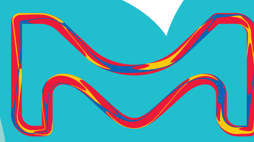
MERCK

THE RESEARCHER'S GUIDE TO RNAi Screening

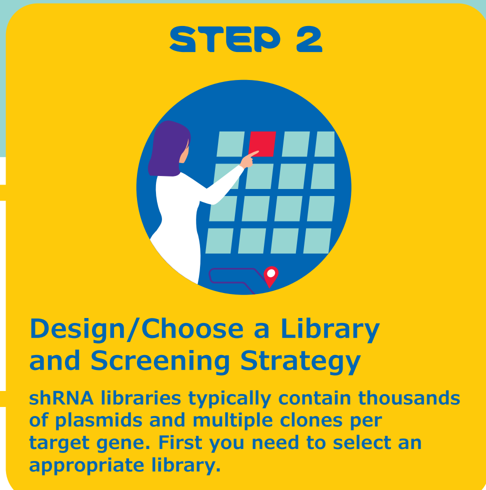


STEP 1

Determine Cell Line

Identify which cell lines will work for your requirements.

1. Ensure the cell line is a good model in terms of relevance, biological process & genotype
2. Do you need a primary, transformed or stem cell platform?
3. Determine if the cell line can be adapted to your workflow
4. Consider the doubling time and ploidy of the cell line

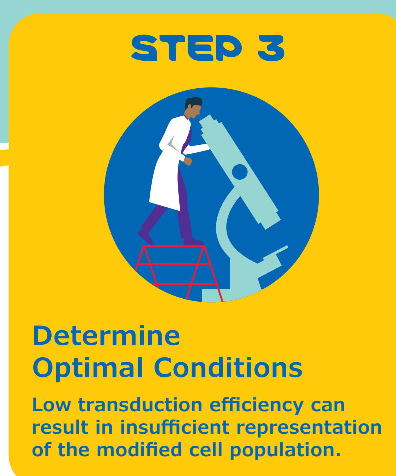


STEP 2

Design/Choose a Library and Screening Strategy

shRNA libraries typically contain thousands of plasmids and multiple clones per target gene. First you need to select an appropriate library.

1. Are you interested in the whole genome or a more focused pathway?
2. Lentivirus shRNA or siRNA?
3. Pooled or arrayed? (also see step 5)
   - Pooled: maximize the number of shRNAs per gene target (minimum of 3 advised)
   - Arrayed: optimize the shRNA selection (we have the largest collection of validated shRNA clones including TRC1, 1.5 and 2.0 libraries)
4. Controls: Use non-targeting shRNA clones and consider controls for enrichment and depletion depending on your screening approach
5. Use optimal designs for shRNA and—if designing your own libraries—spread them to avoid clusters (5′ UTR, exon, 3′ UTR)

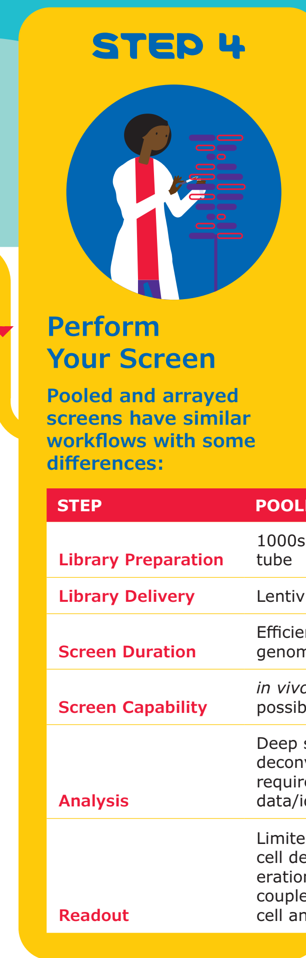


STEP 3

Determine Optimal Conditions

Low transduction efficiency can result in insufficient representation of the modified cell population.

1. Perform a kill curve to determine the concentration of selection antibiotic needed to kill un-transfected/un-transduced cells
2. Determine the functional titer in your intended cell line using:
   - A colony forming unit assay based on antibiotic resistance or
   - A vector containing a fluorescence marker like GFP
3. Use a control vector to optimize the multiplicity of infection (MOI)
   - Use the lowest MOI that offers one gRNA per cell
4. Need a custom vector to fit your needs? We can help by making custom vectors with your choice of promoters, fluorophores or selection markers



STEP 4

Perform Your Screen

Pooled and arrayed screens have similar workflows with some differences:

STEP	POOLED	ARRAYED
Library Preparation	1000s shRNAs per tube	1 shRNA per well
Library Delivery	Lentivirus required	Multiple format options
Screen Duration	Efficient whole genome screening	Time to screen increases with the no. clones
Screen Capability	*in vivo* screening possible	*in vivo* screening not possible
Analysis	Deep sequencing/ deconvolution required to analyze data/identify hits	NGS is not required to understand results
Readout	Limited options (e.g. cell death or proliferation) but can be coupled with single cell analysis	Multiple options e.g. fluorescence, luminescence, high content imaging

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