Reclone.org: a Global Collaboration for Equitable Access to Biotechnology with Open DNA Collections

Yan Kay Ho[1,2], Felipe Xavier Buson[2,3], Isaac Núñez[2,4], Tamara Matute[2,4], Edjohn Aaron Macauyag[1,2], Cibele Zolnier[2], Scott Pownall[2,5], Fernán Federici[2,4,6], Jenny Molloy[1,2,*]

[1]University of Cambridge, [2]Reclone – the Reagent Collaboration Network, [3]University of Bristol, [4]Millennium Institute for Integrative Biology (Chile), [5]Open Science Network Society, [6]Pontificia Universidad Católica de Chile, *Email: coordination@reclone.org

Abstract

Access to affordable enzymes is essential for molecular biology and yet **is often not readily accessible** to researchers **in low- and middle-income countries** (LMICs). Supply chains can be long and expensive, sometimes resulting in weeks to months of delays. This limits the type, scale, and quality of research that LMIC researchers can undertake and ultimately **worsens inequities in global biotechnology innovation**.

Reclone – the Reagent Collaboration Network – co-created the **Open DNA Collections (ODC) as a solution** to this problem: a **community-owned**, **accessible** set of **physical and digital resources** that biotechnological researchers, innovators, and commercial enterprises can use for local manufacturing of critical proteins and enzymes.

The FreeGenes Initiative[1,2] and Addgene[3] already enabled us to **share these reagents to over 500 researchers in 50 countries** and we now aim to extend our reach, providing documentation and guidance to support our users, thereby reducing the barriers to molecular biology. Together, we hope to forge **a future with equitable access** to tools **for all researchers everywhere**.

Reclone Vision and Missions

Our Vision is to see a future where all biologists have **equitable access to the reagents** and tools they need to discover, build, and innovate with biology.

We are working towards our vision through our three primary **Missions**:

Regional Hubs

Establish **Regional Reagent Distribution Hubs** to easily share DNA parts and collections, enabling local researchers to make affordable, reliable enzymes, reagents, and other enabling biotechnologies for research and innovation.

Community

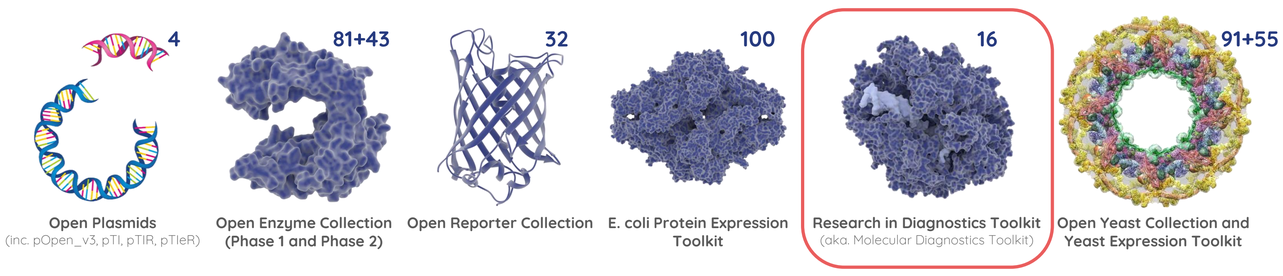
Build a **Globally-Connected Community of Researchers** who wish to collaborate and share ideas and open-source tools that can shape an equitable and sustainable future for biotechnology to benefit science, our people, and our planet.

Data Repository

Co-create a **Repository of Open Access Education and Training Resources** for sharing experimental protocols, open software, hardware designs, knowledge, and experiences – provided by community members, for community members.

The Open DNA Collections

What collections are available for use and distribution?



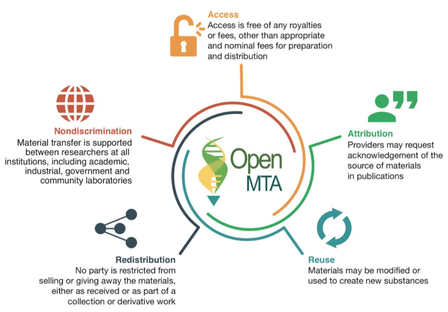
The collections form a library of interchangeable DNA parts, which can be mixed-and-matched together to form transcriptional units using Golden Gate Assembly[4].

Whilst most the individual parts are stored in an open-source cloning vector pOpen_v3, the **Research in Diagnostics Toolkit** (**RiDT**; circled) consist of **"ready-to-express"** constructs: each part within RiDT is a gene with an IPTG-inducible promoter, has a His tag for protein purification, and is stored within the open-source pTI expression vector.

Where can I get the collections and what can I do with them?

Part Types	Examples
Promoters	pDawn: light inducible
	cI857_PL/PR: heat inducible
Affinity Tags	R5: silica binding
	CipA: cellulose binding
Reporters	fuGFP: green fluorescence
	AmilCP: blue chromoprotein
Cleavage	TEV site: TEV protease
	HutMCM2aa: salt-cleaved intein
Terminators	TZ: strong triple terminator
	T7term: late T7 terminator
Coding Seq	OpenVent, Bst-LF, HIV-RT, MMLV-RT, RNaseH, HRP, T7 RNA Polymerase, Bsu, gp32, UvsX, UvsY, PBCV-1ligase

All collections are under OpenMTA[5,6] and free to (re)use:



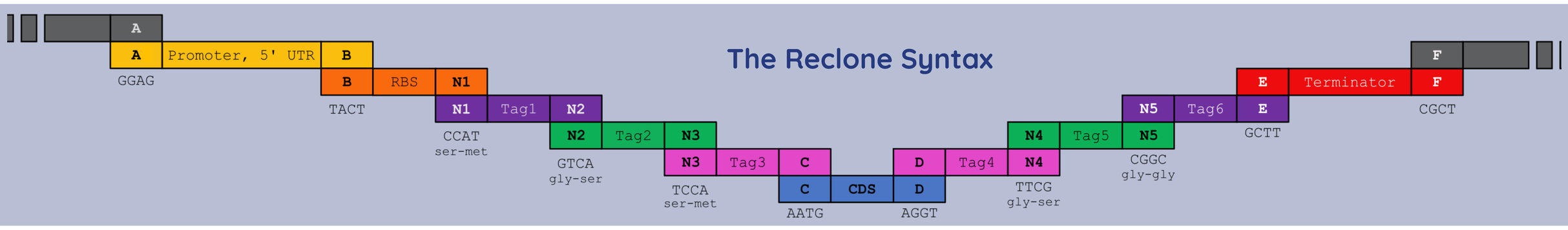
Distributors

Reclone.org, UNCUYO Universidad Nacional de Cuyo, Ashesi University, Ateneo de Manila University, iGEM, addgene, FREE GENES

Note: FreeGenes is currently on hiatus but the members within the Bionet may be open for peer-to-peer exchange of functional biomaterials and associated data.
See refs 13-14 for additional ODC information.

Acknowledgement of contributors:
The original contributions to the Open DNA Collections (ODC: OP, OEC, ORC, ePET, RiDT, OYC, and Bsub PST; 2021) were a concerted effort of the Open Bioeconomy Lab at University of Cambridge (Jenny Molloy, Chiara Gandini – who also developed the Syntax), the Lab de Tecnologías Libres at iBio/PUC Chile (Fernán Federici, Isaac Núñez, Tamara Matute, Anibal Arce), the FreeGenes Project (Drew Endy, Keoni Gandall, Hannah Verdonk, Grace Li-Na Su, Isaac Larkin) and Open Science Network Society (Scott Pownall). During 2023-24, the Open Bioeconomy Lab (Felipe Buson) and members of the Federici group developed the RiDT into ready-to-express constructs for use in the open-source pTI expression vector, whilst the extended OEC and yPET were expanded upon by sourcing from the Reclone Community and further curated by the Open Science Network (Scott Pownall, Ian Caven), the Open Bioeconomy Lab (Jenny Molloy, Yan Kay Ho), PUC Chile (Fernán Federici), Stanford University (Drew Endy), and the BioBricks Foundation (Brian Schulz) (as funded by UKRI grants awarded to the Open Bioeconomy Lab).

The Reclone Syntax

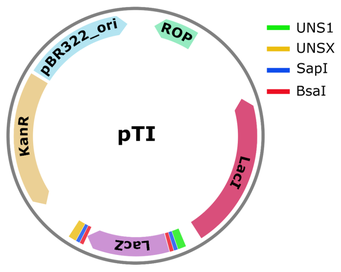


About the Syntax

- A toolbox of enzyme expression that can be easily adapted to different setups and able to be optimised by combinatorial assembly.
- Compatible with: CIDAR MoClo[7], Phytobrick/MoClo common syntax[8,9], Loop/uLoop[10,11].
- New sticky ends picked to ensure high fidelity and avoid erroneous cross-assemblies (see figure above), but CDS parts need to remove instances of BsaI and SapI.
- Scars generate glycine/serine codons to minimise impact in protein structure.
- Some parts are grouped up for ease of use (e.g. RBS + His tag).

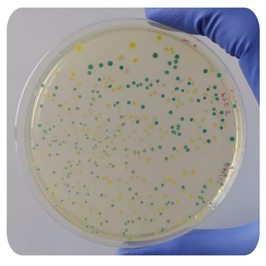
About the Open Expression Vector pTI

pTI[12] is an improved expression vector replacing the expression vector pOpen_v4. It has two variants: pTIR and pTIeR with RFP and eforRED respectively, instead of LacZ.



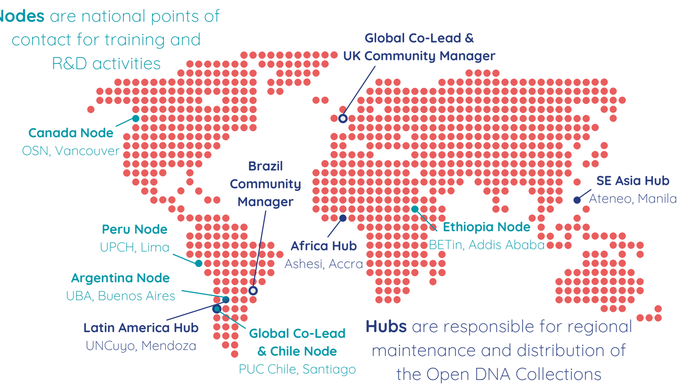
Left: A schematic of the expression vector pTI, which includes a LacI expression cassette, the odd acceptor uLoop schema (with Golden Gate enzyme recognition sites and counter selection LacZ cassette), a KanR casstte, and the pBR322-Rop low copy replicon.

Right: Bright field image of control assembly carried out in the pTI vector in BL21(DE3), in which blue-green colouration of the colonies that did not incorporate the insert can be seen. Plate contains LB supplemented with X-Gal and IPTG to activate the LacZ cassette.



Reclone in Numbers

Nodes are national points of contact for training and R&D activities



Hubs are responsible for regional maintenance and distribution of the Open DNA Collections

277	E.coli DNA Plasmids
148	Yeast DNA Plasmids
250+	Scientists Trained
200+	Community Members
50+	Countries with the Collections
5	Reclone Nodes
3	Reclone Distribution Hubs
2	Community Managers
2	Global Co-Leads

Get Involved and Give Feedback

To request the Collections and/or contribute to adding to the Data Repository, go to:

Order the ODC from Reclone Hubs
(https://forms.gle/oZZ59yokdZMxx3mT8)

Collection Info & Raise Issues (GitHub)
(https://github.com/Reclone-org/Open-DNA-Collections)

Open Discussions on the Reclone Forum
(https://forum.reclone.org/)

References

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14. Additional information on OYC and yPET: https://technology.igem.org/yeast/ouc

In Collaboration with

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