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

Heidelberg iGEM team 2009

Overview

I. The Method
II. Patentability

Overview

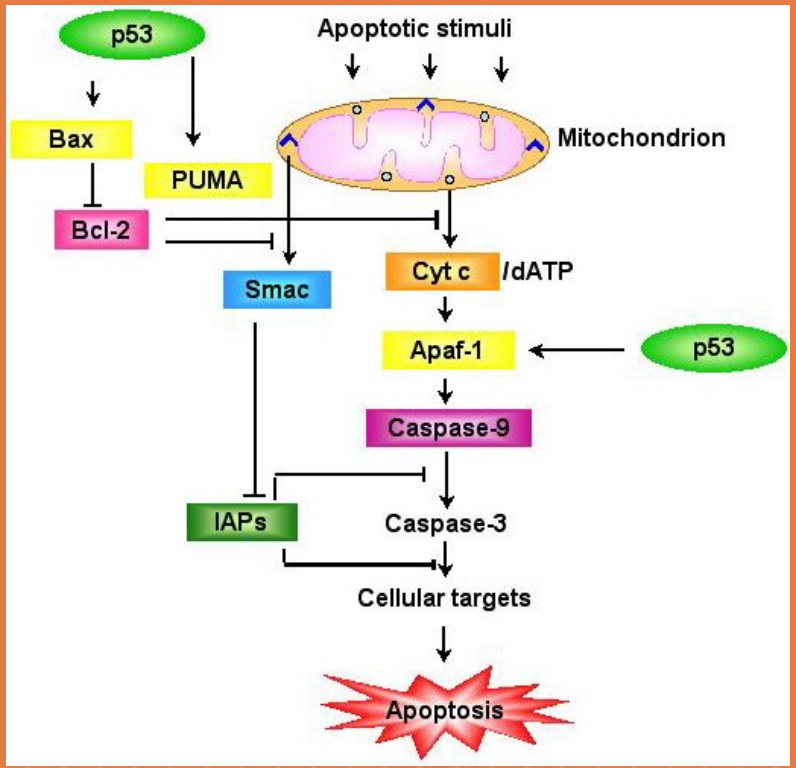
I. **The Method**
   i. Rationale
   ii. How it works
   iii. Results
II. Patentability

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**

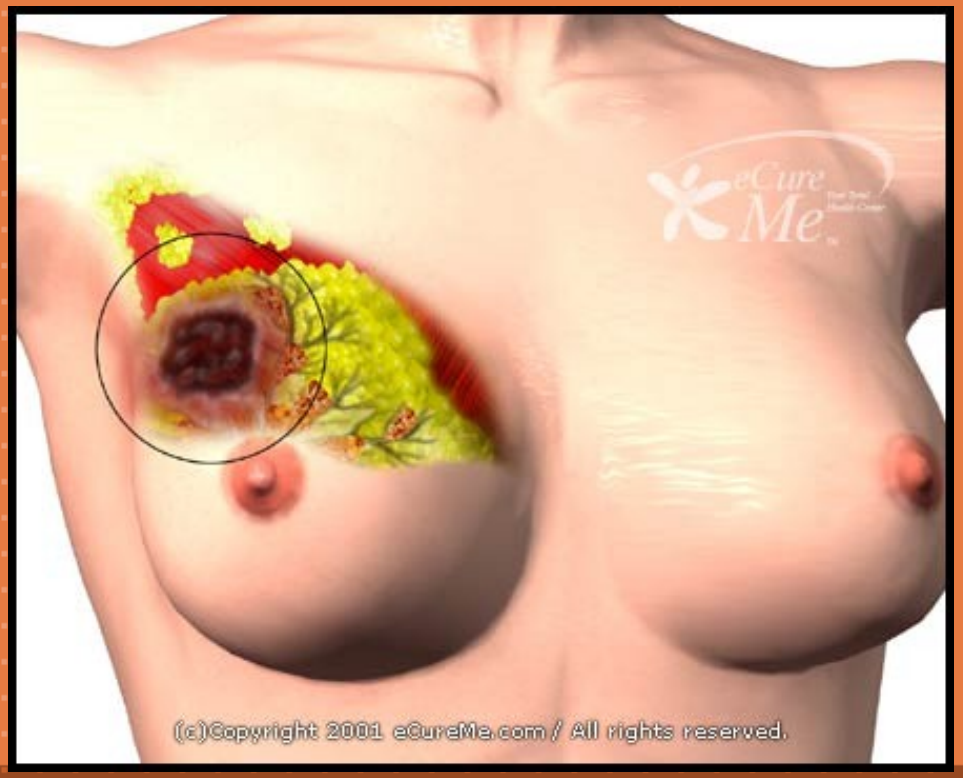
Method – Application examples

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



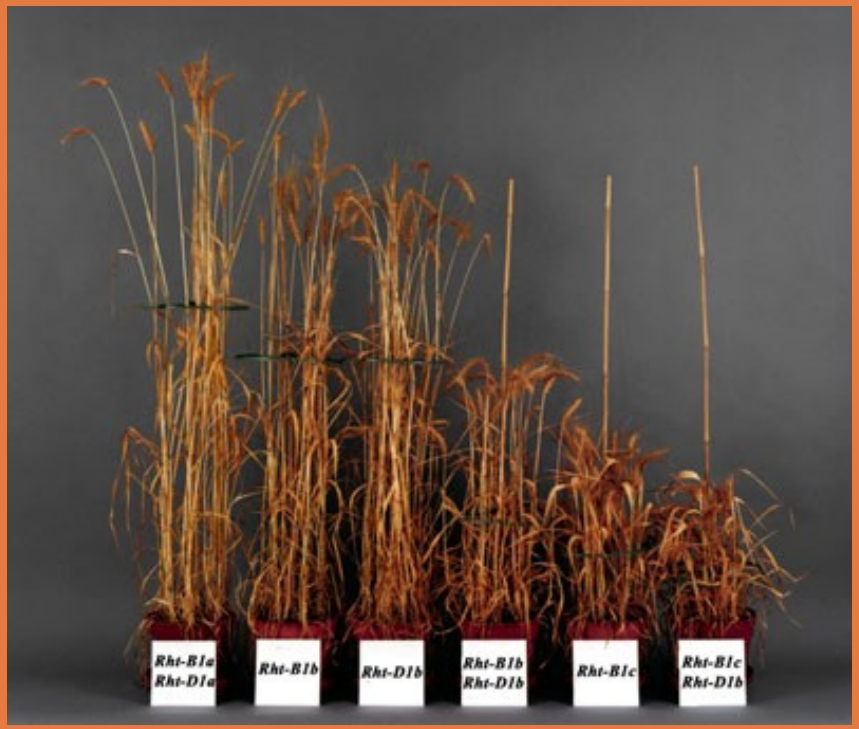
Method – Application examples

- **Virotherpay**
- 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

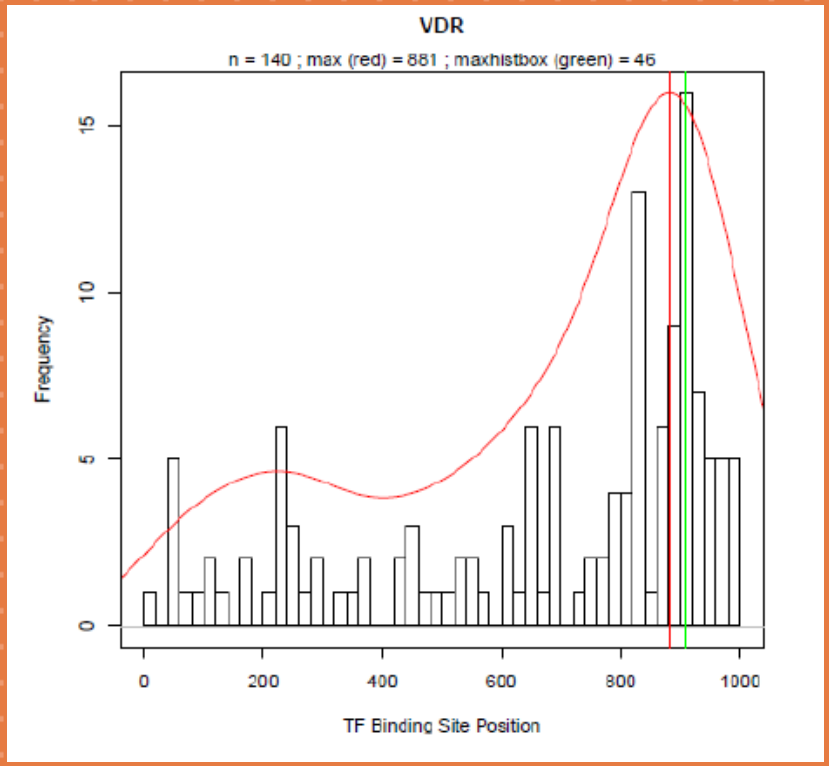
We claim to have invented a standard method for the construction of *any* synthetic promoter:

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



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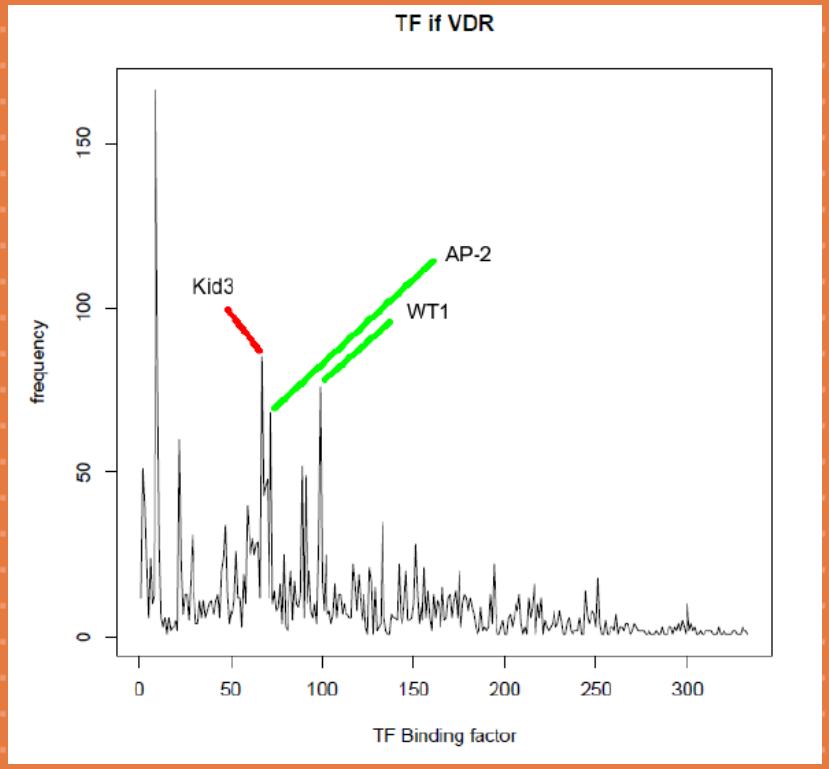
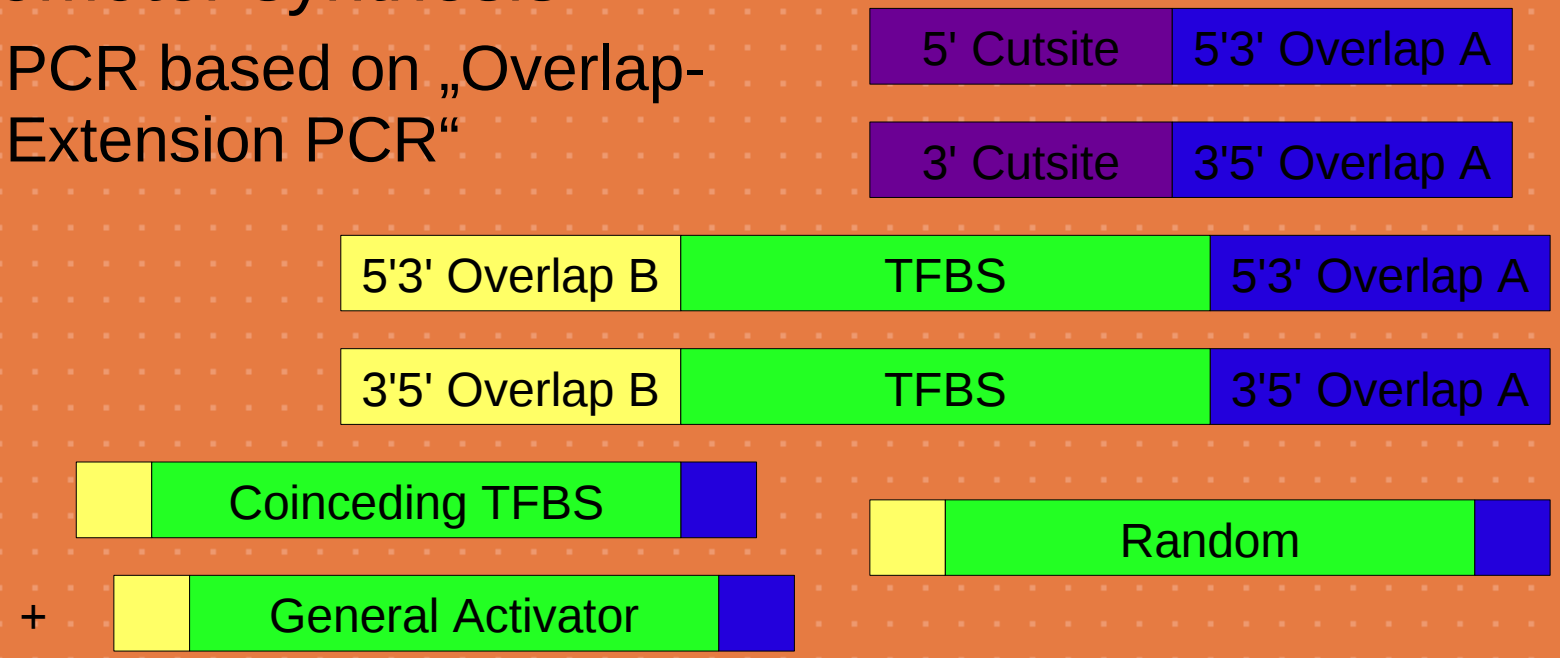
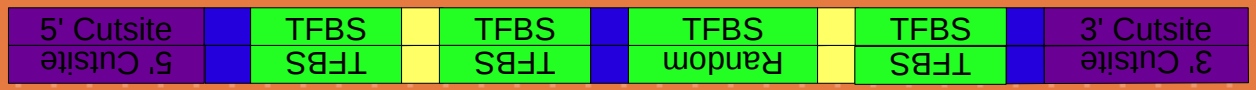


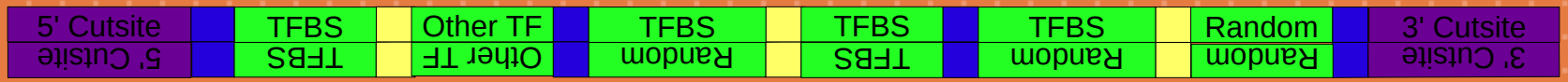
Method – how it works

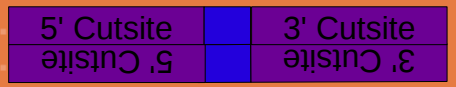
2) Promoter synthesis
   - PCR based on „Overlap-Extension PCR“



Method – how it works







Method – how it works

2) Promoter synthesis
   - Oligo concentration regulates promoter length, TFBS numer 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, variours 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 downregulted.

Method - Results

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 apptication

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

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