***[Strapline: One or two words to describe the general subject area. What about this? Or feel free to suggest an alternative]***

**Cancer genomics**

***[Main title: It should be no more than 40 characters, including spaces, should not include punctuation (including colons), and should be easily understandable for non-specialists How about this shortened, variation on yours, which just fits? Or feel free to suggest another]***

**Less is more in the hunt for driver mutations**

***[Standfirst: 190-225 characters, including spaces, to outline the new results for a general audience. The aim is to entice readers to reader on. How about this? Please amend as needed, bearing the aforementioned restrictions in mind]***

**An analysis of 360 breast-cancer genomes has identified nine cancer-driving promoters in non-coding DNA sequences that regulate gene expression, hinting at the prevalence of such drivers in cancer genomes. See Article p.XXX**

**Sushant Kumar & Mark Gerstein**

***[This opening paragraph is great. I’ve just made a few tweaks to fit our house style, which dictates that the opening sections should be simple and provide a teaser of the new results — is this OK?] [[OK]]***

A typical cancer genome contains thousands of mutations ***[OK? To avoid having to define somatic][[OK]]***, the overwhelming majority of which are in non-protein-coding sequences. Classical models of tumour evolution posit that cancer progression is driven by only a few of these. But almost all known driver mutations are in coding sequences1,2, raising the question of how many drivers lurk in non-coding regions. In a paper online in *Nature*, Rheinbay *et al.*3 make a foray towards the answer.

Identification of non-coding drivers is challenging, owing to the vastness of the genome and the difficulty of characterizing the precise location of non-coding elements (eg regulatory regions that modulate gene expression), which might contain drivers ***[Ok to add? To clarify what non-coding elements are and to introduce the roles of promoters and enhancers, which need defining at some point] [[OK]]***. Drivers in coding regions are easier to identify, because we have a better understanding of the boundaries of these regions and of the impact that mutations in them might on the production and function of proteins ***[Simplification OK?][[OK]]***. However, our better understanding potentially creates an ascertainment bias toward coding drivers, the drunk-looking-under-the-lamppost phenomenon in cancer genomics. ***[Simplification OK?][[reverted back part of it]]***. Consequently, with the whole-genome analysis of cancers there has been interest in identifying non-coding drivers4 ***[OK to shorten? As we don’t go on to discuss methods] [[OK]]***. Previous studies have provided a few examples5–7, but our understanding is far from complete. ***[Changes to shorten this section OK? Interested specialists can look to the papers to find the gene names][[OK]]***.

Rheinbay *et al.* set out to identify coding and non-coding driver mutations in an unbiased fashion, using samples from a cohort of 360 people with breast cancer. To find the non-coding ones, they identified non-coding elements harbouring significantly more mutations than expected, or that contained clusters of mutations around transcription-factor binding sites (known locations for regulating proteins to bind to). [[OK]]***[OK to move up? To begin the results by explaining how they set out to find non-coding drivers. Also, expanded explanations OK? Please amend as needed for accuracy]***.

***[OK to delete a sentence? To avoid repetition with that about hotspots later in this paragraph]*** The authors identified putative driver mutations in nine promoters, and showed that three of these significantly altered gene-expression levels (those associated with the *FOXA1, RMRP* and *NEAT1* genes). Their analysis of mutational hotspots (recurrent mutations at a single site) ***[Expansion OK?]***) [[was confusing so reverted]] indicated that those in promoters are as common as those in coding genes. Furthermore, they found that the per-base mutation rate of promoters with drivers was similar to that of coding regions with drivers. This suggests that that fewer drivers have been found in promoters than in coding regions ***[OK?][[fixed slightly]]*** simply because their "functional territory" is smaller.

This work describes the state-of-the-art in identifying non-coding drivers, but there is more to do. ***[OK to move the following sentence down? To lead into the discussion on power]*** The authors’ power analysis — statistical calculations estimating the sample numbers needed to detect an effect of a given size ***[Definition of a power analysis OK?]*** — indicated that their sample of 360 could be used to reliably identify drivers only if they occurred in at least 10% of patients in the cohort. To understand the directions for improvement, it is worth considering how non-coding elements are defined, and how this plays into statistical power (Fig. 1).

[[SK2MG: This green block of text to be included]]

Currently, many non-coding elements are annotated as being fairly large. ***[OK? Or please could you provide a number to give an idea of what fairly large means in this context?]***. However, this is partly because our techniques for determining the positions of these elements are imprecise, and the real functional territory of a regulatory element is considerably smaller than annotated. For instance, transcription-factor binding sites are often called as 1-kb "peaks" from a noisy cross-genome binding signal, when in fact the actual "functional" site of factor binding might only measure in tens of nucleotides. Thus, aggregating mutational recurrence across over-sized regions instead of actual functional territories can dilute the true signal of positive selection and hinder driver identification. ***[Simplification OK?] [[ok]]***.

One approach to better define the precise functional territory of a non-coding element is identifying evolutionary conserved portions, which are likely more functionally important. Moreover, non-coding elements, like genes, often consist of discontinuous blocks of functional territories. The connections between these are well understood for genes. That is, coding exons are joined up around splice junctions during processing of messenger RNA ***[Simplification OK?]***. But the connections between non-coding elements and between these and the genes are less well understood, and potentially complex — genes can be connected to multiple promoters and enhancers, and one enhancer can affect multiple genes.

After defining the functional territory of a non-coding elements, the next step involves testing for mutational burden over many elements. The more elements that one tests over will exact a multiple-testing penalty on the resulting statistics. Thus, one can increase power through making the element set as small and accurate as possible. Thus, the best way to increase the power of driver detection in non-coding elements is, perhaps non-intuitively, not to investigate every base in the genome. Rather, it is to analyse a compact and highly accurate annotation set containing as few elements as possible, in which each element corresponds as closely as possible to an underlying functional territory.

An additional difficulty with non-coding mutations is evaluating their functional impact. Currently, it is unclear whether each potential substitution of a nucleotide in a regulatory region has an equal impact

In some circumstances, it is clear what effect a mutation will have — if it breaks a transcription-factor binding site or creates a new one, for instance8. Nonetheless, better metrics of functional impact are needed over the whole genome to find non-coding equivalents of the coding mutations known to alter protein production or behaviour. Finally, the power to detect drivers in non-coding regions is dependent on how uniform of the underlying background mutation rate is. However, this is not the case for wide expanses of the genome9, so the approach will require further refinement ***[OK to shorten? To avoid defining chromatin and explaining how the signals causes mutational changes] [[ok]]***.

An exhaustive but effective approach to deal with some of these challenges is sequencing many patients.

This approach is feasible only through large-scale collaborations ***[OK to shorten? As it’s not clear why one in particular should be singled out here]***. Such efforts will generate comprehensive catalogues of non-coding variants, which give us better statistics that can be leveraged to detect more driver mutations. However, these large-scale studies require the assembly of uniform cohorts, which can be challenging owing to the highly heterogeneous nature of cancer. An alternative approach is developing a more more compact functional annotation of the non-coding genome by better and more precisely defining functional territories. Here, ***[What do you mean by large scale in this context? That they look at many tissues? Are genome-wide? Or something else?] systematic*** annotation compendiums such as the ENCODE project10 have a vital role to play. Thus, in the pursuit of more drivers we may be actually be served by less annotation.

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2. Tamborero, D. *et al.* Comprehensive identification of mutational cancer driver genes across 12 tumor types. *Sci. Rep.* **3,** 2650 (2013).

3. Rheinbay *et al. Nature* XXX (2017).

3. Khurana, E. *et al.* Role of non-coding sequence variants in cancer. *Nat. Rev. Genet.* **17,** 93–108 (2016).

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9. Dunham, I. *et al.* An integrated encyclopedia of DNA elements in the human genome. *Nature* **489,** 57–74 (2012).

***[Thanks for your figure suggestion. I like part a, and I think that visualizing the genome in this way will help readers get to grips with the concepts in the piece. However, I propose that we use just part a. To explain, the power analysis is a bit complex for a News & Views — the graph would require quite a bit of explanation and readers don’t need to understand how the graphs work to follow the piece as a whole. Instead, we can simply state in the caption how power can be increased. In addition, I propose that we omit the CRE peak — again, we’d need to explain to readers what it represents, which isn’t needed to follow this piece. The zoom-in you’ve included that highlights the size of the functional territory will be enough for readers to get the idea. Is this OK?]***

***[I’ve made changes to your figure caption to take into account my proposed modifications and our house style. This dictate that captions should stand alone from the main text and mention everything depicted. Please amend further as needed, bearing these restrictions in mind]***

**Figure 1| Improving discovery of cancer-driving mutations in the non-coding genome.** Genes contain coding sequences called exons, the links between which are well established — the messenger RNA that they encode is amalgamated after transcription. Gene expression is regulated by non-coding elements, including nearby promoters and distant enhancers. The links between these regulatory elements and genes are less well understood. Rheinbay *et al.*3 conducted a systematic, unbiased analysis of 360 breast-cancer genomes to identify genetic mutations in non-coding sequences that drive cancer progression, and found nine such mutations in promoters. In the future, more non-coding drivers could be found by analysing more sequences, or by better understanding the links between non-coding elements and genes. In addition, regions annotated as non-coding elements are often much larger than the actual regulatory sequence within the element. Limiting the regions analysed could improve driver identification.