

Claims

1. A method of producing double stranded RNA of from 21 to 23 nucleotides in length comprising:
 - (a) combining double-stranded RNA with a soluble extract that mediates RNA interference, thereby producing a combination; and
 - (b) maintaining the combination of (a) under conditions in which the double-stranded RNA is processed to double stranded RNA of from 21 to 23 nucleotides in length.
2. The method of claim 1 further comprising isolating the double stranded RNA of from 21 to 23 nucleotides in length from the combination.
3. A method as claimed in any one of claims 1 or 2 wherein the double stranded RNA corresponds to a sequence of a gene to be degraded; and the RNA of from 21 to 23 nucleotides in length mediates RNA interference of mRNA of the gene to be degraded, thereby producing double stranded RNA of from 21 to 23 nucleotides in length that mediates RNA interference of the mRNA of the gene.
4. A method of mediating RNA interference of mRNA of a gene in a cell or organism comprising:
 - (a) introducing double stranded RNA of from 21 to 23 nucleotides in length and corresponding to a sequence of the gene which targets the mRNA of the gene for degradation into the cell *in vitro* or a cell of the organism *in vitro* and returning the cell of the organism to the organism;

- (b) maintaining the cell or organism produced in (a) under conditions under which degradation of the mRNA occurs, thereby mediating RNA interference of the mRNA of the gene in the cell or organism.
5. A method of examining the function of a gene in a test cell or test organism, comprising mediating RNA interference of mRNA of a gene in a cell or organism as claimed in claim 4 wherein the organism is a non-human organism and the cell and organism are a test cell and test organism respectively; and
- (c) observing the phenotype of the test cell or test organism produced in (b) and, optionally, comparing the phenotype observed to that of an appropriate control cell or control organism, thereby providing information about the function of the gene.
6. A method as claimed in claim 4 further comprising:
- (a) combining double-stranded RNA that corresponds to a sequence of the gene with a soluble extract that mediates RNA interference, thereby producing a combination;
 - (b) maintaining the combination produced in (a) under conditions under which the double stranded RNA is processed to RNA of from 21 to 23 nucleotides in length, thereby producing double stranded RNA of from 21 to 23 nucleotides in length;
 - (c) isolating the double stranded RNA of from 21 to 23 nucleotides in length produced in (b) prior to the step of introducing RNA isolated in (c) into the cell or organism.

7. A method of assessing whether an agent acts on a gene product comprising mediating RNA interference of mRNA of a gene in a cell or organism as claimed in claim 4 wherein the organism is a non-human organism and;
 - (c) introducing the agent into the cell *in vitro* or the cell of the organism *in vitro* of (b) prior to returning the cell to the organism; and
 - (d) determining whether the agent has an effect on the cell or organism, wherein if the agent has no effect on the cell or organism then the agent acts on the gene product or on a biological pathway that involves the gene product.
8. A method of assessing whether a gene product is a suitable target for drug discovery comprising mediating RNA interference of mRNA of a gene in a cell or organism as claimed in claim 4 wherein the organism is a non-human organism and degradation of the mRNA results in decreased expression of the gene; and:
 - (c) determining the effect of the decreased expression of the gene on the cell or organism, wherein if decreased expression has an effect, then the gene product is a target for drug discovery.
9. The method of any one of claims 4-8, wherein the gene encodes a cellular mRNA or a viral mRNA.
10. A method of producing knockdown cells, comprising introducing into cells in which a gene is to be knocked down isolated double stranded RNA of from 21 to 23 nucleotides in length and corresponding to a sequence of the gene, that targets the mRNA corresponding to the gene and maintaining the resulting cells under conditions under which RNAi occurs, resulting in degradation of the mRNA of the gene, thereby producing knockdown cells.

11. A method of identifying target sites within mRNA that are efficiently cleaved by the RNAi process, comprising combining double stranded RNA of from 21 to 23 nucleotides in length and corresponding to a sequence of a gene to be degraded, labeled mRNA corresponding to the gene and a soluble extract that mediates RNA interference, thereby producing a combination; maintaining the combination under conditions under which the double stranded RNA is degraded and identifying sites in the mRNA that are efficiently cleaved.
12. A method of identifying 21-23 nucleotide RNAs that efficiently mediate RNAi, wherein said 21-23 nt RNAs span the target sites identified within the mRNA by performing the method of claim 11, and further comprising identifying a 21-23 nucleotide RNA that spans target sites.
13. A method as claimed in any one of claims 1-12 wherein the double stranded RNA is obtained from double-stranded RNA that has been cleaved into fragments of from 21 to 23 nucleotides in length.
14. A method as claimed in any one of claims 1-13 wherein the double stranded RNA comprises a terminal 3' hydroxyl group.
15. A method as claimed in any one of claims 1-14 wherein the double stranded RNA is chemically synthesized RNA.
16. A method as claimed in any one of claims 1-15 wherein the RNA comprises one or more non-standard nucleotides, including non-naturally occurring nucleotides.
17. A method as claimed in any one of claims 1-16 wherein the double stranded RNA is isolated using a method selected from gel electrophoresis, non-denaturing methods, or non-denaturing column chromatography.

18. A method as claimed in any one of claims 1-17 wherein the double stranded RNA is complementary to one or more of mammalian cellular mRNA, human mRNA or viral mRNA.

19. The method of any one of claims 1-3, 6, 11, or 12 wherein the soluble extract is derived from syncytial blastoderm *Drosophila* embryos.