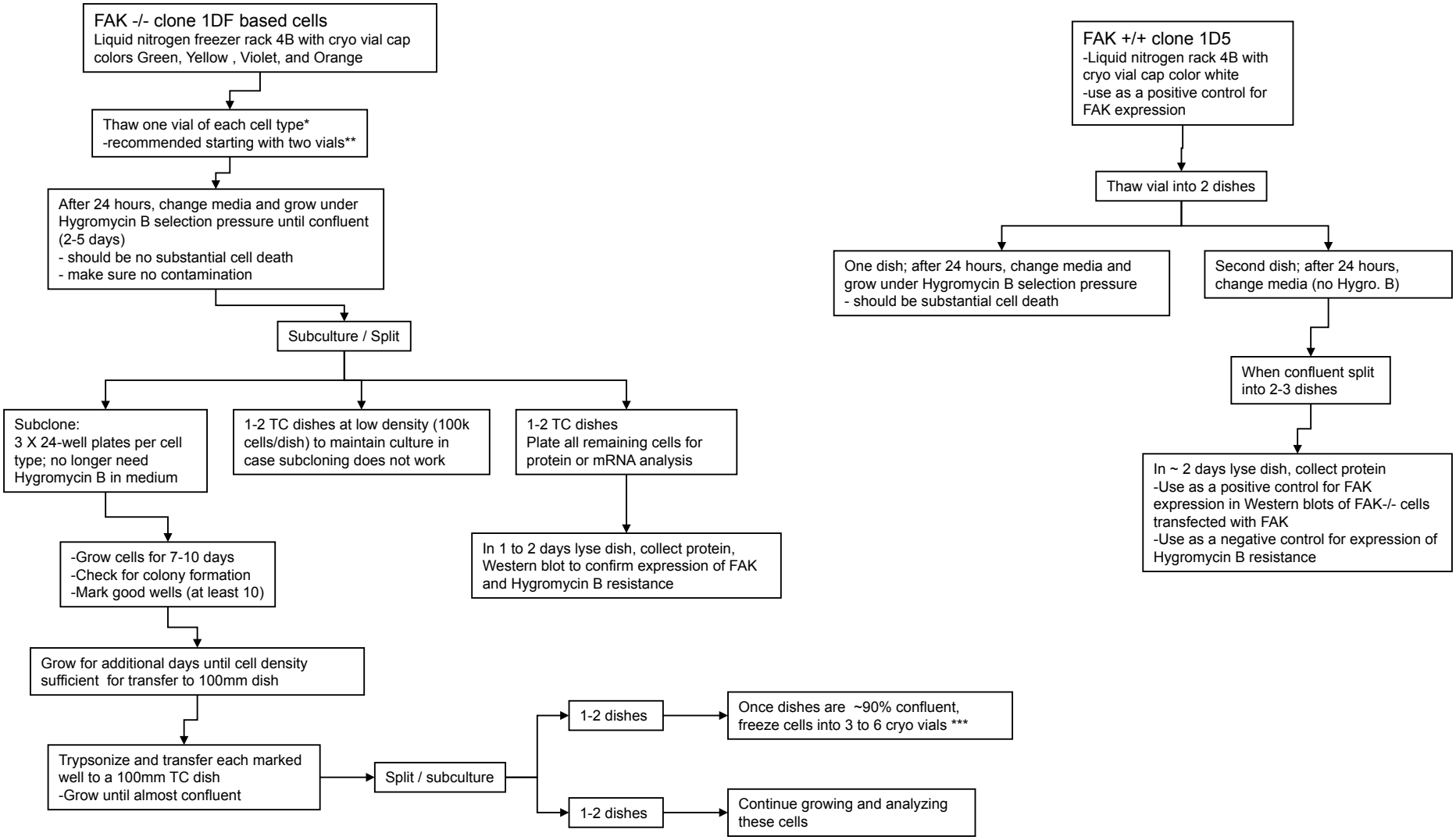
# Suggested Outline for Developing Subclones of Transfected FAK -/- cells




* It is recommended that subcloning of only two types of cells is performed at a time since the final stages of the process will result in 30 + tissue culture dishes per cell type. There are four types of cells developed from FAK -/- clone 1Df. The FAK -/- 1Df cells transfected with the empty pcDNA 3.1 Hygro + vector only ("vector control" cells) have green cryovial caps and express the Hygromycin B resistance gene only. The FAK -/- 1Df cells transfected with pcDNA 3.1 Hygro + wtFAK have violet cryo vial caps and express the Hygromycin B resistance and wild type murine FAK. In a similar way the cryovials with yellow caps express FAK Y397F mutant (tyrosine 397 change to phenylalanine) and Hygro B resistance, and the cryo vials with orange caps express FAK Y925F and Hygro B resistance.

** It is also recommended that you perform this process with the FAK -/- 1Df cells that are expressing vector only (green) and wtFAK (violet). The wtFAK cells should be the easiest to analyze for the expression of FAK and the vector control cells can be used as a negative control for FAK expression.

*** Several vials of each subcloned cell type should be frozen at the lowest passage number possible. These cells should only be used in the future for growing and freezing more cells (sort of like a farmer's "seed" stock of a certain plant).

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FAK -/- clone 1E6 based cells
Liquid nitrogen freezer rack 4B with cryo vial cap colors Pink, Gray, Blue, and Tan

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Thaw vial
-Note these cells are at a lower density than FAK -/- clone 1Df based cells

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After 24 hours, change media and grow under Hygromycin B selection pressure for a minimum of 15 days
- These cells need the additional time under selection pressure to ensure they are stably transfected
- make sure no contamination

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Subculture / Split

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Follow procedure given for FAK -/- 1Df cells on previous slide for subcloning etc.

**Simple illustration of subcloning process**