

ULTimate Y2H™ Screening Process

> Bait design

Before launching your project one of our ULTimate Y2H experts looks carefully at your protein and based on our experience and with the help of BSOL, he/she advises you on the optimal bait to be screened.

> Bait cloning (4 to 6 weeks*)

- > Design and order of PCR primers
- > Sequence verification of the 5' and 3' flanking regions of the given insert
- > PCR amplification of the bait and subsequent cloning in the appropriate bait vector
- > Complete sequence validation of the newly cloned bait

> Optimization of screening conditions (2 weeks*)

Before performing an ULTimate Y2H screen, each bait is screened on a small scale against the library to evaluate the best screening conditions:

- > Bait transformation in yeast
- > Small scale screen
 - Bait toxicity test
 - Bait autoactivation test and choice of the best screening medium
 - Estimation of the number of positive clones

Please note that about 15% of baits cannot be screened using the standard bait vector (LexA-based) due to autoactivation and toxicity issues. In these cases an inducible bait vector may be used, which will decrease the failure rate to approximately 5%; however, the amount of time needed to deliver the results will inevitably increase.

> ULTimate Y2H™ screen (2 to 3 months*)

- > Full scale 'cell-to-cell mating' screen
- > 5p and 3p sequencing of all positive prey clones.
- > Automatic prey analysis
- > Final quality control : random bait and prey reselection to certify the results of the screen

> Results delivery

- > The results will be sent to you on a CD-ROM that contains the following documents:
- > a Results Summary (a pdf file with prey identification, interaction domains and confidence scores),
- > an Excel® spreadsheet (raw data)
- > DomSight® (graphical comparison of the minimal interacting domain with the functional domains present in the preys).

****The times given are averages for each step and are therefore only indicative.***
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