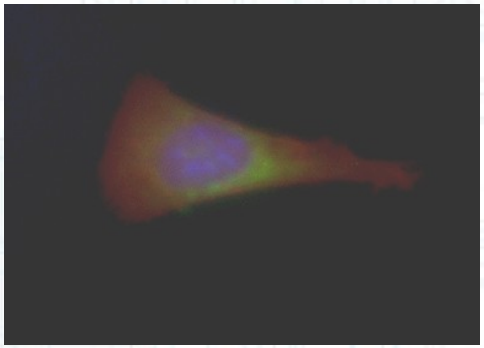
Project standing 8-25

Cell culture

- Transient transfections:
  - Work best in HELA and U2-OS
  - Co-transfections with up to three plasmids work



- Stable cell line
  - Expanding a promising clone of HeLa
  - Picked U2-OS foci
  - Restarted MCF-7
- Characterization of integration site
  - In contact with Manfred Schmidt (LAM-PCR)

Synthetic promoters

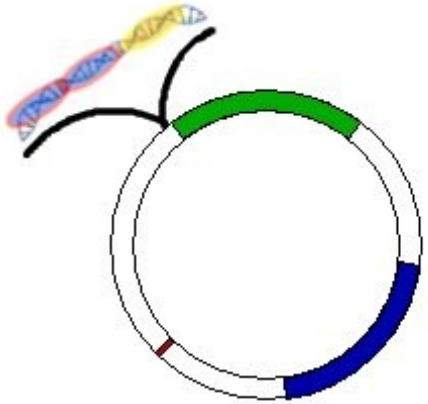
Strategy one: "Random assembly"

1) Synthesize DNA by a random overlap exstension protocol

repeats of Transcription Factor binding site, plus random nucleotides (plus "generally activating" TFs)

core promoter

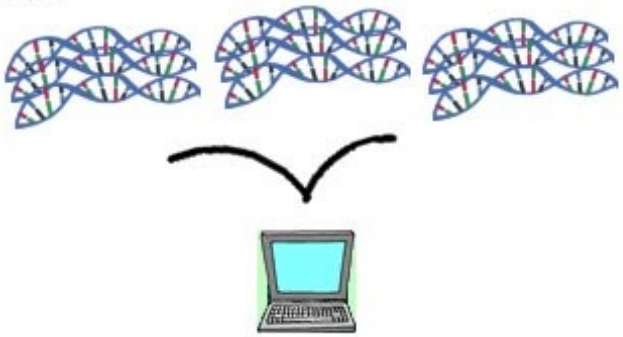
2) Clone into reporter gene plasmid



3) Screen for promoters that behave as desired

Strategy two: "Sequence prediction"

1) Screen many natural promoters for TF-Binding sites



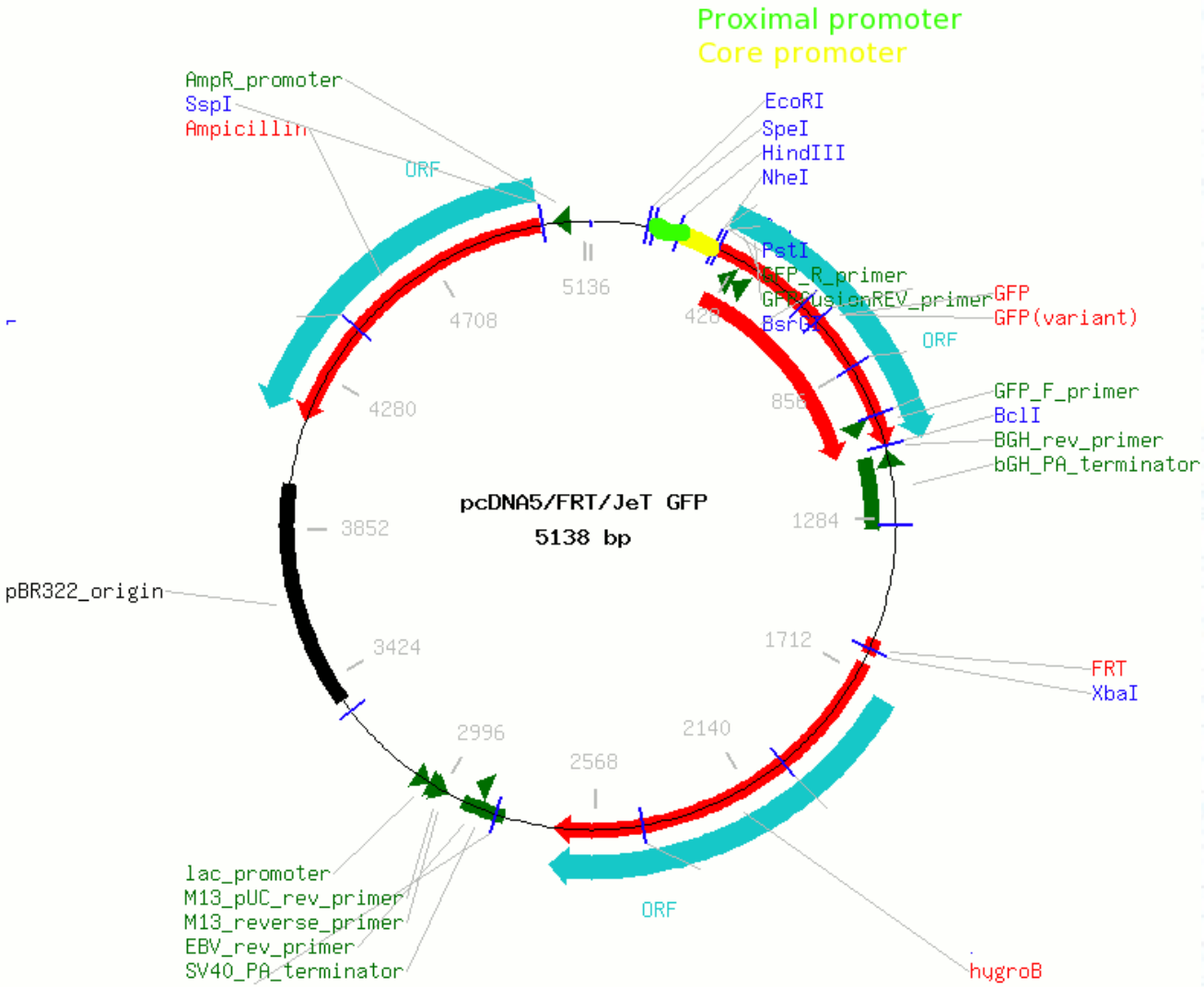
2) Develop a statistics that tells you how a certain transcription factor regulates a it target genes

3) Develop an algorithm that creats "junk" DNA that contains no TF-Binding sites to fill up the spaces between predicted TF-Binding sites

4) Synthesize the DNA as predicted

...ACGTACCGTGTGCAGTCATGACTAG
GTCAGTGACGTAAACTTTGTGACTGC
AGTCCCCCAGTGTATATATAAGCCGA...

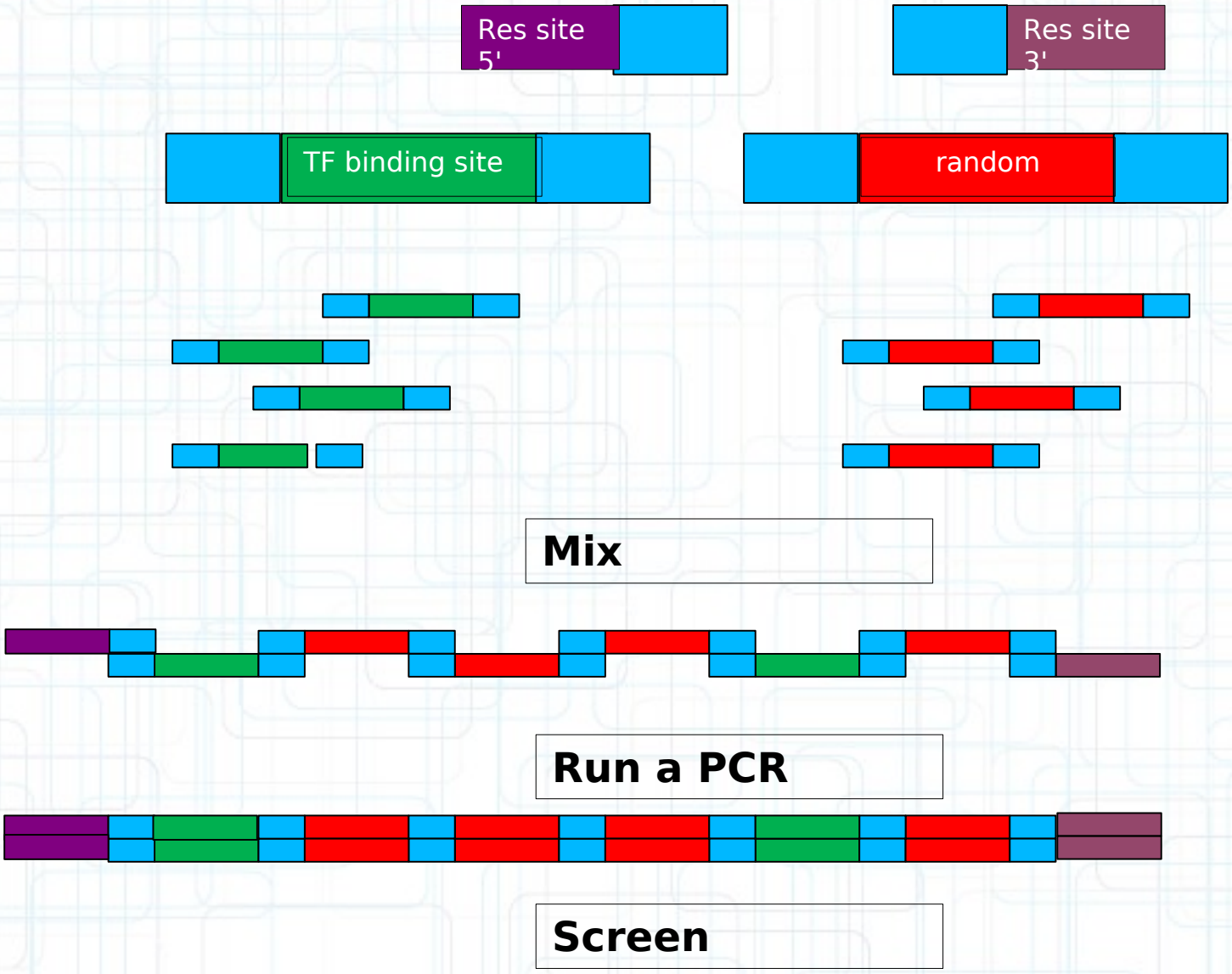
Synthetic promoters



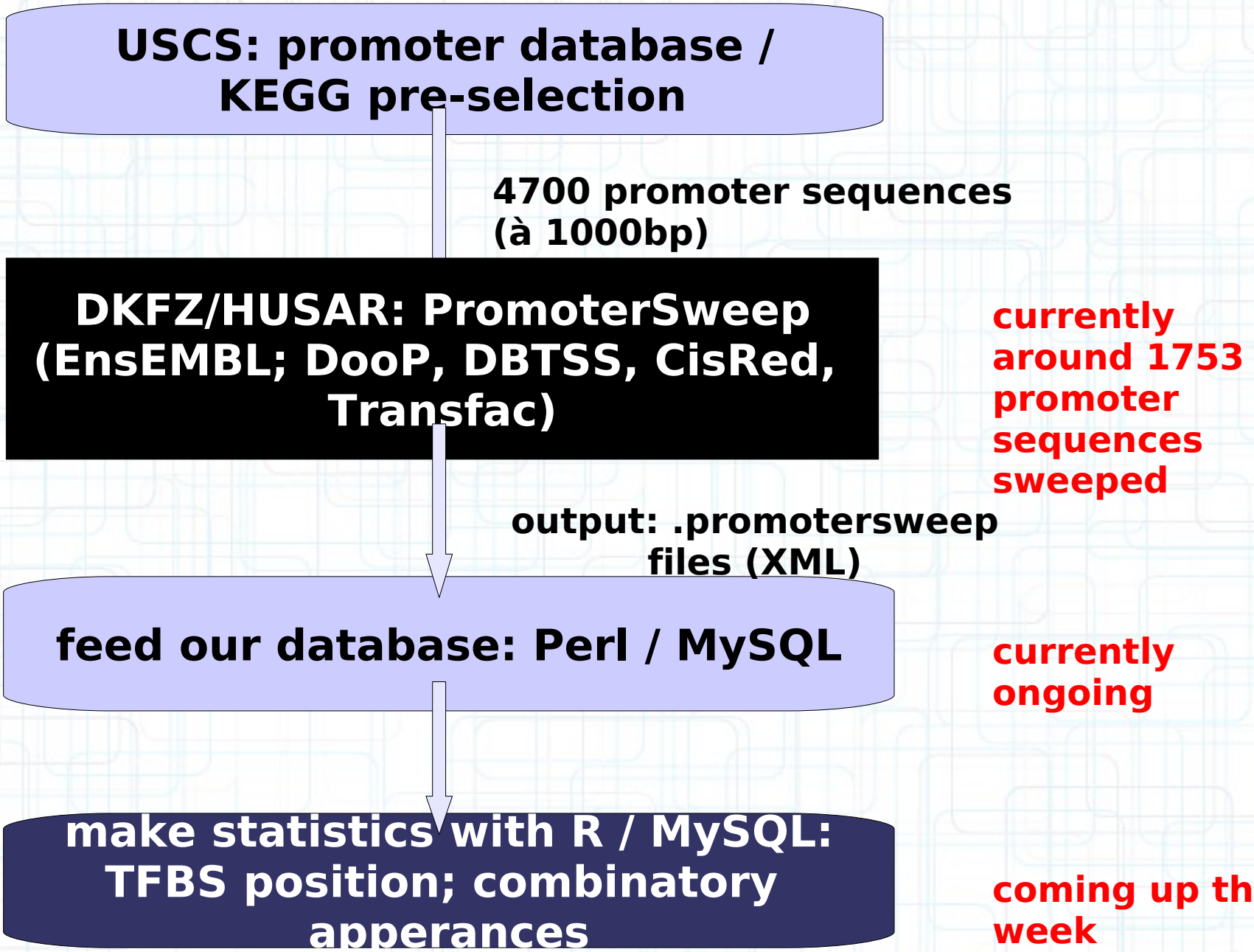
Synthetic promoters

- ✓ Screening plasmid works
- ✓ 2 core promoters, 2 proximal promoters (constitutively active) → all work
- ➔ Quantify existing promoters (gold medal requirement)
- ➔ Synthesize DNA containing HIF1a, NF-kB and p53 binding sites
- ➔ Clone in front of both core promoters, screen

Synthetic promoters



In Silico Promoter prediction



HEARTBEAT database

(HEidelberg ARtificial Transcription factor Binding sites Engineering and Assembly Tool)

```
+------------------------+
| Tables_in_Heartbeat_DB |
+------------------------+
| Gene_Info              |
| Main_Info              |
+------------------------+
```

- **MySQL** is a programming language to create and organise databases
- **HEARTBEAT** contains a main table with all necessary information concerning each Transcription factor binding site

- **Gene_Info** provides additional information about the respective Gene
- Distribution into tables necessary to save storage space

```
+-------------------+----------+------+-----+---------+-------+
| Field             | Type     | Null | Key | Default | Extra |
+-------------------+----------+------+-----+---------+-------+
| refseqID          | char(15) | NO   | PRI |         |       |
| TF_Name           | char(50) | NO   | PRI |         |       |
| TF_Position_Start | int(12)  | NO   | PRI | 0       |       |
| TF_Position_End   | int(12)  | YES  |     | NULL    |       |
| TF_Motive         | char(30) | YES  |     | NULL    |       |
| TF_Score          | char(6)  | YES  |     | NULL    |       |
| BS_Quality        | char(20) | YES  |     | NULL    |       |
+-------------------+----------+------+-----+---------+-------+
```

HEARTBEAT contains currently *1591* different TF binding sites from *196* promoters

Example: Gene_Info

refseqID	entrezID	Gene_Symbol	EnsembleID	TSS_Doop	TSS_DBTSS	TSS_EPD	TSS_MPromDB
NM_000416	3459	IFNGR1	ENSG00000027697	NA	1045	1072	1026

Example: Main_Info

refseqID	TF_Name	TF_Position_Start	TF_Position_End	TF_Motive	TF_Score	BS_Quality
M_000282	Hand1:E47(V$HAND1E47_01)	612	616	tgagtgt	0.949	conserved
M_000282	ZF5(V$ZF5_01)	972	976	atttactg	0.900	conserved

- Coming up: Statistical Analysis
  - selection upon “interesting” pathways
  - plot “histograms” of Tfs
  - appearance in combination

- To do after the promoter design... (September / October)
- Modelings:
-

“exclusive promoters”

- modeling behaviour of activation of single pathways by a single / multiple TF(s) – synergistic effects? 1 + 1 = 2?
- possible modeling layers:
  - (a) affinity
  - (b) gene expression
  - (c) activity
  - (d) dynamics over time

“error checking”

- check affinity (prediction score / TFBS reliability)
- phenotypic measurement

Natural promoters

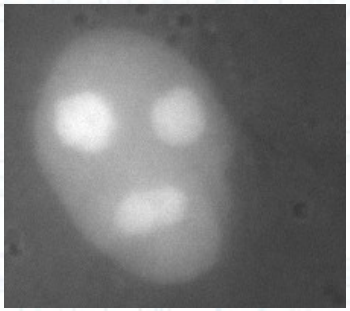
Gene	Pathway	Why?	Induce by	Status
ApoAIV	pPARα	Diabetis meltius	Insulin-synthesizer (drug)	Cloned from gDNA
cyp1A1	AHR	Drug response	Dioxin	To be clone from gDNA
PUMA	p53	DNA damage	Camptotecine	Ordered from AddGene
LDL-R	SREBP	Obesity	Lipoprotein-free medium	Ordered from AddGene
HMG CoA synthase	SREBP	Obesity	Lipoprotein-free medium	Ordered from AddGene

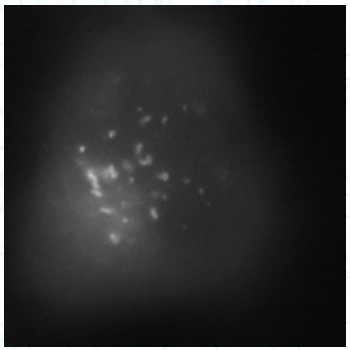
Natural promoters

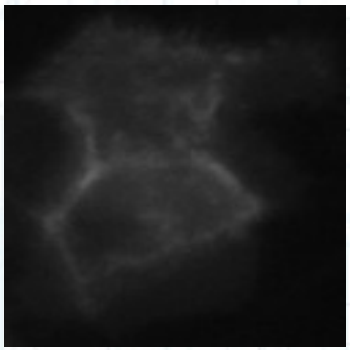
Gene	Pathway	Why?	Induce by	Status
SMAD-RE	SMAD	Cancer	TGF-β	Ordered from AddGene
RARE	Retinoic acid	Vertebrate development	Retinoic acid	Ordered from AddGene
c-jun	EGF	Cancer	EGF	Ordered from AddGene
5*NFkB	NFkB	Cancer	TNF	Obtained from dkfz

Output

- Chose color combination optimal for use under the microscope (YFP, CFP, mCherry or GFP/mCherry)
- Running mutagenesis PCR for target amplicons to remove BBb restriction sites.
- Amplifying/amplified targeting sequences and localization sequences (LS) needed for construction of variants of FP-LS.
- Waiting for BBb/FRT vector to ligate our amplicons into it.







Gold, Silver, Bronze

The requirements to earn a Bronze Medal are:

- Register the team, have a great summer, and have fun attending the Jamboree. ✔
- Successfully complete and submit a Project Summary form. ✔
- Create and share a Description of the team's project via the iGEM wiki (see TUDelft 2008 for a great example). ✔
- Present a Poster and Talk at the iGEM Jamboree (watch the Heidelberg 2008 video for a great example). ✘
- Enter information detailing at least one new standard BioBrick Part or Device in the Registry of Parts ✔ / ✘ (Part OK, information tbd)

Gold, Silver, Bronze

The requirements to earn a Silver Medal are (in addition):

- Demonstrate that at least one new BioBrick Part or Device of your own design and construction works as expected. ✔
- *Characterize the operation of at least one new BioBrick Part or Device and enter this information on the Parts or Device page via the Registry of Parts (see BBa_F2620 for an exemplar).* ✘ (qRT-PCR, cell line characterization to be done)

The requirements to earn a Gold Medal, in addition to the Silver Medal requirements, are any one OR more of the following:

- *Characterize or improve an existing BioBrick Part or Device and enter this information back on the Registry.* ✘ (qRT-PCR of CMV promoter to be done)
- Help another iGEM team by, for example, charactering a part, debugging a construct, or modeling or simulating their system. ✘
- Develop and document a new technical standard t(...) ✘ (BBB characterization)

Measurement

- To calculate PoPS: qRT-PCR:

Heidelberg 2009: Best Experimental measurement

GFP mRNA population

Steady state: PoPS = degration

Therefore: Use Actinomycine D to block transcription

Measure degration

DNA copy no: qPCR (?)

- Relative characterization:
  - FACS (?)
  - Microscopy, ImageJ (?)

Real-time RT PCR ---Current status

- Quantitative measurement of the activity of promoters: CMV, JeT, CMV-JeT
- Housekeeping genes as internal reference: GAPDH, G6PD, 18s rRNA, TUBG2, ACTB
- Fluorescent protein: eGFP, mCherry

- Primer & probe design
- Kits order

Real-time RT PCR ---Work plan

- 2-3 weeks: establishment of the measurement

  -> 1 time point, 6 replicates

- Later: scale-up

  -> more time points, more replicates

  -> reduced variation

- PoPS (Polymerase per second) ???