

All 23-mer subsequences within the target mRNA

100%

Step 1

Selection of functional siRNAs

Satisfies functional siRNA rule

No 75.3%

Yes 14.7%

Step 2

Reduction of seed-dependent off-target effects

T_m in the seed-target duplex

< 21.5 °C

No 11.7%

Yes 3.0%

Step 3

Elimination of near-complete matched genes

At least 2 mismatches against any non-targeted mRNAs

No 0.9%

Yes 2.1%

Functional, seed-dependent off-target reduced, and target-specific siRNA candidates

