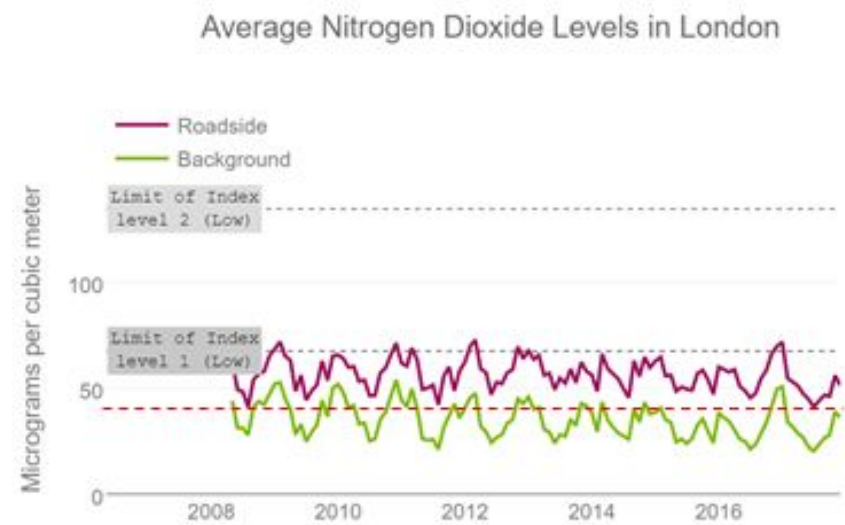# NOxIOUS

## CLEANING OUR AIR

TEAM 4: Will Beardall, Harry Beaven, Julia Berry, Tina Drobnič, Miroslav Gašpárek, Matthew Sargent

# THE PROBLEM

- Air pollution is global issue
- Many different pollutants, we focus on nitrous oxides No$_x$, such as NO and NO$_2$
- Negative health effects: asthma, COPD, lung cancer
- Indoor air pollution; unvented combustion e.g. gas stoves and improved insulation in first world countries→build up of noxious gases

# WHAT'S BEING DONE?



- Number of car users is increasing in densely populated areas such as cities
- Home air filtration/ detection systems are bulky, expensive, unsightly
- No solution currently for developing countries

# THE NOxIOUS SOLUTION

- Our solution will be safe, inexpensive, gives real-time quantitative readout of levels of nitrogen oxides present in the air and once over a threshold level triggers a detoxification pathway to improve air quality
- Ideal for smaller scale air filtering e.g. in the home

# REGULATIONS AND RISK ASSESSMENT

- The European Commission has deemed the current GMO risk assessment methodologies to be sufficient - as such this project will follow the Genetically Modified Organisms (Contained Use) Regulations (2014)

- For a synthetic biology device to be realised, there needs to exist one or more of the following barriers between the organism and the user:
  - Physical
  - Biological
  - Chemical

# REGULATIONS AND RISK ASSESSMENT

The device should have an accompanying risk assessment and as per the aforementioned regulations should consider:

(a) identification of any potentially harmful effects;
(b) characteristics of the proposed activity;
(c) the severity of any potentially harmful effects;
(d) the likelihood of them occurring; and
(e) disposal of waste and effluent

# REGULATIONS AND RISK ASSESSMENT

| Event | Cause/Effect | L | S | RPN | Mitigation |
|---|---|---|---|---|---|
| Release of GMO | Destruction of container, leakage - harm to user | 3 | 5 | YES | - Chemical/mechanical and biological kill switches<br>- Chassis selection<br>- Ultrafiltrate membranes |
| Release of harmful chemicals | Destruction of container, leakage, incomplete breakdown - harm to user | 2 | 5 | YES | - Denitrifying pathway selection<br>- Fast conversion of intermediates<br>- Active pumping of air to prevent back diffusion |
| Death of bacteria | Insufficient nutrients, high metabolic burden, | 2 | 2 | YES | - Use of a nutrient rich broth like LB<br>- User can 'feed' bacteria by spraying nutrients into filter<br>- The denitrifying pathway can be split between different strains |
| Inaccurate sensor | Incorrect rate constants, model not being correct - user may be misled as to the quality of their air | ? | 1 | N/A | - This would be confirmed in wet labs and tweaked accordingly |
| Filtration does not occur | Incorrect rate constant, model not being correct - air quality does not change | ? | 2 | N/A | - This would be confirmed in wet labs and tweaked accordingly |
| Dual Use | Having a product readily available for civilian purchase - harvesting of intermediates | 1 | 5 | YES | - Intermediates are fully converted and cannot diffuse out<br>- Kill switches ensure bacteria cannot be harvested and reincubated<br>- Intermediates are in very low concentrations |

# PHYSICAL IMPLEMENTATION

The device provides a physical barrier between the user and the bacteria - an ultrafiltrate membrane allows particle flow but keeps the bacteria contained

The compartmentalised filtration units allows for minimal final intermediate concentrations and the implementation of a mechanical kill switch
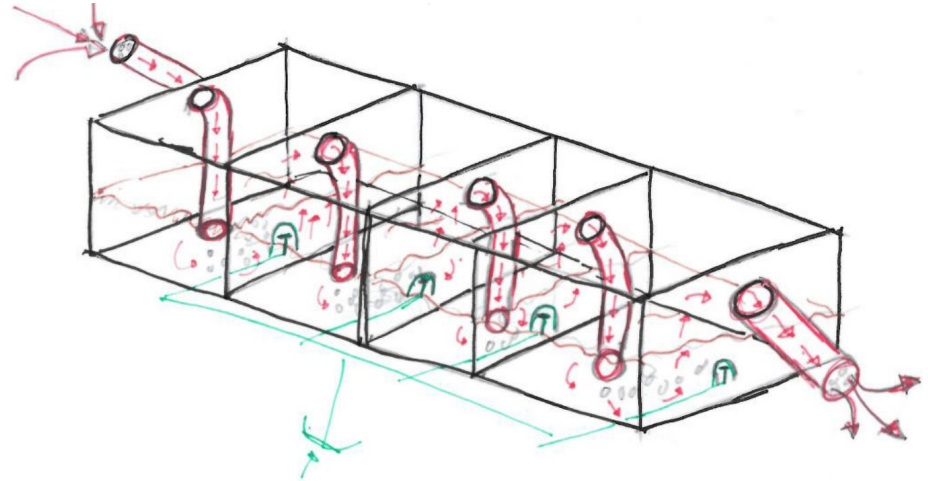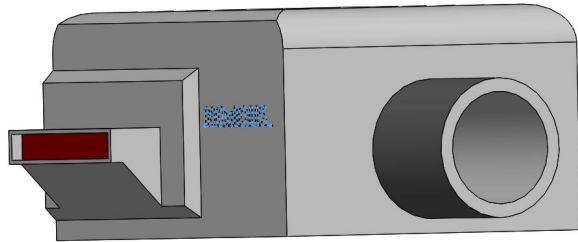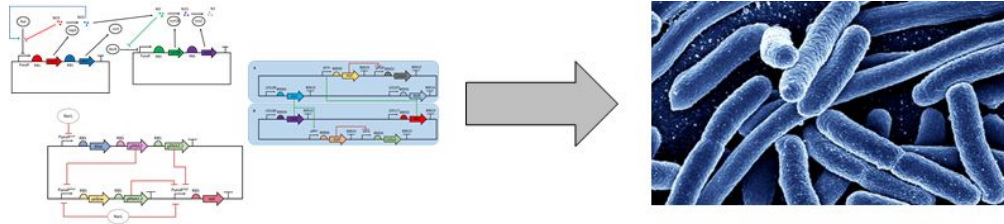
# THE CHASSIS - _E. coli_

**The chassis chosen must satisfy a variety of criteria:**

- Gram-negative
- Non-pathogenic
- Able to hold a large synthetic circuit in the genome

**A future chassis - Cyanobacteria**

- Photosynthetic
- Currently poor characterisation as a chassis

# MULTIPLE CHASSIS

Two chassis with a codependent kill switch

- Lightens metabolic load on the bacteria
- Reduces levels of genomic integration required
- Conforms to the required standards of a biological barrier

**Kill switch 1**

**$NO_3$, $NO_2$ metabolism**

**Kill switch 2**

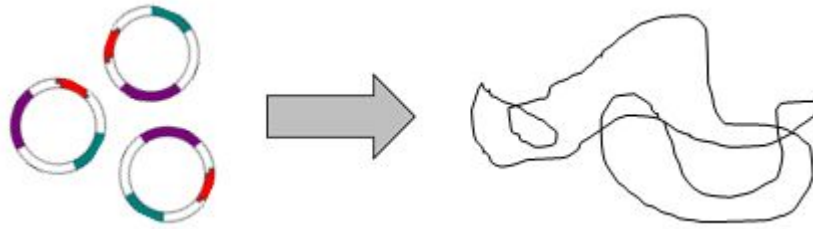**NO, $N_2$ metabolism**
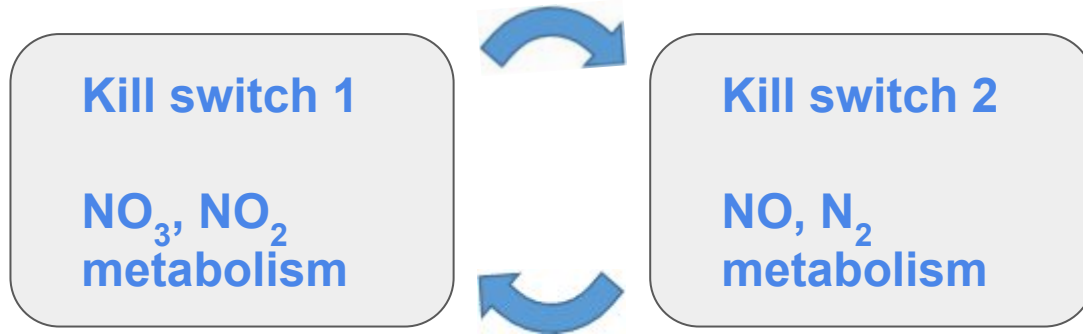
A third Chassis with biosensor activity will also be included.

# GENOMIC INTEGRATION

<u>Plasmid based systems are not an option:</u>

- Stability
- Copy Number
- Require Antibiotic regulation

Genomic integration provides greater stability and regulation of copy number

# Biofiltering: Circuit layout and parts

- Process of **denitrification** by four enzymes (produced in R. denitrificans)

- Feedback from enzyme-producing machinery to gas concentration

- **Inputs:** NO3, NO2, NO, **Output:** N2
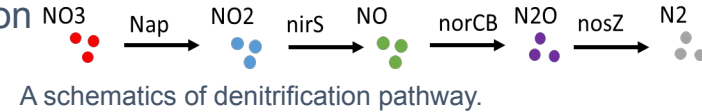
- **Plasmid 1:** NO3 and NO2 conversion enzymes

- **Plasmid 2:** NO and N2O conversion enzymes

- **Assembly of the circuit on two plasmids:**
  (due to size of the circuit)

A schematics of denitrification pathway.
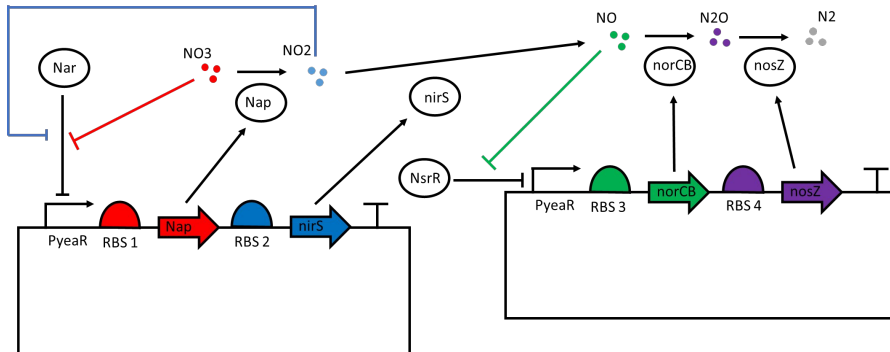
A two-plasmid denitrification circuit schematics.

**Parts to be used:**

NarL repressor - BBa_K1682018 (length: 2831 bp)
NsrR repressor - BBa_K1682011 (length: 426 bp)

**Plasmid 1:**
PyeaR promoter - BBa_K216005 (length: 100 bp)
Ribosome Binding Site 1 – BBa_B0034 (length: 12 bp)
napA enzyme ORF - BBa_K896007 (length: 3127 bp)
Ribosome Binding Site 2 – BBa_B0034 (length: 12 bp)
nirS enzyme ORF - BBa_K1356003 (length: 1707 bp)
Terminator BBa_B0014 (length: 95 bp)

**Plasmid 2:**
PyeaR promoter - BBa_K216005 (length: 100 bp)
Ribosome Binding Site 3 – BBa_B0034 (length: 12 bp)
norCB enzyme ORF - BBa_K1356004 & BBa_K1356005 (length: 441 bp & 1401 bp)
Ribosome Binding Site 4 – BBa_B0034 (length: 12 bp)
nosZ enzyme ORF - BBa_K1356006 (length: 1911 bp)
Terminator BBa_B0014 (length: 95 bp)

**Length of Plasmid 1:** 5053 bp
**Length of Plasmid 2:** 3972 bp

# Biofiltering: Modeling

- Deterministic modelling in MATLAB

- 27 reactions (nonlinear ODEs), 39 parameters, 24 species

- Parameters values are in the Appendix A

- **Modelling results demonstrate that cells can successfully convert the average environmental values of NOx species into N2, if ~ $10^{11}$ - $10^{12}$ E.coli are used -> ~ 0.1-1 liter volume of E. Coli**

- The required volume justifies the use of device in households
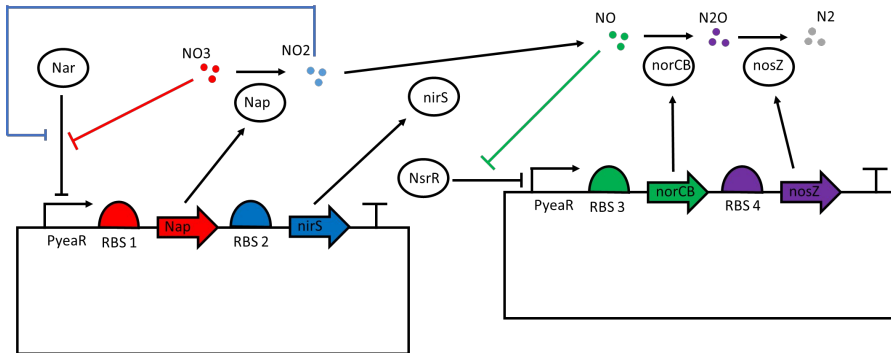


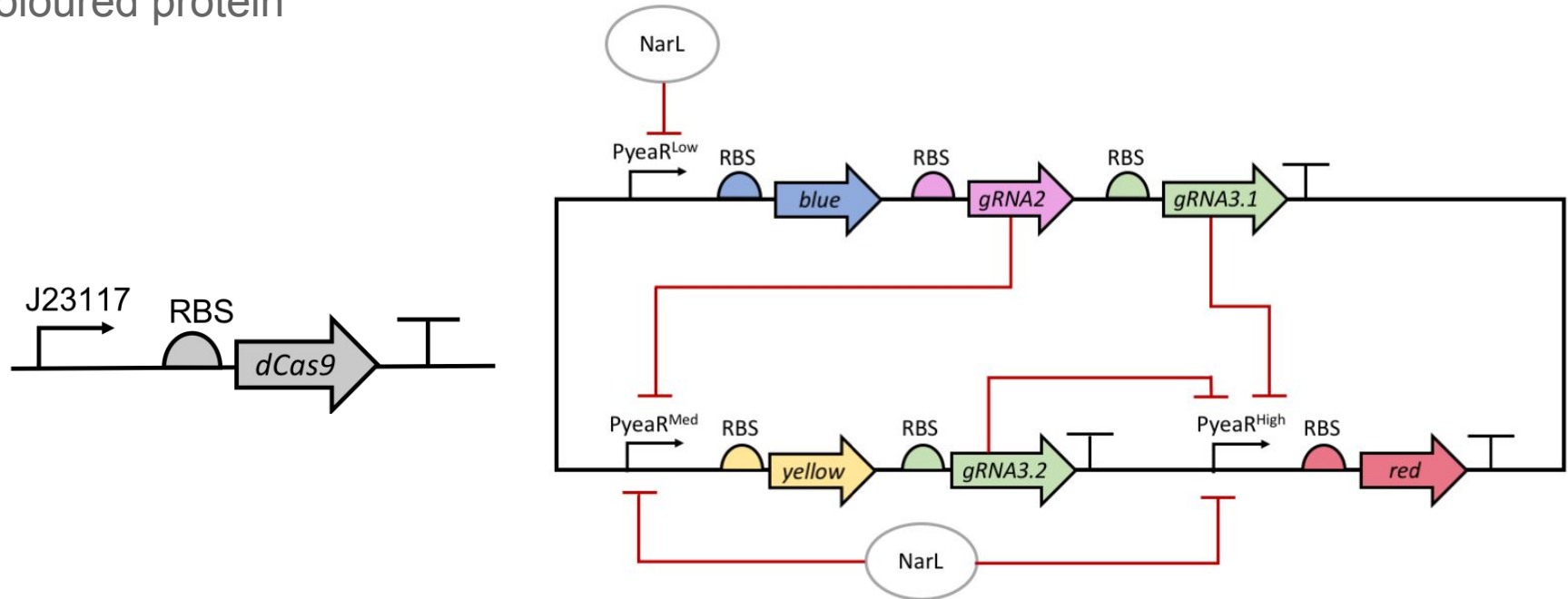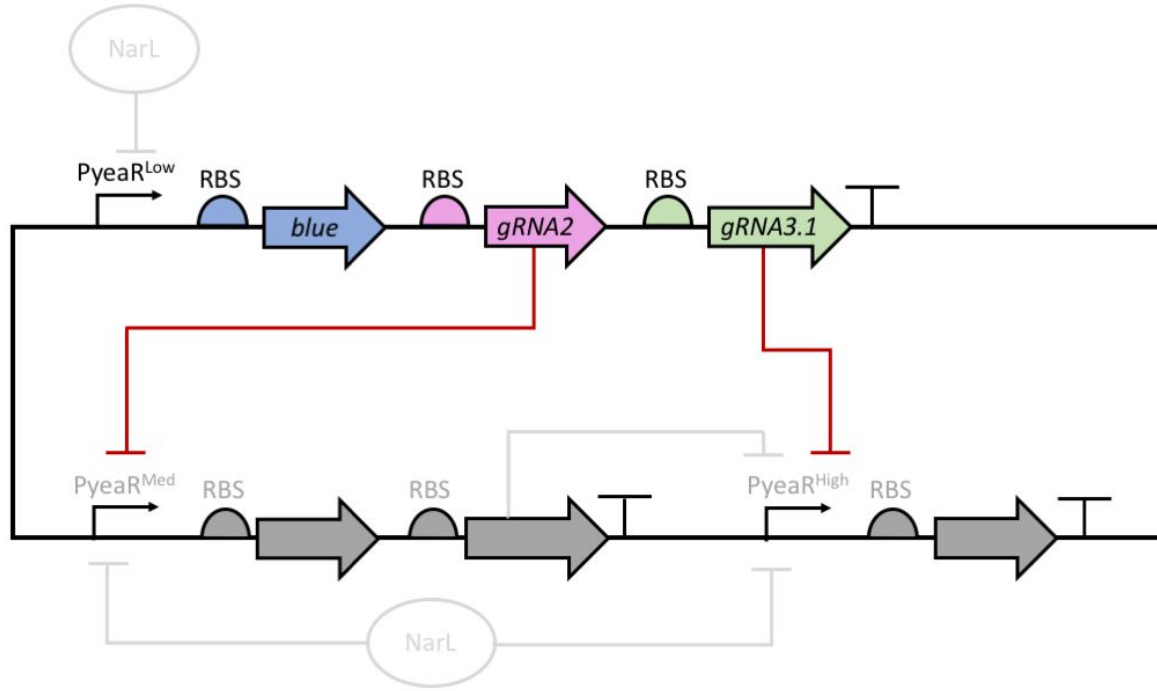A two-plasmid denitrification circuit schematics.
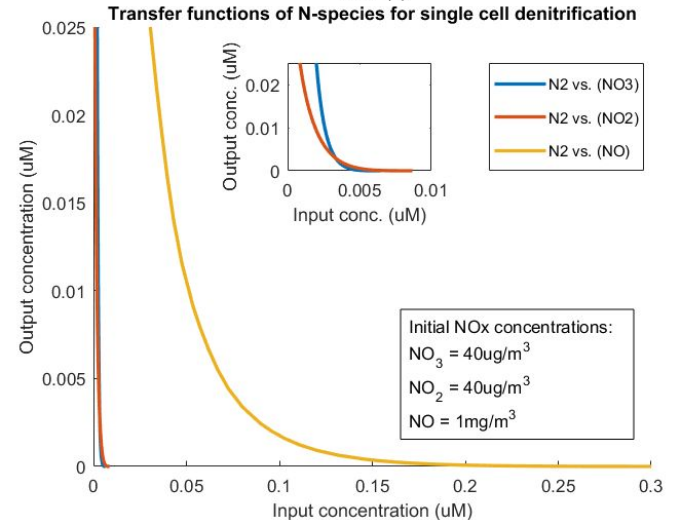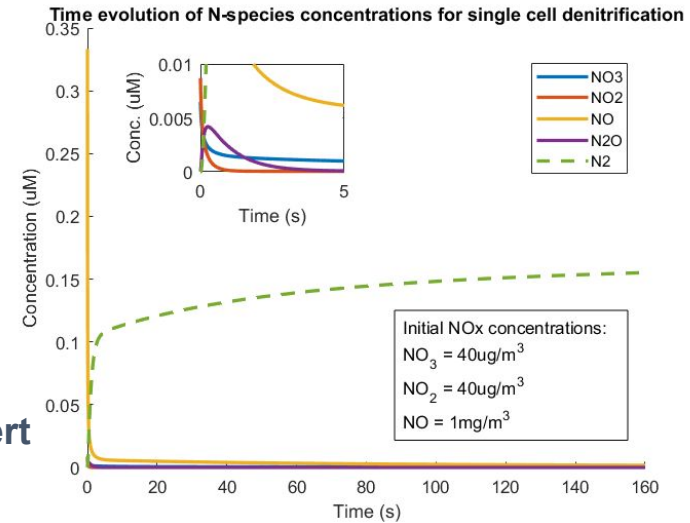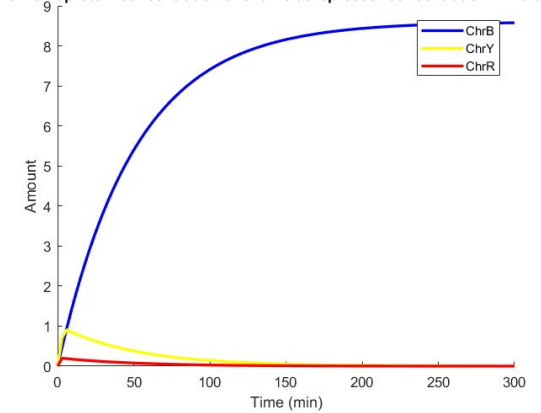
# SEMI-QUANTITATIVE BIOSENSOR

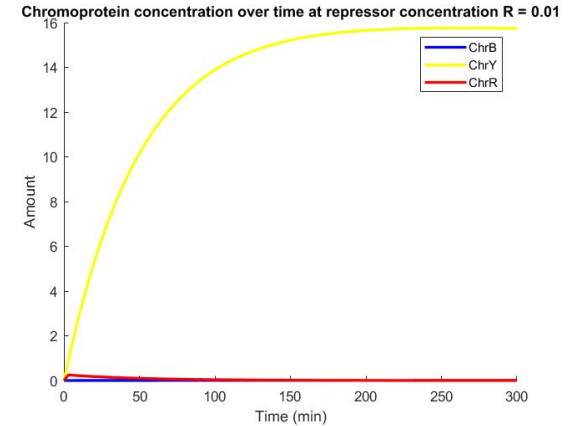Depending on NOx concentration in the medium, E. coli would express different coloured protein

# SEMI-QUANTITATIVE BIOSENSOR
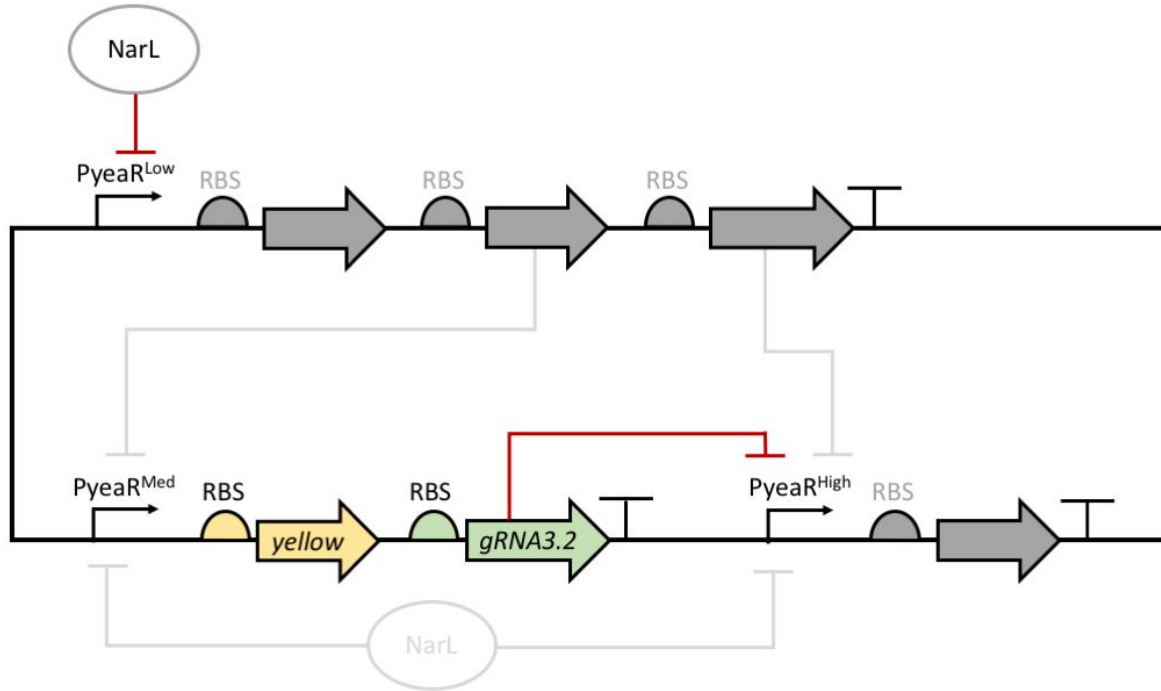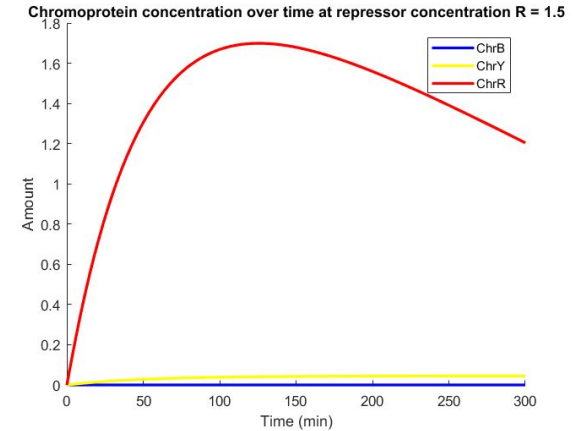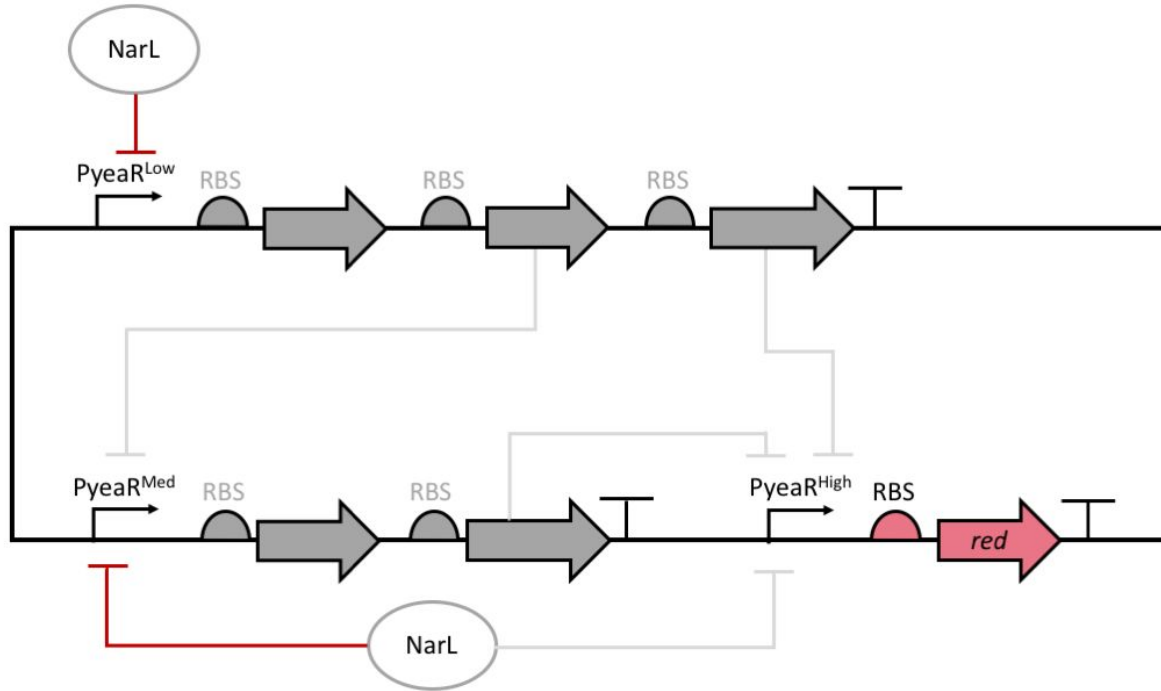
# SEMI-QUANTITATIVE BIOSENSOR
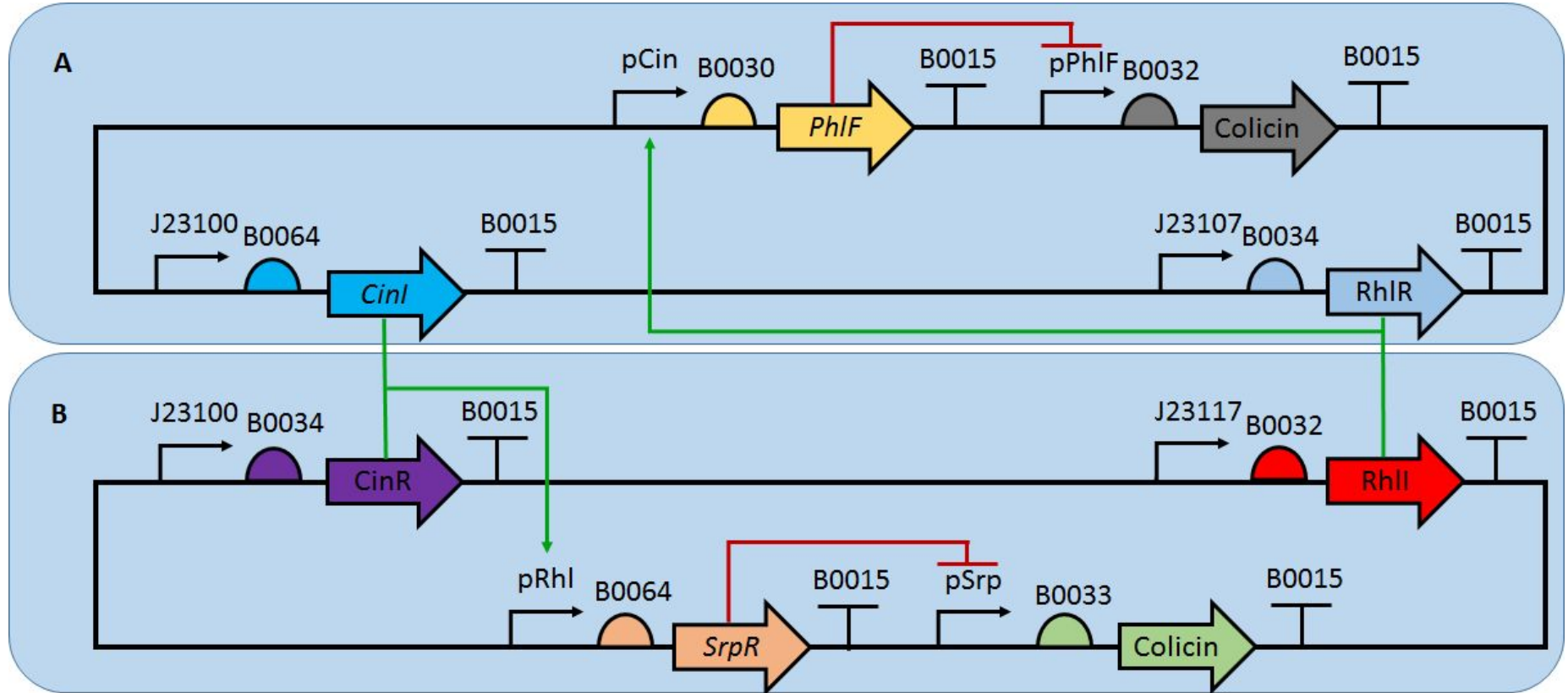
# SEMI-QUANTITATIVE BIOSENSOR

# BIOSENSOR MODEL

Varying affinities of NarL for PyeaR promoters modelled by Hill equation with increasing $K_m$ values

General rates of transcription, translation, and degradation for *E. coli*
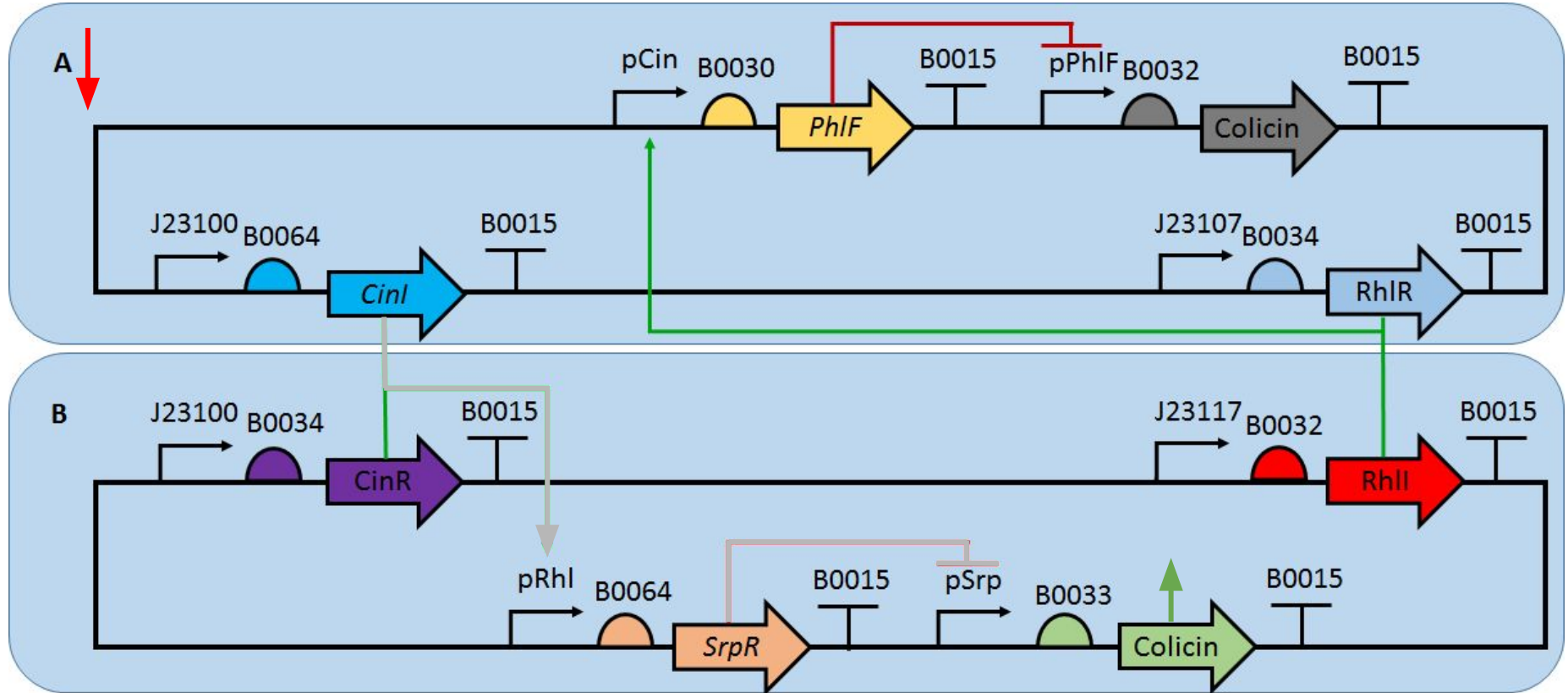
gRNAs silence expression by CRISPRi

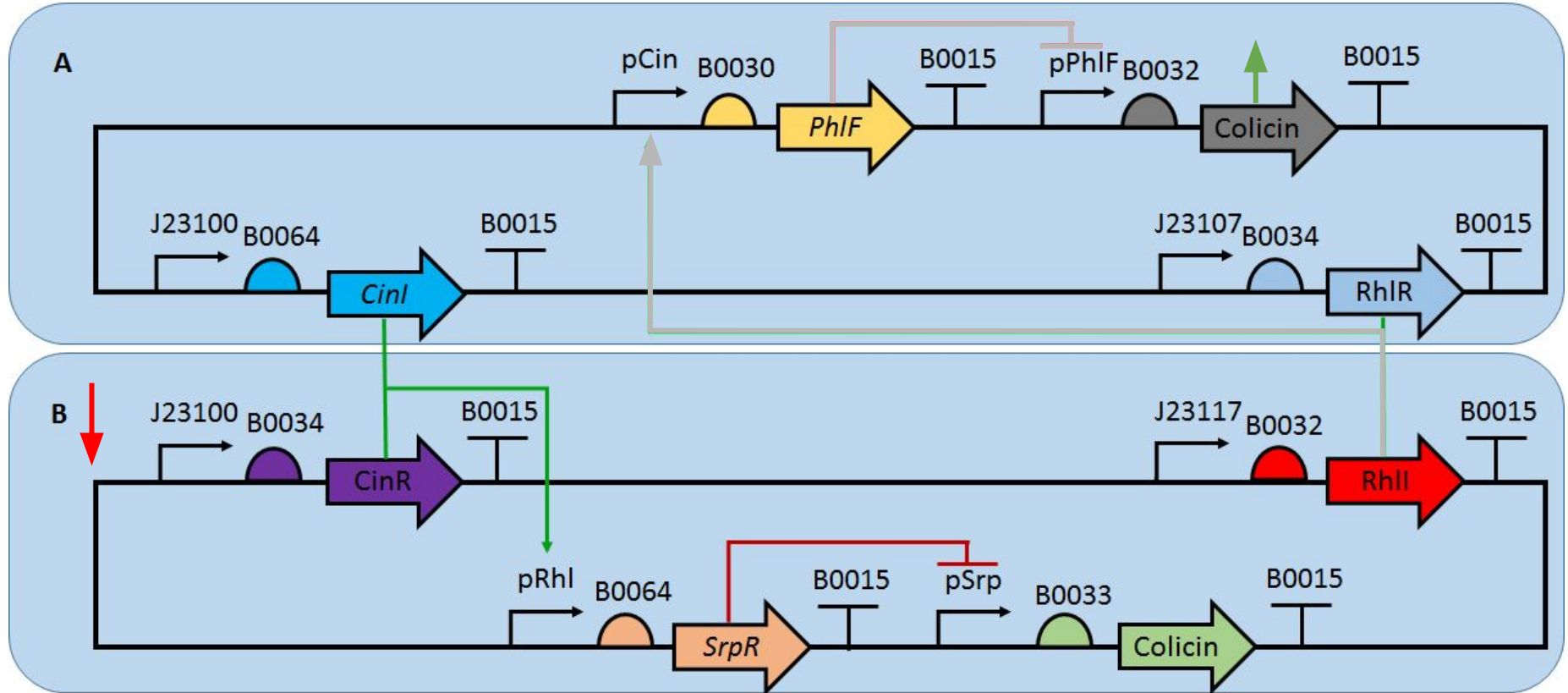dCas9 constitutively expressed on different plasmid

# KILL SWITCH BLOCKS

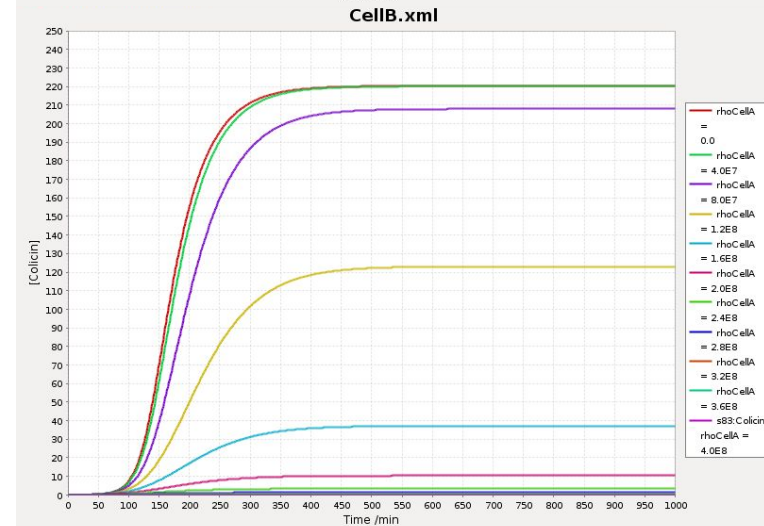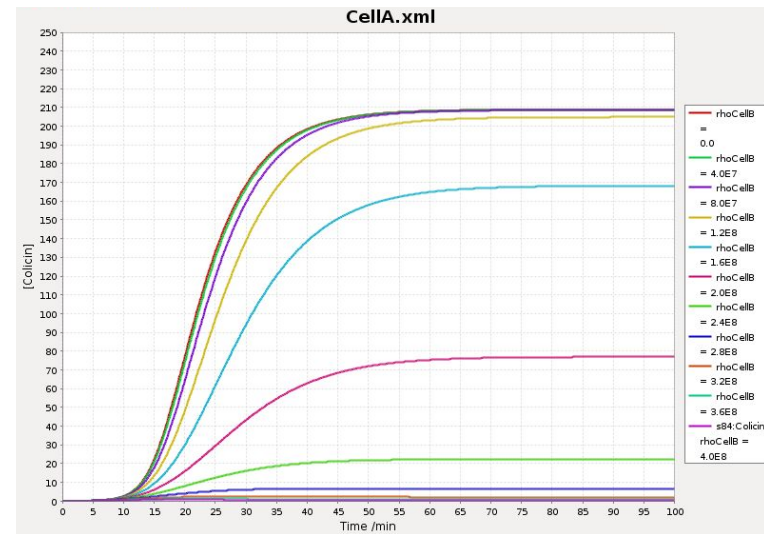# KILL SWITCH BLOCKS

# KILL SWITCH BLOCKS

# KILL SWITCH MODEL

- Deterministic modelling in CellDesigner
- 2 mutually dependent strains modelled
- Optimised for $4*10^8$ cells of each species per ml
- RBS and promoter strengths tuned with model
- RBS and promoters from Anderson set
- Repressors from Stanton et al, 2014 and Glasgow
- Colicin used in both strains
- Colicin toxicity: 14-200 molecules per cell

  Development considerations
- Low-level antitoxin if pPhlF and pSrp (repressors) leaky
- Risk of mutation increasing antitoxin production
- Upper density limit if food control insufficient

# KILL SWITCH TOPOLOGY
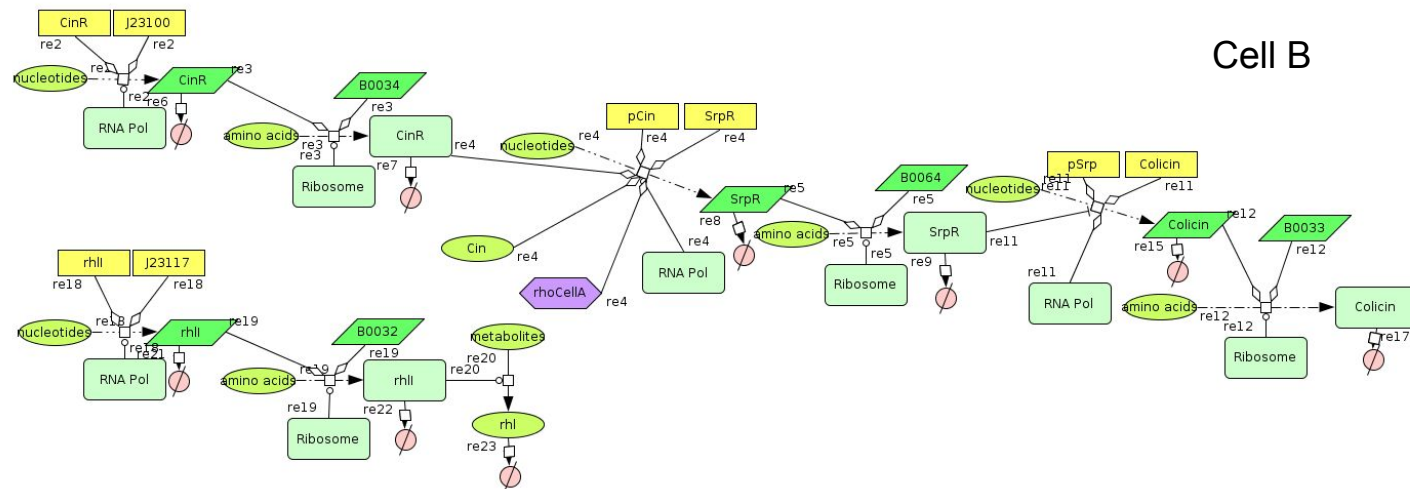
# CONCLUSION

NOx pollution:

- Linked with respiratory disease
- A rising concern in the home

Current methods of NOx removal are too expensive
and unsuitable for the average home

Cheap, effective and safe way to detect reduce NOx levels in the home that
can be scaled to the industrial level

| Air pollutant | Concentration of exposure | Effect | Reference |
|---|---|---|---|
| $SO_x$ | | Infant deaths | 36 |
| $NO_x$ | | Respiratory tract symptoms | 40,56 |
| | | Asthma exacerbations | 41 |
| | | Reduced lung function growth | 42 |
| | | Lymphoma (especially Hodgkin's) incidence | 46 |

TABLE I. Health effects of air pollution on children
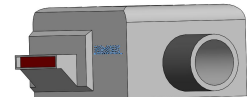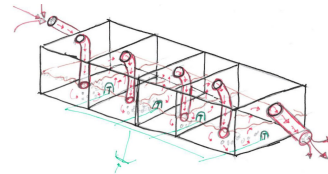
# Appendix A: Modelling of Denitrification process

**Denitrification**

(Reactions modelled using Michaelis-Menten kinetics and mass-action kinetics )

1) $NO_3 \xrightarrow{Nap} NO_2$,   reaction rate: $v = k_{cat1}[Nap]\frac{[NO_3]}{K_{m1}+[NO_3]}$

2) $NO_2 \xrightarrow{nirS} NO$,   reaction rate: $v = k_{cat2}[nirS]\frac{[NO_2]}{K_{m2}+[NO_2]}$

3a) $NO + NO \longleftrightarrow 2NO$,   reaction rate: $v = k_{dimf}[NO]^2 - k_{dimr}[2NO]$

3b) $2NO \xrightarrow{norCB} N_2O$,   reaction rate: $v = k_{cat3}[norCB]\frac{[NO]}{K_{m3}+[NO]}$

4) $N_2O \xrightarrow{nosZ} N_2$,   reaction rate: $v = k_{cat4}[nosZ]\frac{[N_2O]}{K_{m4}+[N_2O]}$
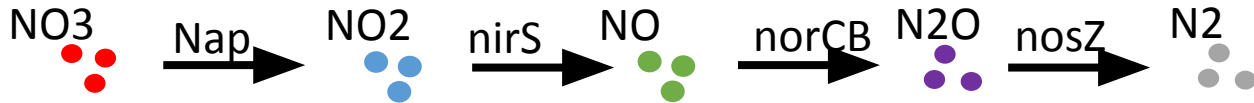
**Sequestration of the promoter and repression of repressors**

(Reactions modelled using mass-action kinetics)

5) $NO_3 + Nar \longleftrightarrow NO_3{:}Nar$,   reaction rate: $v = k_{5f}[NO_3][Nar] - k_{5r}[NO_3:Nar]$

6) $NO_2 + Nar \longleftrightarrow NO_2{:}Nar$,   reaction rate: $v = k_{6f}[NO_2][Nar] - k_{6r}[NO_2:Nar]$

7) $NO + NsrR \longleftrightarrow NO{:}NsrR$,   reaction rate: $v = k_{7f}[NO][NsrR] - k_{7r}[NO:NsrR]$

8) $NsrR + PyeaR \longleftrightarrow NsrR{:}PyeaR$,   reaction rate: $v = k_{8f}[NsrR][PyeaR] - k_{8r}[NsrR:PyeaR]$

9) $Nar + PyeaR \longleftrightarrow NsrR{:}PyeaR$,   reaction rate: $v = k_{9f}[Nar][PyeaR] - k_{9r}[NsrR:PyeaR]$

**Transcription**

(Reactions modelled using mass-action kinetics)

10) $RNAP + PyeaR \longleftrightarrow RNAP{:}PyeaR$,   reaction rate: $v = k_{10f}[RNAP][PyeaR] - k_{10r}[RNAP:PyeaR]$

11) $RNAP{:}PyeaR \longrightarrow mRNA{:}Nap + RNAP + PyeaR$,   reaction rate: $v = k_{11f}[RNAP:PyeaR]$

12) $RNAP{:}PyeaR \longrightarrow mRNA{:}nirS + RNAP + PyeaR$,   reaction rate: $v = k_{12f}[RNAP:PyeaR]$

13) $RNAP{:}PyeaR \longrightarrow mRNA{:}norCB + RNAP + PyeaR$,   reaction rate: $v = k_{13f}[RNAP:PyeaR]$

14) $RNAP{:}PyeaR \longrightarrow mRNA{:}nosZ + RNAP + PyeaR$,   reaction rate: $v = k_{14f}[RNAP:PyeaR]$

**Translation**

(Reactions modelled using mass-action kinetics)

15) $mRNA{:}Nap \longrightarrow Nap + mRNA{:}napA$,   reaction rate: $v = k_{15f}[mRNA:Nap]$

16) $mRNA{:}nirS \longrightarrow nirS + mRNA{:}nirS$,   reaction rate: $v = k_{16f}[mRNA:nirS]$

17) $mRNA{:}norCB \longrightarrow norCB + mRNA{:}norCB$,   reaction rate: $v = k_{17f}[mRNA:norCB]$

18) $mRNA{:}nosZ \longrightarrow norZ + mRNA{:}norZ$,   reaction rate: $v = k_{18f}[mRNA:norZ]$

**mRNA Decay**

(Reactions modelled using mass-action kinetics)

19) $mRNA{:}Nap \longrightarrow null$,   reaction rate: $v = k_{19f}[mRNA:Nap]$

20) $mRNA{:}nirS \longrightarrow null$,   reaction rate: $v = k_{20f}[mRNA:nirS]$

21) $mRNA{:}norCB \longrightarrow null$,   reaction rate: $v = k_{21f}[mRNA:norCB]$

22) $mRNA{:}nosZ \longrightarrow null$,   reaction rate: $v = k_{22f}[mRNA:nosZ]$

**Enzyme/protein dilution**

(Reactions modelled using mass-action kinetics)

23) $Nap \longrightarrow null$,   reaction rate: $v = k_{23f}[Nap]$

24) $nirS \longrightarrow null$,   reaction rate: $v = k_{24f}[nirS]$

25) $norCB \longrightarrow null$,   reaction rate: $v = k_{25f}[norCB]$

26) $nosZ \longrightarrow null$,   reaction rate: $v = k_{26f}[nosZ]$
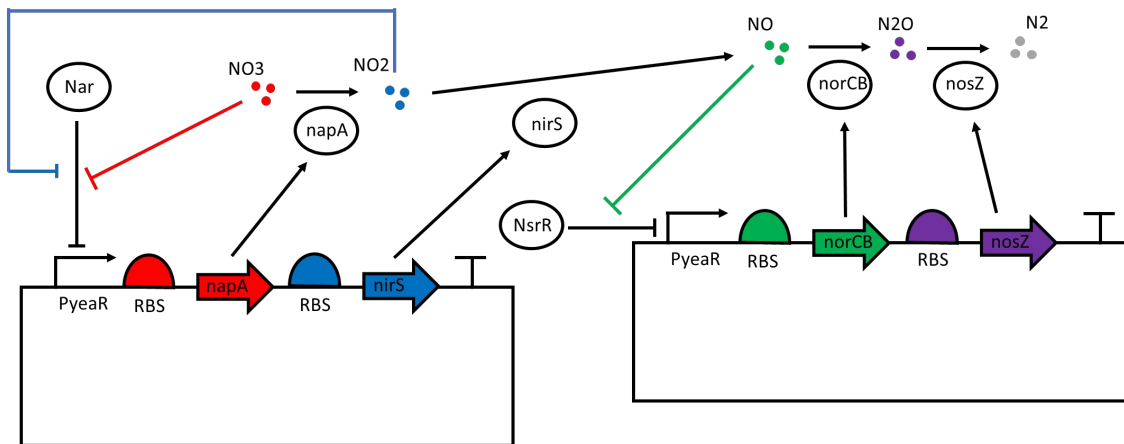
Chemical equations of the model with the rates.



A schematics of denitrification pathway.



A two-plasmid denitrification circuit schematics.

# Appendix B: Values of parameters

| Parameter | Value | Units | Reference |
|---|---|---|---|
| Km1 | 45 | uM | [1] |
| kcat1 | 2.5 | 1/s | [1] |
| Km2 | 12 | uM | [2] |
| kcat2 | 74 | 1/s | [2] |
| Km3 | 35 | uM | [2] |
| kcat3 | 81.2833 | 1/s | [2] |
| Km4 | 6.7 | uM | [2] |
| kcat4 | 264 | 1/s | [2] |
| kdimf | 28 | 1/(uM*s) | [3] *approximated |
| kdimr | 0.022 | 1/s | [3] *approximated |
| k5f | 28 | 1/(uM*s) | [3] *approximated |
| k5r | 0.022 | 1/s | [3] *approximated |
| k6f | 28 | 1/(uM*s) | [3] *approximated |
| k6r | 0.022 | 1/s | [3] *approximated |
| k7f | 28 | 1/(uM*s) | [3] *approximated |
| k7r | 0.022 | 1/s | [3] *approximated |
| k8f | 28 | 1/(uM*s) | [3] *approximated |
| k8r | 0.022 | 1/s | [3] *approximated |
| k9f | 28 | 1/(uM*s) | [3] *approximated |
| k9r | 0.022 | 1/s | [3] *approximated |

| Parameter | Value | Units | Reference |
|---|---|---|---|
| k10f | 1 | 1/(uM*s) | [3] *approximated |
| k10r | 1 | 1/s | [3] *approximated |
| k11f | 0.016 | 1/s | [4] * approximated calculated for average gene size of 1000 bp |
| k12f | 0.016 | 1/s | [4] * approximated calculated for average gene size of 1000 bp |
| k13f | 0.016 | 1/s | [4] * approximated calculated for average gene size of 1000 bp |
| k14f | 0.016 | 1/s | [4] * approximated calculated for average gene size of 1000 bp |
| k15f | 0.024 | 1/s | [4] * approximated calculated for average protein size of 1000 bp |
| k16f | 0.024 | 1/s | [4] * approximated calculated for average protein size of 1000 bp |
| k17f | 0.024 | 1/s | [4] * approximated calculated for average protein size of 1000 bp |
| k18f | 0.024 | 1/s | [4] * approximated calculated for average protein size of 1000 bp |
| k19f | 0.0018 | 1/s | [5] * approximated |
| k20f | 0.0018 | 1/s | [5] * approximated |
| k21f | 0.0018 | 1/s | [5] * approximated |
| k22f | 0.0018 | 1/s | [5] * approximated |
| k23f | 0.000014 | 1/s | [5] * approximated |
| k24f | 0.000014 | 1/s | [5] * approximated |
| k25f | 0.000014 | 1/s | [5] * approximated |
| k26f | 0.000014 | 1/s | [5] * approximated |

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