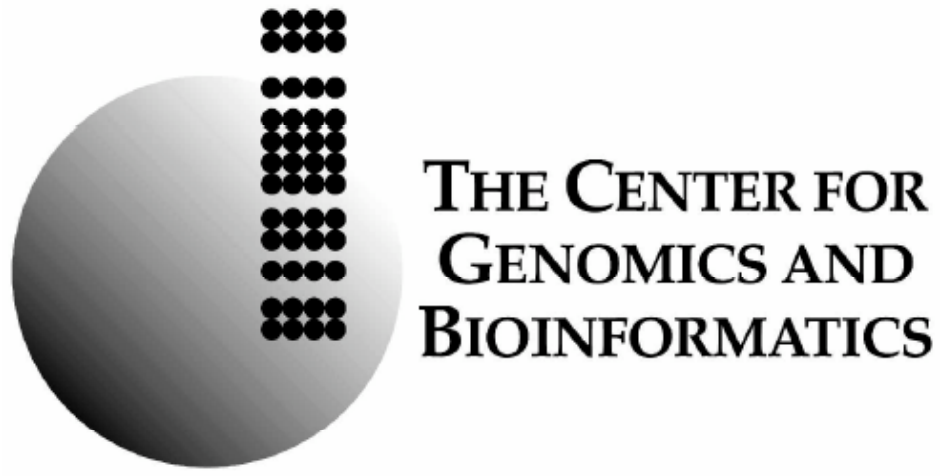

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DGRC Vector Standard Operating Procedure 1.0: Processing Clones for Whatman® FTA® Discs

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DGRC Vector Standard Operating Procedure 1.0: Processing Clones for Whatman® FTA® Discs

(adapted from the Whatman protocol)

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Please process clones within a month of receipt for best results:

Items needed:

1X sterile TE
Thawed competent cells on ice -
* We recommend DH5alpha or an equivalent strain
* Cells should have a transformation efficiency of 1 x 10^7 cfu/ug or higher
1 ml LB media aliquots
LB (+ appropriate antibiotic) plates (see p. 2 for additional considerations)

To process:

1. Add 50 µl of 1X sterile TE to the microfuge tube with the clone disc and pipet up and down quickly 2X - REMOVE the "TE" IMMEDIATELY. This is the critical step **– do not leave the TE on the disc for more than one or two seconds (see note below).**

Please note: **The TE step is necessary to wash off the chemical that Whatman has placed on the filter – it is this chemical that facilitates the lysis of the bacterial clones and allows the DNA to stick. Failure to do the TE wash step will inhibit transformation. However, it is important to note that if the TE sits on the disc for more than one or two seconds, the DNA will elute off and be lost, resulting in a failed transformation.**

2. Place tube on ice

3. Add 50 µl of competent cells and incubate on ice for 30 minutes. Transformation efficiency will increase if you vortex the competent cells/disc mixture half way through the ice incubation step and once more before the heat shock. Vortexing should be for one second only and return immediately to ice.

4. Heat shock cells/disc for 2 minutes at 37°C

5. Transfer cells only (leave disc behind) to 1 ml of LB media and incubate with shaking at 37°C for one hour

6. Plate 100µl and 200µl on LB (+antibiotic) plates**. For GOLD and EST clones we recommend plating the entire transformation.**

Ideally you will obtain approximately 1 colony for every microliter of the transformation plated. However, there will be considerable variability among clones.

If you have followed these procedures exactly and did not obtain colonies, please contact Kris Klueg (dgrc@cgb.indiana.edu).

Other important considerations:

Subcloning efficiency competent cells are **not** recommended. If you are purchasing cells commercially, we recommend library efficiency. If you are making your own cells, the transformation protocol on our website typically gives cells that are 1 x 10^7 cfu/ug.

For chloramphenicol resistant clones, do not use XL10 GOLD competent cells. These cells carry a chloramphenicol-resistance gene on the F' episome

Chloramphenicol – the concentration requirement in plates (for transformation) versus media (for large scale growth) differs. Please see our FAQ page on our website for the concentrations

cDNAs from the *RE library* (REXXXXX) give low DNA yields when grown on ampicillin. For large scale DNA preps, we recommend you use carbenicillin (an ampicillin analog) at 75 µg/ml. (Using carbenicillin for the initial transformation is not necessary).

We recommend using carbenicillin for large scale DNA preps of many of the common vectors (pUAST deriviatives, pCaSpeR derivatives, and Gateway).

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