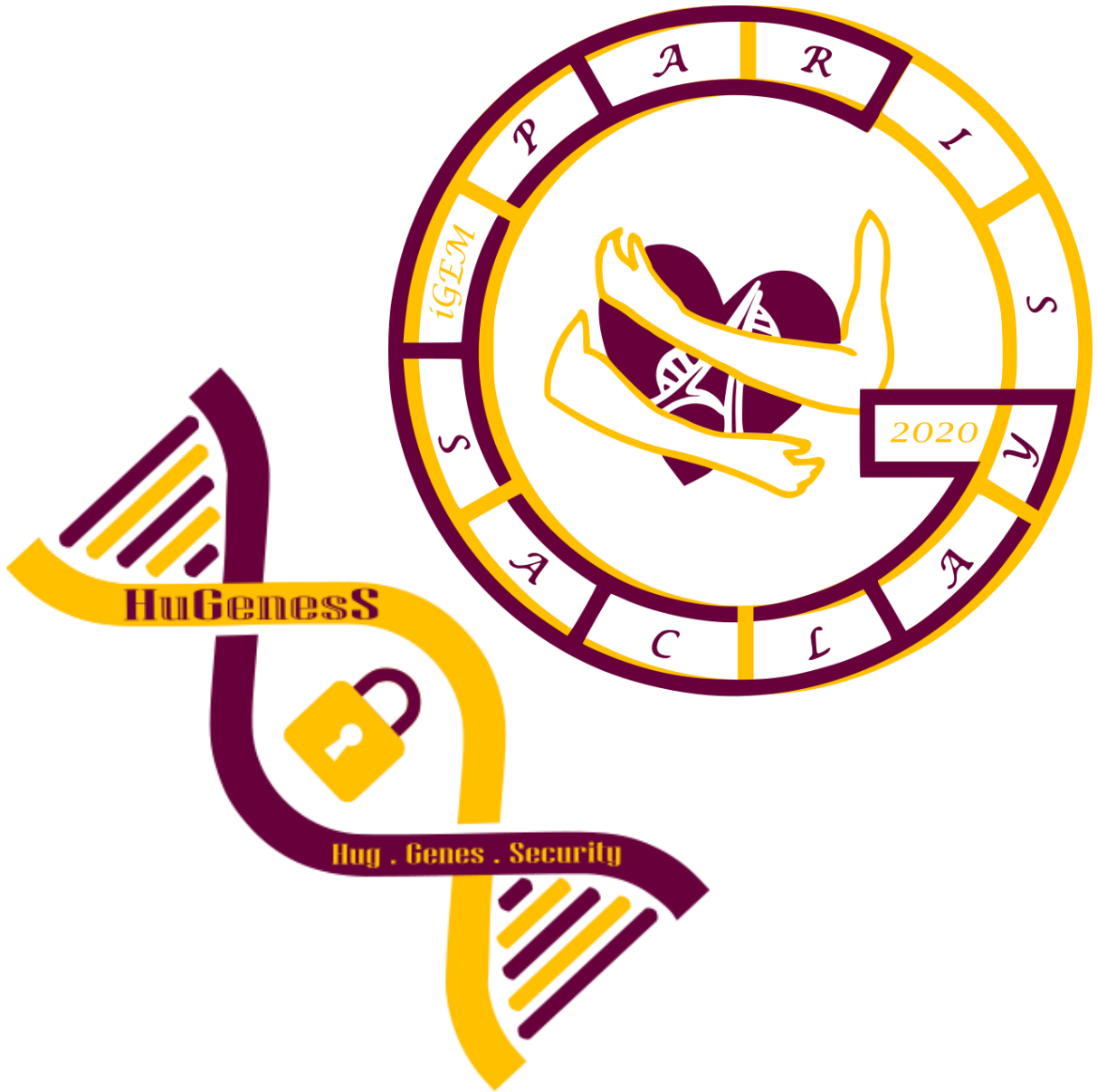



# HuGenesS

*Genetic security for a safer future*

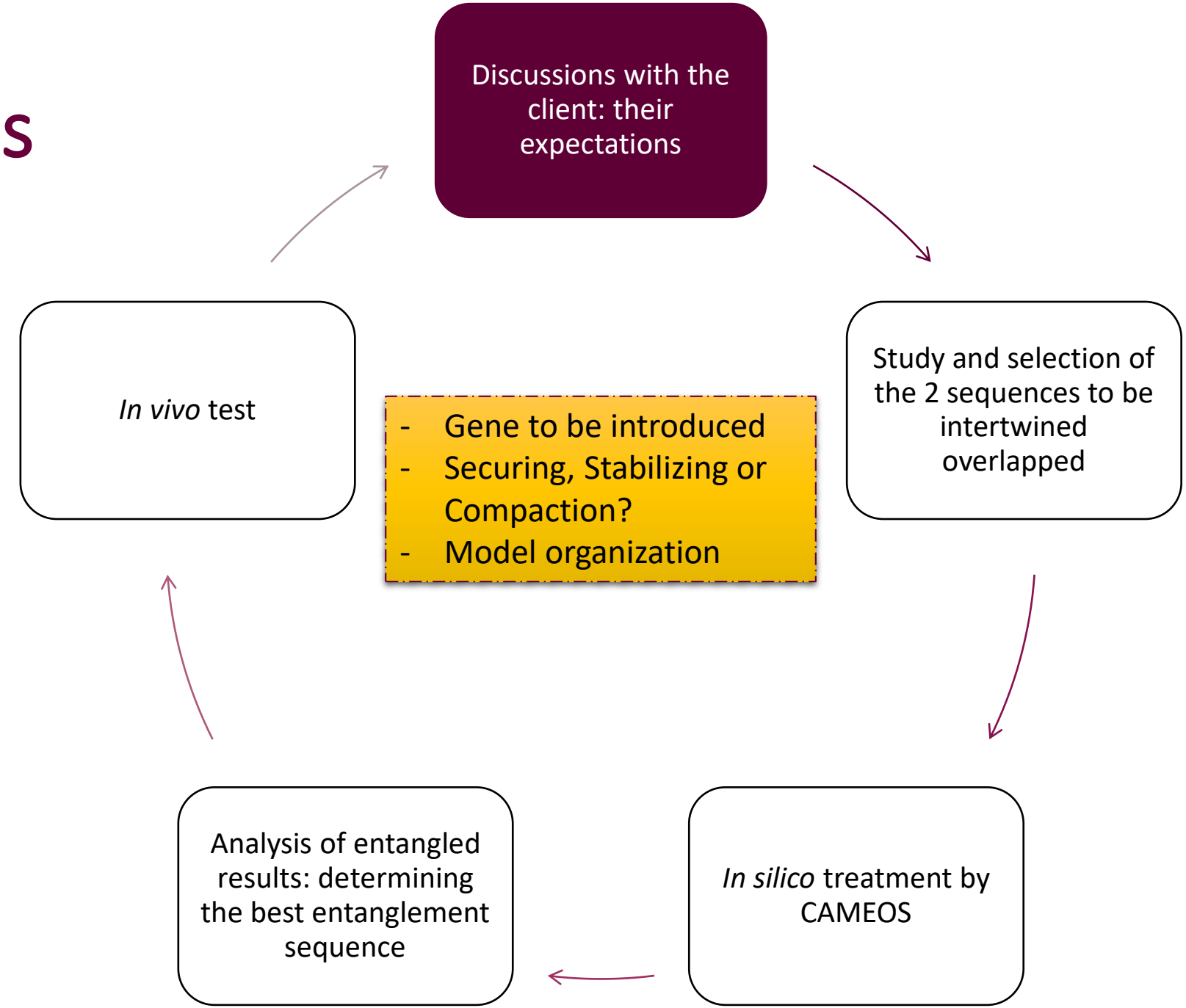
# Comparison of the market offer

| Methods<br>Features | HuGenesS _ Design n°1 = Gene of interest entangled with an essential gene | HuGenesS _ Design n°2 = Gene of interest entangled with a toxin-antitoxin system | Toxin/Antitoxin Kill Switch | Synthetic Auxotrophy | Minimal genomes |
|---|---|---|---|---|---|
| Impact of the design on cell 'health' and genetic cost | The design of entangled genes have only minimal impact on cell physiology. | Part of the population not expressing sufficient antitoxin is likely to die and/or present a severe growth defect. | Part of the population not expressing sufficient antitoxin is likely to die and/or present a severe growth defect. | Auxotrophy in itself may a growth-limiting factor. | The minimal genome strategy may be associated with a decrease in growth rate. |
| Ease of use | Once the strain is obtained, there is no need for a specific growth medium. Usual working conditions can be used. | Once the strain is obtained, it must be maintained in permissive conditions. | Once the strain is obtained, it must be maintained in permissive conditions. | Necessity to bring the compound that is no longer synthesized by the strain to maintain its growth. | This depends on the growth properties and requirements of the strain containing a minimal genome. The growth may be limitated to very specific growth conditions. |
| Potential target genetic material | Viruses, Archaeae, Bacteria, Eukaryotes | Viruses, Archaeae, Bacteria, Eukaryotes | Viruses, Archaeae, Bacteria, Eukaryotes | Archaeae, Bacteria, Eukaryotes (most viruses do not encode function related to metabolism) | Archaeae, Bacteria (for the moment, the genomes of eukaryotes are too large to envisage a minimal approach; viruses are already some kind of 'sub-minimal' genomes i.e. molecular parasites of cells) |
| Context of use | Various - Can be used in closed (labs) or open environments. | Only under permissive conditions (present generally only in laboratories) | Only under permissive conditions (present generally only in laboratories) | Only under permissive conditions (present generally only in laboratories). | The minimal genome does not offer the 'versatility' of complete genomes, meaning that it encodes less possibilities of potential of adaptation to various environmental conditions. |
| Genetic stability | This is the great strength of the technique: the gene of interest cannot be lost (all without the need to maintain selection pressure such as the addition of an antibiotic). Reversion is very improbable. | The gene of interest will tend to be more difficult to lose, but mutations (e.g. in the promoter driven the expression of the toxin) could be enough to revert the confined nature of the strain. | A few mutations (e.g. in the promoter driven the expression of the toxin) could be enough to revert the confined nature of the strain. | Depending on strain design, risk of the establishment of compensatory pathways in the strain over generations (genetic drift). | Classically, minimal genome are devoid of mobile genetic elements and repeats that could lead to genome instability. |
| Horizontal gene transfer (HGT, from the strain of interest to environmental strain) | HGT are still possible, but with limited risk since it was not necessary to use selection markers (of the antibiotic resistance type), the transfer of which would be dangerous. | HGT rather impossible since the strain would transfer the gene encoding the toxin without the gene encoding the antitoxin.=> The HGT is toxic. | HGT rather impossible since the strain would transfer the gene encoding the toxin without the gene encoding the antitoxin.=> The HGT is toxic. | HGT still possible, but limited since the strain has a limited lifespan in the environment, and the prototrophic gene can be used as a marker (instead of selection markers such as the antibiotic resistance markers, the transfer of which would be dangerous. | HGT still possible |
| Cost considerations related to the cloning of the synthetic strain | Relatively reasonable strain development cost (CAMEOS design, gene synthesis, introduction by homologous recombination, assays to assess the functionality of encoded function) | Relatively reasonable strain development cost (CAMEOS design, gene synthesis, introduction by homologous recombination, assays to assess the functionality of encoded function) | Relatively reasonable strain development cost (design of the kill- switch, introduction within the target strain) | Relatively reasonable strain development cost (design of a system for deletion of a target gene after homologous recombination with a template DNA) | DNA synthesis, Gibson assembly, assembly within the yeast, genome transplantation |
| Time | Less than 8 weeks | Less than 8 weeks | Less than 4 weeks | Less than 4 weeks | Several months |
| Other cost Considerations | No specific cost to maintain the strain ad infinitum (other than those related to the classical culture of microorganisms) | Price link to the cost associated to permissive conditions | Price link to the cost associated to permissive conditions | Cell growth has a cost that is related to the price of the compound to be added to ensure the growth of the auxotrophic strain. | Price link to the price of the medium required of the groxth of the strain with a minimal genome |

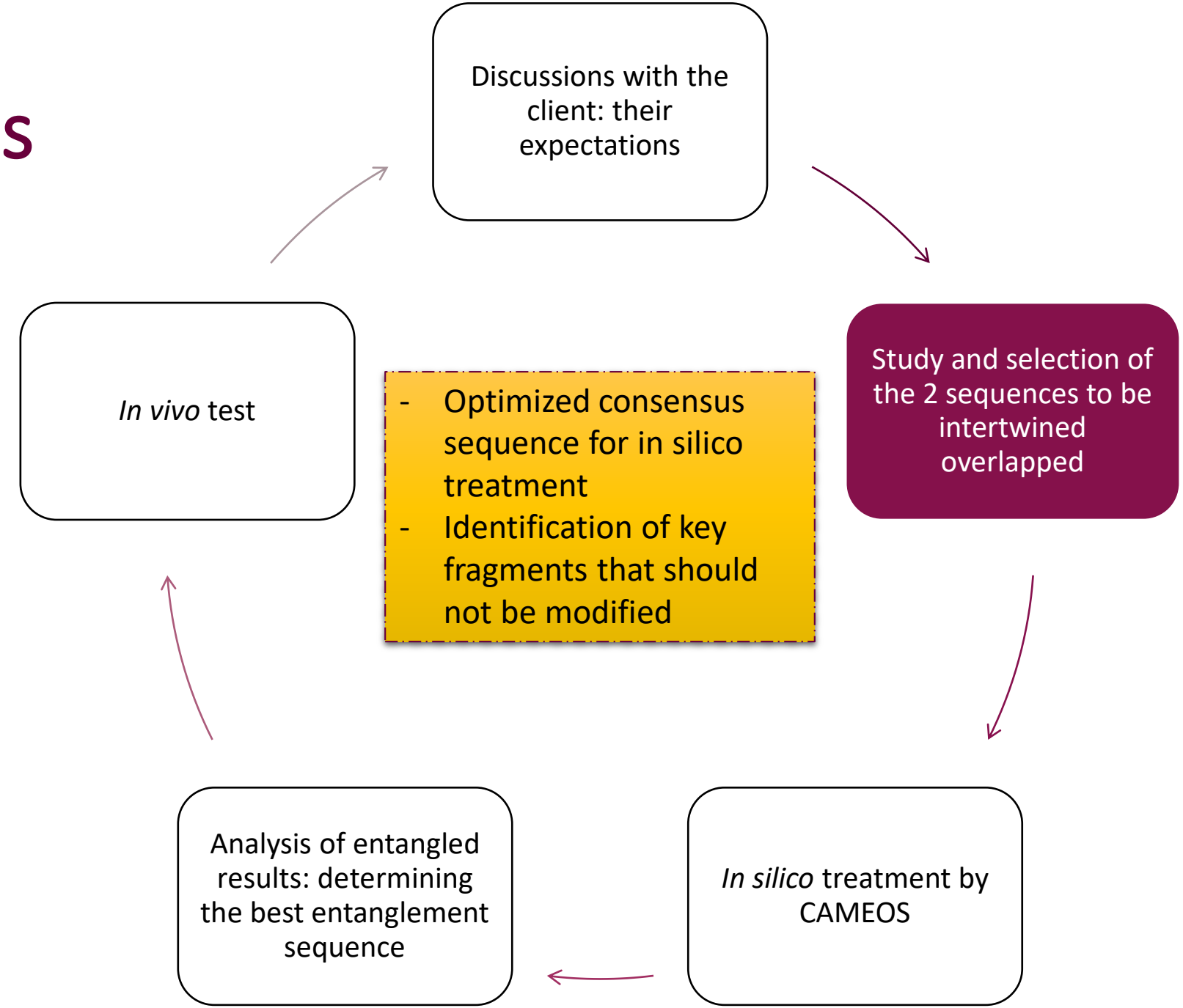
# Comparison of the market offer

| Methods<br>Features | HuGenesS _ Design n°1 = Gene of interest entangled with an essential gene | HuGenesS _ Design n°2 = Gene of interest entangled with a toxin-antitoxin system | Toxin/Antitoxin Kill Switch | Synthetic Auxotrophy | Minimal genomes |
|---|---|---|---|---|---|
| Impact of the design on cell 'health' and genetic cost | 9 | 6 | 5 | 6 | 6 |
| Ease of use | 10 | 3 | 3 | 3 | 5 |
| Potential target genetic material | 10 | 10 | 10 | 6 | 4 |
| Context of use | 10 | 6 | 6 | 3 | 6 |
| Genetic stability | 10 | 6 | 2 | 5 | 7 |
| Horizontal gene transfer (HGT, from the strain of interest to environmental strain) | 6 | 10 | 10 | 6 | 2 |
| Cost considerations related to the cloning of the synthetic strain | 7 | 7 | 8 | 8 | 3 |
| Time | 6 | 6 | 8 | 8 | 1 |
| Other cost Considerations | 9 | 6 | 6 | 3 | 5 |
| Score total | **77** | **60** | **58** | **48** | **39** |

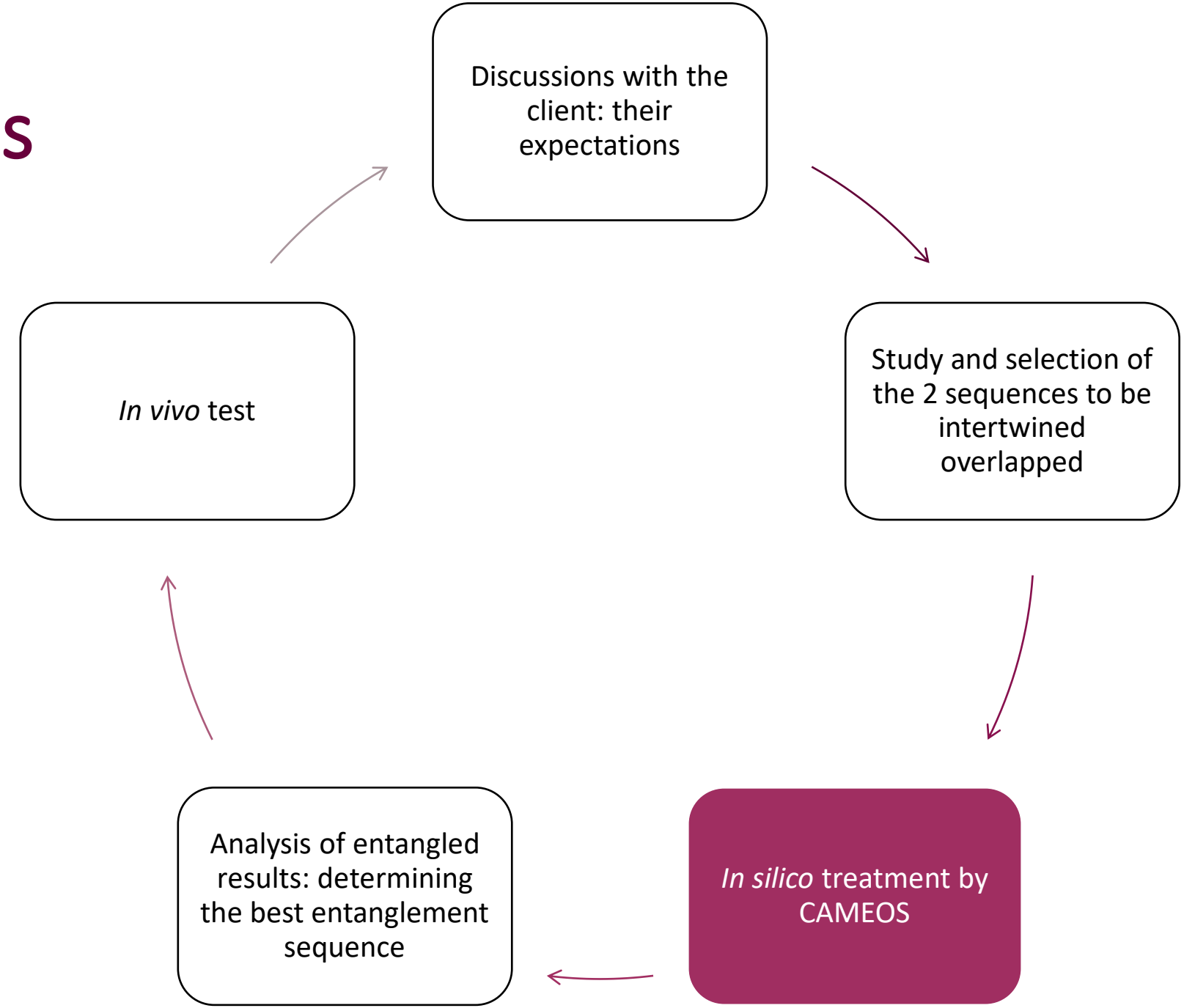
# Process


Discussions with the client: their expectations
Study and selection of the 2 sequences to be intertwined overlapped
- Gene to be introduced
- Securing, Stabilizing or Compaction?
- Model organization
*In silico* treatment by CAMEOS
Analysis of entangled results: determining the best entanglement sequence
*In vivo* test

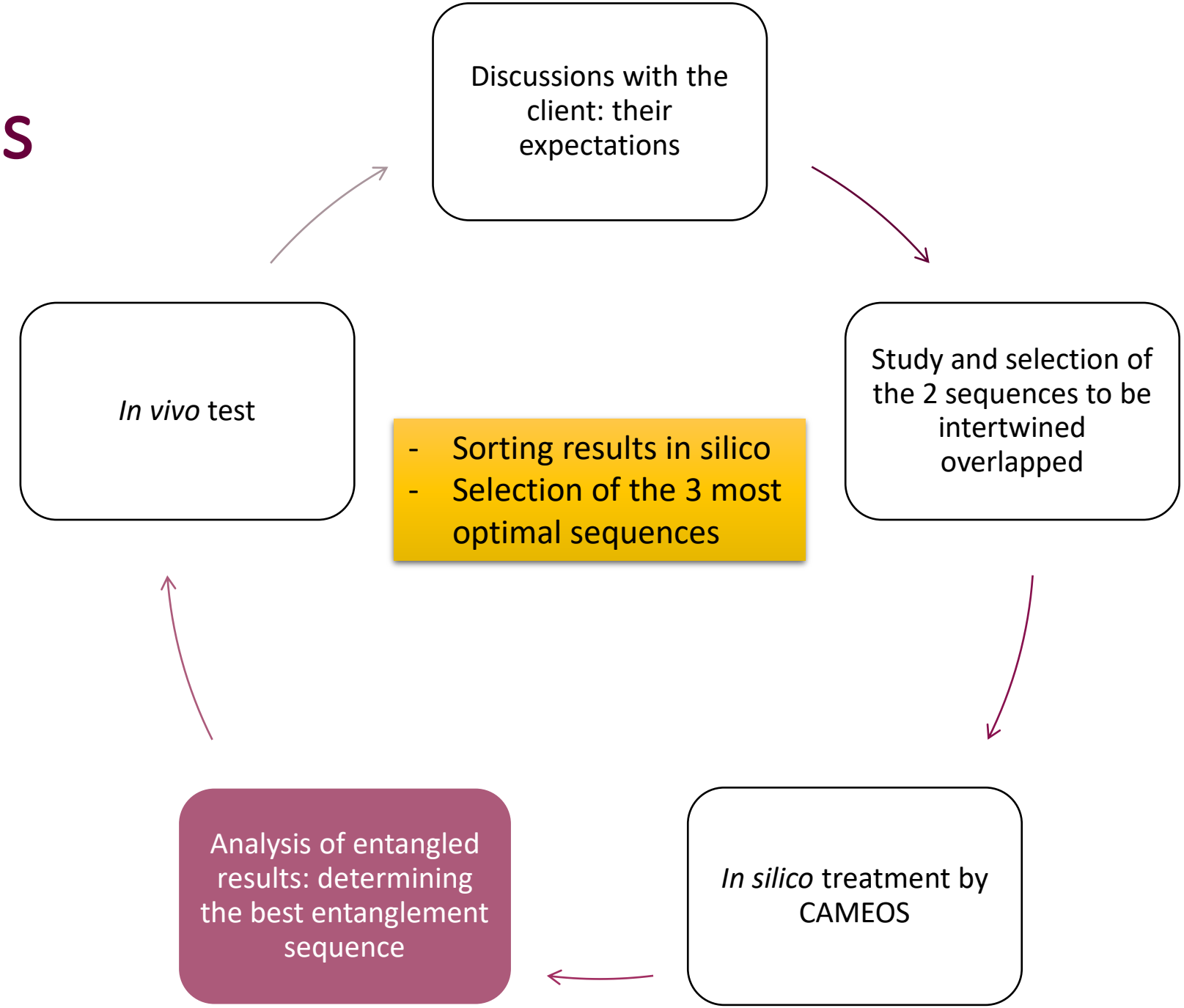
# Process


Discussions with the client: their expectations
Study and selection of the 2 sequences to be intertwined overlapped
- Optimized consensus sequence for in silico treatment
- Identification of key fragments that should not be modified
*In silico* treatment by CAMEOS
Analysis of entangled results: determining the best entanglement sequence
*In vivo* test

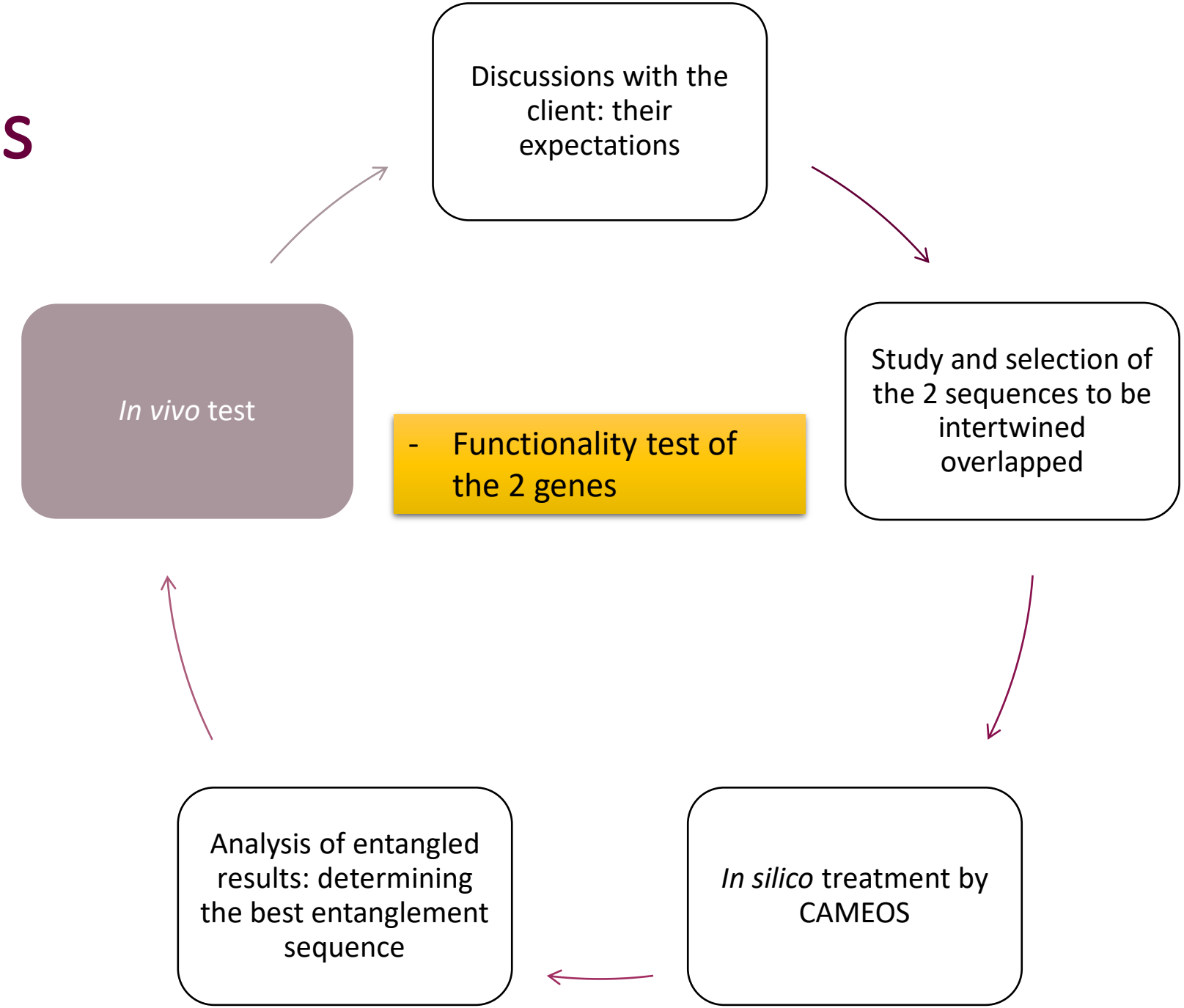
# Process


Discussions with the client: their expectations
Study and selection of the 2 sequences to be intertwined overlapped
*In silico* treatment by CAMEOS
Analysis of entangled results: determining the best entanglement sequence
*In vivo* test

# Process


Discussions with the client: their expectations
Study and selection of the 2 sequences to be intertwined overlapped
*In silico* treatment by CAMEOS
Analysis of entangled results: determining the best entanglement sequence
*In vivo* test
- Sorting results in silico
- Selection of the 3 most optimal sequences

# Process


Discussions with the client: their expectations
Study and selection of the 2 sequences to be intertwined overlapped
*In silico* treatment by CAMEOS
Analysis of entangled results: determining the best entanglement sequence
*In vivo* test
- Functionality test of the 2 genes

# Mix 4P

**Produit**

- DNA sequence of 2 entangled genes

**Place**

- Internet
- Delivery

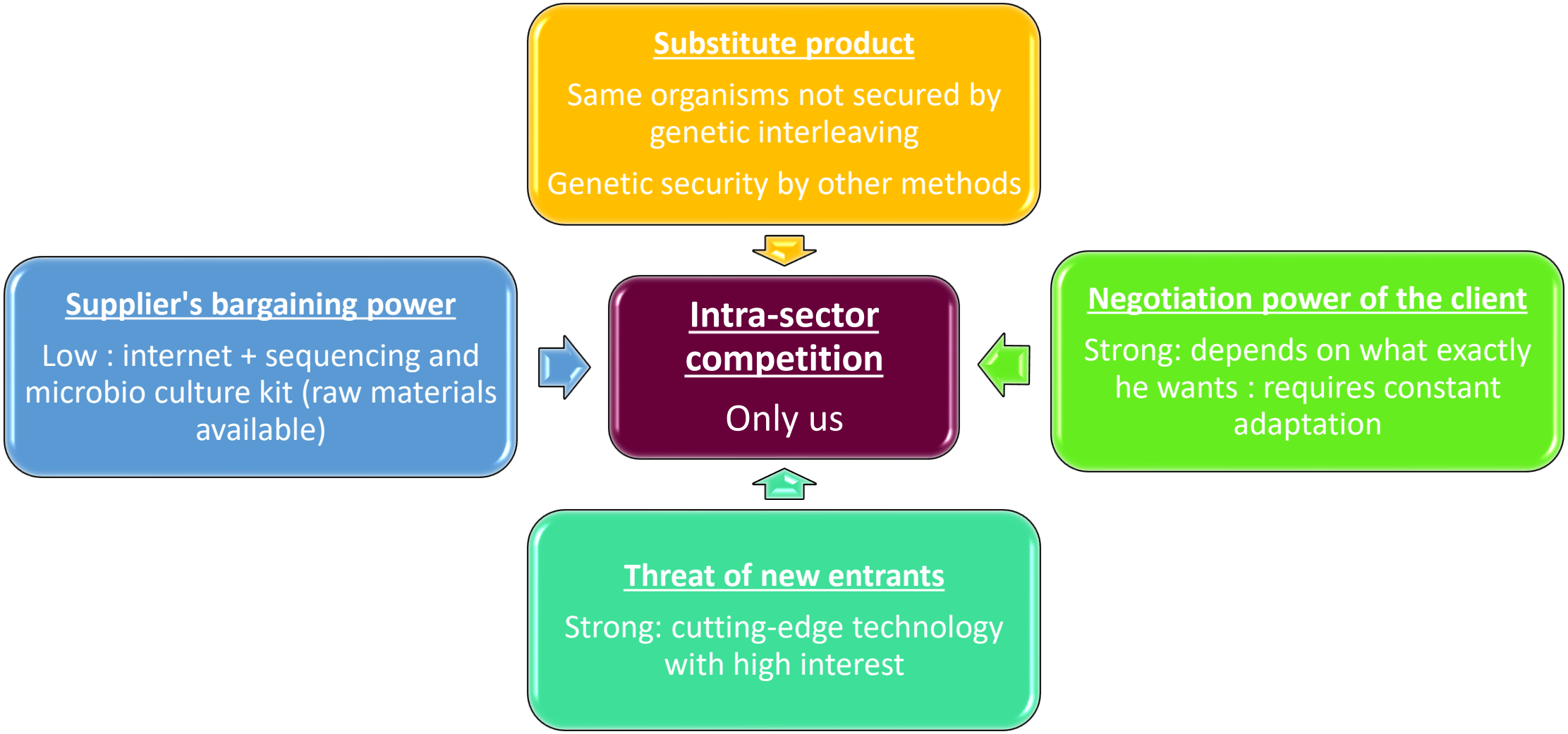
**Prix**

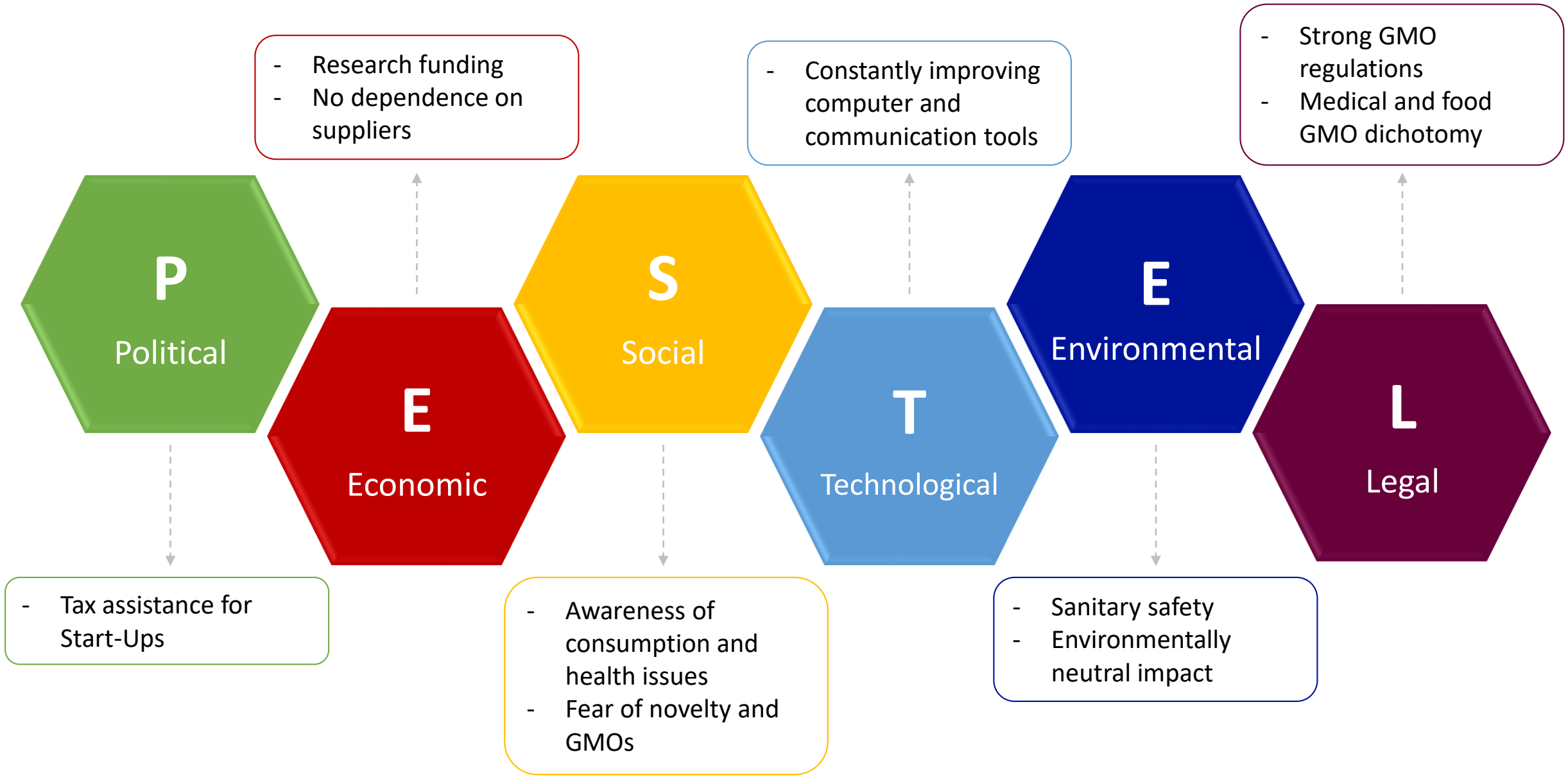
- Design and testing
- DNA synthesis and shipping

**Promotion**

- Technology education for the general public
- Summit

# Porter's five forces analysis




- Research funding
- No dependence on suppliers
- Constantly improving computer and communication tools
- Strong GMO regulations
- Medical and food GMO dichotomy
P
Political
E
Economic
S
Social
T
Technological
E
Environmental
L
Legal
- Tax assistance for Start-Ups
- Awareness of consumption and health issues
- Fear of novelty and GMOs
- Sanitary safety
- Environmentally neutral impact