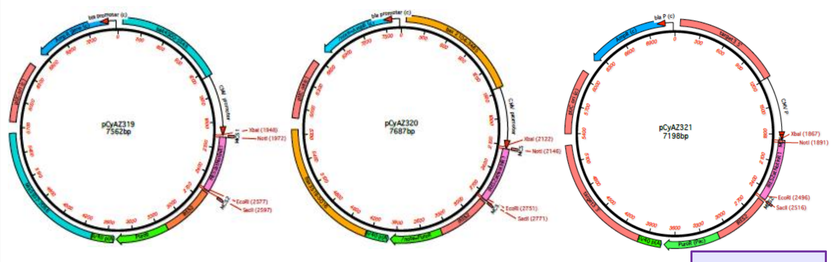
Schematic structure of the commercial dicistronic mammalian expression vector (4 independent expression cassettes)



Hypothesis for the encountered issues

- The entire construct DNA may split before integration into the genome → Selection agents may not select GOI cassettes at the same time
- No genome integration targeting sites → random integration into CHO genome → integration is not stable

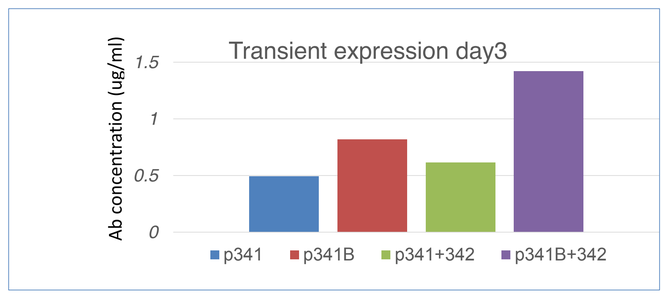
Another set of vectors to address these issues is to use a single selection agent



The transcript is:

Cap-5'UTR-GOI1-IRES2-GOI2-IRES2-puroR-polyA tail

Therefore, only puromycin is needed for selection.



p341 = p1-hpLC-IRES-DHFR-p(A)—p2-hpHC-IRES2-puroR-p(A)
p342 = p1hpLC-IRES-DHFR-p(A)—p2-hpLC-IRES2-puroR-p(A)
p341B = p1-hpLC-IRES2-hpHC-IRES2-puroR-p(A)

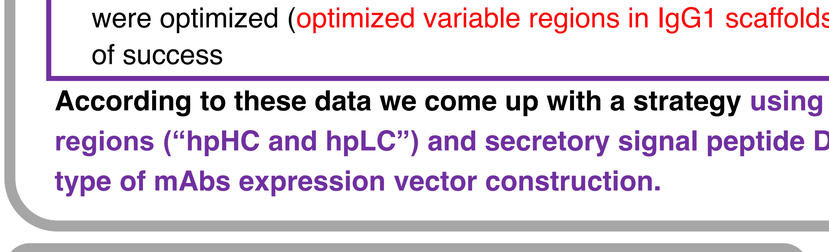
The approach of linking GOI1, GOI2 and puroR *via* two IRESs increases productivity in transient expression.

Comparison of commercial vectors vs. Cytovance vectors + scaffold approaches

	Commercial vectors	Cytovance vectors + scaffold
# of CHO-S cells to be transfected for cloning	3.00E+07	3.00E+06
# of 96-well plates seeded for mini-pools after phase1 selection	Not Applicable	3
# of 96-well plates seeded for cloning after phase 2 selection	50	Not Applicable
# of identified clones or mini-pools	800 clones	24 mini-pools
# of high producer clones (>10 pg/c/d) or mini-pools (>20 pg/c/d)	7 clones	5 mini-pools
Productivity of the best clones or mini-pool (pg/c/d)	20-30	> 100
# of stable clones (>20 pg/c/d)	2	In-process
# of stable high producer clones per # of total clone (%)	0.25	In-process

The preliminary data suggest that Cytovance vectors + scaffold approach is superior to the commercial vectors.

Cytovance's mammalian expression vectors address the above issues



Transcript structures:

Cap-5'UTR-GOI1-IRES1-DHFR-polyA tail
Cap-5'UTR-GOI2-IRES2-puroR-polyA tail

Consecutive expression cassettes address selection issue

All the GOIs were the optimized variable regions of HC and LC inserted into IgG1 scaffolds (hpHC, hpLC).

Experimental results

CHO-S cells were transfected with a cocktail of linearized mAb expression constructs on three different vector backbones.

- The transfectant pools showed dose response to selection stringencies

- In the first round of selection, out of 24 mini-pools, 5 produced between 30-100 pg/c/d
- Second round of selection/amplification is underway

Conclusions

- Using our IgG1 HC and LC scaffolds to develop IgG1 type monoclonal mAb cell lines requires fewer pairs of optimized synthetic mini-genes and therefore reduces the required time, labor, and cost
- Preliminary data suggest that Cytovance's mammalian expression vectors + scaffold system has great potential to:
  A. reduce the labor for cloning and the time for delivery of sufficient materials for characterization
  B. increase the odds of getting stable clonal high producers
  C. enhance the specific productivity of the clonal cell lines
  D. express other types of therapeutic proteins (double or single chain proteins) in addition to mAbs