

M-ROE-PCR
synthetic promoters for
research, gene therapy &
plant biotech

Heidelberg iGEM team 2009

Overview

- I. The Method
- II. Patentability

Overview

I. The Method

- i. Rationale
- ii. How it works
- iii. Results

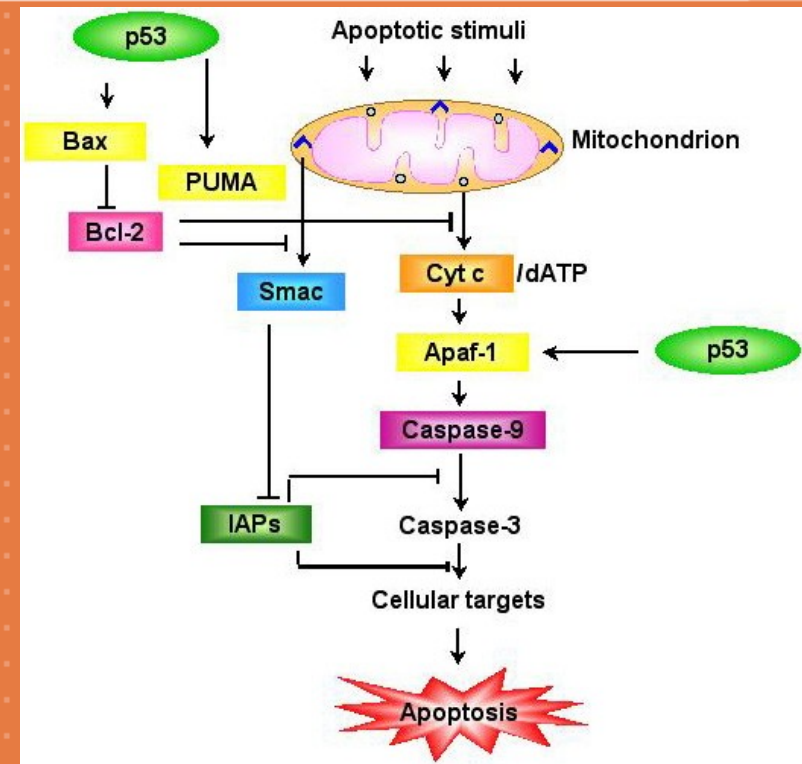
II. Patentability

Method - Rationale

- Natural eukaryotic promoters sense wide variety of conditions
- Synthetic promoters can be constructed to sense specific conditions
- **Examples to demonstrate value of synthetic promoters**

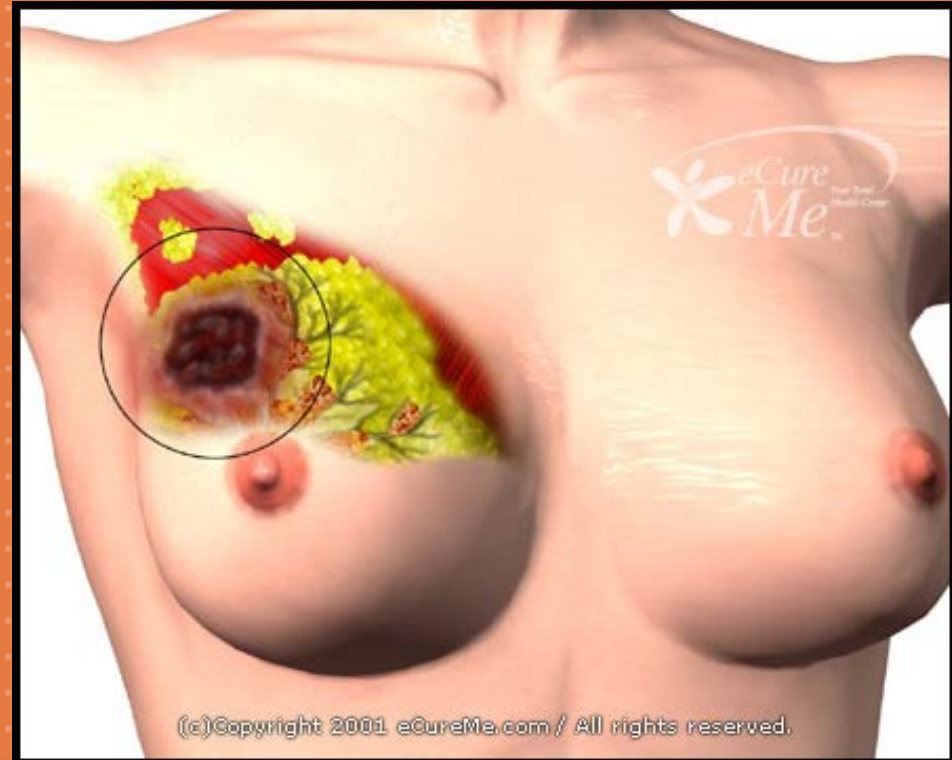
Method – Application examples

- **Transcription-based assays**
- Measuring pathway activity
- No standard method for assay construction exists



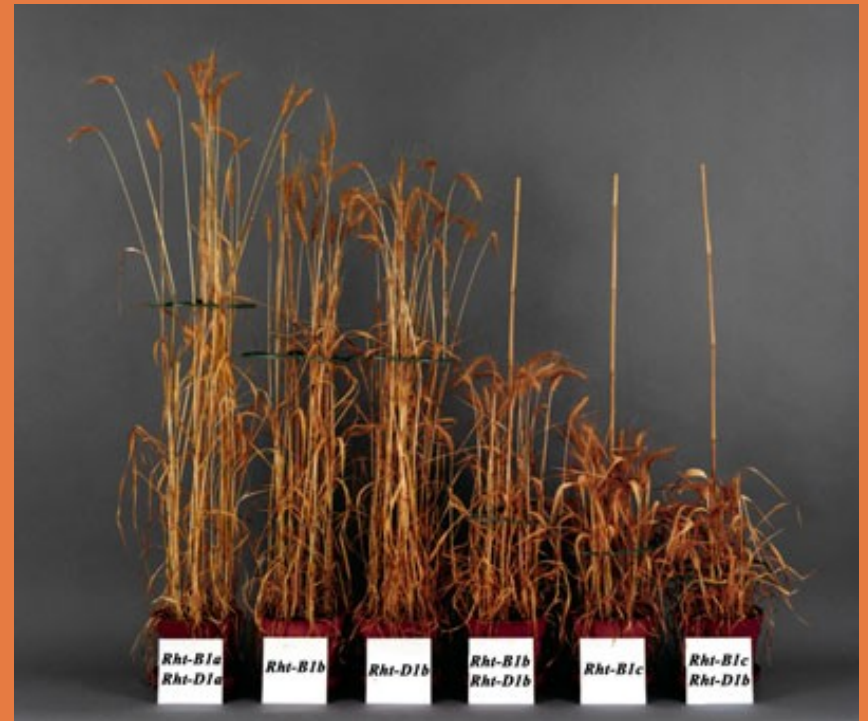
Method – Application examples

- **Virotherapy**
- Synthetic promoter could be constructed to sense elevated estrogen receptor levels + radiation
- Only radiated cancer cells affected



Method – Application examples

- **Plant biotechnology**
- Always in need for new promoters
- e.g. promoters active only during shoot development, fruit ripening or post harvest



Method – how it works

We claim to have invented a standard method for the construction of *any* synthetic promoter:

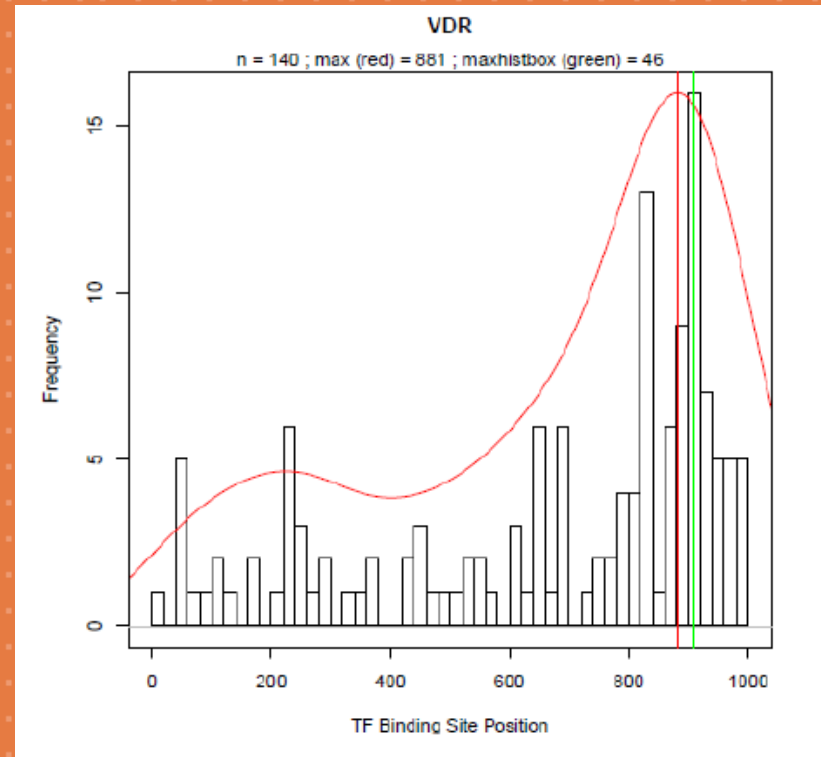
Model-guided Random Overlap Extension PCR

A PCR-based, bioinformatics guided promoter production pipeline

Method – how it works

1) The model

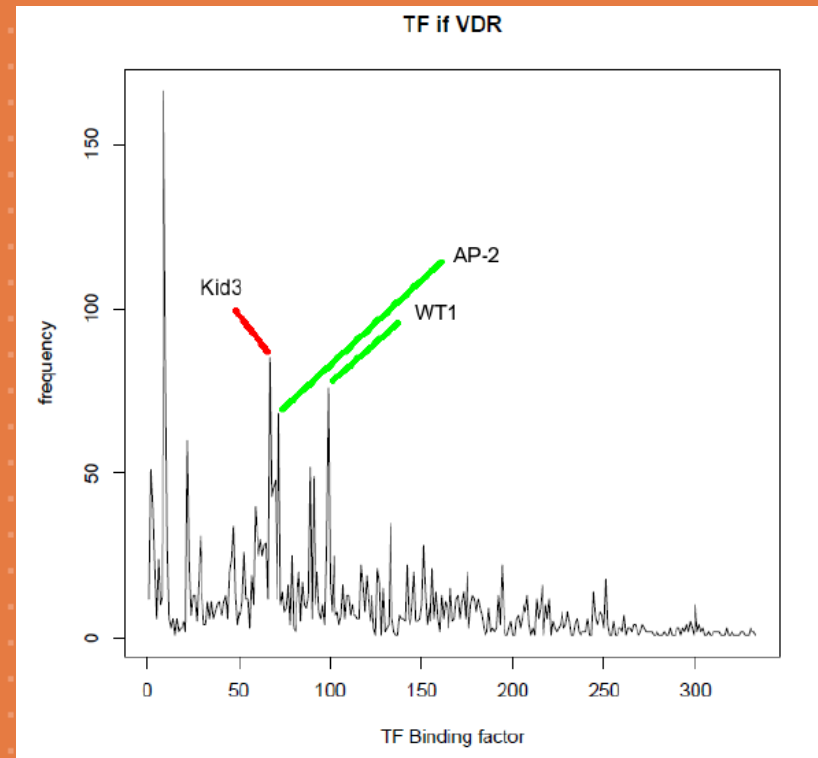
- Genome-wide analysis of Transcription Factor Binding Sites **localization** and coincidence



Method – how it works

1) The model

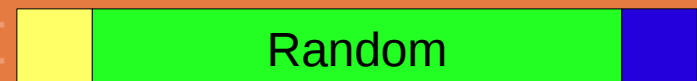
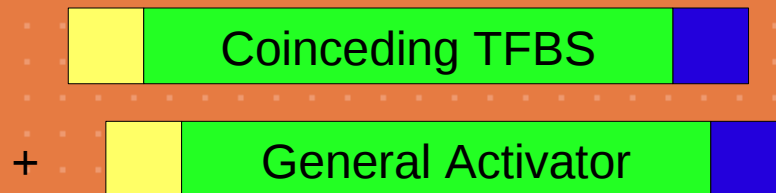
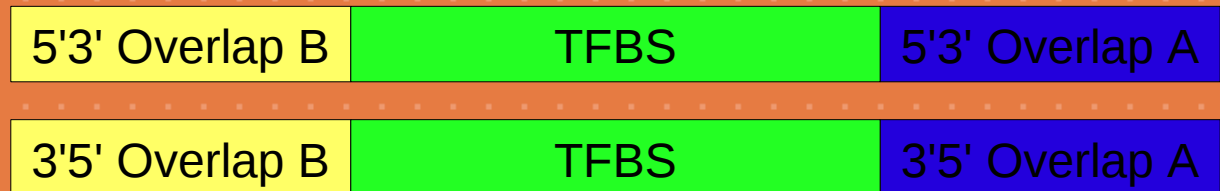
- Genome-wide analysis of Transcription Factor Binding Sites localization and **coincidence**



Method – how it works

2) Promoter synthesis

- PCR based on „Overlap-Extension PCR“



Method – how it works

5' Cutsite		TFBS		TFBS		TFBS		TFBS		3' Cutsite
5' Cutsite		TFBS		TFBS		Random		TFBS		3' Cutsite

5' Cutsite		TFBS		Other TF		TFBS		TFBS		TFBS		Random		3' Cutsite
5' Cutsite		TFBS		Other TF		Random		TFBS		Random		Random		3' Cutsite

5' Cutsite		3' Cutsite
5' Cutsite		3' Cutsite

Method – how it works

2) Promoter synthesis

- Oligo concentration regulates promoter length, TFBS number and ratios
- Clone 5' of a core promoter
- If the model shows a decisive peak, compatible cutters can be used to place TFBS further away from the core promoter or in a certain order – **or a sequence can be ordered / synthesized directly**

Method – how it works

3) Screening

- Pick colonies – each colony represents a unique promoter!
- Transfect in 96 well plates under desired conditions, various control conditions
- Screen by automated methods (e.g. Plate reader)

Method – Results

1	1125.2	1224.3	2	1102.6	1352.3	3	1047.8	1229.9	4	1076.7	1201.9
5	974.56	1045.8	6	1224.3	1255.9	7	1085.6	1159.7	8	1440.8	1491.9
9	1690	1454.9	10	1174.1	1138.7	11	1111.1	1563.4	12	1017.7	1243.2
13	1299.2	1368.2	14	1286.8	1219	15	1306.6	1299.2	16	1069.2	1211.1
17	1232.2	1287.3	18	1209.3	1230.4	19	1272.2	1331.3	20	1147.3	1245.6
21	1274.6	1419	22	1715	1629	23	1412.4	1389.2	24	1193.1	1276.9
25	1169.4	1344.1	26	1146.4	1201.8	27	1181	1178.2	28	1090	1212.7
29	1141	1172.3	30	1055.2	1191.9	31	1343.9	1503	32	1190.6	1405
33	1045.9	990.89	34	1027.1	1010.2	JeT	1274.7	1195.7			

Out of 34 putative NfkB responsive clones, 12 are upregulated by NfkB (between 10 and 100%) and 1 is downregulated.

Method - Results

```
CONST  
  
Const L 1  
ACTAGGGGTGACGGGTTACCCCTGAAACGGGCGATCGGCAGATAGGGCACTTCCGGGTGACGGGTTCAATGACCGATCAGC  
GATCGGCAGATAGGGGATTTCCGGGTGACGGGTTTCAACAACAGACAGCGATCGGCAGATATCCAGTGACGTCA GGGTGAC  
GGGTTACCCGCATACAGCGCGATCGGCAATCACTBAGTGACGTCA GGGTGACGGGTTTCACTAAGCTT  
  
Const L 4  
ACTAGTGGGTGACGGGTTACGCTGTGCGTGCGGATCGGCAGATAGSSAACTTCGGGTGACGGGTTTACAGCTTAGTCAGC  
GATCGGCAGATAGGGGATTTCCGGGTGACGGGTTCACTCACGCGCGATCGGCAGATCATGACTCAGGGTGACGGGT  
CAGTTGGGACCATGCGATCGGCAGATCATGACTCAGGGTGACGGGTTCACTAAGCTT  
  
const L 5  
ACTAGTGGGTGACGGGTTACAGTCCACACACCGGATCGGCAGATCACTATTGACGTCA GGGTGACGGGTTTCACTAAGCTT  
  
const S 5  
ACTAGTGGGTGACGGGTTCAATTGTTAAAGCGGATCGGCAGATCAGGGGTCCCCGGGTGACGGGTTCACTTAGTCAGGTGCCA  
TCGGCAGATCATGACTCAGGGTGACGGGTTCACTAAGCTT  
  
const S 10  
ACTAGTGGGTGACGGGTTTACTAGCGCAAACCGGATCGGCAGATAGSSGATTTCCGGGTGACGGGTTTCAAGGCAGTCTGAGC  
GATCGGCAGATCATTTGACGTGACGTCA GGGTACGGGTTTCAACATGCGTAGCGCGATCGGCAGATCAGGGCAGGGGGGGGGTACGG  
GTTCAACATGCTTTGGCGATCGGCAGATCAGATGGTGACGTCA GGGTGACGGGTTCAATTAAC TCCAGCGATCGGCAGATCAG  
GGACTTTCCGGGTGACGGGTTTACTAGACTAACGGGCGGATCGGCAGATCATGACTCAGGGTGACGGGTTCACTAAGCTT  
  
const S 4  
ACTAGTGGGTGACGGGTTTCAATTGACAAGATCGGATCGGCAGATCAATTGTCGTCA GGGTGACGGGTTTCACTACACACAGGGCA  
TCGGCAGATAGGGGAACCCC GGGTGACGGGTTTCAACACAGGATCGGCAGATCACTATTGACGTTGGGGTACGGGTT  
CACTAAGCTT  
  
Ap1 Nbs CRISPR Random empty Sp1
```

We created 10
constitutive
expression vectors
of different strength
(measurement TBD,
between 0.8 and
0.1* CMV)

Patentability

- I. The Method
- II. Patentability
 - i. Technizität
 - ii. Novelty
 - iii. Inventive Step
 - iv. Industrial application

Patentability - Technizität

- Technische Lehre/Technizität: Technisch ist eine Lehre zum planmäßigen Handeln unter Einsatz beherrschbarer Naturkräfte zur Erreichung eines kausal übersehbaren Erfolgs. (BGH: Rote Taube 1969)
- M-ROE-PCR is a man made method, it does not copy from nature and is therefore technical



Patentability – Novelty, prior art

- **Closest prior art:**
 - Ogawa in *Biotechniques* 42, 628-632: „Random cis-acting element elongation“
 - **Random Ligation of transcription factor binding sites (ds-DNA)**
 - Venter in *Trends in Plant Science* 12, 118-24: „Synthetic promoters: genetic control through cis-motif engineering“
 - **Computer models of synthetic promoters**
- **Other similar research efforts:**
 - By „rational design“ (creating response elements repeats etc) → no scale-up capability
 - Prior Art: Tornoe in *Gene* 297, 21-32; Kotarsky in *Analytical Biochemistry* 288, 209–215; industry: panomics, Luciferase Reporter Vectors (and others)
 - By randomization of spacer sequences, removing response elements etc
 - Prior Art: Tornoe in *Gene* 297, 21-32; Edelman in *PNAS* 97, 3038-3043; Ellis in *Nature Biotechnology* (online, 19.4.2009)

Patentability – Inventive step

- We are the first to combine prediction with screening to get a many functional clones from one experiment
- We did more than just combining the two papers shown:
 - Our method is cheaper and faster, requires only reagents and machinery existing in every lab
 - Our method allows for more randomization (e.g. if badly described TFs are investigated)



Patentability – Industrial application

- Compare examples above

