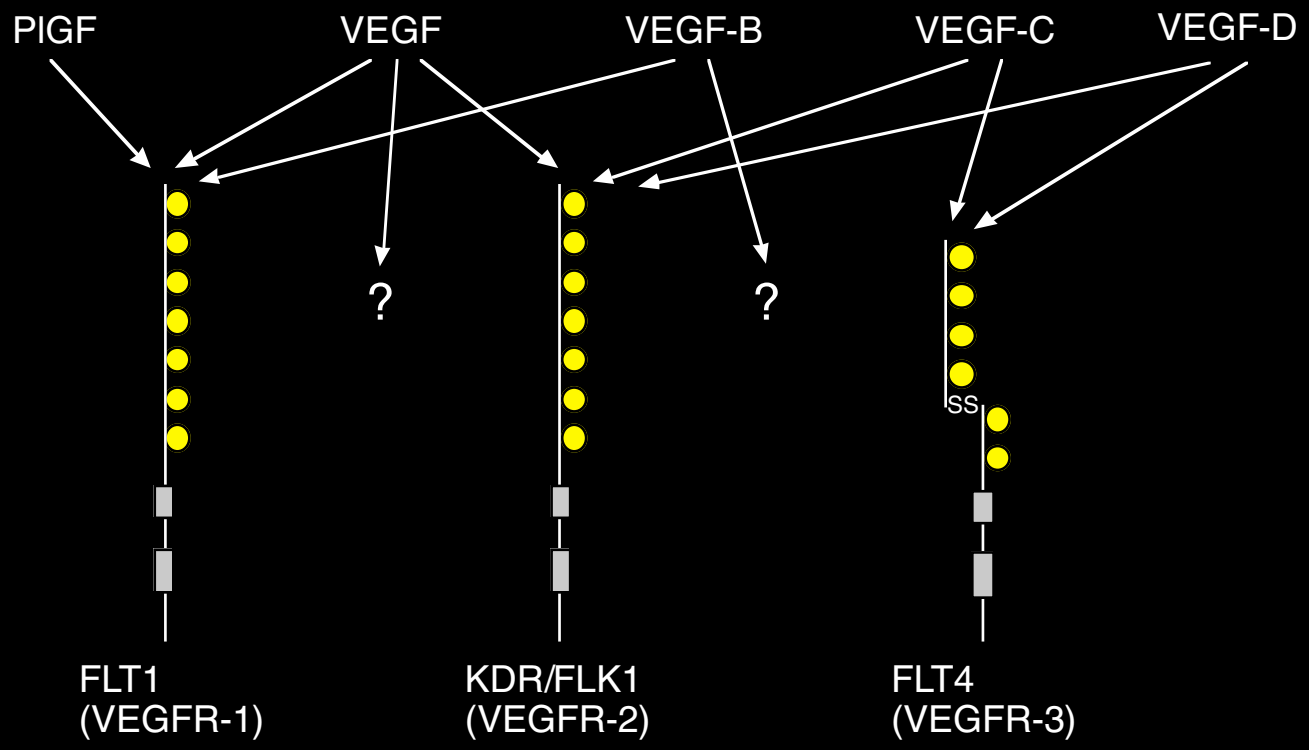


Important research goals that I achieved during the past 2 years:

1. The neon lamps at my bench were repaired.
2. Get a bad reputation in order to work in peace.
3. Aquiring a PAGE apparatus and making 15-well combs

Major future plans:

1. Get cardboard boxes for Eppendorf tube storage and racks for 15- and 50-ml-Falcon tubes and Eppendorf racks
2. Catch somebody in the very act of pouring boiling agarose onto a gel tray and beating him half to death to set an example and in revenche for what happend to Yassir El Maryod.

Possible strategies to archieve goal #1:

- Ally with Birgitta and try to have a reasonable discussion.
- Bribing with the bottle Champagne I brought from Germany.
- Ordering myself and pay with my own money.

Possible strategies to archieve goal #2:

- “The soldier-and-three-bushes”-problem.

| | | | |
|---|---|---|---|
| species | Spodoptera frugiperda (fall armyworm) | | Trichoplusia ni (cabbage looper) |
| cell lines | Sf9 | Sf21 | High Five |
| appearance | spherical, regular size | spherical, different sizes, but generally larger than Sf9 | mix of round and spindel shape |
| | loose attachment | | firm attachment |
| suspension culture | no adaptation required | | adaptation required |
| doubling time | 18-22 h | ~24 h | |
| growth media | Sf900II, TNM-FH | | Ex cell 400 (monolayer), Ex cell 405 (suspension culture), TNM-FH |
| plaque formation | supported | | not supported |
| protein production | 1-500 mg/litre (protein-dependent) | | 5-10 x higher than Sf |

time (d)

1. Transfection of insect cells 2-5

⇓

2. Analysis of transfection supernatant 4
(IP/)Western, NTA-Ni2+ affinity purification
& Ag staining; functional assay

⇓

3. First amplification of selected clones 2-5

⇓

4. Second amplification of selected clones 2-3

⇓

5. Test infection with high titre stock 2

⇓

6. Analysis of conditioned medium 2
protein detection

⇓

7. Prepare large scale stock virus 3-5
(5 big bottles/~200 ml)

⇓

8. Growing cells for large scale infection 7
(~40 big bottles)

⇓

9. Large scale infection 2
(800 ml conditioned medium 0.8-4 mg
DNDC-melSP-VEGF-C/D-H6)

⇓

10. Analysis of conditioned medium 2
like step 6

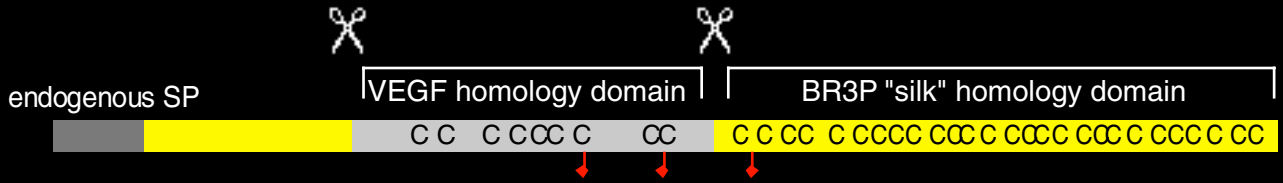
⇓

11. Concentration of medium,
purification & desalting 2-3

⇓

12. Analysis of purified protein 3
Quantitation: Coomassie staining & functional assay
(determining the biological activity)

total amount of time 33-43




Exchange of endogenous signal peptide against honeybee melittin signal peptide

Trimming of putative N- and C-terminal propeptides

Introduction of a C-terminal histidine tag

VEGF-CxVEGF-D heterodimer formation in HF cells

| | MOI | | | | | |
|---|---|---|---|---|---|---|
| VEGF-C-FL | 0 | 0 | 10 | 50 | 90 | 100 |
| VEGF-D-FL-$H_6$ | 0 | 100 | 90 | 50 | 10 | 0 |

IP with $H_5$-Ab (1:1, Dianova/Qiagen)

1. Western with VEGF-C-Ab (882)
2. Silver-staining/metabolic labelling (size difference)

# Problems with baculovirus expression system

## 1. Generating clonal virus stocks

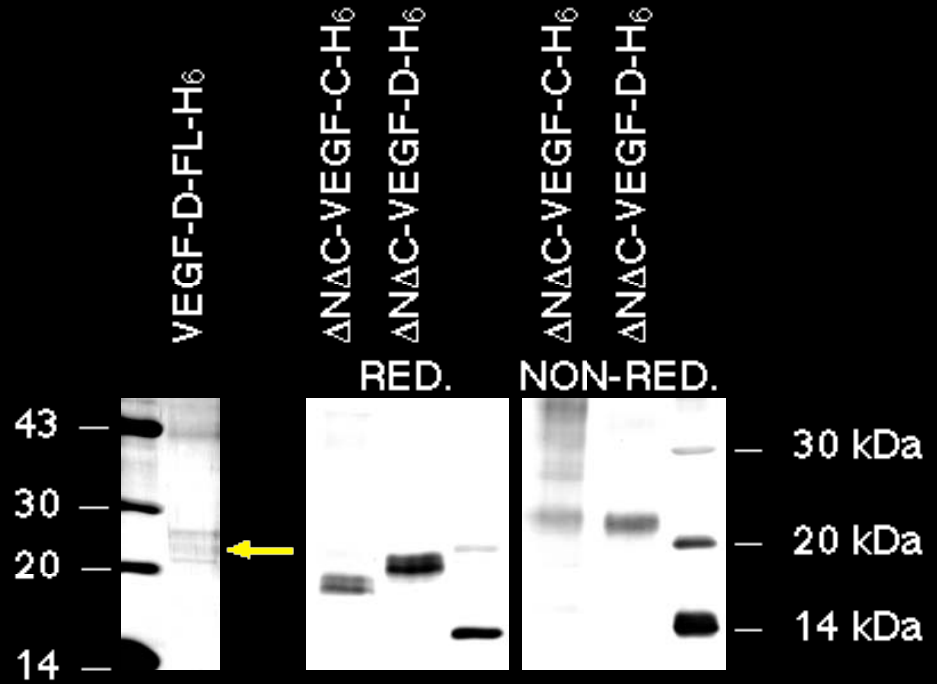
- Plaque purification
- FastBac system only: Retransformation of E.coli DH10B with isolated Bacmid-DNA

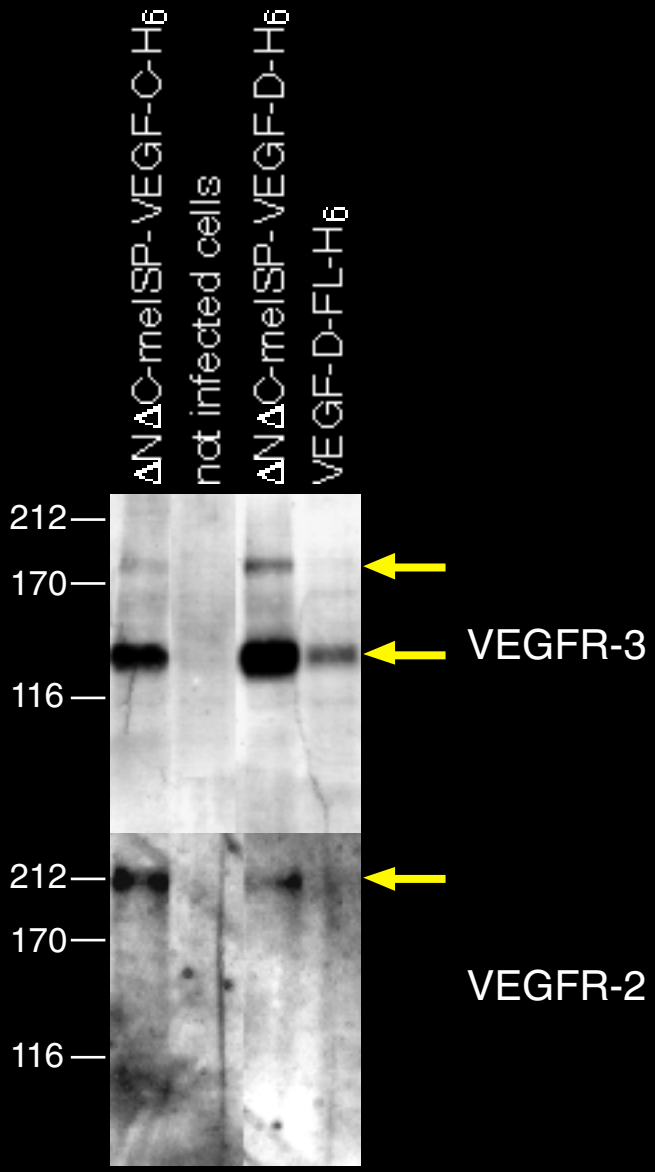
## 2. Protein-heterogeneity

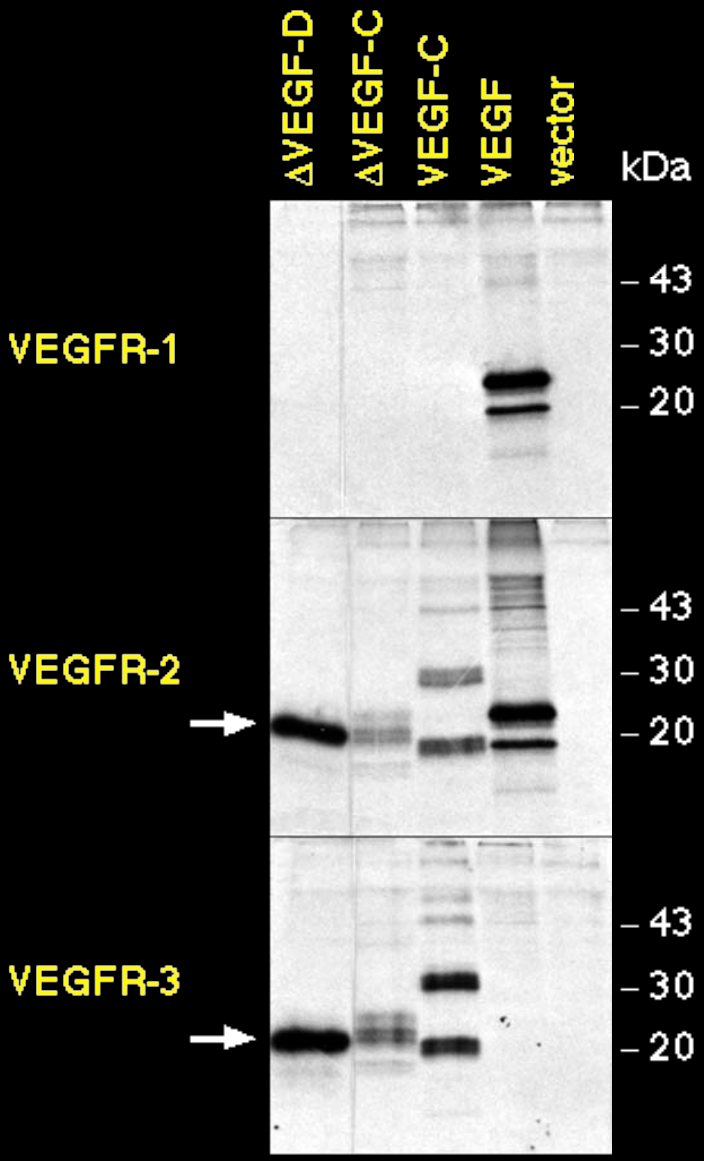
- partial proteolytic processing
- heterogenous cleavage by the signal peptidase
- differential glycosylation

## 3. Expression level

- Generation of high titre stocks: DIPs
- Drop in infectivity due to storage (UV)
- Condition of insect cells: viability, too high passage number, heat-shock protein vs. recombinant protein



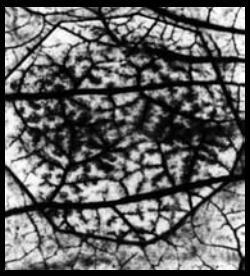




control

$VEGF_{121}$

$VEGF_{165}$



$VEGF_{121}$ x $VEGF_{165}$

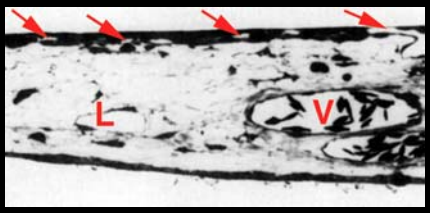
PlGF-1

PlGF-2

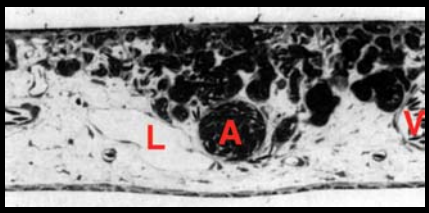
$VEGF_{121}$ x PlGF-1

$VEGF_{165}$ x PlGF-2

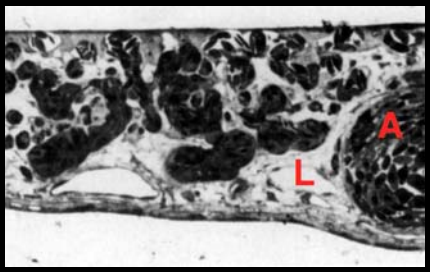
VEGF-C



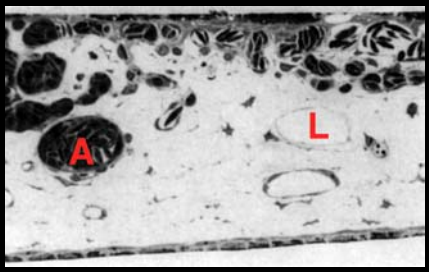

control




$VEGF_{121}$

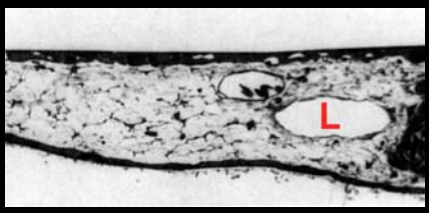



$VEGF_{165}$

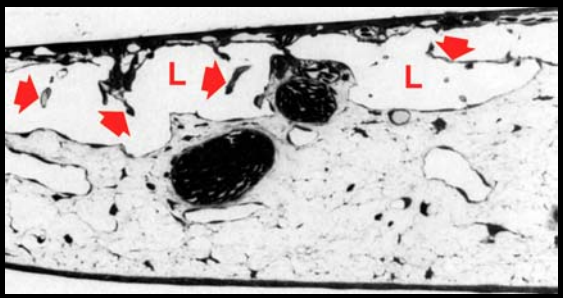



$VEGF_{121}$ x $VEGF_{165}$

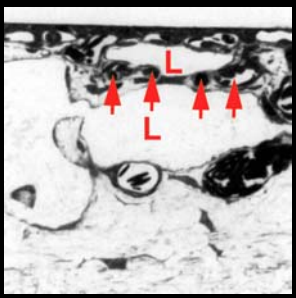

PlGF-1



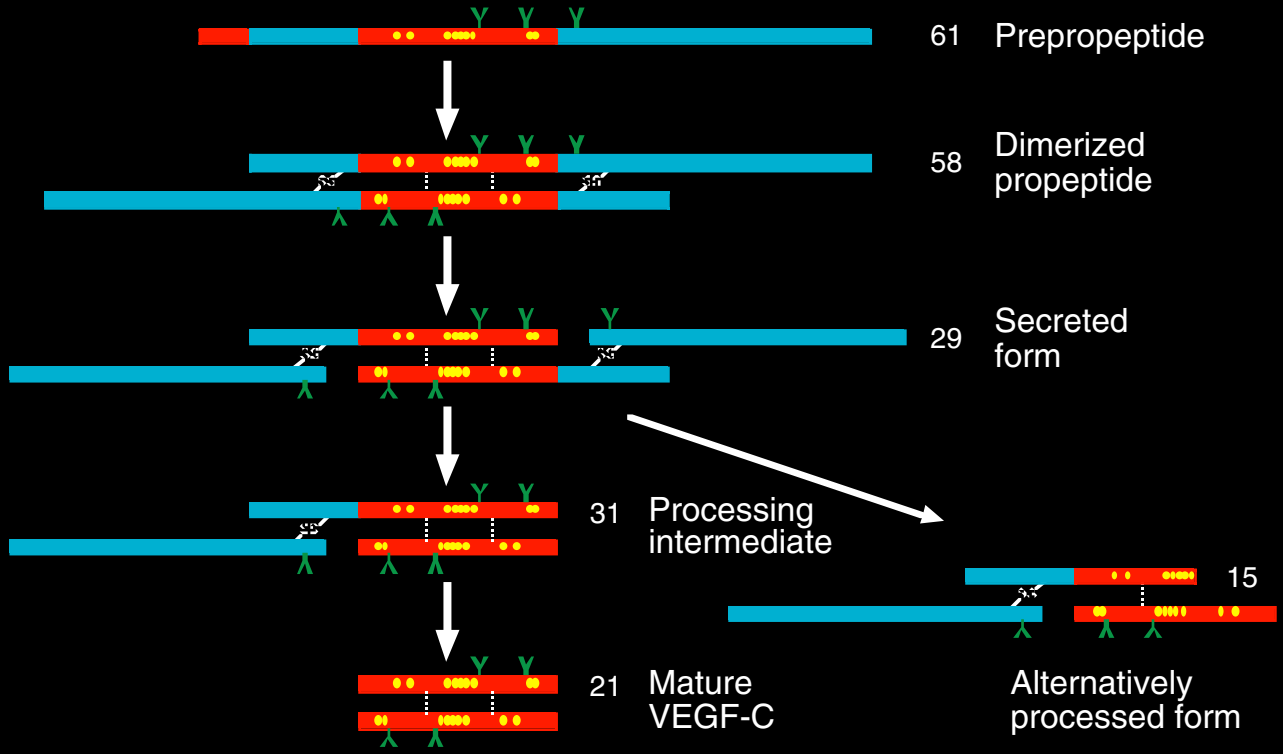

PlGF-2

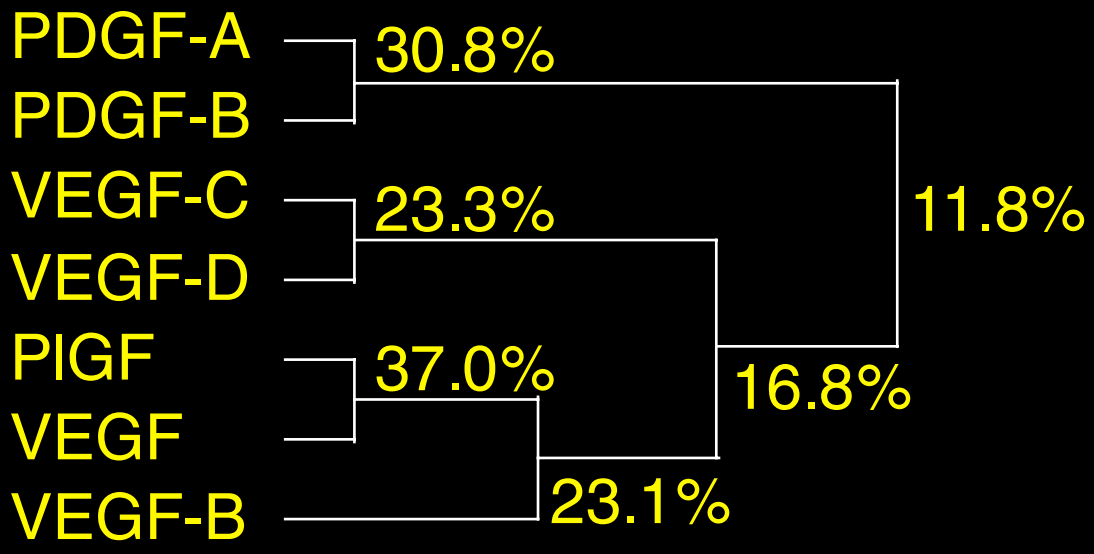



VEGF-C




VEGF-C



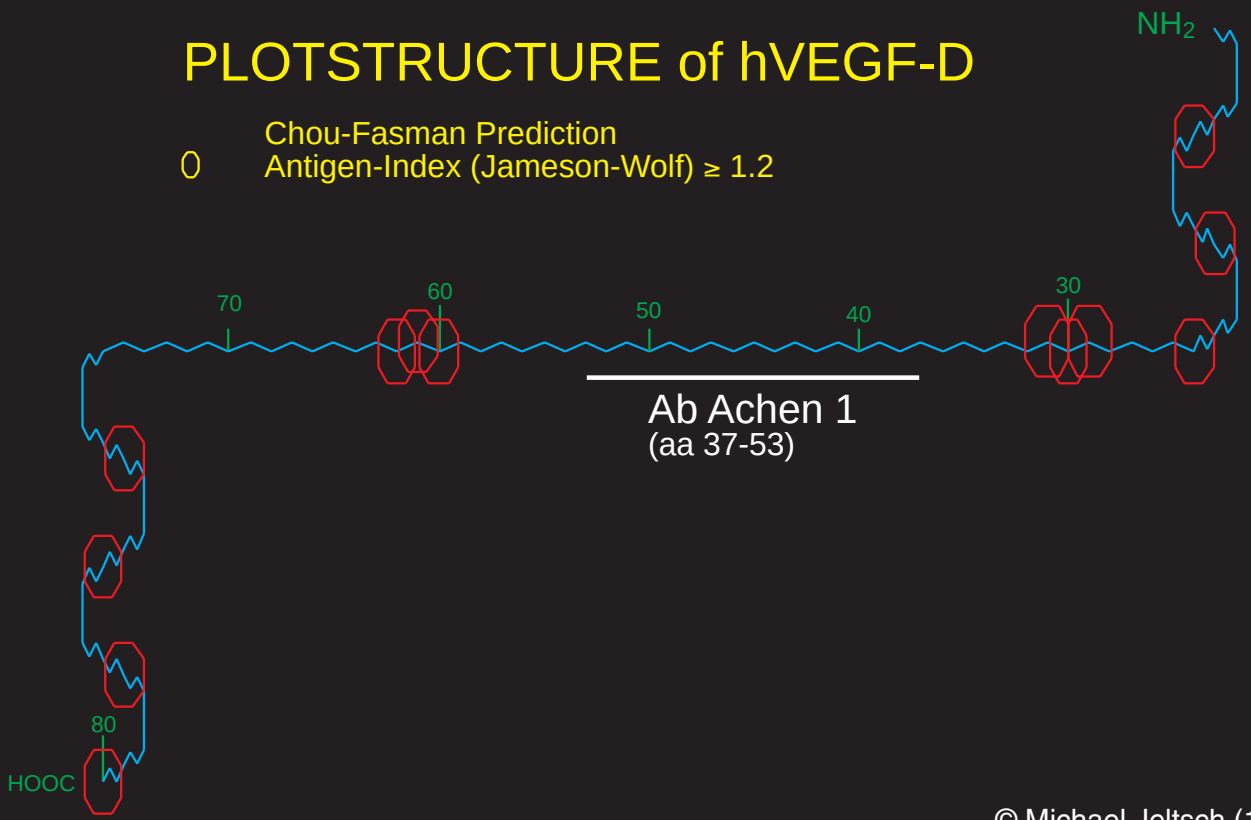


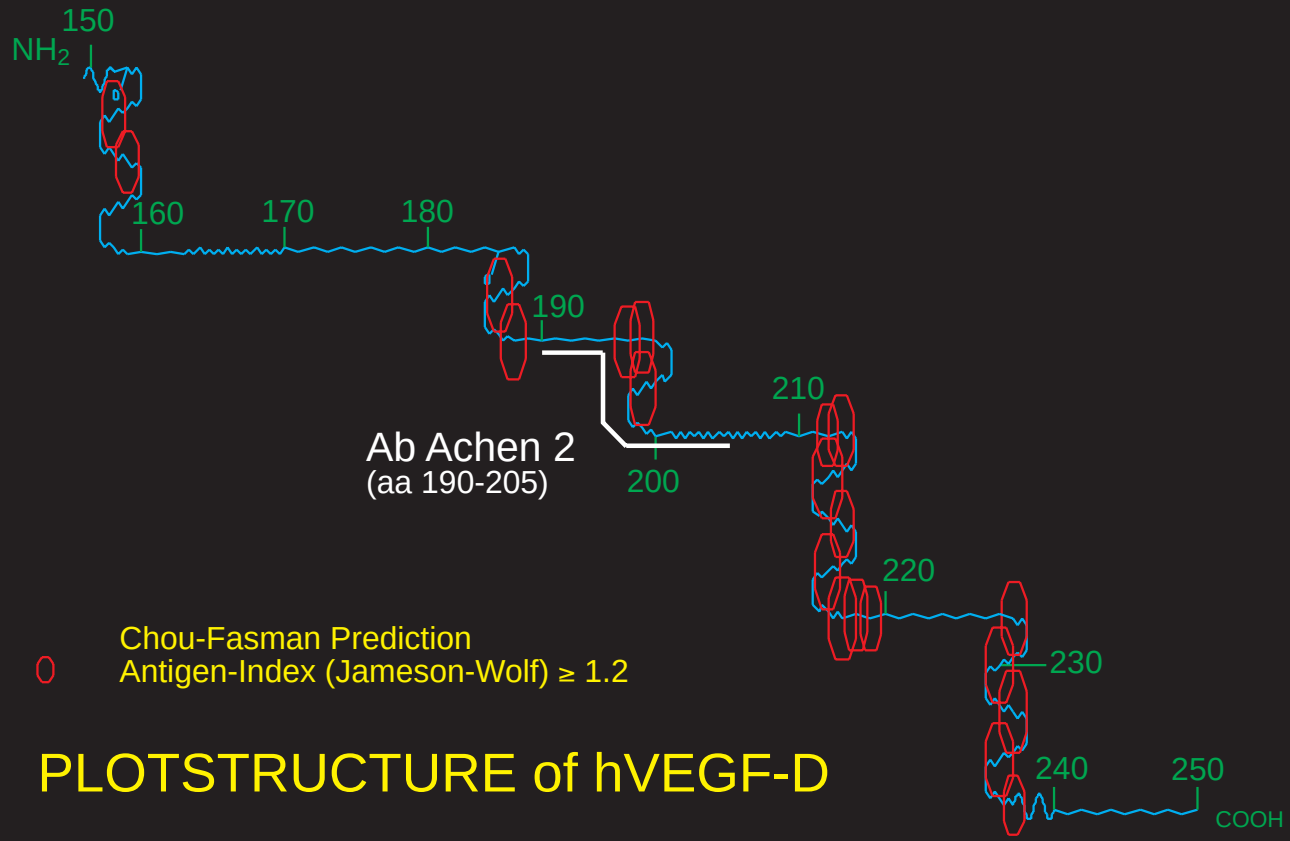
```
          1                                                            50
hVEGF-C   MHLLGFFSVA CSLLA..AAL LPGPREAPAA AAAF...... ESGLDLSDAE
hVEGF-C   MHLLCFLSLA CSLLA..AAL IPSPREAPAT VAAF...... ESGLGFSEAE

hVEGF-D   ..MYREWVVV NVFMMLYVQL VQGSSNEHGP VK.....RSS QSTLERS...
mVEGF-D   ..MYGEWGMG NILMMFHVYL VQGFRSEHGP VKDFSFERSS RSMLERS...

          51                                                           100
hVEGF-C   PDAGEATAYA SKDLEEQLRS VSSVDELMTV LYPEYWKMYK CQLRKGGWQH
hVEGF-C   PDGGEVKAFE GKDLEEQLRS VSSVDELMSV LYPDYWKMYK CQLRKGGWQ.
                              Ab Achen 1
hVEGF-D   .......... ....EQQIRA ASSLEELLRI THSEDWKLWR CRLRLKSF..
mVEGF-D   .......... ....EQQIRA ASSLEELLQI AHSEDWKLWR CRLKLKSL..

          101                 Ab 882                                   150
hVEGF-C   NREQANLNSR TEE..TIKFA AAHYNTEILK SIDNEWRKTQ CMPREVCIDV
hVEGF-C   ...QPTLNTR TGD..SVKFA AAHYNTEILK SIDNEWRKTQ CMPREVCIDV
                              Ab 928 & 929
hVEGF-D   ....TSMDSR SASHRSTRFA ATFYDIETLK VIDEEWQRTQ CSPRETCVEV
mVEGF-D   ....ASMDSR SASHRSTRFA ATFYDTETLK VIDEEWQRTQ CSPRETCVEV

          151                                                          200
hVEGF-C   GKEFGVATNT FFKPPCVSVY RCGGCCNSEG LQCMNTSTSY LSKTLFEITV
hVEGF-C   GKEFGAATNT FFKPPCVSVY RCGGCCNSEG LQCMNTSTGY LSKTLFEITV
hVEGF-D   ASELGKSTNT FFKPPCVNVF RCGGCCNEES LICMNTSTSY ISKQLFEISV
mVEGF-D   ASELGKTTNT FFKPPCVNVF RCGGCCNEEG VMCMNTSTSY ISKQLFEISV

                                        ↓C1HIS     ↓C2HIS
          201                                                          250
hVEGF-C   PLSQGPKPVT ISFANHTSCR CMSKLDVYRQ VHSIIRRSLP .ATLPQCQAA
hVEGF-C   PLSQGPKPVT ISFANHTSCR CMSKLDVYRQ VHSIIRRSLP .ATLPQCQAA
                                   Ab Achen 2
                                        ↓C1HIS     ↓C2HIS
hVEGF-D   PLTSVPELVP VKVANHTGCK CLPTAP..RH PYSIIRRSIQ IPEEDRCSHS
mVEGF-D   PLTSVPELVP VKIANHTGCK CLPTGP..RH PYSIIRRSIQ TPEEDECPHS

          251                                                          300
hVEGF-C   NKTCPTNYMW NNHICRCLAQ EDFMFSSDAG DDSTDGFHDI CGPNKELDEE
hVEGF-C   NKTCPTNYVW NNYMCRCLAQ QDFIFYSNVE DDSTNGFHDV CGPNKELDED
hVEGF-D   KKLCPIDMLW DSNKCKCVLQ EENPL.AGTE DHS....... .....HLQE.
mVEGF-D   KKLCPIDMLW DNTKCKCVLQ DETPL.PGTE DHS....... .....YLQE.

          301                                                          350
hVEGF-C   TCQCVCRAGL RPASCGPHKE LDRNSCQCVC KNKLFPSQCG ANREFDENTC
hVEGF-C   TCQCVCKGGL RPSSCGPHKE LDRDSCQCVC KNKLFPNSCG ANREFDENTC
hVEGF-D   .......... .......... .......... .....PALCG PHMMFDEDRC
mVEGF-D   .......... .......... .......... .....PTLCG PHMTFDEDRC

          351                                                          400
hVEGF-C   QCVCKRTCPR NQPLNPGKCA C.ECTESPQK CLLKGKKFHH QTCSC.....
hVEGF-C   QCVCKRTCPR NQPLNPGKCA C.ECTENTQK CFLKGKKFHH QTCSC.....
                                   Ab Achen 3
hVEGF-D   ECVCKTPCPK DLIQHPKNCS CFECKESLET CCQKHKLFHP DTCSCEDRCP
mVEGF-D   ECVCKAPCPG DLIQHPENCS CFECKESLES CCQKHKIFHP DTCSCEDRCP

                                                  ↓C3HIS
          401                                                          
hVEGF-C   .YRRPCTNRQ KACEPGFSYS EEVCRCVPSY WKRPQMS*
hVEGF-C   .YRRPCANRL KHCDPGLSFS EEVCRCVPSY WKRPHLN*
hVEGF-D   FHTRPCASGK TACAKHCRFP KEKRAAQGPH SRKNP*..
mVEGF-D   FHTRTCASRK PACGKHWRFP KETR.AQGLY SQENP*..
```

# PLOTSTRUCTURE of hVEGF-D

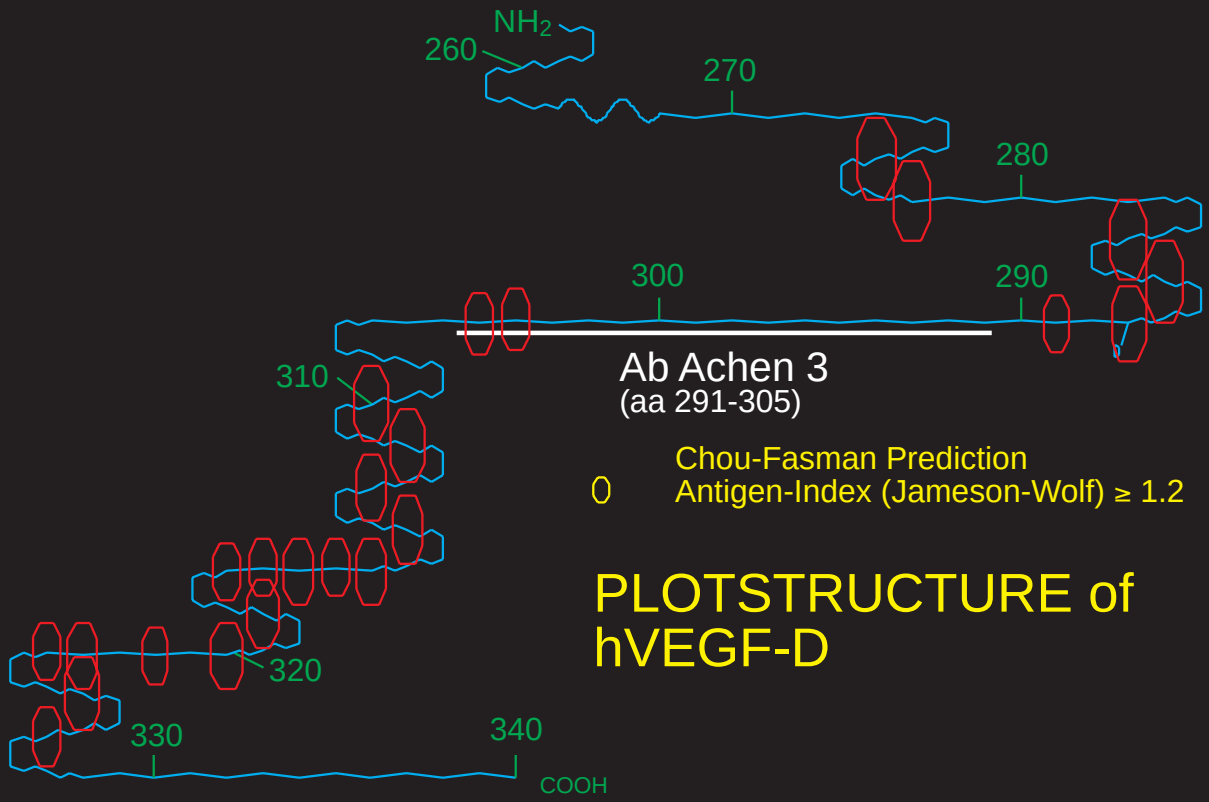
Chou-Fasman Prediction

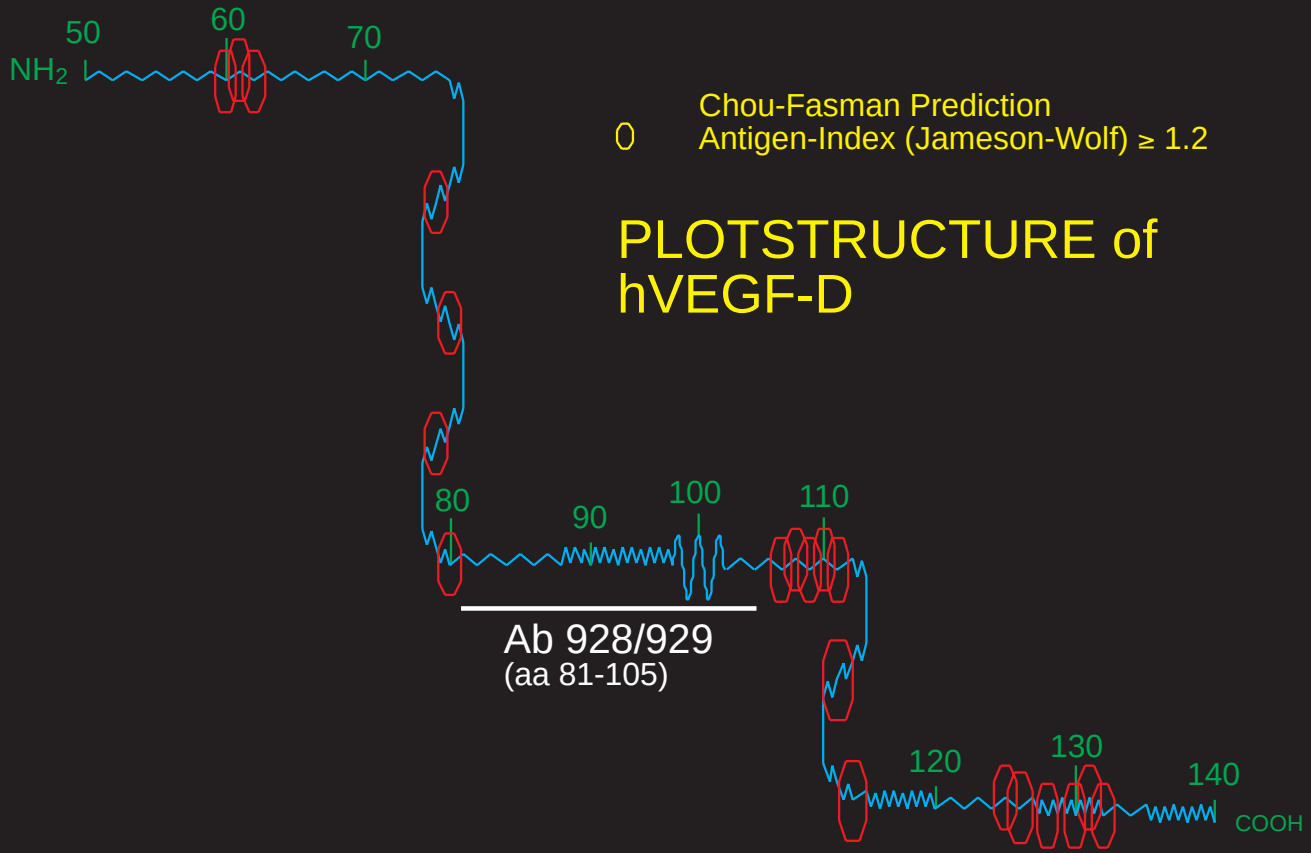
Antigen-Index (Jameson-Wolf) ≥ 1.2

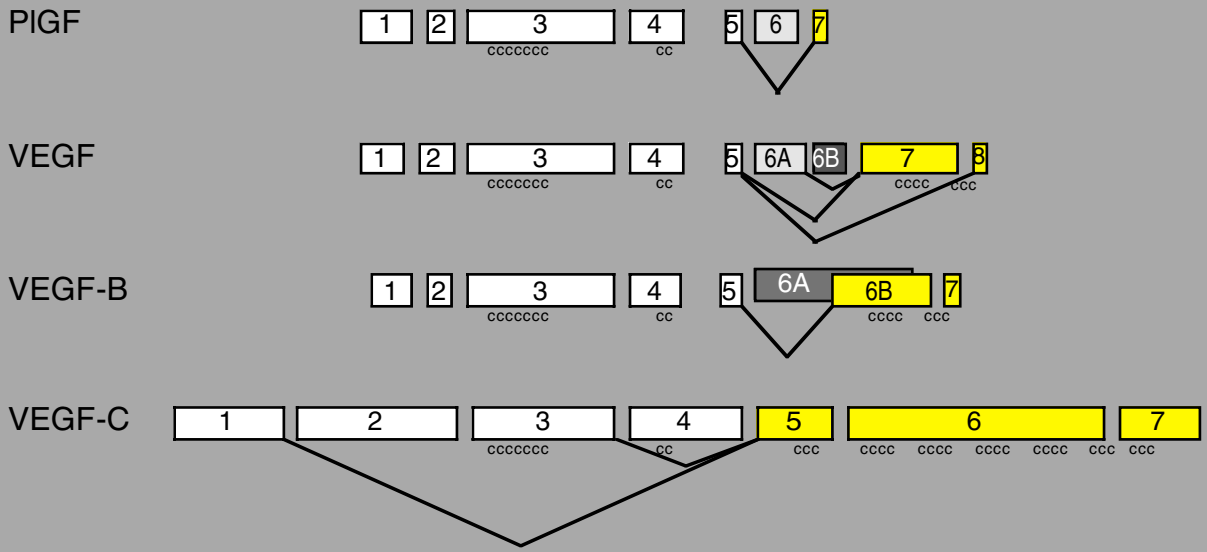




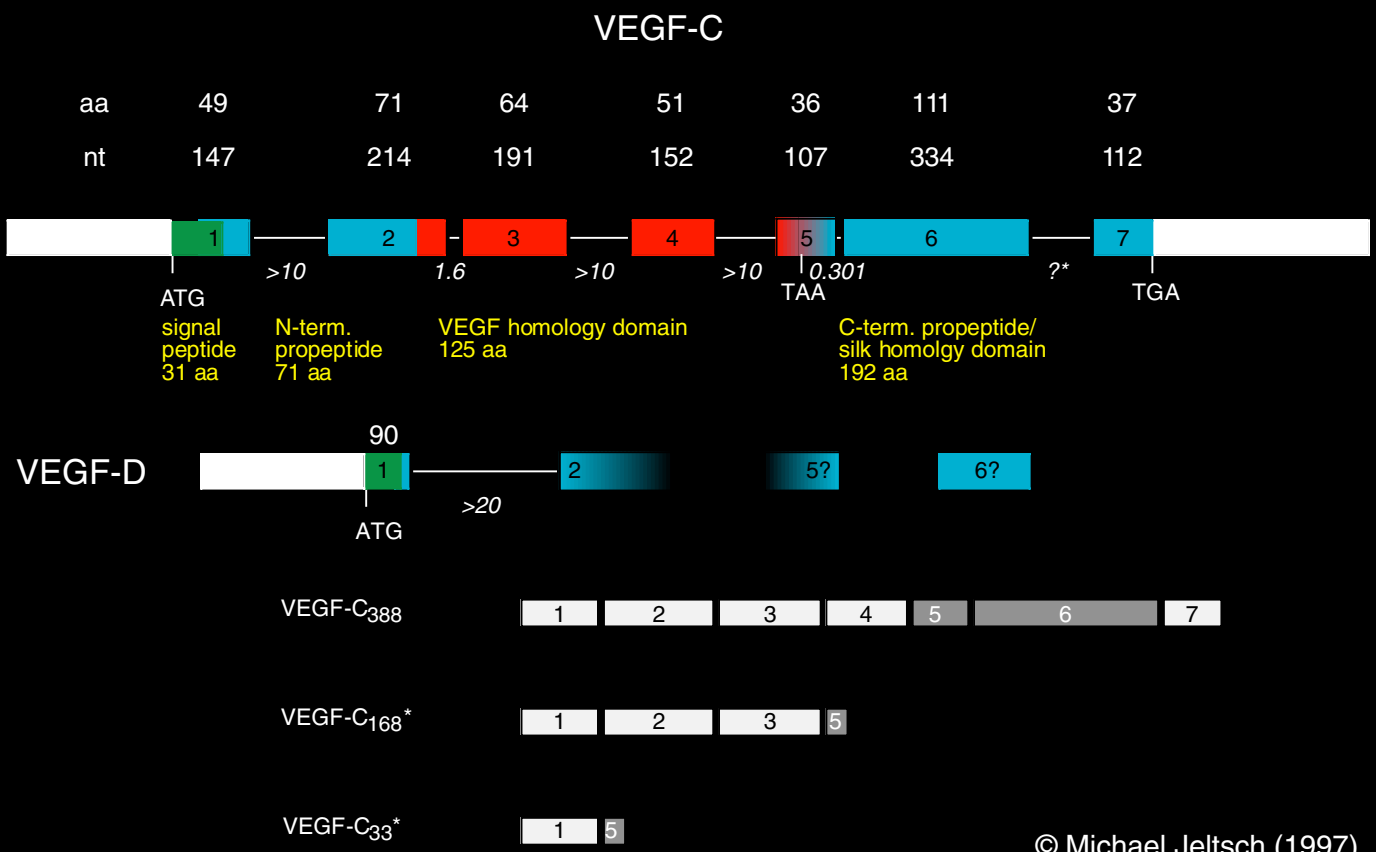

# PLOTSTRUCTURE of hVEGF-D








| | |
|---|---|
| cccccccc cc | 8 conserved cysteine residues of the cystine knot consensus sequence (PDGF family subtype): C(24-26X)C(5X)CXXCC(6X)C(32-36)CXC |
| cccc | 4 conserved cysteines of the BR3P consensus sequence: C(10-14X)CXCXC |
| ccc | 3 conserved cysteines of an incomplete BR3P consensus sequence: C(10-14X)CXC |

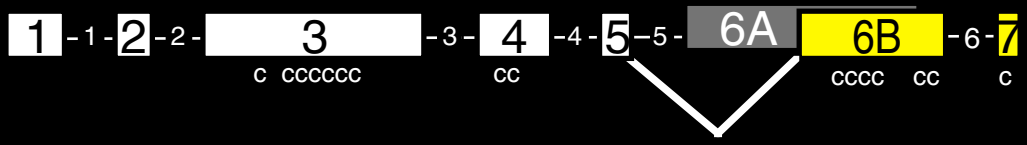

VEGF-C
aa 49 71 64 51 36 111 37
nt 147 214 191 152 107 334 112
1 2 3 4 5 6 7
>10 1.6 >10 >10 0.301 ?*
ATG
TAA
TGA
signal peptide 31 aa
N-term. propeptide 71 aa
VEGF homology domain 125 aa
C-term. propeptide/ silk homolgy domain 192 aa
VEGF-D
90
1 2 5? 6?
>20
ATG
VEGF-C388
1 2 3 4 5 6 7
VEGF-C168*
1 2 3 5
VEGF-C33*
1 5
© Michael Jeltsch (1997)

## Organization of the human VEGF-B gene

| *mistake* | *Grimmond et al.* | *Olofsson et al.* | *Dima's genomic VEGF-B clone* |
|---|---|---|---|
| length of intron 1 | 564 bp | ~760 bp | 565 bp |
| length of intron 2 | 313 bp | 275 bp | 312 bp |
| exon 3-intron 3 donor | CAGgtcctgggca | CAGgtactgggca | like Olofsson's data |
| length of intron 3 | 246 bp | 244 bp | 244 bp |
| intron 4-exon 5 acceptor | tactttccagACC | tacttttcagACC | like Olofsson's data |
| exon 6B-intron 6 donor | CAGgtgaggcgtc | CAGgtaggtttgg | like Grimmond's data |
| length of intron 6 | ~800 bp | 736 bp | 700 bp |

## Additional information obtained while investigating the above mentioned differences:

- length of intron 4: 756 bp ± 2 bp
- length of intron 5: 197 bp (186) and 298 bp (167)
- mistakes in promoter sequence: a PstI site (nt 1030) and a BssHII site (nt 1084) are present in the promoter, GGG instead of GG at nt 1040



# Copy number

- Founder mice (F0) ⇒ apparent copy number reflects both actual copy number per diploid genome and the fraction of cells containing the transgene; approx. 20-30% of all founders are believed to be mosaics (Wilkie et al., 1986)
- Apparent copy number of founder mice has to be confirmed by Southerns of the F1 and F2 generation, before PCR analysis alone is sufficient
- Southern blot (evaluation by eye in comparison with a standard curve) or dot blot (szintillation counting)
- Copy number does often, but not always correlate with transgene expression level! ⇒ quantitation of transgene product or phenotype
- PCR analysis is prone to produce false positives, whereas Southern and dot blot analysis is prone to produce false negatives, especially with low copy numbers
- Determination of DNA concentration (RNA, uncompletely dissolved DNA; measurement after digestion is more accurate then before digestion due to the high viscosity of undigested DNA; duplicate measurements are recommended)
- Internal standard is possible by probing for an endogenous gene (any single copy gene)
- Southern blot results are not as reliable than dot blot results (more handling steps increase variance)
- Controls: Standard curve of 1, 10 and 100 copies of the TGU mixed with neg. tail DNA

# Homozygocity

- Homozygocity is reached **b**y **b**ackcrossing and this term should not **b**e used for mice **w**ho inherited the transgene from t**w**o different founders; most "heterozygous" transgenes are hemizygous
- Approx. 5-15% of all transgene integration events produce recessive lethal mutations (Hogan et al., 1994)

## Identifying homozygous mice

*Methods requiring the knowlegde of the genomic flanking sequences (require integration into non-repetitive DNA)*

1. PCR **w**ith flanking primers
2. Southern **b**lot **w**ith a genomic flanking pro**b**e

*Methods not requiring the knowledge of the genomic flanking sequences*

1. Test **b**reeding
2. In situ hy**b**ridization to interphase nuclei
3. Transgene quantitation **b**y Southern or dot **b**lot
4. Transgene product quantitation

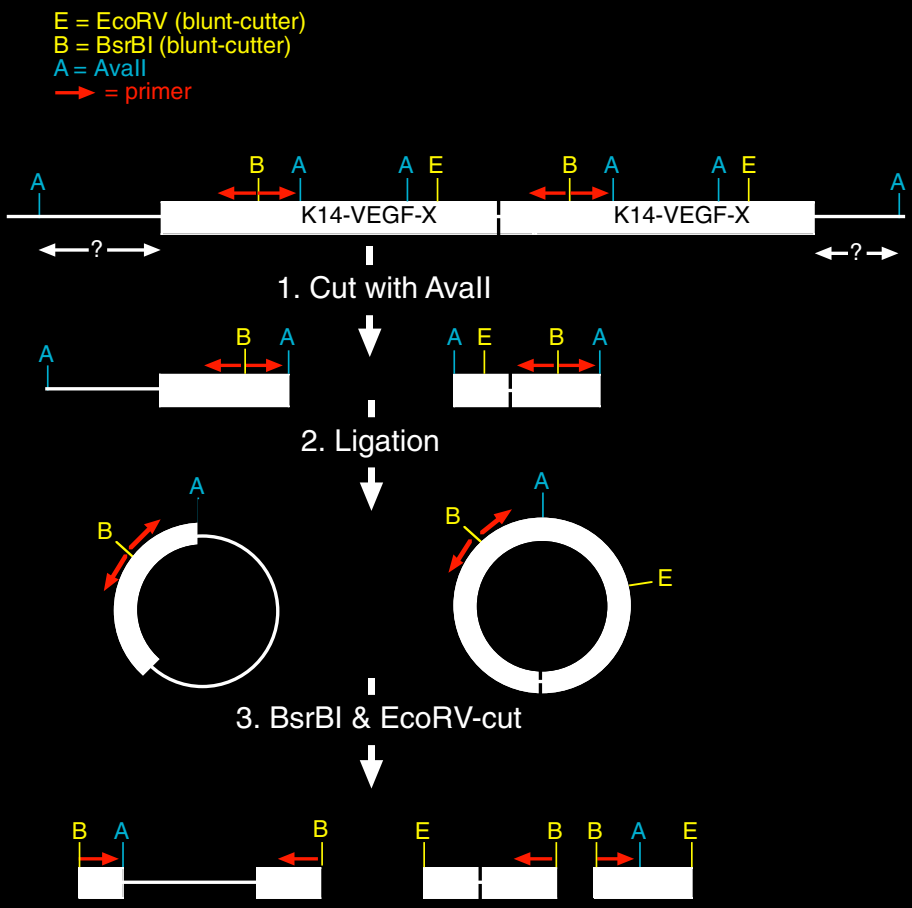
## Identifying mice with transgenes from two different founders

*Methods requiring the knowlegde of the genomic flanking sequences*

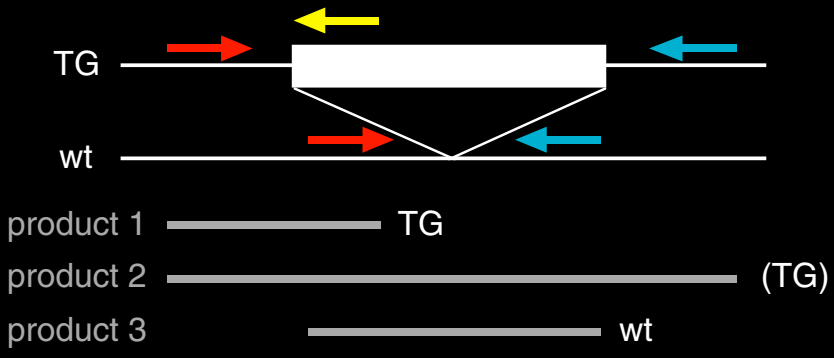
1. PCR with flanking primers
2. Southern blot with a genomic flanking probe

*Methods not requiring the knowledge of the genomic flanking sequences*

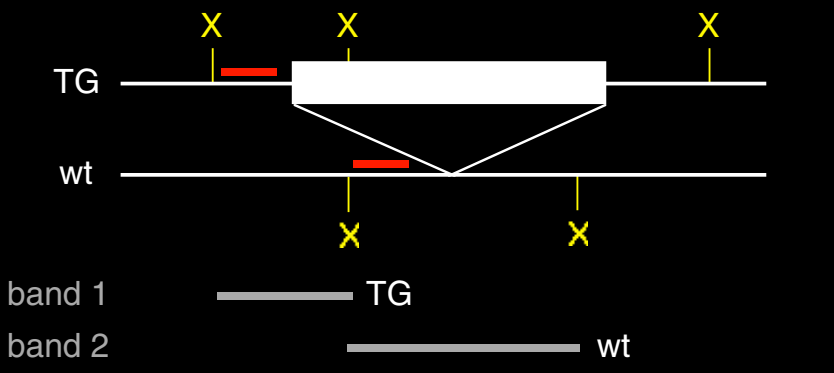
1. Southern blot and junction fragments: first and last fragments of a transgene array are often not suitable to identify transgenics with two integration sites
2. Test breeding
3. In situ hybridization to interphase nuclei
4. Transgene quantitation by Southern or dot blot: only possible, if the copy number of the two different transgenes is similar



## PCR




## Southern blot



# Expression pattern of VEGF-C and VEGF-D: A comparison

| | *hVEGF-D* | *mVEGF-D* | *hVEGF-C* | *mVEGF-C* |
|---|---|---|---|---|
| *transcript* | *2.2 kb* | *2.4 & 3.7 kb* | *2.0 &2.4 kb* | *2.0 & 2.4 kb* |
| *source(s)* | *Yamada et al., 1997* | *Yamada et al., 1997* | *Joukov et al., 1996* | *Kukk et al., 1996* |
| *lung* | +++ | +++ | +++ | +++ |
| *heart* | +++ | + | +++ | +++ |
| *small intestine* | ++ | n.d. | +++ | n.d. |
| *skeletal muscle* | +++ | + | + | + |
| *colon* | ++ | n.d. | + | n.d. |
| *pancreas* | + | n.d. | + | n.d. |
| *kidney* | - | + | - | + |
| *liver* | + | n.d. | - | + |
| *placenta* | - | n.d. | +++ | n.d. |
| *brain* | - | + | - | + |
| *PBL* | - | n.d. | - | n.d. |
| *ovary* | + | n.d. | +++ | n.d. |
| *testis* | + | + | + | - |
| *prostate gland* | + | n.d. | + | n.d. |
| *thymus* | - | n.d. | - | n.d. |
| *spleen* | + | + | + | - |
| *fetal kideny* | + | n.d. | +++ | n.d. |
| *fetal liver* | - | n.d. | - | n.d. |
| *fetal lung* | +++ | n.d. | +++ | n.d. |
| *fetal brain* | - | n.d. | - | n.d. |