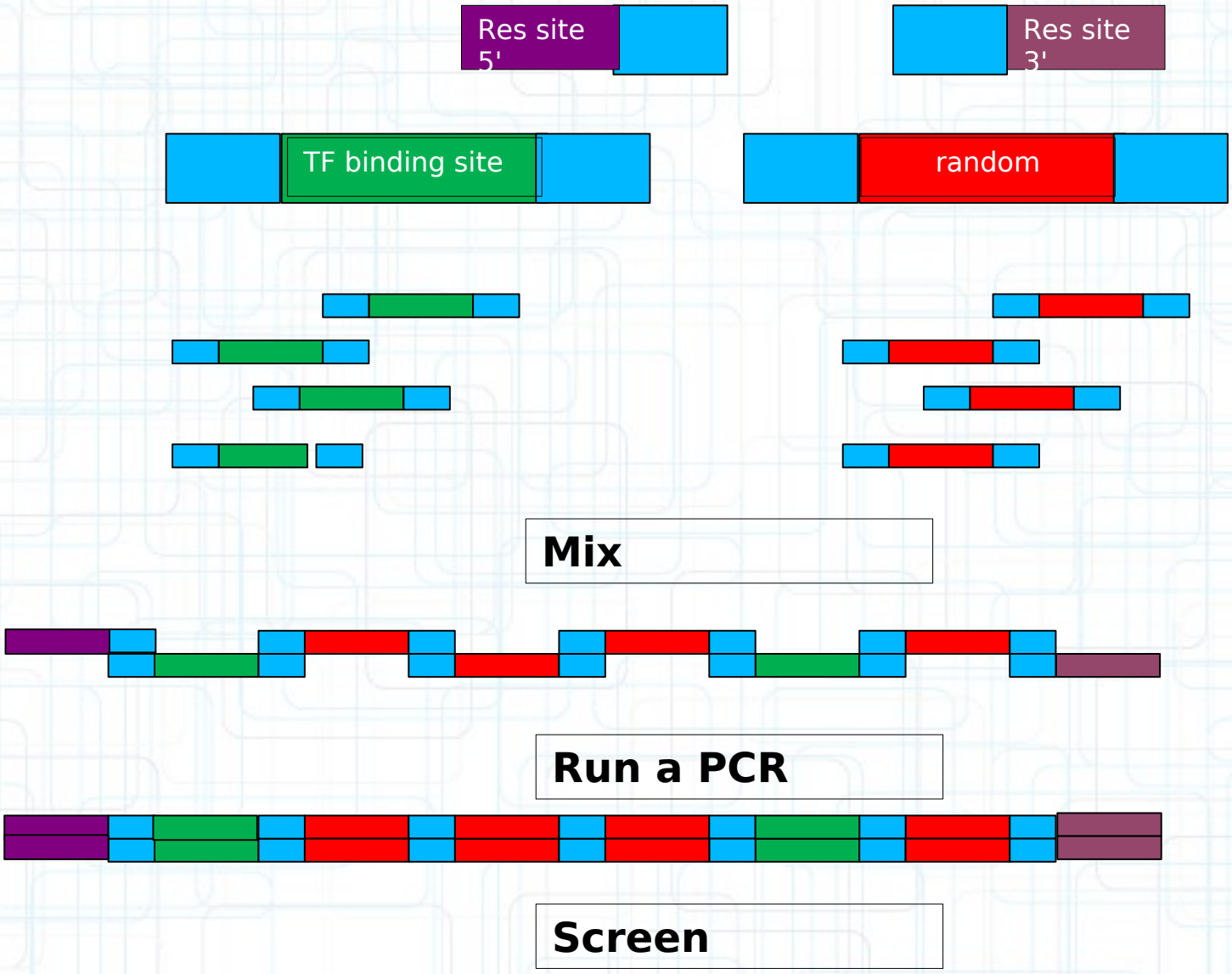
Project standing 9-8

Cell culture

- HeLa/ MCF-7 issue
  - HeLa cells don't like effectene
  - HeLas react too fast to drugs
- Stable cell line
  - One clone of Hela cells
  - Several clones for U2-OS
  - Restarted MCF-7
- Characterization of integration site
  - Meeting with Manfred Schmidt at 2 pm today

Synthetic P.: ROE-PCR



ROE-PCR: 1x 2x 3x promoters

1st set of synthetic promoters of our own making!

CONST

Const L 1

ACTAgtGGGTGACGGGTTCACCCCTGAAACGGGCGATCGGCAGATCAGGGGACTTTGCCGGGTGACGGGTTCAATGACCGATCAGC
GATCGGCAGATCAGGGGACTTTGCCGGGTGACGGGTTCACTCAACAGACAGCGATCGGCAGATCATCCAGTGACGTCAGGGTGAC
GGGTTCACCGCATACAGCGCGATCGGCAATCACTGAGTGACGTCAGGGTGACGGGTTCACTAAGCTT

Const L 4

ACTAGTGGGTGACGGGTTCAGCTGTGCGTGGGCGATCGGCAGATCAGGGGACTTTGCCGGGTGACGGGTTCACAGCTTAGTCAGC
GATCGGCAGATCAGGGGATTTCCCGGGTGACGGGTTCACGTTACACGGCGCGATCGGCAGATCATGACTCAGGGTGACGGGTT
CAGTTGGGACCATGCGATCGGCAGATCATGACTCAGGGTGACGGGTTCACTAAGCTT

const L 5

ACTAGTGGGTGACGGGTTCAGGTCCACACACGCGATCGGCAGATCACTTATTGACGTCAGGGTGACGGGTTCACTAAGCTT

const S 5

ACTAGTGGGTGACGGGTTCATATTGTTAAAGGCGATCGGCAGATCAGGGGGTCCCCCGGgtGACGGGTTCACTTAGTCAGGTGCGA
TCGGCAGATCATGACTCAGGGTGACGGGTTCACTAAGCTT

const S 10

ACTAGTGGGTGACGGGTTCACTAGCGCAAACGCGATCGGCAGATCAGGGGATCTCCCGGGTGACGGGTTCAGGGCAGTCTGAGC
GATCGGCAGATCATTGACTGACGTCAGGgtGACGGGTTCAACATGCGTAGCGCGATCGGCAGATCAGGGGCGGGGCGGGTGACGG
GTTCACACATGCTTTGGCGATCGGCAGATCAGATGGTGACGTCAGGGTGACGGGTTCAATTAACTCCCAGCGATCGGCAGATCAGG
GGACTTTGCCGGGTGACGGGTTCATAGACTAACGGGCGATCGGCAGATCATGACTCAGGGTGACGGGTTCACTAAGCTT

const S 4

ACTAgtGGGTGACGGGTTCATTTGACAAGATGCGATCGGCAGATCATTTGTTGACGTCAGGGTGACGGGTTCACATACACACAGGCGA
TCGGCAGATCAGGGGAACCCCCGGGTGACGGGTTCACAACCAGGgATGCGATCGGCAGATCAGTTATTGACGTCAGGGTGACGGGTT
CACTAAGCTT

Ap1 NfkB CREB Random empty Sp1

ROE-PCR: Inducible Promoters

ROE-PCR: Screening

- ✔ Two promising candidate for p53 inducible promoters
- ✗ Need to optimize conditions for HIF screening (HeLa die too fast)
- ✗ Protocol is very sensitive to small variations in oligo concentration
  - ➔ Remade p53 and NfkB inducible promoters with different concentrations

ROE-PCR: Screening

HIF

HIF L 8

ACTAGT**GGGTGACGGGTTCA**Tgtagtct ATT***GCGATCGGCAGAT***CATCTGTACGTGACCACA**GGGTGACGGGTTCA**AAATGTagagt***GCGAT***
CGGCagaTCATcTgTACgtgCCACAGggtnaCGGGTtcacCTATATAAGC***GCGATcGGCAGAT***CAGTCTACgTGCGgaC**GGgtgacGGGtTCA**CActgaT
GCAcGACGGaTn***GCGATCGGcaGaT***CAGTCTACGTGCGGAC**GGGTGACGGGTTCAc**tAAGCTT

HIF S 23

ACTAgt**GGgtGACGGGTTCA**AAACCAGGCAC***GCGATCGGCAGAT***CATCTGTACGTGACCACA**GGGTGACGGGTTCA**AATTCGGCCAC***G***
CGATCGGCAGATCATCTGTACGTGACCACA**GGGTGACGGGTTCA**CCCACCATTGA***GCGATCGGCAGAT***CATCTGTACGTGACCACA**GG**
GTGACGGGTTCAACCGACGAAA***GCGATCGGCAGAT***CAGTCTACGTGCGGC**GGGTGACGGGTTCA**CTAAGCTT

HIF S 3

ACTagt**GGGTGACGGGTTCA**CACTGATGCACGACGGATC***GCGATCGGCAGAT***CATCTGTACGTGACCACA**GGGTGACGGGTTCA**CACT
GATGCACGACGGATC***GCGATCGGCAGAT***CACCTCCTGACGTCA**GGGTGACGGGTTCA**AATCAACGGCC***GCGATCGGCAGAT***CATCTGTA
CGTGACACA**GGGTGACGGGTTCA**CTAAGCTT

Ap1
HIF
CREB
Random
empty
Sp1
NFY

ROE-PCR: Screening

p53

p53 S 8
ACTAgtGGGTGACGGGTTCACTAAGCTT

p53 S 12
ACTAgt**GGGTGACGGGTTCA**TCTCACGCTGC***GCGATCGGCAGAT***CATGACTCA**GGgtGACGGGTTCA**GCACCATCATA*GCGATCGGCAG*** ***AT***CAGAACATGTCTGACATGCTG**GGGTGACGGGTTCA**ACACGTTATTT***GCGATCGGCAGAT***CATTCCAATTG**GGGTGACGGGTTCA**CT
AAGCTT

p53 S 23
ACTAGTG**GgtGACGGGTTCA**GATGGCCAAAA***GCGATCGGCAGAT***CATGACTCAG**GGTGACGGGTTCA**TTGCACAAGCA*GCGATCGGCA*** ***GAT***CACGTACTGACGTCA**GGGTGACGGGTTCA**CAGCCTATCGC***GCGATCGGCAGAT***CATACCAATTCG**GGTGACGGGTTCA**CTAAGCT
T

p53 S 24
ACTAGTG**GGTGACGGGTTCA**CTTGTACAGGATCGTACGAC***GCGATCGGCAGAT***CATGACTCAG**GGTGACGGGTTCA**TCGCAG***GCGAG*** ***CGATCGTCAT***CAGGGGCGGGGCG**GGTGACGGGTTCA**AGGCGTGGCCA***GCGATCGGCAGAT***CATTCATTGACGTCA**GGGTGACGGGTT**
CATGAAGCTGCGC***GCGATCGGCAGAT***CATACCAATAGG**GGTGACGGGTTCA**CAGGAAGCATC***GCGATCGGCAGAT***CATTTCATGACGTC**
AG**GGTGACGGGTTCA**CTTGTACAGGTACGTACGAC***GCGATCGGCAGAT***CAGAAACATGTCTGTGCATGCTGG**GGTGACGGGTTCA**CT
AAGCTT

p53 L 7
ACTAgtG**GgtgACGGGtTCA**TGCCGCAGgaG***GCGATCGGCAGATC***ATTCCAATCC**GGgtGaCGGGTTCA**CTAagCTT

p53 L 19
ACTAgtG**GGTGACGGGTTCA**CttgtACAGAATCGTACGAC***GcgnTCGGCAGATCA***GAACAtgtCTgtgCATgcTG**GGgtgACGGGTTCA**ctAAGCtT

p53 L 22
ACTAgt**GGGTGACGGGTTCA**CTTgTACAGGAACGTACGAC***GCGATCGGCAGAT***CATTCCAATGG**GGGTGACGGGTTCA**CTAAGCtT

Ap 1 p53 CREB Random empty Sp 1 NFY

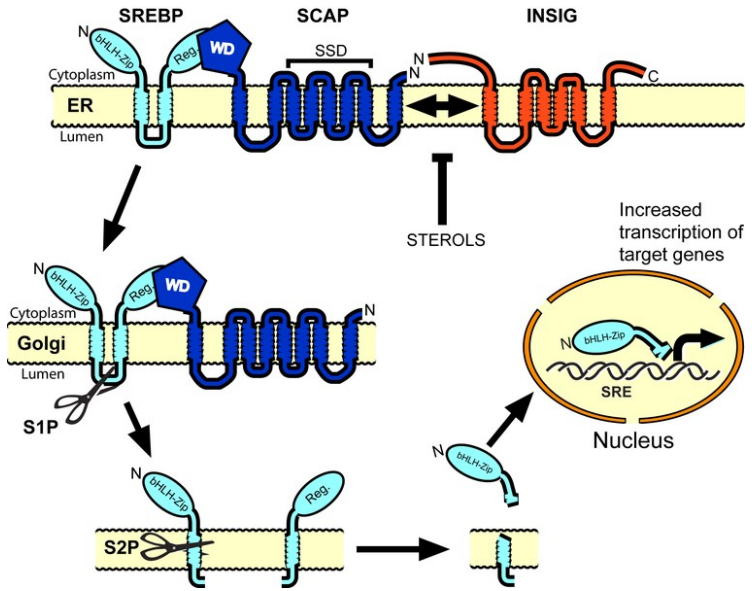
HEARTBEAT

(Heidelberg Artificial Transcription Factor Binding Site Engineering and Assembly Tool)

- HEARTBEAT contains 4395 promoter sequences with altogether 29966 entries
- allowing
  - histograms / density plot of TFBS distribution
  - combinatory appearance
  - multiple queries

Transcription Factors of Interest

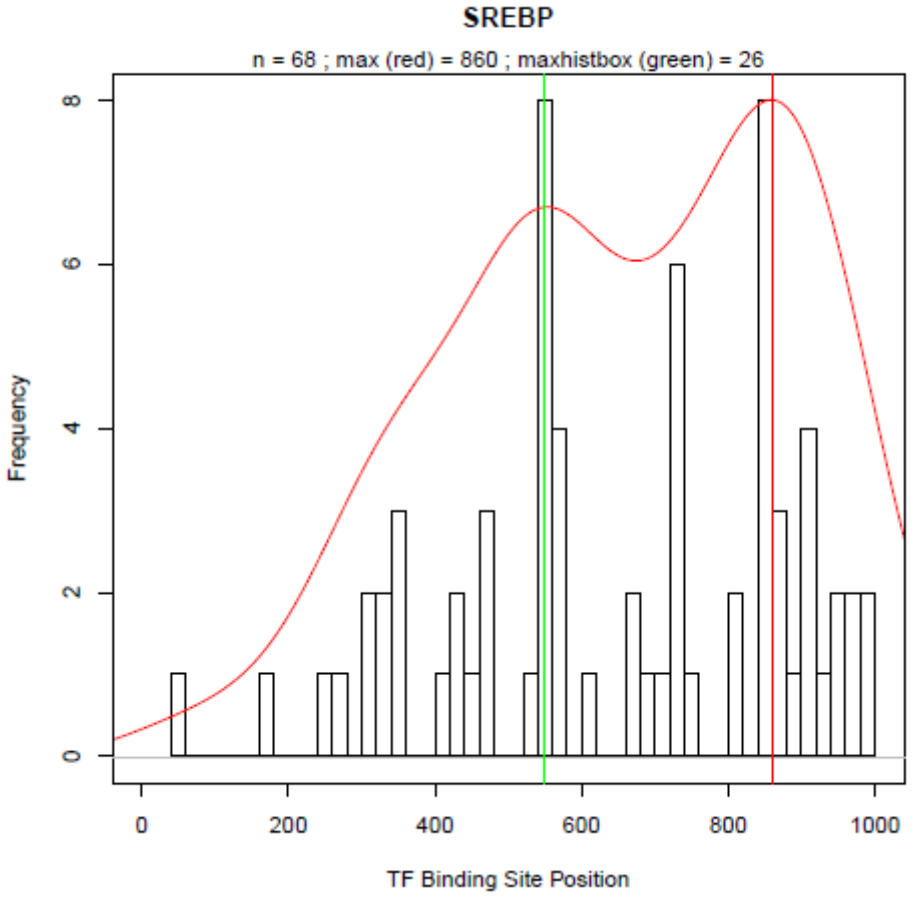
- **SREBP**
  (Sterol Regulatory Element Binding Protein)
  - upregulating sterol biosynthesis
  - negative feedback loop by sterols

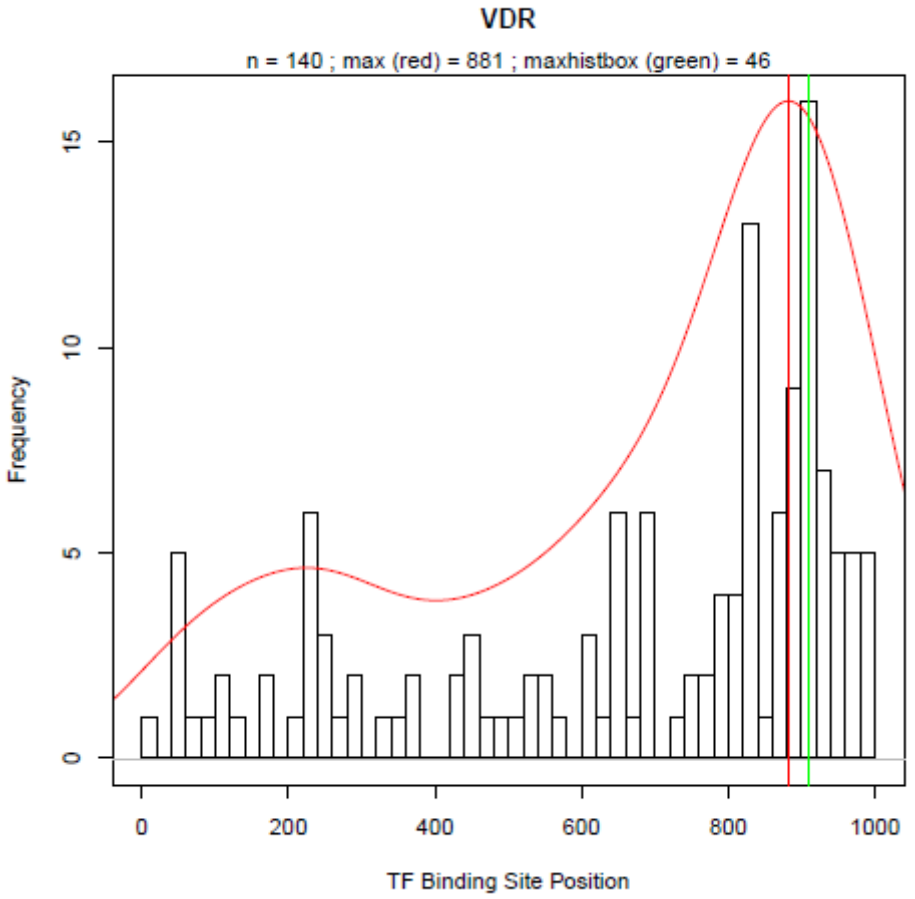


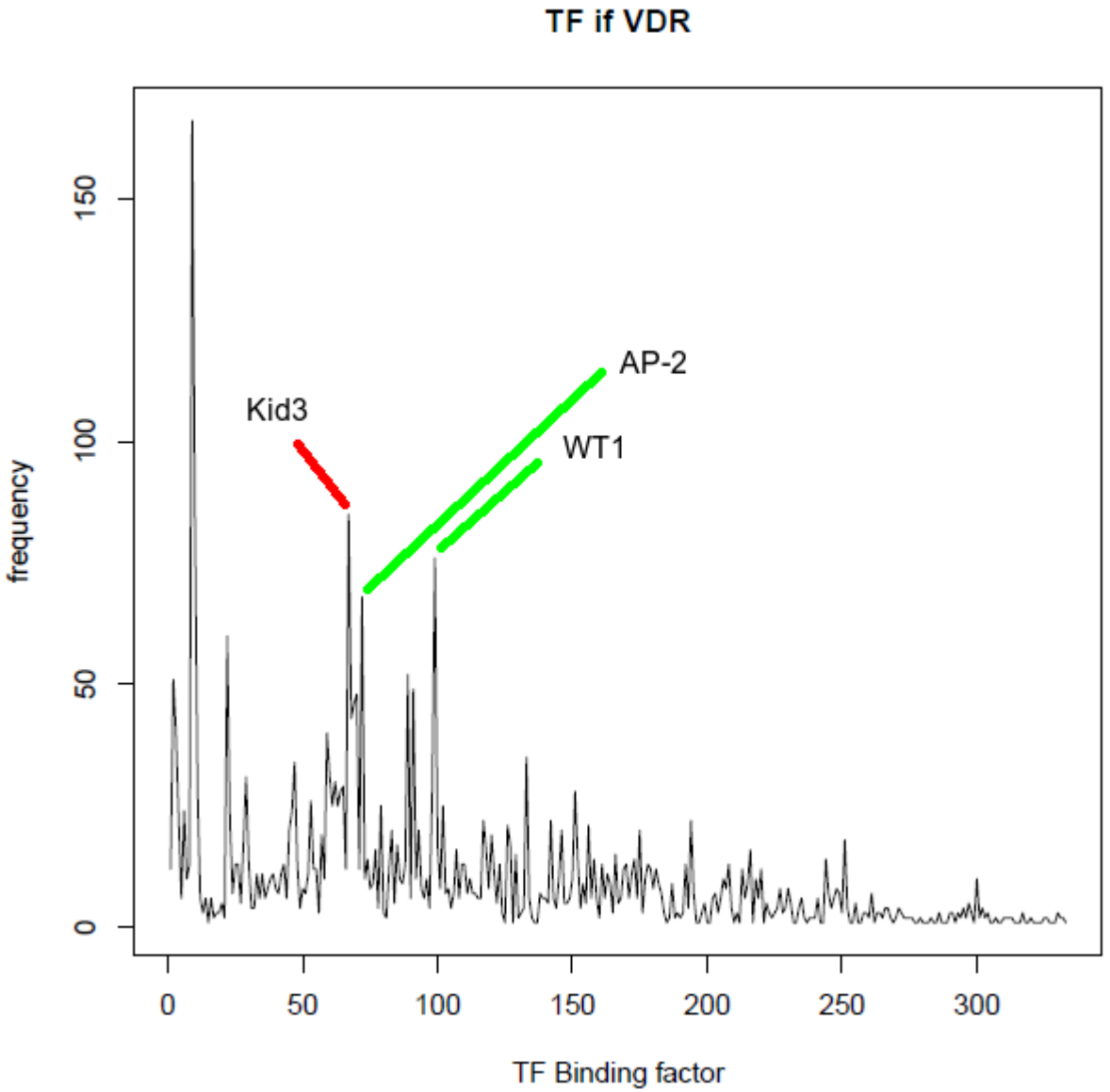
from Brown & Goldstein 1997, PMID 9150132.

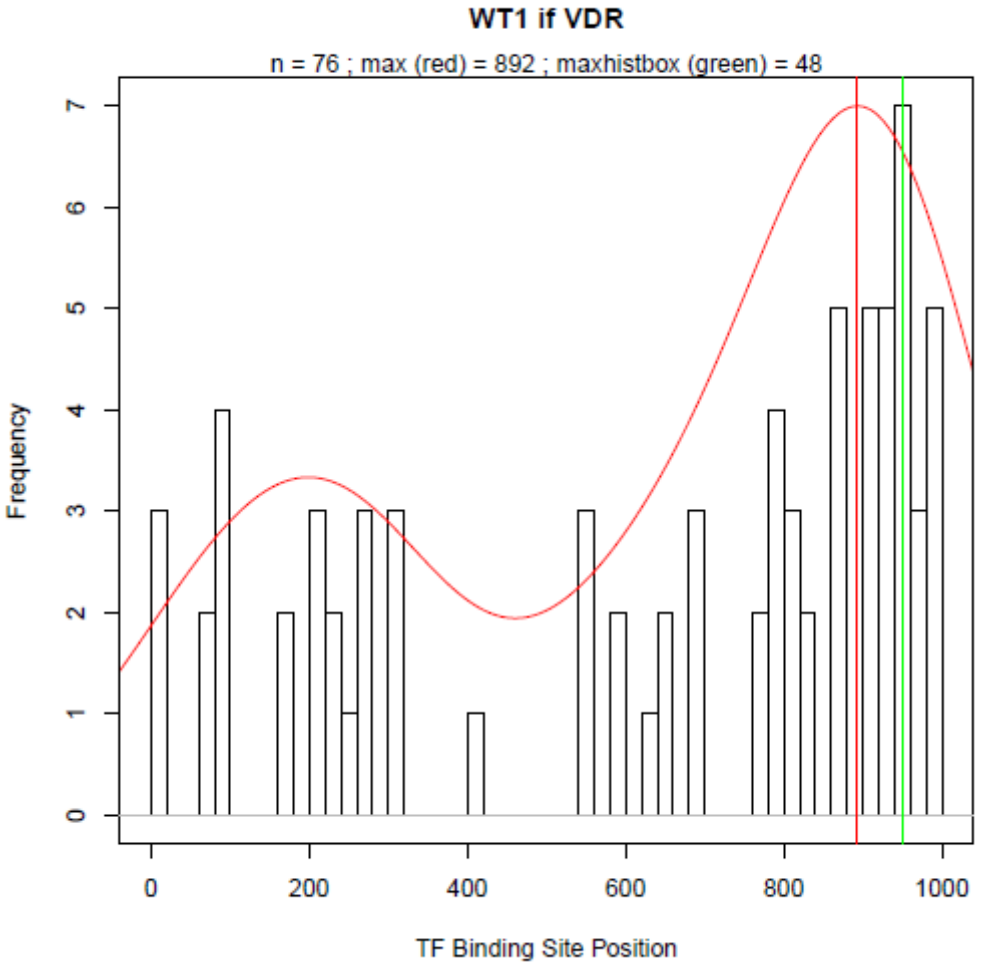
- **VDR**
  (Vitamin D receptor, calcitriol receptor, NR1I1)
  - heterodimerization with RXR (retinoid-X receptor)
  - binding to hormone response elements
  - involved in metabolic pathways:
    - mineral metabolism
    - immune response
    - cancer

Heartbeat - Output

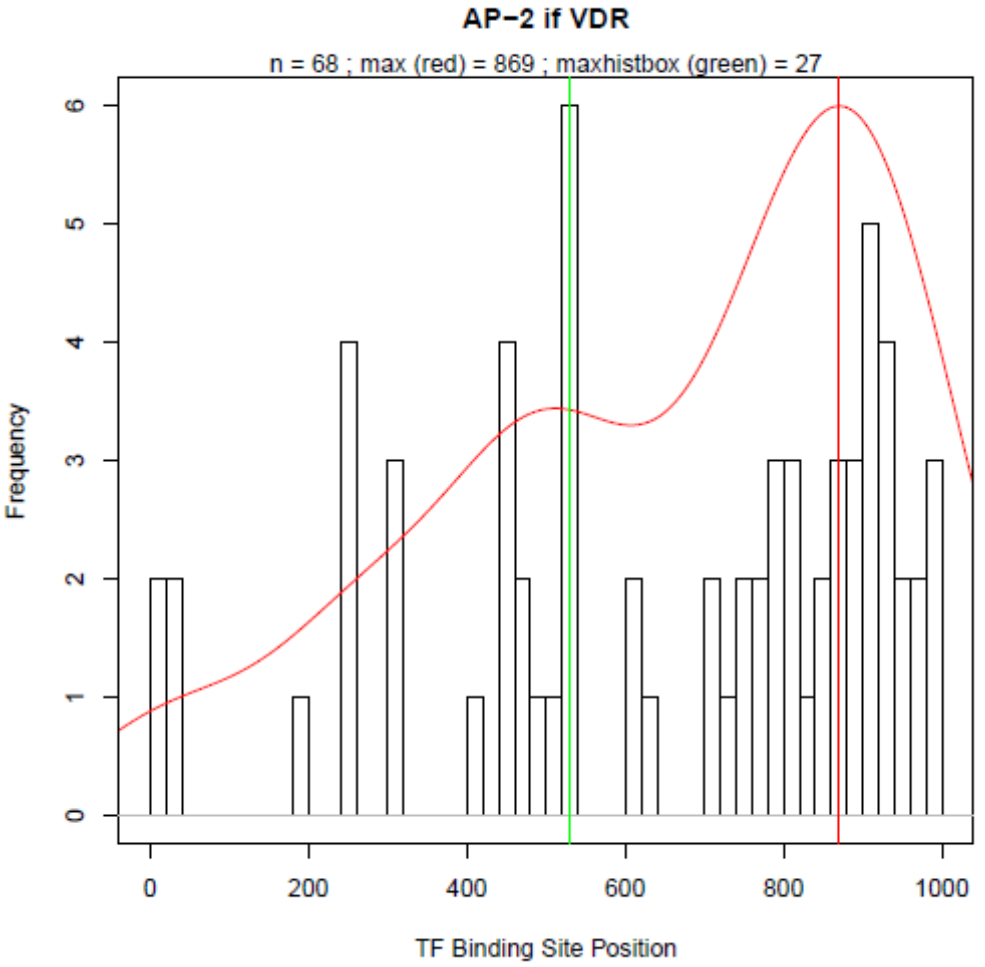





TF if VDR
frequency
0
50
100
150
Kid3
AP-2
WT1
0
50
100
150
200
250
300
TF Binding factor



AP-2 = Activating Protein 2
Regulates gene expression during early development



WT1 = Wilms tumor 1
Contributes to development of the urogenital system

The check-list:

- checking for maxima of the density curve
- searching for additional TF binding sites
- searching for restriction sites
- creation of control sequence

VDR_1.1
single TF, only one binding site

```
-172 AAAAAAATTCAGCCTGCGCTAG
-150 GGCTAGGAATAGCTGGGCAGTAAATGAAAGAAGCGCATGTAAAACTATTC
-100 CTCCGACCCGGTTAGTATCGACCGTACAGGGGAAGCAAGGACACAAGCAC
-50  ATAGTTGCGCAGGCCTACGAAGCAGAATAGACACACGGGCCGCTCCAGGG
0    TSS
```

TransFac: free
Restriction Sites: free

VDR_1.2
single TF, two binding sites

```
-172 GCGCTAGGGCTAGGAATAGCTG
-150 GGCAGTAAATGAAAGAAGCGCATGTAAAACTATTCCTCCGACCCGGTCAA
-100 GCACATAGTTGCTAGTATCGACCGTATTGGGGAAGCAAGGACAGCAGGCC
-50  TACGAAGCGACCGTATTGGGGAAGCAAGGACAGCAGGCCTACCTCCAGGG
0    TSS
```

TransFac: free
Restriction Sites: free

← the BLUE series

The GREEN series →

VDR_3.3
triple TF: VDR + AP2 + WT1
(WT1: additional 200 = -800 -> -738)

```
-800 TCTCACAGAAAAAAATTCAGCCTGCGCTAGGGCTAGGAATAGCTGGGCAG
-750 TAAATGGCGAGCCCTCCTCCAGCGCATGTAAAACTATTCCTCCGACCCGG
-700 TTAGTATCGACCGTACACAAGCACATAGTTGCGCAGGCCTACGAAGCAGA
-650 ATAGACACACGGGCCGCTCCAGGGTAGTTATTTGATCCTCAGTTCGCTAT
-600 CTCACAGAAAAAAATTCAGCCTGCGCTAGGGCTAGGAATAGCTGGGCAGT
-550 AAATGAAAGAAGCGCATGTAAAACTATTCCTCCGACCCGGTTAGTATCGA
-498 CCGTACACAAGCACATAGTTGCGCAGGCCTACGAAGCAGAATAGACACAC
-450 GGGCCGCTCCAGGGTAGTTATTTGATCCTCAGTTCGCTATCTCACAGAAA
-400 AAGCCCCCTGGCGGCGCTAGGGCTAGGAATAGCTGGGCAGTAAATGAAAG
-350 AAGCGCATGTAAAACTATTCCTCCGACCGACCGTATTGGGGAAGCAAGGA
-300 CAGCAGGCCTACTCGACCGTACACAAGCACATAGTTGCGCAGGCCTACGA
-250 AGCAGAATAGACACACGGGCCGCTCCAGGGTAGTTATTTGATCCTCAGTT
-200 CGCTATCTCACAGAAAAAAATTCAGCCTGCGCTAGGGCTAGGAATAGCTG
-150 GGCAGTAAATGAAAGAAGCGCACGACCGTATTGGGGAAGCAAGGACAGCA
-100 GGCCTACGTTGCTAGTATAAGAAGCGCACGAAGCCCCCTGGCGCCTACGA
-50  TGCGAAGCGACCGTATTGGGGAAGCAAGGACAGCAGGCGAGCCCCCCTGC
0    TSS
```

TransFac: free
Restriction Sites:

VDR_2.3.1
double TF, additional AP-2 at 869 = -131

```
>V$AP2_Q6
>consensus:MKCCCSCNGGCG

-412 TATCTCACAGAA
-400 AAAAATTCAGCCTGCGCTAGGGCTAGGAATAGCTGGGCAGTAAATGAAAG
-350 AAGCGCATGTAAAACTATTCCTCCGACCGACCGTATTGGGGAAGCAAGGA
-300 CAGCAGGCCTACTCGACCGTACACAAGCACATAGTTGCGCAGGCCTACGA
-250 AGCAGAATAGACACACGGGCCGCTCCAGGGTAGTTATTTGATCCTCAGTT
-200 CGCTATCTCACAGAAAAAAATTCAGCCTGCGCTAGGGCTAGGAATAGCTG
-150 GGCAGTAAATGAAAGAAGCGCACGACCGTATTGGGGAAGCAAGGACAGCA
-100 GGCCTACGTTGCTAGTATAAGAAGCGCACGAAGCCCCCTGGCGCCTACGA
-50  TGCGAAGCGACCGTATTGGGGAAGCAAGGACAGCAGGCCTACCTCCAGGG
0    TSS
```

TransFac: free
Restriction Sites: free

← The VIOLET series

Outlook

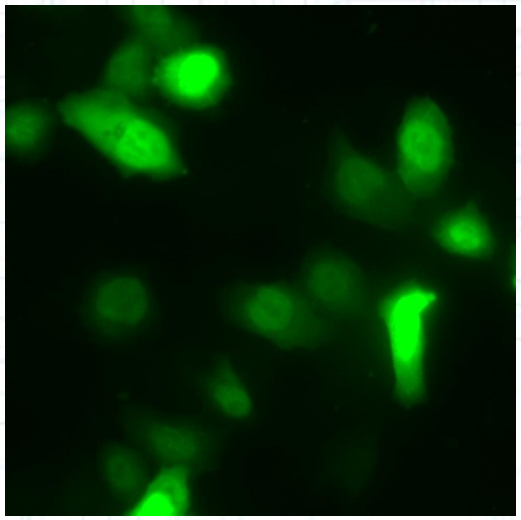
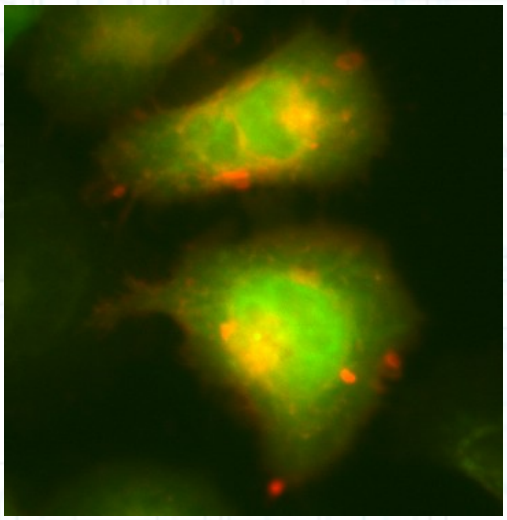
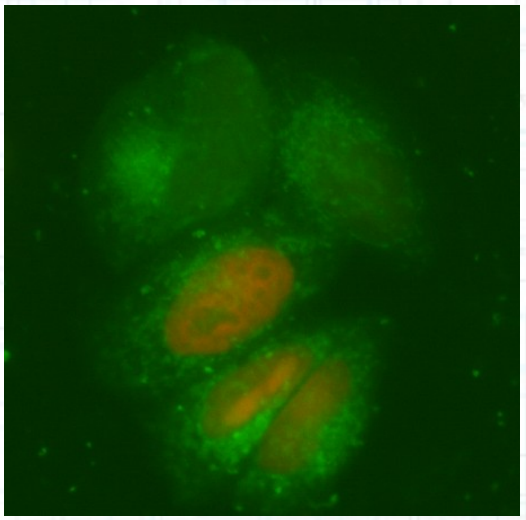
- Development of a GUI which provides all applications to get a synthetic promoter sequence
- Modify Heartbeat_DB
    - longer Sequences
    - check for systematic errors
- Modelling promoter activity

Natural promoters

- **Cyp1A1** for gDNA
- **NFkB** from Oakes group – *Ready for test digest today*
- **HSP70**
- **NFAT** – *plasmid obtained, waiting for primers*
- Other promoters ordered from Addgene

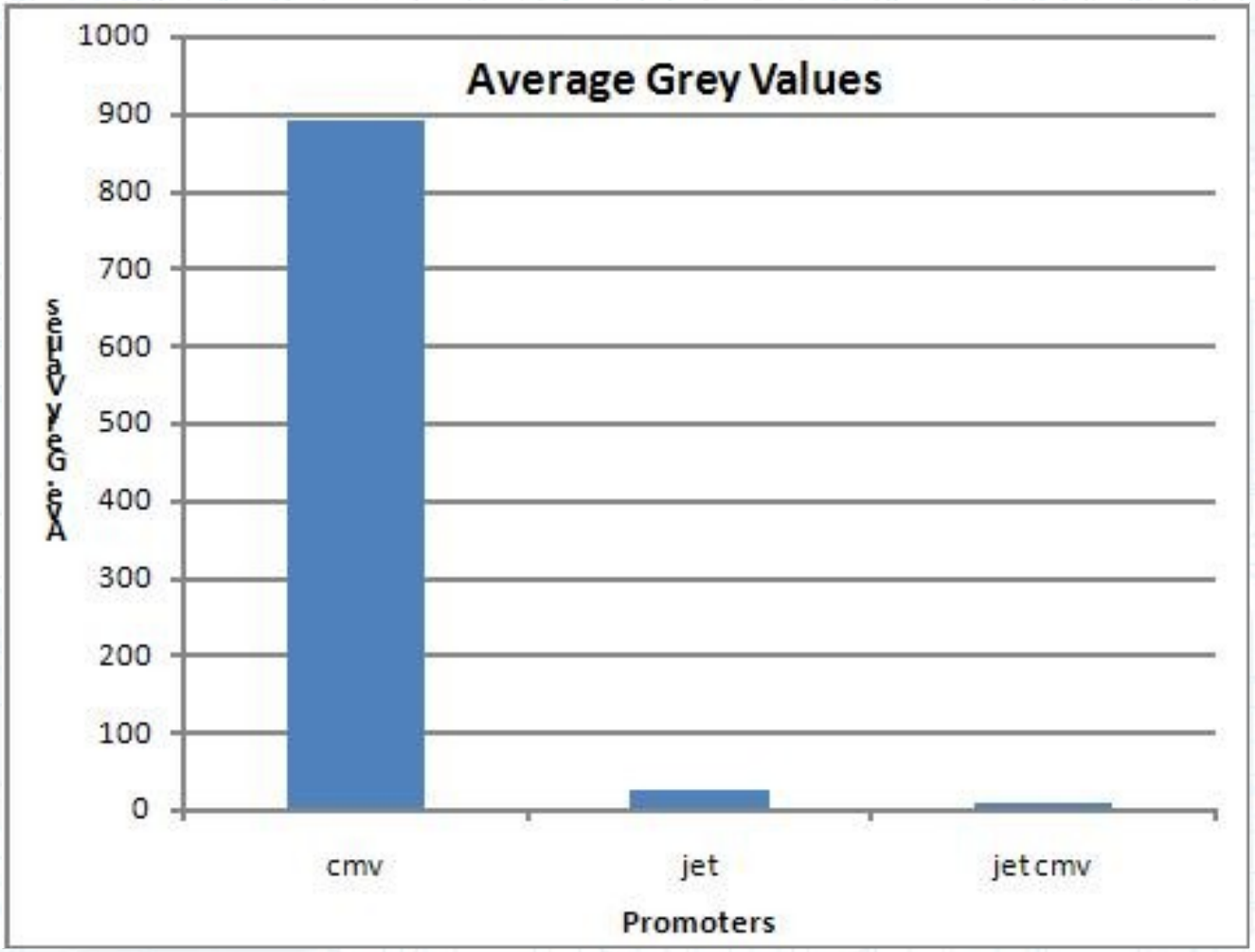
Measurement: Microscopy

- Nikon Eclipse 90i upright automated microscope in Nikon imaging center
- Samples are fixed using 4% Pfa
- Comparative analysis using ImageJ

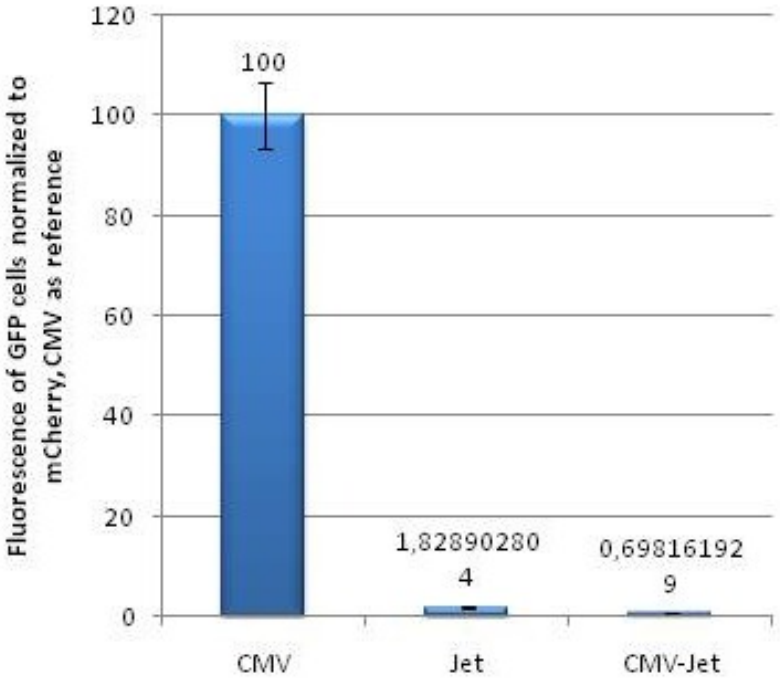
Measurement: Microscopy

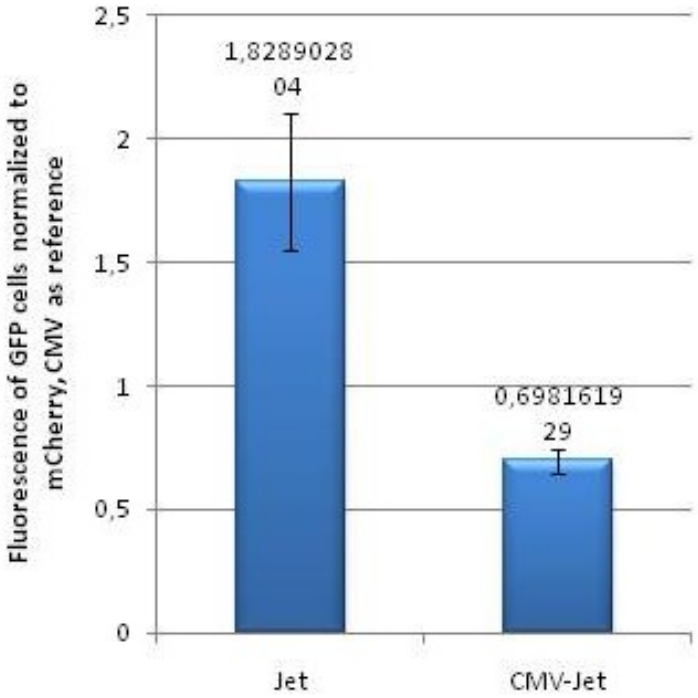
Initial results



Measurement: FACS

CMV as reference

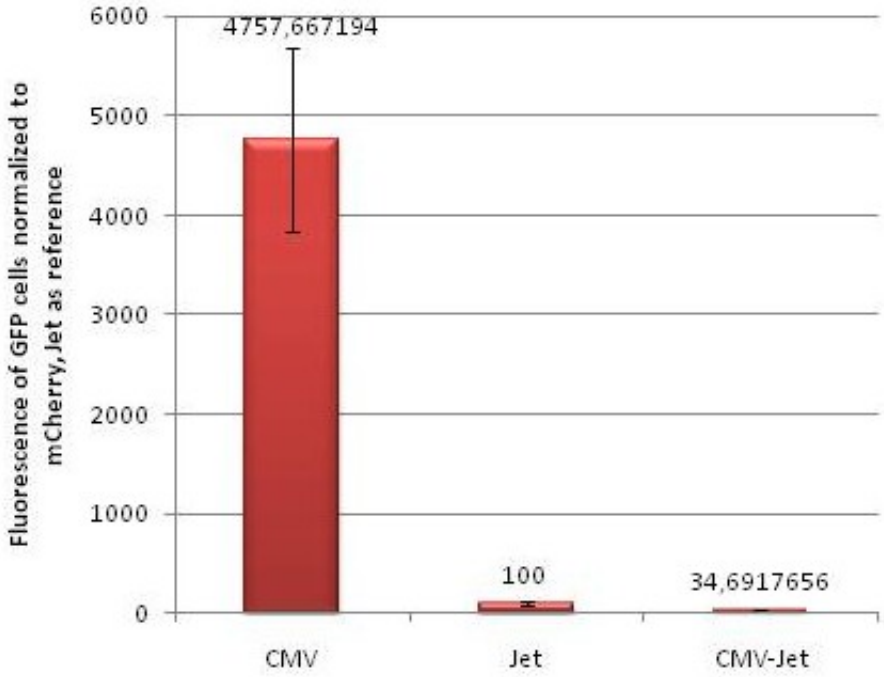


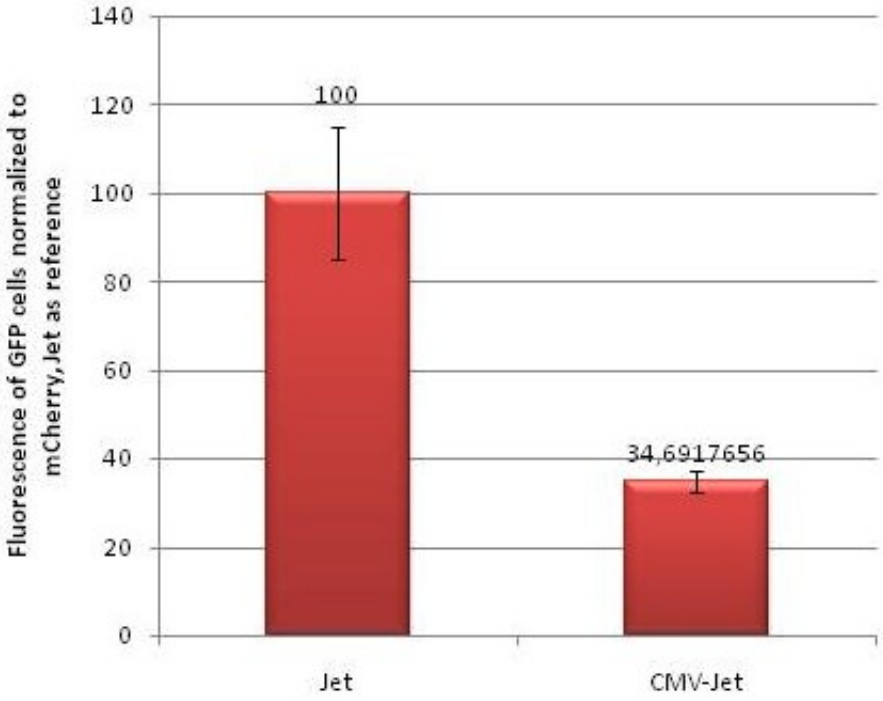


Ratio	CMV : Jet	55:1
	CMV : CMV-Jet	143:1
	Jet : CMV-Jet	2,6:1

Measurement: FACS

JeT as reference





Ratio	CMV : Jet	48:1	(55:1)
	CMV : CMV-Jet	137:1	(143:1)
	Jet : CMV-Jet	2,9:1	(2,6:1)

Real-time RT PCR ---Current status

- RNA extraction:

  satisfactory yield

  10^6 cells => 15 ug

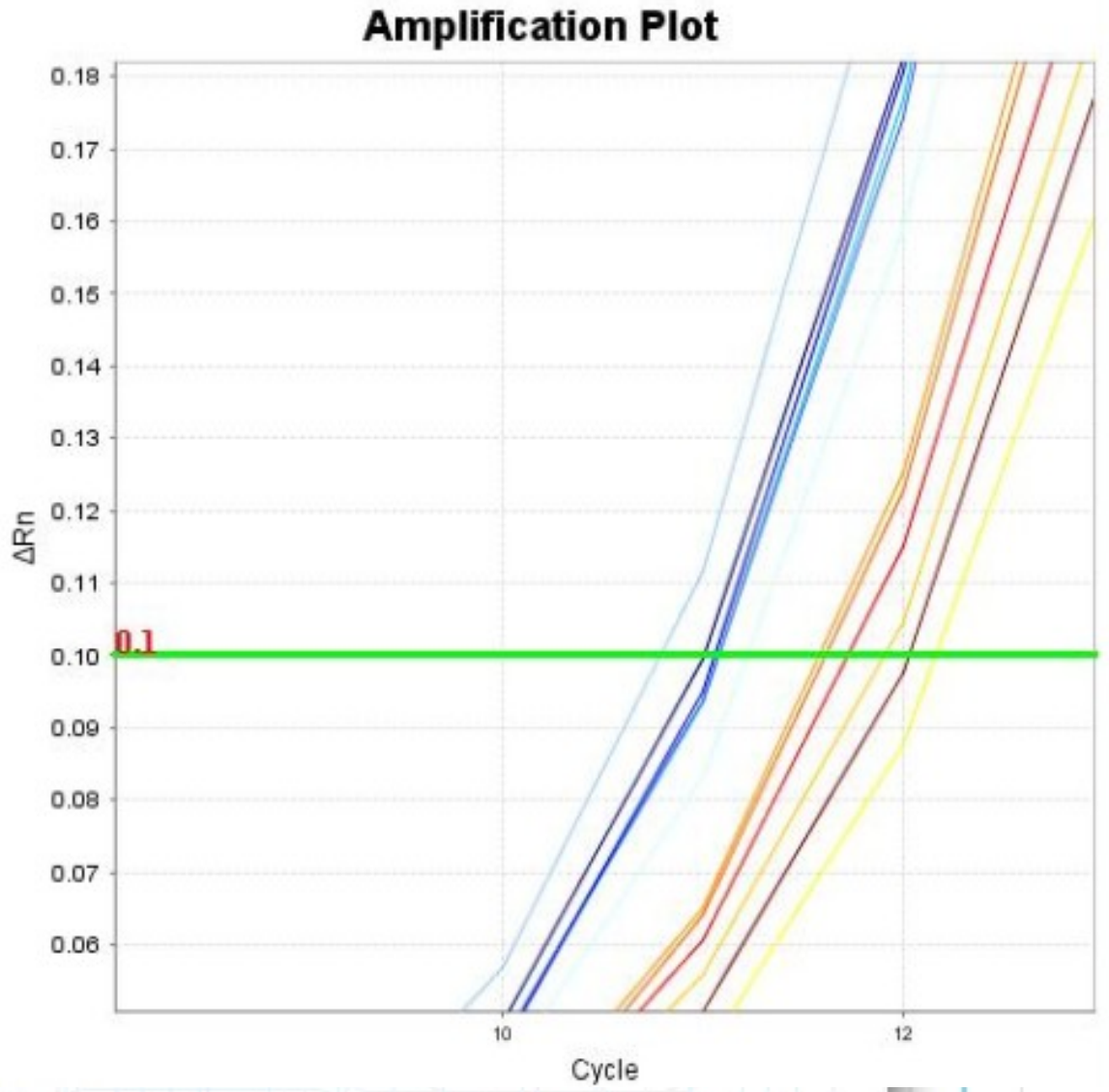
- qRT-PCR

  blue: CMV

  yellow: JeT

CMV:JeT=1.73:1

-> T test



Real-time RT PCR ---Work plan

- standard curve

  ->eGFP: plasmid number?
- 6 replicates

  more time point
- difficulty in cell culture