Criteria for selecting non-target shRNA as a general KD control

Criteria: In line with the following conditions

① **No apparently deleterious effect on infected cells monitoring by cell survival;**

② **Uploaded siRNA predominantly derived from one of the strands of shRNA (increase the probability of off-target if both strands are uploaded onto RISC);**

③ **The profile of transcriptome altered by shRNA is minimal.**

Procedures for selecting non-target shRNA as a general KD control

1. **Selection of candidate shRNAs for study**
   6 newly designed or 33 non-target shRNAs picked from TRC control plate that do not cause apparent cell death
2. **Solexa analysis**
   Two pooled lentiviruses (newly designed or TRC control shRNAs) were prepared and transduced into A549 cells, respectively. After 72 hrs p.i., total RNAs were purified for Solexa analysis. The amounts and frequency of strand-specific expression of the shRNA were analyzed. The shRNA with predominantly expressing one of the strands were selected for subsequent experiment
3. **Sample preparation for Microarray analysis**
   Selected shRNAs were packed into lentiviruses and Huh7, A549 and HeLa cells were infected by such viruses, respectively. Five days later, total RNAs were isolated for microarray analysis.
4. **Microarray analysis**
   Compared to mock control, the fold change of cellular gene expression(up or down regulated) were calculated. The shRNAs with less effect on altering the numbers of cellular gene expression were chosen as negative control shRNAs.

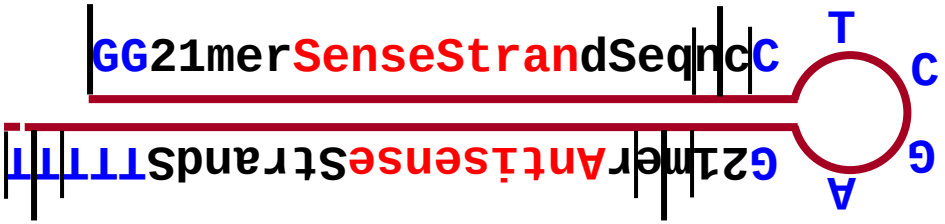
The profile of strand-specific expression of shRNAs

(39 shRNAs were analyzed)

Name	Number and (%) of Reads		
	Sense strand	Antisense strand	Total NGS reads
shLuc 221*	335,128 (98.5%)	5,315 (1.5%)	1^{st} = 2.3X10^7
pVOID#	8 (18.6%)	43 (81.4%)	
shLacZ1339	1,050,766 (99.91%)	916 (0.09%)	2^{nd} = 4X10^7
shLuc976	156 (0.13%)	116,187(99.87%)	

*: Current control shRNA in the selling list

#: VOID stands for vector out of interfering determinants



Gene expression profile

A549	26,009 RefSeq probes		
	Up> 2FC	Down > 2FC	No Change <2FC
shLuc221	4	115	19,558
pLKO.1.nullT	8	135	20,803
pVoid	0	0	22,615
shLacZ1339	3	14	21,756
shLuc976	19	52	21,541
HeLa	**26,009 RefSeq probes**		
	Up> 2FC	**Down > 2FC**	**No Change <2FC**
pVoid	3	0	20371
shLacZ1339	0	0	20,481
shLuc976	0	1	20,505
HuH7	**26,009 RefSeq probes**		
	Up> 2FC	**Down > 2FC**	**No Change <2FC**
pVoid	0	0	19971
shLacZ1339	3	5	21,477
shLuc976	0	0	20,442

FC: Fold change

Clone Detailed Information

Name	Clone ID	Vector	Gene Symbol	Plate	Well locatoion	Target Sequence
shLuc221	TRCN0000072246	pLKO.1	LUCIFERASE	CTR01	F9	CAAATCACAGAATCGTCGTAT
shLuc976	TRCN0000072249	pLKO.1	LUCIFERASE	CTR01	F12	GCGGTTGCCAAGAGGTTCCAT
	TRCN0000231719	pLKO_TRC005	LUCIFERASE	CTR21	F12	GCGGTTGCCAAGAGGTTCCAT
shLacZ1339	TRCN0000072224	pLKO.1	lacZ	CTR01	D11	CGCGATCGTAATCACCCGAGT
	TRCN0000231722	pLKO_TRC005	lacZ	CTR21	D11	CGCGATCGTAATCACCCGAGT

Summary

1. The pVOID shRNA barely expressed siRNA in A549 cells. This may account for the extremely low off-target effect in A549, Huh7 and HeLa cells upon transduced with pVoid-generated lentivirus. Thus, pVoid may function as a lentivirus control.
2. The siRNAs derived from shLacZ1339 and shLuc976 predominantly processed from one strand of shRNA. The level and numbers of gene expression altered by these shRNAs are minimal compared to shLuc221, a control shRNA being sold currently in the Core.

Proposal

1. The pVOID shRNA is proposed to replace pLKO.1.nullT, a lentivirus control sold by the Core currently.
2. shLacZ1339 and shLuc976 are proposed to replace shLuc221 control shRNA.