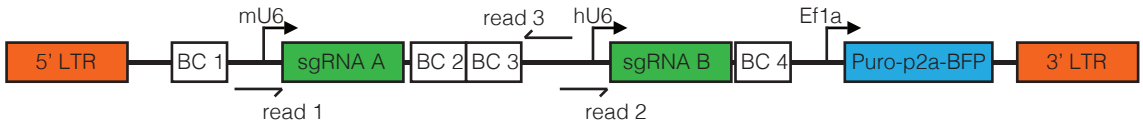
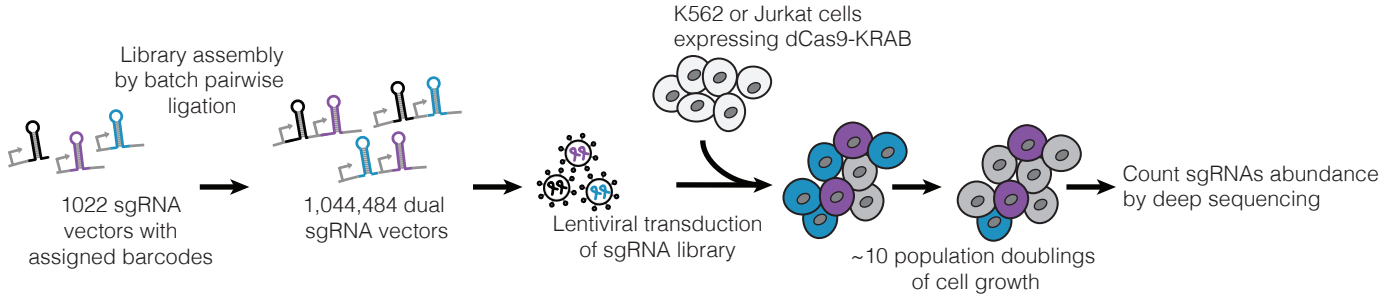
A

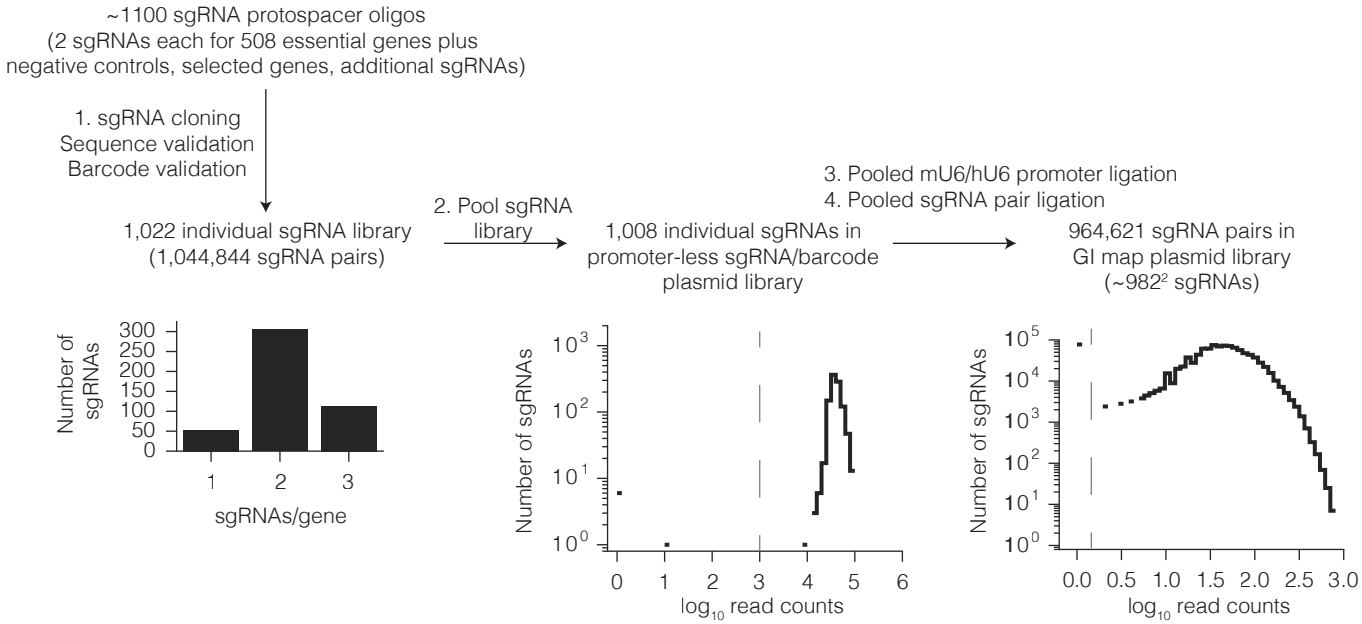
triple sequencing strategy



B



C



D

Count sgRNA sequence/barcode representation

- Align sgRNA and barcode sequences to expected library (tolerate 1 mismatch)
- Discard reads where sgRNA and corresponding barcode identities do not match
- Count sgRNA pairs

↓

Calculate sgRNA pair phenotypes

- sgRNA pair phenotype (γ) = $\log_2$ enrichment (Endpoint/T0) / # cell doublings - median negative control sgRNA pair phenotype
- sgRNA single γ = mean of sgRNA pair γ's of sgRNA with non-targeting controls
- require single sgRNAs to have median 35 reads across all pairs

↓

Compute sgRNA-level genetic interactions

- Average replicate phenotypes and AB/BA pair phenotypes
- GI score = deviation from fit of sgRNA single γ's and pair γ's (see Supplementary Figure 4)
- Average GI scores for each query-sample/sample-query pair

↓

Compute gene-level genetic interactions

- Average all sgRNA pair GI scores corresponding to each gene pair

	K562		Jurkat	
Replicate	1	2	1	2
Plasmid library pairs present	964,621			
T0 pairs >35 reads	954985*	958022*	932656	918041
Endpoint pairs >35 reads	771605	763812	747659	770970
Pairs with scored phenotypes	762129	743044	695556	722500
Single sgRNAs	873	862	834	850
Pairs after replicate averaging	739600		693889	
Unique pairs after AB/BA averaging	370230		334153	
Pairs after phenotype filter (Jurkat only)			203841	
Unique gene pairs	100576		75466	
Single genes	448		388	
Shared K562/Jurkat genes	378			