BBF RFC #: Categorization of non-coding RNA In Part Registry

Eric Ming Fung CHEUNG, Raul Guillermo MEDINA CUELLAR and King L. CHOW

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1. Purpose

Over the years, the number of non-coding RNAs in Part Registry has increased steadily over time and many have been made available to end users. More than 400 entries in the Part Registry are related to RNA devices (updated 25-12-2014). Based on different mode of actions and natures of non-coding RNAs (ncRNAs), they can be grouped under different categories. However, the Part Registry currently does not have a catalog page, categorizing methods or guidelines to organize and curate existing ncRNAs. Some of them are simply grouped under type "RNA", while others are not. This is not useful for looking up and utilizing them. For example, BBa_K145013, which is a part for antisense LuxI, could be used to add an extra layer of control to the widely utilized quorum sensing Lux pathway but it has not been extensively reused. It is likely that Part Registry users are unaware of its existence because there has yet to be an information hub for ncRNA.
We would like to solve this problem by designing a list of category tags based on ncRNAs review by Qi et al.[1], as well as a guideline, so that automated display of non-coding RNAs by the <parttable> function can be facilitated. By doing so, we hope that we can assist other users to find and use those parts efficiently. Ultimately we want to facilitate the implementation of the philosophy of Part Registry, which is to get and give (and share), through a better organization of ncRNA parts in the registry.

2. Relation to other BBF RFCs

BBF RFC # does not update or replace any earlier BBF RFC.

3. Copyright Notice

4. Methods

4.1 Explanation

Different types of ncRNAs have different functions and working mechanisms, as such one should be able to classify different types of ncRNA based on their differences. However, composite ncRNA devices might share more than one type of characteristics,

functions or natures, and belong to more than one class, so it is more helpful to give ncRNA parts classification tags than strictly classifying them under particular types. Here we attempt to exhaustively produce a list of fundamental natures and working mechanisms for different types of ncRNAs. By fundamental we mean that a property is so small that it can hardly be further decomposed into smaller functional properties. Tags have also been defined in a way such that an ncRNA will carry a tag if and only if it fulfils all the descriptors defined in the tag.

The procedures for tagging an ncRNA BioBrick, illustrated by Figure 1, is as follow:

1. Assign tag for function of parts. Note that this ncRNA may possess functions that are not described in this document.
   i. If the function of the ncRNA BioBrick is Regulation, go to 2;
   ii. Otherwise go to 3.

2. Assign tag for "Level of Regulation".

3. Compare the functional properties of the BioBrick against those of different types of ncRNA listed in this document.
   i. If some or parts of the functional properties carried by a BioBrick fulfil all the descriptors defined in a particular tag, attach that tag to the BioBrick of interest.
   ii. If none of the combinations of the functional properties carried by that BioBrick fulfil all the descriptors defined in that particular tag, move on to the next tag.

4. Check if the BioBrick has been compared against all the tag.

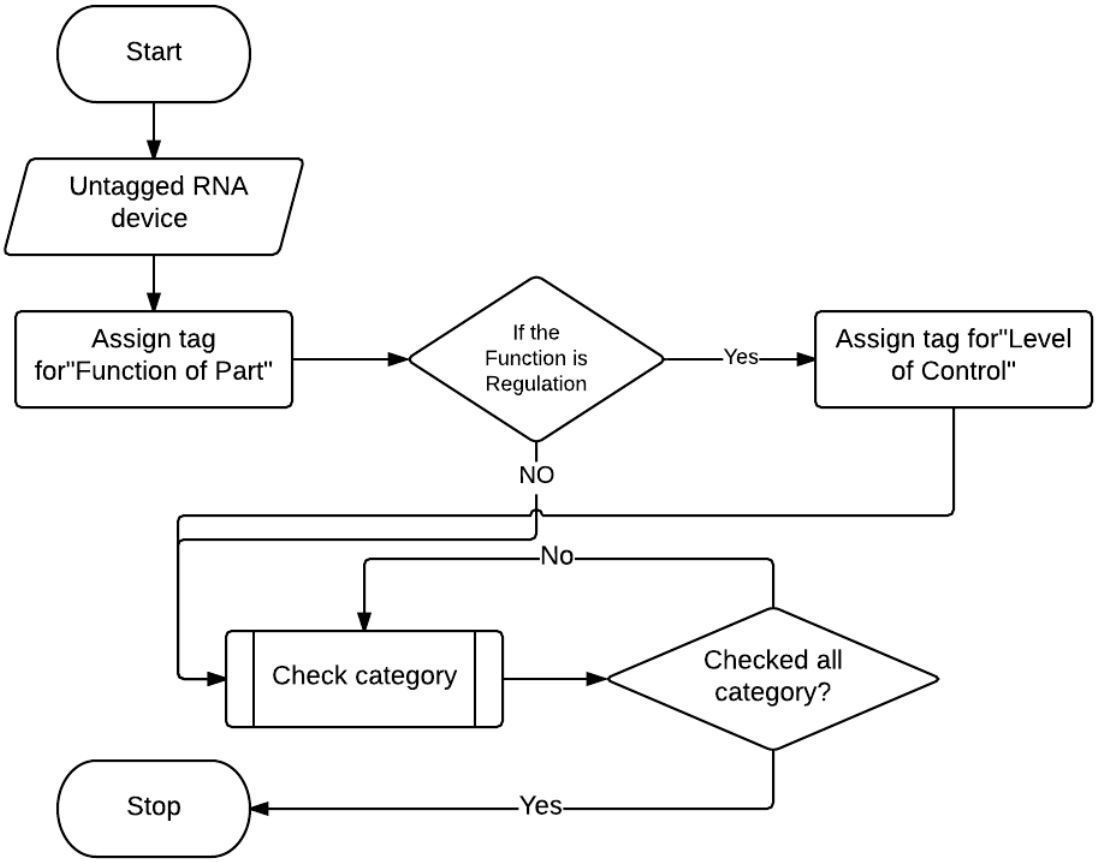

Figure 1. General Workflow for assigning tags to a BioBrick containing ncRNA.
To assign appropriate tags to a particular BioBrick containing ncRNA, the category "Function of part" SHOULD be assigned first. If the function of the ncRNA is to regulate gene expression, an appropriate category for "level of control" SHOULD also be specified. Then the ncRNA device being inspected SHOULD be compared against the fundamental properties of different type of RNA devices until it has been compared with all categories.

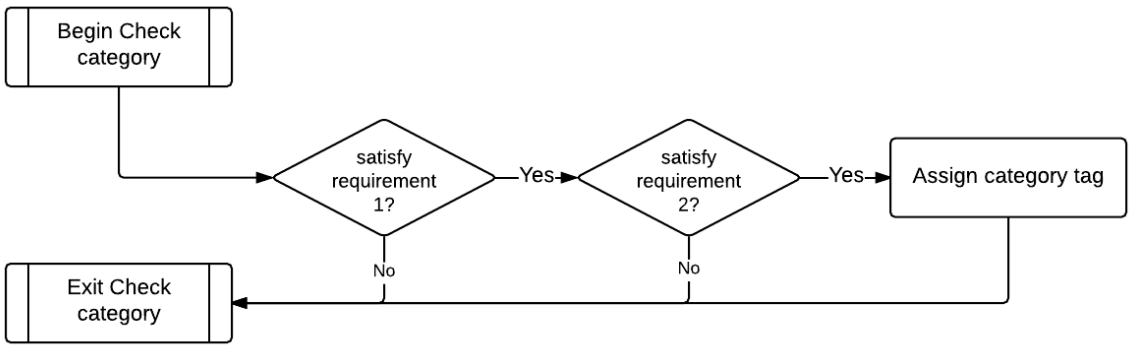

Figure 2. Flow of inspection of a BioBrick containing ncRNA against an ncRNA category with 2 declared fundamental characteristics. Only when the ncRNA being examined fulfil all listed requirement for a particular type of ncRNA will the category tag be attached.

4.2 Table of suggested categories:

TYPE OF CATEGORY	*THE PROPOSED CATEGORIES:*
LEVEL OF REGULATION	//RNA/ncRNA/regulation_level/DNA
LEVEL OF REGULATION	//RNA/ncRNA/regulation_level/RNA
LEVEL OF REGULATION	//RNA/ncRNA/regulation_level/protein
FUNCTION OF PART	//RNA/ncRNA/function/insulating
FUNCTION OF PART	//RNA/ncRNA/function/stability_control
FUNCTION OF PART	//RNA/ncRNA/function/colocalization_scaffold
FUNCTION OF PART	//RNA/ncRNA/function/reporter
FUNCTION OF PART	//RNA/ncRNA/function/regulation
NATURE OF PART	//RNA/ncRNA/nature/RNA_aptamer
NATURE OF PART	//RNA/ncRNA/nature/CRISPR
NATURE OF PART	//RNA/ncRNA/nature/RNA_IN_OUT/RNA_OUT
NATURE OF PART	//RNA/ncRNA/nature/RNA_IN_OUT/RNA_IN
NATURE OF PART	//RNA/ncRNA/nature/riboregulator/cis_repressive
NATURE OF PART	//RNA/ncRNA/nature/riboregulator /trans_activating
NATURE OF PART	//RNA/ncRNA/nature/riboswitch
NATURE OF PART	//RNA/ncRNA/nature/asRNA
NATURE OF PART	//RNA/ncRNA/nature/RNAi
NATURE OF PART	//RNA/ncRNA/nature/aptazyme
NATURE OF PART	//RNA/ncRNA/nature/ribozyme
NATURE OF PART	//RNA/ncRNA/nature/pT181
NATURE OF PART	//RNA/ncRNA/nature/others
NATURE OF PART	//RNA/ncRNA/nature/complex
NATURE OF PART	//RNA/ncRNA/nature/ligand
NATURE OF PART	//RNA/ncRNA/nature/target_sequence

4.3 Functions of ncRNAs:

//RNA/ncRNA/function/insulating
Description: ncRNA processes RNA into fragments with clear boundaries. One example of ncRNAs with this function is the self-cleaving ribozyme (BBa_K598000).

//RNA/ncRNA/function/stability_control
Description: ncRNA stabilizes or destabilizes an RNA molecule, typically by forming hairpin structures that block or facilitate access of ribonucleases to the RNA itself. Other examples include the poly-A signaling sequence on a eukaryotic mRNA.

//RNA/ncRNA/function/colocalization_scaffold
Description: ncRNA recruits more than one element and bring them into close proximity to achieve colocalization of the elements.

//RNA/ncRNA/function/reporter
Description: ncRNA displays information and report biological activities. One example is the Spinach RNA aptamer (BBa_K734002)

//RNA/ncRNA/function/regulation
Description: ncRNA regulates gene expression (such as riboswitches and riboregulators) • ncRNAs under this categories SHOULD also be tagged with appropriate categories specifying the level(s) of regulation (such as //RNA/ncRNA/regulation_level/DNA)

4.4 Fundamental Natures for Different Types of ncRNAs:

RNA Aptamer

//RNA/ncRNA/nature/RNA_aptamer
I. Binds to specific target molecules not through Watson-Crick base pairing
Description: An RNA aptamer can fold into a tertiary confirmation that binds with strong affinity and high specificity to small molecules through non-Watson-Crick base pairing[2].

CRISPR

//RNA/ncRNA/nature/CRISPR
I. Associates with Cas nuclease or their derivatives
Description: CRISPR/Cas RNAs are guide RNAs that direct Cas proteins to their spacers. They can be a 2-subunit RNA comprising CRISPR RNA (crRNA) and trans-acting crRNA (tracrRNA). They can also be a single guide RNA by fusing the above 2 functional domains together through RNA linkers. They associate with Cas proteins or their derivatives and guide them to DNA containing complementary sequence to crRNA[3].

Antisense RNA

//RNA/ncRNA/nature/asRNA
I. Functions as a single stranded RNA
II. Contains long complementary regions to messenger RNA
Description: asRNAs are single stranded RNAs that usually form base pair extensively with the target sense RNA / DNA. They can block translation, interfere with transcription, or module RNA stability. A review by Thomason and Storz provided detailed descriptions to their properties and functions[4].

RNA-IN / RNA-OUT

//RNA/ncRNA/nature/RNA_IN_OUT/ RNA_OUT	//RNA/ncRNA/nature/RNA_IN_OUT/RNA_IN
I. Contains antisense sequence to RNA-IN	I. Forms complementary base pairing with the loop of RNA-OUT
II. Contains a stem and a loop, where the loop interacts with RNA_IN	
III. Forms complementary base pairing with RNA-IN	
IV. Acts in *trans*	
Description: RNA-IN RNA-OUT regulates translation on the RNA level. RNA-IN is located upstream of a RBS whereas RNA-OUT is a small ncRNA which forms complementary bases with RNA-IN. RNA-OUT, when introduced, will hybridize with RNA-IN and block the RBS and the start codon, with the RBS locked up, translation cannot take place[5-6].	

Riboregulator

//RNA/ncRNA/nature/riboregulator /trans_activating	**//RNA/ncRNA/nature/riboregulator/cis_repressive**
I. Acts in *trans*	I. Locates at 5' end of mRNA
II. Contains complementary sequences to cis-repressive sequence	II. Contains complementary sequence to RBS
III. Contains stem and loop, often sequesters part of the complementary sequence	III. Forms secondary structure to sequester RBS
IV. Functions to expose the cis-repressed RBS	
Description: Riboregulators regulate translation by two elements: a cis-repressive sequence upstream of a RBS, and a trans-activating RNA[7]. In absence of the latter, the cis-repressive sequence promotes formation of a hairpin at the RBS. With the RBS locked up, translation cannot take place. Trans-activating RNA, when introduced, can form complementary bases to cis-repressive sequence, promotes disassembly of the hairpin, and expose the RBS for ribosomal binding, allowing translation to occur.	

RNA interference

//RNA/ncRNA/nature/RNAi
I. 19-25 nucleotides long
II. Participates in the RNAi pathway
III. Targets mRNA through complementary sequences
Description: Small interfering RNAs (siRNAs) and micro RNAs (miRNAs) function through the RNA interference (RNAi) pathway. siRNAs are usually produced by "dicing" exogenous, long double stranded RNA into 21-nucleotides small fragments. Whereas miRNAs usually have an endogenous origin and started as hairpin transcripts. Processed siRNAs or miRNAs associates with Argonaute in the RNA-induced silencing complex (RISC). The complex then search for RNA targets using the siRNA/miRNA, and in most cases degrades the latter, resulting in inhibition or reduction of gene expression[8-9,12].

Riboswitch

//RNA/ncRNA/nature/riboswitch
I. Contains an aptamer domain site
II. Contains an expression platform
III. Undergoes conformational changes upon binding of ligand. Change in conformation should result in change in gene expression
Description: A riboswitch is a segment on the mRNA that has the ability to detect small molecules or physical changes (like change in temperatures), and regulates gene expression in an on or off manner. Riboswitches usually contain sensor domains that bind small molecules and regulatory domains that regulate gene regulation. Riboswitches are therefore also aptamers in nature. Upon binding of a suitable ligand in the sensor domain, riboswitches undergo conformational changes that can lead to different outcomes like translation inhibition or mRNA degradation[10].

Ribozyme

//RNA/ncRNA/nature/ribozyme
I. Possesses catalytic activity on its own
Description: A ribozyme is an RNA molecule with intrinsic catalytic activity, which is usually a cleavage or ligation activity[11].

Aptazyme

//RNA/ncRNA/nature/aptazyme
I. Senses small molecules
II. Binds small molecule that leads to ribozyme-mediated cleavage
Description: RNA aptazymes, as the name suggests, are RNAs that carry properties of both aptamers and ribozymes[13]. They are capable of sensing small molecules. Ribozyme-mediated cleavage is triggered upon ligand binding. • For a part to be qualified as an aptazyme, the aptamer domain and the ribozyme domain SHOULD be functionally coupled. An RNA that contains aptamer domains and ribozyme domains without functional relationships is merely a composite RNA device.

pT181

//RNA/ncRNA/nature/pT181
I. Acts in *trans*
II. Binds to target mRNA and induces formation of a premature terminator
III. Derived from pT181
Description: The pT181-RNAI is a special class of ncRNAs derived from elements in the *Staphylococcus aureus* pathogenicity plasmid pT181. A specific 5' UTR region would normally form an anti-termination loop. pT181-RNAi, when introduced, induces formation of a premature terminator loop instead, which results in early termination an incompletely transcribed mRNA[14].

Complex

//RNA/ncRNA/nature/complex
I. Consists of more than one type of ncRNA
Description: This tag describes ncRNA BioBricks containing 2 or more ncRNAs.

Target sequence

//RNA/ncRNA/nature/target_sequence
I. Serves as a target sequence for other ncRNA devices
Description: This tag describes segments of ncRNAs that are purposefully designed to serve as recognizable targets by other ncRNAs.

Other

//RNA/ncRNA/nature/others
I. This tag describes ncRNAs that do not belong to any types of ncRNA listed above.

5. Examples

5.1 Spinach Aptamer (BBa_K734002)

Type of Category	THE PROPOSED TAGS	Justification
FUNCTION OF PART	//RNA/ncRNA/function/reporter	It is a reporter marking a particular RNA species. It requires the fluorophore dimethoxy-HBI (DMHBI).
LEVEL OF CONTROL	None	It is not involved in regulating gene expression.
NATURE OF PART	//RNA/ncRNA/nature/aptamer	Spinach RNA aptamer folds into specific tertiary structure that shows high affinity to a specific molecule. This classifies it as an aptamer.
Chassis	//Chassis/prokaryote/ecoli //Chassis/eukaryote/human	There are evidences that this part is functional in the two chassis.

5.2 Riboregulator

Riboregulator Lock 1 (BBa_J01010)

Type of Category	THE PROPOSED TAGS	Justification
FUNCTION OF PART	//RNA/ncRNA/function/regulation	It is involved in regulating gene expression.
LEVEL OF CONTROL	//RNA/ncRNA/regulation_level/RNA	It regulates gene expression on the RNA level.
NATURE OF PART	//RNA/ncRNA/nature/riboregulator/cis_repressive	Cis-repressive sequence binds to the 5'UTR, including the RBS by Watson-Crick base pairing. The sequestration of RBS represses translation.
Chassis	//Chassis/prokaryote/ecoli	There are evidences that this part is functional in the chassis.

Riboregulator key 1 (BBa_J01008)

Type of Category	THE PROPOSED TAGS	Justification
FUNCTION OF PART	//RNA/ncRNA/function/regulation	It is involved in regulating gene expression.
LEVEL OF CONTROL	//RNA/ncRNA/regulation_level/RNA	It regulates gene expression on the RNA level.
NATURE OF PART	//RNA/ncRNA/nature/riboregulator/trans_activating	Trans-activating sequence binds to the cis-repressive sequence by Watson-Crick bas paring. The exposure of RBS allows translation.
Chassis	//Chassis/prokaryote/ecoli	There are evidences that this part functional in the chassis.

5.3 Anti-theophylline Aptazyme (BBa_K1442006)

Type of Category	THE PROPOSED CATEGORIES	**Justification**
FUNCTION OF PART	//RNA/ncRNA/function/regulation	It is involved in regulating gene expression.
LEVEL OF CONTROL	//RNA/ncRNA/regulation_level/RNA	It regulates gene expression on the RNA level.
NATURE OF PART	//RNA/ncRNA/nature/aptazyme	It is capable of sensing theophylline and upon binding ribozyme-mediated cleavage is triggered.
Chassis	//Chassis/prokaryote/ecoli	There are evidences that this part is functional in the chassis.

5.4 Guide RNA target for RFP (BBa_K1062000)

Type of Category	THE PROPOSED CATEGORIES	**Justification**
FUNCTION OF PART	//RNA/ncRNA/function/regulation	It is involved in regulating gene expression.
LEVEL OF CONTROL	//RNA/ncRNA/regulation_level/DNA	It regulates gene expression on the DNA level.
NATURE OF PART	//RNA/ncRNA/nature/CRISPR	It binds to Cas9, and is involved in the CRISPR pathway.
Chassis	//Chassis/prokaryote/ecoli	There are evidences that this part is functional in the chassis.

6. Author's Contact Information

Eric Ming Fung CHEUNG: mfcheungaa@connect.ust.hk
Raul Guillermo MEDINA CUELLAR: rgmc@connect.ust.hk
King L. CHOW: bokchow@ust.hk

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