Converting rabbit hybridoma into recombinant antibodies with effective transient production in an optimized human expression system

Presented at HAH Belfast 2016 by

Dr. Tim Welsink

Molecular Biology
Transient Gene Expression

CASE STUDY

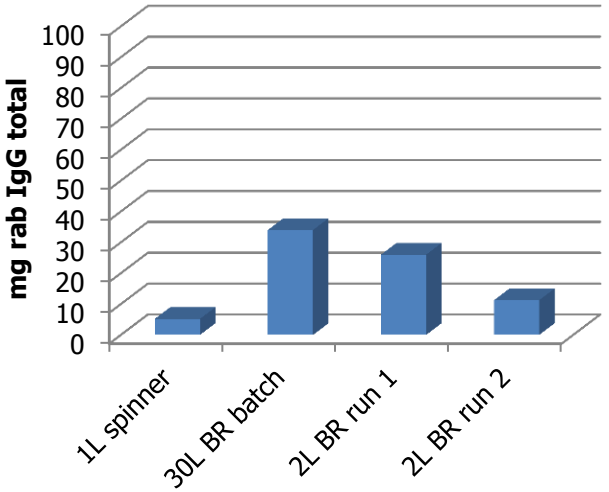
RAB-MAB CONVERSION

monoclonal rabbit hybridoma AB

recombinant rabbit AB produced by TGE

1) cDNA sequencing
2) Vector design, preparation
3) Transient production
4) Purification
5) QC

Hybridoma rab. AB yield



CASE STUDY

Sequence alignment HC variable region:

```
AK6.P16-20.HC.K2 QSVEESGGRLVTPGTPLTLTCTVSGIDLSSYKMSWVRQAPGKGLEWIGVISRTGITIYAS 60
AK6.P16-20.HC.K3 QSVEESGGRLVTPGTPLTLTCTVSGIDLSSYKMSWVRQAPGKGLEWIGVISRTGITIYAS 60
AK6.P16-20.HC.K4 QSVEESRGRLVTPGTPLTLTCTVSGIDLSSYKMSWVRQAPGKGLEWIGVISRTGITIYAS 60
AK6.P18-20.HC.K1 QSLEESGGRLVTPGTPLTLTCTVSGIDLSSYKMSWVRQAPGKGLEWIGVISRTGITIYAS 60
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AK6.P18-20.HC.K4 QSLEESGGRLVTPGTPLTLTCTVSGIDLSSYKMSWVRQAPGKGLEWIGVISRTGITIYAS 60

AK6.P16-20.HC.K2 WAKGRFTISRTSTTVDLKITSPTTEDTATYLCARGGDGGWGYGHIWGPGTLVTISS 116
AK6.P16-20.HC.K3 WAKGRFTISRTSTTVDLKITSPTTEDTATYLCARGGDGGWGYGHIWGPGTLVTISS 116
AK6.P16-20.HC.K4 WAKGRFTISRTSTTVDLKITSPTTEDTATYLCARGGDGGWGYGHIWGPGTLVTVSS 116
AK6.P18-20.HC.K1 WAKGRFTISRTSTTVDLKITSPTTEDTATYLCARGGDGGWGYGHIWGPGTLVTVSS 116
AK6.P18-20.HC.K2 WAKGRFTISRTSTTVDLKITSPTTEDTATYLCARGGDGGWGYGHIWGPGTLVTISS 116
AK6.P18-20.HC.K4 WAKGRFTISRTSTTVDLKITSPTTEDTATYLCARGGDGGWGYGHIWGPGTLVTISS 116
```

Complementarity Determining Regions (CDRs) are colored, mismatches are highlighted

Primer sequences from
Seeber et al., 2014 & Rader et al., 2000

CASE STUDY

Sequence alignment LC variable region:

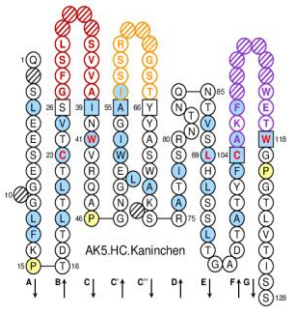
```
AK6.P8-13.LC.K2   ELVLTQTPASVEAAVGGTVTIKCQASQSISNLLAWYQQKPGQRPKLLIYYASNLASGVSS 60
AK6.P9-11.LC.K1   ELDLTQTPASVEAAVGGTVTIKCQASQSISNLLAWYQQKPGQRPKLLIYYASNLASGVSS 60
AK6.P9-11.LC.K2   ELDMTQTPASVEAAVGGTVTIKCQASQSISNLLAWYQQKPGQRPKLLIYYASNLASGVSS 60
AK6.P9-11.LC.K3   ELDLTQTPASVEAAVGGTVTIKCQASQSISNLLAWYQQKPGQRPKLLIYYASNLASGVSS 60
AK6.P10-13.LC.K1  ELDLTQTPASVEAAVGGTVTIKCQASQSISNLLAWYQQKPGQRPKLLIYYASNLASGVSS 60

AK6.P8-13.LC.K2   RFKGSGSGTEYTLTISGVQCADAATYYCQSYYYSSTSNYAFDFGGGTEVVVK 112
AK6.P9-11.LC.K1   RFKGSGSGTEYTLTISGVQCADAATYYCQSYYYSSTSNYAFDFGGGTELEI- 111
AK6.P9-11.LC.K2   RFKGSGSGTEYTLTISGVQCADAATYYCQSYYYSSTSNYAFDFGGGTELEI- 111
AK6.P9-11.LC.K3   RFKGSGSGTEYTLTISGVQCADAATYYCQSYYYSSTSNYAFDFGGGTELEI- 111
AK6.P10-13.LC.K1  RFKGSGSGTEYTLTISGVQCADAATYYCQSYYYSSTSNYAFDFGGGTELEI- 111
```

Complementarity Determining Regions (CDRs) are colored, mismatches are highlighted

Primer sequences from
Seeber et al., 2014 & Rader et al., 2000

CASE STUDY

- cDNA Design
- IgG gene synthesis
- Cloning into expression vector
- Low-endotoxin plasmid preparation
- Transient gene expression

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ACT GTC ACC ATT AAG TGT CAA GCG AGT CAG AGC ATA TCC AAC CTG TTG GCC TGG TAT CAG CAG AAA
CCT GGG CAA AGA CCG AAA CTG CTC ATC TAT TAC GCC TCT AAT CTT GCT TCA GGC GTT TCC AGT CGG
TTC AAA GGG TCA GGA AGC GGT ACC GAG TAT ACA CTG ACC ATT TCT GGC GTC CAG TGT GCA GAT GCT
GCC ACC TAT TAC TGC CAG TCC TAC TAC TAC TCT AGC ACT TCC AAC TAT GCG TTC GAC TTT GGA GGA
GGG ACA GAA GTG GTG GTA AAG GGC GAT CCA GTG GCA CCC ACA GTC CTC ATC TTT CCT CCA GCA GCA
GAC CAG GTA GCC ACC GGT ACT GTC ACA ATC GTG TGT GTG GCC AAC AAG TAC TTT CCC GAC GTT ACG
GTG ACT TGG GAG GTT GAC GGC ACA ACT CAG ACG ACA GGC ATT GAG AAC AGC AAG ACA CCC CAG AAT
AGT GCC GAT TGC ACC TAT AAC CTG TCA AGC ACC CTT ACC CTG ACT TCA ACC CAG TAC AAT AGC CAC
AAG GAG TAC ACA TGC AAA GTG ACT CAA GGA ACC ACG TCT GTC GTG CAG AGC TTC AAT AGG GGC GAT
TGC TAA TAG GTT TAA ACT TAA TTA AAA GCT T
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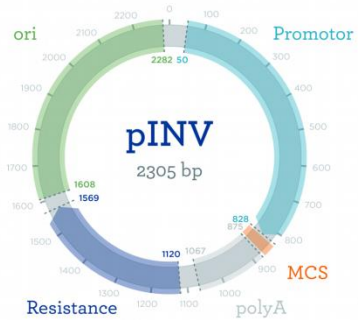
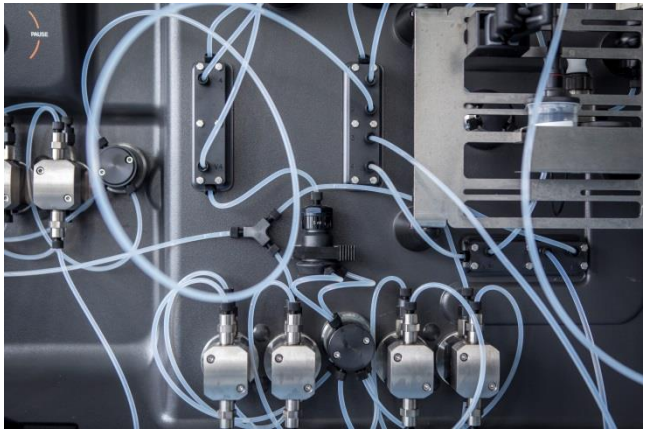


CASE STUDY

- Transient gene expression
  - Cell line: HEK or HEK-INV
  - Transfection protocol: optimized
  - Culture protocol: optimized
  - Scale: 50 to 1,500 ml
  - Production for 7 days
  - Harvest by centrifugation

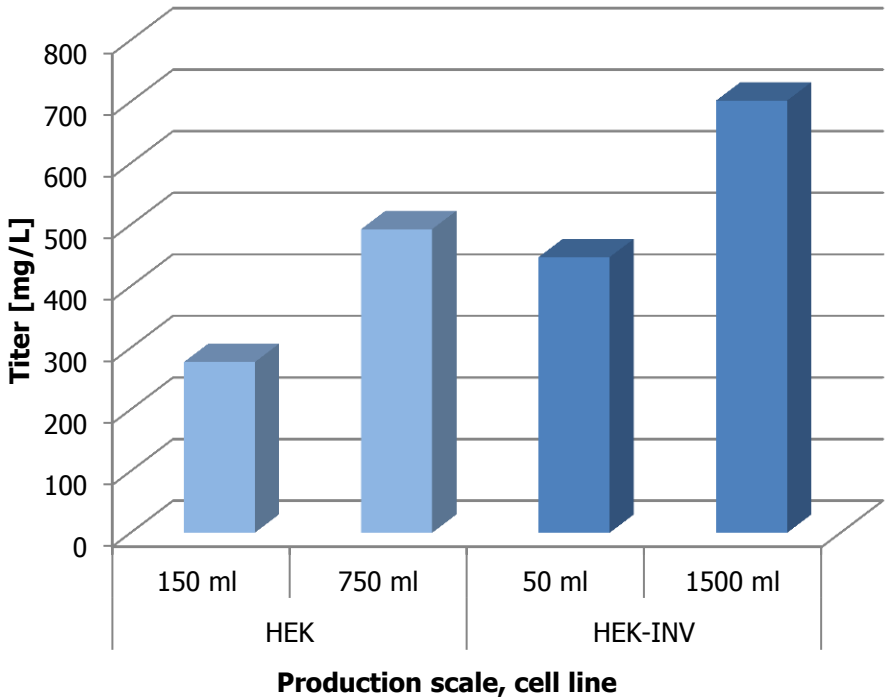
- Purification
  - AF-rProtein A-650F (Tosoh Bioscience)
  - Äkta chromatography system
  - Elution by low pH
  - Dialysis to PBS pH 7.4



CASE STUDY

RESULTS

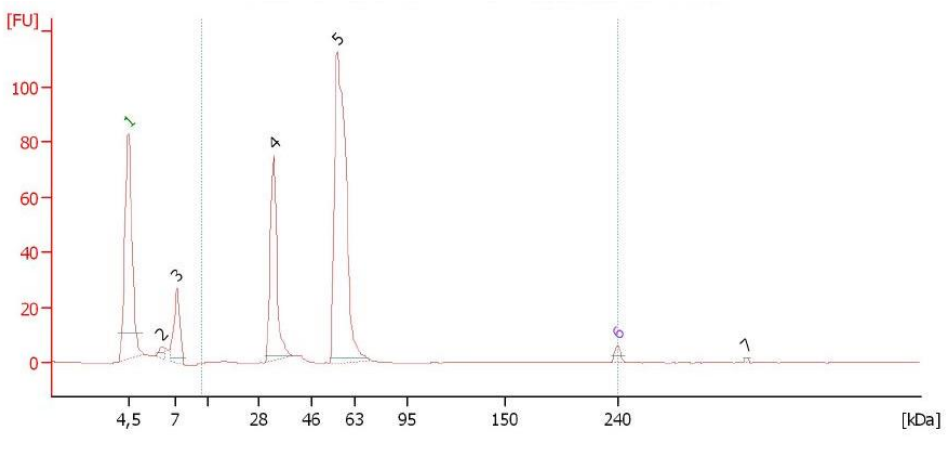
- 4 Batches produced
- Different production scale
- 2 cell lines



CASE STUDY

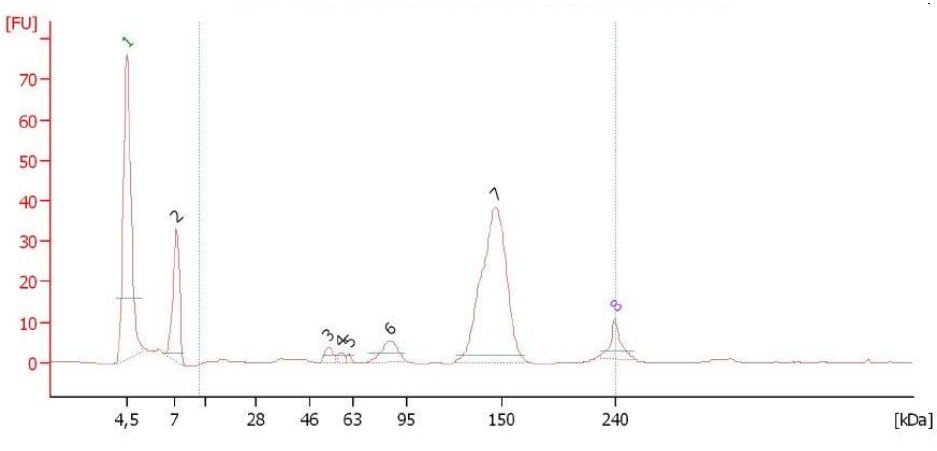
- QC: capillary gel electrophoresis for purity

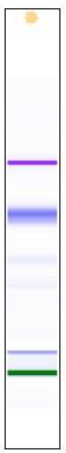
Reducing conditions, Agilent 2100-BioAnalyzer, Protein 230 Kit



Purity ≥ 98%

Non-reducing conditions, Agilent 2100-BioAnalyzer, Protein 230 Kit

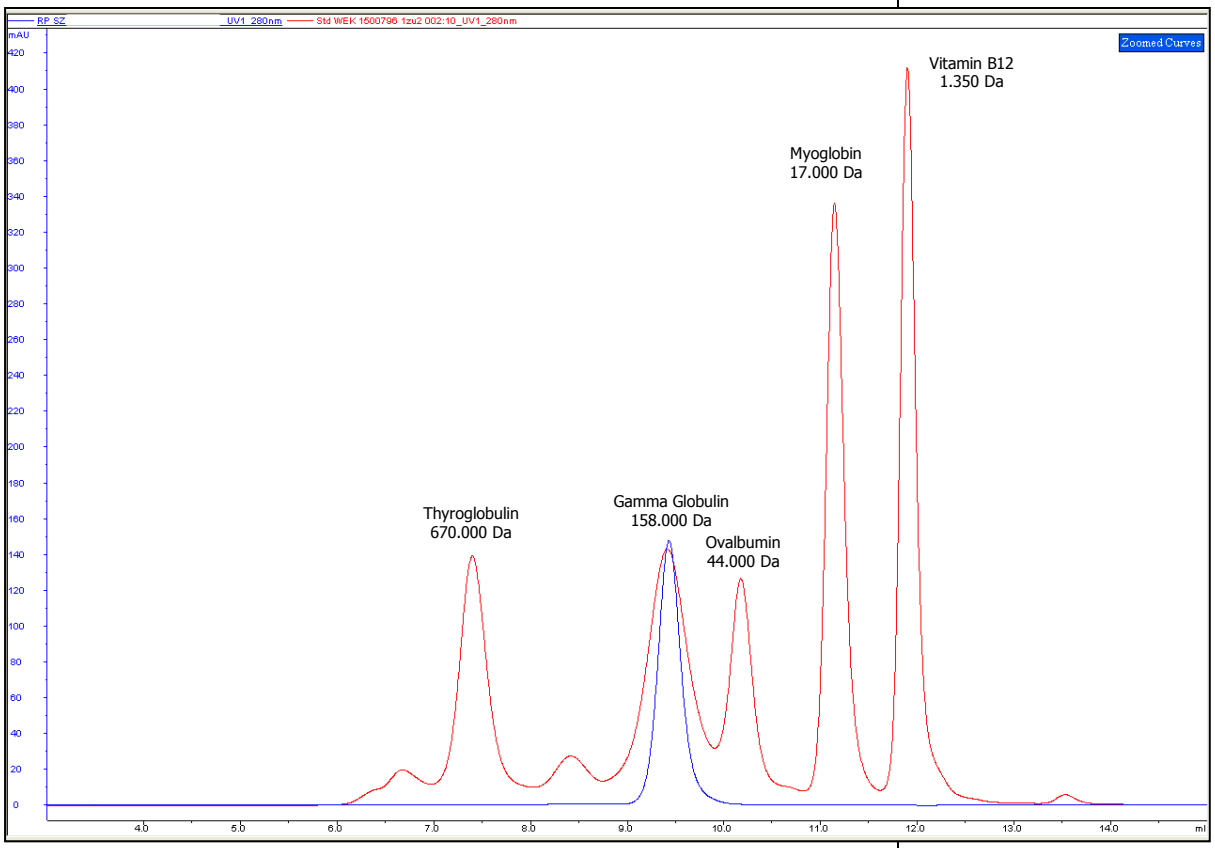




Purity ≥ 85%

CASE STUDY

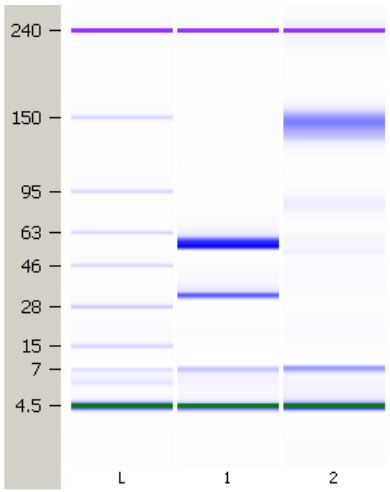
- Analytical SEC for determination of aggregates



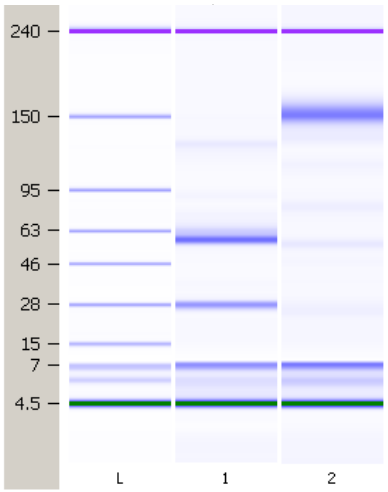
AppliChrom® ABOA-ProteSep S-L 5µ, 300mm x 8mm

CASE STUDY

Recombinant rab-mab

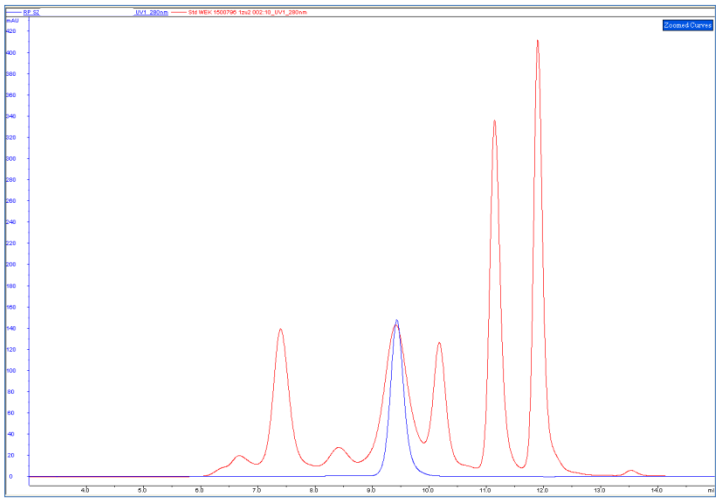


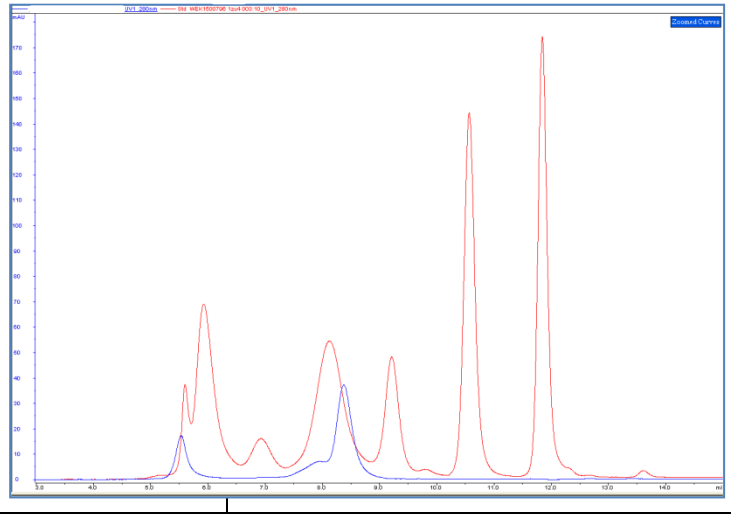
Hybridoma rabbit AB



Right: CGE

Below: analyt. SEC





Summary

- We had to address various needs to aim the goal
- Improvement of established methods
- New methods developed

  Sequencing of Fv, production platform
- Case study rab-mab conversion & production
- Production and purification of 4 independent batches

Outstanding:

- Detailed results upon activity of the recombinant vs. hybridoma rab-mab

Conclusion:

New rab-mab production system allows to generate mg to g scales recombinant rabbit IgGs by transient gene expression within weeks.

ACKNOWLEDGEMENTS

INVIVO

InVivo BioTech Services GmbH

Dr. Wolfgang Weglöhner

Sebastian Püngel
Penélope Soto Villegas
Vanessa Vater

Dr. Sabrina Schindler
Mohamad Eidi

Molecular Biology Lab

Downstream Processing Lab

Cooperation partners:

emp BIOTECH

xcell

QUESTIONS

Please leave your question in our linkedin group transient-transfection
or write use our contact form