Support protocol for using AssemblX with hosts different from *S. cerevisiae* or *E. coli*

If you wish to maintain your final Level 1 or Level 2 assembly in a host different from *E. coli* or yeast, please follow one of the following three options (**Figure S1**). For all three options, it is necessary to linearize the expression vector for the intended host. This may be done by restriction digestion or preferably by PCR amplification. Once the vector is linearized, one of the following three options may be used.

Option 1: Convert your expression vector into an AssemblX compatible Level 2 vector

- For this option, make sure that your expression vector does not contain a *Pac*I site.
- Design primers that amplify the yeast replication origin and the appropriate pair of homology regions from one of the available AssemblX Level 2 vectors. As a template, choose the same Level 2 vector you would have used if yeast was your final host for expression. If you are not sure, take a look at the "*Level 2*" section in the AssemblX protocol that you received after submitting your assembly to the webtool.
- Equip primers with overlaps compatible to your linearized expression vector and use these primers to amplify the cassette described above.
- Combine the following in an appropriate assembly reaction (e.g. SLiCE or Gibson assembly; do **not** use TAR as your vector does not yet contain a selection marker):
    - Linearized expression vector
    - PCR-amplified vector conversion cassette
- Identify positive clones by colony PCR and restriction analysis.
- Use the modified expression vector instead of the designated AssemblX Level 2 vector for the final TAR mediated Level 2 assembly.
- Proceed with construct verification and isolation. Transform verified constructs into your final host.

Option 2: Subclone final AssemblX Level 2 module into your expression vector by recombination.

- For this option, make sure that your expression vector does not contain a *Pac*I site.
- Design primers that amplify the appropriate pair of homology regions from one of the available AssemblX Level 2 vectors. As a template, choose the same Level 2 vector you would have used if yeast was your final host for expression. If you are not sure, take a look at the "*Level 2*" section in the AssemblX protocol that you received after submitting your assembly to the webtool.
- Equip primers with overlaps compatible to your linearized expression vector and use these primers to amplify the cassette described above.
- Combine the following in an appropriate assembly reaction (e.g. HiFi DNA assembly or SLiCE):
    - Linearized expression vector
    - PCR-amplified recombination cassette
- Identify positive clones by colony PCR and restriction analysis.
- Use the modified expression vector to subclone your AssemblX Level 2 construct. For this, digest your verified Level 2 construct with I-*Sce*I, purify the fragment and perform

in-vitro recombination (e.g. HiFi DNA assembly, SLiCE) with *Pac*I digested modified expression vector.

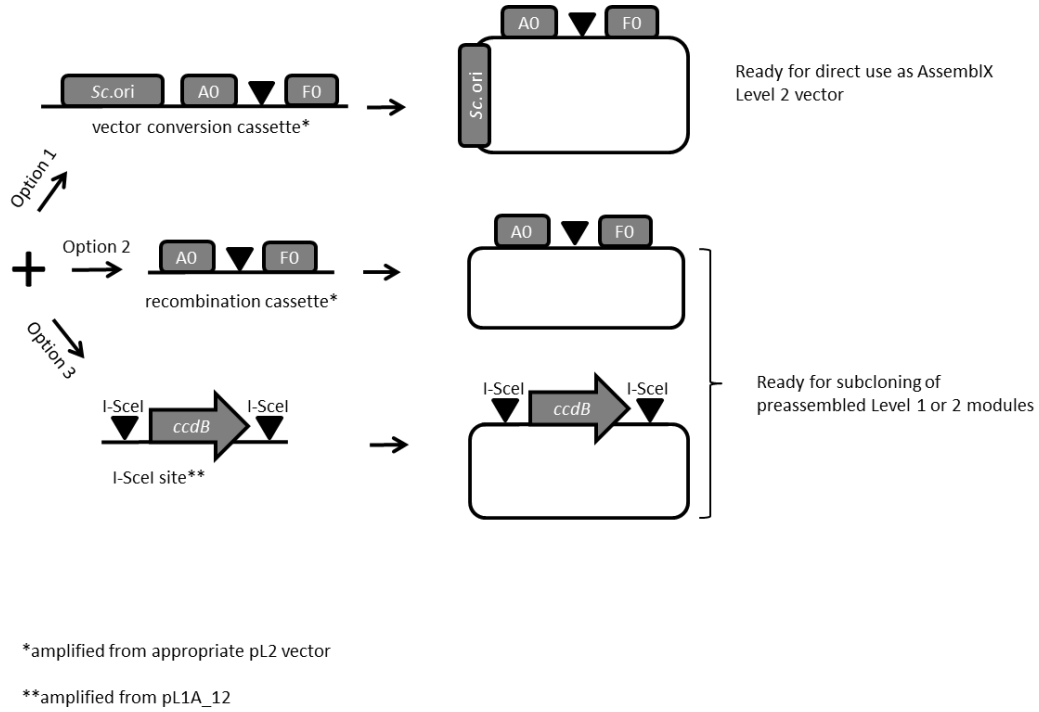
- Identify positive clones by colony PCR and restriction analysis and proceed with transformation in your final host.

Option 3: Subclone AssemblX Level 1 or 2 module into your expression vector via restriction & ligation.

- For this option, make sure that your expression vector does not contain an I-*Sce*I site.
- Design primers that amplify the I-*Sce*I - *ccdB* - I-*Sce*I cassette from pL1A_12.
- Equip primers with overlaps compatible to your linearized expression vector and use these primers to amplify the cassette described above.
- Combine the following in an appropriate assembly reaction (e.g. HiFi DNA assembly or SLiCE):
    - Linearized expression vector
    - PCR-amplified I-*Sce*I - *ccdB* - I-*Sce*I cassette

      For this step, make sure to use ccdB survival cells.

- Identify positive clones by colony PCR and restriction analysis.
- Use the modified expression vector to subclone your AssemblX Level 1 or 2 construct. For this, digest your verified construct with I-*Sce*I, purify the fragment and perform a ligation with your I-*Sce*I digested modified expression vector.
- Identify positive clones by colony PCR and restriction analysis and proceed with transformation in your final host.



*amplified from appropriate pL2 vector

**amplified from pL1A_12

Figure S1: Three different possibilities to convert any expression vector into an AssemblX compatible vector. Option 1: Equip expression vector with a yeast replication origin and use the converted vector directly in a TAR mediated Level 2 Assembly. The appropriate cassette can be amplified from the existing AssemblX Level 2 vectors. Option 2: Equip expression vector with a recombination cassette compatible to your Level 2 construct. The appropriate recombination cassette can be amplified from the existing AssemblX Level 2 vectors. Following the vector conversion, the modified vector can be used to subclone an *I-SceI* released Level 2 module via overlap based cloning. Option 3: Equip expression vector with an I-*Sce*I-*ccdB*-I-*Sce*I cassette amplified from vector pL1A_12. The modified vector can be digested with I-*Sce*I and ligated to any I-*Sce*I released fragment from any AssemblX Level 1 or 2 construct.