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Dark side of Molecular Techniques: How to fix it ?

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Abstract: Many fundamental molecular techniques (PCR, Microarray, Southern and northern hybridization, siRNA, CRISPR/Cas9 etc,) developed so far shows errors. I wish to highlight these molecular techniques are developed on basis of Watson-Crick DNA model, ignoring the concept of parallel stranded DNA. Through this opinion article I wish to highlight specificity and accuracy of these molecular techniques can be enhanced by considering both parallel and anti-parallel hybridization of DNA. Hopefully my views will also solve issue of irreproducibility in life science research.

Keywords: parallel DNA; antiparallel DNA; PCR; CRISPR; nucleic acid hybridization; microarray; siRNA

Introduction

Literal meaning of science is 'to know' and none of us in this world want to know false facts. Everyone is searching the ultimate truth, the knowledge which can be applied for betterment of society. Today worldwide research in field of biology, not merely depends on "Hypothesis" only but it is more technology driven. After the discovery of molecular structure of DNA (Watson-Crick double helical model), there has been a lot of progress in field of molecular biology and many molecular techniques (PCR, Microarray, Southern and northern hybridization, siRNA, CRISPR/Cas9 etc) have been developed. Today these molecular techniques are widely used to study fundamental principles of life and many research papers published in life science fields are based on these molecular techniques. In 2012, the biotechnology company Amgen reported that only 10% of researches published in top journals by world best labs are reproducible (1). It is an alarming situation that $28 billion dollar is wasted in life science field every year in USA alone (2). What is total wastage world-wide? In a recent survey on reproducibility in various fields, "More than 70% of researchers reported that they have tried and failed to reproduce another scientist's experiments, and more than half have failed to reproduce their own experiments. 52% of those surveyed agreed that there is a significant 'crisis' of reproducibility, less than 31% think that failure to reproduce published results means that the result is probably wrong"(3). It clearly indicates that now we are moving into dark phases. As it is difficult for a common man to search things in darkness, it will be difficult for scientists to search true findings among false ones. You must have heard the story in which a group of blind men when investigated an elephant comes to different opinions after touching the elephant (4). Every scientific finding is based on previously published literatures which are commonly cited as reference within an article. If we consider, 90% of research articles are irreproducible then there will be a very high probability that every finding in life science research is discussed/based on much irreproducible research papers directly or indirectly. Whatever knowledge we generate today, have to be used by next generation of young scientists. Will it not confuse many young scientists for next many hundred years? Will it not make situations of young scientists as equal to blind men? Many labs are publishing lot of findings. It will become difficult to find absolute novel usable research among large number of research papers. In last few years, Phase II success rate for new development projects has decreased by 10% and this will definitely increase the cost of new drugs and decrease the trust of the public, Government and funding agencies in scientific activities (5). I wish to highlight one reason behind this is that many molecular techniques shows errors and there is a little progress in making them error free. You may read various published reports which have shown that various molecular techniques like Microarray (6,7,8,9,10), siRNA (11,12,13,14,15,16), CRISPER (17,18,19,20)

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shows errors. Even you must have experienced non-specific amplification in a PCR reaction and non-specific hybridization in Southern and northern hybridization experiments.

Recently We have raised an issue that much of research published in molecular biology field is based on 'Watson-Crick' anti-parallel DNA model (21) ignoring the concept of parallel stranded DNA model (22,23). Even various molecular techniques developed so far are based on antiparallel DNA model. We strongly believe that errors reported in various bio-techniques can be resolved by considering both parallel and anti-parallel hybridization of DNA (24). Recently, we have developed a PD-PCR technique by applying the knowledge of both parallel and anti-parallel hybridization of DNA (25). In 2008, Lestienne *et al.* has also reported that Triple helix primer (THP) bounded to the duplex DNA in a parallel orientation can initiate DNA synthesis (26). In different studies, it has been already reported that Southern hybridization reaction can be performed using parallel complementary probe and gene silencing can be applied using parallel complementary RNA (27,28,29). There are no drastic differences in nearest neighbour base pairs interactions between parallel and antiparallel DNA having mixed AT/GC composition. In parallel stranded DNA, both A-T and G-C pairs with two hydrogen bonds via reversed Watson-Crick base pairing rule (30). The minor groove becomes shallow and wide in parallel DNA. Thus, parallel complementary DNA contains groves of equal size in contrast to dissimilar grooves (major and minor grooves) found in antiparallel B-form of DNA. Various DNA binding drugs and dyes have been reported to show different molecular interaction with parallel stranded DNA in comparison to the antiparallel stranded DNA (31,32,33). In 2000, LeProust *et al* studied "GAA GAA G AAG AAG" and its complementary "CTT CTT C TTC TTC" mirror repeats sequence. Their results indicated that such type of sequences have a possibility to form triplex DNA, antiparallel duplex DNA and Parallel duplex DNA based on pH as confirmed by various physical, chemical and enzymatic probing methods. When such duplex mirror sequences were subjected to electrophoresis at pH 8.3, they separated into two bands. The faster migrating band (FM) corresponded to parallel DNA while the slower one represented antiparallel duplex DNA (34). In 1988, RamSing and Jovin have also reported that parallel duplex sequence migrates faster than antiparallel duplex (22).

In sum, complementary in DNA can be defined by two ways "Parallel Complementary" and "Antiparallel Complementary". In Current scenario, there is a need to develop molecular techniques (siRNA technology, Microarray, CRISPER genome editing etc) free of non-specific reactions then only we will be able to know root causes of various diseases. There is a science behind non-specific reactions in various molecular techniques. If we know the reason behind errors then only we can rectify it. We strongly believe applying knowledge by considering all possibilities (parallel and antiparallel hybridization of DNA, Non-watson Crick pairing rules) will minimize errors in molecular techniques and will take us towards a new light which will make us more reproducible in life science research. There will be more progress in life science if we come out of dark phase in lesser time and devote more time in light phase of life science. Molecular techniques without errors will increase reproducibility in research worldwide.

"Science is based on experiment, on a willingness to challenge old dogma, on an openness to see the universe as it really is. Accordingly, science sometimes requires courage- at the very least the courage to question the conventional wisdom" Carl Sagan

Competing interests: The author declare no competing interests

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