|  |  |  |  |
| --- | --- | --- | --- |
|  | Living organism: | Living Modified Organism: | Obtained through modern biotechnology: |
|  | Capable of transferring or replicating genetic material | Possesses a novel combination of genetic material | In vitro nucleic acid techniques | Overcome natural physiological reproductive or recombination barriers and are not techniques used in traditional breeding and selection  |
| Total *in vitro* synthesis ofgenomes or individual chromosomes , e.g. *Mycoplasma mycoides, Mycoplasma* derived minimal cellandSc2.0 | ✔ | ✔ | ✔ | ✔(majority of the cases) |
| ?1 |
| Gene editing techniques  | ✔ | ✔ | ✔ | ? 1 |
| Cisgenesis | ✔ | ✔ | ✔ | ?1 |
| Xenobiology, e.g. organisms contacting non-natural nucleotides and aminoacids | ✔ | ✔ | ✔ | ✔ |
| ×2 |
| *De novo* engineered proteins, RNAs, signaling and metabolic pathways, incl. such without analogs in nature | ✔ | ✔ | ✔ | ✔ |
| RNA/DNA-based manipulations | ✔ | ?3 | ✔ | ? |
| Epigenetic engineering | ✔ | ×4 | ✔5 | ?6 |
| Protocells | ?7 | ✔ | ✔ | ✔ |
| Cell-free systems | × | ✔8 | ✔ | ✔ |

1 When only minimal changes, which could have been obtained through traditional breeding techniques, have been introduced

2 When auxotrophic mutants are fed with non-natural nucleotides or amino acids

3 Those nucleic acids are only transiently present in the cell, but during that time there can be expression of genetic information

4 Assuming that genetic material does not extend to epigenetic modifications, which in some cases can be inherited for some generations.

5 Only in some cases. Epigenetic changes can be introduced through pharmacological agents as well

6 It is unclear whether epigenetic changes can be considered overcoming natural reproductive or recombination barrier

7 No protocell capable of transferring genetic material exists at present to the best of my knowledge; replication can happen is some model systems, but it’s more like a cell-free system

8 Usually in such cell-free systems recombinant nucleic acid molecules are used, e.g. systems for cell-free expression of proteins

**Note:** The table above is prepared assuming that “natural physiological reproductive or recombination barriers” envisioned are not absolute. Horizontal gene transfer can occur in principle between any two organisms and genes with any sequence can evolve if necessary and if unlimited time is available. In this sense modern biotechnology overcomes barriers not so much by making impossible things possible, but rather by allowing us to do things that would take an unrealistic amount of time if we used other methods.