Fact-checking of nucleotide sequences in life science publications: the *seek & blastn* tool

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Automatic detection of questionable research papers

Scientific ethics

- Plagiarism, auto-plagiarism, content reuse...
- $N - grams$ signature (hashing functions).

Non-sense detection

- Paper generator (SCIgen, physic-gen, MathGen...)
- Authorship detection (inter-textual distance).

Need to detect questionable scientific results

- Fabrications (making up data or results)
- Falsification (manipulating data or results)
- False or unsupported affirmations
- Genuine errors

$\Longrightarrow$

- Error spreading
- Wrong belief
- Research irreproducibility

Starting point : striking similarities, obvious errors

Jennifer Byrne:

- First reported *TPD52L2* (20 years ago)
- 5 Publications with obvious errors!

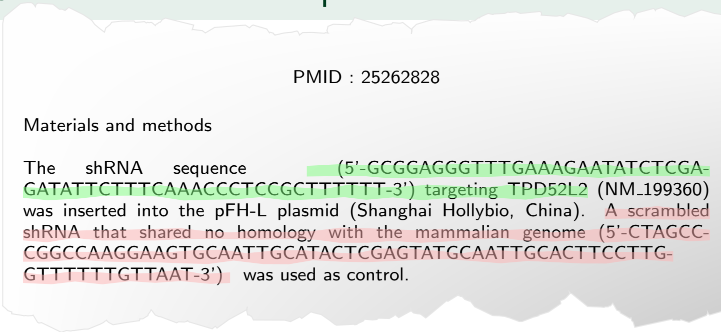
5 Publications from China:

- Single gene knockdown experiments.
- Human cancer cell lines.

Conclusions highlight potential therapy

- ...TPD52L2... novel therapeutic target for glioma treatment.
- ...TPD52L2... novel clues for oral squamous cell carcinoma therapy.
- ...TPD52L2... therapeutic approach for the treatment of breast cancer.
- ...TPD52L2 is indispensable in gastric cancer proliferation.
- ...TPD52L2 could be a novel therapeutic target for human liver cancer.

Obvious errors: example

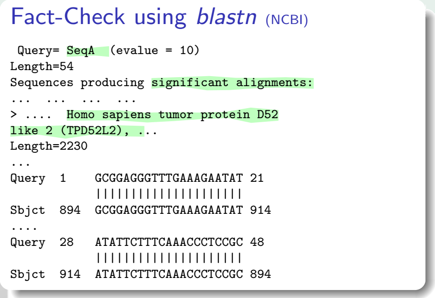


PMID : 25262828

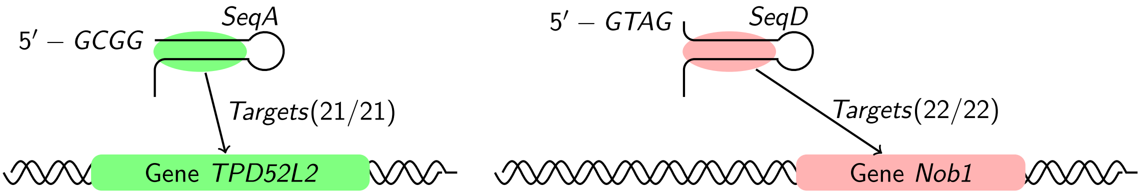
Materials and methods

The shRNA sequence (5'-GCGGAGGGTTTGAAAGAATATCTCGA-GATATTCTTTCAAACCCTCCGCTTTTTT-3') targeting TPD52L2 (NM_199360) was inserted into the pFH-L plasmid (Shanghai Hollybio, China). A scrambled shRNA that shared no homology with the mammalian genome (5'-CTAGCC-CGGCCAAGGAAGTGCAATTGCATACTCGAGTATGCAATTGCACTTCCTTG-GTTTTTTGTTAAT-3') was used as control.

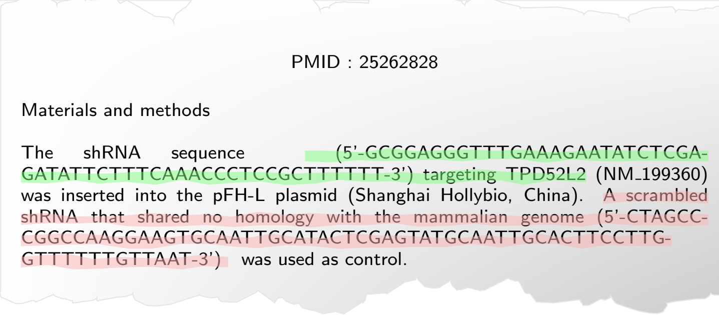
Fact-Check using *blastn* (NCBI)



```
 Query= SeqA  (evalue = 10)
Length=54
Sequences producing significant alignments:
...  ...  ...  ...
> ....  Homo sapiens tumor protein D52
like 2 (TPD52L2), ...
Length=2230
...
Query  1    GCGGAGGGTTTGAAAGAATAT 21
            |||||||||||||||||||||
Sbjct  894  GCGGAGGGTTTGAAAGAATAT 914
....
Query  28   ATATTCTTTCAAACCCTCCGC 48
            |||||||||||||||||||||
Sbjct  914  ATATTCTTTCAAACCCTCCGC 894
```



Obvious errors: example



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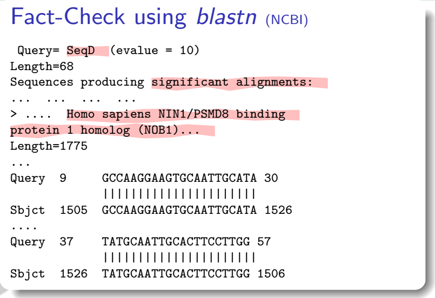
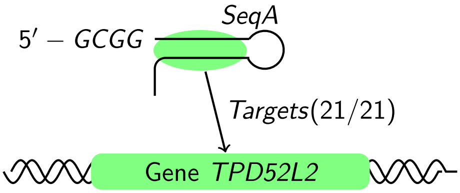
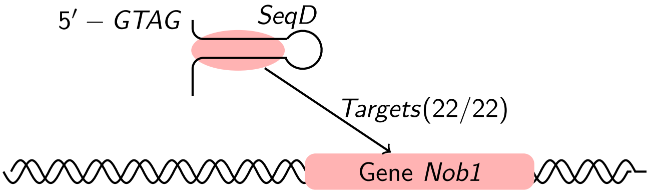


Fact-Check using *blastn* (NCBI)

```
 Query= SeqD  (evalue = 10)
Length=68
Sequences producing significant alignments:
...  ...  ...  ...
> ....  Homo sapiens NIN1/PSMD8 binding
protein 1 homolog (NOB1)...
Length=1775
...
Query  9     GCCAAGGAAGTGCAATTGCATA 30
             ||||||||||||||||||||||
Sbjct  1505  GCCAAGGAAGTGCAATTGCATA 1526
....
Query  37    TATGCAATTGCACTTCCTTGG 57
             |||||||||||||||||||||
Sbjct  1526  TATGCAATTGCACTTCCTTGG 1506
```





Nucleotide sequence by *Status* (targeting vs non-targeting)

Targeting

Primers:

Two sets of primers were used for PCR: β-actin (ACTB) forward, 5'-GTGG...AGAC-3' and reverse, 5'-AAAG...AACTA-3'; NOB1 forward, 5'-GAAAG....TGGAG-3' and reverse, 5'-CAGCCTTGAGATGACCTAAGC-3'.

Silencing:

shRNA targeting the NOB1 (CCGGGCTGAACA...TTGTTCAGCTTTTTG).

Positive control:

A NOB1 positive control (5'-CCG...TT-3') was used ...

Non-Targeting

Negative control:

... and negative control (TTCTC...CACGT) sequences were cloned into...

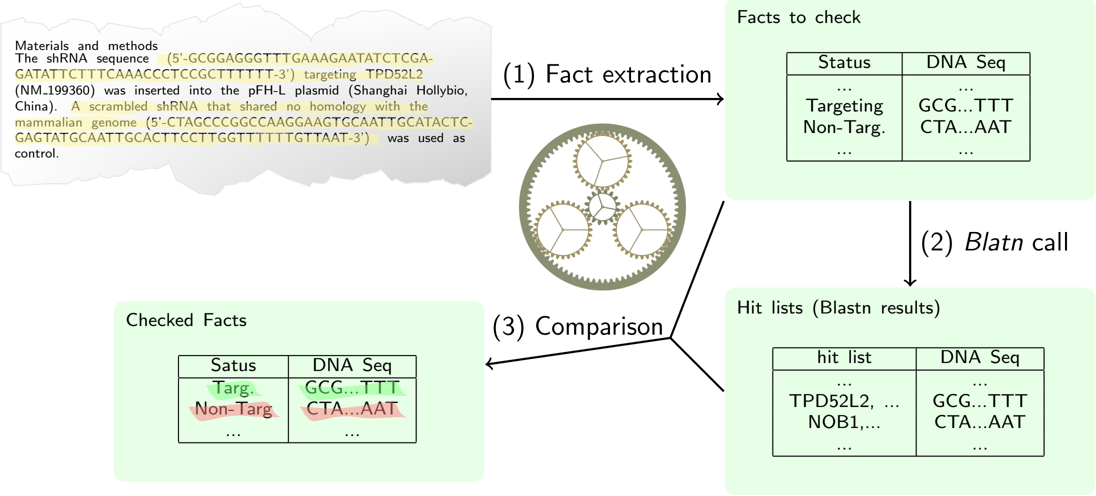
Non-targeting:

Non-targeting shRNA sequence (5-CTAGCC ... TTGTTAAT-3) was used as a control.

Scrambled:

A scrambled sequence (5'-GCGGA ... CTTTTTT-3') that has no significant homology with human gene sequences was used as a negative control.

Seek & Blastn at a glance



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(1) Fact extraction

Facts to check

Status	DNA Seq
...	...
Targeting	GCG...TTT
Non-Targ.	CTA...AAT
...	...

(2) *Blatn* call

Hit lists (Blastn results)

hit list	DNA Seq
...	...
TPD52L2, ...	GCG...TTT
NOB1,...	CTA...AAT
...	...

(3) Comparison

Checked Facts

Satus	DNA Seq
Targ.	GCG...TTT
Non-Targ	CTA...AAT
...	...

Seek & Blastn steps

(1) Fact extractions

Named entity recognition techniques (thesaurus and rules):

- identifies gene names, contaminated cell lines.

Sequences containing DNA sequences are analyzed (*Finite-state machines*):

- extract nucleotide sequences (15-90 bases),
- assign a status *targeting* or *non-targeting*.

(2) Blastn call

NCBI software gives the hit list for each sequence.

(3) Blastn analysis vs text-extracted information

Set of rules to check whether or not Blastn results are compatible with affirmation detected in the text.

Used Corpora

Problematic Papers (CorpusP)

- A cohort of highly similar cancer research publications.
- 38/48 (79%) included nucleotide sequence(s) that did not match their experimental use (according to *blastn*).

Unknown papers (CorpusU)

154 papers, automatically retrieved using papers from CorpusP and the "PubMed similar" functionality. Mostly open-access[a].

[a] because when fee-based, automatically download is not permitted

Tests and results

Seek & Blastn performances

- In CorpusP and CorpusU, nucleotide sequences were extracted from 48/48 (100%) and 111/154 (73%) papers.
- Claims were not (correctly) identified for 19/341 (5.6%) sequences in CorpusP.
- Identification of the 38/48 (79%) papers in CorpusP incorrectly use nucleotide sequence.

Error detection in scientific literature

- 38/48 (79%) papers in CorpusP appear to have incorrectly employed nucleotide sequence.
- *Seek & Blastn* predicted that 30/154 (19%) CorpusU papers may have incorrectly employed nucleotide sequence reagent(s) but roughly half of them are.

Results suggest ...

that in addition to the "knock down" series, there may exists a "migration series", a "prognosis series", ...

Conclusion

Automatic detection, related works

- Detection of statistically flawed papers
- Fake news detection

Seek & Blastn perspectives

- Online tool : `http://scigendetection.imag.fr/TPD52`
- Avoid false positive.
- Tests of more in-depth analysis of sentences.

How Seek & Blastn could be use

- Pre- and post-publication checking
- Contribute to publishing guidelines
    - Inclusion of sequences within publications, Nucleotide sequence formatting
- Identification of other forms of misconduct