

Polyclonal selection of recombinant *L. tarentolae* strains

- As soon as the 10 ml o/n cultures obtained from the transfection experiments (**capt. 4.4.** of LEXSY Expression Kit manuals) start to get slightly turbid (OD₆₀₀ 0.4; ca. 10⁷ cells/ml; usually approx. 20 h after electroporation), add the appropriate selective **LEXSY antibiotic(s)** to the recommended final concentration(s) and continue incubation as static suspension culture at 26°C. **Don't let the cultures overgrow before selection, since it will take longer to kill non-recombinant cells!**
- Follow the status of the cultures microscopically and visually until you start seeing the difference to the cells electroporated without DNA under the same conditions (negative control). Recombinant cells are motile, of drop-like shape and grow as a "cloudy" suspension culture whereas the cells in the negative control begin to die during the selection period and appear as spherical or irregular forms without flagella under the microscope. Visually, however, the negative control may also appear as a turbid suspension
- It usually takes one consecutive transfer into fresh medium with the appropriate antibiotic(s) at 1:10 inoculation rate to get a turbid culture of the antibiotic-resistant recombinant cell line and a clear negative control (absence of growth). This passage should be performed within 7 days after first drug addition, usually at day 5. Do not wait for a longer period to passage the culture even if the negative control also got turbid
- if you feel that the transfected cultures do not start to grow under selection within the first week post electroporation, centrifuge cells 3 min at 2000g, resuspend pellet carefully in fresh medium with selective antibiotic(s) and continue incubation. Also, you may combine 1:10 dilution with medium renewal of the remaining culture at day 5 of selection
- If at the time of first addition of selective antibiotic the cultures were already too dense, the negative control may not be dead after the first 1:10 passage. In this case another (or even more) subsequent 1:10 dilution(s) into fresh medium with selective antibiotic may be needed to get a turbid recombinant culture and a clear negative control

Please, refer to **capt. 5.2.** of LEXSY Expression Kit manuals for more details. **Capt. 5.1.** of the manuals describes also an alternative protocol for clonal selection.