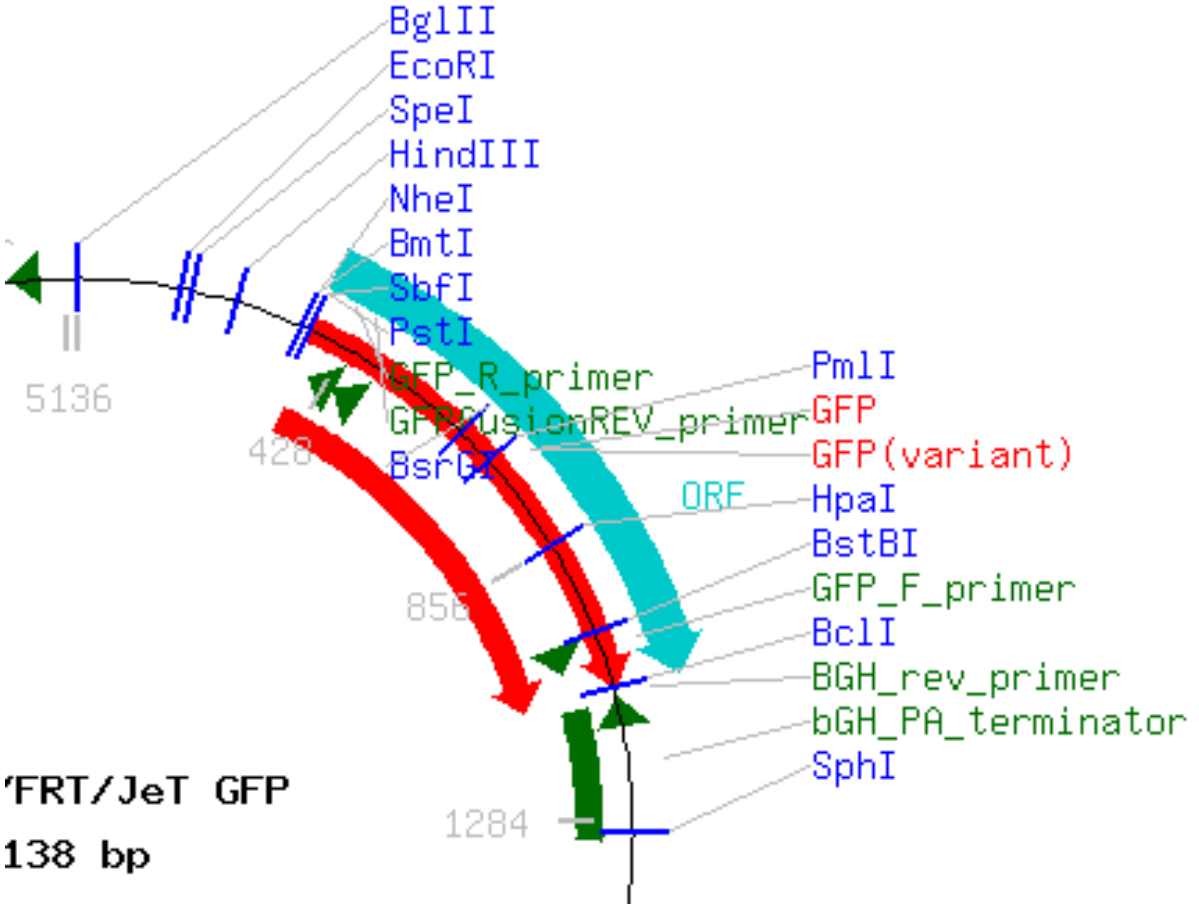
Synthetic promoters

Converting strategies into operations

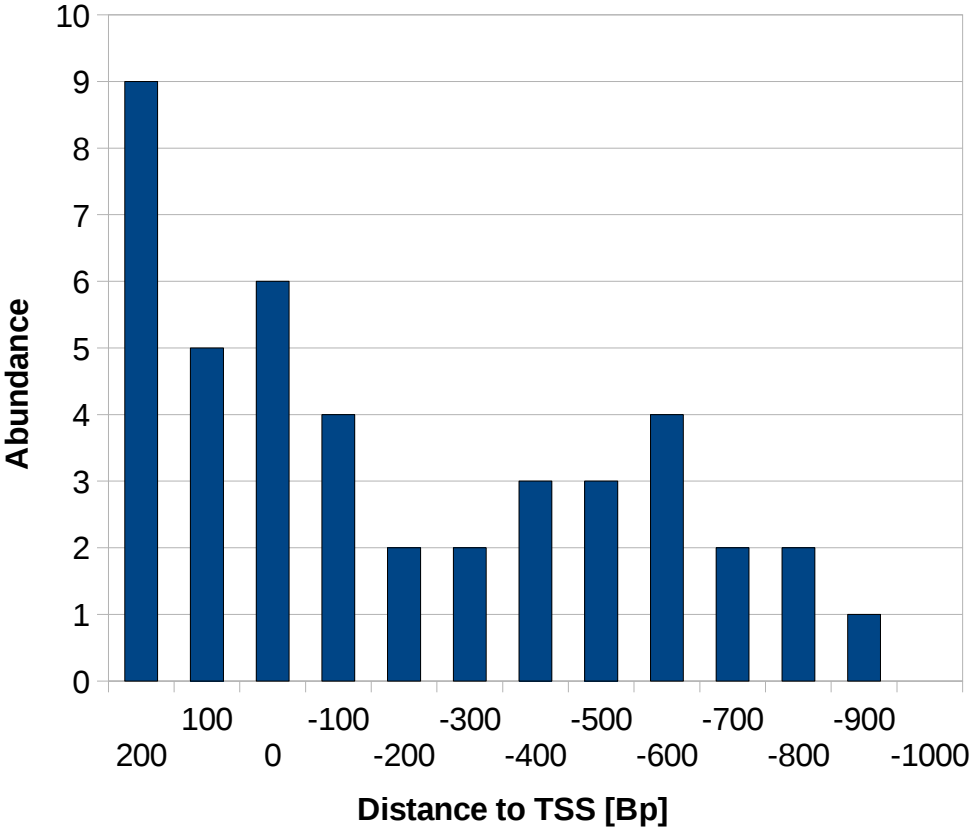
Assembly of a standard vector for promoter testing, genome insertion and assembly

- Relies on FRT site for Genome integration
- Therefore: NO BBa
- Use Tom Knight BBb standard



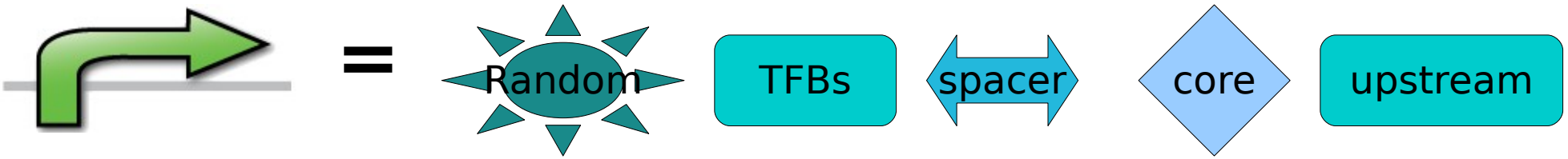
Development of a program for promoter prediction

- Analysis of HIF-1 binding sites by hand: Approach promising
- Features required: Spacing of promoter elements relative to TSS, analysis of cis-regulatory motivs („Coinceding TFs“)



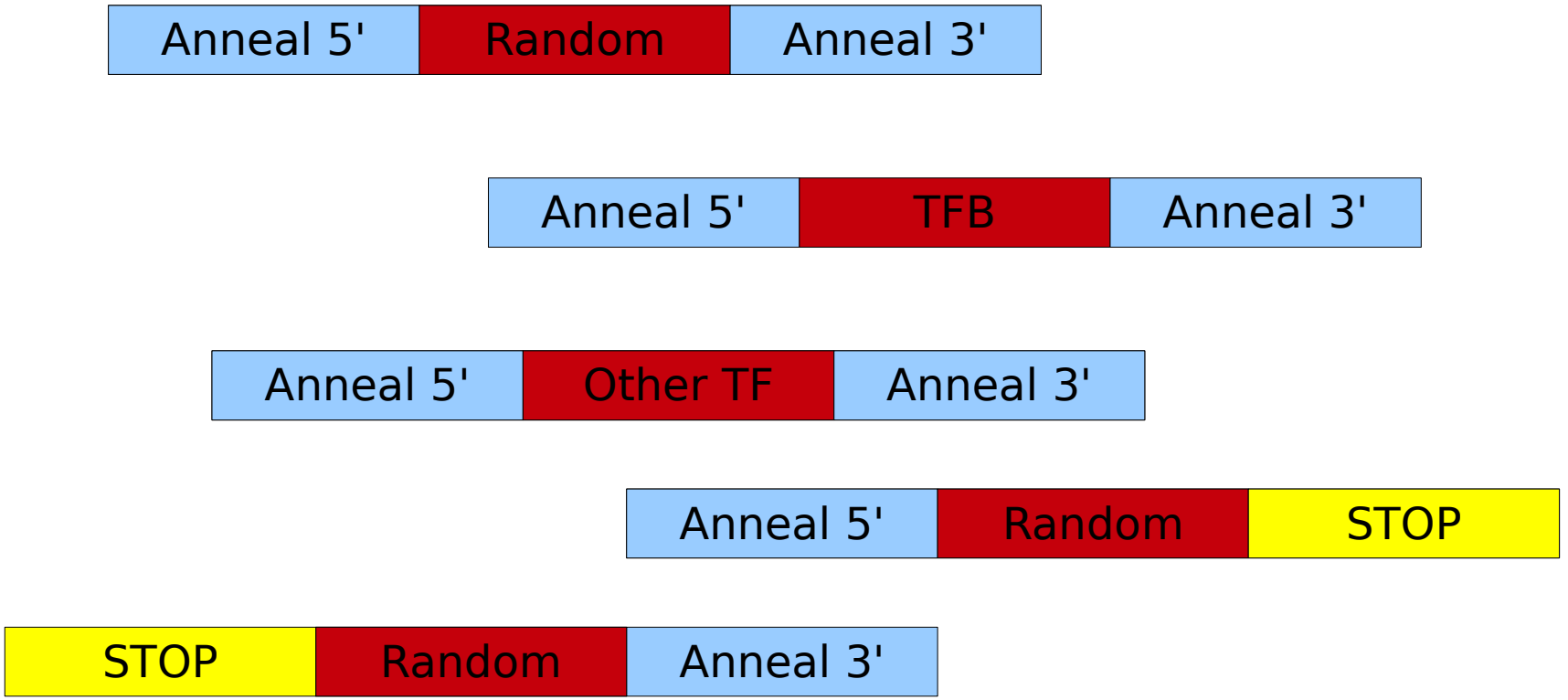
Modularized promoter assembly

- TF-Binding sites + Spacers + Core-Promoter + Random DNA all cloned together in the Bbb standard
- Promoters:



Randomized promoter assembly

- As a control: Oligo-self-assembly



Time schedule

JUNE	•Vector assembly •Establishing a mutagenesis protocol •Establishing a Gene synthesis protocol ✔ •Synthesis of core promoters ✔
JULY	•Testing of core promoters, protocol establishing •Establishing of a randomized assembly protocol •Assembly of a HIF and NfKB random promoter •Prediction program development •BioBricking of Spacers, core promoters
AUGUST	•Prediction Program development •BioBricking of TF-BS oligomers, randomers •Assembly of promoters •Promoter screening
SEPTEMBER	•Assembly of promoters •Promoter screening
OCTOBER	•Quantitative analysis of promoter quality