Discovery of Novel Tumor Suppressor p53 Response Elements Using Information Theory

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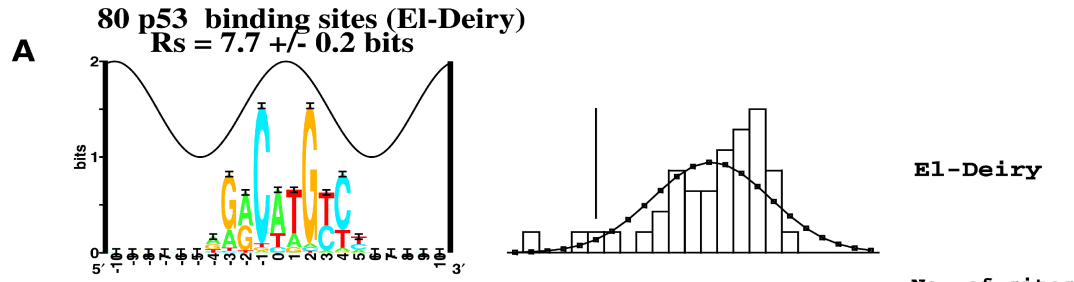Center for Cancer Research Nanobiology Program
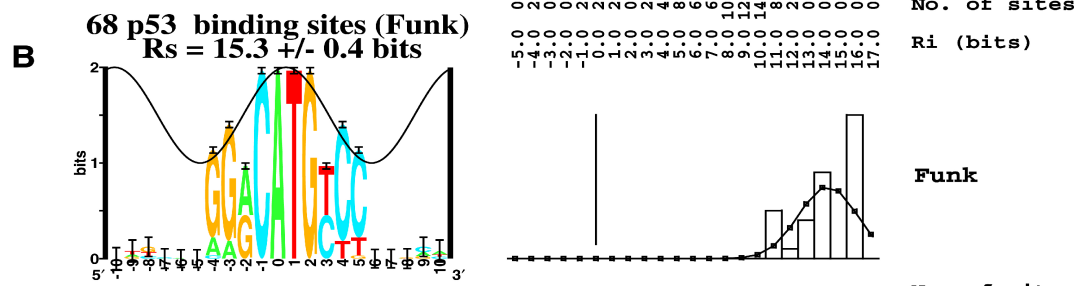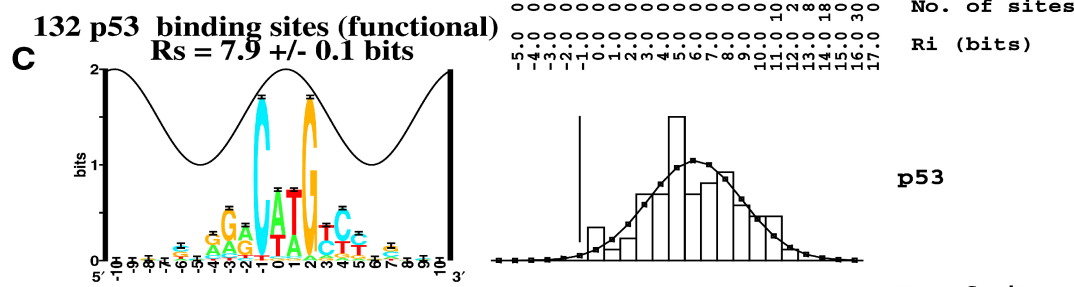
Molecular Information Theory Group

NCI-Frederick

CCRNP

Decameric and Flexible p53 models

A

80 p53 binding sites (El-Deiry)
Rs = 7.7 +/- 0.2 bits

El-Deiry

- El-Deiry *et al.* (Selection of human genomic fragments by immunoprecipitaion)

B

68 p53 binding sites (Funk)
Rs = 15.3 +/- 0.4 bits

Funk

- Funk *et al.* (Cyclic Amplification and Selection of Targets (CASTing)
Selection of strong artificial sites

C

132 p53 binding sites (functional)
Rs = 7.9 +/- 0.1 bits

p53

- Our collection of experimentally proven naturally occurring sites

D

number of occurances
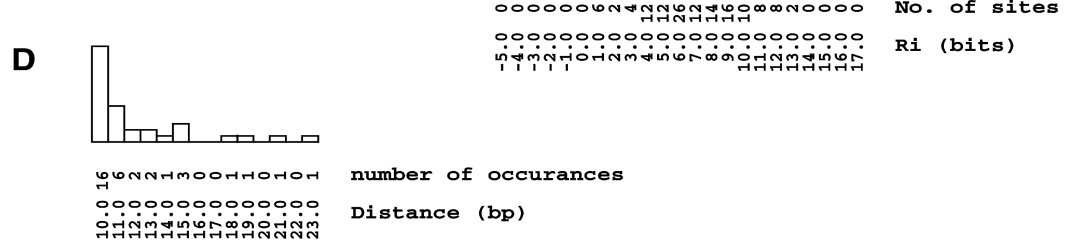
Distance (bp)

# Scanning of human chromosome 1 and 2 with the flexible p53 model

- Average information content of the flexible p53 model is 12.3+/-3.1 bits.

- 50% of the calculated distances between a p53 RE and a promoter are less that 300 bp

- Range: -300 to +100 from identified promoters on human chromosomes 1 and 2

- Rs cutoff for the flexible model is 12 bits

- Rs cutoff for the decameric model is 5 bits

Calcium-activated ion channel protein (CLCA2)

UDP glycosyltransferase (UGT1A6)

Hypothetical protein (FLJ38753)

Hypothetical protein (FLJ43374)

Potassium channel protein (KCNA2)

Prominin 2 (PROM2)

Hexose-6-phosphate dehydrogenase (H6PD)

Ribosomal protein S8 (RPS8)

DEAQ box polypeptide 1 (RNA-dependent ATPase) (DQX1)

Transmembrane protein sorting (VPS24)

Retinol dehydrogenase (RDH14)

U5 snRNP-specific protein, RNA helicase (U5-200KD)

Hypothetical protein (MGC955)

S100 calcium binding protein A6 (calcyclin) (S100A6)

Proteoglycan, potential growth suppressor (LEPRE1)

Basic leucine zipper protein (BZW1)

Confirmation of predicted p53REs by Electromobility Shift Assay (EMSA)
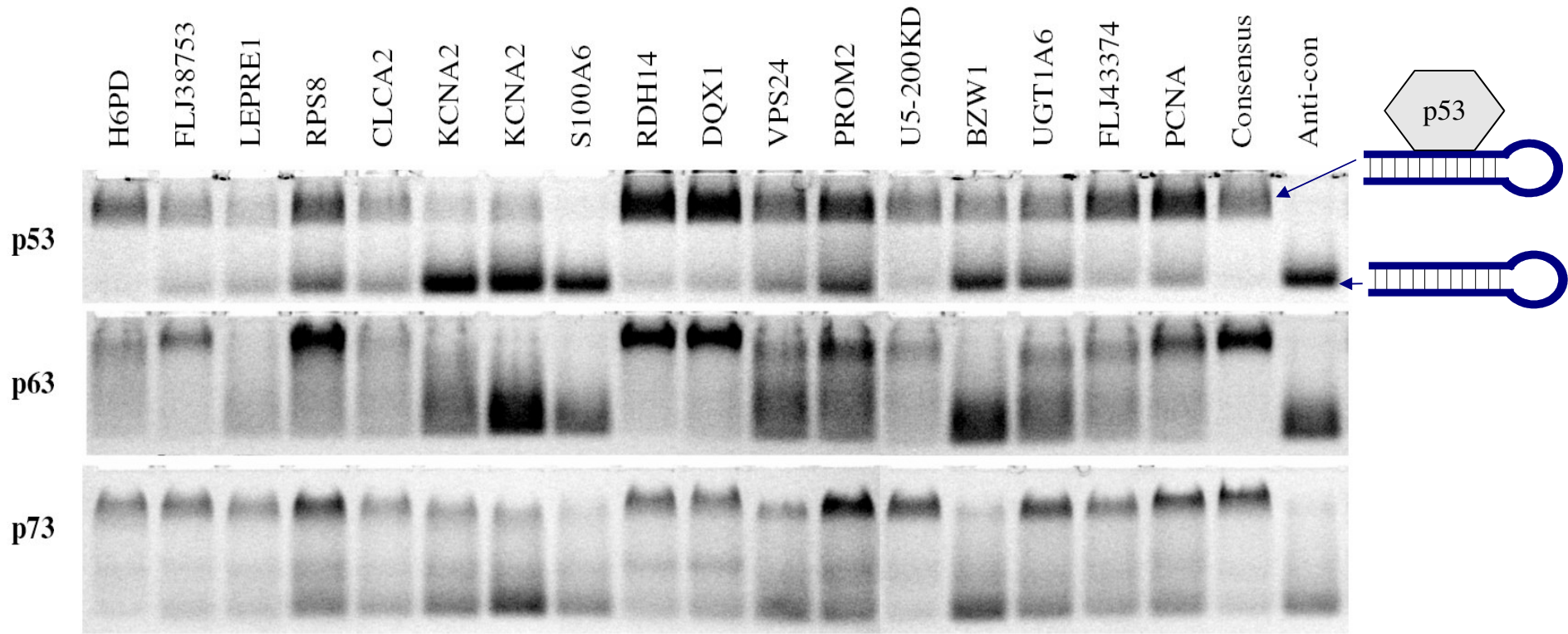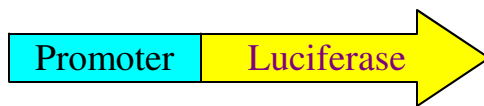

Electrophoretic mobility shift assays (EMSA) with hairpin oligonucleotides containing predicted p53 binding sites using the p53, p63 and p73 proteins.

Confirmation of predicted p53REs in human cell culture
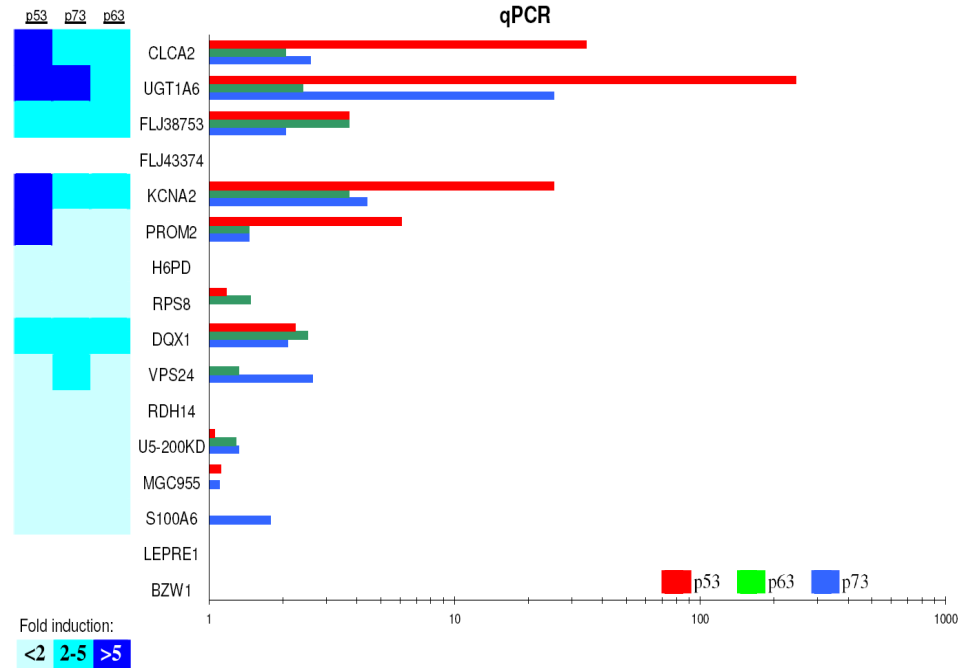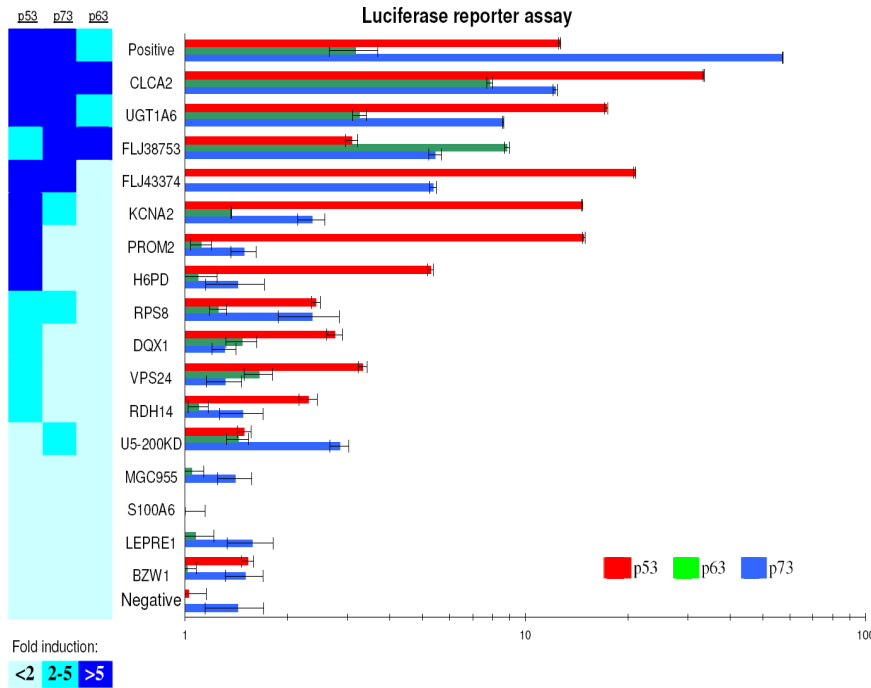
Transcriptional regulation of genes containing the predicted binding sites by p53, p63 and p73.

Conclusions

The flexible p53 binding model was created.
Human chromosomes 1 and 2 were scanned and 16 p53REs were predicted.
The predicted sites were confirmed by EMSA, reporter assays and qPCR

94% (15/16) of the predicted sites showed activity

94% (15/16) bind p53 in vitro
75% (12/16) bind p63 in vitro
81% (13/16) bind p73 in vitro

Luciferase reporter assay:
12 are activated by p53, p63 or p73 more than 2 fold
7 are activated by p53, p63 or p73 more than 5 fold

qPCR:
7 are activated by p53, p63 or p73 more than 2 fold
4 are activated by p53, p63 or p73 more than 5 fold

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