

Methoden der Krebsforschung zur Identifikation von Onkogenen

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Inhalt

Zusammenfassung des Artikels „Cancer and Genomics“

Erläuterung von Forschungsmethoden aus dem Jahre 2001 und davor

Definition von CRISPR/Cas9

CRISPR/Cas9 als Werkzeug der Gentechnik

- Beispiele der Anwendung

Fazit

Cancer and Genomics

- Was sind Onkogene?
- Was sind „paralogues“?
- im Artikel angewandte Forschungsmethoden:
 - Gen-Mapping
 - paraloge Gene
 - „draft genome“



Census

GRCh38 · COSMIC v86

- Overview
- Cancer Gene Census
- Breakdown
- Abbreviations

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Overview

The Cancer Gene Census (CGC) is an ongoing effort to catalogue those genes which contain mutations that have been causally implicated in cancer. The original census and analysis was published in [Nature Reviews Cancer](#).

The census is not static, instead it is updated when new evidence comes to light. In particular we are grateful to Felix Mitelman and his colleagues in providing information on more genes involved in uncommon translocations in leukaemias and lymphomas. Currently, more than 1% of all human genes are implicated via mutation in cancer. Of these, approximately 90% contain somatic mutations in cancer, 20% bear germline mutations that predispose an individual to cancer and 10% show both somatic and germline mutations.

Census tiers

Genes in the Cancer Gene Census are divided into two groups, or tiers.

Tier 1

To be classified into Tier 1, a gene must possess a documented activity relevant to cancer, along with evidence of mutations in cancer which change the activity of the gene product in a way that promotes oncogenic transformation. We also consider the existence of somatic mutation patterns across cancer samples gathered in COSMIC. For instance, tumour suppressor genes often show a broad range of inactivating mutations and dominant oncogenes usually demonstrate well defined hotspots of missense mutations. Genes involved in oncogenic fusions are included in Tier 1 when changes to their function caused by the fusion drives oncogenic transformation, or in cases when they provide regulatory elements to their partners (e.g. active promoter or dimerisation domain).

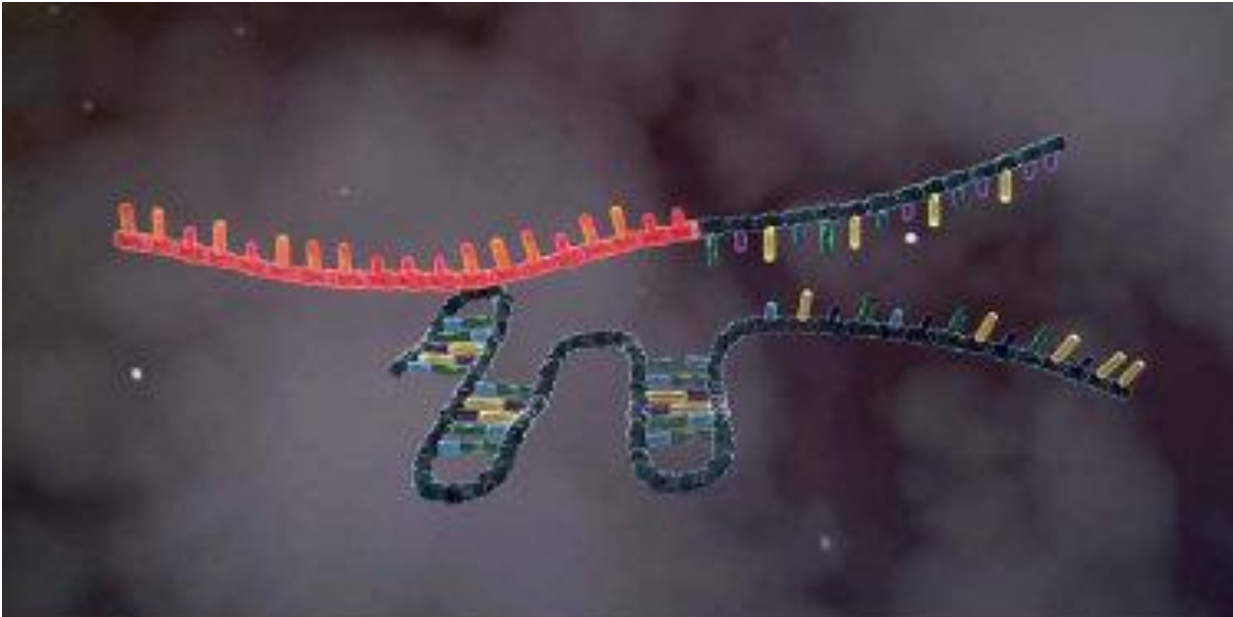
https://cancer.sanger.ac.uk/census#cl_overview

CRISPR/Cas9 (1)

Clustered **R**egularly Interspaced **S**hort **P**alindromic **R**epeats

- Was ist das CRISPR/Cas9 – System?
- Funktionsweise
- Anwendung in der Forschung

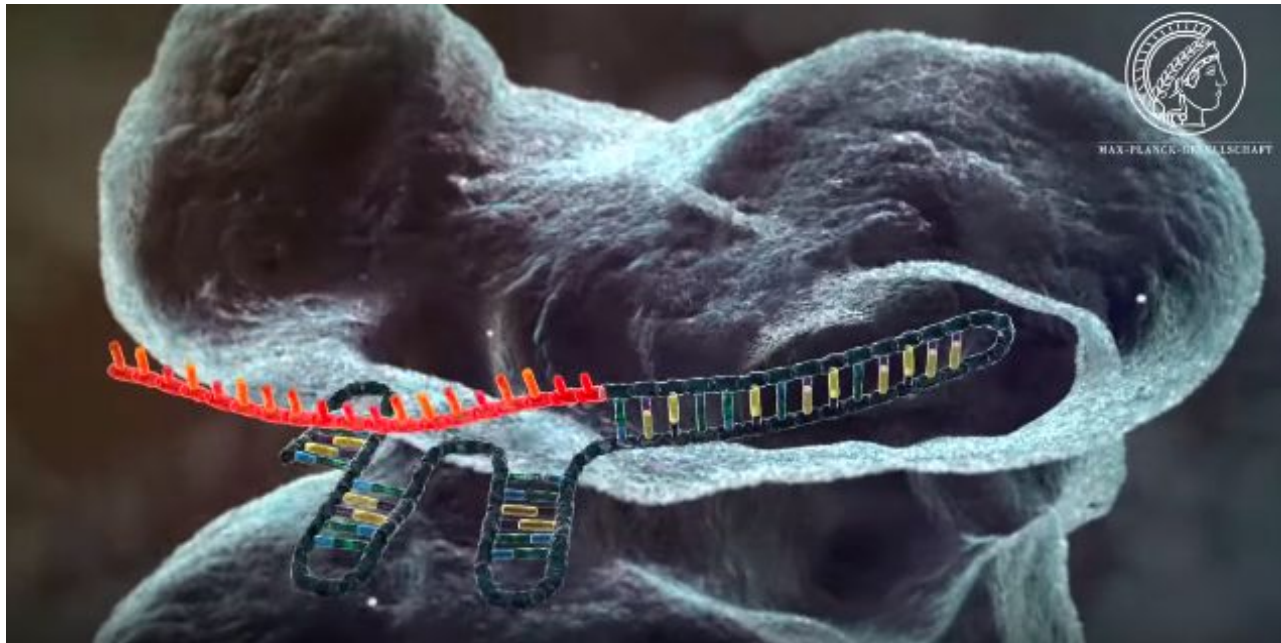
CRISPR/Cas9 (2)



- Oben: CRISPR-RNA mit einem komplementären Abschnitt
- Unten: tracr-RNA

<https://www.youtube.com/watch?v=ouXrsr7U8WI>

CRISPR/Cas9 (3)



Verbindung der CRISPR-
und tracr-RNA mit dem
Cas9-Protein

<https://www.youtube.com/watch?v=ouXrsr7U8WI>

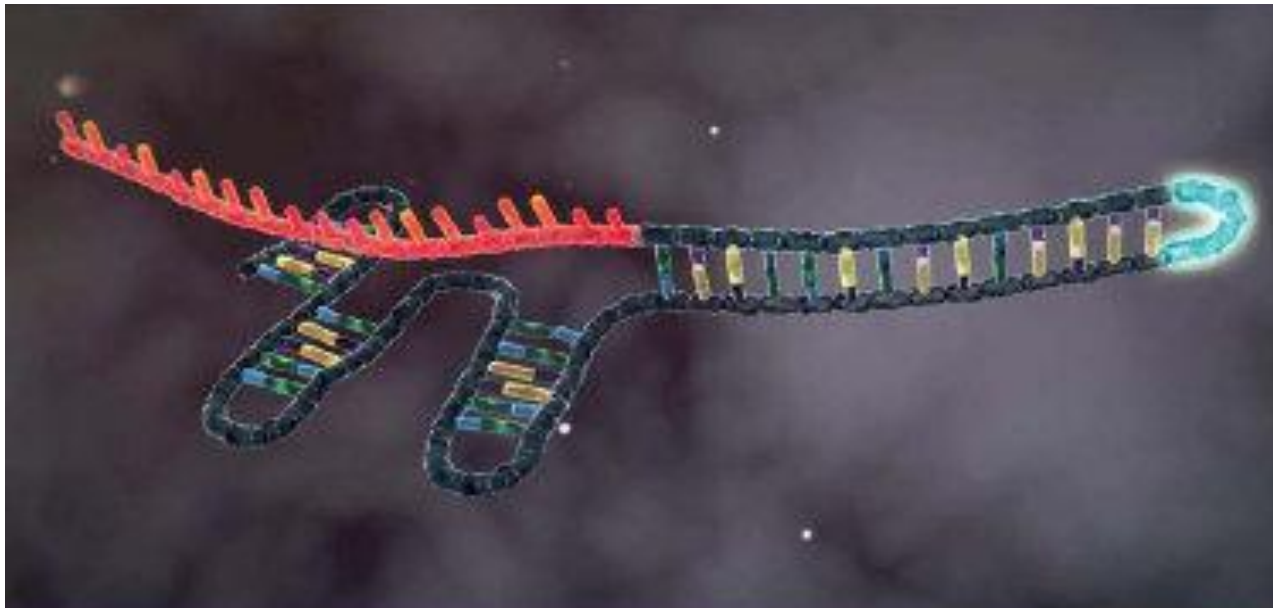
CRISPR/Cas9 (4)



- Anlagerung der CRISPR-RNA an die komplementäre Sequenz
- Bestimmung der Schnittstelle

<https://www.youtube.com/watch?v=ouXrsr7U8WI>

CRISPR/Cas9 (5)



Fusionierung der CRISPR-
und tracr-RNA

<https://www.youtube.com/watch?v=ouXrsr7U8WI>

CRISPR/Cas9 (6)



- Wiederaufbau des DNA-Strangs

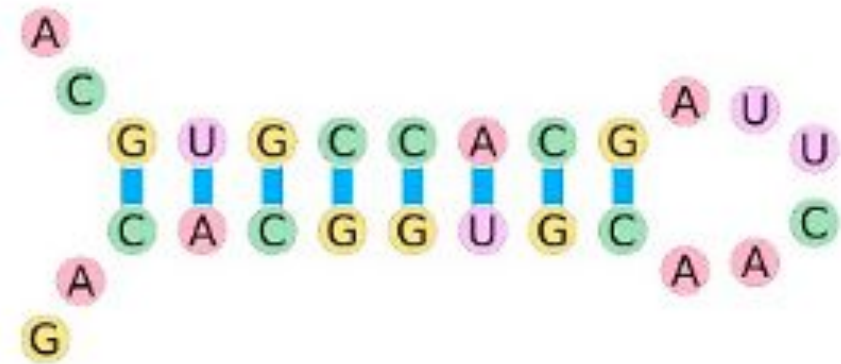
<https://www.youtube.com/watch?v=ouXrsr7U8WI>

CRISPR/Cas9 als Werkzeug der Gentechnik

- Fusion von CRISPR- und tracr-RNA
- Genausschaltung (gezieltes „knock-out“)
- Anwendung in der Krebsforschung

CRISPR/Cas9 als Werkzeug der Gentechnik

- „Drop-out Screens“ = „Loss of function – Experimente“
- shRNA = small hairpin RNA



Quelle:

<https://www.vitascientific.com/news/article/using-dropout-screens-to-better-understand-breast-cancer?cpath=22>

Fazit

- Im Artikel genannte Methoden haben ihre Schwächen
- Es wird immer schwieriger neue Onkogene zu finden
- CRISPR/Cas9 etabliert sich als neue Technik in der Krebsforschung

Danke für eure Aufmerksamkeit!

AND ENJOY: <HTTPS://WWW.YOUTUBE.COM/WATCH?V=K99BMTG4ZRK>