# Project Logi·col[i]:
## Terminator/Attenuator Anti-Sense Logic (T/AasL)


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## What is genetic logic?

- Uses both natural and synthetic regulatory elements to control transcription and translation in the cell.
- Emphasizes process control and decision making.
- May combine both analog and digital responses.
- Permits us to engineer novel behavior in cells.

The integrated circuit is a major milestone of modern technology. Electrical circuits that once occupied an entire room can now able be placed on a single micro-chip the size of a postage stamp. The implementation of Boolean logic (AND, OR, NOT, etc.) using integrated electronic circuits forms the basis for modern day computers.

Levels of integration like those found in electronics have yet to be realized in biological systems. While electronic devices are inherently connectible due to their common currency of electrons, biological circuits lack similar connectivity. This poses a significant challenge to the development of truly programmable biosystems. Solving this challenge should open the door for development of biological devices just as integrated circuits led to an explosion of electronic devices.

Potential applications for logical circuitry in organisms include almost any sensory, patterning or information processing scenario. The uses could range from combining simple sensory circuits to indicate the presence of a molecule, to having synthetic tissue make patterning decisions that form biological shapes never seen in nature. Trying to describe the potential applications of programming behavior into living organisms is as difficult as describing the computer, cell phone or HDTV to a society which has just invented the light bulb. Although we may be able to scratch the surface of the potential, it is certain that many unforeseen inventions will be generated in the future.

## The Problem

- Genetic logic is not readily connectible (output from one circuit/gate acting as input to the next).

  Electronic devices are inherently connectible due to their familiar flow of electrons – biological circuits lack this universal connectivity.

- Genetic logic is not easily extensible (the development of each new logic circuit is a new scientific experiment).

  For example, both the circuits of Elowitz[1] and of Collins[2] utilize well-known cI, lacZ and tet *cis* regulatory elements and corresponding repressor proteins. Extending this to include additional gates would require the design and fabrication of novel DNA operator sequences, as well as repressor proteins and novel input small molecules.

## Guide to the Illustrations

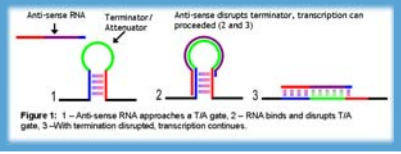



**Figure 1:** 1 – Anti-sense RNA approaches a T/A gate, 2 – RNA binds and disrupts T/A gate, 3 –With termination disrupted, transcription continues.

## Transcription regulation with terminator/attenuators (T/As)

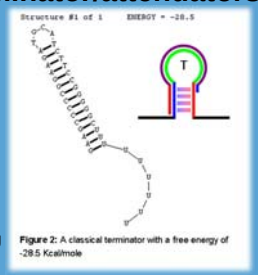



**Figure 2:** A classical terminator with a free energy of -28.5 Kcal/mole

- RNA stem-loop structures.
- Followed by UA rich sequence.
- Causes RNA polymerase to dissociate from DNA, terminating transcription.
- Transcriptional terminators are found at the 3' end and stop transcription at the end of a gene.
- Transcriptional attenuators are identical, but are at the 5' end of a gene, and stop transcription prematurely.
- Stability of RNA secondary structures can be estimated using software (e.g RNAStructure).

## Anti-sense disruption

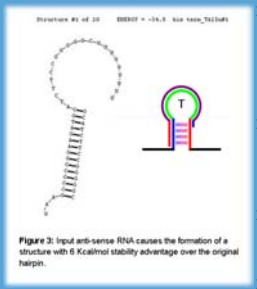



**Figure 3:** Input anti-sense RNA causes the formation of a structure with 6 Kcal/mol stability advantage over the original hairpin.

- Design anti-sense RNA oligonucleotides complementary to a portion of the TA structure.
- Bimolecular stability of mRNA plus anti-sense RNA > intramolecular stem-loop stability.
- Results in disruption of terminator's hairpin structure.
- Transcription proceeds.

## Connectivity

➢Genetic circuits can be connected in this approach since the output of one circuit can easily be used as the input for other circuits.
➢Hammerhead ribozymes liberate the RNA signal from the whole transcript
➢Anti-sense RNA diffuses throughout the cell.

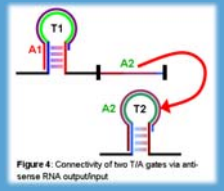



**Figure 4:** Connectivity of two T/A gates via anti-sense RNA output/input

## T/AasL logic functions

**AND**
We have designed the AND logic function by placing two independent T/As in the regulatory region of the gene. The T/A structures will attenuate transcription unless **both** are disrupted by input anti-sense signals.

**OR**
We have designed the OR logic function using terminator (T) and related anti-terminator (aT) and anti-anti-terminator structures (aaT). These structures are similar to the terminator stem-loop but are not immediately followed by a UA-rich sequence, thus they form stable hairpins but do not terminate transcription. Structures are designed with stabilities such that aaT>aT>T. When the aaT is formed, it hinders formation of the less stable aT and permits T to be formed, attenuating transcription. When aaT is disrupted by an anti-sense signal, aT is now permitted to form, disrupting the less stable T and permitting transcription to proceed. Alternatively, direct disruption of T by a different anti-sense input also turns the gate ON.

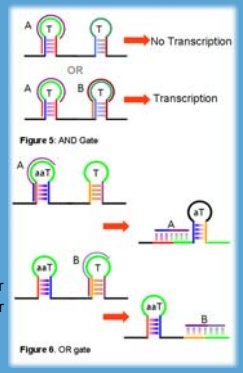



**Figure 5:** AND Gate



**Figure 6.** OR gate

## Device construction

➢Plasmid construction follows BioBrick Foundation Standards with extra restriction sites for testing convenience.
➢Output test harness part contains a fusion of the Tac promoter, a logic gate, an RBS and the LacZα cds. [$P_{Tac}$::TAn::LacZα].
➢For convenience, BamHI and NcoI sites have been placed on either side of the logic gate. This permits the easy replacement of a gate in the harness with only a single digestions and ligation step.
➢The input test harness permits us to provide any input anti-sense signal to the logic gate(s) in the cell. [AraC::PBAD::HH1::TAnIn::HH2].

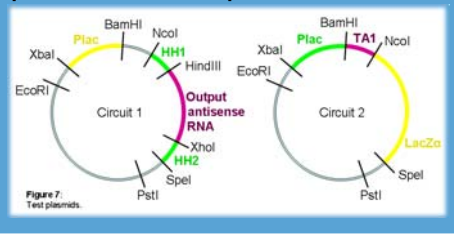



**Figure 7:** Test plasmids.

## Device characterization

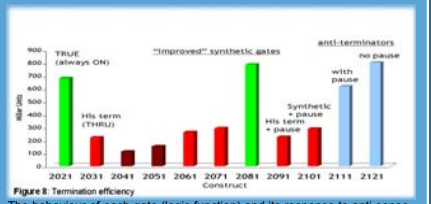
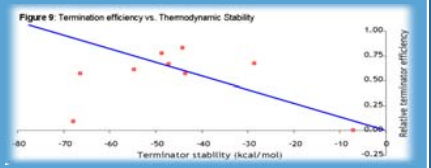



**Figure 8:** Termination efficiency

The behaviour of each gate (logic function) and its response to anti-sense input can be measured using the LacZ assay in a 96-well plate format. The assay is carried out in XL1-Blue cells in LB media supplemented with antibiotics to select for the presence of the appropriate plasmids. Four replicates of each gate are measured in the presence or absence of an input signal.




Contrary to what we expect, termination efficiency is not directly correlated with thermodynamic stability.


$$\text{Activation ratio} = \frac{\text{activation by correct input}}{\text{activation by "null" input}}$$

## Conclusions

**1) Terminator/Attenuator stem-loop structures and disrupting anti-sense input signals can be used to regulate transcriptional activity.**

**2) Combinations of terminators, anti-terminators and anti-anti-terminators can be designed to act as simple logic gates (AND, OR, NAND).**

**3) This combination solves the interconnectivity problem in genetic logic, allowing the output of one functional gate to affect the state of another functional gate.**

**4) We can rationally engineer a large number of orthogonal gates using computer analysis. This makes the logic functions extensible.**

**5) Early tests demonstrate that simple gates can be designed, but optimization is required. More complex binary functions have been designed and are in the process of being constructed for testing.**


[1] Elowitz, M.B. and Leibler, S., 2000, A synthetic oscillatory network of transcriptional regulators, *Nature*, 403, 335.
[2] Gardner, T.S., Cantor C.R., Collins J.J., 2000, Construction of a genetic toggle switch in Escherichia coli, *Nature*, 403, 339