UNIVERSIDAD CATÓLICA DE VALENCIA SAN VICENTE MÁRTIR

ETHICAL ISSUES OF SYNTHETIC BIOLOGY: A PERSONALIST PERSPECTIVE

Tesis doctoral

Presentada por:

Dª. LUCÍA GÓMEZ TATAY

Dirigida por:

DR. D. JOSÉ MIGUEL HERNÁNDEZ ANDREU

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ABSTRACT

Synthetic Biology is a scientific area that combines biology and engineering to build new biological systems that could provide solutions to a wide range of social needs. Multiple and promising applications are expected from this discipline. However, Synthetic Biology also raises several ethical concerns that need to be addressed, not only to protect those values that may be threatened by the different applications of this discipline, but also because failure to fully confront them could be, together with social rejection, an obstacle to the realization of these applications.

This work has been carried out under the hypothesis that a detailed study of the current state of Synthetic Biology from a personalist perspective will highlight the main bioethical issues that could be a threat for a genuine development, respectful of human life and dignity, and provide solutions for it to become a reality.

The main objective of this thesis is to assess the bioethical issues raised by Synthetic Biology from a specific bioethical approach, personalism, specifically ontological personalism, a philosophy that shows the objective value of the person on the basis of its ontological structure. The person, as a being endowed with reason, freedom and awareness, has a special value which is above that of other beings.

This thesis has been divided into three main phases: contextualization (explanation of what Synthetic Biology is); identification and definition of the ethical issues raised by this discipline; and approach to bioethical issues from an ontological personalist framework. A literature search was therefore carried out to define the state of the question, covering the development of this discipline, the main advances achieved and the applications in this field, and to identify the ethical concerns that have been associated with Synthetic Biology. The bioethical principles derived from ontological personalism

(Sgreccia, 2012) have been applied to give an answer from this ethical framework to the different issues raised.

From this critical analysis, different conclusions have been drawn regarding the questions raised, as well as proposals that are in accordance with the personalist framework. Thus, the implications of the personalist principles for each of the ethical issues identified have been discussed and then examined in more depth by evaluating the different branches of Synthetic Biology separately. Finally, directions for future research have been outlined.

RESUMEN

INTRODUCCIÓN

La Biología Sintética es un área científica que combina la biología y la ingeniería para construir nuevos sistemas biológicos que podrían brindar soluciones a una amplia gama de necesidades sociales. Se esperan múltiples y prometedoras aplicaciones de esta disciplina. Sin embargo, la Biología Sintética también plantea varias inquietudes éticas que deben abordarse, no solo para proteger los valores que pueden verse amenazados por las diferentes aplicaciones de esta disciplina, sino también porque un abordaje deficiente de estas cuestiones éticas puede ser, junto con el rechazo social. , un obstáculo para la realización de estas aplicaciones.

HIPÓTESIS

Este trabajo se ha llevado a cabo bajo la hipótesis de que un estudio detallado del estado actual de la Biología Sintética desde una perspectiva personalista sacará a relucir los principales problemas bioéticos que podrían ser una amenaza para un desarrollo genuino, respetuoso con la vida y la dignidad humana, y proporcionará soluciones para que se convierta en una realidad.

OBJETIVOS

El objetivo principal de este trabajo es evaluar las cuestiones bioéticas planteadas por la Biología Sintética desde un enfoque bioético específico, el personalismo, específicamente el personalismo ontológico, una filosofía que muestra el valor objetivo de la persona sobre la base de su estructura ontológica.

METODOLOGÍA

Se han realizado tres fases principales de trabajo: contextualización (definición de la Biología Sintética y del estado de la cuestión); identificación y definición de las cuestiones éticas planteadas por esta disciplina; y abordaje de las cuestiones bioéticas

desde un marco personalista ontológico. Así, se ha realizado una búsqueda en la literatura para definir el estado de la cuestión, abarcando el desarrollo de esta disciplina, los principales avances logrados y las aplicaciones en este campo, y para identificar las cuestiones éticas asociadas con la Biología Sintética. Los principios bioéticos derivados del personalismo ontológico (Sgreccia, 2012) se han aplicado para dar una respuesta desde este marco ético a las diferentes cuestiones planteadas. A partir de este análisis crítico, se han extraído diferentes conclusiones sobre las cuestiones planteadas, así como propuestas de actuación acordes al marco personalista.

RESULTADOS Y DISCUSIÓN

Evaluación desde el personalismo de las cuestiones bioéticas planteadas por la Biología Sintética.

La evaluación personalista de las cuestiones bioéticas planteadas por la Biología Sintética encuentra diferentes implicaciones para cada una de las cuestiones evaluadas:

-Con respecto a la cuestión del impacto de la Biología Sintética en el concepto de vida y su creación, ninguna rama de esta disciplina se ha encontrado inevitablemente contraria a los principios personalistas. Sin embargo, las interpretaciones mecanicistas o reduccionistas del concepto de vida, que podrían derivarse de estos enfoques, podrían amenazar los valores defendidos por el *principio de protección del ecosistema y el medio ambiente* o incluso el *principio de protección de la vida y la identidad genética de cada individuo humano*.

-Respecto al valor moral de los organismos sintéticos, sus intereses deben ser tenidos en cuenta. Se pueden derivar algunas conclusiones adicionales. En primer lugar, las consideraciones sobre el posible florecimiento dañado de los organismos sintéticos debido a su diseño no tienen relevancia moral desde nuestro punto de vista. En segundo lugar, deben observarse las características de los organismos sintéticos para determinar

sus intereses o requerimientos y preservarlos en la medida de lo posible. En tercer lugar, no existe un deber moral de salvaguardar la conservación de las especies sintéticas, ya que no forman parte de la biodiversidad que debe protegerse bajo el *principio de protección del ecosistema y el medio ambiente*. Finalmente, siempre se debe observar la primacía de la persona, teniendo en cuenta que, desde nuestro marco ético, los intereses humanos prevalecen sobre los intereses de los organismos vivos no humanos. Además, usar el término "máquina" para describir los organismos sintéticos puede ser peligroso. Si bien hoy en día los únicos organismos sintéticos que pueden producirse son microorganismos, cuyos requisitos de manipulación no tienen impacto a nivel moral, esto podría cambiar cuando se produzcan organismos más complejos. Nombrarlos adecuadamente desde el principio garantizaría que los intereses de los organismos sintéticos más complejos se respeten en el futuro.

-Con respecto a los problemas de *biosafety*, hay algunas características de esta disciplina que justifican mejoras en las medidas de *biosafety* para salvaguardar los principios personalistas. Además, la legislación debe tener un papel en su regulación, incluidas las acciones de los biohackers, tanto a nivel nacional como internacional, teniendo en cuenta la naturaleza de los riesgos y los beneficios esperados. Se ha encontrado un punto débil en el desarrollo de los cinco principios personalistas aplicados en esta tesis. En consecuencia, parece que el *principio de la competencia de la comunidad* debería reformularse para incluir específicamente y describir el p*rincipio de libertad de investigación*. Por lo tanto, el principio podría ser el *principio de libertad de investigación y competencia de la comunidad*. Su contenido debe incluir explícitamente el principio de libertad de investigación, su subordinación a los principios 1 y 2, de los cuales se deriva la investigación responsable, y la necesidad de tener en cuenta las opiniones de todos los interesados y la sociedad.

- También son necesarias mejoras en las medidas de *biosecurity* para responder a los riesgos, incluyendo el establecimiento de autoridades para la toma de decisiones y el desarrollo de normas, regulaciones y políticas de financiamiento para promover la seguridad, así como la implementación de formas eficientes y eficaces de controlar las secuencias genéticas distribuidas y las diseminación del conocimiento de alto riesgo, entre otras.

- La posibilidad de aplicar la Biología Sintética a los seres humanos ha sido sugerida por importantes representantes en este campo, y podría servir para introducir grandes modificaciones en nuestro genoma (bioingeniería) o para sintetizarlo artificialmente (genómica sintética). Incluso bases no canónicas podrían introducirse en nuestro genoma (xenobiología). Según los principios empleados en este trabajo, la modificación del genoma humano es aceptable solo cuando es necesaria para el tratamiento de una enfermedad de otro modo incurable. Por lo tanto, el diseño y la síntesis de genomas humanos mejorados o empeorados y su introducción hipotética en ovocitos enucleados para iniciar el programa de desarrollo humano es éticamente inaceptable. Sin embargo, en el caso de que se produjeran seres humanos sintéticos, estos tendrían un estatus moral completo y estarían sujetos a los mismos derechos y privilegios que el resto de seres humanos. El término *subhumano* es, por lo tanto, injustificado y contrario a la dignidad humana.

- Otras cuestiones éticas relacionadas con la Biología Sintética son la justicia, la percepción pública y la comunicación, y los derechos de propiedad intelectual. El principio más relevante para discutir estos temas es el *principio de la competencia de la comunidad*, que reconoce la necesidad de promover el bien común. Esto fundamenta tanto la distribución justa de los riesgos y beneficios de esta disciplina, como la combinación de un sistema de propiedad intelectual con otro sistema de acceso abierto a la

investigación. No se pueden derivar reglas más específicas desde nuestro marco ético, pero esta posición se presenta como una base para el desarrollo, a partir del bioderecho, de medidas específicas acordes.

Sin embargo, cabe señalar que la idea de promover el interés del bien común se desarrolla mucho mejor en el *principio de socialidad y subsidiariedad*. Dado que el *principio de la competencia de la comunidad* parece derivarse del *principio de libertad y responsabilidad*, parece apropiado incluir un sexto principio para la evaluación bioética de la ingeniería genética (y otras aplicaciones de la biotecnología): el *principio de sociabilidad y subsidiariedad*.

Finalmente, se ha encontrado un elevado nivel de ignorancia social respecto a la Biología Sintética. De acuerdo con el *principio de la competencia de la comunidad*, se debe hacer un esfuerzo informativo para aumentar la conciencia pública sobre esta disciplina. En este sentido, la cobertura del tema por los medios de comunicación debe ser lo suficientemente comedida para ayudar a desarrollar una imagen realista del campo.

Evaluación personalista de las diferentes ramas de la Biología Sintética.

Se ha encontrado que muchas de las cuestiones bioéticas planteadas están asociadas con todas las ramas de la Biología Sintética (excepto para la Biología Sintética *in silico*), aunque de diferentes maneras. Teniendo en cuenta estas especificaciones y la evaluación personalista de las diferentes cuestiones éticas, la evaluación de cada rama de la Biología Sintética puede continuar.

-Este análisis no encuentra objeciones insuperables al desarrollo de la bioingeniería. Por el contrario, esta rama debe fomentarse en virtud de los beneficios que puede aportar a la sociedad. Sin embargo, se deben tomar precauciones, especialmente en relación con

los riesgos de *biosafety* y *biosecurity*, la justicia y el desarrollo de conceptos erróneos sobre la vida y los seres vivos.

-Aunque la genómica sintética tampoco presenta actualmente inconvenientes insuperables, se ha encontrado que los riesgos de la investigación de doble uso y/o la diseminación del conocimiento en esta área podrían justificar eventuales restricciones a este respecto. Además, la posible producción de seres humanos cuyo genoma ha sido diseñado es éticamente inaceptable.

- La Biología Sintética de proteocélulas no es, por el momento, controvertida. A pesar de que varios aspectos éticos también están asociados con esta rama, la asociación es tenue, ya que es un campo en el que aún no se han alcanzado grandes hitos, y aún quedan algunas incógnitas con respecto a su futuro desarrollo y riesgos de *biosafety*. Por este motivo, la evaluación de riesgos y el desarrollo de estrategias de prevención deben acompañar la investigación en esta área. Si las protocélulas se convierten en organismos vivos en el futuro, serán sujetos de valor moral, pero sus intereses no serán moralmente relevantes (dada la simplicidad definitoria de estos organismos). Finalmente, esta es la rama que parece plantear más preocupaciones sobre los biólogos sintéticos excediendo ciertos límites, considerando si pueden o deben *crear* vida. Desde el personalismo, este enfoque no viola ninguno de los principios éticos. No obstante, se ha encontrado que el uso de alternativas al término crear no solo describe mejor lo que se está haciendo, sino que también puede ayudar a evitar preocupaciones injustificadas. De la misma manera, se desaconseja el uso de la fórmula "jugar a ser Dios". Los avances en este campo deben explicarse de manera realista al público.

-Las principales preocupaciones planteadas por la xenobiología se relacionan con la *biosafety*. La investigación en este sentido debe ser paralela al progreso en este área, así como la comunicación pública. En relación con esto, los investigadores deben realizar

su actividad de manera responsable, y las regulaciones podrían ser necesarias en el futuro. Además, un concepto mecanicista de la vida podría llevar a despreciar el valor moral de los organismos sintéticos. Otras preocupaciones se relacionan con la *biosecurity* y el transhumanismo, pero las posibilidades en este sentido son actualmente remotas. Finalmente, las cuestiones relacionadas con la justicia y los derechos de propiedad intelectual no pueden excluirse de las implicaciones éticas de esta rama, pero, como en el caso de las protocélulas, no exigen una respuesta inmediata. En general, no existe un obstáculo ético definitivo para el desarrollo de este campo.

-El principal problema ético relacionado con el movimiento DIYbio es la *biosafety*, ya que el riesgo de accidentes aumenta en un contexto de dudosas medidas de prevención y con profesionales que pueden no tener la debida formación. La *biosecurity* también es una cuestión relevante, aunque por el momento los riesgos a este respecto parecen ser menores. A pesar de la fuerte naturaleza de investigación libre de este movimiento, de acuerdo con el marco ético personalista se necesitan regulaciones y estrategias de supervisión a este respecto, para salvaguardar valores más fundamentales. Los problemas secundarios, que surgen de los primeros, son la justicia, la percepción pública y la responsabilidad de la investigación. Sin embargo, no se ha encontrado que ninguna de estas cuestiones sea irremediablemente opuesta a los principios personalistas, sino que se pueden tomar medidas al respecto para salvaguardar estos principios.

Direcciones futuras.

Del estudio realizado, se pueden destacar diferentes áreas donde la investigación futura puede contribuir al desarrollo de la Biología Sintética, tanto en términos de sus beneficios como de su seguridad. Además, se deben desarrollar nuevas fórmulas de regulación, participación social y patentes.

En primer lugar, la traducción de la investigación en este campo a aplicaciones de la vida real se ve dificultada no solo por los impedimentos económicos y logísticos, sino también por las limitaciones éticas y sociales. Por lo tanto, se deben dedicar esfuerzos para actuar sobre estos diversos factores, facilitando esta traducción para liberar los beneficios que se esperan de esta disciplina.

Además, dos cuestiones apremiantes en el campo de la Biología Sintética son la *biosafety* y la *biosecurity*. Si bien existen algunas medidas disponibles para mitigar los riesgos, persisten algunas lagunas en el conocimiento que deben ser cubiertas por la investigación, como el potencial impacto ambiental de la introducción de organismos sintéticos en el medio ambiente, las implicaciones de la de-extinción y el *gene drive*, el desempeño ambiental de los procesos y productos sintéticos, etc. Además, se deben desarrollar estrategias adicionales para la biocontención, incluidas estrategias basadas en la propia Biología Sintética, el control de las secuencias genéticas distribuidas, el reconocimiento de ataques y su atribución. Además, se deben realizar investigaciones sobre nuevas contramedidas médicas, que también pueden ser apoyadas por las posibilidades de la Biología Sintética. Por otro lado, se deben establecer regulaciones adecuadas al respecto, incluso para el movimiento DIYbio, promover acciones educativas y desarrollar guías específicas.

Con respecto al público y otras partes interesadas, se debe fomentar la información y la participación, para lo cual es necesario trabajar para establecer plataformas de diálogo efectivas, así como cuidar el lenguaje informativo, que debe ser accesible y realista.

Finalmente, en vista de los inconvenientes de la aplicación del sistema de patentes actual en este campo, se deben buscar nuevas fórmulas para la protección de la propiedad intelectual, que se adapten a la realidad biotecnológica actual.

CONCLUSIONES

1. Diez cuestiones bioéticas generales están asociadas con la Biología Sintética: 1) impacto en el concepto de vida y su creación; 2) valor moral de los productos sintéticos; 3) riesgos de *biosafety*; 4) riesgos de *biosecurity*; 5) transhumanismo; 6) justicia; 7) percepción pública y comunicación; 8) derechos de propiedad intelectual; 9) regulación; y 10) investigación responsable

Recomendación 1: Las cuestiones bioéticas planteadas por las diferentes ramas de la Biología Sintética no son idénticas. Por lo tanto, las especificidades de cada rama de esta disciplina deben tenerse en cuenta en las discusiones bioéticas al respecto..

2. Con respecto al impacto de la Biología Sintética en el concepto de vida y su creación, ninguna rama de esta disciplina se ha encontrado inevitablemente contraria a los principios personalistas. Sin embargo, las interpretaciones mecanicistas o reduccionistas del concepto de vida, que podrían derivarse de estos enfoques, podrían amenazar los valores defendidos por el *principio de protección del ecosistema y el medio ambiente* o incluso el *principio de protección de la vida y la identidad genética de cada individuo humano*.

Recomendación 2: Deben evitarse las interpretaciones mecanicistas y reduccionistas del concepto de vida, a fin de salvaguardar el *principio de protección del ecosistema y el medio ambiente* y el *principio de protección de la vida y la identidad genética de cada individuo humano*.

Recomendación 3: El uso de los términos *diseño*, *construcción* o *recreación* de la vida se recomienda sobre el uso del término *creación*, ya que son más apropiados para describir lo que se hace en Biología Sintética y pueden prevenir preocupaciones injustificadas..

3. Las entidades sintéticas que conservan las características comúnmente reconocidas en lo vivo son organismos, independientemente de su origen y de los propósitos humanos involucrados en su producción. Por lo tanto, en virtud del principio de protección del ecosistema y el medio ambiente, son moralmente valiosos y deben tenerse en cuenta sus intereses.

Recomendación 4: Las características de los organismos sintéticos deben ser observadas para determinar sus requerimientos y preservarlos en la medida de lo posible, teniendo en cuenta que los intereses humanos son moralmente más importantes que los intereses de cualquier otro ser y, por lo tanto, prevalecen al decidir un curso de acción.

Recomendación 5: El uso del término "máquina" para describir los organismos sintéticos no es aconsejable. Nombrarlos adecuadamente desde el principio puede ayudar a respetar los intereses de organismos sintéticos más complejos en el futuro, tal vez incluso de los humanos.

4. No existe el deber moral de salvaguardar la conservación de las especies sintéticas, ya que no forman parte de la biodiversidad que debe protegerse bajo el *principio de protección del ecosistema y el medio ambiente*. Además, según el personalismo, no existe una obligación moral de sintetizar organismos para aumentar la biodiversidad.

5. Todas las ramas de la Biología Sintética presentan riesgos de *biosafety* que deben evitarse en la medida de lo posible para preservar el *principio de protección de la vida y la identidad genética de cada individuo humano* y el *principio de protección del ecosistema y el medio ambiente*.

Recomendación 6: Para enfrentar los desafíos planteados por la Biología Sintética, deben continuar las mejoras en las medidas de *biosafety* existentes, que incluyen:

auxotrofias sintéticas, cortafuegos xenobiológicos o circuitos genéticos sintéticos; investigación y desarrollo continuos en *biosafety*; caracterización de la función de las partes biológicas, estandarización de la información presentada a los evaluadores de riesgos, revisión y adaptación de las medidas de protección de los trabajadores; educación en *biosafety*; y el establecimiento de diferentes formas de regulaciones internacionales y nacionales, incluidas leyes obligatorias.

6. La mayoría de las ramas de la Biología Sintética, con la excepción de las protocélulas, plantean riesgos de *biosecurity* que deben evitarse en la medida de lo posible para preservar el *principio de protección de la vida y la identidad genética de cada individuo humano* y el *principio de protección del ecosistema y el medio ambiente*.

Recomendación 7: Para enfrentar los desafíos planteados por la Biología Sintética, deben continuar las mejoras en las medidas de *biosecurity* existentes, que incluyen: el establecimiento de autoridades para la toma de decisiones, políticas de financiamiento y regulaciones; posible restricción de la difusión del conocimiento de doble uso; control de las secuencias genéticas sintetizadas, solicitadas y distribuidas; educación y sensibilización sobre los riesgos del doble uso; medidas necesarias para hacer frente a un ataque; estrategias para reconocer y atribuir el ataque; y el desarrollo de capacidades de gestión de consecuencias.

7. De acuerdo con el *principio de la competencia de la comunidad*, todos los interesados, así como el público, deben participar en el debate sobre la Biología Sintética y sus riesgos de *biosafety* y *biosecurity*, así como otros aspectos controvertidos relacionados con la Biología Sintética, como el transhumanismo.

Recomendación 8: El debate sobre las diferentes cuestiones relacionadas con la Biología Sintética debe involucrar a todos los interesados, así como al público. Se

deben proporcionar plataformas de diálogo efectivas que involucren a científicos, legisladores, el público en general y todas las partes interesadas.

8. Desde la aplicación práctica de los cinco principios personalistas al caso de la Biología Sintética, se han detectado algunas debilidades en el marco ético:

a. El *principio de la competencia de la comunidad* contiene otro principio, el *principio de la libertad de investigación*, cuya jerarquía y contenido en el marco de los otros principios no se clarifica. De la disertación anterior de Sgreccia, se deduce que está subordinado a los principios 1 y 2. La reformulación del principio para incluir y describir explícitamente la libertad de investigación facilitaría la aplicación directa de los principios de Sgreccia a la evaluación bioética de la biotecnología.

b. El *principio de la competencia de la comunidad* establece la necesidad de promover el interés del bien común. Sin embargo, esta idea está mucho mejor desarrollada en el *principio de sociabilidad y subsidiariedad*, perteneciente a la lista inicial de principios. El contenido de este principio en el tema en cuestión es altamente relevante, y en la lista inicial es independiente del *principio de libertad y responsabilidad* (de donde nace el *principio de la competencia de la comunidad*).

Recomendación 9: El *principio de la competencia de la comunidad* debe reformularse como el *principio de libertad de investigación y competencia de la comunidad*. Su contenido debe incluir explícitamente la libertad de investigación, su subordinación a los principios 1 (el *principio de protección de la vida y la identidad genética de cada individuo humano*) y 2 (el *principio de protección del ecosistema y el medio ambiente*), la investigación responsable y la necesidad de tener en cuenta los puntos de vista de todas las partes interesadas y de la sociedad.

Recomendación 10: El *principio de sociabilidad y subsidiariedad* debe incluirse como un sexto principio en la lista de principios desarrollados para la ingeniería genética.

9. La posibilidad de alterar el genoma humano con propósitos transhumanistas o para producir subhumanos es contraria al p*rincipio de protección de la vida y la identidad genética de cada individuo humano* y al *principio terapéutico*.

Recomendación 11: Las técnicas de Biología Sintética no deben aplicarse al genoma de individuos humanos a menos que sea con fines terapéuticos.

10. Con respecto a las cuestiones de justicia y derechos de propiedad intelectual, el *principio de competencia de la comunidad* requiere la promoción del interés del bien común.

Recomendación 12: Debe apoyarse tanto la distribución equitativa de los riesgos y beneficios de la Biología Sintética como la combinación de un sistema de propiedad intelectual con otro sistema de acceso abierto a la investigación.

11. De acuerdo con el *principio de la competencia de la comunidad*, las poblaciones necesitan información y compartir responsabilidades, pero existe una baja conciencia social sobre la Biología Sintética.

Recomendación 13: Se debe hacer un esfuerzo informativo para aumentar la conciencia pública de esta disciplina. La cobertura del tema por parte de los medios de comunicación debe ser lo suficientemente comedida para ayudar a desarrollar una imagen realista del campo.

12. Desde el personalismo, no se han encontrado objeciones insuperables al desarrollo de la bioingeniería. La *biosafety* y la *biosecurity* son los problemas más apremiantes asociados con esta rama de la Biología Sintética.

Recomendación 14: La bioingeniería debe fomentarse en virtud de los beneficios que puede aportar a la sociedad. Sin embargo, se deben tomar precauciones, especialmente en relación con los riesgos de *biosafety* y *biosecurity* y la justicia. A tal fin, se deben establecer las regulaciones apropiadas. El valor moral de los organismos sintéticos debe ser reconocido.

13. Desde el personalismo, la genómica sintética tampoco presenta inconvenientes insuperables en su estado actual. Sin embargo, la producción hipotética de seres humanos cuyo genoma está diseñado es éticamente inaceptable.

Recomendación 15: Cuando los riesgos de la investigación de doble uso y/o la difusión del conocimiento en esta área son demasiado altos, se deben aplicar restricciones a este respecto.

14. Desde el personalismo, la Biología Sintética de protocélulas no viola ningún principio ético. Las principales preocupaciones giran en torno a los biólogos sintéticos *creando* vida. Además, quedan varias incógnitas sobre los riesgos futuros de bioseguridad que plantean las protocélulas.

Recomendación 16: La evaluación de riesgos y el desarrollo de estrategias de prevención deben acompañar la investigación en este campo. El público debe ser informado sobre el progreso en esta área, tratando de evitar preocupaciones innecesarias. Con este objetivo, se recomiendan alternativas al término *crear*, mientras que se desaconseja el uso de la fórmula "jugar a Dios".

15. Desde el personalismo, no hay un obstáculo ético definitivo para el desarrollo de la xenobiología. Las principales preocupaciones están relacionadas con las incertidumbres relacionadas con los riesgos de *biosafety*.

Recomendación 17: La investigación sobre los riesgos de *biosafety* de la xenobiología debe ser paralela al progreso en esta área, así como la comunicación pública.

16. No se ha encontrado que ninguna de las cuestiones relacionadas con el DIYbio se oponga irremediablemente a los principios personalistas. La principal cuestión ética a este respecto es la *biosafety*, seguida de la *biosecurity*.

Recomendación 18: El movimiento DIYbio también debe estar sujeto a regulaciones.

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1 INTRODUCTION

Many definitions have been suggested for Synthetic Biology, also known as SynBio, but none is universally accepted (annex 1). This is an interdisciplinary discipline, which combines elements from biotechnology, chemistry and engineering, among others (Raimbault, Cointet, & Joly, 2016; Shapira, Kwon, & Youtie, 2017). "In the simplest terms, synthetic biology is an emerging discipline that combines both scientific and engineering approaches to the study and manipulation of biology" (National Academy of Engineering and National Research Council, 2013, p. 2).

There are several features of Synthetic Biology that make it different to the technologies from which it has emerged. One of the original characteristics of this discipline is the pursuit of knowledge through doing, not just analyzing: "synthetic biologists seek to assemble components that are not natural (therefore synthetic) to generate chemical systems that support Darwinian evolution (therefore biological). By carrying out the assembly in a synthetic way, these scientists hope to understand non-synthetic biology" (Benner & Sismour, 2005, p. 533). Importantly, synthetic biologists apply engineering principles in their work, such as standardization, decoupling and abstraction (Endy, 2005), giving a different perspective in the use of biological parts and systems (National Academy of Engineering and National Research Council, 2013).

- Standardization: "devising a broad consensus on the composition of parts, devices, and systems so that they may be used reliably in any setting" (National Academy of Engineering and National Research Council, 2013, p. 12). As explained by Endy (2005), standards are useful for the definition, description and characterization of both biological components and their optimal conditions of use. Furthermore, standards can be a valuable tool to guide different activities

within the field of Synthetic Biology, such as marking the synthetic DNA so that it is easily recognizable, or sharing genetic parts, for which legal standards are necessary. In this respect, the Open Material Transfer Agreement (OpenMTA) has been developed, which is a legal standard "for sharing biological materials as broadly as possible without undue restrictions, while respecting the rights of creators and promoting safe practices and responsible research" (Kahl et al., 2018, p. 923).

- Decoupling: "de-linking the requirements for design from requirements for manufacture to allow non-biologists to use biological components in various applications" (National Academy of Engineering and National Research Council, 2013, p. 12).
- Abstraction: "a system for managing biological complexity by eliminating unnecessary details" (National Academy of Engineering and National Research Council, 2013, p. 12).

Additionally, a report signed by three European non-food Scientific Committees (the Scientific Committee on Consumer Safety [SCCS], the Scientific Committee on Health and Environmental Risks [SCHER] and the Scientific Committee on Emerging and Newly Identified Health Risks [SCENIHR]) finds that Synthetic Biology entails easier and faster design and manufacturing than traditional genetically modified organisms (GMO), although it is difficult to accurately and unambiguously differentiate the two disciplines due to the lack of quantifiable and measurable criteria (SCENIHR, SCCS, & SCHER, 2014). Synthetic Biology also differs from genetic engineering in that it involves the design of novel genomes combining multiple parts, instead of the mere transfer of one gene between species (Andrianantoandro, Basu, Karig, & Weiss, 2006; Tucker & Zilinskas, 2006). Porcar and Peretó (2012) propose some criteria to differentiate

Synthetic Biology from other biotechnological fields, namely "lack of design, use of assay/error tuning strategies, and lack of orthogonality and/or modularity" (p. 82), arguing that "ad-hoc strategies and standard-free approaches are incompatible with canonical engineering" (p. 82). De Lorenzo (2010) explains that this discipline "embodies and recapitulates much of what has been done in the past and is still done under the frame of Genetic Engineering, although it subsequently takes different directions" (p. 926), and he identifies eight stages of transition between naturally-occurring organisms and wholly synthetic microbes (Figure 1).

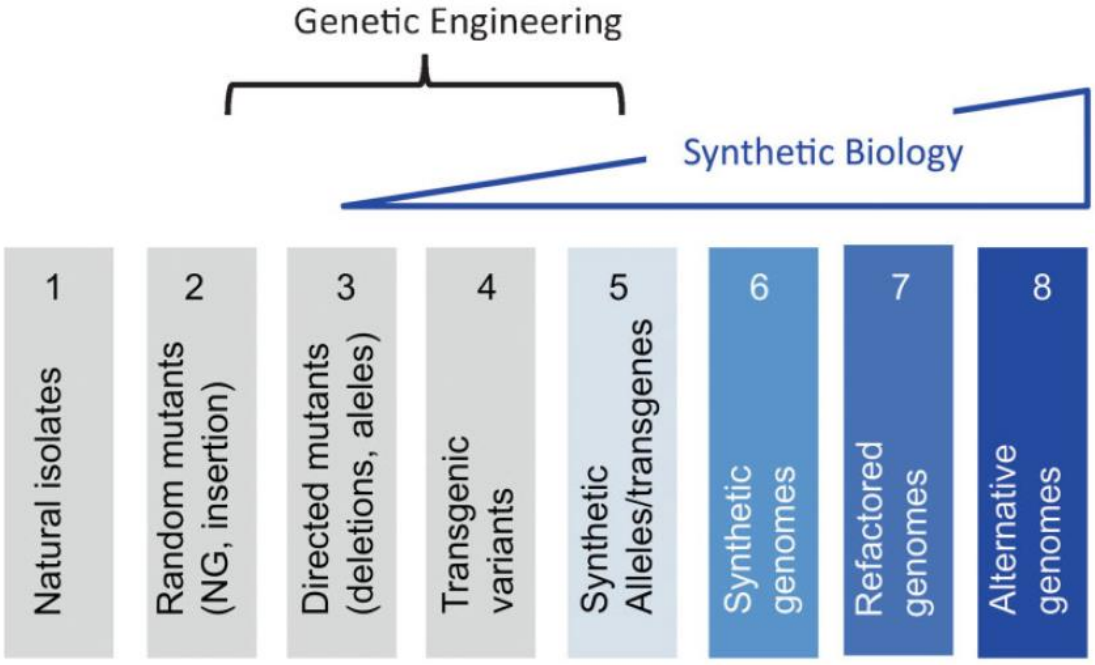

***Figure 1*. Different stages of transition between naturally-occurring organisms and wholly synthetic microbes:** 1) naturally occurring microorganisms and natural isolates, such as fermentations; 2) random changes in the DNA; 3) directed changes in the DNA using short synthetic oligonucleotides; 4) transgenesis with natural genes; 5) transgenesis with synthetic genes; 6) synthesis of copies of entire natural genomes; 7) synthesis of entire genomes extensively modified to fulfill a given purpose; and 8) cells bearing alternative genomes, for example with alternative nucleic acids. Modified from de Lorenzo (2010).

1.1 HISTORICAL OVERVIEW

In the same way that its description is not clearly defined, the origins of Synthetic Biology are somewhat diffuse. The reason for this is twofold: on the one hand, as

Synthetic Biology is an interdisciplinary field, its origins are traced back to several disciplines (Table 1). On the other, the term appeared in some publications well before it became a practical concept, while applications came later. However, it has been more than a century since some pioneering scientists began to work on the idea of obtaining life in the laboratory as a means of understanding living beings (Peretó, 2016).

Table 1.

Synthetic Biology Tools and Technology Timeline*. Modified from National Academy of Engineering and National Research Council (2013).*

Year	***Scientific development***
1941	First functional program-controlled computer (Konrad Zuse)
1953	Crick and Watson describe the double helix structure of DNA
1960	First computer-aided drafting (CAD) program (Sketchpad)
1961	Discovery of mathematical principles in gene regulation
1971	First genetically modified organism (*Escherichia coli*)
1972	First synthetic gene (*yeast*)
1973	Cohen, Boyer and Berg create first genetically engineered organism (*E. Coli*)
1974	First U.S. patent on recombinant DNA (Stanley Cohen and Herbert Boyer)
1975	Asilomar Conference on Recombinant DNA Early genome sequencing techniques established
1976	First biotechnology firm founded (Genetech) NIH guidelines for Recombinant DNA
1978	Term "bioinformatics" coined Synthetic insulin gene inserted into *E. Coli*
1980	In Diamond v. Chakrabarty, the U. S. Supreme Court rules that "a live, human-made micro-organism is patentable subject matter"
1982	U.S. Food and Drug Administration (FDA) approves use of synthetic insulin
1983	Development of the polymerase chain reaction (PCR) DNA amplification technology
1984	First commercialized genetically modified food (Flavr Savr tomato)
1990	Human Genome Project (HGP) launched
1991	First public availability of the World Wide Web
2000	International Human Genome Sequencing Consortium announces "working draft" of human genome Genetic oscillators and toggle switches published

The first experiments in the synthesis of biological compounds were carried out in the field of organic chemistry, such as the synthesis of urea (Wöhler, 1828). Additionally, since the structure of DNA was first described (Watson & Crick, 1953), several advances

have facilitated the synthetic approach in genetics, including the discovery of mathematical logic in gene regulation (Jacob, Perrin, Sánchez, & Monod, 1960; Monod & Jacob, 1961), the appearance of recombinant DNA (Jackson, Symons, & Berg, 1972), the design of DNA sequencing techniques (Maxam & Gilbert, 1977; Sanger, Donelson, Coulson, Kössel, & Fischer, 1973), and the discovery of the polymerase chain reaction (PCR) (Mullis et al., 1986).

Stephane Leduc first used the term Synthetic Biology in his *Biologie Syntetique* (Leduc, 1912). However, practical applications took decades to arrive, until a 207-base pair (bp) DNA sequence was synthesized in 1976 (Khorana et al., 1976). These and other events constitute the "origins of the field" (Cameron, Bashor, & Collins, 2014), a stage in which the modular vision of the cell and its genetics was consolidated and which extended to 1999.

Years 2000-2003 can be considered as the "foundational years" (Cameron et al., 2014), in which the first genetic circuits were engineered. Synthetic genetic regulatory networks were first assembled in 2000 (Elowitz & Leibler, 2000; Gardner, Cantor, & Collins, 2000). Later, in 2003, the first international Genetically Engineered Machine (iGEM) competition[1] took place at Massachusetts Institute of Technology (MIT).

After that—and until 2007—the field started to grow and technical advances were achieved, although progress was moderate since several bottlenecks remained, such as the lack of methods to effectively assemble and characterize genetic parts (Cameron et al., 2014). Since 2008, however, an era of "increase in pace and scale" has begun, in which applications in various areas are constantly appearing, as the genetic circuits designed have increased their complexity and variety of functions, in parallel with the

[1] See http://igem.org/About

development of a wide repertoire of increasingly better characterized parts (Cameron et al., 2014). The growing impact of this scientific discipline is reflected in the steady rise in the number of publications in this field (Figure 2). Today, Synthetic Biology has an important and growing presence in markets, with great relevance in the global bioeconomy (Clarke & Kitney, 2016; Flores Bueso & Tangney, 2017; Si & Zhao, 2016).

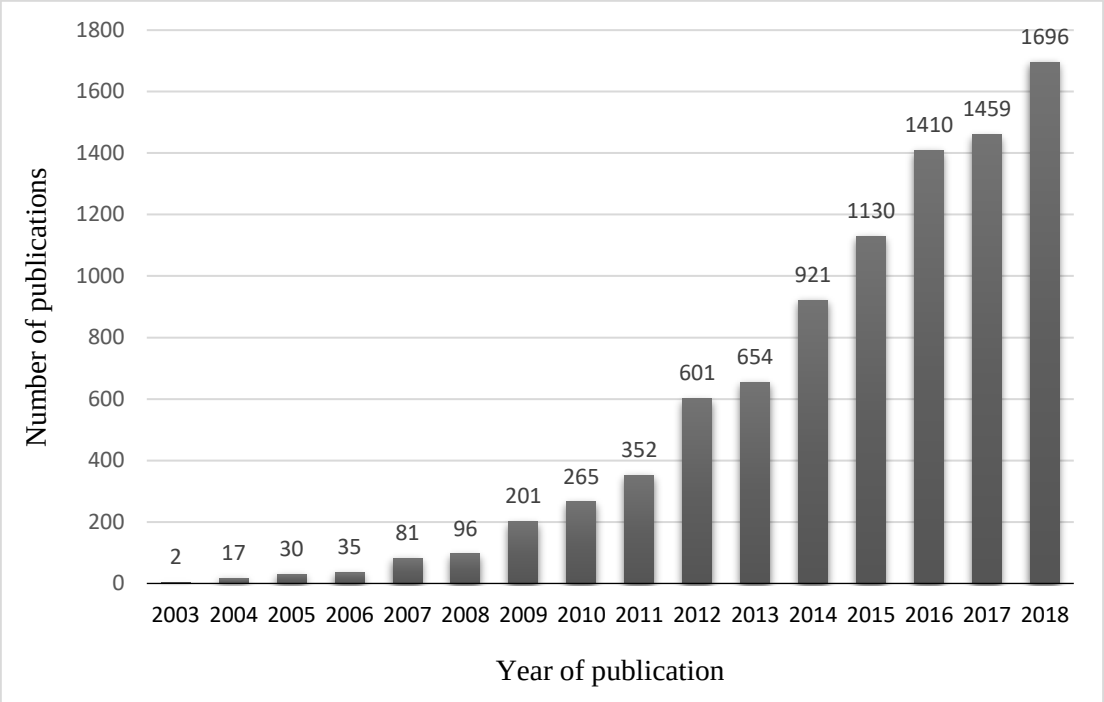

Figure 2. **Number of publications by year in PubMed when searching for "synthetic biology".**
Source: Prepared by the author based on the results available in PubMed.

1.2 DIFFERENT APPROACHES WITHIN SYNTHETIC BIOLOGY

Due to the diversity of approaches that can be followed within the field of Synthetic Biology, it is difficult to find an exact and definitive definition of this discipline (Newson, 2011). Several authors have proposed different subdivisions within the field. For example, Benner and Sismour (2005) distinguish two broad classes of synthetic biologists: "One uses unnatural molecules to reproduce emergent behaviors from natural

biology, with the goal of creating artificial life. The other seeks interchangeable parts from natural biology to assemble into systems that act unnaturally" (p. 533).

Endy (2005) classifies synthetic biologists into four different groups, namely biologists, chemists, 're-writers' and engineers:

> "for biologists, the ability to design and construct synthetic biological systems provides a direct and compelling method for testing our current understanding of natural biological systems; disagreements between expected and observed system behaviour can serve to highlight the science that is worth doing. For chemists, biology is chemistry, and thus synthetic biology is an extension of synthetic chemistry; the ability to create novel molecules and molecular systems allows the development of useful diagnostic assays and drugs, expansion of genetically encoded functions, study of the origins of life, and so on. For 're-writers', the designs of natural biological systems may not be optimized for human intentions (for example, scientific understanding, health and medicine); synthetic biology provides an opportunity to test the hypothesis that the genomes encoding natural biological systems can be 're-written', producing engineered surrogates that might usefully supplant some natural biological systems. Finally, for engineers, biology is a technology; building upon past work in genetic engineering, synthetic biology seeks to combine a broad expansion of biotechnology applications with [...] an emphasis on the development of foundational technologies that make the design and construction of engineered biological systems easier" (p. 449).

De Lorenzo, Serrano, and Valencia (2006) differentiate two approaches: the 'deconstruction' of life and the 'construction' of life. The first "dissects biological

systems in the search for simplified and minimal forms that will help us understand the adaptation and evolution of natural processes" (p. 127), while the second aims to "build systems that inspired by general biological principles, use biological or chemical components to reproduce the behavior of live systems. [...] The general underlying notion is to combine autonomous, modular, robust and reusable components" (p. 127).

Forster and Church (2007) divide Synthetic Biology projects (SBP) into two classes: *in vivo* and *in vitro*: "In vivo SBPs mostly involve bacterial engineering, have diverse goals, and are generally more suited than in vitro SBPs for large-scale production/conversion of materials" (p. 1). In vitro SBPs involve cell-free applications, such as the "synthesis of genes from oligos made on chips" (p. 2).

O´Malley, Powell, Davies and Calvert (2008) identify three categories of Synthetic Biology: DNA-based device construction, genome-driven cell engineering and protocell creation. Similarly, Deplazes (2009) establishes five categories: bioengineering, synthetic genomics, protocell Synthetic Biology, unnatural molecular biology and *in silico* approaches. The different branches present differences based on several criteria: the scientific background, the vision, and the techniques employed (Table 2). Similar classifications have also been used by other authors (SCENIHR, SCCS, SCHER, 2014; Porcar & Peretó, 2012; Schmidt, 2009), and some have included citizen science or do-it-yourself bio (DIYbio) as an additional branch within Synthetic Biology (SCENIHR, SCHER, & SCCS, 2015a). The advantage of this classification is that it can ease ethical and societal assessments by delimiting specific issues in each category:

> "For societal and ethical assessments of synthetic biology, it is important to consider both the differences and the similarities between the branches of synthetic biology in order to distinguish between questions that affect the

field as a whole and those that are specific to individual categories. [...] These categories should help to make societal assessments of synthetic biology more specific and precise by clarifying which issues concern which branches" (Deplazes, 2009, p. 428).

For this reason, and given the analytical dimension of this work as regards the ethical implications of this discipline, this classification has been chosen as the starting point from which to develop the assessment.

Table 2.

Overview of the various approaches to Synthetic Biology. *Modified from Deplazes (2009).*

Approach	***Scientific background***	***Vision***	***Technique***
Bioengineering	Engineering, biotechnology	Making biology an engineering discipline	Standardized and elaborated genetic engineering
Synthetic genomics	Molecular biology, chemistry	Chassis organisms	DNA synthesis
Protocell synthetic biology	Chemistry, biochemistry	Synthetic cells	Chemical synthesis of a cell
Unnatural molecular biology	Chemistry, biochemistry, molecular biology	´Parallel life`	Synthesis of unnatural genomes and biological adaptation of the cell
***In silico* synthetic biology**	Computer science, engineering	Designed organisms	Computer technology
All synthetic biology approaches	—	New forms of life, designed life	—

1.2.1 Bioengineering

This branch of Synthetic Biology aims to overcome the complexity of biological systems by defining and delimiting the function of genetic parts so that they can be used in the design of genetic circuits in a predictable, fast and effective way. To that end,

standardization of the design, assembly, characterization and data sharing processes is essential. Although many useful advances are being made in this regard, there is still a long way to go (Decoene et al., 2018).

This is a highly active branch within Synthetic Biology (Deplazes, 2009), with a multitude of publications constantly appearing that report the design of genetic circuits with the most diverse applications, such as therapeutic uses (Flores Bueso, Lehouritis, & Tangney, 2018), biosensing (Ito-Harashima et al., 2017), biofuel production (Jagadevan, et al., 2018), bioplastics production (Darvishi, Ariana, Marella, & Borodina, 2018), and bioremediation (Geva, Kahta, Nakonechny, Aronov, & Nisnevitch, 2016).

A relevant early example of this approach was the production of artemisinic acid, the anti-malarial drug precursor, in both bacteria and yeast (Anthony et al., 2009; Ro et al., 2006; Tsuruta et al., 2009; Westfall et al., 2012). Another remarkable achievement in this field was the production of the first synthetic species (Moreno, 2012). Introduction of a designed genetic circuit into the genome of *Drosophila melanogaster* led to reproductive isolation between the modified and *wild type* organisms (Figure 3). Since the criterion of being able to reproduce with one another (but not with other organisms) is used to group organisms within the same species (Coyne & Orr, 2004), reproductive isolation justifies the possibility of referring to a new species, in this case *Drosophila synthetica*. Importantly, this reproductive isolation for synthetic species may serve to preserve natural species by preventing natural organisms from interbreeding with their synthetic counterparts (Moreno, 2012).

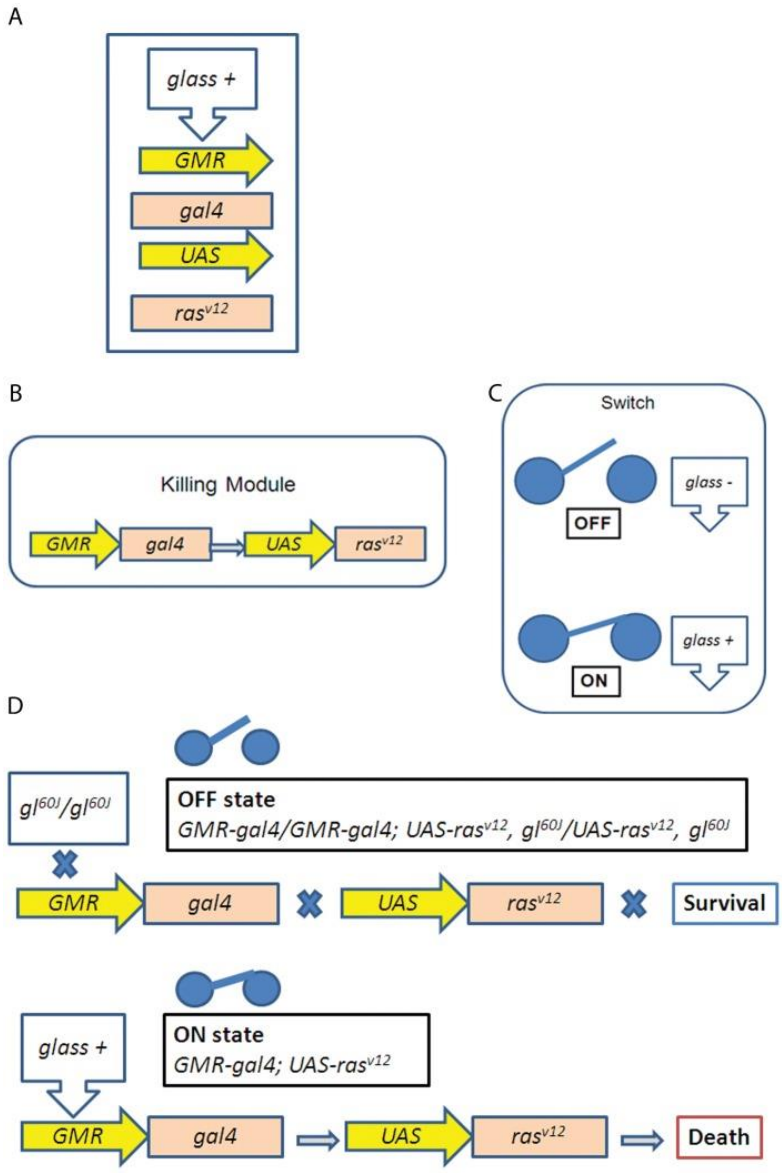

***Figure 3*. "Design of a genetic circuit with selected components that form a synthetic species barrier.** (A) The 5 genetic elements used: transcription factor *glass*, enhancer *GMR*, transcription factor *gal4*, enhancer *UAS* and a constitutively activated form of *ras*. (B, C) Arrangement of the genetic elements in two modules, a killing module (B) composed by two independent transgenes, *GMR-gal4* and *UAS-rasv12*, and a switch that depending on the presence or absence of the transcription factor *glass* can switch the killing module ON and OFF (C). (D) In the absence of *Glass*, activation of the killing module is not possible and the flies survive. However, in the presence of *Glass*, expression of the constitutively active form of *ras* kills the animal". *Retrieved from Moreno (2012).*

Additionally, a very illustrative example of the bioengineering approach is the iGEM Registry of Standard Biological Parts[2], a growing collection of over 20,000 documented genetic parts, called BioBricks™, which adapt to certain assembly standards to allow their combination in the design and construction of genetic circuits. The most commonly used assembly standard in the Registry of Standard Biological Parts is BioBrick RFC(10)[3], which provides a set of specifications (summarized in Table 3) that the registered genetic sequences must meet in order to be compatible with the 3A (three antibiotic) Assembly method[4]. This method allows the easy assembly of the parts that fulfill the RFC(10) Standard, so that users of the Registry can easily combine the available parts according to the functions they seek to obtain. Briefly, the Standard specifies that the genetic parts do not contain certain sequences that are the target site of certain restriction enzymes. These sequences are included, instead, as prefixes and suffixes of the main sequence, which will allow those enzymes to be used to cut and bind the sequences of the Registry.

In the same vein, the BioBricks Foundation[5] Free Genes project[6] adapts synthesized sequences to be compatible with the MoClo (modular cloning) assembly system (Weber, Engler, Gruetzner, Werner, & Marillonnet, 2011), with the aim of making standardized open access libraries whose synthetic DNA parts can be easily and quickly cloned for any desired application, drastically lowering costs.

[2] http://parts.igem.org/Main_Page
[3] https://openwetware.org/wiki/The_BioBricks_Foundation:BBFRFC10
[4] http://parts.igem.org/Help:Assembly/3A_Assembly
[5] https://biobricks.org/
[6] https://biobricks.org/freegenes/

Table 3

RFC(10) standard: required sequence properties for a Biobrick™ standard biological part. *Source: Prepared by the author based on the information provided in* https://openwetware.org/wiki/The_BioBricks_Foundation:BBFRFC10

Standard sequence property	Details
Biobrick parts should not contain certain subsequences	Sequences not allowed: EcoRI site: GAATTC; XbaI site: TCTAGA; SpeI site: ACTAGT; PstI site: CTGCAG; NotI site: GCGGCCGC
A specific Biobrick Suffix is required	Each Biobrick part must contain precisely this sequence immediately following the 3' end of the part: T ACTAGT A GCGGCCG CTGCAG
A specific Biobrick Prefix is required	a. Non-coding Biobrick parts must contain precisely the following sequence immediately 5' of the part: GAATTC GCGGCCGC T TCTAGA G b. Biobrick parts coding for proteins must contain precisely the following sequence immediately 5' of the ATG start of the coding region: GAATTC GCGGCCGC T TCTAG Parts containing start codons other than ATG must be modified to use ATG as the start codon.
Biobrick parts must be supplied in plasmids compliant with specific constraints	a. Antibiotic resistance: All plasmids must carry at least one of the following antibiotic resistance markers: Ampicillin; Chloramphenicol; Kanamycin; Tetracycline Parts delivered to the registry must be in strains which do not convey resistance to these markers. b. Sequencing primers: Plasmids which omit or misplace the following primers cannot be sequenced: VF2: TGCCACCTGACGTCTAAGAA VR: ATTACCGCCTTTGAGTGAGC
Submission strains must or are recommended to fulfill some specifications	The registry encourages submissions in frozen bacteria. The bacterial strain must be a K-12 cloning strain (endA-). Strains must be BSL-1. Strains such as Top10, DH10B, and DH5a are recommended, while submissions in MC4100, BL21 and similar strains are not recommended.

Within this branch of Synthetic Biology, so-called cell-free Synthetic Biology is gaining importance. This approach is based on the activation of biological processes *in vitro,* bypassing the cell membrane barriers and thus allowing greater engineering flexibility, which has several advantages (Table 4). Thus far, there are three types of well-developed cell-free transcription-translation (TX-TL) systems: extract-based systems, composed of crude extract obtained from different organisms; purified systems, which have different components purified from different organisms; and synthetic enzymatic pathway systems (Lu, 2017).

These systems provide a useful platform to engineer proteins and metabolic pathways (Karim & Jewett, 2016; Lu, 2017; Martin et al., 2018). They also allow for rapid prototyping of genetic circuits (Moore, MacDonald, & Freemon, 2017), and to study non-model microbes (Moore et al., 2017; Moore, et al., 2018). Additionally, they can be used as rapid, low-cost biosensors, for example to detect Zika virus (Pardee et al., 2016). Finally, cell-free Synthetic Biology has shown promise for the construction of protocells (see section 1.2.3 Protocells) by encapsulating the *in vitro* system within a membrane (Caschera, Lee, Ho, Liu, & Jewett, 2016; Jewett & Forster, 2010; Scott et al., 2016; van Nies et al., 2018).

Table 4.

Comparison of in vitro cell-free systems and traditional in vivo cell systems. *Retrieved from Lu (2017).*

Feature	*In vitro cell-free system*	*In vivo cell system*
Manipulation of transcription and translation	Easy to control in an open environment	Hard because of cell membrane as the barrier
Post-translational modification	Hard	Easy
Self-replication	Hard	Easy
DNA template	Plasmids or PCR products	Plasmids or genomes
Synthesis of membrane proteins and complex proteins	Easy synthesis by adding surfactants or adjusting the system environment	Hard synthesis due to limited intracellular environment
Incorporation of unnatural amino acids into proteins	Easy	Hard
Ability to only produce the desired products	Easy achievement by focusing on the target metabolic pathways	Hard achievement due to complicated cellular metabolism
Toxic tolerance	High	Low
Integration with materials	Easy	Hard
Design-build-test-learn cycle	Two days	Two weeks
Biomanufacturing	High production rate High product yield Easy purification process without cell lysis	Modest production rate Modest product yield Cell lysis prior to product purification
Cost	Modest to high	Low to modest

1.2.2 Synthetic Genomics

This involves the chemical synthesis of functional genomes which are then transplanted into living cells. Its ultimate goal is to produce minimal genomes that contain only the genes that are indispensable for life, in order to gain insight into the understanding of the concept of life (Glass, Merryman, Wise, Hutchison, & Smith, 2017). Furthermore, the elimination of redundant DNA would facilitate the process of synthesis and assembly, and minimal genomes have the potential application of constituting chassis genomes, to which different genes with desired functions can be attached (Mol, Kabra, &

Singh, 2018; Sung, Choe, Kim, & Cho, 2016), for example with the intention of "transforming a bacterial-like cell in a cell-bioreactor to produce bio-fuels or any molecule of interest for industry" (Murtas, 2009). These "minimal genomes" would have several advantages, such as their predictability and easiness for modeling their metabolism, the facility to manipulate them genetically, the lower expense and greater precision in the synthesis of the genome, as well as the saving in energy use and transcription and translation costs (Choe, Cho, Kim, & Cho, 2016). Additionally, research on essential genes may serve to develop new antimicrobials, as bacteria need them to survive (Juhas, Eberl, & Church, 2012).

However, unlike engineering, in biology the functionality of the parts depends to a large extent on their context, which, in addition, is dynamic. This poses a challenge to the notion of a chassis genome (Nuño, 2016; Porcar et al., 2011). whose functionality can be completely isolated from that of the added parts. Another challenge in terms of the applicability of these chassis is that genetic simplicity can generate problems in terms of the rate of growth of the organism and its ability to survive, factors that must be refined in the development of these organisms (Porcar et al., 2011).

The ultimate goal of the synthetic genomics approach to Synthetic Biology was first approximated by researchers at the J. Craig Venter Institute, who besides synthesizing a simple bacterial genome (although it is not the simplest one), successfully introduced it into a bacterium (Gibson et al., 2010). This experiment is a milestone in Synthetic Biology history, since it came closer than ever before to the Synthetic Biology aim of "creating" life. In this experiment, a 1079-kilobase pair (kbp) genome based on the genome of *Mycoplasma mycoides* was completely synthesized *in vitro* (Figure 4). Importantly, the synthetic genome, called JCV-syn1.0, showed correct functioning when introduced into a *M. capricolum* recipient cell.

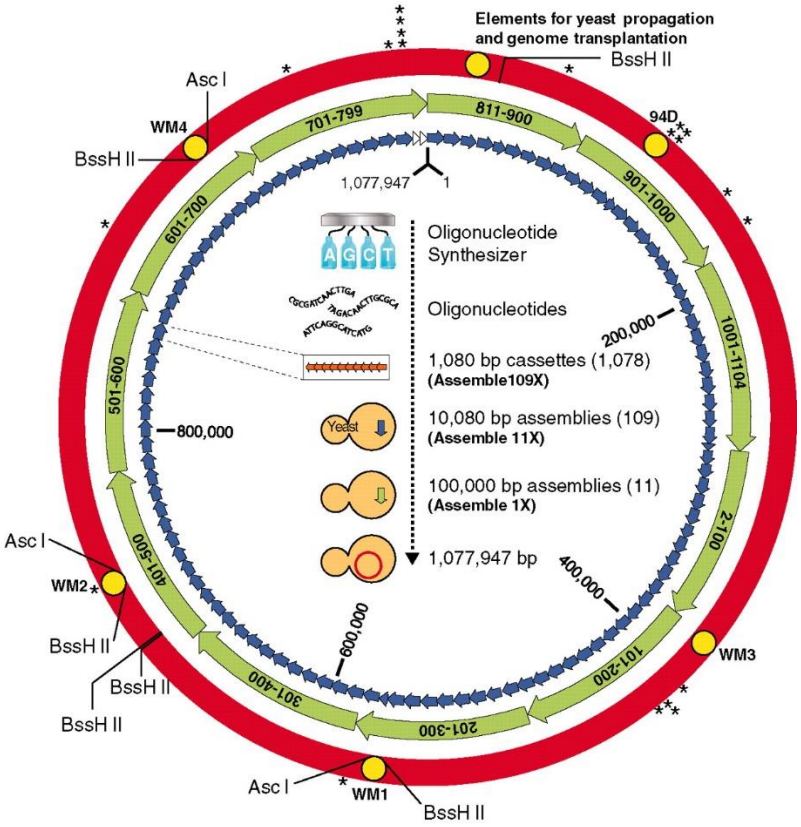

***Figure 4*. "The assembly of a synthetic *M. mycoides* genome in yeast**. A synthetic *M. mycoides* genome was assembled from 1078 overlapping DNA cassettes in three steps. In the first step, 1080-bp cassettes (orange arrows), produced from overlapping synthetic oligonucleotides, were recombined in sets of 10 to produce 109 ~10-kb assemblies (blue arrows). These were then recombined in sets of 10 to produce 11 ~100-kb assemblies (green arrows). In the final stage of assembly, these 11 fragments were recombined into the complete genome (red circle). With the exception of two constructs that were enzymatically pieced together in vitro (white arrows), assemblies were carried out by in vivo homologous recombination in yeast. Major variations from the natural genome are shown as yellow circles. These include four watermarked regions (WM1 to WM4), a 4-kb region that was intentionally deleted (94D), and elements for growth in yeast and genome transplantation. In addition, there are 20 locations with nucleotide polymorphisms (asterisks). Coordinates of the genome are relative to the first nucleotide of the natural *M. mycoides* sequence. The designed sequence is 1,077,947 bp. The locations of the Asc I and BssH II restriction sites are shown. Cassettes 1 and 800-810 were unnecessary and removed from the assembly strategy (11). Cassette 2 overlaps cassette 1104, and cassette 799 overlaps cassette 811". *Retrieved from Gibson et al. (2010).*

Six years after the construction of JCVI-syn1.0, Craig Venter and his team achieved a new milestone within this branch of Synthetic Biology, obtaining an organism with the smallest genome of any known cell life form, called JCVI-syn3.0 (Hutchison et al., 2016). JCVI-syn3.0 is a reduced version of JCVI-syn1.0 containing 473 genes (531 kb), some of which are genuinely essential (necessary for the organism to live) and some of which are quasi-essential (required for robust growth). Unexpectedly, 149 of these genes have been shown to be necessary but are of unknown function. Even so, this is the closest that scientists have come to obtaining a cell in which the function of each gene is known. Its construction scheme is very similar to that of JCV-syn1.0 (Figure 5).

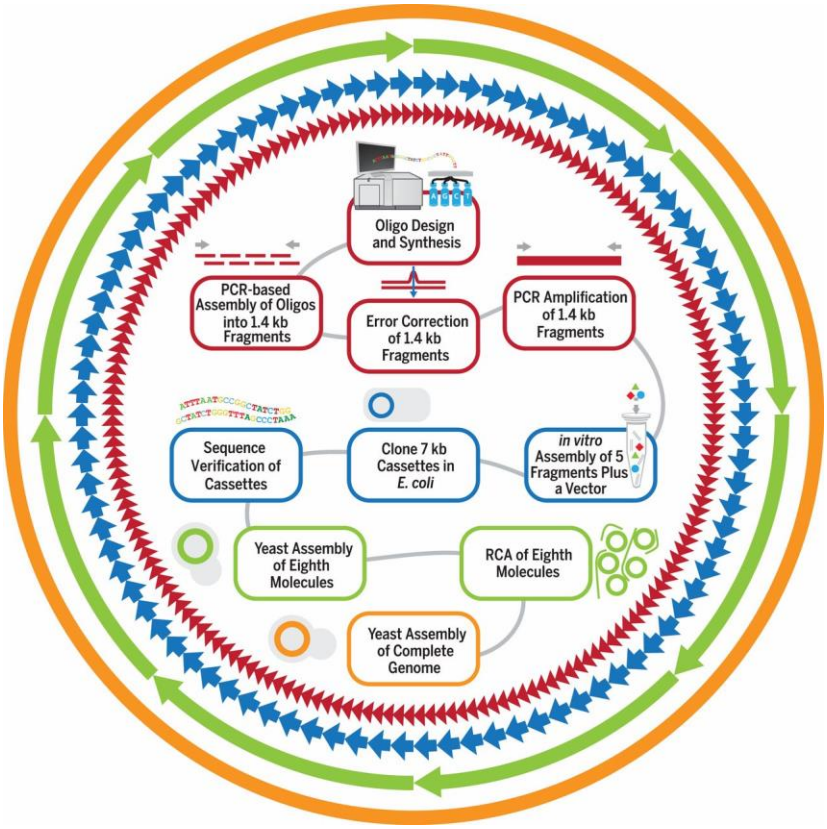

***Figure 5*. Construction of JVC-syn3.0.** Overlapping oligonucleotides (oligos) were designed, chemically synthesized and assembled into 1.4 kbp fragments (red). After correction of errors and PCR amplification, five fragments were assembled in 7 kbp cassettes (blue). The cassettes were sequentially checked and then assembled into yeast to generate eight molecules (green). The eight molecules were amplified by PCR and then assembled in yeast to generate the complete genome (orange). *Modified from Hutchison et al. (2016).*

However, a new methodology for the construction of simple or minimal genomes has subsequently been developed: chemical synthesis rewriting of genomes (Venetz et al., 2019). This method consists of rewriting protein-coding sequences to obtain synonymous sequences that lack surplus genetic elements (such as alternative reading frames or control elements) while maintaining their coding function. The advantage over the aforementioned studies—which preserve gene sequences as they occur in the natural organism, introducing only small modifications to facilitate its operation—is that their rewriting could provide a greater understanding of the biological functions of the different genes. In their work, Venetz et al. (2019) start from the genome of *Caulobacter crescentus* to construct a new, rewritten, synthetic minimal genome, called *Caulobacter ethensis-2.0* (*C. eth-2.0*). The new genome contains 530 genes and 123,562 codons were rewritten by introducing 133,313 base substitutions. The design changes include, among others, the elimination of synthesis constraints, which results in greater ease of the subsequent production process, and the elimination of various restriction sites, to facilitate assembly. After completing the design, the next step was the chemical synthesis of the genome, which was carried out in four successive steps. Starting with 236 DNA blocks, these were assembled into increasingly larger segments. Finally, the proper function of the rewritten genes was tested in *C. crescentus* by transposon mutagenesis. Segments of *C. eth-2.0* genome were introduced in *C. crescentus*. If the rewritten genes were functional, native genes were no longer essential and acquired disruptive transposon insertions. On the contrary, if rewritten genes were not functional, their native counterpart did not tolerate those insertions. Results showed that 432 rewritten genes (81.5%) were functional. As for the remaining 98 genes, the authors argue that "it is reasonable to conclude that these genes are misannotated or contain hitherto unknown essential genetic elements embedded

within their CDS [coding sequence]. Alternatively, it is also possible that a subset of these genes encode for RNA rather than protein-coding functions" (p. 8).

Another important advance in this area was the construction of the first synthetic eukaryotic chromosome in 2014. It was called synIII and is based on the 316,617–bp native *Saccharomyces cerevisiae* chromosome III. The synthetic variant is composed of 272,871 bp and includes changes such as TAG/TAA stop-codon replacements, deletion of subtelomeric regions, introns, transfer RNAs, transposons, and silent mating loci. In spite of these changes, synIII was proven to be functional in *S. cerevisiae*. (Annaluru et al., 2014). At least five more chromosomes have been synthesized to date, as part of the Sc2.0 project[7], which aims to obtain a full synthetic version of the 16-chromosome genome of *S. cerevisiae* (Kannan & Gibson, 2017), and a strain has already been achieved in which three of its chromosomes are completely synthetic and functional (Mitchell et al., 2017).

Scientists have great expectations regarding the applications of synthetic yeast. Thus, in the goals' section on the webpage of the Sc2.0 project they state that:

> "[t]he synthetic yeast genome can be used to answer a wide variety of profound questions about fundamental properties of chromosomes, genome organization, gene content, function of RNA splicing, the extent to which small RNAs play a role in yeast biology, the distinction between prokaryotes and eukaryotes, and questions relating to genome structure and evolution. The availability of a fully synthetic genome will allow direct testing of evolutionary questions not otherwise approachable. The eventual "synthetic yeast" being designed and refined could eventually play an important

[7] http://syntheticyeast.org/sc2-0/

> practical role. Yeasts, and *Saccharomyces cerevisiae* in particular, are preeminent organisms for industrial fermentations, with a wide variety of practical uses including ethanol production from agricultural products and by-products" (Synthetic Yeast 2.0, n.d.)[8].

A relevant project that can also be classified within this branch of Synthetic Biology is the Human Genome Project-Write (HGP-write)[9], which aims to synthesize human genomes artificially (Boeke, Church, Hessel, Kelley, & The GP-Write Consortium, 2016). Proponents justify the interest of the project in that it would contribute to the advancement of genome-building technology, with the consequent benefits in biological studies. Thus, research on constructing the human genome and other large genomes is expected to bring increased knowledge of genetic blueprints, as well as new and more efficient methods and tools for genetic synthesis and editing (Boeke et al., 2016).

For the time being, however, a lack of funds and technical difficulties represent a challenge for the successful development of this project, whose future remains uncertain (Servick, 2017). In fact, the organizers of the project have decided to temporarily postpone this objective and focus on a sub-objective, the creation of a virus-resistant human cell line. In addition to the specific applications of these cells, which could be used in industry without risk of viral contamination, it is expected that the knowledge and technical development derived from this work will serve the first objective of the project (Dolgin, 2018).

[8] syntheticyeast.org/sc2-0/goals/
[9] https://engineeringbiologycenter.org/

1.2.3 Protocells

Protocells are intended to be simple forms of life constructed completely from scratch (Bedau, Parke, Tangen, & Hantsche-Tangen, 2009). The aim of this research field is that protocells will be capable of reproducing themselves, and that protocell populations will adapt and evolve (Rasmussen et al., 2009). Similarly, according to Murtas (2009), protocells should achieve three properties: self-maintenance, self-reproduction and evolvability. In other words:

> "a protocell is a self-assembling and self-reproducing chemical system, with the following three properties:
>
> 1. It maintains its identity over time by spatially localizing its components in some form of container.
>
> 2. It utilizes free energy from its environment and digests environmental resources in order to maintain itself, grow, and ultimately reproduce. This use of energy and materials is a form of metabolism.
>
> 3. The containment and metabolism are under the control of replicable and inheritable chemical information that can be "mutated" when the protocell reproduces. This informational chemistry functions as a programmable genetic system.
>
> The proper chemical integration of these three properties enables protocells to reproduce themselves, and a population of them could adapt and evolve by natural selection" (Bedau et al., 2009, p. 67).

In the literature, this approach has sometimes been referred to as "bottom up", in contrast to "top down" approaches:

> "Most of the best-known work in synthetic biology is 'top down' in the sense that it starts with some pre-existing natural living system and then re-

> engineers it for some desired purpose [...]. Another approach to engineering novel biological systems works strictly from the 'bottom up' in the sense that it attempts to make new simple kinds of minimal chemical cellular life, using as raw ingredients only materials that were never alive" (Bedau, et al., 2009, p. 65).

Murtas (2009) identifies two main routes to do so: One is to synthesize the necessary biochemical mechanisms to construct a self-replicating system in a cell assembly, with the aim of better understanding certain cellular processes, as well as to improve *in vitro* methods for the synthesis of therapeutics, biopolymers, and biosensors. The second aims to construct models for the first forms of cellular life. This approach is based on the idea that the current complexity found in even the simplest unicellular organisms is not strictly necessary for life:

> "The biochemical complexity of a cell is in large part the result of millions of years of evolution based on competition struggles. This has produced plenty of defence mechanisms, enzymes and nucleic acid redundancies, security loops and the development of a series of reactions that would have probably not occurred in a more permissive historical environment. The other argument is based simply on the consideration that the first early cells, that started the origin of life, could not have been possibly so complex from the very start, they must have been, conceivably, much simpler" (Murtas, 2009, p. 1293).

Early attempts at this approach were limited to the encapsulation of a cell-free expression system (usually an *E. coli* extract) with some plasmid genes (Figure 6), achieving the expression of a protein for a very limited period of time (Noireaux & Libchaber, 2004) or the proper functioning of very basic genetic networks (Ishikawa, Sato, Shima, Urabe, & Yomo, 2004).

At present, genes and enzymes have been incorporated inside lipid vesicles, and several advances have been made in the encapsulation of proteins, ribosomes (D'Aguanno et al., 2015) and genetic circuits (Adamala, Martin-Alarcon, Guthrie-Honea, & Boyden, 2017); even the photosynthetic reaction center has been embedded in an artificial lipid membrane (Altamura et al., 2017). The *de novo* formation and growth of phospholipid membranes has also been achieved (Bhattacharya, Brea, Niederholtmeyer, & Devaraj, 2019), but many authors agree that current protocells are not alive (Buddingh' & van Hest, 2017; Rasmussen, Constantinescu, & Svaneborg, 2016; Xu, Hu, & Chen, 2016; Yewdall, Mason, & van Hest, 2018) (Figure 7).

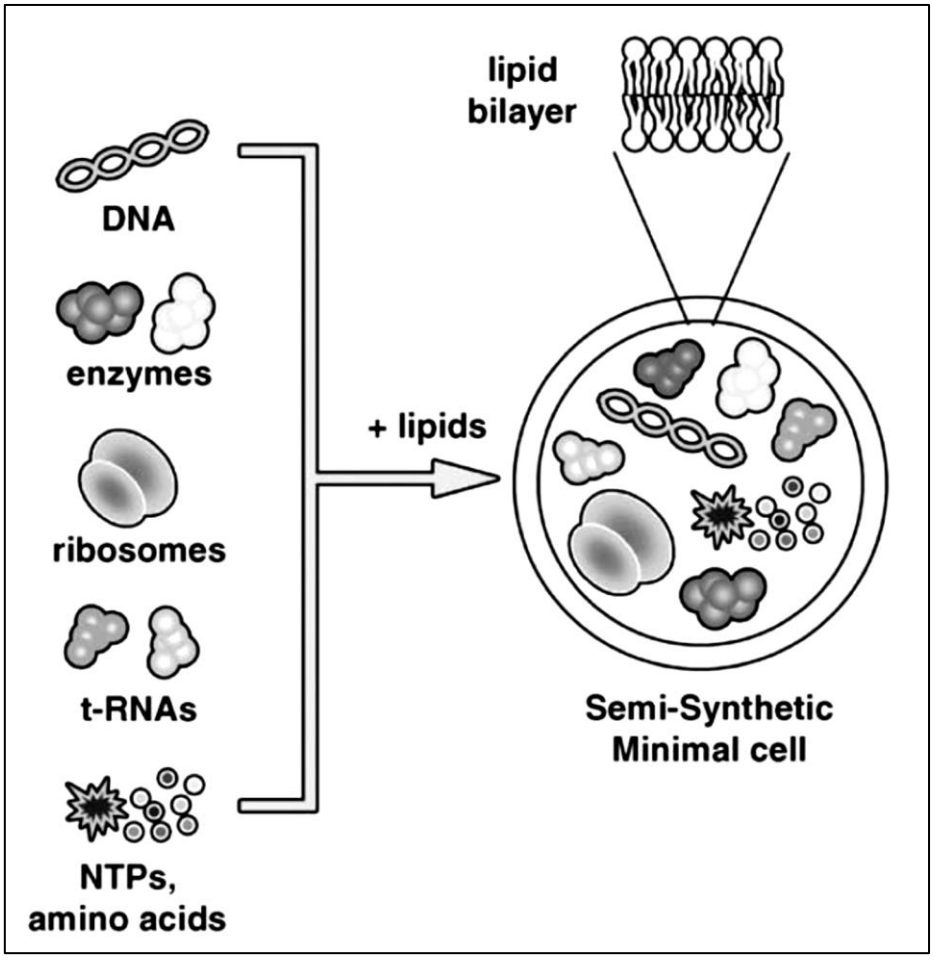

Figure 6. **Semi-synthetic minimal cell.** "Semi-synthetic minimal cells are composed of the minimal number of genes, enzymes, ribosomes, tRNAs and low molecular weight compounds that are encapsulated within a synthetic compartment". *Retrieved from Chiarabelli, Stano, & Luisi (2009)*.

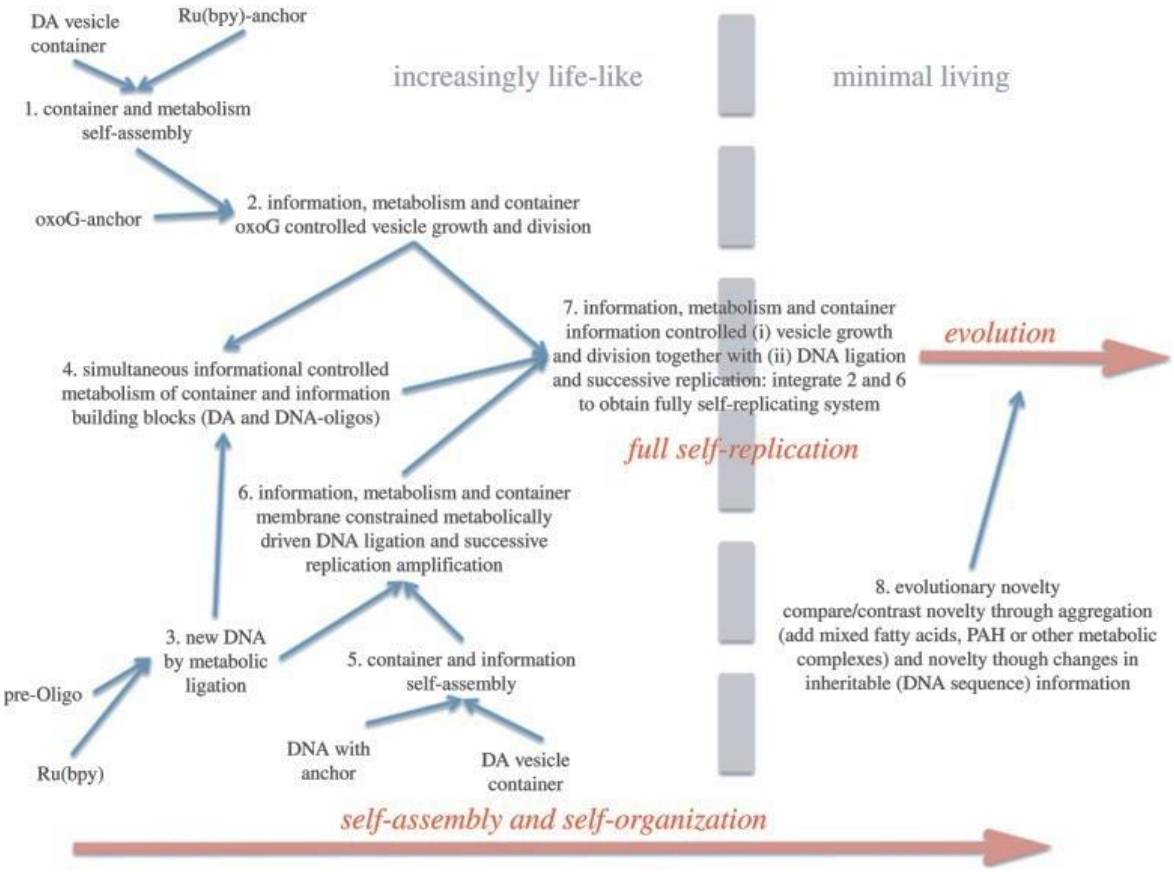

Figure 7. **Overview of the implementation of experimental systems and envisioned systems integration**: "(i) self-assembly of a decanoic acid container; (ii) anchoring to the container a metabolic ruthenium complex as well as (iii) a conjugated nucleic acid information complex; (iv) container feeding and growth; (v) metabolically driven container replication; (vi) metabolically driven information ligation (part of replication); (vii) one-pot metabolic production of both amphiphilic molecules and ligated oligomers, new information molecules. These are all key milestones towards the construction of a minimal living system. One key milestone is not yet reached, however, before full protocell integration can occur: implementation of an effective DNA self-replication process based on template-directed ligation of two smaller oligomers". *Retrieved from Rasmussen et al. (2016)*.

Some of these structures are called cell mimics (Majumder & Liu, 2017; Yoo, Irvine, Discher, & Mitragotri, 2011) or 'non-typical' artificial cells (Xu et al., 2016). These are engineered materials that mimic some features of biological cells, such as morphology, surface characteristics or functions (Xu, et al., 2016). These simplified cell-like structures have defined compositions and are used to study specific aspects of cell biology in isolation (Figure 8) (Salehi-Reyhani, Ces, & Elani, 2017).

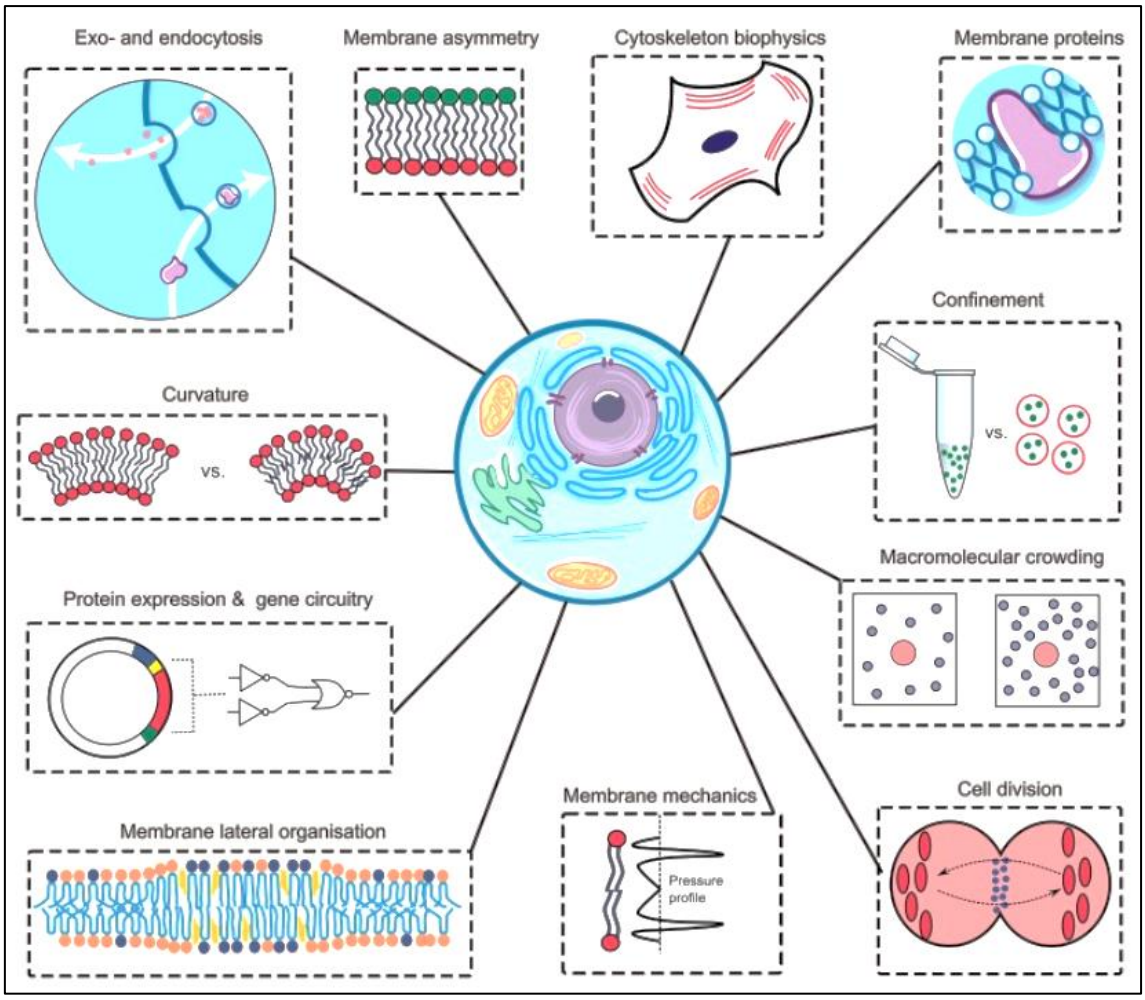

***Figure 8*. "Schematic summarizing some of the cellular components and biological phenomena that have been studied using artificial cell mimics".** *Retrieved from Salehi-Reyhani et al. (2017).*

Mimics of eukaryotic cells have been produced with an artificial porous polymer membrane containing an artificial hydrogel compartment, where the genetic material of the cell mimics is retained. These cell mimics "are able to communicate through diffusive protein signals, activate gene expression in neighboring cell-mimics, and display

collective responses to cell-mimic density similar to bacterial quorum sensing" (Niederholtmeyer, Chaggan, & Devaraj, 2018). This approach sometimes converges with cell-free Synthetic Biology, as is the case of the "multi-compartment encapsulation of communicating droplets and droplet networks in hydrogel as a model for artificial cells" (Bayoumi, Bayley, Maglia, & Sapra, 2017).

Bedau et al. (2009) predicted that, within a period of five to ten years, fully autonomous protocells would be produced. Ten years later, this expectation has not been fulfilled and we are not even close to it (Rasmussen, et al., 2016). Therefore, additional predictions based on that statement (that protocells "could survive in the natural environment outside of the laboratory within the next ten to twenty years. On that same timescale, we expect that protocells will also be ready for commercial applications" (Bedau et al., 2009, p. 66)) will in turn be deferred. However, with several groups working on it, and millions of Euros invested in this approach, some scientists expect to obtain the first artificial cells in less than a decade (Powell, 2018).

The envisioned applications of protocells include: study of the origin of life and cellular processes, fuel production, drug synthesis (which could occur *in situ*), replacement or supplementation of deficient cells, implementation of new functions, directed evolution, protein synthesis and *in vivo* diagnostics or biosensing (Bally et al., 2010; Elani, Law, & Ces, 2015; Fujii et al., 2014; Lagny & Bassereau, 2015; Ma & Feng, 2015; Miller et al., 2006; Thomas, Friddin, Ces, & Elani, 2017; Xu, et al., 2016).

However, due to the reduced complexity of protocells, they have very limited capabilities. For this reason, a variation of the approach has been investigated consisting of the encapsulation of living cells in artificial vesicles, thus allowing artificial cells to take advantage of the cellular biochemistry (Figure 9) (Elani et al., 2018).

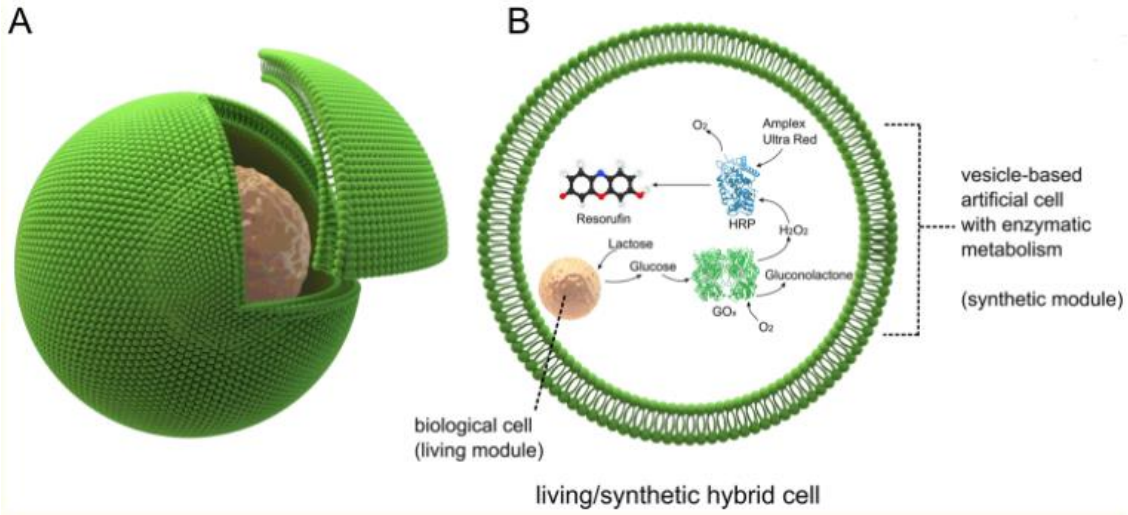

Figure 9. "**Living/Synthetic hybrid cells**. (A) Schematic of a biological cell encapsulated inside a vesicle-based artificial cell. (B) The encapsulated cell serves an organelle-like function in the vesicle reactor, processing chemical elements which are then further metabolised downstream by a synthetic enzymatic cascade co-encapsulated in the vesicle". *Retrieved from Elani et al. (2018).*

1.2.4 Xenobiology

Schmidt, Pei and Budisa (2018) define xenobiology as a branch of Synthetic Biology whose particular characteristic is that it aims to redesign life not only by means of new genetic modules or new combinations of these modules, but through a change in the chemical composition of the genetic modules themselves. This branch of Synthetic Biology thus aims to expand the genetic code in order to exceed the standard chemical composition of cells. There are two possible avenues to do this: by introducing new nucleotides, or xeno-nucleotides (Benner, 2004; Benner & Sismour, 2005; Chin et al., 2003; Hamashima, Kimoto, & Hirao, 2018; Saito-Tarashima & Minakawa, 2018; Wang, Brock, Herberich, & Schultz, 2001), or by repurposing natural codons (Anderson et al., 2004; Lajoie et al., 2013; Xie & Schultz, 2006).

An important milestone reached for the first approach was the construction of a semi-synthetic strain of *E. coli* that not only harbors an unnatural base pair (UBP), formed

by two xeno-nucleotides, dNaM and dTPT3, but also transcribes it and incorporates the non-canonical amino acids (NCAAs) PrK and pAzF into a protein (Zhang et al., 2017) (Figure 10). These elements need to be provided externally, as the cells are unable to produce them themselves, which is a safety mechanism in case of accidental escapes from the laboratory. Modified tRNAs are produced which are capable of reading the new codons and carrying the NCAAs.

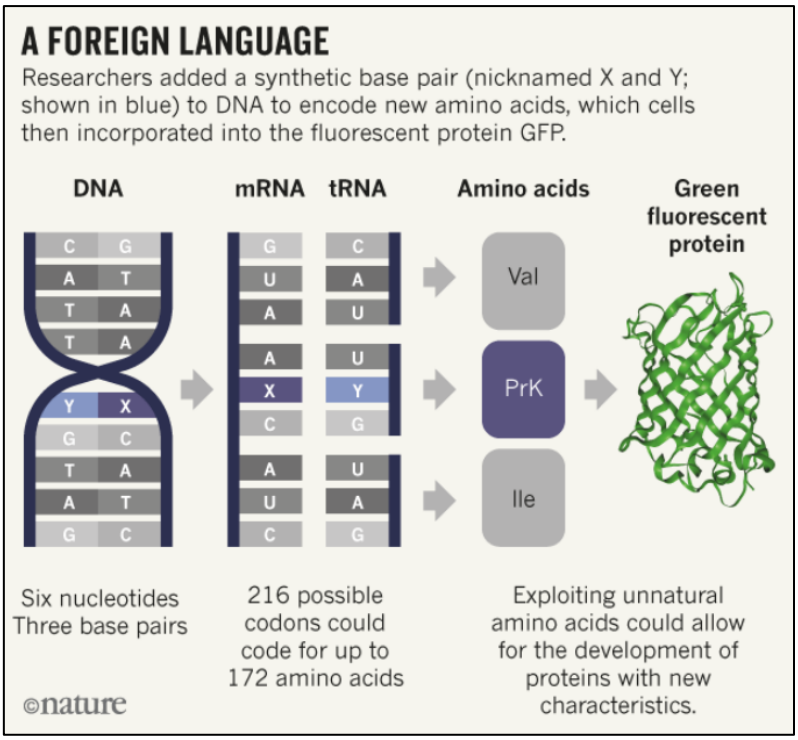

***Figure 10*. Successful protein incorporation of a non-canonical amino acid coded by an unnatural base pair.** *Retrieved from Callaway (2017).*

Scientists are currently working on refining the system and expanding the repertoire of UBPs (Dien, Morris, Karadeema, & Romesberg, 2018). A recent advance is the genetic reprogramming of the *E. coli* replisome in order to avoid progressive UBP loss, increasing its retention and achieving its incorporation into the bacterial chromosome (Ledbetter, Karadeema, & Romesberg, 2018). Additionally, a genetic system of eight nucleotides, four natural and four unnatural, has been constructed (Hoshika, et al., 2019). This system has been called "hachimoji" ('eight letters' in Japanese) and the two new base-pairs are

also formed by hydrogen bonds. According to the authors, “hachimoji DNA has potential applications in bar-coding and combinatorial tagging, retrievable information storage, and self-assembling nanostructures”.

Engineered genetic codes involve the modification of cellular machineries in order to incorporate NCAAs into the proteome. There are two main routes to do this. On the one hand, NCAAs can be incorporated in a proteome-wide manner, forcing organisms to take up the amino acids and isolating the mutants (Figure 11); on the other, NCAAs can be incorporated into specific sites by means of orthogonal tRNA/aminoacyl-tRNA synthetase pairs (Figure 12) (Lin, Yu, & Chan, 2017).

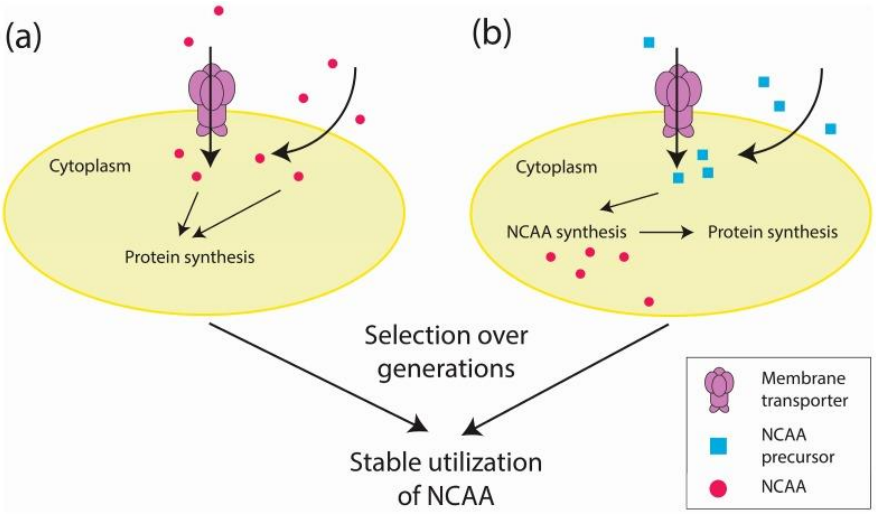

Figure 11. “**An overview of proteome-wide approaches to incorporate NCAAs.** (a) The NCAA enters a cell via membrane transporters or diffusion across the membrane. (b) The NCAA precursor similarly enters a cell in which it will be used to synthesize NCAAs. Following several generations of propagation with either the NCAA or its precursor, cells that can stably utilize the NCAA are selected”. *Retrieved from Lin et al. (2017).*

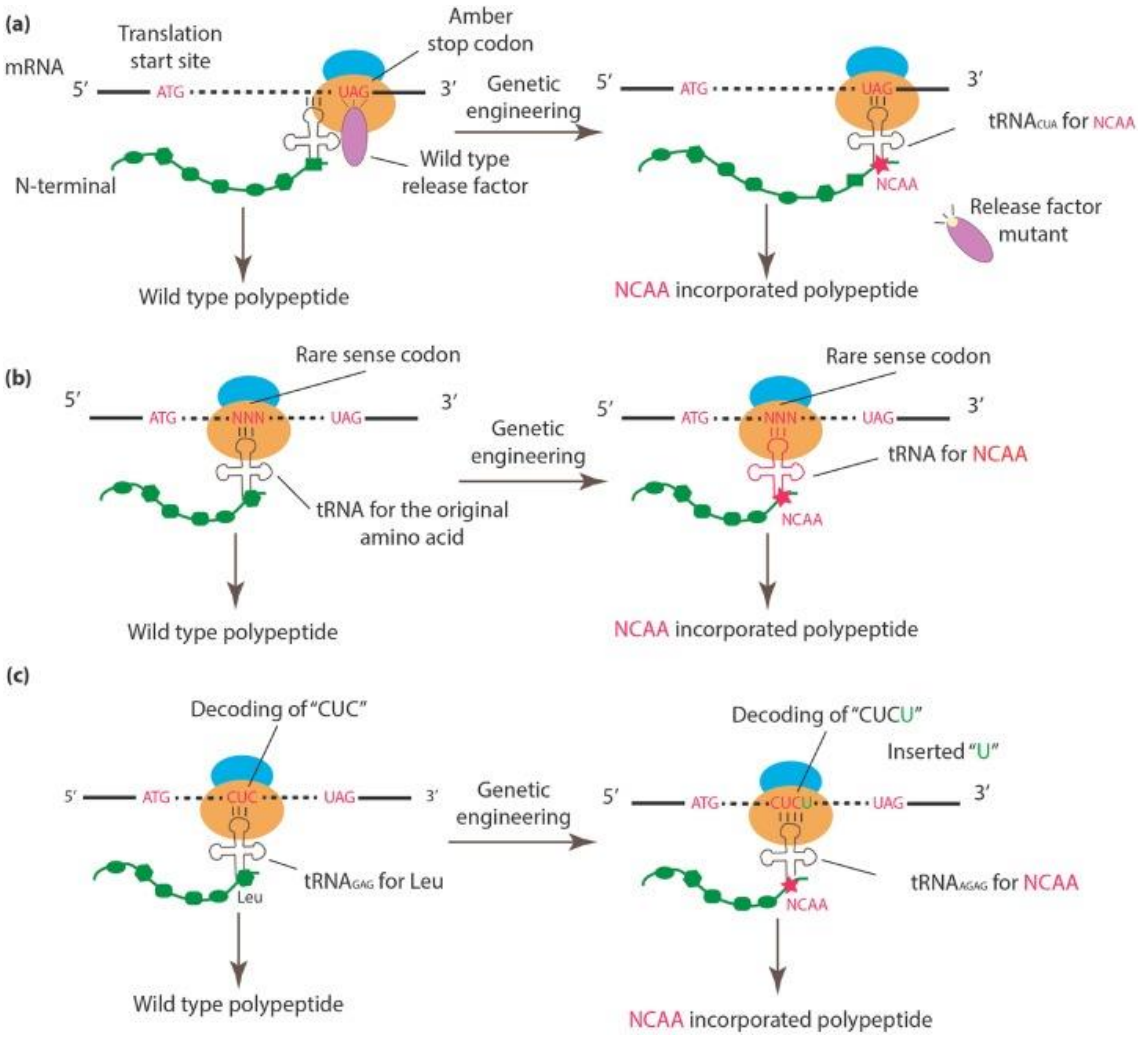

Figure 12. "**An overview of approaches to incorporate NCAAs into specific sites**. (a) The wild-type release factor is mutated or knocked out, allowing the newly introduced $tRNA_{CUA}$ to read through the stop codon, followed by NCAA incorporation with assistance from the compatible aminoacyl-tRNA synthetase. (b) The tRNA and corresponding tRNA synthetase for a rare sense codon are genetically engineered to confer the ability to encode NCAA. (c) A single-base is inserted after the canonical codon (e.g. "CUC" for Leu). The newly introduced quadruplet tRNA (e.g., $tRNA_{AGAG}$) can encode NCAA by targeting the quadruplet codon "CUCU."" *Retrieved from Lin et al. (2017)*.

Synthetic recoding of organisms serves several applications, such as: to improve or expand protein functions, to establish proteomic signatures in order to identify synthetic organisms, to improve biocontainment to provide virus resistance to industrially relevant strains ("[a] bacterial strain that cannot recognize a common sense codon should be unable to translate essentially any phage gene" (Kuo et al., 2018)), or to learn fundamental biology. Additionally, it can serve to develop diagnostic tools ("[d]iagnostic molecular

beacons with fluorescent dye linked to the unnatural bases can serve as molecular diagnostic tools, for example, to target infectious diseases of interest" [Schmidt et al., 2018]), to generate high affinity and specificity DNA aptamers, with potential applications for diagnostics and therapeutics, and to develop quantitative PCR methods (Hamashima et al., 2018).

1.2.5 *In silico* approach

This consists of the development of the computational tools that are used in the other branches of Synthetic Biology (Carbonell et al., 2016; Deplazes, 2009; MacDonald et al., 2011; Madec et al., 2017; Marchisio & Stelling, 2008). Advances in computer aided design (CAD) are continuously being developed, which is fundamental for the application of Synthetic Biology on an industrial scale (Nowogrodzki, 2018). Table 5 shows different examples of these tools.

> "Next generation gene sequencing machines now provide faster and less expensive methods for indexing genetic code. Currently, synthetic biologists have the ability to design genetic code to elicit a specific function, pre-test the code for functionality using computer modeling, order the relevant genetic material from a commercial or open-source gene synthesis facility, and insert the material into a cell body in order to test real world functionality. Some DNA designs are now working the first time they are tested, replacing what has historically been a tedious trial-and-error based approach to engineering novel phenotypes" (National Academy of Engineering and National Research Council, 2013, p. 10).

Table 5.

Software for Synthetic Biology design. *Modified from Kelwick, MacDonald, Webb and Freemont (2014).*

SOFTWARE TOOL	DESCRIPTION
PATHWAY AND CIRCUIT DESIGN	
AutoBioCAD	Automated design of gene regulatory circuits
Cell designer	Modeling of biochemical networks
Genetic engineering of cells (GEC)	Biological programming language and visual simulator of biological systems
GenoCAD	GenoCAD is an open-source computer-assisted-design (CAD) application for synthetic biology
Genome compiler – iGEM edition	Cloud based genetic design tool that is optimized for BioBrick assembly and the iGEM competition
MATLAB: Simbiology	SimBiology® provides an application and programmatic tools to model, simulate, and analyze dynamic biological systems
Operon calculator	Rational design of bacterial operons to control protein expression
OptCom	A modeling framework for the flux balance analysis of microbial communities
ProMoT	Process Modeling Tool, software for the construction and manipulation of complex technical and biological systems
BIOPART DESIGN	
CaDNAno	Simplifies the process of designing three-dimensional DNA origami nanostructures
COOL	Codon Optimization OnLine (COOL): a web-based multi-objective optimization platform for synthetic gene design
mfold/UNAfold	Prediction of nucleic acid secondary structure
NUPAC	Prediction and design of nucleic acid secondary structure
Promoter calculator	*E. coli* σ^E – In development
RBS calculator	The Ribosome-Binding Site (RBS) Calculator is a design method for predicting and controlling translation initiation and protein expression in bacteria
RBS designer	Computational design of synthetic ribosome-binding sites (RBS) to control gene expression levels
RNA designer	Designs RNA secondary structure
Rosetta	Tools for structure prediction, design, and remodeling of proteins and nucleic acids
UTR designer	Predictive design of mRNA translation initiation region to control prokaryotic translation efficiency
MISCELLANEOUS	
R2oDNA designer	Designs orthogonal biologically neutral linker sequences for DNA assembly and other uses
SBOL	SBOL core provides an interoperable data format to transfer biopart characterization data between software programs and tools
SBOLv	SBOL visual defines a standardized way to visually denote bioparts through symbols

1.2.6 DIY Synthetic Biology

DIYbio consists of the practice of Synthetic Biology by individuals outside of institutional settings, at home or in community labs (Scheifele & Burkett, 2016). Although this movement is considered as a branch of Synthetic Biology by some authors (SCENIHR, SCHER, & SCCS, 2015a), rather than a specific type of activity different from that carried out in other branches, DIYbio is about conducting these activities in a different place and on the practitioner's own account. Genspace[10], for example, a Biosafety Level One facility in Brooklyn, New York, is the first-ever community biotechnology laboratory. As an illustration of what these kinds of facilities can deliver, Genspace offers hands-on courses to the public, provides extracurricular experiences for students, encourages scientific entrepreneurship and hosts a variety of talks, workshops, and cultural events. Since this community-based laboratory was set up in 2010, at least 84 similar spaces have been established (Sleator, 2016). Although we assume that the number of facilities will have increased today, it has not been possible to find more up-to-date data.

DIYbio is characterized by five features: a) interdisciplinarity; b) primarily a not-for-profit endeavor; c) design and use of cost effective tools and equipment; d) focusing on open source and open science innovation; and e) democratization and self-empowerment (Seyfried, Pei, & Schmidt, 2014).

The core idea of this movement is to bring biotechnology closer to the lay public with the aim of promoting scientific progress. A well-known Biopunk Manifesto written by Meredith Patterson, a leading figure in the movement, states that:

[10] See http://genspace.org/page/About

> "We reject the popular perception that science is only done in million-dollar university, government, or corporate labs; we assert that the right of freedom of inquiry, to do research and pursue understanding under one's own direction, is as fundamental a right as that of free speech or freedom of religion" (Patterson, 2010).

For the moment, DIYbio is not expected yield great discoveries, but is a new way of doing science, emphasizing transparency and the sharing of knowledge, promoting citizen science and decentralized access to biotechnology. This is achieved to a great extent through the implementation of cheap and accessible devices to carry out the same procedures that until now required professional and expensive equipment (Table 6). A good example of this is Bento Lab[11], a portable DNA analysis laboratory that comes with a PCR thermocycler, centrifuge, gel electrophoresis box and a transilluminator, costs only €1179, and is smaller than a laptop. In addition, second-hand laboratory equipment can be found at low prices on the Internet, in pages such as eBay, and a large number of instruments have become available at very low costs that allow research laboratories to be set up outside the academic realm (Landrain, Meyer, Perez, & Sussan, 2013).

[11] See https://www.bento.bio

Table 6.

DIYbio alternatives for major experimental steps and lab equipment needed to realize Synthetic Biology projects. *Retrieved from Landrain et al. (2013).*

Experimental steps	***Necessary equipments/ consumables***	***DIYbio solutions***	***Saving ratio***
Cell culture	Incubator—$130	Styrofoam insulated box with heating pads, computer fans and a thermostat—recycled material—$10	13×
	Bioreactor—$3,000	Aquarium air pumps connected to plastic bottles with fluorescent tubes—recycled material—$100	30×
Microscopy	400× optical microscope with camera—$130	Webcam optical microscope 400×—$10	13×
Centrifuges	Benchtop centrifuge—$2,000	Dremelfuge (requires 3D printer, drill)—$100	20×
Water bath	Water bath—$400	Home-made water bath (aquarium heater + bucket)—$40	10×
Magnetic stirrer	Magnetic stirrer—$70	DIY magnetic stirrer—$10	7×
Spectrophotometer	Spectrophotometer—$150	DIY spectrophotometer—$10	15×
Sterile work	Autoclave—$1,000	Pressure cooker—$70	14×
		Microwave—$50	20×
	Glove box—$10,000	DIY Glove box—$500	20×
	Sterile hood—$2,000	Custom sterile hood—$200	10×
Electrophoresis	Gel box—$400	Home-made plastic gel box—$25	8×
		Pearl gel box kit (with UV transilluminator)—$199	7×
	UV Transilluminator—$1,000	DIY UV transilluminator—$100	10×
	Blue light transilluminator—$1,000	The blue note project—$30	33×
	Power supply—$1,000	DIY power supply—$40	25×
PCR	Thermocycler—$2,500	Open PCR—$500	5×

		Lava Amp—$300-$500	5×
		Thermotyp—$400	6×
		Personal PCR—$199	7×
		Bulb PCR—$25	100×
DNA purification	Miniprep kits—$1 per miniprep	DIY buffers + Regeneration of silica columns—reusable 10–20 times—$0.2 per miniprep	5×
DNA digestion/DNA ligation/PCR/DNA assembly	Enzymes—Taq polymerase $0.3 per Unit	DIY purification of recombinant proteins—Taq polymerase $0.001 per Unit	300×
Make cells competent + transfo rmation	Long protocol using Ice and CaCl2 salts—ice difficult to procure	Fast one-step method using PEG-3,350 (laxative) + MgSO4(Epsom salts)—easy to find	1×
Transformation	Gene-gun—$17,000	DIY Gene-gun—$200	85×
Sequencing	1 single read—5$	No cheaper alternative	1×
Quantitative PCR diagnostics	qPCR—$10,000	Amplino—$200	50×
Bioprinting	Non existing commercial solutions	Hacked inkjet printer for printing layers of bacteria	N/A

1.3 APPLICATIONS OF SYNTHETIC BIOLOGY

Synthetic Biology has multiple and varied applications in a wide range of fields. In therapeutics, it is expected to provide new means for the production of drugs, their delivery, and for the treatment of serious diseases such as cancer. It can also be used to construct useful biosensors, for example to detect a particular contaminant in the environment or a molecule in the human body. Another important application of Synthetic Biology is the production of biofuels and other products. Additionally, this discipline shows promise in biodiversity conservation, as well as bioremediation and pollution control.

1.3.1 Therapeutics

A largely pursued application of Synthetic Biology is the production of biopharmaceuticals (Breitling & Takano, 2015). To this end, bacteria are very useful organisms (Flores Bueso et al., 2018), but yeasts have also been employed in this area to produce a wide range of medically-relevant compounds (Table 7) (Walker & Pretorius, 2018). Mammalian Synthetic Biology is also being explored for the production of biopharmaceuticals, as well as for other medical applications (Kis, Pereira, Homma, Pedrigi, & Krams, 2015). Emerging therapeutic approaches include the application of Synthetic Biology to engineer phages (Lemire, Yehl, & Lu, 2018), the human microbiota or different probiotics (Bober, Beisel, & Nair, 2018; Dou & Bennett, 2018; Mays & Nair, 2018). Interestingly, Synthetic Biology provides other uses beyond the mere production of drugs, constituting an aid for the different steps during the drug development (DD) process (Figure 13) (Trosset & Carbonell, 2015).

A wide variety of treatments are being developed, including treatments for infectious diseases, metabolic disorders and cancer (Chien, Doshi, & Danino, 2017; Krishnamurthy, Moore, Rajamani, & Panchal, 2016; Ozdemir, Fedorec, Danino, & Barnes, 2018; Planson, Carbonell, Grigoras, & Faulon, 2012; Wu, Bethke, Wang, & You, 2017) (Table 8). Other medical applications include disease mechanism investigation, vaccine development, diagnosis and prevention (Abil, Xiong, & Zhao, 2015; Folcher & Fusseneger, 2012; Weber & Fussenegger, 2012).

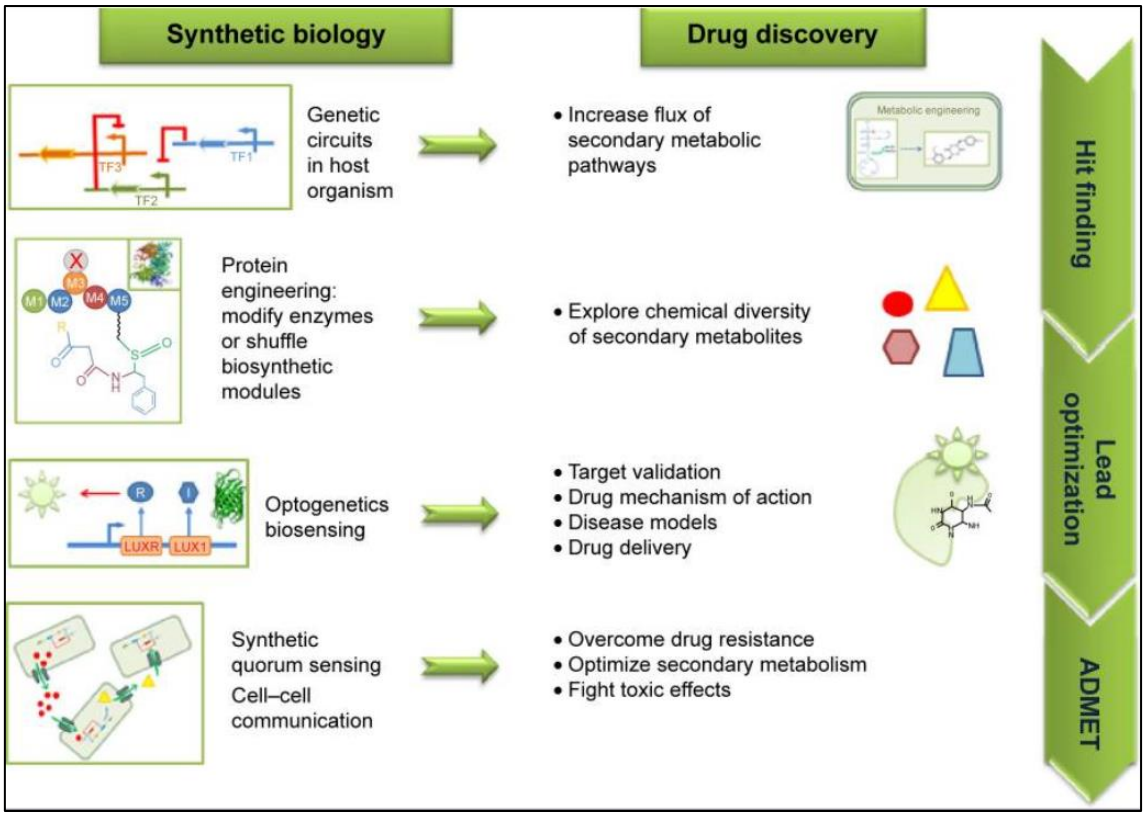

Figure 13. **"Synthetic biology tools in various steps of drug discovery.** Abbreviations: ADMET, absorption, distribution, metabolism, excretion, and toxicity; TF, transcription factor". *Retrieved from Trosset & Carbonell (2015).*

Table 7.

Biopharmaceutical compounds produced by engineering yeast. *Modified from Walker and Pretorius (2018).*

Compound	***Application***	***Compound Class***	***Chassis Organism***	***Titre***	***Natural Source***
Agkisacutacin (Snake venom)	Antithrombotic	Protein	*Pichia pastoris*	100 mg/L	*Agkistrodon acutus* (Pit viper)
Apidaecin Ia	Antimicrobial	Antimicrobial peptide	*P. pastoris*	>700 mg/L	*Apis* (Honeybee)
Artemisinic acid	Artemisinin (anti-malaria) precursor	Sesquiterpene	*Saccharomyces cerevisiae*	25 g/L	*Artemisia annua* (Sweet wormwood)
Astaxanthin	Antioxidant	Carotenoid	*Kluyveromyces marxianus*	1 mg/g DCW	Various, including krill and shrimp
Breviscapine (Scutellarin and apigenin-7-*O*-glucuronide)	Chinese medicine. Cardiovascular and cerebrovascular disease.	Flavanoid	*S. cerevisiae*	105 and 185 mg/L	*Erigeron breviscapus*
Carnosic acid	Antioxidant	Diterpene	*S. cerevisiae*	18 mg/L	*Rosmarinus officinalis* (Rosemary) and *Salvia officinalis* (Sage)
β-Carotene	Antioxidant	Carotenoid	*Yarrowia lipolytica*	6.5 g/L (90 mg/g)	Various, including carrots
Hydrocodone	Pain relief (opioid)	Benzylisoquinoline alkaloids (BIA)	*S. cerevisiae*	<1 µg/L	N/A (Semi-synthetic from Codeine)
Lycopene	Antioxidant, anti-cancer	Carotenoid	*S. cerevisiae*	55.56 mg/g DCW	*Solanum lycopersicum* (Tomato)

Anti-Ebola monoclonal antibodies	Antiviral	Monoclonal antibody	*P. pastoris*	1 to 10 mg/L	N/A
Noscapine	Anticancer	Benzylisoquinoline alkaloids (BIA)	*S. cerevisiae*	2.2 mg/L	*Papaver somniferum* (Poppy plant)
Penicillin	Antibiotic	Beta-lactam nonribosomal peptide	*S. cerevisiae*	14.9 ng/mL	Penicillium fungi
Pisiferic acid	Antimicrobial agent	Diterpene	*S. cerevisiae*	2.65 mg/L	*Chamaecyparis Pisifera* (Sawara cypress)
Resveratrol	Several; antioxidant	Stilbenoid	*S. cerevisiae*	800 mg/L	*Polygonum cupidatum* (Japanese knotweed)
Salviol	Established bioactivity, awaiting further evaluation	Diterpene	*S. cerevisiae*	15 mg/L	*Salvia miltiorrhiza* (Chinese sage)
Strictosidine	Intermediate	Monoterpene indole alkaloid	*S. cerevisiae*	0.8 mg/L	N/A (chemical synthesis)
Taxadiene	Anticancer Taxol precursor	Diterpenoid	*S. cerevisiae*	72.8 mg/L	*Taxus brevifolia* (Pacific yew)
Δ^9-tetrahydrocannabinolic acid	Tetrahydrocannabinol precursor	Cannabinoid	*P. pastoris*	3.05 g/L	*Cannabis sativa* (Cannabis)
Thebaine	Opioid precursor	Benzylisoquinoline alkaloids (BIA)	*S. cerevisiae*	<1 µg/L	*Papaver somniferum* (Poppy straw)
Theophylline	Anti-asthma medication	Methylxanthine	*S. cerevisiae*	61 µg/L	*Camellia sinensis* (Tea) and *Theobroma cacao* (Cocoa)
Vindoline	Anticancer (vinblastine and vincristine) precursor	Monterpenoid indole alkaloid	*S. cerevisiae*	2.7 mg/L	*Catharanthus roseus* (Madagascar periwinkle)
Violacein	Antibiotic	Bis-indole pigment	*S. cerevisiae*	16.8 mg/L	*Chromobacterium violaceum*

Table 8

Complex Live Biotherapeutic Systems. *Retrieved from Ozdemir et al. (2018).*

Target	Location	Model Organism	Chassis	Mechanism	Reference
Cancer	liver	mouse	*E. coli* Nissle 1917	engineered strain secretes an enzyme to cleave a substrate that can be detected in urine	(Danino et al., 2015)
Cancer	subcutaneous and liver	mouse	*S. typhimurium*	synchronized population lysis to release triple combination of cancer therapeutics	(Din et al., 2016)
Cancer	liver	mouse	*S. typhimurium*	quorum sensing to only produce protein when population threshold has been reached, reducing off-target therapeutic delivery	(Swofford, Dessel, & Forbes, 2015)
Cancer	subcutaneous	mouse	*S. typhimurium*	inducible expression of FlaB in tumor tissue to stimulate an immune response	(Zheng et al., 2017)
Colitis	GI tract	mouse	*E. coli*	use *inv* to invade intestinal mucosal cells and deliver therapeutic under control of inflammation-inducible promoter	(Castagliuolo et al., 2005)
Enterohemorrhagic E. coli	–	*Galleria mellonella*	bacteriophage	delivery of CRISPR-Cas9-based, targeted antimicrobial	(Citorik, Mimee, & Lu, 2014)
Fever	subcutaneous and GI tract	mouse	*E. coli*	use thermo-sensitive promoters to detect fever and remote-control gene expression using ultrasound	(Piraner, Abedi, Moser, Lee-Gosselin, & Shapiro, 2017)
Inflammation	GI tract	mouse	*E. coli*	phage-lambda-based memory circuit to record markers of inflammation, stable for 200 days in vivo	(Riglar et al., 2017)
Inflammation	GI tract	mouse	*E. coli* Nissle 1917	detection of inflammation using tetrathionate and thiosulfate sensors	(Daeffler et al., 2017)
Inflammation and glycosuria	–	–	*E. coli*	thresholding, digitizing, and amplifying circuit for the sensitive detection of nitrogen oxides and glucose in pathological samples	(Courbet, D, Renard, Molina, & Bonnet, 2015)
P. aeruginosa	GI tract	Caenorhabditis elegans and mouse	*L. casei*	sense quorum molecule and produce bacteriocin and dispersin B for lysing	(Hwang et al., 2017)
S. aureus	skin	mouse	bacteriophage	delivery of CRISPR-Cas9-based, targeted antimicrobial	(Bikard et al., 2014)

A recent and striking example is the work of Shao et al. (2017), who designed a cellular glucose production system in diabetic mice, consisting of hydrogel capsules containing the engineered cells together with wirelessly powered light-emitting diodes. The genetic system is activated in response to a light stimulus (specifically far-red light signals), which in turn responds to glucose levels detected by a glucometer that sends these data via Bluetooth to a smartphone. Depending on the glucose levels detected, a light signal of a certain intensity is emitted, which activates glucose production by means of a genetic circuit (Figure 14). This example is framed within the growing application of optogenetics, a science based on genetic induction by light, in Synthetic Biology (Kolar & Weber, 2017; Mansouri, Strittmatter, & Fussenegger, 2018).

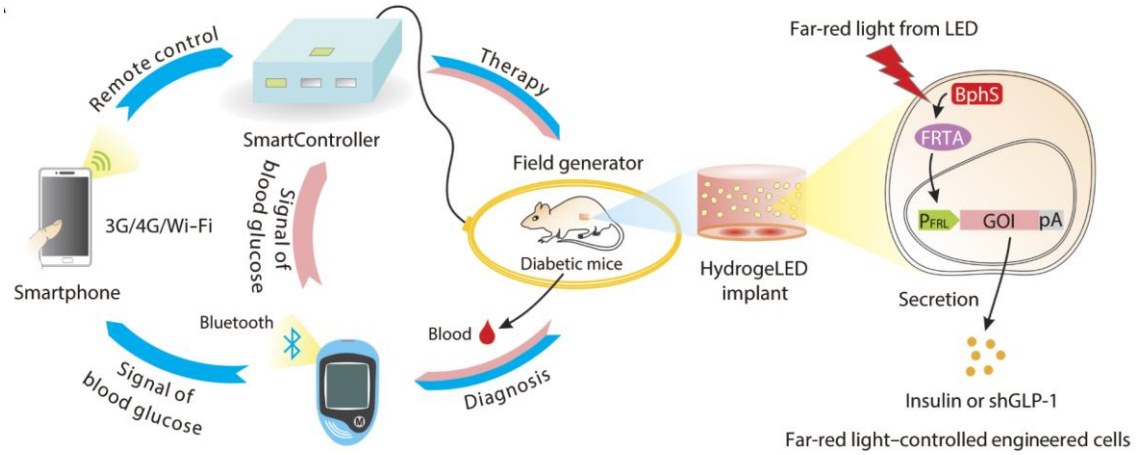

***Figure 14*. "Abstract diagram showing smartphone-controlled engineered cells enabling semiautomatic point of care for combating diabetes".** *Retrieved from Shao et al. (2017).*

Another case showing the potential of Synthetic Biology to offer new therapeutic solutions is the work of Schukur, Geering, Charpin-El Hamri, & Fussenegger (2015), which is paradigmatic of the capability of this discipline to provide automated treatments in diseases whose onset is not immediately detected, in this case psoriasis. Thus, researchers designed a genetic circuit that expresses the anti-inflammatory cytokines IL4 and IL10 in the presence of psoriasis biomarkers (tumor necrosis factor [TNF] and

interleukin 22 [IL22]). The system was tested in psoriatic mice models, where it prevented psoriatic onset, improved skin lesions and stopped acute psoriasis.

As mentioned above, a growing therapeutic approach in Synthetic Biology is the engineering of probiotic organisms (Bober et al., 2018; Dou & Bennett, 2018; Mays & Nair, 2018). Recent research in this area includes the development of potential treatments for conditions as varied as Crohn's disease (McKay et al., 2018), colorectal cancer (Ho et al., 2018), phenylketonuria (Isabella et al., 2018) and microbial infections (Hwang et al., 2017).

Synthetic Biology also provides toolkits for drug production and screening, which enable the generation of libraries of genetic variants that can be easily screened to find the best-producing variants and optimize producer strains (Chen et al., 2018). As an example, Reider Apel et al. (2017) applied a Cas9-based toolkit in yeast to optimize the production of taxadiene synthase, a precursor of the anticancer drug paclitaxel. They built an expression context library of 23 Cas9-sgRNAs plasmids, 37 promoters and 10 protein-localization, degradation and solubility tags. After performing the screening, a 25-fold improvement in the production of the enzyme was achieved.

Additionally, Synthetic Biology provides new means for drug delivery. In a recent article, artificial lipid-based vesicles were used to produce anticancer proteins inside tumors. The “synthetic cells” contained the template DNA, amino acids and energy-supplying molecules, constituting an encapsulated cell-free protein synthesis (CFPS) system. As the authors describe, these cell-mimicking particles can interact with their environment and exchange nutrients, showing promise for the development of therapeutic platforms that allow the delivery of useful proteins at the disease site (Krinsky et al., 2018).

1.3.2 Biosensing

Synthetic biosensors are composed of two parts: the sensitive elements and the transducer modules. The former recognize and bind analytes, while the latter transmit and report signals. Importantly, they also allow cellular responses to be regulated by engineering different components at the transcriptional, translational and post-translational levels (Khalil & Collins, 2010; Marchisio & Rudolf, 2011).

For instance, *S. cerevisiae* has been engineered to identify the presence of cadmium contamination in environmental water and soil (Ito-Harashima et al., 2017). Strains were established carrying plasmids with several yeast promoters connected to the bacterial *lacZ* reporter gene, and it was found that the *JLP1* promoter produced more sensitive strains. Importantly, the cost of sample analysis with this system is very low. Yeast biosensors have also been developed to detect antibiotics. Weaver, Halweg, Joyce, Lieberman and Goodson (2015) designed a paper-based device that used engineered yeast to detect antibiotics in the tetracycline family. The paper-based format is advantageous for use in developing countries or resource-poor settings. Additionally, the fact that it is yeast-based implies some advantages over the more commonly used bacteria, such as tolerance to pH and temperature fluctuations, established procedures for long-term storage, and the ability to survive for long periods of time in a dried state.

Biosensing can also converge with therapeutics. To illustrate, Mimee et al. (2018) reported the construction of biosensor *E. coli* Nissle cells which, in combination with miniaturized electronics inside semipermeable membranes, can detect a certain signal in a difficult-to-access environment, the gastrointestinal tract, and communicate with an external device. The device, called IMBED (ingestible micro-bio-electronic device), was able to diagnose gastrointestinal bleeding in swine through the engineering of bacteria to generate light in response to the presence of extracellular heme. Photodetectors embedded

in the electronic device detected the light and wirelessly communicated the corresponding signal. Additionally, alternative biosensors were integrated, demonstrating the extensibility of the platform to sense thiosulfate (a biomarker of gut inflammation) and acyl-homoserine lactone (molecular signature of particular bacteria).

1.3.3 Production of biofuels and other chemicals

Regarding the production of biofuels, as a renewable alternative to fossil fuels, Synthetic Biology showed great promise at an early stage (Wang, Wang, Zhang, & Meldrum, 2012). Thus, different hosts can be engineered to produce various biofuels and increase yield and tolerance to them. Among bacteria, *E. coli* stands out as a host organism for the production of fatty acid-, alcohol- and terpenoid-based biofuels (Wang, Pfleger, & Kim, 2017). Research on other organisms, such as yeasts, is also underway (Tsai, Kwak, Turner, & Jin, 2015). For example, the production of 1-octanol was achieved in *S. cerevisiae* through the combination of a previously engineered yeast fatty acid synthase with carboxylic acid reductase from *Mycobacterium marinum* and phosphopantetheinyl transferase Sfp from *Bacillus subtilis* (Henritzi, Fischer, Grininge, Oreb, & Boles, 2018). Cyanobacteria are also gaining popularity in this field of application for the synthesis of hydrocarbons, since, due to their photosynthetic capacity, they can convert CO_2 directly into chemicals, have a higher growth rate than plants and microalgae, and their genomes can be more easily manipulated (Xie, Wang, Zhang, Chen, & Lu, 2017). Research in this area of application is also being conducted on microalgae (Jagadevan et al., 2018) and plants (Mortimer, 2018).

Synthetic Biology can be used to produce other chemicals as well. For example, *S. cerevisiae* has been engineered to produce aromas and compounds such as vanillin, raspberry ketone, cinnamaldehyde (flavor and aroma of cinnamon), geraniol (rose-like

aroma), linalool (floral aromas), and limonene, among others (Kutyna & Borneman, 2018). Some precursors of biodegradable plastics have also been produced (Darvishi et al., 2018; Song et al., 2018).

1.3.4 Biodiversity and environmental conservation

Synthetic Biology is expected to bring invaluable benefits for biodiversity and environmental conservation (European Commission, 2016; Piaggio et al., 2017; Redford, Adams, & Mace, 2013) (Table 9). Examples of these benefits are the production of pest-resistant crops, which would make the use of pesticides unnecessary, avoiding their negative effects on other species; the protection of biodiversity through the control of climate change by developing renewable energy sources; obtaining useful products of animal or vegetable origin in engineered microorganisms; providing different species with certain genetic resistance to guarantee their survival; control of disease vectors or invasive species through gene drive systems; or even the restoration of extinct species (European Commission, 2016).

Synthetic Biology likewise shows great promise in the application area of bioremediation (de Lorenzo et al., 2018; Dvořák, Nikel, Damborský, & de Lorenzo, 2017; Solé, 2015; Solé, Montañez, & Duran-Nebreda, 2015). By way of illustration, *S. cerevisiae* has been engineered to express human MT2 and GFP genes under the activation of copper inducible promoters. MT2 produces a protein with metal-binding abilities, resulting in an enhanced ability for yeast copper ion bioremediation (Geva et al., 2016). In other experiments, *Pseudomonas putida* has been engineered through the insertion of several genes to enhance its oxygen-sequestering capability and simultaneously degrade organophosphates, pyrethroids, and carbamates, as well as providing real-time monitoring (Gong et al., 2018). A set of Synthetic Biology tools has

also been developed to engineer *Comamonas testosteroni*, whose features (such as its capacity to degrade pollutants and to colonize diverse environments) make it a promising chassis for bioremediation (Tang, Lu, & Liu, 2018). This application can result in a benefit for biodiversity, by eliminating substances that can threaten the survival of the species that inhabit the affected areas (European Commission, 2016).

Additionally, a great hope in this field is the modification of extremophilic bacteria and archaea for use in metal and radionuclide remediation (Marques, 2018). Other perspectives include the use of Synthetic Biology to degrade phenol and its derivatives (Rucká, Nešvera, & Pátek, 2017), and to absorb mercury (Tay, Nguyen, & Joshi, 2017). Furthermore, not only organisms can be engineered for bioremediation, but cell-free systems may also be useful for these purposes (Karig, 2017). De Lorenzo, Marlière and Solé (2016) have even greater aspirations:

> "beyond the classical concept of bioremediation – conceptualized as the mere removal of pollutants from given sites with biological agents [...] we envision a more ambitious goal that will require novel engineering perspectives and a highly interdisciplinary research effort with the global environmental microbiome at its core" (p. 619-620).

Table 9

Synthetic Biology potential solutions to different conservation issues. *Modified from Piaggio et al. (2017).*

Conservation issues	***Biodiversity issues***	***Synthetic Biology solutions***
Invasive species	Mice and rats on islands	Insertion of a male-determining gene (*Sry*) into a natural gene drive system present on chromosome 17, so that practically all the derived offspring will be, at least phenotypically, male
	Brown tree snake (*Boiga irregularis*) in Guam	Use Y chromosome alterations and gene drives to stop reproduction in this species
Pathogens	Avian blood parasites in Hawaiian birds	Use gene drives to spread a dominant female-lethal gene to eradicate avian malaria mosquito vector
	Fungal pathogens: white-nose syndrome in North American bats and chytrid fungus in amphibians and snakes	Engineer genetic resistance to fungal diseases
	Plague in black-footed ferrets	Use CRISPR/Cas9 to cut out part of genome that is susceptible to disease and replace with genetic code for disease resistance
Habitat conversion	Palm oil	Use other plants or systems to produce man-made palm oil and take pressure off current production methods, and thus reduce tropical forest conversion
	Productivity of soils reduced from pesticides and herbicides or by mining practices such as gold or strip mining	Synthetically restore microbiome of soils for habitat restoration, engineer plants that require less pesticides/herbicides for production
	Extraction and use of fossil fuels	Provide alternative solutions and thus alleviate pressures on such resources and the damage they cause, such as habitat loss and pollution. Create and modify microorganisms to consume hydrocarbons to clean up oil spills
Loss of biodiversity	Agriculture and its limitations to feed and house(forests) a growing human population	New food sources or ways to produce food without pesticides and large tracts of arable land

	Loss of faunal and floral biodiversity	Create ecological proxies, restore ecological functions
	Revive and restore extinct species	'De-extinction' (e.g., woolly mammoth): the use of an existing species(e.g., elephant) whose genome is altered to incorporate genetic code from the extinct species, thereby creating a proxy species that hopefully fills the same ecological role as the extinct species
Overexploitation	Rhino horn ivory and deep sea sharks for squalene	Produce a material that is a substitute and can be man-made
	Pet trade and feral domestic animals	Produce sterile pets
	Fish species	Improve aquaculture for higher protein production
Pollution	Replacing things made from petroleum and synthetic rubber	Engineer plants to make the same products
	Pesticide use	Increase resistance to pests
	Emissions of CO_2 or other greenhouse gases	Biofuels from synthetic algae
	Pharmaceuticals in the environment	Create or modify microorganisms to consume or degrade pharmaceuticals
	Micro-plastics in oceans and soils	Create or modify microorganisms to consume or degrade micro-plastic
	Water pollution	Create and modify algal or bacterial species that consume or degrade pollutants
	Coral reef bleaching	Alter the coral reef genome for resistance by borrowing pathways from coral species that withstand increased temperature and/or acidity

1.4 ETHICAL ASPECTS RELATED TO SYNTHETIC BIOLOGY

In spite of its useful applications and great potential, Synthetic Biology also raises several ethical concerns that need to be addressed, not only to protect those values that may be threatened by the different applications of this discipline, but also because an inadequate approach to these ethical issues can be, together with social rejection, an obstacle to the translation of basic science into real-world practice (Heidari Feidt, Ienca,

Elger, & Folcher, 2019). Various issues have been identified and have been classified differently.

In a review commissioned by the Biotechnology and Biological Sciences Research Council (BBSRC), the areas of ethical concern identified were the creation of artificial life, uncontrolled release of synthetic organisms into the environment, bioterrorism, patenting and justice (Balmer & Martin, 2008).

Additionally, in an opinion report by the European Group on Ethics in Science and New Technologies (EGE) to the European Commission (Capurro, Kinderlerer, Silva, & Rosell, 2009), conceptual-ethical issues "related to the ethical legitimacy of manufacturing living organisms" (p. 60) are distinguished from specific ethical issues, which concern the different applications of this discipline. In this report, so-called 'conceptual-ethical issues' include the potential impact of Synthetic Biology on the concept of life, the value of synthetic organisms, and our relationship with them. 'Specific ethical issues' mainly refer to biosafety and biosecurity, but justice and intellectual property issues are also discussed. Governance and public perception are mentioned as relevant to the ethical dimension of Synthetic Biology, but are treated separately from the ethical aspects.

Another distinction made is between physical and non-physical harms (Parens, Johnston, & Moses, 2009). Physical harms are "those that might be done to the health of persons or the environment if a synthesized molecule or organism mutated or escaped and contaminated someone or something outside of the controlled research setting" (p. 15). According to the authors, ethical considerations on physical harms should include discussions about responsible research, freedom of research and governance, since they are aimed at protecting us from these harms. Non-physical harms, on the other hand, could include surpassing possible moral limits in the creation of life, to contravene the

appropriate relationship that humans ought to have with nature, or to promote injustices and inequalities.

Other publications on this topic reserve the category "ethics" to group some of these issues, while the rest are treated separately. Thus, Schmidt et al. (2008) include the creation of life from scratch, the concept of life, justice and transhumanism as ethical issues, while biosafety, biosecurity, intellectual property rights, regulation and governance, public perception and communication are each treated as independent categories. Similarly, Bhutkar (2005) distinguishes patentability and regulation from ethics, a category in which the author includes the distinction between engineered machines and living organisms, the moral value of synthetic products, the concept of life and the responsibilities of researchers in this area.

In contrast, Kaebnick (2010) identifies biosafety and biosecurity concerns with ethics: "the most significant moral problems associated with synthetic biology have to do with its potential outcomes. [...] accidents and deliberate misuse also pose undeniable risks" (p. 49). Similarly, Douglas & Savulescu (2010) advocate that "the most important issue for ethicists to examine is the risk that knowledge from synthetic biology will be misused, for example, in biological terrorism or warfare" (p. 687), a concern that falls in the category of biosecurity issues; and Anderson et al. (2012) argue that "the ethical issues that most warrant consideration relate to the possible risks of releasing synthetic entities into the environment" (p. 588), which is related with biosafety.

Deplazes (2009) also addresses biosafety and biosecurity issues independently. Interestingly, this author suggests that the ethical evaluation of Synthetic Biology may be enriched if the relevant differences between its various branches are taken into account, and identifies different societal impacts with the different approaches existing within this discipline (Table 10). In relation to this, it has been argued that "it would be wise for

synthetic biologists, ethicists and policymakers to be familiar with ethical issues in synthetic biology throughout its subfields and abstraction hierarchies, and how different areas of research may interrelate" (Heavey, 2015, p. 126).

Table 10.

Ethical issues associated with different approaches to Synthetic Biology. *Modified from Deplazes (2009).*

Approach*	Notable societal impact
BIOENGINEERING	Biosafety: interaction with environment Ethics: turning organisms into machines
SYNTHETIC GENOMICS	Biosecurity: synthesis of pathogens
PROTOCELL SYNTHETIC BIOLOGY	Ethics: *in vitro* synthesis of life
UNNATURAL MOLECULAR BIOLOGY	Ethics: *in vitro* synthesis of life Biosafety: resistance to viruses
IN SILICO SYNTHETIC BIOLOGY	Only as applied to other approaches

**According to Deplazes (2009), all Synthetic Biology approaches pose biosafety and biosecurity issues depending on applications, as well as ethical issues "related to the impact on society and related to dealing with life".*

1.4.1 Different approaches to the bioethical analysis of Synthetic Biology

Several authors have approached the bioethical study of Synthetic Biology, but only a few examples have been found in which a rationally developed philosophical theory is used as a basis for the ethical study, or in which at least a set of principles is used as a reference to make ethical assessments and derive recommendations.

The report of the EGE (Capurro et al., 2009) points out, in the first place, the relevance of the international framework on ethics and human rights, formed by the legally binding Oviedo Convention (Council of Europe, 1997) and other non-legally binding documents, such as the Universal Declaration on the Human Genome and Human

Rights (UNESCO, 1997), the Universal Declaration on Bioethics and Human Rights (UNESCO, 2006), the Declaration of Helsinki (World Medical Association [WMA], 1964), and the European Charter of Fundamental Rights (European Union, 2012). Secondly, the report works on the basis of certain principles: the respect for human dignity, as a fundamental principle; the principle of safety, which requires protecting human health and the environment from harms caused by research or applications; the principle of sustainability; the principle of justice; the principle of precaution, of particular relevance for addressing biosafety issues; the principle of freedom of research, which is subject to the principle of safety; and the principle of proportionality, which requires that the goals pursued are important and that there are no other less risky alternatives to achieve them.

Moreover, the Presidential Commission for the Study of Bioethical Issues (PCSBI) carried out a study requested by then president of the United States Barack Obama in 2010, in which the implications of Synthetic Biology were examined and several recommendations were proposed (PCSBI, 2010). Although the use of an ethical framework was considered crucial to carry out the study, no suitable example was found. The Commission therefore identified five ethical principles as "relevant to considering the social implications of synthetic biology" (p. 24): public beneficence, responsible stewardship, intellectual freedom and responsibility, democratic deliberation and justice and fairness. They "are intended to illuminate and guide public policy choices to ensure that new technologies, including synthetic biology, can be developed in an ethically responsible manner" (p. 4). Thus, the PCSBI uses these principles in order to derive several recommendations to guide the development of Synthetic Biology.

Heavey (2013) presents two possible positions to address the ethical assessment of Synthetic Biology: consequentialism, which in general terms assesses the balance of good

and bad consequences that will be derived from this technology, and deontologism, which, instead of looking at the consequences, aims to determine if the technology is good in itself or not. Given that Synthetic Biology seems to have great potential for both good and evil, in this paper, the author opts for the deontological evaluation of this discipline. As he states, his aim is to "examine the ethics of synthetic biology from some broadly mainstream deontological perspectives, evaluating how synbio relates to the integrity of nature, the dignity of life, and the relationship of God and his creation" (Heavey, 2013, p. 442). Having answered these three questions, he concludes that Synthetic Biology is, from a deontological point of view, ethically acceptable. Accordingly, regarding the integrity of nature, the author argues that what Synthetic Biology does "is simply a significant technological advance on techniques which have been used for millennia. [...] With proper care, synthetic biology may yield great benefits without damaging nature's integrity" (p. 444). In relation to the impact of this discipline on the dignity of life, Heavey (2013) considers that Synthetic Biology "may lead to some negative attitudes; it could also be applied in ways that are injurious to life; but that is not to say that synbio per se challenges the dignity of life" (p. 445). Regarding the third question, the author reviews the available literature and concludes that "for a significant part of mainstream religious thought, synthetic biology does not appear to be, in itself, a usurpation of God's creative role" (p. 450).

However, the author points out that this conclusion that Synthetic Biology is good in itself is not of much practical use, since it does not provide criteria to guide its development. On the other hand, consequentialism, which does not allow us to determine if this discipline is good or bad, does provide criteria to cautiously steer the advance of Synthetic Biology. In this way, the two ethical approaches complement each other. In the words of Heavey (2013):

> "Neither approach provides adequate ethical guidance. However, combining the two approaches suggests that synbio per se is ethical, and it is good to proceed with it, albeit with stringent safeguards and precautions. Consequentialism's evaluation of potential benefits and dangers provides a useful road map for research directions and governance. The deontological and consequentialist approaches complement each other in this case, and their combination seems essential to obtain an adequate ethical analysis" (p. 452).

In a subsequent publication, the author builds on this idea, focusing on the ethical implications of Synthetic Biology from a consequentialist point of view. Thus, the potential effects of this discipline in different areas, such as agriculture or medicine, are analyzed, as well as biosafety and biosecurity issues arising in this field. The author reaffirms the conclusion that consequentialism is incapable of clarifying the moral nature of Synthetic Biology, although determining the consequences that could result from this technology is useful to establish measures that properly guide its development:

> "a consequentialist analysis is invaluable in determining the immediate potential benefits and dangers of synbio and in giving guidance to ethicists and policymakers as to how to respond in the short term. However, paradoxically, it is of no value in determining whether synbio is ultimately ethical and whether humanity should take this step. Consequentialism fails in a scenario such as this, where consequences cannot be predicted in any meaningful way beyond the short term" (Heavey, 2017, p. 221-222).

The author indicates this conclusion as a sign of the theoretical invalidity of this ethical framework:

> "If a topic as important as synbio cannot be dealt with meaningfully by consequentialism, then the usefulness, and indeed the validity, of the theory comes into question. If consequentialism fails in this important and testing scenario, then it must be questioned whether it is valid in any scenario. It appears to be flawed at its conceptual roots. [...] this analysis suggests that consequentialism is not a fully correct description of the "moral universe," although it may offer useful approximate guidance in some cases" (p. 222).

The ethics of Synthetic Biology have also been approached from a utilitarian perspective (Smith, 2013). Similar to the previous work, this author observes that an analysis of the outcomes is complicated, since they are very difficult to foresee. Therefore, and based on the potential benefits that are expected from this technology, the author proposes that the ethically correct position with regard to the development of this discipline must be a *laissez-faire* stance. He points out, however, that in those specific cases in which a realistic risk-benefit balance can be made, the ethical duty of not continuing with that action could be determined.

Principlism has also been confronted with the ethical questions posed by Synthetic Biology (Yearley, 2009). The author of this paper argues that principlism, which is the theoretical model that prevails in US biomedicine practice, does not provide the elements needed to carry out the ethical assessment of Synthetic Biology. He argues that the four principles of principlism (beneficence, non-maleficence, autonomy and justice) are not enough to address political and sociological concerns. "They are worries about whom to believe and how to check the power of the mighty and secretive. Precisely because the outlook of principlism is non-political, it fails to attend to this dimension of people's concerns" (p. 564). Moreover, the author argues that these principles are hardly applicable to such an innovative area, whose regulatory demands are very different from those of

biomedicine: “in the case of the environmental release of synthetic organisms the main public concern is not the ethics of the matter but the uncertainty and unpredictability of environmental impacts” (p. 564). The author concludes that an ethical review of Synthetic Biology must integrate social and ethical reflection, rather than only focus on ethics, and that “that review should be conducted in broader terms than those offered by the comfortable language of principlism” (p. 564).

1.5 PERSONALISM AS AN ETHICAL FRAMEWORK

The approach chosen to carry out this thesis is personalism, specifically ontological personalism, a philosophy that shows the objective value of the person on the basis of its ontological structure. The person, as a being endowed with reason, freedom and awareness, has a special value which is above that of other beings. Ontological personalism “emphasizes that there is an existence and an essence, a body-soul composite, at the foundation of subjectivity itself. [...] In man personhood consists in an individuality constituted by a body animated and structured by a spirit” (Sgreccia, 2012. p. 57).

Personalist philosophy can be defined as the philosophical current or currents originating in the 20th century that possess the following characteristics: 1) they are structurally constructed around a modern concept of person; 2) a modern concept of person means the anthropological perspective that thematizes or emphasizes all or part of these elements: the person as I and who, the affectivity and subjectivity, interpersonal and community character, corporality, tripartition of the person at a somatic, psychic and spiritual level, the person as male and female, primacy of love, freedom as self-determination, narrative character of human existence, transcendence as a relationship with a You, etc.; 3) some of the main philosophers of reference are the following:

Mounier, Maritain, Nédoncelle, Scheler, Von Hildebrand, Stein, Buber, Wojtyla, Guardini, Marcel, Marias and Zubiri (Burgos, 2012).

From this philosophy, specifically from ontological personalism, personalist bioethics is derived, which provides a set of principles to guide the ethical evaluation of various scientific facts in accordance with the safeguarding of human life and dignity (Sgreccia 2012). The ethical principles derived from this philosophy are always at the service of every human life: "From the moment of conception until death, in every situation of suffering or health, the human person is the reference point and standard for distinguishing licit from illicit" (Sgreccia, 2012, p.58). This ethical theory has been the starting point for addressing those bioethical issues that appear around the different branches of Synthetic Biology.

This approach has two remarkable strengths. In the first place, the fact of providing ethical principles is of great practical utility for carrying out ethical evaluations of different scientific facts. This feature is also present in the well-known principlist model of Beauchamp and Childress (Beauchamp & Childress, 2013). However, these principles lack an anthropological foundation that allows the establishment of a hierarchy of values and the avoidance of some unsolvable conflicts between principles. In this sense, the second strength of the personalist model is its anthropological foundation:

> "There is a tendency to develop ethics *without reference to man*, in a way that is disconnected from anthropology, from the idea of man as he is and how he would have to be if he were to realize his essence and attain his end—that is, ethics without an idea of the nature and end of man. [...] Contemporary ethics knows the speaking, dialoguing, communicating subject, but not the existing person with his or her individual substance, freedom, spirituality, and totality.

> It is necessary to combine ethics and anthropology and to recognize that ethics goes beyond the foundation of norms. Without a connection between the doctrine of man and the doctrine of norms, the latter seem unintelligible. No procedural practice can compensate for what lacks on the level of the real perception of the good and of man's nature" (Sgreccia, 2012. p. 174).

Recently a new current has been proposed within personalism, modern ontological personalism, which differs in some aspects from classical ontological personalism and could provide new anthropological bases to the bioethics of Sgreccia (Bermeo Anturi, 2019). Thus, regarding the concept of human life, Sgreccia welcomes the Aristotelian-Thomistic tradition, which uses the analogical method to describe this concept, so that it starts from general categories that are applied, by analogy, to different entities, among them the human being. The problem is that this can lead to include human life as a mere variant within the different existing lives. Modern ontological personalism, on the other hand, considers that "the understanding of human life should not be reduced to a biological dimension, nor to metaphysical concepts applicable to all entities, but should highlight human being's distinctive features, to avoid falling into the common elements between living beings" (p. 167). As a solution, it is proposed "to think of the human being from categories designed from him and for him, to highlight the irreducible and unique that he possesses, without denying the commonality of his corporeal structure" (p. 168). The same problem arises with the concept of human nature, against which modern ontological personalism proposes the concept of *humanity*, which "aims to refer to the way of being of human beings, to the ontological equality of all people, to the structural elements that each individual of the human species possesses" (p. 194). Finally, while classical ontological personalism does not include subjectivity as something objective and fundamental to the person, for modern ontological personalism "subjectivity, like the

ontological structure, is indispensable for the understanding of the person" (p. 201). Additionally, several dimensions of the person are defined: freedom and self-determination, affectivity and sexuated condition of the person.

Modern ontological personalism brings new elements that strengthen personalist philosophy. Even so, the bioethical principles proposed by Sgreccia (2012) are still valid to guide scientific practice based on respect for human life.

1.5.1 Personalist principles

The best-known principles of Sgreccia (2012) were developed for their application in ethical discussions regarding medical interventions on human life. These principles are: 1) the principle of defense of physical life (this principle is hierarchically superior to the rest); 2) the principle of freedom and responsibility; 3) the principle of totality or the therapeutic principle; and 4) the principle of sociality and subsidiarity. For the case of Genetic Engineering, Sgreccia (2012) proposes an adaptation of these principles, which results in the following five:

> 1- *Protecting the life and genetic identity of every human individual*: "Any intervention involving the destruction of the physical individuality of a human subject [...] constitutes an offense against the fundamental value of the human person because it deprives the human subject of the fundamental value on which all others rest: the value of bodily life. [...] Therefore the genetic inheritance of the human individual should also be considered untouchable except in the case of the therapeutic principle" (Sgreccia, 2012. p. 321).
>
> 2- *The therapeutic principle*: "It is licit to carry out even an invasive procedure for the benefit of the living subject in order to correct a defect or

eliminate an otherwise incurable condition. As with every therapy, gene therapy has its foundation and justification here" (Sgreccia, 2012. p. 322).

3- *Protecting the ecosystem and the environment*: "The justification of this principle is twofold: first of all because the environment, which is made up of a set of individual ecosystems that constitute the global ecosystem, is necessary for the life and health of man; second, in the creational understanding of the universe, the created world is indeed ordered to the good of man, who is its center and steward, yet serving man's welfare is not its only reason for being: it is still a good that has its reason for being in God. [...] the existence of other living beings is not exhausted in being an instrument" (Sgreccia, 2012. p. 322).

4- *The ontological and axiological difference between man and other living beings*: "While recognizing the bond of intimate and vital exchange between living beings and man, it is nonetheless impossible to overlook the real and profound difference in man by virtue of his capacity for reflective knowledge, freedom, and responsibility- in short, his being endowed with a spirit. [...] This fact prevents the use of the same criterion for interventions on man and on other living beings, such as the criterion of *feeling pain*" (Sgreccia, 2012. p. 322).

5- *The competence of the community*: "The search for solutions to the problem of interventions on the genetic patrimony of human beings and other living beings as well cannot be entrusted only to certain experts, whether scientists or politicians: it is a question that in certain ways regards humanity as a whole. The future of humanity often demands the responsible participation of the community. This is why the principle of freedom of science and research

should be recognized but also combined with the fact that populations need information and share in responsibility" (Sgreccia, 2012. p. 323).

These principles are also applicable to the case of Synthetic Biology (as well as to other fields of biotechnology), given the similarities between this discipline and Genetic Engineering, regarding both the scientific fact and the anthropological implications. They have therefore been used in the ethical assessment of the various issues raised in this field.

2 HYPOTHESIS AND OBJECTIVES

2.1 SUMMARY OF THE BACKGROUND

Synthetic Biology is a scientific area that combines biology and engineering to build new biological systems that could provide solutions to a wide range of social needs. The activities carried out in this field are very diverse, and thus several branches can be differentiated within Synthetic Biology, such as bioengineering, synthetic genomics, protocells and xenobiology (Deplazes, 2009; SCENIHR, SCCS, SCHER, 2014; Schmidt, 2009). Among the most important milestones reached in this discipline are the synthesis of the 1079-kbp *M. mycoides* genome, as well as its self-replication when introducing it in a *M. capricolum* recipient cell (Gibson et al., 2010); the production of artemisinic acid, the anti-malarial drug precursor, in both bacteria and yeast (Anthony et al., 2009; Ro et al., 2006; Tsuruta et al., 2009; Westfall et al., 2012); the production of an organism with the smallest genome of any known cell life form (Hutchison et al., 2016); the construction of the first synthetic eukaryotic chromosome (Annaluru et al., 2014); and the construction of a semi-synthetic strain of *E. coli* which harbors an unnatural base pair and is capable of transcribing it and incorporating NCAAs into a protein (Zhang et al., 2017).

Progress in this discipline promises numerous and important applications in different fields (Khalil & Collins, 2010), such as medicine, biosensing, biofuel production, and bioremediation. Nevertheless, Synthetic Biology also raises several ethical questions that must be addressed in order to ensure that biotechnological progress is oriented towards authentic human development. Although some authors have approached the bioethical study of Synthetic Biology, there is a paucity of literature in this regard. In addition, there appears to be a generalized absence of a basic philosophical framework that serves to develop ethical arguments.

2.2 HYPOTHESIS

A detailed study of the current state of Synthetic Biology from a personalist perspective will highlight the main bioethical issues that could be a threat for its genuine development, respectful of human life and dignity, and provide solutions for it to become a reality.

2.3 GENERAL AND SPECIFIC OBJECTIVES

General objective:

- The main objective of this work is to assess the bioethical issues raised by Synthetic Biology from a specific bioethical approach, personalism.

Specific objectives:

- To review the development and current status of Synthetic Biology.
- To identify the ethical issues arising from this discipline.
- To elaborate on these issues, delimiting the different implications of the various branches of Synthetic Biology.
- To apply the principles of personalist bioethics to derive answers to the different questions.
- To propose actions to follow that are consistent with personalist values.

3 MATERIALS AND METHODS / METHODOLOGICAL ASPECTS

This study was carried out following a working plan consisting of three main phases:

- Contextualization. Explanation of what Synthetic Biology is: definition, history, applications, etc.

- Identification and definition of the ethical issues raised by this discipline.

- Approach of bioethical issues from an ontological personalist framework.

First, a literature search was carried out to define the state of the question, covering the development of this discipline, the main advances achieved and the applications in this field, and to identify the ethical concerns that have been associated with Synthetic Biology. Second, the bioethical principles derived from ontological personalism (Sgreccia, 2012) were applied to give an answer from this ethical framework to the different issues raised. For a better understanding of personalism I have used the book by Burgos (2012), '*Introducción al personalismo*'.

3.1 LITERATURE SEARCH METHODOLOGY

Information regarding the origins, applications, risks and challenges of Synthetic Biology and bioethical literature on this field was collected with the aim of forming a consistent basis on which to develop a relevant ethical argument.

Searches were carried out in PubMed. The main searches performed are detailed below. The date shown is the last time that each search profile was reviewed. For each search, the titles and abstracts of the papers obtained were reviewed, and articles that did

not serve the purpose of the search or were written in a language other than English were discarded. The full text of the remaining papers was then reviewed, discarding those that did not contain useful information or were redundant.

- **("synthetic biology"[Title]) AND (history OR origin OR emergence OR development OR advances OR definition) / 8 June 2018**

The objective of this search was to gather information regarding the definition and characteristics of Synthetic Biology, the origin of this discipline and its development. The search produced 473 items, from which 11 articles were finally selected and served as a basis to construct the first sections of the introduction:

1. Decoene T, De Paepe B, Maertens J, Coussement P, Peters G, De Maeseneire SL, De Mey M. Standardization in synthetic biology: an engineering discipline coming of age. Crit Rev Biotechnol. 2018 Aug;38(5):647-656. doi: 10.1080/07388551.2017.1380600. Epub 2017 Sep 27. PubMed PMID: 28954542.
2. Flores Bueso Y, Tangney M. Synthetic Biology in the Driving Seat of the Bioeconomy. Trends Biotechnol. 2017 May;35(5):373-378. doi: 10.1016/j.tibtech.2017.02.002. Epub 2017 Feb 27. PubMed PMID: 28249675.
3. Shapira P, Kwon S, Youtie J. Tracking the emergence of synthetic biology. Scientometrics. 2017;112(3):1439-1469. doi: 10.1007/s11192-017-2452-5. Epub 2017 Jul 1. PubMed PMID: 28804177; PubMed Central PMCID: PMC5533824.
4. Peretó J. Erasing Borders: A Brief Chronicle of Early Synthetic Biology. J Mol Evol. 2016 Dec;83(5-6):176-183. Epub 2016 Nov 30. Review. PubMed PMID: 27900404.
5. Clarke LJ, Kitney RI. Synthetic biology in the UK - An outline of plans and progress. Synth Syst Biotechnol. 2016 Oct 17;1(4):243-257. doi: 10.1016/j.synbio.2016.09.003. eCollection 2016 Dec. Review. PubMed PMID: 29062950; PubMed Central PMCID: PMC5625736.
6. Raimbault B, Cointet JP, Joly PB. Mapping the Emergence of Synthetic Biology. PLoS One. 2016 Sep 9;11(9):e0161522. doi: 10.1371/journal.pone.0161522. eCollection 2016. PubMed PMID: 27611324; PubMed Central PMCID: PMC5017775.

7. Si T, Zhao H. A brief overview of synthetic biology research programs and roadmap studies in the United States. Synth Syst Biotechnol. 2016 Sep 4;1(4):258-264. doi: 10.1016/j.synbio.2016.08.003. eCollection 2016 Dec. Review. PubMed PMID: 29062951; PubMed Central PMCID: PMC5625737.
8. Sleator RD. Synthetic biology: from mainstream to counterculture. Arch Microbiol. 2016 Sep;198(7):711-3. doi: 10.1007/s00203-016-1257-x. Epub 2016 Jun 17. Review. PubMed PMID: 27316777.
9. Cameron DE, Bashor CJ, Collins JJ. A brief history of synthetic biology. Nat Rev Microbiol. 2014 May;12(5):381-90. doi: 10.1038/nrmicro3239. Epub 2014 Apr 1. Review. PubMed PMID: 24686414.
10. Andrianantoandro E, Basu S, Karig DK, Weiss R. Synthetic biology: new engineering rules for an emerging discipline. Mol Syst Biol. 2006;2:2006.0028. Epub 2006 May 16. PubMed PMID: 16738572; PubMed Central PMCID: PMC1681505.
11. Benner SA, Sismour AM. Synthetic biology. Nat Rev Genet. 2005 Jul;6(7):533-43. Review. PubMed PMID: 15995697.

❖ **"essential genes" AND "synthetic biology" / 2 October 2018**

This search was carried out to complete subsection 1.2.2 Synthetic genomics. 76 items were obtained, from which 5 articles were selected to complete this section:

1. Peng C, Lin Y, Luo H, Gao F. A Comprehensive Overview of Online Resources to Identify and Predict Bacterial Essential Genes. Front Microbiol. 2017 Nov 27;8:2331. doi: 10.3389/fmicb.2017.02331. eCollection 2017. Review. PubMed PMID: 29230204; PubMed Central PMCID: PMC5711816.

2. Sung BH, Choe D, Kim SC, Cho BK. Construction of a minimal genome as a chassis for synthetic biology. Essays Biochem. 2016 Nov 30;60(4):337-346. Review. PubMed PMID: 27903821.

3. Hutchison CA 3rd, Chuang RY, Noskov VN, Assad-Garcia N, Deerinck TJ, Ellisman MH, Gill J, Kannan K, Karas BJ, Ma L, Pelletier JF, Qi ZQ, Richter RA, Strychalski EA, Sun L, Suzuki Y, Tsvetanova B, Wise KS, Smith HO, Glass JI, Merryman C, Gibson DG, Venter JC. Design and synthesis of a minimal bacterial genome. Science. 2016 Mar 25;351(6280):aad6253. doi:

10.1126/science.aad6253. Erratum in: ACS Chem Biol. 2016 May 20;11(5):1463. PubMed PMID: 27013737.

4. Choe D, Cho S, Kim SC, Cho BK. Minimal genome: Worthwhile or worthless efforts toward being smaller? Biotechnol J. 2016 Feb;11(2):199-211. doi: 10.1002/biot.201400838. Epub 2015 Sep 10. Review. PubMed PMID: 26356135.

5. Juhas M, Eberl L, Church GM. Essential genes as antimicrobial targets and cornerstones of synthetic biology. Trends Biotechnol. 2012 Nov;30(11):601-7. doi: 10.1016/j.tibtech.2012.08.002. Epub 2012 Aug 30. Review. PubMed PMID: 22951051.

❖ **"human genome" AND write project / 24 October 2018**

This search was carried out to complete subsection 1.2.2 Synthetic genomics. 32 items were obtained, from which 2 articles were selected to complete this section:

1. Servick K. Genome writing project confronts technology hurdles. Science. 2017 May 19;356(6339):673-674. doi: 10.1126/science.356.6339.673. PubMed PMID: 28522476.
2. Boeke JD, Church G, Hessel A, Kelley NJ, Arkin A, Cai Y, Carlson R, Chakravarti A, Cornish VW, Holt L, Isaacs FJ, Kuiken T, Lajoie M, Lessor T, Lunshof J, Maurano MT, Mitchell LA, Rine J, Rosser S, Sanjana NE, Silver PA, Valle D, Wang H, Way JC, Yang L. GENOME ENGINEERING. The Genome Project-Write. Science. 2016 Jul 8;353(6295):126-7. doi: 10.1126/science.aaf6850. Epub 2016 Jun 2. PubMed PMID: 27256881.

❖ **xenobiology / 31 October 2018**

This search was carried out to complete subsection 1.2.4 Unnatural molecular biology. 21 items were obtained, from which 4 articles were selected to complete this section, as well as to enrich the section on Biosafety:

1. Hamashima K, Kimoto M, Hirao I. Creation of unnatural base pairs for genetic alphabet expansion toward synthetic xenobiology. Curr Opin Chem Biol. 2018 Oct;46:108-114. doi: 10.1016/j.cbpa.2018.07.017. Epub 2018 Jul 27. Review. PubMed PMID: 30059833.
2. Whitford CM, Dymek S, Kerkhoff D, März C, Schmidt O, Edich M, Droste J, Pucker B, Rückert C, Kalinowski J. Auxotrophy to Xeno-DNA: an exploration of

combinatorial mechanisms for a high-fidelity biosafety system for synthetic biology applications. J Biol Eng. 2018 Aug 14;12:13. doi: 10.1186/s13036-018-0105-8. eCollection 2018. Review. PubMed PMID: 30123321; PubMed Central PMCID: PMC6090650.

3. Schmidt M, Pei L, Budisa N. Xenobiology: State-of-the-Art, Ethics, and Philosophy of New-to-Nature Organisms. Adv Biochem Eng Biotechnol. 2018;162:301-315. doi: 10.1007/10_2016_14. Review. PubMed PMID: 28567486.
4. Schmidt M. Xenobiology: a new form of life as the ultimate biosafety tool. Bioessays. 2010 Apr;32(4):322-31. doi: 10.1002/bies.200900147. Review. PubMed PMID: 20217844; PubMed Central PMCID: PMC2909387.

❖ **("synthetic biology"[Title]) AND application*[Title] / 20 September 2018**

The objective of this search was to gather information regarding the different applications of Synthetic Biology. 98 items were obtained, from which 10 articles were selected as a basis to construct the section on "Applications of Synthetic Biology" (1.3):

1. Chen B, Lee HL, Heng YC, Chua N, Teo WS, Choi WJ, Leong SSJ, Foo JL, Chang MW. Synthetic biology toolkits and applications in Saccharomyces cerevisiae. Biotechnol Adv. 2018 Nov 15;36(7):1870-1881. doi: 10.1016/j.biotechadv.2018.07.005. Epub 2018 Jul 18. Review. PubMed PMID: 30031049.

From this review, four additional references were recovered:

- Reider Apel, A., d'Espaux, L., Wehrs, M., Sachs, D., Li, R.A., Tong, G.J., Garber, M., Nnadi, O., Zhuang, W., Hillson, N.J., Keasling, J.D., Mukhopadhyay, A., 2017. A Cas9-based toolkit to program gene expression in Saccharomyces cerevisiae. Nucleic Acids Research 45(1), 496-508.
- Ito-Harashima, S., Mizutani, Y., Nishimura, M., Kim, H.J., Kim, Y.J., Kim, H.S., Bae, J.H., Koedrith, P., Kawanishi, M., Seo, Y.R., Yagi, T., 2017. A pilot study for construction of a new cadmium-sensing yeast strain

carrying a reporter plasmid with the JLP1 promoter. Journal of Toxicological Sciences 42(1), 103109.

- Weaver, A.A., Halweg, S., Joyce, M., Lieberman, M., Goodson, H.V., 2015. Incorporating yeast biosensors into paper-based analytical tools for pharmaceutical analysis. Analytical and Bioanalytical Chemistry 407(2), 615-619.
- Geva, P., Kahta, R., Nakonechny, F., Aronov, S., Nisnevitch, M., 2016. Increased copper bioremediation ability of new transgenic and adapted Saccharomyces cerevisiae strains. Environmental Science and Pollution Research 23(19), 19613-19625.

2. Lemire S, Yehl KM, Lu TK. Phage-Based Applications in Synthetic Biology. Annu Rev Virol. 2018 Jul 12. doi: 10.1146/annurev-virology-092917-043544. [Epub ahead of print] PubMed PMID: 30001182.
3. Walker RSK, Pretorius IS. Applications of Yeast Synthetic Biology Geared towards the Production of Biopharmaceuticals. Genes (Basel). 2018 Jul 6;9(7). pii: E340. doi: 10.3390/genes9070340. Review. PubMed PMID: 29986380; PubMed Central PMCID: PMC6070867.
4. Bober JR, Beisel CL, Nair NU. Synthetic Biology Approaches to Engineer Probiotics and Members of the Human Microbiota for Biomedical Applications. Annu Rev Biomed Eng. 2018 Jun 4;20:277-300. doi: 10.1146/annurev-bioeng-062117-121019. Epub 2018 Mar 12. PubMed PMID: 29528686; PubMed Central PMCID: PMC6100750.
5. De Lorenzo V, Prather KL, Chen GQ, O'Day E, von Kameke C, Oyarzún DA, Hosta-Rigau L, Alsafar H, Cao C, Ji W, Okano H, Roberts RJ, Ronaghi M, Yeung K, Zhang F, Lee SY. The power of synthetic biology for bioproduction, remediation and pollution control: The UN's Sustainable Development Goals will inevitably require the application of molecular biology and biotechnology on a global scale. EMBO Rep. 2018 Apr;19(4). pii: e45658. doi: 10.15252/embr.201745658. Epub 2018 Mar 26. PubMed PMID: 29581172; PubMed Central PMCID: PMC5891403.
6. Kis Z, Pereira HS, Homma T, Pedrigi RM, Krams R. Mammalian synthetic biology: emerging medical applications. J R Soc Interface. 2015 May 6;12(106). pii: 20141000. doi: 10.1098/rsif.2014.1000. Review. PubMed PMID: 25808341; PubMed Central PMCID: PMC4424663.

7. Abil Z, Xiong X, Zhao H. Synthetic biology for therapeutic applications. Mol Pharm. 2015 Feb 2;12(2):322-31. doi: 10.1021/mp500392q. Epub 2014 Aug 13. Review. PubMed PMID: 25098838; PubMed Central PMCID: PMC4319687.
8. Folcher M, Fussenegger M. Synthetic biology advancing clinical applications. Curr Opin Chem Biol. 2012 Aug;16(3-4):345-54. doi: 10.1016/j.cbpa.2012.06.008. Epub 2012 Jul 21. Review. PubMed PMID: 22819494.
9. Weber W, Fussenegger M. Emerging biomedical applications of synthetic biology. Nat Rev Genet. 2011 Nov 29;13(1):21-35. doi: 10.1038/nrg3094. Review. PubMed PMID: 22124480.
10. Khalil AS, Collins JJ. Synthetic biology: applications come of age. Nat Rev Genet. 2010 May;11(5):367-79. doi: 10.1038/nrg2775. Review. PubMed PMID: 20395970; PubMed Central PMCID: PMC2896386.

- **("synthetic biology"[Title/Abstract]) AND (bioremediation OR remediation[Title/Abstract]) / 28 September 2018**

The objective of this search was to gather information regarding the application of Synthetic Biology for bioremediation, as little had been found with the previous search. 76 items were obtained, from which 9 articles were selected to complete this sub-section on "Applications of Synthetic Biology" (1.3.4):

1. Tang Q, Lu T, Liu SJ. Developing a Synthetic Biology Toolkit for Comamonas testosteroni, an Emerging Cellular Chassis for Bioremediation. ACS Synth Biol. 2018 Jul 20;7(7):1753-1762. doi: 10.1021/acssynbio.7b00430. Epub 2018 Jun 12. PubMed PMID: 29860823.
2. Gong T, Xu X, Dang Y, Kong A, Wu Y, Liang P, Wang S, Yu H, Xu P, Yang C. An engineered Pseudomonas putida can simultaneously degrade organophosphates, pyrethroids and carbamates. Sci Total Environ. 2018 Jul 1;628-629:1258-1265. doi: 10.1016/j.scitotenv.2018.02.143. Epub 2018 Feb 20. PubMed PMID: 30045547.
3. Marques CR. Extremophilic Microfactories: Applications in Metal and Radionuclide Bioremediation. Front Microbiol. 2018 Jun 1;9:1191. doi:

10.3389/fmicb.2018.01191. eCollection 2018. Review. PubMed PMID: 29910794; PubMed Central PMCID: PMC5992296.

4. De Lorenzo V, Prather KL, Chen GQ, O'Day E, von Kameke C, Oyarzún DA, Hosta Rigau L, Alsafar H, Cao C, Ji W, Okano H, Roberts RJ, Ronaghi M, Yeung K, Zhang F, Lee SY. The power of synthetic biology for bioproduction, remediation and pollution control: The UN's Sustainable Development Goals will inevitably require the application of molecular biology and biotechnology on a global scale. EMBO Rep. 2018 Apr;19(4). pii: e45658. doi: 10.15252/embr.201745658. Epub 2018 Mar 26. PubMed PMID: 29581172; PubMed Central PMCID: PMC5891403.
5. Dvořák P, Nikel PI, Damborský J, de Lorenzo V. Bioremediation 3.0: Engineering pollutant-removing bacteria in the times of systemic biology. Biotechnol Adv. 2017 Nov 15;35(7):845-866. doi: 10.1016/j.biotechadv.2017.08.001. Epub 2017 Aug 5. Review. PubMed PMID: 28789939.
6. Tay PKR, Nguyen PQ, Joshi NS. A Synthetic Circuit for Mercury Bioremediation Using Self-Assembling Functional Amyloids. ACS Synth Biol. 2017 Oct 20;6(10):1841-1850. doi: 10.1021/acssynbio.7b00137. Epub 2017 Aug 2. PubMed PMID: 28737385.
7. Rucká L, Nešvera J, Pátek M. Biodegradation of phenol and its derivatives by engineered bacteria: current knowledge and perspectives. World J Microbiol Biotechnol. 2017 Sep 6; 33(9):174. doi: 10.1007/s11274-017-2339-x. Review. PubMed PMID: 28879631.
8. Karig DK. Cell-free synthetic biology for environmental sensing and remediation. Curr Opin Biotechnol. 2017 Jun; 45:69-75. doi: 10.1016/j.copbio.2017.01.010. Epub 2017 Feb 20. Review. PubMed PMID: 28226291.
9. De Lorenzo V, Marlière P, Solé R. Bioremediation at a global scale: from the test tube to planet Earth. Microb Biotechnol. 2016 Sep;9(5):618-25. doi: 10.1111/1751-7915.12399. Epub 2016 Aug 4. Review. PubMed PMID: 27489146; PubMed Central PMCID: PMC4993180.

❖ **(“synthetic biology”[Title/Abstract]) AND ethic*[Title/Abstract] / 7 November 2018**

The objective of this search was to define in broad terms the current bioethical landscape with respect to Synthetic Biology. 107 items were obtained, from which 19 articles were selected to complete the section on the "Ethical aspects related to Synthetic Biology" as well as different subsections in "Results":

1. Heavey P. Consequentialism and the Synthetic Biology Problem. Camb Q Healthc Ethics. 2017 Apr;26(2):206-229. doi: 10.1017/S0963180116000815. PubMed PMID: 28361719.
2. Heavey P. Integrating ethical analysis "into the DNA" of synthetic biology. Med Health Care Philos. 2015 Feb;18(1):121-7. doi: 10.1007/s11019-014-9588-3. PubMed PMID: 25185871.
3. Smith K. Synthetic biology: a utilitarian perspective. Bioethics. 2013 Oct;27(8):453-63. doi: 10.1111/bioe.12050. PubMed PMID: 24010857.
4. Heavey P. Synthetic biology ethics: a deontological assessment. Bioethics. 2013 Oct;27(8):442-52. doi: 10.1111/bioe.12052. PubMed PMID: 24010856.
5. Boldt J. Do we have a moral obligation to synthesize organisms to increase biodiversity? On kinship, awe, and the value of life's diversity. Bioethics. 2013 Oct;27(8):411-8. doi: 10.1111/bioe.12051. PubMed PMID: 24010852.
6. Link HJ. Playing God and the intrinsic value of life: moral problems for synthetic biology? Sci Eng Ethics. 2013 Jun;19(2):435-48. doi: 10.1007/s11948-012-9353-z. Epub 2012 Mar 3. PubMed PMID: 22389208.
7. Deplazes-Zemp A. The conception of life in synthetic biology. Sci Eng Ethics. 2012 Dec;18(4):757-74. doi: 10.1007/s11948-011-9269-z. Epub 2011 Apr 12. PubMed PMID: 21484320.
8. Anderson J, Strelkowa N, Stan GB, Douglas T, Savulescu J, Barahona M, Papachristodoulou A. Engineering and ethical perspectives in synthetic biology. Rigorous, robust and predictable designs, public engagement and a modern ethical framework are vital to the continued success of synthetic biology. EMBO Rep. 2012 Jun 29;13(7):584-90. doi: 10.1038/embor.2012.81. PubMed PMID: 22699939; PubMed Central PMCID: PMC3389334.
9. Bubela T, Hagen G, Einsiedel E. Synthetic biology confronts publics and policy makers: challenges for communication, regulation and commercialization. Trends

Biotechnol. 2012 Mar;30(3):132-7. doi: 10.1016/j.tibtech.2011.10.003. Epub 2011 Nov 25. PubMed PMID: 22119159.

10. Newson AJ. Current ethical issues in synthetic biology: where should we go from here? Account Res. 2011 May;18(3):181-93. doi: 10.1080/08989621.2011.575035. Review. PubMed PMID: 21574073.
11. Douglas T, Savulescu J. Synthetic biology and the ethics of knowledge. J Med Ethics. 2010 Nov;36(11):687-93. doi: 10.1136/jme.2010.038232. Epub 2010 Oct 8. PubMed PMID: 20935316; PubMed Central PMCID: PMC3045879.
12. Kaebnick GE. Synthetic biology, analytic ethics. Hastings Cent Rep. 2010 Jul-Aug;40(4):49. PubMed PMID: 20669782.
13. Bedau MA, Parke EC, Tangen U, Hantsche-Tangen B. Social and ethical checkpoints for bottom-up synthetic biology, or protocells. Syst Synth Biol. 2009 Dec;3(1-4):65-75. doi: 10.1007/s11693-009-9039-2. Epub 2009 Oct 10. PubMed PMID: 19816801; PubMed Central PMCID: PMC2759431.
14. Deplazes A, Huppenbauer M. Synthetic organisms and living machines: Positioning the products of synthetic biology at the borderline between living and non-living matter. Syst Synth Biol. 2009 Dec;3(1-4):55-63. doi: 10.1007/s11693-009-9029-4. Epub 2009 Oct 10. PubMed PMID: 19816800; PubMed Central PMCID: PMC2759422.
15. Dabrock P. Playing God? Synthetic biology as a theological and ethical challenge. Syst Synth Biol. 2009 Dec;3(1-4):47-54. doi: 10.1007/s11693-009-9028-5. Epub 2009 Oct 10. PubMed PMID: 19816799; PubMed Central PMCID: PMC2759421.
16. Schmidt M, Ganguli-Mitra A, Torgersen H, Kelle A, Deplazes A, Biller-Andorno N. A priority paper for the societal and ethical aspects of synthetic biology. Syst Synth Biol. 2009 Dec;3(1-4):3-7. doi: 10.1007/s11693-009-9034-7. Epub 2009 Oct 10. PubMed PMID: 19816794; PubMed Central PMCID: PMC2759426.
17. Yearley S. The ethical landscape: identifying the right way to think about the ethical and societal aspects of synthetic biology research and products. J R Soc Interface. 2009 Aug 6;6 Suppl 4:S559-64. doi: 10.1098/rsif.2009.0055.focus. Epub 2009 May 15. Review. PubMed PMID: 19447816; PubMed Central PMCID: PMC2843963.
18. Schmidt M, Torgersen H, Ganguli-Mitra A, Kelle A, Deplazes A, Biller-Andorno N. SYNBIOSAFE e-conference: online community discussion on the societal

aspects of synthetic biology. Syst Synth Biol. 2008 Jun;2(1-2):7-17. doi: 10.1007/s11693-008-9019-y. Epub 2008 Sep 18. PubMed PMID: 19003430; PubMed Central PMCID: PMC2671589.

19. Bhutkar A. Synthetic biology: navigating the challenges ahead. J Biolaw Bus. 2005;8(2):19-29. PubMed PMID: 16538811.

❖ **"synthetic biology" AND biosafety / 28 June 2018**

The objective of this search was to gather information regarding biosafety issues related to Synthetic Biology. 54 items were obtained, from which 19 articles were selected to complete section 4.3 "Biosafety":

1. Lee JW, Chan CTY, Slomovic S, Collins JJ. Next-generation biocontainment systems for engineered organisms. Nat Chem Biol. 2018 May 16. doi: 10.1038/s41589-018-0056-x. [Epub ahead of print] Review. PubMed PMID: 29769737.
2. Heavey P. Consequentialism and the Synthetic Biology Problem. Camb Q Healthc Ethics. 2017 Apr;26(2):206-229. doi: 10.1017/S0963180116000815. PubMed PMID: 28361719.
3. Howard J, Murashov V, Schulte P. Synthetic biology and occupational risk. J Occup Environ Hyg. 2017 Mar;14(3):224-236. doi: 10.1080/15459624.2016.1237031. PubMed PMID: 27754800.
4. Torres L, Krüger A, Csibra E, Gianni E, Pinheiro VB. Synthetic biology approaches to biological containment: pre-emptively tackling potential risks. Essays Biochem. 2016 Nov 30;60(4):393-410. Review. PubMed PMID: 27903826; PubMed Central PMCID: PMC5264511.
5. Sleator RD. Synthetic biology: from mainstream to counterculture. Arch Microbiol. 2016 Sep;198(7):711-3. doi: 10.1007/s00203-016-1257-x. Epub 2016 Jun 17. Review. PubMed PMID: 27316777.
6. Schmidt M, de Lorenzo V. Synthetic bugs on the loose: containment options for deeply engineered (micro)organisms. Curr Opin Biotechnol. 2016 Apr;38:90-6. doi: 10.1016/j.copbio.2016.01.006. Epub 2016 Feb 10. Review. PubMed PMID: 26874261.

7. Chan CT, Lee JW, Cameron DE, Bashor CJ, Collins JJ. 'Deadman' and 'Passcode' microbial kill switches for bacterial containment. Nat Chem Biol. 2016 Feb;12(2):82-6. doi: 10.1038/nchembio.1979. Epub 2015 Dec 7. PubMed PMID: 26641934; PubMed Central PMCID: PMC4718764.
8. Wright O, Delmans M, Stan GB, Ellis T. GeneGuard: A modular plasmid system designed for biosafety. ACS Synth Biol. 2015 Mar 20;4(3):307-16. doi: 10.1021/sb500234s. Epub 2014 May 22. PubMed PMID: 24847673.
9. Mandell DJ, Lajoie MJ, Mee MT, Takeuchi R, Kuznetsov G, Norville JE, Gregg CJ, Stoddard BL, Church GM. Biocontainment of genetically modified organisms by synthetic protein design. Nature. 2015 Feb 5;518(7537):55-60. doi: 10.1038/nature14121. Epub 2015 Jan 21. Erratum in: Nature. 2015 Nov 12;527(7577):264. PubMed PMID: 25607366; PubMed Central PMCID: PMC4422498.
10. Rovner AJ, Haimovich AD, Katz SR, Li Z, Grome MW, Gassaway BM, Amiram M, Patel JR, Gallagher RR, Rinehart J, Isaacs FJ. Recoded organisms engineered to depend on synthetic amino acids. Nature. 2015 Feb 5;518(7537):89-93. doi: 10.1038/nature14095. Epub 2015 Jan 21. Erratum in: Nature. 2015 Nov 12;527(7577):264. PubMed PMID: 25607356; PubMed Central PMCID: PMC4590768.
11. Seyfried G, Pei L, Schmidt M. European do-it-yourself (DIY) biology: beyond the hope, hype and horror. Bioessays. 2014 Jun;36(6):548-51. doi: 10.1002/bies.201300149. Epub 2014 Apr 29. PubMed PMID: 24782329; PubMed Central PMCID: PMC4158858.
12. Committee on Science, Technology, and Law, Policy and Global Affairs, Board on Life Sciences, Division on Earth and Life Sciences, National Academy of Engineering, National Research Council. Positioning Synthetic Biology to Meet the Challenges of the 21st Century: Summary Report of a Six Academies Symposium Series. Washington (DC): National Academies Press (US); 2013 Aug 5. PubMed PMID: 24872969.
13. Wright O, Stan GB, Ellis T. Building-in biosafety for synthetic biology. Microbiology. 2013 Jul;159(Pt 7):1221-35. doi: 10.1099/mic.0.066308-0. Epub 2013 Mar 21. PubMed PMID: 23519158.
14. De Lorenzo V. Environmental biosafety in the age of synthetic biology: do we really need a radical new approach? Environmental fates of microorganisms

bearing synthetic genomes could be predicted from previous data on traditionally engineered bacteria for in situ bioremediation. Bioessays. 2010 Nov;32(11):926-31. doi: 10.1002/bies.201000099. Epub 2010 Oct 8. PubMed PMID: 20936643.

15. Schmidt M, Ganguli-Mitra A, Torgersen H, Kelle A, Deplazes A, Biller-Andorno N. A priority paper for the societal and ethical aspects of synthetic biology. Syst Synth Biol. 2009 Dec;3(1-4):3-7. doi: 10.1007/s11693-009-9034-7. Epub 2009 Oct 10. PubMed PMID: 19816794; PubMed Central PMCID: PMC2759426.
16. Schmidt M. Diffusion of synthetic biology: a challenge to biosafety. Syst Synth Biol. 2008 Jun;2(1-2):1-6. doi: 10.1007/s11693-008-9018-z. Epub 2008 Jul 9. PubMed PMID: 19003431; PubMed Central PMCID: PMC2671588.
17. Schmidt M, Torgersen H, Ganguli-Mitra A, Kelle A, Deplazes A, Biller-Andorno N. SYNBIOSAFE e-conference: online community discussion on the societal aspects of synthetic biology. Syst Synth Biol. 2008 Jun;2(1-2):7-17. doi: 10.1007/s11693-008-9019-y. Epub 2008 Sep 18. PubMed PMID: 19003430; PubMed Central PMCID: PMC2671589.
18. Race MS, Hammond E. An evaluation of the role and effectiveness of institutional biosafety committees in providing oversight and security at biocontainment laboratories. Biosecur Bioterror. 2008 Mar;6(1):19-35. doi: 10.1089/bsp.2007.0048. PubMed PMID: 18386970.
19. Tucker JB, Zilinskas RA. The promise and perils of synthetic biology. New Atlantis. 2006 Spring;12:25-45. PubMed PMID: 16832953.

❖ **"synthetic biology" AND biosecurity / 11 July 2018**

The objective of this search was to gather information regarding biosecurity issues related to Synthetic Biology. 36 items were obtained, from which 12 articles were selected to complete section 4.4 "Biosecurity":

1. mSphere. 2018 Mar 7;3(2). pii: e00079-18. doi: 10.1128/mSphere.00079-18. eCollection 2018 Mar-Apr. Re-creation of Horsepox Virus. Imperiale MJ.
2. mSphere. 2018 Mar 7;3(2). pii: e00040-18. doi: 10.1128/mSphere.00040-18. eCollection 2018 Mar-Apr. A Critical Analysis of the Scientific and Commercial Rationales for the De Novo Synthesis of Horsepox Virus. Koblentz GD.

3. Ahteensuu M. Synthetic Biology, Genome Editing, and the Risk of Bioterrorism. Sci Eng Ethics. 2017 Dec;23(6):1541-1561. doi: 10.1007/s11948-016-9868-9. Epub 2017 Jan 10. PubMed PMID: 28074376.
4. Koblentz GD. The De Novo Synthesis of Horsepox Virus: Implications for Biosecurity and Recommendations for Preventing the Reemergence of Smallpox. Health Secur. 2017 Nov/Dec;15(6):620-628. doi: 10.1089/hs.2017.0061. Epub 2017 Aug 24. PubMed PMID: 28836863.
5. DiEuliis D, Carter SR, Gronvall GK. Options for Synthetic DNA Order Screening, Revisited. mSphere. 2017 Aug 23;2(4). pii: e00319-17. doi: 10.1128/mSphere.00319-17. eCollection 2017 Jul-Aug. PubMed PMID: 28861521; PubMed Central PMCID: PMC5566836.
6. Evans NG, Selgelid MJ. Biosecurity and Open-Source Biology: The Promise and Peril of Distributed Synthetic Biological Technologies. Sci Eng Ethics. 2015 Aug;21(4):1065-83. doi: 10.1007/s11948-014-9591-3. Epub 2014 Sep 24. PubMed PMID: 25248872.
7. Jefferson C, Lentzos F, Marris C. Synthetic biology and biosecurity: challenging the "myths". Front Public Health. 2014 Aug 21;2:115. doi: 10.3389/fpubh.2014.00115. eCollection 2014. PubMed PMID: 25191649; PubMed Central PMCID: PMC4139924.
8. Edwards B. Taking stock of security concerns related to synthetic biology in an age of responsible innovation. Front Public Health. 2014 Jul 9;2:79. doi: 10.3389/fpubh.2014.00079. eCollection 2014. PubMed PMID: 25072048; PubMed Central PMCID: PMC4088943.
9. Colussi IA. Synthetic biology between challenges and risks: suggestions for a model of governance and a regulatory framework, based on fundamental rights. Rev Derecho Genoma Hum. 2013 Jan-Jun;(38):185-214. PubMed PMID: 24340832.
10. Sture J, Whitby S. Preventing the hostile use of the life sciences and biotechnologies; fostering a culture of biosecurity and dual use awareness. Conclusions. Med Confl Surviv. 2012 Jan-Mar;28(1):99-105. PubMed PMID: 22606764.
11. Kelle A. Ensuring the security of synthetic biology-towards a 5P governance strategy. Syst Synth Biol. 2009 Dec;3(1-4):85-90. doi: 10.1007/s11693-009-

9041-8. Epub 2009 Oct 10. PubMed PMID: 19816803; PubMed Central PMCID: PMC2759433.

12. Kelle A. Synthetic biology and biosecurity. From low levels of awareness to a comprehensive strategy. EMBO Rep. 2009 Aug;10 Suppl 1:S23-7. doi: 10.1038/embor.2009.119. Review. PubMed PMID: 19636299; PubMed Central PMCID: PMC2725994.

❖ **"synthetic biology" AND "intellectual property" / 8 January 2019**

The objective of this search was to gather information regarding intellectual property issues related to Synthetic Biology. 29 items were obtained, from which 3 articles were selected to complete section 4.6.3 "Intellectual property rights":

1. Minssen T, Rutz B, van Zimmeren E. Synthetic biology and intellectual property rights: six recommendations. Biotechnol J. 2015 Feb;10(2):236-41. doi: 10.1002/biot.201400604. Epub 2015 Feb 9. PubMed PMID: 25676051.

2. Van den Belt H. Synthetic biology, patenting, health and global justice. Syst Synth Biol. 2013 Sep;7(3):87-98. doi: 10.1007/s11693-012-9098-7. Epub 2012 Oct 30. PubMed PMID: 24432146; PubMed Central PMCID: PMC3740100.

3. Saukshmya T, Chugh A. Intellectual property rights in synthetic biology: an anti-thesis to open access to research? Syst Synth Biol. 2010 Dec;4(4):241-5. doi: 10.1007/s11693-011-9067-6. Epub 2011 Feb 20. PubMed PMID: 22132050; PubMed Central PMCID: PMC3065585.

3.2 BIOETHICAL ANALYSIS

Once the current state of Synthetic Biology was defined and information was collected on the ethical questions raised by this discipline, delimitations were established, so that the different issues were related to the different branches within Synthetic Biology,

allowing a deeper and more focused view of these, as recommended by some authors (Deplazes, 2009; Heavey, 2015). Thus, when different branches have different implications, for example the different biosecurity implications of bioengineering and xenobiology, these have been delimited.

The ethical evaluation of the different questions was then carried out by comparing the scientific facts with the principles derived from ontological personalism:

1- Protecting the life and genetic identity of every human individual

2- The therapeutic principle

3- Protecting the ecosystem and the environment

4- The ontological and axiological difference between man and other living beings

5- The competence of the community

From this critical analysis, different conclusions were drawn regarding the questions raised, as well as proposals that are in accordance with the personalist framework.

4 RESULTS AND DISCUSSION

From the literature review carried out on the ethics of Synthetic Biology, a list of thematical issues can be derived, which include:

1. Ethical legitimacy of manufacturing living organisms, creation of life from scratch, impact on the concept of life.
2. Distinction between engineered machines and living organisms, the (moral) value of synthetic products.
3. Biosafety risks.
4. Biosecurity risks.
5. Transhumanism.
6. Justice, fairness, equality.
7. Public perception and communication.
8. Intellectual property rights, patentability.
9. Regulation and governance.
10. Progress, responsibilities of researchers in this area.

Although the bioethical assessment of Synthetic Biology has been approached by some authors, few of them use a philosophical framework as the basis on which to develop their argument. Here, these issues have been approached from an ethical framework, ontological personalism, using the principles developed by Sgreccia (2012): 1) *protecting the life and genetic identity of every human individual*, 2) *the therapeutic principle*, 3) *protecting the ecosystem and the environment*, 4) *the ontological and axiological difference between man and other living beings*, and 5) *the competence of the community*. The objective of this approach is twofold: to carry out an evaluation of this scientific discipline from personalism, and to derive action proposals in accordance with this ethical framework.

It is worth highlighting some distinctions between these issues. As explained previously, different authors make different classifications. To illustrate, Capurro et al. (2009) distinguish between conceptual or specific issues, while Parens et al. (2009) differentiate physical from non-physical harms. Additionally, some issues can arguably be classified as primary issues, those that are inherent to the scientific activity in question (Synthetic Biology), while others, mainly issues 9 and 10, arise from the first ones, and for that reason are secondary. For example, questions regarding responsibilities of researchers in this area or the need for regulations arise insofar as Synthetic Biology poses safety risks or questions regarding the moral status of its products. Issues 6 and 7 are also secondary to some extent. For example, there is a need to distribute the security risks that appear, but there are also other primary issues such as the just distribution of benefits and non-exploitation of the underdeveloped world. Finally, some of these issues are more specific to the case of Synthetic Biology, although not necessarily exclusive of it, while others are concerns generally raised by new biotechnologies, with some specific characteristics for this field. Issues 1 to 5 are included among the former, and for this reason they are the focus of this work. Secondary issues 9 and 10 will not be treated independently, but together with the primary issues with which they are related.

In what follows, the different questions are discussed. The bioethical debate about each issue is presented and addressed from the personalist framework, applying the ethical principles developed by Sgreccia (2012) in order to obtain answers and recommendations that are consistent with this philosophy, and that can guide the development of this promising scientific discipline. In order to make a deeper and more practical analysis, the different branches of Synthetic Biology are considered separately when appropriate, as recommended by Deplazes (2009) and Heavey (2015).

4.1 CONCEPT OF LIFE AND ITS CREATION

This ethical issue is mainly associated with synthetic genomics and protocell Synthetic Biology, although all the approaches in Synthetic Biology have implications in this sense, as they pursue the common objective of obtaining new forms of life (Deplazes-Zemp, 2012).

4.1.1 Bioengineering

Deplazes-Zemp (2012) argues that, in Synthetic Biology:

> "life turns into a property of the product that is evaluated according to its efficiency, usefulness and suitability, with the possibility to be improved if necessary. It is not really the *given* property of living organisms anymore. This type of evaluation is not only applied to life as a whole but also to the individual features of living organisms. [...] for synthetic biologists, these features are starting points to designing new life forms, which could in turn provide us with more insight about life itself. Life is thus interesting as a property of living organisms and the source of potential useful applications" (p. 768).

According to the author, the ethical concerns that may arise from this conception of life are in regard to the appropriate treatment of synthetic organisms, the possible conformation of an arrogant and disrespectful attitude towards life, and the technological overexploitation of nature.

Similarly, from personalism, this "conception of life as a toolbox" can jeopardize the *principle of protecting the ecosystem and the environment* and the *principle of protecting the life and genetic identity of every human individual*. If organisms are viewed

as machines, they would be outside the respect prescribed by the *principle of protecting the ecosystem and the environment*. This concern will be addressed in greater depth in section 4.2 "Moral status of synthetic entities". Additionally, if this approach were to be applied in humans, the human genome could be used as a raw material on which to develop different improvements. In this regard, the *principle of protecting the life and genetic identity of every human individual* must be taken into account, which argues that the life of all human beings must be defended, and that genetic interventions should only be applied for therapeutic purposes (see section 4.5 Transhumanism).

4.1.2 Synthetic genomics

The chemical synthesis of a minimal genome has been said to be "the culmination of a reductionist research agenda about the meaning and origin of life" (Cho, Magnus, & Caplan, 1999, p. 2089). Reductionism aims to deduce the properties or concepts of a complex scientific domain from a simpler scientific domain (Brigandt & Love, 2017). One way to apply this approach to the understanding of the concept of life is to deduce the meaning of life, the properties shared by all that is alive, from genes.

Synthetic genomics, which aims to construct a minimal genome, raises concerns about the reductionist understanding of life. It supposes that if the traits of an organism are given by its genome, then the special feature of being alive is given by a specific set of genes. The rest of the genome provides additional features to the living organism. It should be noted that the set of minimum genes needed to live can vary between organisms and according to environmental conditions. In this regard, Cho et al. (1999) find two concerns when following this approach to understanding life. First, throughout history there have been cases where reductionist thinking has led to erroneous deductions, such as setting viruses as the phylogenetic precursors to cellular life, and, likewise, "by

devoting far greater effort to understanding the role of the nucleus in the functioning of the cell compared with other cellular elements, which have their own causal roles to play, we can bias our understanding of how cells operate" (p. 2089). Second, the deductions that can be derived from a reductionist study of life, particularly human life, may not fulfill those conceptions of life that include other dimensions besides the physical one, and could undermine the value that we attribute to living beings (Cho et al., 1999). Finally, the authors argue that:

> "[r]educing life to genes has profound implications for several critical societal debates, including what constitutes human life and when life begins. [...] If we extend the reductionism implicit in minimal genome research to a definition of human life, this has implications for the debate about whether stem cells, early embryos, or hybrid embryos combining human DNA with the cellular components of other species are human. Likewise, a genetic definition of when life begins would have implications for the abortion debate" (Cho et al., 1999, p. 2090).

Regarding the influence that defining the concept of life based on genes may have on the debate about when human life begins and about abortion, given that early embryos and fetuses have the same genetic makeup as adults, these concerns are not warranted from personalism. According to this view, human life begins at conception (which is supported by research in embryology [Pearson, 2002]), and every human being is a person:

> "the entire value of the individual human person is ontologically present from the moment of conception for two reasons: (a) *the connection between body and soul* is essential and not accidental –the body is the transcription, manifestation, and instrument of the person and not simply its garment or

> accessory– so the person is a corporeal person, an incarnate "I" and not just an entity that *has* a body, and (b) *personhood* in the human race is identical with the existential *act* that realizes the human nature which is made up of soul and body, psyche and soma. The existential act operates at the very same moment in which the new being is *actuated*" (Sgreccia, 2012. p. 442-443).

Therefore, if life came to be defined on the basis of essential genes, it would not affect personalist views on these topics, but rather would support them: life would still be seen to begin at conception and, in virtue of the *principle of protecting the life and genetic identity of every human individual*, abortion would be illicit as well.

However, even gametes could be considered living beings according to this criterion, which raises the ontological problem of determining how two beings, the gametes, become a single being, the zygote. This confirms that this way of understanding life is excessively reductionist, and that other characteristics must be taken into account. Problems could also arise regarding the other cases mentioned by Cho et al. (1999). Thus, if essential genes are the only necessary elements to define life, a human stem cell could be considered a human being. In fact, although Cho et al. (1999) only mention stem cells, all human cells would be human beings, since they would all have the essential genes. This is contrary to the personalist view of human life beginning at conception.

Additionally, hybrid embryos obtained by transferring the human nuclear genome to an enucleated animal cell would also be considered human beings according to the criterion of essential genes. In this regard, it has been proven that there must be a match between the genome and the other cellular components to give rise to a viable individual (Chung et al, 2009). However, these embryos do live some days, which raises doubts about the nature of these organisms. Therefore, from a personalist point of view, this

practice is unacceptable, since it could be violating human life (it would also be a type of cloning).

Beyond the personalist view of these questions, some more basic considerations can be made. It seems that from the purely biological point of view, the essential genes are not enough to define life, since other cellular elements are necessary; the genome alone is not considered alive. In fact, DNA can be recovered from dead organisms, even if they have long since died. Certainly, defining the concept of life, even if only physiologically, is complex, and a commonly-accepted exact definition has not yet been reached, although different sets of characteristics have been proposed by different authors, of which genetic information is only a part.

For example, Koshland Jr (2002) puts forward what he calls "The seven pillars of life": (1) program, which on Earth is the DNA; (2) improvisation, which is the capacity of organisms to change their program in order to adapt to their environment by a process of mutation plus selection; (3) compartmentalization or confinement of organisms to a limited volume by means of a membrane or skin; (4) energy, since organisms are metabolizing systems; (5) regeneration, in order to compensate the thermodynamic losses. Reproduction is one of these pillars; (6) adaptability, which is part of the program and involves those behavioral responses that allow survival in quickly changing environments; and (7) seclusion, which is mainly based on the specificity of enzymes for their substrates, thus avoiding interference between different metabolic pathways.

Another important contribution in this field is that of Ganti (2003), who proposes "the principles of life" as being necessary and sufficient for life: (1) units of life lose their properties if they are subdivided; (2) units of life perform metabolism; (3) units of life must be inherently stable in spite of environmental changes; (4) units of life contain an informational subsystem necessary for them to function; and (5) units of life have their

processes regulated and controlled. According to this author, growth, reproduction, the capacity for evolution and mortality are potential but not absolute life criteria, which means that all of them together are crucial for populating the planet but not strictly necessary for life.

Another well-known theory to describe life is the theory of autopoiesis. According to this model, organisms are "homeostatic systems that have their own organization as the critical fundamental variable that they actively maintain constant" (Maturana, 1975, p. 318). The concept of autopoiesis has been recently reformulated in order to provide a clearer and more precise definition, easier to apply in practice (Razeto-Barry, 2012). According to this new definition, a system can be deemed autopoietic only if it meets the following conditions:

> "1. It is a network of physical and chemical processes.
>
> 2. This network chemically produces a subset of the components which are parts of the network.
>
> 3. This subset of components, by means of relations among its members and with the components of its surroundings, generates the conditions necessary to maintain the components of the network in physical proximity, collectively forming a spatially discrete individual unit over time" (Razeto-Barry, 2012, p. 557).

Thus, essential genes cannot fulfill the concept of life, much less redefine it. Although the investigation of the essential genes may be relevant in studies on the origin of life or the evolution of organisms, their essentiality only implies that they are necessary, not that they are the only necessary component for life.

Lastly, synthetic genomics is linked to the paradoxical concept of *artificial life*, whose status may not seem clear (see section 4.2 "Moral status of synthetic entities").

4.1.3 Protocells

The protocell approach to Synthetic Biology goes beyond the synthesis of a minimal genome; it is aimed at the *creation* of living organisms completely from scratch, that is, from inanimate materials. For this reason, this approach also raises questions about the concept of life. As stated by Schwille et al. (2018), "by trying to create artificial life out of synthetic chemicals in order to better understand the process of how life evolves coincidently the question arises how such endeavours might change basic epistemological and ontological concepts such as life" (p. 13390). In this regard, Bedau et al. (2009) express that:

> "[c]reating the first bottom-up protocell, however, will mark the first time humans have synthesized life from wholly nonliving materials. When we do gain the ability to design and manipulate protocells to suit defined purposes, this could have profound impacts on our view of life, including human life and our picture of our own place in the universe" (p. 67).

Moreover, this objective, if achieved, could be said to mean the true *creation* of life, which has led several authors to associate the formula "Playing God" with Synthetic Biology (Balmer & Martin, 2008; Dabrock, 2009; Douglas & Savulescu, 2010; ETC Group, 2007; Link, 2013; Schmidt et al., 2008; Taylor, 2009; van den Belt, 2009). From this, one might think that this ethical concern is ascribed to some religious groups, who can see in this goal of Synthetic Biology a desire to supplant the creative role of God. However, as argued by Link (2013):

> "For many theologians, however, thinking of synthetic biologists as tinkering in God's domain is based on a gross misunderstanding (or modern transfiguration) of the term 'creation'. Creation, it is argued, is not to be thought of merely as a causal origination. [...] We humans can participate in God's creation as *cooperatores Dei*, but we simply do not have the potential for a *creatio a novo*. Consequently, we cannot break into a sacrosanct, divine domain" (p. 443).

In this vein, the US Presidential Commission "found this language to be unhelpful at best, misleading at worst" (PCSBI, 2010, p. 156) and that "secular critics of the field are more likely to use the phrase 'playing God' than are religious groups" (PCSBI, 2010, p. 156).

Another reading of the formula, however, can be understood as referring to the caliber of the implications, which may escape our control:

> "The worry here is not that only God could create wholly new forms of life, but that only a being with almost God-like understanding and wisdom would have the moral and scientific insight required to properly and judiciously exercise the capacity to create new forms of life" (Bedau, et al., 2009, p. 70).

This concern mainly falls in the area of biosafety, and also has a place in the discussion about the moral status of synthetic entities.

With regard to the concerns about synthetic biologists *creating* life, there are no bases in personalism on which to sustain an ethical impediment in this respect. Nevertheless, it might be more accurate, as well as responsible, to talk about *designing*, *constructing* or *recreating* life, since what is being done at the moment is recreating existing organisms and constructing new forms of life by changing preexisting organisms.

In fact, the greatest achievement of synthetic biologists to date in this regard is the construction of the genome of *M. mycoides* and its introduction into a *M. capricolum* recipient cell (Gibson et al., 2010). In addition, if one day they were able to obtain a living being from inanimate materials, they would be starting from something pre-existing, those materials, and it would not be *a creatio ex nihilo*. The use of these terms would be more precise, and would avoid unnecessary misconceptions and worries.

As in the case of the other branches within Synthetic Biology, regardless their impact on the concept of life, the *principle of protecting the life and genetic identity of every human individual* and the *principle of protecting the ecosystem and the environment* must be respected.

Lastly, protocells, as well as synthetic genomics, are linked to the concept of artificial life, raising questions about their moral status (see section 4.2 "Moral status of synthetic entities").

4.1.4 Xenobiology

This branch of Synthetic Biology has been also associated with the "Playing God" concern (Schmidt et al., 2018). De Lorenzo (2010) places xenobiological systems in the last stage of the transition spectrum between natural and synthetic organisms, classifying them as wholly synthetic microbes. This may be misleading. In spite of the functional or compositional novelties, probably not all of their genome would be alternative, but it will contain some xeno-nucleotides and/or some repurposed codons. Taking into account these considerations, the approach of the questions on the concept of life and its creation raised by xenobiology can be referred to the discussion exposed for the case of protocells (section 4.1.3). Again, regardless the impact on the concept of life, the *principle of*

protecting the life and genetic identity of every human individual and the *principle of protecting the ecosystem and the environment* must be respected.

4.2 MORAL STATUS OF SYNTHETIC ENTITIES[12]

One of the aims of bioengineering, synthetic genomics, protocells and xenobiology is to obtain new entities that are not found in nature. These entities differ in some of their characteristics with respect to normal living beings, more closely resembling machines in certain aspects. The boundary between organisms and machines thus becomes blurred, and concerns about the moral status of these entities arise (Deplazes & Huppenbauer, 2009).

Defining the moral status or intrinsic value of Synthetic Biology products before they are created is ethically imperative, as it would clarify which treatment they deserve (Bhutkar, 2005; Douglas & Savulescu, 2010), allowing the exercise of scientific activities to be guided according to the ethical standards of research with non-human organisms. The views of some of the few authors who have addressed this topic are explained below.

4.2.1 Some contributions on this topic

Attfield (2012) discusses the bearing of biocentrism on the production of artificial life. He uses the term "moral standing" of organisms, meaning "they warrant moral attention or consideration for their own sake" (p. 2). From this perspective, all living creatures have moral standing because they have a good of their own and, therefore, "their flourishing or attaining their good is intrinsically valuable" (p. 2). The author argues that, guided by the principle of beneficence, humans should avoid inflicting harm and injury

[12] An extract of this section has been published as a paper (annex 7.2.2: Gómez-Tatay L, Hernández-Andreu JM, Aznar J. The Conception of Synthetic Entities from a Personalist Perspective. Sci Eng Ethics. 2017 Oct 26. doi: 10.1007/s11948-017-9994-z).

(where there is no good reason to do so) on any kind of organism, even if it is non-sentient. From this perspective, synthetic organisms are also bearers of moral standing. However, he finds a problem in defining what constitutes the harm (and the flourishing) of synthetic organisms, since there is no point of reference for distinguishing whether their lives are going well or badly. To resolve this question, he presents some arguments. First, synthetic organisms would be bearers of most of the central characteristics of life (metabolism, growth, homeostasis, reproduction, self-organization and/or goal-orientedness). If some of these characteristics are harmed in some way in a synthetic organism, then its flourishing can be said to be impaired. Secondly, observation can also help us to determine the good for these organisms. Later, the author talks about the quality of life of synthetic organisms, which could be wronged (by comparison with that of creatures of familiar kinds) if the genetic modifications led to consequences such as a lack of sentience or a creature that seldom moves.

> "For example, meat producers might wish to produce such a creature to continue producing meat while avoiding charges of causing pain and suffering to sentient creatures. […] So we could intelligibly talk of their quality of life being a deterioration from that of familiar creatures; and this could form the basis of an ethical objection to generating them" (p. 8).

Bedau and Larson (2013) use environmental ethics as a framework to draw conclusions about the intrinsic value of synthetic organisms. Following Sandler (2012), they distinguish three kinds of intrinsic values: intrinsic subjective value, which depends on someone's opinion; intrinsic objective value, which something possesses in and of itself; and inherent worth, which is possessed by virtue of something having a good of its own, having its own interests, purposes or biological needs. With regard to intrinsic subjective values, the authors explain that synthetic organisms can have these, since these

values depend on people's opinion. However, they point out that, due to this subjectivity, the ethical implications of having this kind of value are not to be considered. Regarding intrinsic objective values, they argue that synthetic organisms have those properties that are objectively valuable, such as self-regulation, stability, self-organization or spontaneity, and thus they also possess this kind of value. At this point, they discuss whether the fact that synthetic organisms do not have a natural evolutionary history interferes with the possibility of their having an intrinsic objective value. They provide two reasons to argue that this is not the case. Firstly, directed evolution and evolutionary design of experiments, which mimic adaptive Darwinian evolution, are processes that are used to produce some synthetic organisms, suggesting that "synthetic life-forms could have the intrinsic objective value (if any) that comes from the wisdom of nature" (p. 79). Secondly, if a synthetic organism, once produced, was released into the environment, it could adapt, mutate and evolve. Finally, with regard to inherent worth, the authors conclude that this is also present in synthetic organisms, since they have interests derived from their biological needs in the same way that natural organisms do.

Douglas, Powell and Savulescu (2013) state that what matters when defining the moral status of an organism is the non-genealogical properties that it possesses, such as mental capacities. They define moral status as a special value "typically attributed to beings in virtue of the mental capacities they or normal members of their species possess" (p. 692), such as capacities for consciousness, experiencing pleasure and pain, self-consciousness or rationality. Baertschi (2012) also uses the term moral status, and advocates that some organisms have it and some do not on the basis of their intrinsic properties. The author defines the moral status of an organism as having "a peculiar value grounded in some of his intrinsic properties" (p. 5), which forces moral agents to have moral obligations towards them, so that they cannot be treated in just any way we please.

According to these views, the artificial origin of synthetic organisms would have no impact on their moral status. Synthetic organisms would have moral status if their intrinsic properties were valuable.

Basl and Sandler (2013) argue that even non-sentient organisms have a good on their own, since they are teleologically organized according to the etiological account of teleology, which implies that the goal-directedness of entities is derived from the selection process from which they result. Therefore, they argue, synthetic organisms also have a good of their own. However, they explain that this does not mean that they have moral status, since for an entity to have moral status, it must have interests and those interests must be morally relevant.

Finally, Preston (2013) argues that the intrinsic value of synthetic organisms is diminished relative to that of naturally occurring organisms. The author presents three arguments to defend this view. First of all, the situation that the teleological organization of synthetic organisms is connected to the designer's intentions implies that their good is not entirely their own. Second, in the case of synthetic organisms, the "artifactual final cause" (attributable to the designer) is prior to the "organismal final cause" (attributable to the organism's autonomous functioning). Finally, the condition that synthetic organisms are organisms is an incidental attribute to the intended purpose, which is the essential attribute.

However, these views take it for granted that synthetic entities are organisms. This has to be demonstrated, since synthetic entities, which are not found in nature, share characteristics with the world of life and also with machines. Hence, it has been said that their membership in one of these two groups is not evident, consequently blurring the boundary between them (Deplazes & Huppenbauer, 2009).

4.2.2 Classifying synthetic entities as organisms or machines

Thus, Deplazes & Huppenbauer (2009) compared organisms and machines as opposite ends representing the living and the non-living worlds, and found that these entities differ in four properties: composition (organic material *vs*. inorganic material), origin (uncertain *vs*. clearly defined), development (change *vs.* permanence) and purpose (own purposes *vs.* external, i.e. human, purposes). Taking into account these features, they placed the different products of Synthetic Biology between these ends, where before there was nothing: Synthetic cells, which are the product of the protocell approach, are like organisms with regard to their composition, development and, in principle, purpose, but not as regards their origin, which is completely artificial. Chassis organisms, which are the product of the synthetic genomics approach, are midway between organisms and machines, resembling the former in their composition and development and the latter in their purpose. With respect to their origin, this is partly machine-like and partly organism-like, since a minimal artificial genome is introduced into a natural cell. Finally, bioengineering products are like organisms with regard to their composition, origin and development, but they fulfill a completely human-determined purpose. Table 11 summarizes these distinctions, showing how the boundary between organisms and machines is being blurred. Although the products of xenobiology are not included, they can also be said to be machine-like (Schmidt et al., 2018) in some aspects, mainly with regard to their purpose.

Table 11.

Comparison of Synthetic Biology products with organisms and machines. *Retrieved from Deplazes and Huppenbauer (2009).*

	Machines	Bioengineering products	Chassis organisms	Synthetic cells	Organisms
Composition	++	−−	−−	−−	−−
Origin	++	−	−+	++	−−
Development	++	−	−−	−−	−−
Purpose	++	++	+	−	−−

++ indicates that the feature is machine-like, while − − indicates that it is organism-like. Between these ends, + indicates that the feature is not absolutely machine-like, but more similar to a machine than to an organism; − indicates that the feature is not exactly organism-like, but more similar to an organism than to a machine; − + indicates that the feature is right in the middle of the two ends.

The authors explain that, when positioning Synthetic Biology products as either machines or organisms, it is more important to focus on their purpose than on their origin. They argue that, when naming them as living machines or as synthetic organisms, the noun refers to the purpose of the organism, while the descriptive adjective refers to their origin. Given that the noun is more important, i.e. vital, than the adjective for categorization of the entities, then their purpose predominates over their origin. Thus, those entities whose purpose is external (human) would be better positioned as machines, which would avoid discussions about their instrumentalization. This conclusion is given for the case of very simple forms of life (Deplazes & Huppenbauer, 2009).

According to this view, those Synthetic Biology products which fulfill human purposes would be better positioned as machines, to which no moral status is granted. However, it does not seem appropriate to rely on this characteristic, extrinsic to the entity itself, to define whether it corresponds to one category or another. In fact, Deplazes and Huppenbauer (2009) make their argument on the basis that these synthetic products are very simple forms of life (of which they recognize no moral value), qualifying them as machines for a matter of practical utility, to avoid ethical questions regarding their production and use. Thus, they state that if higher forms of life were discussed "the moral

meaning of their 'self-interest' might have to be revisited and contrasted to machines" (p. 63). In fact, it is contradictory to attend to human purposes in order to determine the moral value of an organism, since it is humans, as unique moral beings, who confer a moral value to organisms and, based on this, limit the fulfillment of their purposes. Thus, their argument does not seem able to resolve questions about the moral status of synthetic products.

Additionally, understanding the term *purpose* as something exclusively human or inherent to the organism is inaccurate, since these two purposes need not be mutually exclusive. Actually, the fact that an entity is designed and constructed in order to fulfill human purposes does not mean that it does not serve its own ends. Along these lines, Nicholson (2013) advocates that if synthetic entities remain intrinsically purposive systems, then they must be classified as organisms, regardless of their artificial origin and the human purposes involved in their design and production. Therefore, the author also considers *purpose* as a differentially defining feature of organisms and machines, but he understands the term in another way, meaning ultimate *telos*. Table 12 summarizes a list of fundamentally different distinctive features of organisms and machines according to this author.

For Nicholson, the most important dissimilarity between organisms and machines is their purposiveness. The former are *intrinsically* purposive systems while the latter are *extrinsically* purposive systems. An organism "acts on its own behalf, towards its own ends. Its *telos* is internal, arising from within, and it ultimately serves no purpose other than to maintain its own organization" (p. 3). Furthermore, the internal organizational dynamics of organisms are characterized by the phenomena of self-formation, self-preservation, self-reproduction and self-restitution. In contrast, a machine "operates towards an end that is external to itself. Its *telos* is imposed from the outside and it is of

use or value to an agent other than itself. A machine does not serve its own interests" (p. 3). Its construction, assembly and maintenance require an external agent.

Table 12.

Main differences between organisms and machines. *Retrieved from Nicholson (2013).*

	Organisms	**Machines**
Purposiveness	Intrinsic	Extrinsic
Organization and production	System itself	Maker
Maintenance and repair	System itself	Maker and/or user
Functional determination	System itself	Maker and/or user
Functional attributions	Parts	Parts and whole
Properties of parts	Dependent on whole	Independent from whole
Structural identity of system	Transitional	Continual
Ontogenic priority	First whole, then parts	First parts, then whole
Division	Preserves unity	Compromises unity
Operation and existence	Interdependent	Independent
Normativity	System itself	Maker and/or user

Nicholson also points out other differences. Unlike machines, organisms do not have a function, since they do not operate to benefit an external agent, but to ensure their continued existence. This does not mean that a human being cannot derive benefits from them. In addition, the nature of the relationship between the parts and the whole is not equal in organisms and machines: "the generation, properties, and functions of the parts of an organism, unlike those of a machine, cannot be understood independently from the whole" (p. 4). Moreover, organisms and machines differ in their structural identity. Organisms exhibit a transitional structural identity, in contrast to the permanent structural identity of machines. Another difference is the fact that "in a machine, the whole only comes into existence after all the parts have been appropriately assembled by its maker"

(p. 4). In contrast, in organisms "the existence of the parts does not precede that of the whole given that the parts only acquire their respective identities *qua* parts as the whole progressively develops from an originally undifferentiated yet already integrated system" (p. 4). Therefore, according to this author, if synthetic products are intrinsically purposive systems, then they are organisms, regardless of their artificial origin.

Apart from the comparisons between organisms and machines, which are based on the distinguishing characteristics between both types of entities, it is worth returning to the characteristics that, apart from comparisons, seem to define life (see section 4.1.2). Certainly, the different Synthetic Biology approaches address different features of living organisms, such as metabolism, genetic program, or the interaction with the environment, in order to obtain new life forms (Table 13) (Deplazes-Zemp, 2012). Nonetheless, while synthetic biologists modify these features, they do not eliminate them. Although there are different definitions of life (Ganti, 2003; Koshland Jr, 2002; Maturana, 1975; Razeto-Barry, 2012), Synthetic Biology does not eliminate any of the features proposed as defining life. Consequently, at least some Synthetic Biology products must be considered as organisms, regardless of their natural or artificial origin and the human purposes involved in their design and production, and can be called synthetic organisms. If the entity still keeps these features, it does not matter in what measure they are artificially modified, the entity will still be an organism. The *Mycoplasma capricolum* cell containing the genome of *Mycoplasma mycoides*, synthesized by researchers at the J. Craig Venter Institute (Gibson et al., 2010), serves as an example of these kinds of organisms.

This view is consistent with that of Sgreccia (2012), who states that "there is a finality throughout the world of living things […]. In inanimate realities, the purpose or end is something external; it does not reside in the thing but rather in the mind of the planner. […] On the contrary, the purpose is immanent within animate realities" (p. 81).

Relatedly, engineering conceptions of life have been criticized insofar as they would not be realistic. According to Nuño (2016), the application of the principles of engineering to the organic universe constitutes a utopia, as informed by contemporary evolutionary biology. Thus, the author questions that Synthetic Biology can progress while trying to ignore the characteristics of the living, its complexity, because this, rather than an obstacle to eliminate is a factor that must be taken into account.

Table 13.

Overview of how synthetic biologists want to design new life forms, starting from the characteristic features of living organisms. *Retrieved from Deplazes-Zemp (2012).*

	''Addressed'' feature of living organisms	**Novelty in 'new life forms'**	**Modifications by a rational design**
Bioengineering	Metabolism	Signalling pathways, regulatory mechanisms Substances produced by the organism Behaviour of the organism Controllability	Optimising life Overcoming life Utilising life
Synthetic genomics	Genetic programme	Synthetically produced genome Size and composition of the genome, Chassis genome	Minimising life Utilising life
Protocell Synthetic Biology	Autopoiesis Interaction with the environment Constant transformation	Synthetically produced cell Simplified version of a cell	Minimising life
Unnatural molecular biology	Genetic programme	Types of nucleotides or genetic code Orthogonal life	Varying life Utilising life
***In silico* Synthetic Biology**	Metabolism	Models, simulations Regulatory mechanisms	Optimising life

4.2.3 What is the moral status of synthetic organisms and how should they be treated?

Questions about the moral status of these new entities must be addressed in order to preserve the *principle of protecting the ecosystem and the environment*, which states that living organisms cannot be treated as mere instruments. In order to do so, a preliminary consideration must be made: What is it meant by moral status?

Certainly, it is not simple to answer this question, since the concept of moral status itself is not clearly defined. Furthermore, there is a plethora of theories regarding the moral status of different organisms, focusing mainly on animals (Cavalieri & Singer, 1994; Regan, 2001; Regan & Singer, 1998; Rollin, 2006; Rowlands, 2009; Ryder, 2000; Singer, 2006), and a consensus is far from being reached. Discussions revolve around having or not having moral status, or placing the moral status of some organisms above that of others, usually sentient over non-sentient organisms (Jaworska & Tannenbaum, 2017).

From a personalist point of view, human beings would have the highest moral status, consistent with the principle of *the ontological and axiological difference between man and other living beings*, which states that there is a profound difference between living beings and man by virtue of his special features (reflective knowledge, freedom and responsibility). But, does it make sense to talk about the "highest moral status"? In this case, we would be assuming that other organisms have a *lower moral status*. Therefore, there would be two options: either that all non-human living beings have the same moral status and this is lower than that of humans, or that the moral status of non-human living organisms differs in degrees (which would have to be established). From our perspective, the first scenario is the most convincing, since the *principle of protecting the ecosystem and the environment* does not make any differentiation between different

organisms (not even among these and the environment). Rather, they are valuable in the same degree.

However, using the same term, *moral status*, for both human and non-human organisms may be misleading, since it does not contain the same definition in both cases. Therefore, by virtue of *the ontological and axiological difference between man and other living beings*, different categories should be established for them. With this aim, moral status and moral value are two concepts that can be distinguished. Moral status appears to be superior to moral value (Steinbock, 2009), so perhaps should be reserved for persons. Thus, moral status can be understood as the moral implications arising from human dignity, and moral value as the quality of an entity that implies that its treatment as a mere medium by a moral agent (person) has negative moral implications. Accordingly, all organisms have a moral value, but so too do other entities, such as natural species, ecosystems, the environment or human corpses.

Once this nomenclature is established, it is debatable whether all non-human organisms have the same moral value, as has already been mentioned above. The *principle of protection of the ecosystem and the environment* does not differentiate between different organisms (not even among these and the environment). Rather, they are valuable in the same degree. However, does this mean that all non-human living beings should be treated in the same way? This would not be consistent with our principle. So, to act as responsible "stewards" of the created world, and in particular, of other living creatures, as established by the principle, the diverse interests of the different organisms must be taken into account. Thus, although different organisms can be said to have the same moral value, their interests may differ, and may morally matter to different degrees. The consequences triggered by actions for the interests of the different organisms are often given by the features of the organism itself (sentience, emotions, consciousness,

etc.). Thus, in spite of the lack of specific determinations regarding the moral status—or value, according to our terminology—of organisms, legislations and guidelines usually govern the use of organisms to satisfy human purposes (for example, in industrial farming and experimentation) based on the features and interests of those organisms. Different organisms are not treated with the same care, since by virtue of their different characteristics, they have different requirements. Bringing these conclusions to the case of Synthetic Biology, attention should be paid to the features of its products, to determine their interests and whether their intended use could wrong those interests. The morally right way to behave towards them can then be defined, preserving their interests as far as possible.

In conclusion, having established that at least some Synthetic Biology products are organisms and not machines or anything intermediate, their moral value must be recognized and their interests taken into account in order to preserve them as far as possible, safeguarding the *principle of protecting the ecosystem and the environment*.

It may happen that human interests are contrary to the interests of other organisms, as often occurs in scientific research. Given *the principle of the ontological and axiological difference between man and other living beings*, human interests morally matter more than the interests of any other being, and thus prevail when deciding a course of action. Therefore, it is ethically appropriate to act in order to accomplish human interests, even if it means frustrating the interests of another organism, provided that such human interests are morally appropriate in themselves.

Apart from this principle, the moral implications of the relationship of the human being with other living beings and with the environment have not been addressed from personalism, much less with respect to synthetic organisms. Expounding this issue goes beyond the objectives of this work. However, it would be very valuable if this area of

research could be developed and studied in more depth from a personalist philosophy. Pending this, I propose to review the contributions of several authors in this field, and to contrast the validity of their claims from a personalist point of view.

First, the perspective of Attfield (2012) exceeds the obligations towards non-human organisms prescribed by personalism, since besides considering that synthetic organisms are bearers of "moral standing" (equivalent to our term of moral value), he argues that it must be weighed whether their artificial characteristics imply an improvement in comparison with that of the natural familiar creatures. The *principle of protecting the ecosystem and the environment* obliges us to preserve biodiversity and the environment and not to interfere with the interests of non-human organisms if it is not necessary to satisfy some human interest (provided that they are morally correct). Thus, from our perspective, one must take into account the interests of the organism as it is, and the consideration of how much better a life it could have lived if it were not synthetic has no moral relevance. Additionally, as it has been explained above, it is ethically permissible to act against the interests of a non-human organism if this is necessary to satisfy human interests, in virtue of the *principle of the ontological and axiological difference between man and other living beings*.

Second, with regard to the view of Bedau & Larson (2013), who conclude that "inherent worth" is present in synthetic organisms, this is correct according to their terminology. However, a point should be made on the consequences derived from the recognition of this intrinsic value. Sgreccia (2012) states that:

> "[t]he attribution of intrinsic value to non-human entities has led to an extension of the boundaries of the moral community beyond the unique category of human beings. This broadening can be considered essentially correct so long as it is interpreted as the need to establish moral duties for

> man not only toward other human beings, but also toward natural entities. Conversely, matters become remarkably problematic and even unacceptable from both the philosophical and scientific standpoints with the affirmation that all natural entities possess the same moral value" (p. 97).

From a personalist point of view, the recognition of these values would not lead us to equate their moral value with that of humans, which is consistent with our distinction between moral value and moral status.

Third, the intrinsic properties suggested by Douglas, Powell, & Savulescu (2013) and Baertschi (2012) as determinant of the moral status of organisms, are, from our ethical framework, only relevant to determine the interests of the organism. These views fail to fulfill the *principle of the ontological and axiological difference between man and other living beings*. By applying the same criteria to humans and animals, the most vulnerable human beings, such as embryos, are left unprotected. Furthermore, this conception of moral status leads to conclusions that are contrary to the *principle of protecting the ecosystem and the environment*, according to which no living organism can be treated as a mere instrument.

Fourth, with regard to the arguments of Basl & Sandler (2013), their differentiation between the fact of having "a good of their own" or having "moral status" is not in accordance with our distinction of moral status and moral value. In the first place, they do not distinguish a higher status in humans, and secondly, only those organisms with certain superior interests would have moral value. Finally, their term of goal-directedness is broader than our consideration on intrinsic purposiveness, thus leading to discordant conclusions, such as the implication that artifacts have a good of their own.

Finally, the arguments of Preston (2013) to advocate that synthetic organisms have a value lower than that of naturally occurring organisms are questionable. First, as Nicholson (2013) argues, even if the synthetic organism serves the interests of its maker or user, it would do so only to some extent; its ultimate purpose will still be to maintain its own organization. Second, in the current state of Synthetic Biology, the technique works on preexisting organisms. Even Venter's artificial bacteria required insertion of the synthetic genome into a natural DNA-damaged bacterium (Gibson et al., 2010). Finally, synthetic biologists design their studies on the basis that they are going to work on organisms, and taking into account the special features of these organisms is critical to achieve successful outcomes (Porcar & Peretó, 2016). From a personalist perspective, also, there are no grounds on which to argue a difference of value among natural organisms and synthetic ones.

Nevertheless, it should be clarified that, although respect for them is prescribed by the *principle of protecting the ecosystem and the environment*, this only obliges us to protect the interests of each specific individual, and not the synthetic species in general, which would not form part of the ecosystems and environment that must be preserved. In fact, it will usually be the case that natural biodiversity and ecosystems must be preserved from interaction with synthetic organisms in order to safeguard their conservation. In this regard, it can be argued that there is a moral obligation to synthesize organisms in order to increase biodiversity (Boldt, 2013). This obligation, however, cannot be deduced from personalist principles, which, as just explained, are limited to the protection of biodiversity integrated into ecosystems. In addition, obtaining synthetic species is not something that anyone can do, so it does not seem right to set out a general moral obligation in this regard. The possibility of restricting this obligation to qualified people does not make sense either, since it would conflict with freedom of research and would

stop the investment of time and resources in other activities that may also be beneficial for man. Accordingly, it seems that in this matter it can only be affirmed that the creation of synthetic species can be positive in some cases, depending on their utilities.

It is thus necessary to address those features of synthetic organisms that determine their interests, in order to define what their interests would be, whether these interests morally matter, and whether the intended use for them could harm those interests. Having made these concretizations, it can be determined how these organisms must be treated.

Although existing laws and guidelines can be useful to guide the ethically acceptable treatment of organisms, preserving their interests as far as possible, it could be the case that the rules or principles established cannot be extrapolated to organisms that are not included among those for which the rule applies. This limitation might be more notable in the case of Synthetic Biology, since unknown organisms or existing organisms with novel features (and perhaps different interests) can appear. For this reason, it is necessary to anticipate these situations, determine the interests of synthetic organisms before producing them, consider them in the context of their intended use, and establish the necessary measures to preserve their interests as far as possible.

Last but not least, when discussing the moral consideration owed to synthetic organisms, an aspect of special interest is the hypothetical case of synthetic humans. Ethical assessments of this special case are developed in section 4.5 “Transhumanism”.

4.3 BIOSAFETY[13]

Biosafety refers to the prevention of risks to public health and the environment that could be produced by unexpected interactions between dangerous biological agents and other organisms or the environment.

> "SynBio Safety is mainly concerned with lab safety and, in the future, also with the deliberative release of synthetic organisms into the environment. While the former basically and for most part relates to the research personnel, the object of the latter– and in severe accidents also the former–is the general public in the vicinity of the company and research sites (such as field trials locations), and the environment" (Ahteensuu, 2017, p. 1545).

Some authors believe that differences between Synthetic Biology and traditional genetic engineering are only quantitative, so the biosafety issues raised by Synthetic Biology are not qualitatively different. Others, on the contrary, consider that differences are qualitative, since the construction of new life forms could become considerably easier or could be based on alternative biological systems. This divergence became apparent in SYNBIOSAFE, the first project carried out in Europe to address the ethical and safety concerns raised by Synthetic Biology with the aim of facilitating socially acceptable development of this discipline. It was also suggested that the risk assessment framework currently in place for GMO may be insufficient to deal with Synthetic Biology from a biosafety standpoint. A second issue regarding the diffusion of knowledge was discussed and, again, opinions were conflicting. Some argued for strict regulation, while others advocated an open source movement (Schmidt et al., 2008).

[13] An extract of this section has been published as a paper (annex 7.2.3: Gómez-Tatay L, Hernández-Andreu. Biosafety and biosecurity in Synthetic Biology: A review. Critical Reviews in Environmental Science and Technology. 2019. DOI: 10.1080/10643389.2019.1579628).

In this regard, de Lorenzo (2010) argues that most of what is done in Synthetic Biology is equivalent to GMO in terms of safety, because synthetic organisms usually have genomes very similar to an already extant genome. Therefore, he advocates that the risks for the environment are low because ecosystems tend to maintain their equilibrium, and changes in the genome are very likely to make the organism less fit. Nevertheless, he concludes that:

> "[u]nlike the earlier cases where it is possible to find precedents to the risk questions and to foresee possible answers, the uncertainties raised by artificial/orthogonal life constitute a completely unknown territory. It is intuitive that such systems should be the safest, because they could not interact or interfere with the extant biological world; however, one can also conceive of plausible threats" (p. 930).

Schmidt & de Lorenzo (2016) identify four aspects of Synthetic Biology that could set it apart from traditional genome engineering in terms of biosafety:

> "First, the ease of DNA synthesis and genome editing and therefore the unprecedented possibility to altogether reprogram the biological agenda of extant organisms. […] Second, SynBio can create live agents that work (entirely or in part) on the basis of a non-canonical biochemistry […]. Third, the envisioned applications consider a scale for releasing synthetic biological agents that goes much beyond the bioreactor, live vaccines or even bioremediation of polluted sites. […] And fourth, SynBio is an activity that could soon be run by amateur biologists in their homes rather than by experts in an academic or industrial setting" (p. 91).

Similarly, one of the reports of the three European Scientific Committees states that:

> "[n]ew challenges in predicting risks are expected due to emergent properties of SynBio products and extensive genetically engineered systems, including, 1) the integration of protocells into/with living organisms, 2) future developments of autonomous protocells, 3) the use of non-standard biochemical systems in living cells, 4) the increased speed of modifications by the new technologies for DNA synthesis and genome editing and 5) the rapidly evolving DIYbio citizen science community, which may increase the probability of unintentional harm" (SCENIHR, SCHER & SCCS, 2015a, p. 5).

One area of special concern is gain-of-function research (GOFR), which involves experimentation that aims to increase the transmissibility and/or virulence of pathogens. The goal of this research is "to improve understanding of disease causing agents, their interaction with human hosts, and/or their potential to cause pandemic" (Selgelid, 2016, p. 923). The main fear is that "a devastating pandemic could potentially result from a laboratory accident involving an especially dangerous pathogen created via GOFR" (Selgelid, 2016, p. 925). Evans, Lipsitch, and Levinson (2015) argue that in scientific areas where expertise is common and procedures are cheap, such as Synthetic Biology, experiments can be performed in many laboratories. This implies that research involving dangerous pathogens could also be carried out in laboratories that lack the necessary biosafety measures, increasing the risk of an accident taking place.

The different branches of Synthetic Biology raise specific concerns regarding biosafety risks (SCENIHR, SCHER, & SCCS, 2015b; Schmidt, 2009).

4.3.1 Bioengineering

Some of the applications of this approach involve the release of synthetic organisms into the natural environment, in the form of biosensors or in bioremediation, or their introduction into the human body, for example to produce a drug. The possible adverse effects that these organisms could generate in the environment or in human health are a biosafety issue.

In this regard, recent advances in the production of synthetic probiotics are of great interest. For example, *E. coli* has been successfully engineered to exhibit prophylactic activity against *Pseudomonas aeruginosa in vivo*, which has been demonstrated both in *Caenorhabditis elegans* and mice (Hwang et al., 2017). It has also been engineered to produce the enzymes that metabolize phenylalanine, with the aim of developing a synthetic probiotic that is effective in the treatment of phenylketonuria, showing promising results in both mice and cynomolgus monkeys (Isabella et al., 2018). Another interesting advance in this field is the construction of synthetic adhesins in the outer membrane of *E. coli*. These proteins allow microorganisms to aggregate, which protects them in hostile environments and may have application in the development of probiotics that can survive in the gut (Lewis et al., 2018). In this regard, clinical trials have already started (ClinicalTrials.gov identifiers NCT03179878, NCT03447730 and NCT03516487), with the first synthetic probiotic tested in humans, SYNB1020, also an engineered *E. coli* strain, which addresses hyperammonemia conditions through the conversion of NH_3 to l-arginine (Kurtz et al., 2019). This has been granted “fast track” designation from the US Food and Drug Administration (FDA) (Roy, 2017), highlighting the potential of these synthetic drugs to fill unmet medical needs. The possibility of introducing synthetic organisms into the human body for therapeutic purposes requires strict and exhaustive control of their safety.

Additionally, the advancement of genetic parts libraries, which is part of this branch of Synthetic Biology, has some biosafety implications that must be controlled. On the one hand, the use of components that have not been adequately characterized could increase, as the introduction of new biological parts in libraries increases exponentially (SCENIHR, SCHER, & SCCS, 2015a). On the other, complex systems constructed through the combination of different genetic parts available in the libraries can develop unexpected emergent functions, which poses a challenge when it comes to predicting risks (SCENIHR, SCHER, & SCCS, 2015a). Similarly, Schmidt (2009) argues that:

> "[e]mergent effects in the creation of synthetic genetic circuits could cause problems in the design process and create new uncertainties [...]. Instead of 'just' having to assess how the new genetic element behaves in the new cell in a particular environment, now it is necessary to assess also the interactions among the many genetic parts themselves, that were inserted into the cell. These interactions will have no comparable counterpart in nature, making it more difficult to predict the cell's full behavioural range with a high degree of certainty" (p. 87).

4.3.2 Protocells

Several advances are being made in the field of protocells, as previously mentioned. Of particular significance regarding biosafety is recent research in which protocells are placed in natural environments, where there are natural cells. Thus, Krinsky et al. (2018) report, for the first time, the synthesis of anticancer proteins by artificial lipid-based vesicles inside tumors in the mammary fat pad of mice, concluding that "such platforms present a new drug delivery approach—the production of therapeutic proteins directly at the disease site. In the future, these platforms may prove effective for synthesizing

biologics encoded to address the patient's personalized needs" (p. 5). Moreover, the work of Ding et al. (2018) describes not only the directed interaction between artificial and natural cells, which had already been demonstrated (Lentini et al., 2014; Lentini et al., 2017), but also how the sensitivity of their synthetic gene networks to the extracellular chemical context can be minimized, which is critical to develop future applications (Ding et al., 2018).

In the final report of the three European Committees, some "gaps in knowledge" regarding protocells were identified that raise biosafety concerns. First, current protocells could interact synergistically with natural organisms, with unknown consequences. Second, if protocells eventually fulfill the necessary characteristics to be considered organisms, we would have no information on how these life forms could interact with natural organisms. Finally, there is the possibility that protocells, if released into the environment, could mimic natural vesicles produced by bacteria and interfere in as yet unknown biological functions (SCENIHR, SCHER, & SCCS, 2015b).

4.3.3 Xenobiology

De Lorenzo (2010) acknowledges that:

> "the uncertainties raised by artificial/orthogonal life constitute a completely unknown territory. […] The way that such cells – assuming they would be viable – could behave when interacting with standard organisms is impossible to predict at this time. Sooner or later these types of questions will have to be added to the risk assessment agenda of future Biotechnology" (p. 930).

Along these lines, the European Committees recommend evaluation of the potential toxicity and allergenicity of xenobiological compounds, and warn that xenobiological organisms could exhibit changes in features such as evolutionary fitness, ecological

competitiveness, degree of horizontal gene flow, susceptibility to viruses, diseases and predation (SCENIHR, SCHER, & SCCS, 2015b).

4.3.4 DIY Synthetic Biology

Amateur Synthetic Biology, or the DIYbio movement, generates new biosafety risks, as Synthetic Biology processes could become easily available in a user-friendly format to anyone. “An unrestricted biohackery scenario could put the health of a biohacker, the community around him or her and the environment under unprecedented risk” (Schmidt, 2008, p. 3). In fact, the study carried out by the PCSBI reported that DIY synthetic biologists pose some potential risks that must be foreseen in order to develop adequate policies in this regard. Indeed, in one of the 18 given recommendations (Recommendation 12), the Commission urges the government to assess the risks arising from DIY research, among others. It concludes that for now there is no reason to stop this movement, but the DIY scientist must be trained in responsibility (PCSBI, 2010).

4.3.5 Synthetic genomics

SCENIHR, SCHER, & SCCS (2015b) and Schmidt (2009) both find that Synthetic Genomics does not pose biosafety risks. In the words of Schmidt (2009), “[o]rganisms with a highly reduced set of genes and physiological functions will by definition be restricted to a very narrow ecological niche. Therefore the minimal organism with a minimal genome is per-se a safe organism as it can only inhabit particular environments and will not be able to exist outside of these” (p. 89). However, virus synthesis experiments could be classified within this branch of Synthetic Biology, and they do pose risks of biosafety (Koblentz, 2017). Nevertheless, given that the consequences of an accidental release to the environment would be the same or lesser than in the case of an

intentional release, the risk of this research is addressed in the biosecurity section (see section 4.4.1).

4.3.6 Biosafety measures

Although the debate on biosafety in Synthetic Biology is open, measures of biocontainment and regulation, among others, have already been proposed in this area.

Biocontainment

Biocontainment measures include physical measures (engineering design of equipment, process and production plant) and biological means, among which inducible systems, auxotrophy and cellular circuits are the most established strategies. In inducible systems the introduced genes are expressed only if a specific inducer is present. The inducer required is not common in the natural environment, so that if the organism escapes the lab, the engineered trait will not be expressed, avoiding any potential advantage given by the genetic construct. Auxotrophy is the inability of the organism to produce a particular vital compound, which must be provided in its media. Cellular circuits are genetic constructs that can lead to cell death when activated (kill switches), or to the death of the new host in the case of horizontal gene transfer (addiction modules). However, these strategies are not effective enough when used in isolation, since the evolutionary cost of bypassing or reverting the containment mechanism is very low. Multiple strategies and targets must therefore be combined (Figure 15), in what is known as multi-layered containment (Torres, Krüger, Csibra, Gianni, & Pinheiro, 2016), although “the higher the complexity of a safety device, the more prone it may be to disturbance and failure” (Wright, Stan, & Ellis, 2013). Furthermore, precautions must be adopted, such as not to incorporate antibiotic-resistance genes as markers for plasmid selection, in order to avoid their propagation (Wright et al., 2013).

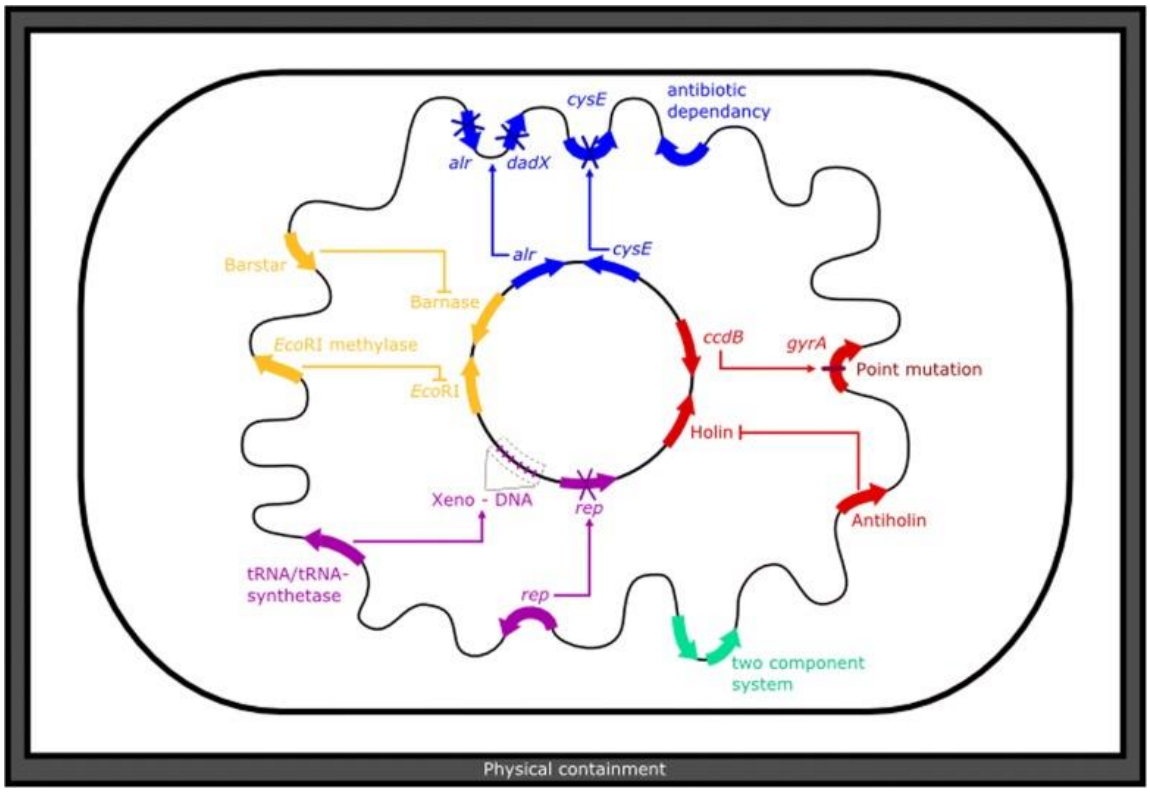

***Figure 15*. Comprehensive combination of biosafety mechanisms in *E. coli*.** "Proposed combination of orthologous biosafety mechanisms. Auxotrophies (blue), TA-systems (red), replication control mechanisms (purple) and self-destruction systems (yellow) could be combined to achieve a high-quality biosafety system. Furthermore, the proposed combination of systems includes physical containment (grey) and a two-component system (green) to enhance the reliability even further. To create artificial auxotrophies, alr, dadX and cysE were deleted in the genome and must be replaced with plasmid-bound gene copies. CcdB and Holin serve as toxins, but their toxicity will only effect wildtype cells. The toxicity of CcdB can be avoided through a single point mutation within the gyrA gene. To neutralize the toxicity of holin, an antiholin-encoding gene is present in the genome of the desired host. By moving the rep gene from the plasmid to the genome, the plasmid can only replicate if Rep is provided in trans. Incorporation of artificial bases into the plasmid (Xeno-DNA) prevents wildtype cells without the corresponding tRNA/tRNA-synthetase to produce any of the encoded proteins. To destroy the plasmid DNA if taken up by wildtype cells, self-destruction systems like barnase and EcoRI are included. Only the desired host possesses the corresponding inhibitors Barstar and EcoRI methylase and hence can counteract the toxicity". *Retrieved from Whitford et al. (2018).*

For example, the PCSBI propose the introduction of "suicide" genes into the genome of synthetic organisms, which would prevent their survival outside of a contained environment (PCSBI, 2010). However, much remains to be investigated before this technique can be safely applied, since its effect may be voided by mutation or genetic interchange processes (Murray, 2012). Ecological modeling of synthetic microorganisms

has also been suggested to study the effect of those synthetic organisms that are expected to be released to the environment (for applications in biosensing or biorremediation) (Tucker & Zilinskas, 2006). Also for this application, it has been proposed that microorganisms be engineered in such a way that their growth and function is linked to both the environment and the microbiota specific to the intended place of release, by means of feedback-circuits (Solé et al., 2015).

Although Synthetic Biology may pose new biosafety challenges, it can also provide new and more effective containment mechanisms (Schmidt, 2010; Schmidt & de Lorenzo, 2012; Torres et al., 2016). For example, organisms that require synthetic compounds for survival (an extended form of auxotrophy), such as non-canonical amino acids, could be produced (Mandell et al, 2015; Rovner et al., 2015). This strategy improves upon traditional auxotrophism because the required synthetic cofactors can never be found in the natural environment. Another possibility is to engineer an organism so that it has an altered genetic code, "either through codon reassignment or by altering the decoding rules […], ensuring functional proteins could only be functional in the engineered host–making the information itself semantically contained" (Figure 16) (Torres et al., 2016, p. 397).

Artificial nucleotides, xeno–nucleotides, can also be produced, which are polymerized into Xenobiotic Nucleic Acids (XNAs). "As replication, transcription or incorporation of an XNA into the genome of GEM's [*genetically engineered microorganisms*] natural counterparts would be prevented, a 'genetic firewall' could be raised between nature and genetic engineering" (Figure 17) (Torres et al., 2016, p. 399).

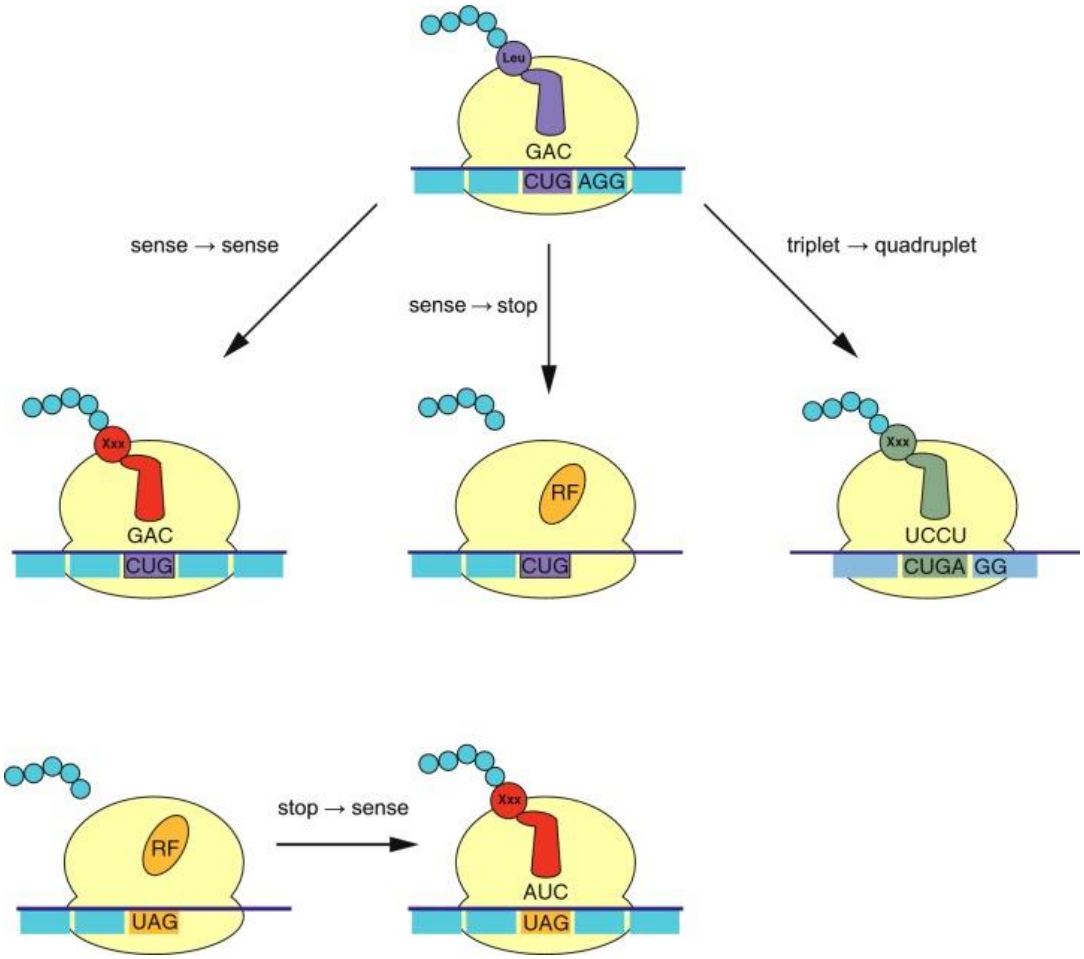

***Figure 16*. "Changing the genetic code for semantic containment.** Any change in the genetic code–sense-to-sense, sense-to-stop or triplet-to-quadruplet (see orthogonal translation as a containment strategy section)–leads to the incorporation of a different amino acid (Xxx) or protein synthesis termination (RF–release factor). As a result, the same messenger RNA (mRNA) message leads to two different proteins under the natural and engineered code. If the reassignment is sufficiently disruptive, such that under the natural code the resulting protein is not functional, then information cannot move from engineered to natural organism". *Retrieved from Torres et al. (2016).*

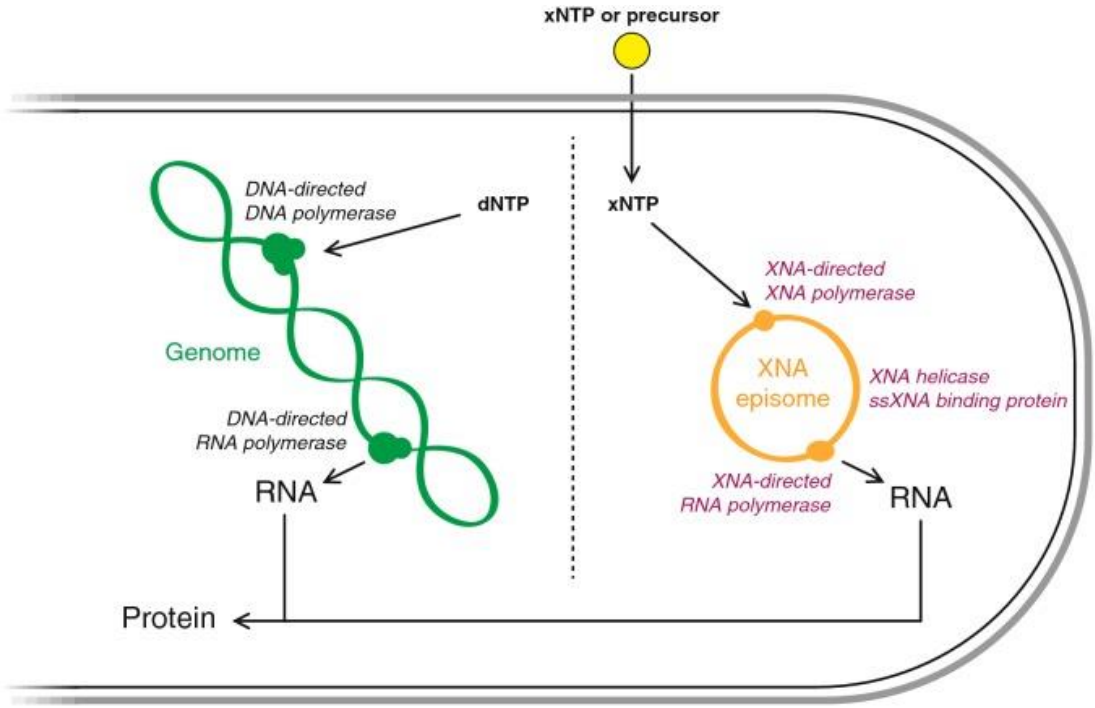

***Figure 17*. "An orthogonal replication system for genetic containment.** An XNA genetic element (in orange) is maintained by the external provision of xenobiotic nucleoside triphosphates (xNTPs) or cell-permeable precursors (yellow) and replicated by means of an engineered XNA-dependent XNA polymerase (or XNA replicase) and accessory proteins (in purple). Selection of the synthetic episome across generations occurs by encoding a vital gene product or functional XNA in the episome itself. In either case, an XNA-directed RNA polymerase is necessary (in purple). Prevention of cross-talk with the natural DNA genetic system (in green) is essential to create a stable XNA system and to establish an effective genetic firewall (dotted line)". *Retrieved from Torres et al. (2016).*

Furthermore, genetic circuits can be designed to kill their hosts outside of the designated environmental conditions. An important advantage of these circuits over the auxotrophy approach is their modularity, which allows them to be readapted for different environmental inputs (Chan, Lee, Cameron, Bashor, & Collins, 2016).

In addition to controlling the proliferation of synthetic organisms outside of the desired environment, horizontal gene transfer must be avoided. To this end, strategies such as toxin-antitoxin pairing systems or conditional origins of replication can be employed (Lee, Chan, Slomovic, & Collins, 2018). For example, Wright, Delmans, Stan,

& Ellis (2015) designed GeneGuard, a plasmid that was reformatted through the combination of three strategies (conditional origins of replication, auxotrophies, and toxin–antitoxin pairs), resulting in plasmids that depend on their designated host but are injurious for different hosts.

Nonetheless, these biocontainment strategies have limitations. Thus, synthetic auxotrophy cannot prevent horizontal gene transfer, gene circuits do not achieve the desired escape rates, and toxin-antitoxin pairs and the use of conditional origins of replication are only applicable to mobile DNA elements, not to chromosomal DNA. For this reason, several strategies are being explored to complement these methods, such as transgene-specific inactivation instead of killing systems to reduce fitness cost to hosts, automated restoration of mutated circuits, mutagenesis reduction systems, compartmentalization in different strains of the synthetic genes necessary to obtain a product (multispecies consortia), additional xenobiological approaches, etc. (Lee et al., 2018).

Further biosafety measures include genetic ‘barcodes’, which can be placed in the synthetic genes, making them easily traceable. Another option is embedding ‘DNA watermarks’ throughout the genome, as done with JCV-syn1.0 (Gibson et al., 2010). The first option is more suitable for tracing synthetic DNA in the environment, since upstream or downstream ‘DNA watermarks’ are more likely to be lost during recombination events than the ‘barcodes’ included in the synthetic genes (Wright et al., 2013).

Imperfect retention is another interesting possibility:

> “i.e. cells that survive for months but not years in the environment, or plasmid-based constructs that are gradually lost after their hosts are deployed. [...] it seems prudent for synthetic biology to be intentionally designing

GMMs [*genetically modified microbes*] with half-lives of days or weeks where the intended application permits" (Wright et al., 2013, p. 1230).

Finally, systems to reproductively isolate synthetic species from wild type species, which may guarantee the preservation of natural biodiversity, are also a promising biocontainment measure (Callaway, 2018; Maselko, Heinsch, Chacón, Harcombe, & Smanski, 2017; Moreno, 2012).

Risk assessment research

The three European Scientific Committees identify major gaps in knowledge for accurate risk assessment, and provide research recommendations in this regard for each branch of Synthetic Biology (Table 14). As a general recommendation, they advise carrying out research "on standardised techniques to monitor biocontainment and survival in environments outside the bioreactor and to generate comparative data for use in quantitative biocontainment assessment" (p. 43). They additionally recommend carrying out research on: impacts from accidental or intentional introduction of SynBio organisms into the environment, with emphasis on the effects on habitats, food webs and biodiversity; the difference in physiology of natural and synthetic organisms; vertical or horizontal gene flow; survival, persistence, ecological fitness and rate of evolutionary change; de-extinction and the debate around it; containment strategies to prevent unintentional release of or exposure to organisms resulting from SynBio techniques; the environmental performance of SynBio processes and products, considering the full product life cycle; and gene drives (SCENIHR, SCHER, & SCCS, 2015b).

In relation to this, the International Union for Conservation of Nature (IUCN) is currently examining the potential benefits and risks that Synthetic Biology poses for the

conservation of species, with the ultimate goal of developing a policy that constitutes an adequate guide in this regard (IUCN, 2016).

Table 14.

Gaps in knowledge for accurate biosafety risk assessment of Synthetic Biology approaches and related research recommendations. *Modified from SCENIHR, SCHER, & SCCS (2015b).*

Branch of Synthetic Biology	Gaps in knowledge	Research recommendations
Bioengineering	Tools for predicting emergent properties of complex biological systems may not be sufficiently accurate or may not be available to risk assessors. The methods for submitting genetic modification data and genetic parts information to risk assessors is yet unstandardised across EU member states and internationally and are largely natural language submissions. Such practices could limit the sophistication of quantitative analyses, data evaluation, efficiency and effectiveness of risk assessment. The increased speed of modifications might pose challenges to risk assessment mainly because administrative procedures might not be able to cope with a large number of rapidly created engineered organisms.	Support a) research to characterise the interactions between modified and novel parts, b) development of computational tools to predict emergent new properties of SynBio organisms and their potential failure modes, including biological prediction tools that explicitly incorporate the uncertainty of molecular and genetic information and c) broad dissemination of and training in such tools and knowledge resources. Research approaches to streamline and standardise the methods for submitting genetic modification data and genetic parts information, including systems biology models, to risk assessors across EU Member States. Develop guidelines for risk assessors on the evaluation of potential emergent properties of genetically engineered systems. Research on the use of GMOs with a proven safety record as acceptable comparators for risk assessment so that the baseline state of safe organisms can advance step-by-step with the complexity of new modifications.
Synthetic genomics	How to define and engineer biological robustness with the aim to move closer to neutral or even zero evolution.	Research on the introduction of biosafety of modules at the design stage. Further fundamental research on quantifying and qualifying the evolutionary change of phenotypes through time is required to understand and predict how these two demands, increased genetic robustness and decreased environmental robustness, can be simultaneously satisfied.
Protocells	There is little to no information about the behaviour, impact and evolutionary ramifications of interactions of systems consisting of organisms and chemical non-living systems. Unknown hazardous properties of future autonomous, replicating chemical systems, including, allergenicity, pathogenicity, biological stability. Lack of knowledge on behaviour of "natural protocells" i.e. lipid vesicles produced by bacteria and loaded with	More information is needed to assess the implications, as well as the environmental and evolutionary consequences of a collaborative interaction between non-living protocells and living organisms, including the host range and the specificity of collaborative interactions between protocells and natural cells. If protocells become life-like entities, it will be necessary to develop methods to assess the risk of allergenicity, pathogenicity and biological stability. More research is necessary to learn and increase knowledge about the ecological and

	peptides, RNA, DNA, which may be a comparator to synthetic protocells	evolutionary role of natural vesicles containing peptides, RNA and DNA.
Unnatural Synthetic Biology	Unknown effects of non-standard biochemical molecules/systems, e.g., XNA, alternative base pairs, etc., in living cells. Unknown potential toxicity and allergenicity of novel xenobiological compounds. Lack of data supporting risk assessment such as change in evolutionary fitness, ecological competitiveness, degree of horizontal gene flow, susceptibility to viruses, diseases or predation. Lack of a clear and reliable metric to measure the escape frequency of different types of semantic containment (e.g., the use of different genetic codes, or alternative biochemistries of key informational biopolymers such as nucleic acids or amino acids). Insufficient mechanistic understanding of underlying principles of semantic containment, to allow for a reliable prediction of the strength of semantic containment strategies is missing.	Each individual chemical class of xeno-compounds (e.g., HNA, GNA) should initially be characterised and tested comprehensively (e.g., toxicity and allergenicity), including a risk assessment for emergent properties. Establish a methodology to quantitatively and qualitatively characterise xenobiologic organisms with respect to evolutionary fitness, ecological competitiveness, degree of horizontal gene flow, susceptibility to viruses, diseases and predation. Develop a clear and reliable metric to measure the escape frequency associated with different types of semantic containment. Improve the mechanistic understanding of underlying principles of semantic containment to allow for a reliable prediction of the strength of semantic containment strategies.
Citizen science	Knowledge gap whether citizen scientists reliably comply with the established biosafety rules	Development of strategies to further increase and maintain the compliance of citizen scientists with harmonised European biosafety rules and codes of ethics, including collaboration with acknowledged institutions and training.

Regulatory measures

Regarding the international regulation with implications on the biosafety risks posed by Synthetic Biology, the Convention on Biological Diversity (CBD) stands out (Bellver, 2016), whose objectives "are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources" (United Nations [UN], 1992, p. 3). For the application of this convention, two international agreements have been developed that are already in force, the Cartagena protocol, "which aims to ensure the safe handling, transport and use of living modified organisms (LMOs) resulting from modern biotechnology that may have adverse effects on biological diversity, taking also into

account risks to human health"[14], and the Nagoya protocol, "which aims at sharing the benefits arising from the utilization of genetic resources in a fair and equitable way"[15]. The governing body of the Convention, The Conference of the Parties (COP)[16], holds periodic meetings in order to take decisions that advance implementation of the CBD. As explained by Bellver (2016), it is in the eleventh meeting of the COP (COP, 2012) that Synthetic Biology appears for the first time as a specific discipline that can have an impact on biodiversity and must be evaluated. Subsequently, in the twelth meeting of the COP (COP, 2014), the establishment of an Ad Hoc Technical Expert Group on Synthetic Biology (AHTEG) was decided, in order to inform the Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA), a scientific body that provides the COP "with timely advice relating to the implementation of the Convention"[17]. These three entities work jointly to comply with the CBD in what Synthetic Biology is concerned.

In terms of the regulatory measures suggested to avoid biosafety risks, the precautionary principle has been said to be a useful tool to follow Synthetic Biology developments, which must be continuously evaluated in order to properly adapt policy (Capurro et al., 2009), and in fact it is the approach of the CBD. However, there are also those who advocate the opposite view, the pro-actionary approach. The former in principle consider the potential risks of emerging sciences and propose a very cautious advance, while the latter consider new technologies acceptable (i.e. good), unless proven otherwise. There are also those who find themselves between these positions (Parens et al., 2009).

[14] http://bch.cbd.int/protocol
[15] https://www.cbd.int/abs/
[16] https://www.cbd.int/cop/
[17] https://www.cbd.int/sbstta

In a democratic society, one way to resolve this conflict would be for risk-taking strategies to reflect the vision of society, as proposed by Selgelid (2016). According to this author, in a democracy, the risk-taking strategy employed by policy-making should arguably reflect the risk-taking strategies of the people. The author develops a framework for GOFR decision- and policy-making regarding the funding and conduct of GOFR. It is comprised of eight principles (Table 15) that constitute a framework to place different GOFR cases on an ethical spectrum, in order to determine to what extent they approach the ideal of research without ethical drawbacks or to what extent they move away from this ideal. "The aim should be that any GOFR pursued (and/or funded) should be as far as possible towards the former end of the spectrum" (Selgelid, 2016, p. 960). Arguably, this framework is applicable to any scientific research involving biosafety risks.

Gronvall (2014) advocates national-level biosafety norms:

> "The next time there is concern about GOF or some other potentially concerning research, it would be helpful to know that the research took place in an environment where there are national standards for the work, including for equipment maintenance, worker safety training, health monitoring, surveillance, and other myriad activities to help keep the researchers and the larger public safe, and that the nation has an adequate surveillance system in place to identify and limit potential outbreaks that could result from such accidents" (p. 1-2).

Table 15.

Principles for GOFR decision- and policy-making regarding the funding and conduct of GOFR. *Source: prepared by the author based on Selgelid (2016).*

PRINCIPLE	DEFINITION OF THE PRINCIPLE
Research Imperative	"The ethical acceptability of GOFR posing extraordinary risks partly depends on the importance of the research question it aims to address"
Proportionality	"The ethical acceptability of extraordinarily risky GOFR partly depends on the extent to which there is reasonable expectation that the research in question will (1) yield answers to the target public health question and (2) ultimately result in benefits that outweigh risks involved"
Minimization of Risks	"Other things being equal, the ethical acceptability of a GOFR study is a function of the degree to which (1) there is confidence that no less risky forms of research would be equally beneficial and (2) reasonable steps have been made to minimize risks of the GOFR study in question"
Manageability of Risks	"Other things being equal, the more manageable the risks of a GOFR study, the more ethically acceptable the study would be. Conversely, the more important/beneficial a GOFR study is expected to be, the more we should be willing to accept potentially unmanageable risks"
Justice	"Because justice requires fair sharing of benefits and burdens, the ethical acceptability of GOFR partly depends on the degree to which (1) risks fall on some people more than others, (2) risks fall on those who are unlikely to benefit, and/or (3) any resulting harms are uncompensated"
Good Governance—Democracy	"GOFR decision- and policy-making should (insofar as possible) reflect the ultimate values, value weightings, and risk-taking strategies of public citizens"
Evidence	"Decision- and policy-making regarding GOFR should be based on more/better evidence regarding risks, benefits, (means of) risk minimization, who is likely to benefit or be harmed by research, and the values, value weightings, and risk-taking strategies of public citizens"
International Outlook and Engagement	"Because risks and benefits of GOFR (can) affect the global community at large, the ethical acceptability of GOFR partly depends on the extent to which it is accepted internationally. Decision- and policy-making regarding GOFR should (insofar as possible) involve consultation, negotiation, coordination, and related forms of active engagement with other countries"

Evans et al. (2015) propose to extend the application of the bioethical principles that govern experimentation with humans to the evaluation of those experiments in which, although the human being is not the direct object of the study, human health is threatened by the risks associated with the experiment. In particular, the authors highlight two factors that should be considered when proposing scientific investigations that pose risks for human beings: the humanitarian importance of the aims pursued and the lack of alternatives to achieve these aims:

> "Specifically we highlight the Nuremberg Code's requirements of 'fruitful results for the good of society, unprocurable by other methods', and proportionality of risk and humanitarian benefit, as broad ethical principles that recur in later documents on research ethics and should also apply to certain types of research not involving human subjects" (Evans et al., 2015, p.1).

Regarding the DIY movement, biohackers themselves have shown concern for safety (Kuiken, 2016), since they are proactive in the promotion of safety information display[18], codes of conduct[19] and seeking professional advice from biosafety experts[20]. However, there are also examples of overly risky and careless behavior. Thus, the initiators of the Glowing Plant project, whose aim was to produce glowing plants with the ultimate goal of achieving sustainable natural lighting, were willing to send seeds to those who would support them in their crowdfunding campaign, which would have led to the uncontrolled spread of these synthetic organisms. The project eventually failed[21], but the rejection of the precautionary principle has also been expressed in the previously

[18] See http://igem.org/Safety
[19] See https://diybio.org/codes/
[20] See http://ask.diybio.org/
[21] See https://www.kickstarter.com/projects/antonyevans/glowing-plants-natural-lighting-with-no-electricit/posts/1786250

mentioned manifesto: “We reject outright the admonishments of the precautionary principle, which is nothing more than a paternalistic attempt to silence researchers by inspiring fear of the unknown” (Patterson, 2010).

In relation to this, it seems that only the public who is actively involved is taken into account. “If the right to decide about an issue devolves upon the ‘doers’ and the active elements of the public, then this automatically entails a disenfranchising of the less active part of the public and of those who are indirectly affected” (Keulartz & van den Belt, 2016, p. 14). In fact, many biohackers, including Patterson, advocate a model of ‘do-ocracy’:

> “It stands for an ethic of selforganization in which anyone who decides to do something is empowered to do it, and to make the decisions about how to do it… This is a simple, powerful form of practical anarchy that works well for getting things done. However, it doesn’t work well for resolving conflicts between people who want different things to happen; it doesn’t protect people who have less ability to do things because of unequal access to time, or to resources, or unequal physical ability; and it is no help to people who believe that certain things just shouldn’t be done at all” (Worden, 2012, p. 14-15).

Schmidt (2009) argues that measures to regulate the actions of biohackers should be taken, such as laws, codes of conduct, voluntary measures, access restrictions to key materials, institutional embedding and mandatory reporting to Institutional Biosafety Committees.

Other biosafety measures

Other biosafety measures or improvements are considered necessary to adapt to the development of Synthetic Biology.

Regarding the risks for workers, Howard, Murashov, & Schulte (2017) advocate the need to improve worker protection measures, since a greater number and variety of people are expected to be exposed to Synthetic Biology processes in the future. Suggested measures include disease surveillance, proactive risk assessment, design of effective biocontainment strategies, Synthetic Biology-specific safety guidance, post-exposure prophylaxis for lentiviral vectors, and greater government involvement.

As regards risk assessment methodologies, the three European Scientific Committees argue that some improvements must be made to deal with Synthetic Biology developments:

> "1) support the characterisation of the function of biological parts and the development of computational tools to predict emergent properties of SynBio organisms, 2) streamline and standardise the methods for submitting genetic modification data and genetic parts information to risk assessors, 3) encourage the use of GMOs with a proven safety record as acceptable comparators for risk assessment, 4) aim to ensure that risk assessment methods advance in parallel with SynBio advances, and 5) support the sharing of relevant information about specific parts, devices and systems with risk assessors" (SCENIHR, SCHER, & SCCS, 2015a, p. 6).

Also, with regard to the DIYbio movement, chemists, engineers, physicists and computer scientists who work in Synthetic Biology are generally newcomers to biology, and are usually untrained in biosafety rules. Thus, the inclusion of biosafety education in the interdisciplinary curricula of Synthetic Biology has been recommended (Schmidt, 2009).

4.3.7 Application of personalist principles to biosafety issues

Following the *principle of protecting the life and genetic identity of every human individual*, it is necessary to ensure that Synthetic Biology will not infringe on human life and integrity, either directly, by means of medical applications, or indirectly, due to interactions with synthetic organisms accidentally released into the environment. Furthermore, according to the *principle of protecting the ecosystem and the environment*, the environment and natural organisms must also be preserved from damaging interactions with Synthetic Biology products. Therefore, the risks that could derive from unexpected interactions between Synthetic Biology products and humans, other organisms or the environment must be addressed.

Accordingly, all available containment measures are desirable, as well as research and continuous improvement in this field and in risk assessment strategies to enhance the safeguarding of these principles

This view is incompatible with the pro-actionary approach, which gives more importance to scientific development and freedom of inquiry than to the protection of life, which is the fundamental value according to personalism. On the other hand, the precautionary approach does seem appropriate to ensure the safeguarding of personalist principles. Such an approach requires preventive measures to be established. In order to guarantee the application of these measures, these must be compelled by law; if not, compliance with the decisions would be optional, an inadequate feature in this case, given the importance of the values at stake. The protection of life prevails over freedom of investigation according to our ethical framework. However, given that freedom of research is also a personalist principle, the precautionary principle does not imply a prohibition of the advancement of this discipline, but rather the establishment of

appropriate barriers when fundamental values are threatened. Therefore, according to this framework, neither scientific self-regulation alone nor the 'do-ocracy' model advocated by some biohackers are legitimate options to manage the biosafety risks posed by Synthetic Biology.

The implications of biosafety for Synthetic Biology can-not be considered in isolation in each nation. This is a matter of international concern, since in the case of biological agents, their action can spread rapidly from one geographical location to another. Accordingly, international agreements, directives and guidelines are necessary. National laws are also essential to implement these recommendations in each country, according to the different legislative codes.

Thus, the Selgelid proposal needs some specifications if it is to be extended to any form of research that implies biosafety risks. Although the importance of the research in question and the expected benefits must be taken into account, the nature of the risks and benefits must also be considered. In this way, risks to human life can be weighed against benefits for human life, but not against other kinds of benefits, since it would not be correct, according to our ethical framework, to carry out research that poses risks to people's lives when the expected benefit is merely economic, for example. This consideration is aligned, in a way, with the proposal of Evans et al. (2015).

Another principle must be also considered here: the *principle of the competence of the community*. According to the personalist ethics developed by Sgreccia (2012):

> "[t]he search for solutions to the problem of interventions on the genetic patrimony of human beings and other living beings as well cannot be entrusted only to certain experts, whether scientists or politicians: it is a question that in certain ways regards humanity as a whole. The future of

humanity often demands the responsible participation of the community. This is why the principle of freedom of science and research should be recognized but also combined with the fact that populations need information and share in responsibility" (p. 323).

Thus, decision-making processes can-not be entrusted only to politicians or specific committees. Instead, all the stakeholders must be involved, as well as the public. Scientists (and biohackers) must inform society about the factors involved in biosafety, such as the risks of releasing synthetic organisms (biodiversity damage, horizontal gene transfer or unexpected side-effects for the environment and other organisms), advances made in safety systems (physical containment or engineering synthetic organisms to limit their survival to specific conditions), the achievements of the DIYbio movement, etc., so that society can participate in the debate and help in the establishment of appropriate regulations.

4.4 BIOSECURITY[22]

Laboratory biosecurity can be defined as "the protection, control and accountability for valuable biological materials [...] within laboratories, in order to prevent their unauthorized access, loss, theft, misuse, diversion or intentional release" (WHO, 2006). However, it is not only biological materials that must be controlled, but processes, practices, equipment, information and knowledge associated with perilous biological materials must be equally overseen (Sture, Whitby, & Perkins, 2013).

[22] An extract of this section has been published as a paper (annex 7.2.3: Gómez-Tatay L, Hernández-Andreu. Biosafety and biosecurity in Synthetic Biology: A review. Critical Reviews in Environmental Science and Technology. 2019. DOI: 10.1080/10643389.2019.1579628).

As an issue of international interest, an important treaty in this regard is the Biological Weapons Convention (BWC), the first multilateral disarmament treaty banning the development, production and stockpiling of an entire category of weapons of mass destruction (United Nations Office for Disarmament Affairs [UNODA], 1972). In order to give effect to the BWC, states are required to adopt penal, biosafety, biosecurity and enforcement measures, and must adopt import and export controls and both domestic and international cooperation and assistance measures (Verification Research, Training and Information Centre [VERTIC], 2002). Additionally, the Australia Group (AG) is "an informal forum of countries which, through the harmonization of export controls, seeks to ensure that exports do not contribute to the development of chemical or biological weapons" (AG, 2017), and its work complements that of the BWC.

The possibilities of misuse of Synthetic Biology are, therefore, a biosecurity matter. The distinction between biosafety and biosecurity issues, together with the fact that there is a record of misuse of new technologies in the past, and that the biosecurity community has identified Synthetic Biology as an area of concern, warrant the separate biosecurity analysis of this discipline (Kelle, 2009a). Examples of potential misuse of Synthetic Biology are bioterrorism, biowarfare or bioattacks motivated by individual desires (revenge, crimes of passion, economic disputes, etc.). Thus, organisms could be modified to produce toxins or to increase their pathogenicity, and novel pathogens could be synthesized *de novo*. Another possibility of misuse is the illegal production of drugs (Oye, Lawson, & Bubela, 2015; Schmidt, 2009).

Thus, scientific research can sometimes be used for either beneficial or malevolent ends, making it questionable whether or not to pursue such investigations. This is known as the dual-use dilemma. In 2001, a group of Australian researchers engineered a strain of the mousepox virus, obtaining a new, much more lethal strain (Jackson et al., 2001).

While this research could contribute to the understanding of poxviruses, which are genetically very similar to them and some of which are transmissible in humans, it also posed a risk, since it could be used to engineer human-transmissible poxviruses into far more lethal viruses (Miller & Selgelid, 2007). This case is a prime example of a possible dual-use research of concern (DURC). Subsequent examples of this type of research are the *de novo* synthesis of the poliovirus (Cello, Paul, & Wimmer, 2002) and the Spanish influenza virus (Tumpey et al., 2005), the modification of the highly pathogenic avian influenza H5N1 virus for transmission between ferrets (Herfst et al., 2012; Imai et al., 2012), the generation of a virus composed of avian influenza virus segments with high homology with the 1918 pandemic influenza virus (Watanabe et al., 2014) and, very recently, the synthesis of the horsepox virus (closely related to the smallpox virus) (Kupferschmidt, 2017; Noyce, Lederman, & Evans, 2018).

Some aspects of Synthetic Biology set it apart from traditional genome engineering in terms of biosecurity as well. According to Ahteensuu (2017), these are three developments introduced by this discipline: "(1) a spread of the required know-how, (2) improved availability of the techniques, instruments and biological parts, and (3) new technical possibilities such as 'resurrecting' disappeared pathogens" (p. 1542). Similarly, MacIntyre (2015) identified acceleration in DURC, public availability of methods for DURC and difficulty differentiating between natural and unnatural outbreaks as characteristics of Synthetic Biology that pose new challenges for biosecurity. Likewise, in a recent study conducted by the National Academies of Sciences, Engineering, and Medicine (NAS) at the request of the US Department of Defense on the biosecurity concerns raised in this field (National Academies of Sciences, 2018), a framework was developed for assessing the biosecurity implications of Synthetic Biology capabilities. It consists of four factors (usability of the technology, usability as a weapon, requirements

of actors, and potential for mitigation) along with descriptive elements within each factor. The report concludes that "Synthetic biology expands what is possible in creating new weapons. It also expands the range of actors who could undertake such efforts and decreases the time required" (p. 164), and ranks biosecurity concerns raised by Synthetic Biology according to this framework (Figure 18).

These concerns are raised by different branches of Synthetic Biology. As stated by Kelle (2009b):

> "It should be self-evident that the different subfields of synthetic biology have different kinds of security implications, which are already relevant or will become so at different points in time. Clearly, the potential security implications of synthetic genomics—with its capacity to generate rapidly large DNA molecules—are of more immediate concern than those of some future minimal cell construct that could act as a chassis for nefarious applications even further down the line" (Kelle, 2009b, p. 23).

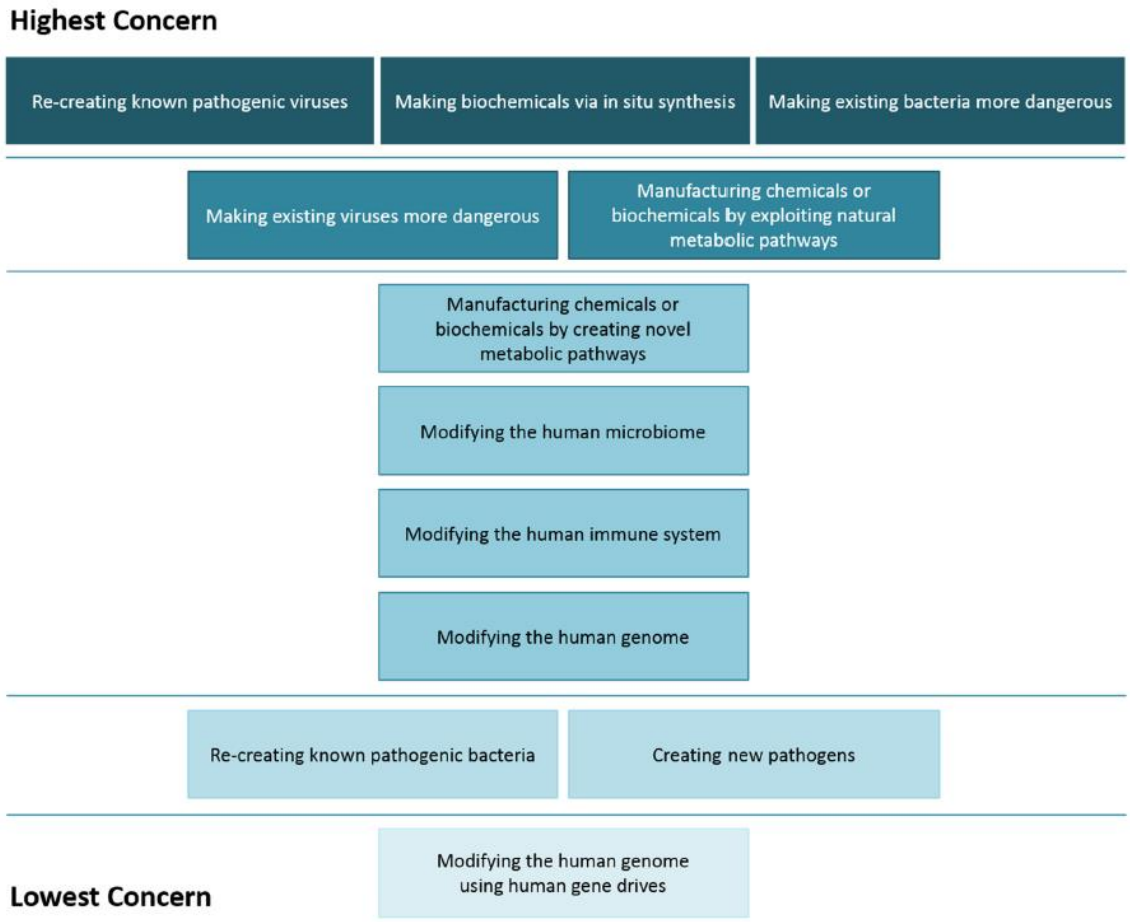

***Figure 18*. "Relative ranking of concerns related to the synthetic biology–enabled capabilities analyzed. At the present time, capabilities toward the top warrant a relatively higher level of concern while capabilities toward the bottom warrant a relatively low level of concern".** *Retrieved from National Academies of Sciences (2018).*

4.4.1 Synthetic genomics

The study by the NAS (2018) points to the possibility of re-creating known pathogenic viruses as the most important biosecurity concern raised by Synthetic Biology. The *de novo* synthesis of the poliovirus was achieved in 2002 (Cello et al., 2002), and the Spanish influenza virus was synthesized in 2005 (Tumpey et al., 2005). Today, breakthroughs made in this subfield, which have greatly increased our capacity for both DNA synthesis and assembly, mean that "building the genome of virtually any virus—either in the form of the genome itself for a DNA virus or as a cDNA of an RNA virus that can be transcribed into the viral genome—is now possible" (National Academies of

Sciences, 2018, p. 50). A notable example is the synthesis of the horsepox virus (as mentioned, closely related to the smallpox virus), which has been achieved very recently (Noyce et al., 2018).

The advances made by Synthetic Biology in DURC, especially with the recent synthesis of the horsepox virus, are a matter of concern for many in terms of biosecurity (Imperiale, 2018). To illustrate, Koblentz (2017) argues that:

> "The technologies and techniques used to resurrect the horsepox virus are directly applicable to the synthesis of variola virus. This research demonstrates that the risk of synthetic biology being used to generate a strain of smallpox that could be reintroduced into the human population has moved from being a theoretical possibility to a plausible threat to global health security. The reemergence of smallpox—due to a laboratory accident or an intentional release—would be a global health disaster" (p. 1).

Re-creating pathogenic bacteria is also a worrisome possibility, although it is much more challenging compared to that of re-creating viruses, since the larger size of the bacterial genome represents a considerable technical barrier; making it work successfully in a cellular container also constitutes a technical hurdle (National Academies of Sciences, 2018).

4.4.2 Bioengineering

This branch of Synthetic Biology poses a vast range of biosecurity issues. Accordingly, the report of the National Academies of Sciences (2018) includes the potential to make existing bacteria more dangerous and making harmful biochemicals via *in situ* synthesis in humans among the most disturbing capabilities enabled by Synthetic

Biology. Additional bioengineering-enabled possibilities that pose serious risks are the prospect of making existing viruses more dangerous or developing harmful chemicals or biochemicals by engineering natural metabolic pathways. Other possibilities that seem less feasible today, but must nevertheless be taken into account, are the possibility of manufacturing chemicals or biochemicals by designing and constructing novel metabolic pathways or modifying the human microbiome, the human immune system or the human genome (National Academies of Sciences, 2018).

4.4.3 Xenobiology

This branch of Synthetic Biology may also raise some biosecurity concerns, mainly in relation to the possibility of building genetic systems that are difficult to detect: "Cells with alternative DNA bases, codons, amino acids, or genetic codes may also be able to evade detection based on standard methods such as polymerase chain reaction (PCR), DNA sequencing, or antibody-based assays" (National Academies of Sciences, 2018, p. 190). Xenobiology could also contribute to the creation of radically new pathogens, although the NAS placew this possibility at "the extreme end of difficulty (and feasibility)" (National Academies of Sciences, 2018, p. 70).

4.4.4 DIY Synthetic Biology

Biotechnology is becoming increasingly accessible to a larger number of people, especially Synthetic Biology, which aims to simplify genetic procedures and enable access to science for students—as in the case of the iGEM competition, with 371 labs currently registered and a further 32 pending approval [23] —and non-professionals (DIYbio). This increases the risk of dual-use (Samuel, Selgelid, & Kerridge, 2009;

[23] https://igem.org/Lab_List

Schmidt, 2008), and challenges traditional security paradigms, making it "unlikely that regulatory devices such as professional codes, export controls, or classification will be effective in the context of a deskilled, deprofessionalized community of practitioners" (Evans, 2014, p. 272).

Miller and Selgelid (2007) predict that:

> "in the not too distant future a would-be terrorist will no longer need to go to an inhospitable region to find a naturally occurring pathogen such as Ebola, or to steal a highly virulent and transmissible pathogen such as smallpox from one of a very small number of very secure laboratories, or even to employ standard recombinant DNA techniques to enhance the virulence and transmissibility of some more readily available pathogen. Rather he or she could buy a bench-top DNA synthesizer and potentially use it to assemble a specified genomic sequence of a highly virulent and transmissible pathogen from readily available raw materials" (p. 525).

Evans and Selgelid (2015) argue that this approach to science makes regulation difficult, giving several reasons for the lack of governability of open-source biology (OSB): first, there is no hierarchy of control over the projects carried out, since the work is uniformly distributed; second, information is openly available online, which means a logistical challenge in the event that a restrictive regulation was to be applied; third, members of the OSB community are expected to oppose any kind of imposed restriction; fourth, an open-source project may lead to several projects arising from it, as groups or individuals diverge in their interests at some point. Also, malevolent users can benefit from the achievement of these projects; and finally, bottom-up regulation is unlikely to ensure the commitment of all members.

The US Federal Bureau of Investigation (FBI) has therefore involved itself in dialogue and cooperation with the DIYbio community, having sponsored the iGEM competition and various biotechnology conferences. This rapprochement is generally accepted among US biohackers, while in Europe they are rather more reticent to welcome this collaboration (Wolinsky, 2016).

However, Jefferson et al. (2014) have another perspective regarding the dual-use issues posed by Synthetic Biology. They argue that the focus commonly adopted on access to biological materials and digital information would be better placed on human practices and institutional dimensions. According to these authors, there are several assumptions (they call them "myths") generally made in discussions about Synthetic Biology and biosecurity that are not completely correct, and "portray speculative scenarios about the future as realities in the present or the near future, when this is not warranted" (p. 1). In broad terms, they argue that Synthetic Biology and current advances in DNA synthesis cannot really, for the time being, make it easy for anybody to engineer biology, let alone to construct biological weapons, and that the DIYbio movement is far from being able to offer anything useful for bioterrorists.

4.4.5 Biosecurity measures

Biosecurity measures in this field encompass prevention measures and measures to deal with an attack once it has already occurred (National Academies of Sciences, 2018). The former include the establishment of decision-making authorities and the development of norms, regulations and funding policies to promote security. As a specific measure to be implemented and regulated, restriction of dissemination of dual-use knowledge is a widely discussed possibility. Additionally, control of the synthesized, ordered and distributed genetic sequences is a rather widespread specific measure, whose regulation

is also being discussed. Finally, education and raising of awareness of dual-use risks are biosecurity measures suggested to aid prevention. Among the measures needed to deal with an attack already produced, strategies to recognize and attribute the attack and the development of consequence management capabilities have been suggested.

Establishment of decision-making authorities

The different options of decision-making authorities to regulate dual-use technologies are summarized by Miller and Selgelid (2007) in an extensive paper: 1) governmental control; 2) institutional control; 3) a hybrid of institutional and government control; 4) control by an independent authority; or 5) control by individual scientists. After evaluating the different possibilities, the authors argue that options 1 and 5 are not the most desirable, since they cannot provide an adequate balance between scientific freedom and biosecurity. Option 2 is less desirable than options 3 and 4 because it does not involve mandatory licensing of technology, mandatory education or mandatory personnel security regulation. Finally, according to the authors, both options 3 and 4 are the most ethically justifiable forms of governance.

The White House has developed two complementary policies regarding DURC: the March 2012 DURC Policy (US Government, 2012) and the United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern (US Government, 2014). These policies, in accordance with option 3 of Miller and Selgelid (2007), are based on the premise that a "comprehensive oversight system must include both the U.S. Government (USG) and institutional oversight processes" (US Government, 2014, p. 3) and acknowledge that "[i]nstitutional oversight of DURC is a critical component [...] because institutions are most familiar with the life sciences research conducted in their facilities and are in the best position to promote and strengthen the responsible conduct and communication of DURC" (US Government, 2014, p. 4). This

policy is limited to certain agents and toxins as well as to specific experiments, and the policy does not apply to institutions that do not receive US government funds for life sciences research (US Government, 2014).

This model is analogue to that developed in order to regulate oversight of recombinant DNA research. It is based on Institutional Biosafety Committees (IBCs) that must follow the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (NIH Guidelines) (NIH, 2016).However, the effectiveness of these Committees has been harshly questioned (Race & Hammond, 2008). Palmer, Fukuyama, & Relman (2015) criticize that the supervision responsibilities fall on the current institutions, since "[l]eadership biased toward those that conduct the work in question can promote a culture dismissive of outside criticism and embolden a culture of invincibility (p. 1472)". They argue that expertise in governance, risk management, and organizational behavior must take priority over scientific and technical expertise, and suggest the creation of "a high-ranking government official position, such as a special assistant to the president, and a coordinating committee, responsible for anticipating and managing risks associated with biotechnology. This committee would be independent from, but work in partnership with, funding bodies" (p. 1472). This model would be an hybrid of options 1 and 4 of Miller and Selgelid (2007).

Regardless of the decision-making authority established, it is generally argued that all stakeholders must have active involvement in decision-making processes (Colussi, 2013; Kelle, 2009b), in order to avoid biases or overly severe restrictions (Schmidt et al., 2009). In this vein, MacIntyre (2015) argues that collaboration between community, health, science, law enforcement and defense agencies is necessary to improve global biosecurity, while Kuhlau, Höglund, Eriksson, & Evers (2013) say that, to govern risks

under uncertainty, deliberation is needed between scientists, security experts and other stakeholders (i.e. public health authorities, ethicists and policy-makers).

Development of regulations and funding policies to promote security

Kelle (2009a) suggests a governance structure based on five policy intervention points (the 5P-strategy): the principal investigator (PI), the project, the premises, the provider of genetic material and the purchaser. The author points out two advantages of the 5P-strategy: First, it considers the research character of Synthetic Biology, overcoming one important limitation of the BWC, which does not take into account research on biological weapons. The 5P-strategy also completes and at the same time simplifies the proposal of governance of Garfinkel et al. (2007), which only covers one of the branches of Synthetic Biology (i.e. synthetic genomics) and focuses on other issues besides biosecurity. In order to identify potential biosecurity measures at each of the five proposed policy intervention points, the author provides a matrix in which these measures are mapped against these points (Figure 19). Different approaches to Synthetic Biology may require different policy measures.

However, for the moment, policy interventions are being very discreet, in particular with regard to the establishment of mandatory regulations and laws (Edwards, 2014). In this respect, Colussi (2015) finds that existing regulations against bioterrorism cannot be automatically extended to the case of Synthetic Biology in some instances. For example, the BWC does not include those branches of Synthetic Biology that produce systems that are fundamentally new, in the same way that the AG's rules only cover organisms that produce the toxins on the list.

Potential bio-security measures	Policy intervention points				
	Principal investigator	Project	Premises	Provider	Purchaser
Awareness raising					
Education/ training					
Guidelines					
Codes of conduct					
Regulation					
Natl laws					
International treaty/ agreement					

***Figure 19*. "Potential bio-security measures in the context of the 5P-strategy".** *Retrieved from Kelle (2009a).*

Furthermore, in the particular case of experiments involving the synthesis of dangerous viruses, it is suggested that additional biosecurity regulations must be developed (Koblentz, 2017). In the US, for example, as "biosecurity preparedness is largely based on access control to a specific list of regulated pathogens, this provides a workaround for nefarious actors" (DiEuliis & Gronvall, 2018, p. 7), and the "exemption of life sciences research that is privately funded is a large, and growing, loophole in the oversight system" (Koblentz, 2018, p. 8). Koblentz (2017) suggests some measures to be applied in this field: to shape an international legal prohibition against the possession of variola virus, with actions such as the enshrinement of the recommendations of the Advisory Committee for Variola Virus Research (ACVVR) on the handling and synthesis of variola virus DNA into international law; to extend World Health Organization (WHO) oversight not only to smallpox but also to other orthopoxviruses; several suggestions for the DNA synthesis industry; and the establishment of legislative measures by national governments, such as the criminalization of possession and synthesis of variola virus; etc.

Different proposals have been issued for the governance of biosecurity risks specific to Synthetic Biology:

- The National Science Advisory Board for Biosecurity (NSABB) (2006) gives some recommendations regarding the synthesis of Select Agents (biological agents and toxins that are listed as potentially posing severe health threats), including the development and dissemination of "harmonized guidance to investigators and nucleic acid/gene/genome providers" (p. 10); the governmental imposition of certain charges to the relevant federal agencies (e. g. "develop a process to be used by providers of synthetic DNA for determining the sequences for which to screen" [p. 11], "develop and promote standards and preferred practices for screening orders and interpreting the results, and require that orders be screened by providers" [p. 11]; "develop standards and practices to be used by providers for retaining records of orders" [p. 11]); or international dialogue and collaboration in this regard.

- The NSABB (2010) recommends that: "[s]ynthetic biology should be subject to institutional review and oversight since some aspects of this field pose biosecurity risks" (p. 13); "[o]versight of dual use research should extend beyond the boundaries of life sciences and academia" (p. 13); "[o]utreach and education strategies should be developed that address dual use research issues and engage the research communities that are most likely to undertake work under the umbrella of synthetic biology" (p. 13); and "[t]he US Government should include advances in synthetic biology and understanding of virulence/pathogenicity in efforts to monitor new scientific findings and technologies" (p. 14).

- Garfinkel et al. (2007) suggest some policy options to enhance both biosafety and biosecurity in DNA processes. These options are divided into three groups according to the intervention point: commercial firms that sell synthetic DNA to users (e.

g. software requirements, client verification or information storage); owners of laboratory 'bench-top' DNA synthesizers (e. g. machine registration, or license requirements); and the users of synthetic DNA as well as their institutions (e. g. education about risks and best practices, extension of the review responsibilities of IBCs or the inclusion of external oversight).

- Maurer, Lucas, and Terrell (2006) give some options that can be implemented through community self-governance, such as the adoption of best practice screening procedures by all commercial gene synthesis houses, the improvement of watch lists and software tools for screening, providing free biosafety and biosecurity expert advice, the investigation and reporting of dangerous behavior, etc.

- Bügl et al. (2007) identified some options for governance of DNA synthesis: development of minimum standards for screening and reporting, development of government points of contact worldwide, development of standards for record keeping, and requirement of industry and consumer best practice as a condition for receiving research funding. They also recommend future research on improving screening software and on the transition from screening based on lists of specific agents to specific sequences.

- Friends of the Earth, CTA, and ETC Group (2012) advocate "mandatory synthetic biology-specific regulations" (p. 4), which "should specify civil and criminal penalties for violations" (p. 4).

- The National Academies of Sciences (2018) argue that strategies such as norms and self-governance, voluntary guidance, regulations, and international bans are useful but insufficient with respect to the misuse of Synthetic Biology, and provide some recommendations to the U.S. Department of Defense (DoD): "continue exploring strategies that are applicable to a wide range of chemical and biodefense threats" (p. 157); "evaluate the national military and civilian infrastructure that informs population-based

surveillance, identification, and notification of both natural and purposeful health threats" (p. 157-158); and "consider strategies that manage emerging risk better than current agent-based lists and access control approaches" (p. 158).

- Schmidt (2009) proposes to establish measures to regulate the actions of biohackers, such as laws, codes of conduct, voluntary measures, access restrictions to key materials, institutional embedding and mandatory reporting to IBCs.

Restriction of dissemination of dual-use knowledge

With respect to this possibility, the modification of the highly pathogenic H5N1 avian influenza virus for transmission between ferrets (Herfst et al., 2012; Imai et al., 2012) is an illustrative case of the conflict that can sometimes arise between the values of knowledge, freedom and openness in sciences, and the risks of disseminating dangerous knowledge (Kuhlau et al., 2013). Research on the H5N1 influenza virus led the NSABB to ask *Nature* and *Science* (the two journals where the research was to be published) to censor part of the content of the articles. It recommended "that the general conclusions highlighting the novel outcome be published, but that the manuscripts not include the methodological and other details that could enable replication of the experiments by those who would seek to do harm" (NIH, 2011). The alleged benefits of this research were that "it will help public health officials understand, detect, and defend against the emergence of H5N1 virus as a human threat, a development that could pose a pandemic scenario" (Collins, 2012), but three factors made its release very risky: the high death rate of H5N1, seasonal flu's rapid transmission, and the uncertainties regarding the possibility of developing effective vaccines and drugs. If the airborne infection that had been obtained in ferrets also occurred in humans, it would be a catastrophic epidemic (Ledford, 2012). This was the first time ever that a journal was asked not to publish an article on the basis of biosecurity, since the risks of misuse were believed to be very high.

This recommendation received criticism from both those who opposed any form of restriction of scientific activity and those who thought it was too soft. Although the NSABB specified that some selected researchers could have access to the full details of the research, there was no guidance on how this would be implemented. Therefore, *Nature* and *Science* stated that they would wait until this problem was solved to make a decision (Ledford, 2012). Finally, in March 2012, the NSABB recommended the publication of the revised articles, although further clarifications should be made in the manuscript submitted to *Science* (NSABB, 2012). Thus, one article was published in *Nature* on 2 May 2012 (Imai et al., 2012) and the other in *Science* on 22 June 2012 (Herfst et al., 2012).

In this regard, Douglas and Savulescu (2010) contend that the risk that knowledge from Synthetic Biology will be misused is the most important issue for bioethicists studying this field. The authors consider that, despite the potential benefits of Synthetic Biology, the possibility of misuse is associated with a number of risks dangerous enough to doubt whether the pursuit and dissemination of some knowledge from this discipline is right at all. They quote Selgelid (2007) to assert that the misuse of knowledge from Synthetic Biology could be more dangerous than that from nuclear technology, given that Synthetic Biology is likely to become quite cheap and that there is a tradition of open access in life sciences that is not present in nuclear technology research. Thus, the authors suggest developing an 'ethics of knowledge' to deal with the dual use dilemma. It would not merely focus on the ethics of how scientific knowledge is produced, but also on the ethics of pursuing and disseminating certain kinds of knowledge. The authors reject arguments against limiting knowledge dissemination by concluding that although downstream solutions, once the knowledge has been disseminated, may often be useful, we can-not be sure that these strategies will work in all cases; it is debatable that scientists

are never morally responsible for the subsequent use of the knowledge they produce, but even if that was true, it would not imply that any investigation was morally right; and even if knowledge has an intrinsic value, its instrumental value must be also considered.

In the same vein, Kuhlau et al. (2013) reason that "compromises may be justified when scientific knowledge threatens other important values, such as the right to health and security. To protect these values, the professional responsibility to do no harm may supersede the responsibility to do good" (p. 12). The authors propose the implementation of an ethics of dual-use knowledge dissemination, which should include three aspects:

> "(i) dual-use awareness, enabling identification of a dual-use dilemma; (ii) precaution, enabling reflection and cautious behavior in situations where dissemination of knowledge may pose serious risks of harmful outcomes; and (iii) acknowledging conflicting values, prompting a recognition that potential harm in certain research circumstances may outweigh expected benefits" (p. 14).

Resnik (2013) maintains that restrictions on publication may be warranted if there are objective probabilities (based on statistical frequencies, mathematical modeling, or scientific analyses) that potential risks outweigh the potential benefits. If there are no objective probabilities for different outcomes, then we cannot use risk-benefit assessment, since it would be based on subjective guesses, which are susceptible to biases and for which there is often insufficient evidence. The author suggests that different strategies should be used for making decisions under ignorance, and recommends the precautionary principle: We should take reasonable measures to avoid, minimize or mitigate serious and plausible harms. According to him, a "measure is reasonable if it balances the different values at stake fairly, is proportional to the nature of the threat, and is effective" (p. 8).

The author also acknowledges that our assessment "depends on how we weigh and consider the different values at stake" (p. 8) (scientific openness, knowledge advancement, respect for autonomy vs. preventing harm to individuals and society, protecting intellectual property or proprietary information). In this case, reasonable measures can range from classification or censorship to publishing papers in redacted form or recommending full publication, depending on the values under threat. With respect to the possibility of making the full papers available to only some selected individuals, the author states that there is no system available for this purpose, and developing it may be difficult: "Government agencies, journals, and scientists from different nations should work together to make redacted publication a viable option for dealing with papers that raise DURC issues" (p. 13).

Similarly, Colussi (2013) argues that censorship might be an appropriate option for those publications whose potential harms outweigh potential benefits.

Control of the distributed genetic sequences

The control of the synthesized, ordered and distributed genetic sequences is a rather widespread specific measure. Different guidelines are available that provide screening frameworks to assist providers in the identification of dangerous genetic sequences.

Thus, the US government developed a guidance document that provides a screening framework to assist providers of synthetic DNA to identify requests of concern, 'Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA' (Department of Health and Human Services [HHS], 2010). According to the guidance, this framework should include both sequence screening and customer screening, and if either of these raises any suspicions, follow-up screening should also be carried out.

Two separate industry groups also published different guidelines in 2009. The International Association Synthetic Biology (IASB) issued 'The IASB Code of Conduct for Best Practices in Gene Synthesis' (IASB, 2009) and the International Gene Synthesis Consortium (IGSC) published 'Harmonized screening protocol: gene sequence & customer screening to promote biosecurity' in 2009 and updated it in 2017 (IGSC, 2017).

The approach of the US government is based on an automated procedure, which matches the requested sequences with the so-called Select Agent list, a list of biological agents and toxins which have been determined to be potentially harmful (HHS & U.S. Department of Agriculture [USDA], 2017). In contrast, the approach of the IASB and the IGSC requires human screeners to participate in the process of identification of dangerous sequences. The first approach is substantially faster and cheaper, but is also less thorough (Fischer & Maurer, 2010; Kaebnick, 2010).

However, this surveillance strategy is not easy. As argued by Samuel et al. (2009), DNA synthesis companies "generate a vast number of different gene sequences—most of which are not associated with biosafety or biosecurity risks—and the additional overheads would almost certainly raise the costs of DNA synthesis. Moreover, oligonucleotide sequences are short, 'non-specific' and difficult to definitively link to pathogenic sequences" (p. 10). Therefore, several improvements in screening processes are claimed to be necessary (Bügl et al., 2007; Garfinkel et al., 2007).

In this regard, Schmidt et al. (2009) suggest three technical solutions: the cooperation of DNA synthesis companies in screening the ordered sequences, the improvement of technical means for DNA screening, and a future balance between security gains and feasibility. DiEuliis, Carter, and Gronvall (2017) also suggest other potential solutions, such as refinement of databases, economic support by the government

to companies for screening, establishing requirements for companies that receive federal funding, investigating different aspects of the current screening situation, or the creation of an international secretariat devoted to these issues. In this vein, a program by the Office of the Director of National Intelligence, called Functional Genomic and Computational Assessment of Threats (Fun GCAT),

> "intends to develop next-generation computational and bioinformatics tools to improve DNA sequence screening, to augment biodefense capabilities through the characterization of threats based on function, and to advance our understanding of the relative risks posed by unknown nucleic acid sequences. These tools will enhance the ability to computationally and functionally analyze nucleic acid sequences, ascribe threat potential to known and unknown genes through comparisons to the functions of known threats, and facilitate the ability to screen and identify sequences of concern, including genes responsible for the pathogenesis and virulence of viral threats, bacterial threats, and toxins" (IARPA, n.d.).

The program has brought in researchers from Battelle Memorial Institute, Harvard University, Signature Science, SRI International and Virginia Tech.

Education and raising of awareness of dual-use risks

Sture and Whitby (2012) suggest education and awareness-raising as two indispensable strategies to prevent the misuse of science, pointing to these measures as a more desirable alternative than legislative restriction, which can have a negative impact on scientific innovation and autonomy.

Despite the relevance of these issues, the biosecurity of Synthetic Biology was not deliberated when Synthetic Biology first emerged, but took a while to materialize and did so mainly in the US. In order to develop an active debate in Europe as well, it was necessary to increase awareness of the dual-use character in Synthetic Biology, which was considerably low among European scientists (Kelle, 2007). For this reason, the biosecurity section of the SYNBIOSAFE e-conference posed the question of how to increase biosecurity awareness among Synthetic Biology practitioners. In response to this question, education was broadly accepted as the tool needed to raise the level of awareness (Schmidt et al., 2008).

Several proposals have been made in this regard, sometimes for the particular case of Synthetic Biology and at other times for new biotechnologies in general:

Edwards and Kelle (2012) propose several strategies to be applied at different levels: i) At the level of individual Synthetic Biology practitioners: biosecurity education for university level students and courses adapted to the background of synthetic biologists should be introduced; ii) At the institutional level: ELSI (Ethical, Legal and Social Implications) and dual-use issues should be included in the Synthetic Biology curriculum; iii) Both at the institutional level, and from a funding agency perspective, priority should be given to those projects that incorporate upstream engagement of social science expertise and the wider public; and iv) Nationally and internationally, funding decisions should be embedded in policies that take into account dual-use risks.

High school bioethics and biosafety education has been also suggested (Evans, 2014), as well as continued professional development to maintain engagement with biosecurity issues (Novossiolova & Sture, 2012).

Revill et al. (2012) concluded, on the basis of different dual-use educational experiences, that "there is no 'one size fits all' approach to the implementation of dual-use education. Rather, initiatives must be tailored to suit the teaching traditions, geographical and historical context in which they are being delivered" (p. 31). In relation to this, Sture, Minehata, and Shinomiya (2012) propose that "each state should produce a national biosecurity action plan, with accompanying resources and materials" (p. 85), and provide a model and guidance to implement it.

Online resources have also been developed to help biosecurity stakeholders become familiar with the implications of the BWC (Bollaert & Whitby, 2012). Moreover, it is also necessary to act in the public sphere, in order to prevent a future backlash of public opinion against Synthetic Biology (Samuel et al., 2009).

Strategies to recognize and attribute the attack

The NAS report argues that Synthetic Biology poses some challenges to the current strategies for determining if a health threat has arisen naturally or by means of an intentional attack and, in the second case, to attribute the attack to the actor responsible. These strategies include epidemiology, laboratory diagnostics, environmental monitoring, disease surveillance and agent identification. Among the new challenges that Synthetic Biology poses in this regard, the report identifies the possibility of developing bioweapons that produce health effects that cannot be immediately associated with a disease outbreak or attack (e. g. by reducing immunity or modifying the human microbiome). Additionally, Synthetic Biology could produce pathogens or toxins that are very different from any known natural agent, making it difficult or even impossible to identify the cause in a biological attack due to lack of a comparator (National Academies of Sciences, 2018):

> "it would not be possible to act to mitigate or contain an outbreak until patients have developed symptoms that trigger a health community response; as a result of this delay, people would become ill before it is possible to know that an attack has occurred" (p. 129).

In this regard, MacIntyre (2015) explains that, in recent years, infectious diseases such as Ebola, Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and avian influenza have emerged following suspicious patterns, although natural emergence has always been assumed. In 1984, 751 people became ill with *Salmonella* in Oregon in the US, in what was initially believed to be a food-borne outbreak caused by unsanitary food handlers. However, a local politician accused a local religious cult of deliberately contaminating salad bars. Health authorities did not believe him, but six months later, the leader of the cult confessed to the attack. The case did not appear in the medical literature until 13 years after the incident (Török et al., 1997).

The National Academies of Sciences, Engineering, and Medicine state that "[a]dditional tools that enable one to detect that a sequence had been genetically manipulated, or tools to analyze features of a sequence or a resulting organism that contribute to actor attribution, would be valuable additions to mitigation strategies" (National Academies of Sciences, 2018, p. 134), and identifies advances in next-generation sequencing as "[o]ne of the most significant developments for identifying agents" (National Academies of Sciences, 2018, p. 135).

Development of consequence management capabilities.

Existing medical countermeasures (i.e. vaccines, drugs, or antibody-based treatments) may be useless against new engineered organisms. Research in this regard is therefore needed. However, Synthetic Biology itself can provide the necessary

countermeasures, given its potential application for diagnostics, vaccine development, drug discovery and drug production (National Academies of Sciences, 2018).

4.4.6 Application of personalist principles to biosecurity issues

The personalist principles with implications in this area are the same as those with implications for biosafety: the *principle of protecting the life and genetic identity of every human individual*, the *principle of protecting the ecosystem and the environment*, and the *principle of the competence of the community*. Nevertheless, these implications have their own specificity for each of the two fields.

In the case of biosecurity, the *principle of protecting the life and genetic identity of every human individual* and the *principle of protecting the ecosystem and the environment* urge the approach of the risks for the human beings, other organisms or the environment that could derive from a bad use, particularly if it is intentional, of this discipline.

Therefore, the establishment of binding legislations that not only penalize the misuse of this technology, but also ensure the cautious development of dual-use technologies, is necessary. Regarding the establishment of decision-making authorities, specific models can-not be derived from these principles, and a recommendation can only be made to establish a model capable of safeguarding these principles. Self-regulation by scientists alone thus does not seem sufficient, and a model that allows for a legal regulation is needed. Additionally, although specifications such as compiling a list of dangerous agents or controlling funding have great practical utility, regulations should expand their range of action enough to cover the possibility of misuse of Synthetic Biology that involves unknown biological agents, or institutions or researchers that do not receive public funds, including amateur scientists. In this regard, regulation in this

field should be not only at national, but also international level. In the words of Heavey (2012),

> "[w]hile strong regulation at the national level, in the countries where synbio is at its most advanced, is necessary, the mobility of research means that such regulation alone may, paradoxically, lead to looser, more permissive regulations for the field overall, as some research moves to places where regulation is least. Ethically contentious research is most likely to follow this path.Therefore, regulation needs to be worldwide in scope" (p. 64).

According to the personalist framework, an oversight strategy to follow DIYbio research is also needed. This movement has great potential to improve science education and, although its capacity to produce scientific breakthroughs remains unclear, it is apparent that it can inspire innovation in unprecedented ways. However, the risk of misuse of the means made available by this movement to citizens cannot been ignored. Government oversight could prevent unfortunate consequences, and would not have to imply the cessation of this movement.

Additionally, the pursuit and dissemination of knowledge may sometimes be too risky. In that case, it must be restricted according to the personalist framework, since, as explained above, the principle of freedom is at the service of the principle of defense of human physical life, and the former may never jeopardize the latter.

With regard to the *principle of the competence of the community*, all the stakeholders, including the public, must participate in important decision-making processes. Therefore, scientists must inform society about the factors involved in biosecurity, such as the risk of misuse (biological terrorist attacks or bio-war), the

advances made on safety systems, etc. Once the public and other stakeholders are well informed, they can make significant contributions in the debate about these issues.

4.5 TRANSHUMANISM

So far, Synthetic Biology has mainly focused on microorganisms. Nevertheless, the possibility of applying Synthetic Biology to humans has already been proposed by leading representatives in this field. Thus, Drew Endy has suggested that it offers the possibility of skipping evolution by designing our own offspring (Specter, 2009), while George Church has written that it could be used to obtain virus-proof humans, or to bring Neanderthals back to life (Church & Regis, 2012).

For now, however, the most that can be expected in this area is the complete synthesis of a human genome and its introduction into a cell, as pursued by the HGP-write, which could still take several years to occur (Servick, 2017). This project has raised several ethical questions:

> "For example, could scientists synthesise a modified human genome that is resistant to all natural viruses? They likely could, for purely beneficial purposes, but what if others then sought to synthesise modified viruses that overcame such resistance? Might doing so start a genome-engineering arms race? And, what of even greater changes that can be imagined? In a world where human reproduction has already become a competitive marketplace, with eggs, sperm and embryos carrying a price, it is easy to make up far stranger uses of human genome synthesis capacities. Would it be OK, for example, to sequence and then synthesise Einstein's genome? If so how many Einstein genomes should be made and installed in cells, and who would get to make them? [...] Given that human genome synthesis is a technology that

> can completely redefine the core of what now joins all of humanity together as a species, we argue that discussions of making such capacities real, like today's Harvard conference, should not take place without open and advance consideration of whether it is morally right to proceed. Pluralistic, public, and deliberative discussions are instead the best appropriate way to frame paths forward" (Endy & Zoloth, 2016, p. 1-2).

The branches of Synthetic Biology that have implications related to this issue are mainly bioengineering and synthetic genomics, to the extent that the human genome could be modified or artificially synthesized. An even more remote possibility is to introduce non-canonical bases into our genome, which would correspond to the branch of Xenobiology. Accordingly, some potential scenarios must be taken into account when considering the use of Synthetic Biology techniques to modify the human genome: using it with the aim of improving humans, or to develop what has been called *subhumans*, humanoid organisms that would serve purposes such as being sources of transplantable tissues and organs, experimental subjects or crash test dummies, and to neutralize landmines (Newman, 2012).

From personalism, the principle of *protecting the life and genetic identity of every human individual* and the *therapeutic principle* establish that it would only be lawful to modify the human genome when it is necessary for the treatment of a disease. Therefore, modification of the genetic composition of human populations in order to promote the reproduction of more desirable traits or to decrease or eliminate some capabilities is rejected from consideration as an ethically acceptable option. The *principle of the competence of the community* comes also into play here. In this regard, transparent and accountable communication must be encouraged, as well as the establishment of effective

dialogue platforms that allow the contributions of various participants, from both specialized areas and the general public.

In this regard, Douglas, Powell, & Savulescu (2013) state that:

> "if synthetic biologists did manage to construct a human embryo entirely from scratch, and this developed into a human person, that person would, intuitively, be entitled to the same rights and privileges as another person, despite her curious origin. What matters, again, is not origin, but mental capacity" (p. 695).

From a personalist perspective, however, a synthetic human would be a person by virtue of their dignity, not of their mental capacity. Additionally, in terms of their interests, the same criteria are not applied when speaking of human beings or non-human beings, as prescribed by the *principle of the ontological and axiological difference between man and other living beings*. Even if a human being has very limited mental capacities, he is subject to the same rights and privileges as other humans. The same would be true in the hypothetical case of synthetic humans.

This special case alerts us to the risk of viewing synthetic organisms as machines. Using the term machine to label synthetic organisms is not only incorrect but also dangerous. If synthetic organisms are deemed machines, then no moral considerations are necessary when dealing with them. Thus, in the case that *subhumans* are obtained, their interests would not be taken into account, and they could be used as a mere means to serve the purposes of normal humans. However, as has been argued in the section on the moral status of synthetic entities (Section 4. 2), they would not be machines, but organisms. Moreover, regardless of the changes made to their genome, they would still

be humans. Consequently, the term *subhuman* is unwarranted, and both its meaning and underlying objectives are contrary to human dignity.

4.6 OTHER ETHICAL ISSUES RELATED TO SYNTHETIC BIOLOGY

4.6.1 Justice

Justice has been deemed as "key to the ethics of Synthetic Biology" (Capurro et al., 2009, p. 68). Additionally, the PCSBI defines the 'principle of justice and fairness' for its ethical assessment of Synthetic Biology, establishing that "every nation has a responsibility to champion fair and just systems to promote wide availability of information and fairly distribute the burdens and benefits of new technologies" (PCSBI, 2010, p. 5). 'Distributive justice' and 'procedural justice' have also been identified as suitable principles to guide the development and regulation of this discipline (Bubela, Hagen, & Einsiedel, 2012). The former relates to the distribution of burdens and benefits, while the latter relates to the participation of all interested parts in decision-making processes.

An important appreciation in this regard is that in communities with traditional economic models, the irruption of innovative models, such as the case of the bioeconomy, based on scientific progress, can be a detriment to their subsistence (Bellver, 2016).

The *principle of the competence of the community* has implications in this regard, since it not only establishes the participation of the different stakeholders in decision-making, but also the need to promote the interest of the common good. This idea is better developed in the *principle of sociality and subsidiarity*. The *principle of sociality* "commits each individual person to self-realization through participation in achieving the good of their neighbours [...] promoting the common good by promoting the good of each

individual" (Sgreccia, 2012. p. 182). According to the *principle of subsidiarity*, "the community on the one hand must help more where the need is greater [...], and on the other hand must not supplant or replace the free initiatives of individuals and groups" (Sgreccia, 2012. pp. 182-183). Given the relevance of the content of this principle to the subject in question, and that, in the initial list of principles, the *principle of sociality and subsidiarity* is independent of that of *freedom and responsibility* (from which the *principle of the competence of the community* is born), it seems appropriate to include the *principle of sociality and subsidiarity* as a sixth principle in the list of principles developed for genetic engineering.

According to this principle, justice considerations have implications for the global distribution and regulation of biosafety and biosecurity risks, as well as for the distribution of benefits, which should not be an obstacle for the economy of developing countries (Wellhausen & Mukunda, 2009) - quite the opposite, in fact.

4.6.2 Public perception and communication

The ethical questions posed by Synthetic Biology mean that the need to inform the public about this discipline was included in the debate on its societal issues, to avoid unwarranted public reactions opposed to its progress (Schmidt et al., 2008). Interestingly, in a review of the reports on Synthetic Biology prepared by different organizations with the objective of informing public opinion, a markedly unilateral presentation of the phenomenon has been observed, always positioned in a concrete vision and without presenting confronted views (Bellver, 2016).

A high level of social ignorance of this scientific field was found, both in the US and in Europe, where, about 10 years ago, more than 80% of the population had heard little or nothing about Synthetic Biology (Gaskell et al., 2010; Hart Research Associates,

2008; Kahan, Braman, & Mandel, 2009; TNS Opinion & Social, 2010). However, the degree of social awareness of this field has increased slightly, at least in the US (Hart Research Associates, 2013). When the participants of the respective surveys were asked about their main concern, the possible risks received the highest score in both territories (Gaskell et al., 2010; Hart Research Associates, 2008).

Interestingly, when European respondents were asked about their approval of Synthetic Biology, 21% did not approve of it, except under very special circumstances, while 17% strongly disapproved (TNS Opinion & Social, 2010). Similarly, 33% of US respondents supported a temporary ban on the discipline until its potential consequences are better defined (Pauwels, 2013).

In this regard, the *principle of the competence of the community* is highly relevant. This principle states that "populations need information and share in responsibility" (Sgreccia, 2012. p. 323). However, as shown, social ignorance of Synthetic Biology is still very high. An informative endeavor must therefore be made to increase public awareness of this field. In this regard, coverage of the topic by the media might be crucial. Exaggerations and fragmentary pictures should consequently be avoided in order to contribute to the development of a realistic view among the public (Bubela et al., 2012). In this sense, it is important to be careful with the language used, so that it reliably describes the reality that is being discussed. Metaphors in this field may have descriptive utility, but they can also lead to confusion (Braun, Fernau, & Dabrock, 2018). As Pauwels (2013) explains,

> "[t]he exploration of the role of mental representations and language in the construction of scientific reality has important implications for policy and public communication. Comparing living organisms to computers implies that we have an understanding of and control over the function, reliability,

and purpose of living organisms. This is a misleading perception that contradicts what experts in biological complexity have attempted to express—the notorious complexity and context dependency of biological systems and the delicate balance that needs to be struck for these systems to be viable" (p. 88).

Importantly, public information should not be conceived with the aim of shaping a directed opinion, but of providing the necessary information on which to generate critical thinking in the population, which includes information on both the scientific discipline and the values at stake.

4.6.3 Intellectual property rights

Discussions regarding this issue center around whether an intellectual property (IP) frame or an open access frame is more appropriate for the case of Synthetic Biology (Saukshmya & Chugh, 2010; Schmidt et al., 2008; van den Belt, 2013).

"The first frame holds that intellectual property rights like patents, copyright and plant breeders rights are a just reward for those who have expended creative effort in realizing inventions, artistic works and other innovative products and that the prospect of such exclusive rights constitutes an indispensible incentive for future innovative activities. The adherents of this frame also assume that you cannot have too much of a good thing too readily, so that if intellectual property is good, more intellectual property is even better. The second frame questions the assumption that exclusive rights are always indispensible for invention and innovation by referring to the contrary experience with free and open-source software in recent decades. It also points to the importance of access to existing knowledge and information as

> essential inputs for further innovation. Its adherents finally hold that human rights (like the right to health, to adequate food, to education and to participation in cultural life and scientific advancement) should never be subordinated to the protection of IP rights. The first frame has dominated the past three decades, but the second frame is in the ascendant" (van den Belt, 2013, p. 88).

Several authors advocate an intermediate situation, in which a patent system is maintained but redefined to favor innovation and research and development (R&D) within Synthetic Biology (de Miguel, 2016; García-Llerena, 2016; Minssen, Rutz, & van Zimmeren, 2015; Rai & Boyle, 2007; Saukshmya & Chugh, 2010), although more elaborate proposals on this idea are scarce. In this vein, the implementation of additional protection systems has been proposed. For example, a report summarizing the recommendations given in an expert meeting on "Synthetic Biology & Intellectual Property Rights", organized by the Danish Agency for Science, Technology and Innovation, concluded that:

> "an optimization of the current patent system and a better governance of granted patent rights are necessary to unleash the full potential of SB [Synthetic Biology]. Although in theory patenting research results does not limit access to the actual information (i.e. disclosure is actually an important requirement of patent law), solutions that could facilitate transparency, access and use of the patented technology could contribute to stimulating R&D and innovation in SB. In proposing creative and innovative solutions, the interests of the different stakeholders involved in SB should be taken into consideration. While patents will remain a crucial aspect of SB, policymakers, legislators and the SB community should also re-consider the

> governance and legal framework for other IPRs that will become increasingly significant for SB, such as trademarks, copyrights and trade secrets" (Minssen et al., 2015, p. 241).

The *principle of the competence of the community* states that the scientific research must take into account the common weal. A very restrictive patent system would benefit only those institutions or private companies involved in the inventions, and would slow down the progress of Synthetic Biology. On the other hand, a completely open access to research could have the same effect by causing inventors to lose the innovative impetus or be reluctant to disclose their results. Accordingly, it seems that both systems must co-exist in order to guarantee the maximum development of Synthetic Biology, as proposed by Saukshmya and Chugh (2010). The specification of this system is by no means simple, and requires the nature of the various products that can be obtained from this discipline to be taken into account. This goes beyond the scope of this work, but it is important to reaffirm the relevance of the *principle of the competence of the community* in this matter (or, even better, the *principle of sociality and subsidiarity*, as explained above). Additionally, the relationship between this issue and the issue of justice is worth noting. In the words of van den Belt (2013):

> "[t]he stakes in this contest are high as issues of global health and global justice are implied. Patents are not simply to be seen as neutral incentives, but must also be judged on their effects for access to essential medicines, a more balanced pattern of innovation and the widest possible social participation in innovative activity. We need moral imagination to design new institutional systems and new ways of practising SB that meet the new demands of global justice" (p. 87).

4.7 CONCLUDING REMARKS

This thesis aims to provide some answers to the ethical questions that arise in the field of Synthetic Biology from a specific ethical framework, personalism, specifically ontological personalism. In the previous sections, each of the issues identified is addressed in detail. Below, the implications of the personalist principles for each of them are summarized, the discussion is continued in more depth by evaluating the different branches of Synthetic Biology separately and directions for future research are outlined.

4.7.1 Personalist assessment of the bioethical issues raised by Synthetic Biology

Concept of life and its creation

Since the objective of obtaining new forms of life is characteristic of Synthetic Biology, questions have been raised regarding the impact of this discipline on the concept of life and its creation. In bioengineering, the view of life "as a property of living organisms and the source of potential useful applications" (Deplazes-Zemp, 2012) could lead to the use of synthetic organisms without taking into account their interests, considering them as mere tools, which is contrary to the *principle of protecting the ecosystem and the environment*. Xenobiology, synthetic genomics and protocells could also lead to viewing novel organisms as machines. However, this is only a possibility, not an inevitable direct consequence of the scientific approach. Moreover, it is predictable and avoidable.

Synthetic genomics raises questions about the advisability of defining the concept of life based on the essential genes, since it could lead to a bias in our understanding of cells, or to question what constitutes human life. Thus, concerns in this sense do not revolve around the scientific fact, but around the interpretation that may derive from the

knowledge obtained. According to personalism, human life begins at conception, when a human sperm fertilizes a human oocyte. The *principle of protecting the life and genetic identity of every human individual* obliges us to protect the life of the human individual from then on. Nevertheless, it is not necessary to resort to personalism to find objections to defining the concept of life based on the essential genes, since there are other elements that participate in the life process.

Protocell Synthetic Biology aims to produce living organisms completely from scratch, that is, from inanimate materials. This could have an impact on the concept of life and its creation, although it seems more appropriate to use the terms *designing*, *constructing* or *recreating* life instead of *creating*. Regardless of the impact of protocells on the concept of life and its creation, though, the *principle of protecting the ecosystem and the environment* and the *principle of protecting the life and genetic identity of every human individual* must be respected.

In spite of the functional or compositional novelties of the products of xenobiology, its composition is not expected to be exclusively artificial. Taking into account these considerations, the approach of the questions on the concept of life and its creation raised by xenobiology can be referred to the discussion exposed for the case of protocells.

In conclusion, regarding the question of the impact of Synthetic Biology on the concept of life and its creation, no branch of this discipline was found to be inevitably contrary to personalist principles. However, mechanistic or reductionist interpretations of the concept of life, which could derive from these approaches, could threaten the values defended by the *principle of protecting the ecosystem and the environment* or even the *principle of protecting the life and genetic identity of every human individual*.

Moral status of synthetic entities

Among the ethical issues that arise from the emerging field of Synthetic Biology, the definition of the moral status of its products is key to ensuring that both research and applications derived from this discipline are carried out in an ethically acceptable way. Given their artificial origin, purpose or development, the debate focuses on the classification of synthetic entities as living beings or machines. From personalism, the most relevant principle here is the *principle of protecting the ecosystem and the environment*, which states that man is not only the beneficiary, but also the steward of the world and, in particular, of other living creatures.

Based on the principle of *the ontological and axiological difference between man and other living beings*, the concepts of moral status (meaning the moral implications arising from human dignity) and moral value (meaning the quality of an entity that implies that its treatment as a mere means by a moral agent [a person] has negative moral implications) are distinguished. According to this terminology, only humans have moral status, while all organisms, as well as ecosystems, have moral value.

Therefore, in order to determine if synthetic entities have moral value, the question of whether they are organisms or machines must be addressed. Defining the concept of life is beyond the scope of this work, but it can be argued that those synthetic entities that retain the characteristics commonly recognized in life (Ganti, 2003; Koshland Jr, 2002; Maturana, 1975; Nicholson, 2013; Razeto-Barry, 2012) can be considered organisms regardless their origin and the human purposes involved in their production. Thus, a cloned individual, whose origin could be considered artificial, or an individual with their genome modified to satisfy a human purpose, does not cease to be perceived as living individuals. Others are the characteristics that seem to define what is alive.

Thus, according to the *principle of protecting the ecosystem and the environment*, synthetic organisms, as living beings, have moral value: they are intrinsically valuable and their interests must be taken into account.

By comparing the contributions of other authors in this field from the standpoint of personalism, some additional conclusions can be derived. First, considerations about the possibly wronged flourishing of synthetic organisms because of their design have no moral relevance from our point of view. Secondly, the characteristics of synthetic organisms must be observed in order to determine their requirements and preserve them as far as possible. Thirdly, an important point is the case that there is no moral duty to safeguard the conservation of synthetic species, since they are not part of the biodiversity that must be protected under the *principle of protecting the ecosystem and the environment*. Finally, the primacy of the person must always be observed, taking into account that, from our ethical framework, human interests prevail over the interests of non-human living organisms.

Furthermore, using the term ‘machine’ to describe synthetic organisms is perilous. While today the only synthetic organisms that can be produced are microorganisms, whose requirements for manipulation have no impact at the moral level, this could change when more complex organisms are produced. Naming them properly from the beginning would ensure that the interests of more complex synthetic organisms are respected in the future.

Biosafety

Biosafety refers to the prevention of risks to public health and the environment that could be produced by unexpected interactions between dangerous biological agents and other organisms or the environment, which must be addressed and prevented in order to

observe the *principle of protecting the life and genetic identity of every human individual* and the *principle of protecting the ecosystem and the environment*.

The main concerns relate to bioengineering, protocells, xenobiology and DIYbio. Accordingly, some bioengineering applications involve the release of synthetic organisms into the natural environment, and genetic parts libraries pose risks due to difficulties in the correct characterization of the parts, as well as in relation to the emergent properties that could appear when combining them. With respect to protocells, current fears are based on several unknowns: the consequences of an eventual interaction between these systems and natural organisms, allergenicity, pathogenicity, etc., or the possibility of protocells interfering with natural vesicles produced by bacteria. As regards xenobiology, again, concerns arise from the uncertainties of the consequences of a possible interaction with natural organisms and the behavioral characteristics of these agents in the environment. Additionally, the rapidly-growing DIYbio movement generates concerns regarding the increased likelihood of accidents, since practitioners with no biosafety training could be manipulating organisms in their homes, and also in terms of the possibility of pursuing reckless projects with no regulation. In all cases the risks could escalate, since these are thriving areas. On the other hand, synthetic genomics poses mainly biosecurity risks, but the organisms derived from this technology that could be used for malicious purposes could also accidentally escape from the laboratory, which implies a biosafety risk.

There are, therefore, some features of this discipline that warrant improvements in biosafety measures. Along these lines, several biocontainment measures have already been developed, and Synthetic Biology itself can provide new effective mechanisms, such as synthetic auxotrophies, xenobiological firewalls or ingenious genetic circuits. Research in this area must continue. Other biosafety measures are also believed to be

necessary, and involve several improvements to current methodologies, e.g. characterization of the function of biological parts, standardization of information submitted to risk assessors, review and adaptation of worker protection measures, etc. In order for these improvements to be implemented, research in this area is also urgent. Biosafety education should also be included in the interdisciplinary curricula of Synthetic Biology.

All these measures are desirable and serve to safeguard both the *principle of protecting the life and genetic identity of every human individual* and the *principle of protecting the ecosystem and the environment*. However, legislation must have a role in their regulation, including the actions of biohackers, both nationally and internationally, taking into account the nature of the risks and the benefits expected; if not, compliance with the decisions would be optional, an inadequate feature in this case, given the importance of the values at stake. The protection of life prevails over freedom of research according to our ethical framework. As Sgreccia (2012) explains when describing the *principle of freedom and responsibility*, "the right to the defense of life precedes the right to freedom. In other words, freedom must mean taking responsibility for one's own life first and foremost, as well as the lives of others" (Sgreccia, 2012. p. 179). Additionally, when describing the *principle of the competence of the community*, Sgreccia (2012) states that "the principle of freedom of science and research should be recognized but also combined with the fact that populations need information and share in responsibility" (Sgreccia, 2012. p. 323), reflecting the relative nature of this principle, which is subordinate to more important ones. Other measures to regulate Synthetic Biology may also be useful, such as codes of conduct or guidelines.

In this sense, there seems to be a weak point in the development of the five personalist principles applied in this thesis. As explained earlier, the principles first

developed by Sgreccia (2012) are: 1) *the principle of defense of physical life*; 2) *the principle of freedom and responsibility*; 3) *the principle of totality or the therapeutic principle*; and 4) *the principle of sociality and subsidiarity*. These principles were later adapted to the case of genetic engineering, obtaining the list of five principles used herein. When raising the issue of establishing mandatory regulations to prevent biosafety risks, it can be observed that the *principle of the competence of the community* contains another principle, that of *freedom of research*, whose hierarchy within the framework of the other principles is not made clear. From Sgreccia's previous dissertation, it follows that this principle is subordinate to principles 1 and 2 (since principle 2 serves the first), but it would be worth explaining this. Accordingly, it seems that the *principle of the competence of the community* should be reformulated to specifically include and describe the *principle of freedom of research*, which would be a more faithful adaptation to the previous *principle of freedom and responsibility*. Therefore, the principle could be the *principle of freedom of research and the competence of the community*. Its content should explicitly include the *principle of freedom of research*, its subordination to principles 1 and 2—from which responsible research is derived—and the need to take into account the views of all stakeholders and society.

This reformulation would facilitate the direct application of Sgreccia's principles to the bioethical evaluation of biotechnology.

It could be argued that this is not necessary, since a combination of the four initial principles and the five specific principles of genetic engineering can be used. Nonetheless, there are two considerations that support this proposal. In the first place, the four initial principles are not exactly general principles, but principles specific to the case of medicine. For example, the *principle of freedom and responsibility* contains several paragraphs explaining the implications of the principle for euthanasia, care of the

mentally ill, refusal of medical treatment or patient consent. Secondly, having a single list of principles that can be applied to the bioethical discussion in all the different areas of biotechnology is a much easier and faster access and application tool, especially for those in the field of bioethics whose training is scientific rather than philosophical.

Finally, according to the *principle of the competence of the community*, all the stakeholders, as well as the public, must be involved in the debate on Synthetic Biology and its risks, so that decision-making processes take into account the views of society.

Biosecurity

The possibilities for misuse of Synthetic Biology are a biosecurity matter. As when discussing biosafety issues, the *principle of protecting the life and genetic identity of every human individual* and the *principle of protecting the ecosystem and the environment* require the prevention of these risks.

The main challenges posed by Synthetic Biology in this regard relate to the recreation of known pathogenic viruses (synthetic genomics), making existing bacteria more dangerous, or producing harmful biochemicals via *in situ* synthesis (bioengineering), among other possibilities. Furthermore, given that Synthetic Biology is intended to be accessible to a large number of people (DIYbio) by simplifying and lowering the cost of the necessary processes and material, it is worrisome that the means are provided to malicious agents to perpetrate a bio-attack, and that regulation is hindered. Finally, xenobiology could also contribute to the creation of radically new pathogens, although this is a remote possibility.

Biosecurity prevention measures in the field of Synthetic Biology include the establishment of decision-making authorities and the development of norms, regulations

and funding policies to promote security. However, the measures that currently govern biosecurity issues are prior to Synthetic Biology. Given the risks posed by this discipline, these regulations must be revisited, updated and, if necessary, extended to implement new specific guidelines and laws. In this regard, the possibility of applying some restrictions to the dissemination of dual-use knowledge is under discussion, although their potential implementation is far from being defined. Additionally, control of the synthesized, ordered and distributed genetic sequences is a rather broad specific measure, whose regulation is also under discussion. Education and awareness-raising of dual-use risks are biosecurity measures suggested to aid prevention. Among the measures needed to deal with an attack already produced, strategies to recognize and attribute the attack as well as the development of consequence management capabilities are needed.

With respect to biosecurity risks, the importance of the *principle of protecting the life and genetic identity of every human individual* and the *principle of protecting the ecosystem and the environment* justify the establishment of national and international regulations that exercise control in this field. These regulations should include institutions or researchers that do not receive public funds, including amateur scientists, and must consider the possibility that unknown biological agents may appear. Importantly, from the personalist framework, restricting the pursuit and dissemination of knowledge is licit when it endangers the *principle of protecting the life and genetic identity of every human individual* and the *principle of protecting the ecosystem and the environment*, since the *principle of freedom of research* is subordinate to them. In this regard, it is worth referring again to the reformulation of the *principle of the competence of the community* as the *principle of freedom of research and the competence of the community*.

Finally, in accordance with the *principle of the competence of the community*, society must be informed about the factors involved in biosecurity, in order to participate in the debate on them.

Transhumanism

The possibility of applying Synthetic Biology to humans has been proposed by leading representatives in this field, and could serve to introduce large modifications in our genome (bioengineering) or to artificially synthesize it (synthetic genomics). Even non-canonical bases could be introduced into our genome (xenobiology).

According to the principle of *protecting the life and genetic identity of every human individual* and the *therapeutic principle*, modification of the human genome is acceptable only when necessary for the treatment of a disease. Hence, the design of *enhanced* or *weakened* human genomes or their their hypothetical synthesis and introduction into enucleated oocytes in order to initiate the human development program is ethically unacceptable. However, in the event that synthetic humans were produced, they would still have full moral status and would be subject to the same rights and privileges as other humans. The term *subhuman* is therefore unwarranted and contrary to human dignity.

Additionally, in accordance with the *principle of the competence of the community*, advances in this field must be reported, and effective dialogue platforms must be provided that engage scientists, legislators, the general public and all stakeholders.

Other ethical issues related to Synthetic Biology

Other ethical issues related to Synthetic Biology are justice, public perception and communication, and intellectual property rights. The most relevant principle for discussing these issues is the *principle of the competence of the community*, which

recognizes the need to promote the interest of the common good. This supports both the fair distribution of the risks and benefits of this discipline, and the combination of an intellectual property system with another system of open access to research. More specific rules cannot be derived from our ethical framework, but this position is presented as a basis for the development (from biolaw) of specific measures according to it.

Nevertheless, it should be noted that the idea of promoting the interest of the common good is much better developed in the *principle of sociality and subsidiarity*. Given that the *principle of the competence of the community* appears to be derived from the *principle of freedom and responsibility*, it seems appropriate to include a sixth principle for the bioethical assessment of genetic engineering (and other biotechnology applications): the *principle of sociality and subsidiarity*.

Finally, a high level of social ignorance of Synthetic Biology has been found. According to the *principle of the competence of the community*, an informative effort must be made to increase public awareness of this discipline. In this regard, coverage of the topic by the media must be sufficiently restrained, in order to help to develop a realistic picture of the field.

4.7.2 Personalist assessment of the different branches within Synthetic Biology

As mentioned above, the division proposed by Deplazes (2009) aims to facilitate bioethical discussion in this area by allowing the association of different ethical issues with different branches of Synthetic Biology. This is particularly important when evaluating a discipline that includes so many varied actions, since the conclusions derived from a generalist evaluation are unlikely to be extendible, appropriate or warranted for all cases. After reviewing the scientific practices and aims of each branch of Synthetic Biology, the different issues related to this discipline have been specified for the different

approaches (Table 16). Interestingly, it has been found that many of the bioethical questions raised are associated with all the branches of Synthetic Biology (except for *in silico* Synthetic Biology), albeit in different ways. Taking into account these specifications and the personalist assessment of the different ethical issues, the evaluation of each branch of Synthetic Biology can proceed.

Table 16

Ethical issues related to Synthetic Biology. *Source: prepared by the author.*

Approach	Ethical issues
BIOENGINEERING	**Concept of life and its creation:** the conception of life as a toolbox **Moral status of synthetic entities** **Biosafety:** applications that involve the release of synthetic organisms into the natural environment, uncharacterized synthetic parts **Biosecurity**: making existing bacteria or viruses more dangerous, producing harmful biochemicals via *in situ* synthesis in humans, development of harmful chemicals or biochemicals, modification of the human microbiome, the human immune system or the human genome **Transhumanism:** modification of the human genome **Justice:** as the branch with the widest range of applications, the benefits must be distributed fairly, without resulting in exploitation of underdeveloped countries. Biosafety and biosecurity risks must be regulated worldwide **Public perception and communication** **Intellectual property rights** **Regulation** **Research responsibility**
SYNTHETIC GENOMICS	**Concept of life and its creation:** reductionist understanding of life, artificial life (synthetic genome) **Moral status of synthetic entities** **Biosafety:** dangerous organisms could escape from the laboratory **Biosecurity:** possibility of re-creating known pathogenic viruses and bacteria **Transhumanism:** artificial synthesis of the human genome **Justice:** biosecurity risks must be regulated worldwide **Public perception and communication** **Intellectual property rights** **Regulation** **Research responsibility**
PROTOCELL SYNTHETIC BIOLOGY	**Concept of life and its creation:** "*creation*" of life, artificial life (completely), "playing God" **Moral status of synthetic entities** **Biosafety:** applications in natural environments, potential interactions with natural organisms, unknown properties that may be dangerous, possible interference with natural biological functions **Justice:** biosafety risks must be regulated worldwide **Public perception and communication** **Intellectual property rights** **Regulation** **Research responsibility**
XENOBIOLOGY	**Concept of life and its creation:** "playing God", artificial life (new and unfamiliar versions of life) **Moral status of synthetic entities** **Biosafety:** unpredictability of potential interactions **Biosecurity:** creation of radically new pathogens, evasion of detection methods **Transhumanism:** modification of the human genome **Justice:** biosafety and biosecurity risks must be regulated worldwide **Public perception and communication** **Intellectual property rights** **Regulation** **Research responsibility**
DIY SYNTHETIC BIOLOGY	**Biosafety:** risk of accidents and lack of control **Biosecurity:** greater risk of dual-use, difficult control **Justice:** biosafety and biosecurity risks must be regulated worldwide **Public perception and communication** **Regulation** **Research responsibility**

Bioengineering

This is the most active branch within Synthetic Biology, from which the largest number of products and applications are expected to be obtained. The most pressing bioethical issues associated with bioengineering are biosafety and biosecurity. Applications involving the release of synthetic organisms into the natural environment, and even into the human body in the form of probiotics, or the use of uncharacterized synthetic parts are the main actions that pose biosafety risks; biosecurity risks in this area are mainly the possibility of making existing bacteria more dangerous or producing harmful biochemicals via *in situ* synthesis in humans. Various measures are already in place and others are being developed to prevent the associated risks. Advances in this regard must continue, and researchers should be mindful of the risks of their work, limiting those actions that may aggravate the dangers. Regulations are also needed, and must take into account expert views and public opinion.

Both the potential for application of bioengineering and the risks it poses have implications for the issue of justice. Benefits and risks must both be distributed without exploiting some human groups in favor of others, seeking the common good and helping those more in need. Additionally, the "conception of life as a toolbox" could lead to a misunderstanding of the moral value of synthetic organisms. Transhumanism has also been associated with bioengineering, but is not a current possibility. Finally, intellectual property rights will need to be defined for bioengineering products.

This analysis finds no insurmountable objections to the development of bioengineering. On the contrary, it should be encouraged by virtue of the benefits it can bring to society. Nevertheless, precautions must be taken, especially regarding biosafety and biosecurity risks, justice, and the development of misconceptions about life and living beings.

Synthetic genomics

Synthetic genomics is the branch of Synthetic Biology that poses the greatest biosecurity concerns, since it enables the re-creation of known pathogenic viruses. Prevention measures must therefore be continuously improved to address the risks posed by this dual-use research. As in the previous case, this branch has also implications related to research responsibility, regulation, public perception and justice. Additionally, the essential genes approach could lead to a reductionist understanding of life and a misunderstanding of the moral value of synthetic organisms. Efforts are also being made to artificially synthesize the human genome, which could lead to the possibility of obtaining human beings whose genome is designed to improve or worsen particular characteristics. Finally, intellectual property right issues can also be associated with Synthetic genomics.

Although this branch of Synthetic Biology does not currently present insurmountable drawbacks either, it has been found that the risks of dual-use research and/or the dissemination of knowledge in this area could justify eventual restrictions in this regard. In addition, the possible production of human beings whose genome has been designed is ethically unacceptable.

Protocells

Protocell Synthetic Biology is, for the moment, relatively uncontroversial. Even though various ethical issues are also associated with this branch, the association is tenuous, since it is a field in which great milestones have not yet been reached, and several unknowns remain as regards its future development and biosafety risks. For this reason, risk evaluation and the development of prevention strategies should accompany research in this area. The approach to related issues such as justice, responsible research,

regulation, or intellectual property rights is not urgent, although discussions must begin. If protocells become living organisms in the future, they will be subjects of moral value, but their interests will not be morally relevant (given the defining simplicity of these organisms) and so the practical implications of this consideration are null. Finally, this is the branch that seems to raise more concerns about synthetic biologists exceeding certain limits, considering whether they can or should create life. From personalism, this approach does not violate any of the ethical principles. Nonetheless, it has been found that using alternatives to the term *create* not only better describes what is being done, but can also help to avoid unjustified concerns. In the same way, the use of the phrase "playing God" is discouraged. Advances in this field must be realistically explained to the public.

Xenobiology

The main concerns posed by xenobiology relate to biosafety, as potential interactions between organisms containing XNA or non-canonical amino acids are unpredictable. Research in this regard must be parallel to progress in this area, as well as public communication. In relation to this, researchers must conduct their activity responsibly, and regulations could be necessary in the future. Moreover, as mentioned above, a mechanistic concept of life could lead to disregard for the moral value of synthetic organisms. Further concerns relate to biosecurity and transhumanism, but the possibilities of the approach in this sense are currently remote. Finally, issues relating to justice and intellectual property rights cannot be excluded from the ethical implications of this branch but, as in the case of protocells, they do not demand immediate response. On the whole, there is no definitive ethical obstacle to the development of this field.

DIY Synthetic Biology

The main ethical issue related to DIYbio is biosafety, since the risk of accidents increases in a context lacking biosafety measures and with practitioners who may have no biosafety training. Biosecurity is also a relevant related issue, although, for the time being, risks in this regard seem to be lower. Despite the strong free research nature of this movement, in accordance with the personalist ethical framework, regulations and oversight strategies are needed in this respect in order to safeguard more fundamental values. Secondary issues arising from the former are justice, public perception and research responsibility. However, none of these issues has been found to be irremediably opposed to personalist principles, but measures can be taken in this regard to safeguard these principles.

4.7.3 Future directions

From the study carried out, different areas can be highlighted where future research can contribute to the development of Synthetic Biology, both in terms of its benefits and its safety. In addition, new formulas for regulation, social participation and patents must be developed.

In the first place, translation of research in this field to real-life applications is failing at present, not only because of economic and logistical impediments, but also due to ethical and social limitations (Heidari Feidt, et al., 2019). Efforts must therefore be devoted to acting on these various factors, facilitating this necessary process of translation in order to unleash the benefits that are expected from this discipline.

Two pressing issues in the field of Synthetic Biology that must also be considered are biosafety and biosecurity. As has been shown in sections 4.3.5 and 4.4.5, several measures are available to mitigate risks. However, several gaps in knowledge remain

which need to be filled by research, such as the potential environmental impact of the introduction of synthetic organisms into the environment, the implications of de-extinction and gene drives, the environmental performance of synthetic processes and products, etc. (SCENIHR, SCHER, & SCCS, 2015b). Additional strategies must be developed for biocontainment too, including strategies based on Synthetic Biology itself, control of the distributed genetic sequences, and attack recognition and attribution. Research on new medical countermeasures is also needed, which can also be supported by Synthetic Biology possibilities. On another note, adequate regulations must be established in this regard, including for the DIYbio movement, educational actions must be promoted, and specific guidelines must be developed, such as the IUCN policy on Synthetic Biology and conservation (IUCN, 2016).

With regard to the public and other stakeholders, information and participation must be encouraged. To this end, work needs to be done to establish effective dialogue platforms, as well as to be mindful of the informative language, which must be accessible as well as realistic.

Finally, in view of the drawbacks of applying the current patent system in this field, new formulas for the protection of intellectual property should be sought, which adapt to the current biotechnological reality.

5 CONCLUSIONS

1. Ten broad bioethical issues are associated with Synthetic Biology: 1) impact on the concept of life and its creation; 2) moral value of synthetic products; 3) biosafety risks; 4) biosecurity risks; 5) transhumanism; 6) justice; 7) public perception and communication; 8) intellectual property rights; 9) regulation; and 10) responsible research.

Recommendation 1: The bioethical questions posed by the different branches of Synthetic Biology are not identical. Therefore, the specificities of each branch of this discipline should be taken into account in bioethical discussions about it.

2. With regard to the impact of Synthetic Biology on the concept of life and its creation, no branch of this discipline has been found to be inevitably contrary to personalist principles. However, mechanistic or reductionist interpretations of the concept of life, which could derive from these approaches, could threaten the values defended by the *principle of protecting the ecosystem and the environment* or even the *principle of protecting the life and genetic identity of every human individual*.

Recommendation 2: Mechanistic and reductionist interpretations of the concept of life must be avoided, in order to safeguard the *principle of protecting the ecosystem and the environment* and the *principle of protecting the life and genetic identity of every human individual*.

Recommendation 3: The use of the terms *designing*, *constructing* or *recreating* life is encouraged over the use of the term *creation*, since they are more appropriate to describe what is done in Synthetic Biology and can prevent unwarranted concerns.

3. Those synthetic entities that retain the characteristics commonly recognized in life are organisms, regardless of their origin and the human purposes involved in their production. Therefore, by virtue of the *principle of protecting the ecosystem and the environment*, they are morally valuable and their interests must be taken into account.

Recommendation 4: The characteristics of synthetic organisms must be observed in order to determine their requirements and preserve them as far as possible, taking into account that human interests morally matter more than the interests of any other being, and thus prevail when deciding a course of action.

Recommendation 5: The use of the term 'machine' to describe synthetic organisms is inadvisable. Naming them properly from the beginning may help in respecting the interests of more complex synthetic organisms in the future, perhaps including humans.

4. There is no moral duty to safeguard the conservation of synthetic species, since they are not part of the biodiversity that must be protected under the *principle of protecting the ecosystem and the environment*. Additionally, according to personalism there is no moral obligation to synthesize organisms in order to increase biodiversity.

5. All branches of Synthetic Biology pose biosafety risks that should be prevented as far as possible to preserve the *principle of protecting the life and genetic identity of every human individual* and the *principle of protecting the ecosystem and the environment*.

Recommendation 6: In order to face the challenges posed by Synthetic Biology, improvements in existing biosafety measures must continue, which include: synthetic auxotrophies, xenobiological firewalls or synthetic genetic circuits; continuous research and development in biosafety; characterization of the function of biological parts, standardization of information submitted to risk

assessors, review and adaptation of worker protection measures; biosafety education; and establishment of different forms of international and national regulations, including mandatory laws.

6. Most branches of Synthetic Biology, with the exception of protocells, pose biosecurity risks that should be prevented as far as possible to preserve the *principle of protecting the life and genetic identity of every human individual* and the *principle of protecting the ecosystem and the environment*.

Recommendation 7: In order to face the challenges posed by Synthetic Biology, improvements in existing biosecurity measures must continue, which include: the establishment of decision-making authorities, funding policies and regulations; potential restriction of the dissemination of dual-use knowledge; control of the synthesized, ordered and distributed genetic sequences; education and awareness-raising of dual-use risks; measures needed to deal with an attack; strategies to recognize and attribute the attack; and the development of consequence management capabilities.

7. According to the *principle of the competence of the community*, all the stakeholders, as well as the public, must be involved in the debate about Synthetic Biology and its biosafety and biosecurity risks, as well as other controversial aspects related to Synthetic Biology, such as transhumanism.

Recommendation 8: The debate about the different issues related to Synthetic Biology must involve all the stakeholders as well as the public. Effective dialogue platforms must be provided that engage scientists, legislators, the general public and all stakeholders.

8. From the practical application of the five personalist principles to the case of Synthetic Biology, some weaknesses have been detected in the ethical framework:

a. The *principle of the competence of the community* contains another principle, the *principle of freedom of research*, whose hierarchy and content within the framework of the other principles is not made clear. From Sgreccia's previous dissertation, it follows that it is subordinate to principles 1 and 2. Reformulation of the principle to explicitly include and describe freedom of research would facilitate the direct application of Sgreccia's principles to the bioethical evaluation of biotechnology.

b. The *principle of the competence of the community* establishes the need to promote the interest of the common good. However, this idea is much better developed in the *principle of sociality and subsidiarity*, pertaining to the initial list of principles. The content of this principle in the subject in question is highly relevant, and in the initial list it is independent of the *principle of freedom and responsibility* (from which the *principle of the competence of the community* is born).

Recommendation 9: The *principle of the competence of the community* should be reformulated as the *principle of freedom of research and the competence of the community*. Its content should explicitly include the freedom of research, its subordination to principles 1 (the *principle of protecting the life and genetic identity of every human individual*) and 2 (the *principle of protecting the ecosystem and the environment*), responsible research, and the need to take into account the views of all stakeholders and society.

Recommendation 10: The *principle of sociality and subsidiarity* should be included as a sixth principle in the list of principles developed for genetic engineering.

9. The possibility of altering the human genome for transhumanist purposes or to produce subhumans is contrary to the principle of *protecting the life and genetic identity of every human individual* and the *therapeutic principle*.

Recommendation 11: Synthetic Biology techniques should not be applied to the genome of human individuals unless it is for therapeutic purposes.

10. Regarding the issues of justice and intellectual property rights, the *principle of the competence of the community* requires the promotion of the interest of the common good.

Recommendation 12: Both the fair distribution of the risks and benefits of Synthetic Biology and the combination of an intellectual property system with another system of open access to research must be supported.

11. According to the *principle of the competence of the community*, populations need information and to share responsibility, but there is low social awareness of Synthetic Biology.

Recommendation 13: An informative effort must be made to increase public awareness of this discipline. Coverage of the topic by the media must be sufficiently restrained, in order to help develop a realistic picture of the field.

12. From personalism, no insurmountable objections have been found to the development of bioengineering. Biosafety and biosecurity are the most pressing issues associated with this branch of Synthetic Biology.

Recommendation 14: This branch of Synthetic Biology must be fostered by virtue of the benefits it can bring to society. However, precautions must be taken, especially regarding biosafety and biosecurity risks and justice. To that end, appropriate regulations must be established. The moral value of synthetic organisms must be recognized.

13. From personalism, synthetic genomics does not present insurmountable drawbacks either in its current state. However, it poses important biosecurity risks related to the re-creation of known pathogenic viruses. Additionally, the hypothetical production of human beings whose genome is designed and synthesized is ethically unacceptable.

    Recommendation 15: When the risks of dual-use research and/or the dissemination of knowledge in this area are too high, restrictions must be applied in this regard.

14. From personalism, protocell Synthetic Biology does not violate any ethical principles. Major concerns revolve around synthetic biologists *creating* life. Additionally, several unknowns remain regarding future biosafety risks posed by protocells.

    Recommendation 16: Risk evaluation and the development of prevention strategies must accompany research in this field. The public should be informed about progress in this area, trying to avoid unnecessary concerns. With this aim, alternatives to the term *create* are recommended, while use of the phrase "playing God" is discouraged.

15. From personalism, there is no definitive ethical obstacle to the development of xenobiology. The main concerns relate to uncertainties regarding biosafety risks.

    Recommendation 17: Research on biosafety risks of xenobiology must be parallel to progress in this area, as well as public communication.

16. None of the issues related to DIYbio have been found to be irremediably opposed to personalist principles. The main ethical issue in this regard is biosafety, followed by biosecurity.

    Recommendation 18: The DIYbio movement must also be subject to regulations.

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7 ANNEXES

7.1 DEFINITIONS OF SYNTHETIC BIOLOGY

Table 17.

Definitions of Synthetic Biology. *Modified from SCENIHR et al. (2014).*

Source	Definition	Key words/focus
European Commission (2005)	Synthetic biology is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems which display functions that do not exist in nature. This engineering perspective may be applied at all levels of the hierarchy of biological structures from individual molecules to whole cells, tissues and organisms. In essence, synthetic biology will enable the design of biological systems in a rational and systematic way.	Engineering Principles applied to biology; Rational design and synthesis of complex (novel) biological systems.
Synthetic Biology 3.0 (2007)	Synthetic biology is a new and rapidly emerging discipline that aims at the (re-) design and construction of (new) biological systems.	(Re-) designing and synthesis of (new) biological systems.
UK Parliamentary Office of Science and Technology (POST, 2008)	Synthetic biology aims to design and build new biological parts and systems or to modify existing ones to carry out novel tasks.	New or modified biological parts and systems for novel tasks.
POST (2008)	[Synthetic biology] describes research that combines biology with the principles of engineering to design and build standardised, interchangeable biological DNA building-blocks. These have specific functions and can be joined to create engineered biological parts, systems and, potentially, organisms. It may also involve modifying naturally occurring genomes to make new systems or by using them in new contexts.	DNA building blocks to engineer biological parts.
Gaisser, Reiss, Lunkes, Müller, & Bernauer (2008)	Synthetic Biology aims at designing biological systems that do not exist in nature using engineering principles or re-designing existing ones to better understand life processes, to generate and assemble functional modular components, and to develop novel applications or processes.	(Re) design of (novel) biological systems; Functional modular components for novel applications and processes.
Capurro et al. (2009)	A definition of synthetic biology should therefore include: 1.The design of minimal cells/organisms (including minimal genomes); 2. The identification and use of biological ‘parts’ (toolkit); 3. The	Identification, design and use of (artificial) biological parts.

	construction of totally or partially artificial biological systems.	
Synthetic Biology Org[24]	Synthetic Biology is (a) the design and construction of new biological parts, devices, and systems, and (b) the redesign of existing, natural biological systems for useful purposes.	Design of new biological parts, devices and systems; Redesign of existing, natural biological systems.
European Commission, & Health and Consumers Directorate General (2010)	Two complementary definitions for SynBio: (a) designing and making biological parts and systems that do not exist in the natural world using engineering principles, and (b) redesigning existing biological systems, again using engineering principles.	Designing new or redesigning the existingbiological systemsthrough engineering processes
PCSBI (2010)	Synthetic biology is the name given to an emerging field of research that combines elements of biology, engineering, genetics, chemistry, and computer science. The diverse but related endeavors that fall under its umbrella rely on chemically synthesised DNA, along with standardised and automatable processes, to create new biochemical systems or organisms with novel or enhanced characteristics.	Combines different Scientific disciplines; uses synthetic DNA to develop new biochemical systems or organisms with novel or enhanced characteristics.
UK Synthetic Biology Roadmap coordination Group (2012)	Synthetic biology is the design and engineering of biologically based parts, novel devices and systems as well as the redesign of existing, natural biological systems.	(Re)design/engineering of biologically based parts, novel devices and systems; Engineering of biologically based parts, novel devices and systems Redesign of existing, natural biological systems.
Balmer & Martin (2008)	Synthetic Biology is the deliberate design of biological systems and living organisms using engineering principles.	Design / engineering of biological systems and organisms.
Blake & Isaacs (2004)	Synthetic biology is advancing rapidly as biologists, physicists and engineers are combining their efforts to understand and program cell function. By characterizing isolated genetic components or modules, experimentalists have paved the way for more quantitative analyses of genetic networks.	Genetic components and module.
De Vriend (2006)	Synthetic biology is a newly emerging scientific field where ICT, biotechnology and nanotechnology meet and strengthen each other. Synthetic biology is a new trend in science and technology and a clear example of converging technologies.	Convergence of various technologies.
Heinemann & Panke (2006)	Synthetic biology is interpreted as the engineering-driven building of increasingly	Engineering driven complex biological

[24] http://syntheticbiology.org/

	complex biological entities for novel applications.	entities for novel applications.
Drubin, Way, & Silver (2007)	Synthetic biology refers to a variety of experimental approaches that either seek to modify or mimic biological systems.	Approaches to modify or mimic biological systems.
ETC Group (2007)	Synthetic Biology (also known as Synbio, Synthetic Genomics, Constructive Biology or Systems Biology) – the design and construction of new biological parts, devices and systems that do not exist in the natural world and also the redesign of existing biological systems to perform specific tasks.	(Re)design and construction of (novel) biological parts, devices, and systems to perform specific tasks.
ETC Group (2007)	Synthetic biology is an emerging area of research that can broadly be described as the design and construction of novel artificial biological pathways, organisms or devices, or the redesign of existing natural biological systems.	(Re)design and construction of (novel) biological pathways, organisms or devices.
Entus, Aufderheide, & Sauro (2007)	Synthetic biology is a useful tool to investigate the dynamics of small biological networks and to assess our capacity to predict their behavior from computational models.	A means to investigate and model biological networks.
Bailey, Metcalf, & Crook (2012)	Synthetic biology is a term used to cover areas of biochemistry research that is involved in the chemical synthesis of DNA, utilising biological agents or their components for potential application across a wide range of industrial sectors.	Manipulation of synthetic DNA in biological systems.
The Royal Academy of Engineering (2009)	Synthetic biology aims to design and engineer biologically based parts, novel devices and systems as well as redesigning existing, natural biological systems. Synthetic biology strives to make the engineering of biology easier and more predictable.	(Re)design/engineer novel systems and devices.
De Lorenzo & Danchin (2008)	The fundamental idea behind synthetic biology is that any biological system can be regarded as a combination of individual functional elements — not unlike those found in man-made devices. These can therefore be described as a limited number of parts that can be combined in novel configurations to modify existing properties or to create new ones.	Novel combinations of biological functional parts.
Benner & Sismour (2005)	[Synthetic biology] attempts to recreate in unnatural chemical systems the emergent properties of living systems ... [the] engineering community has given further meaning to the title… to extract from living systems interchangeable parts that might be tested, validated as construction units, and reassembled to create devices that might (or might not) have analogues in living systems.	Artificial assembly of biological parts.

Erasynbio (n. d.)	Synthetic Biology is the engineering of biology: the deliberate (re)design and construction of novel biological and biologically based parts, devices and systems to perform new functions for useful purposes, that draws on principles elucidated from biology and engineering.	(Re)design/engineer novel systems and devices, new functions.
Bhutkar (2005)	Rather than splicing in a gene from one organism to another, or forcing a mutation in a genome for a specific purpose, synthetic biology mainly concerns designing and building artificial regulatory elements into genomes or constructing a complete genome out of nucleotides.	

7.2 PAPERS PUBLISHED

7.2.1 Paper 1

Gómez-Tatay L, Hernández-Andreu JM, Aznar J. A Personalist Ontological Approach to Synthetic Biology. Bioethics. 2016 Jul;30(6):397-406. doi: 10.1111/bioe.12230.

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A PERSONALIST ONTOLOGICAL APPROACH TO SYNTHETIC BIOLOGY

LUCÍA GÓMEZ-TATAY, JOSÉ MIGUEL HERNÁNDEZ-ANDREU AND JUSTO AZNAR

Keywords
synthetic biology,
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ontological personalism

ABSTRACT
Although synthetic biology is a promising discipline, it also raises serious ethical questions that must be addressed in order to prevent unwanted consequences and to ensure that its progress leads toward the good of all. Questions arise about the role of this discipline in a possible redefinition of the concept of life and its creation. With regard to the products of synthetic biology, the moral status that they should be given as well as the ethically correct way to behave towards them are not clear. Moreover, risks that could result from a misuse of this technology or from an accidental release of synthetic organisms into the environment cannot be ignored; concerns about biosecurity and biosafety appear. Here we discuss these and other questions from a personalist ontological framework, which defends human life as an essential value and proposes a set of principles to ensure the safeguarding of this and other values that are based on it.

7.2.2 Paper 2

Gómez-Tatay L, Hernández-Andreu JM, Aznar J. The Conception of Synthetic Entities from a Personalist Perspective. Sci Eng Ethics. 2019 Feb;25(1):97-111. doi: 10.1007/s11948-017-9994-z.

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ORIGINAL PAPER

The Conception of Synthetic Entities from a Personalist Perspective

Lucía Gómez-Tatay[1,2,3] · José Miguel Hernández-Andreu[1,2] · Justo Aznar[1]

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Abstract Synthetic biology opens up the possibility of producing new entities not found in nature, whose classification as organisms or machines has been debated. In this paper we are focusing on the delimitation of the moral value of synthetic products, in order to establish the ethically right way to behave towards them. In order to do so, we use personalism as our ethical framework. First, we examine how we can distinguish between organisms and machines. Next, we discuss whether the products of synthetic biology can be considered organisms at all and assess what their moral value is and how should we behave towards them. Finally, we discuss the hypothetical case of synthetic humans.

Keywords Synthetic biology · Bioethics · Personalism · Moral value · Moral status

7.2.3 Paper 3

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CRITICAL REVIEWS IN ENVIRONMENTAL SCIENCE AND TECHNOLOGY
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Biosafety and biosecurity in Synthetic Biology: A review

Lucía Gómez-Tatay[a,b,c] and José M. Hernández-Andreu[b,c]

[a]Escuela de Doctorado de la Universidad Católica de Valencia "San Vicente Mártir", Valencia, Spain; [b]Departamento de Ciencias Médicas Básicas, Grupo de Medicina Molecular y Mitocondrial, Facultad de Medicina y Odontología de la Universidad Católica de Valencia "San Vicente Mártir", Valencia, Spain; [c]Instituto de Ciencias de la Vida de la Universidad Católica de Valencia "San Vicente Mártir", Valencia, Spain

ABSTRACT

Synthetic Biology is a growing field which holds great promise in several areas of application. However, it also poses serious risks for human health and the environment that must be anticipated in order to develop effective prevention and management measures. Here, the current situation on biosafety and biosecurity in this scientific field is reviewed. Biosafety concerns mainly relate to the damaging effects for workers and the environment that could result from accidental interactions with dangerous biological agents. On the other hand, biosecurity risks refer to the potential misuses of Synthetic Biology, such as bioterrorism, biowarfare or bioattacks that could derive from the genetic engineering of organisms. In this paper, the specific challenges posed by Synthetic Biology are discussed, and perspectives in the development of measures to guide the safe advance of this discipline are presented.

KEYWORDS

Synthetic Biology; biosafety; biosecurity

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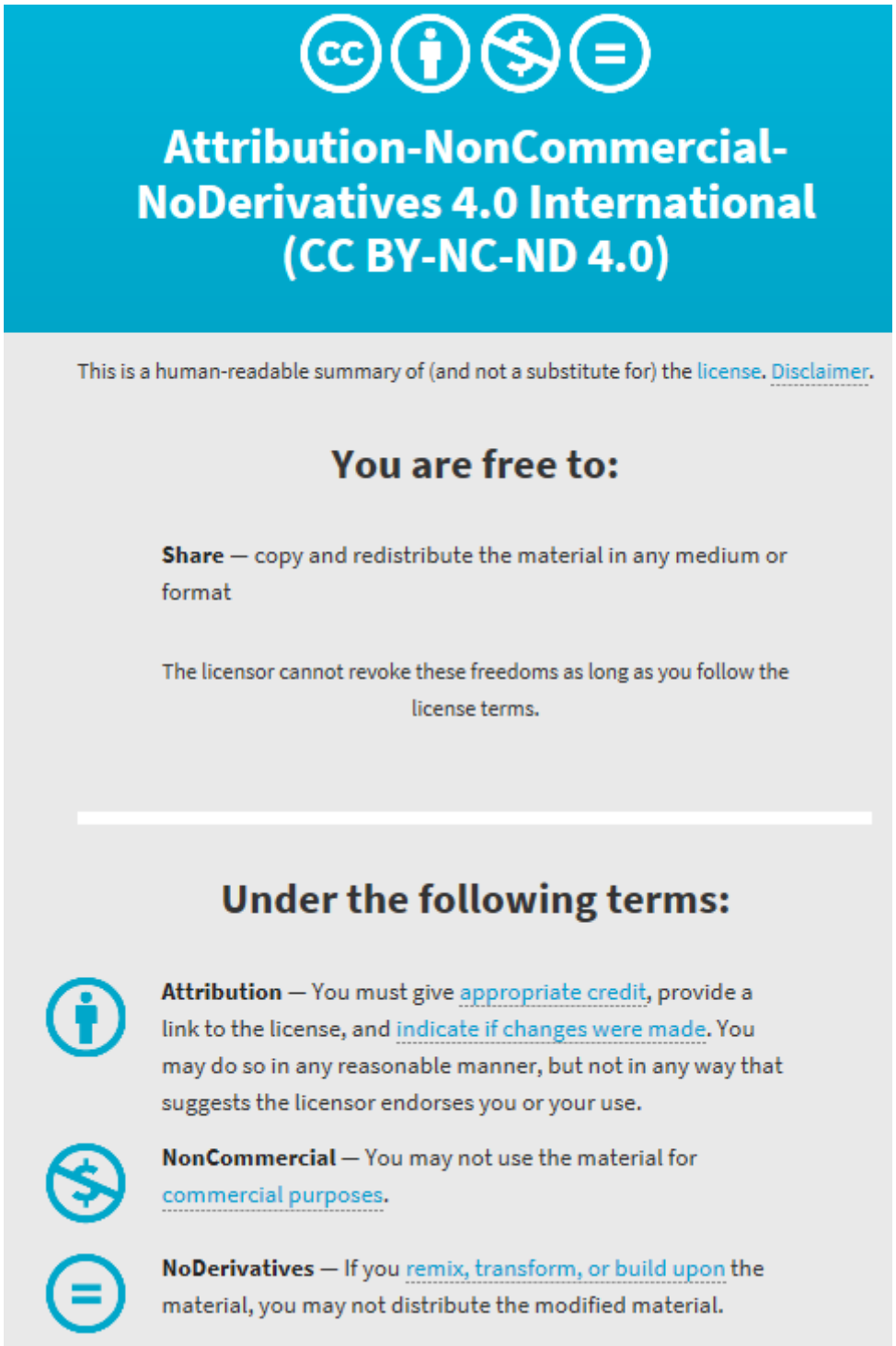

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