

## Clonal selection of recombinant *L. tarentolae* strains

- Withdraw 1 - 4 batches of 2 ml from the transfected 10 ml o/n culture obtained by the standard LEXSY electroporation protocol (**capt. 4.4.** of LEXSY Expression Kit manuals). The remaining culture may be used in parallel for polyclonal selection as described in **capt. 5.2.** of the manuals
- Pellet cells for 3 min at 2000g and room temperature
- Transfer the supernatant back to the culture in the TC flask and resuspend the cells in approx. 50 µl of residual medium
- Carefully spread the resuspended cells onto freshly prepared **LEXSY BHI agar\*** supplemented with the resp. selective antibiotic(s) and with **LEXSY NTC** and **LEXSY Hygro** in case of **T7-TR** strain for inducible expression
- Optionally, you may streak the cells onto nitrocellulose filters (BA85, 0.45 µm, blotting grade) placed on the surface of the agar. Plating is easier on these membranes than directly on the 1% agar, and swarming of colonies, as evtl. observed on soft agar, is diminished. Moreover, plating on membranes allows colony lifts for testing induction profiles of clonal populations e.g. by fluorescence scanning or specific detection methods for the given target protein\*\*. You may also consider to test both plating techniques for your application
- Seal plates with parafilm and incubate bottom up at 26°C
- 5 – 7 days after plating small, defined colonies begin to appear on a slight background lawn.
- After these colonies have grown up to 1 – 2 mm diameter (approx. 7-9 days after plating), they can be transferred to 0.2 ml of selective growth medium in a 96-well plate using a pipette tip
- After 24 hours incubation at 26°C these clones must be expanded into 1 ml selective medium in a 24-well plate. If the colonies are grown for a longer period on the agar plates (they survive on agar plates for ca. 20 days post electroporation and can reach 5 mm diameter), they may be expanded directly into 1 ml selective medium in 24 well plates bypassing the 96 well format. For speeding up growth, the 24.well plate may be agitated on a microplate shaker located in the 26°C incubator
- After another ca. 48 hour incubation at 26°C the cultures are expanded into 10 ml selective medium in TC flasks and can be used for evaluation.

\* For customer convenience, the **LEXSY Plating Kit (Cat. No. ML-451)** contains all components for preparation of plates for clonal selection of LEXSY expression strains. Appendix 8.3. of the LEXSY Expression Kit manuals describes the formulation of solid LEXSY plating media.

\*\* In case of **pLEXSY\_I-blecherry3** clones colony lift technique can be applied for selection supported by monitoring induction through development of a cherry color of the colonies in daylight. To this end fresh **LEXSY BHI agar** plates are prepared containing the inducer Tetracycline in addition to the selection antibiotics if the colonies arrive at ca. 1-2 mm diameter. Following transfer of the membrane with the colonies the plates are incubated for additional 1-3 days. The development of a cherry color of the colonies is based on co-induction of the *blecherry* gene and indicates that the inducible system is intact. In case there are evtl. singular pale colonies those are excluded from further expansion.

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