Visualization of tissue heterogeneity with single cell resolution – mutation analysis *in situ*

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Novel tool for cancer diagnostics

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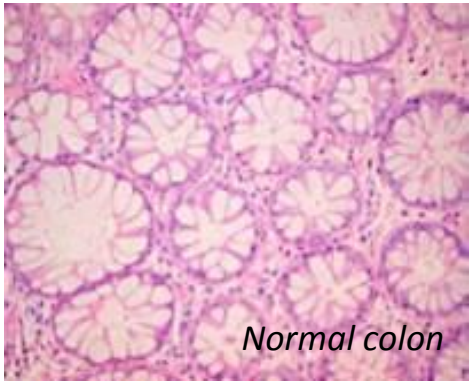
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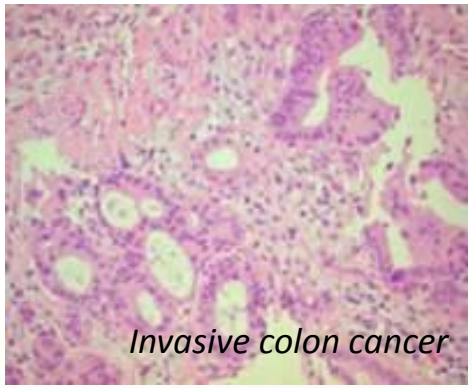
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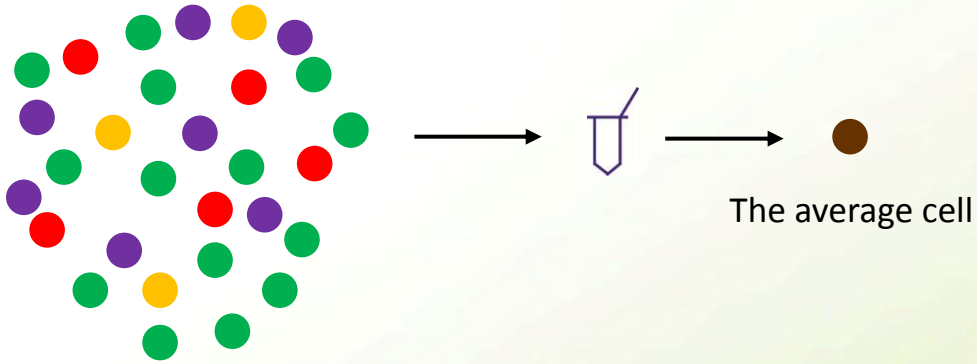
Current diagnostic methods

Pathological examination



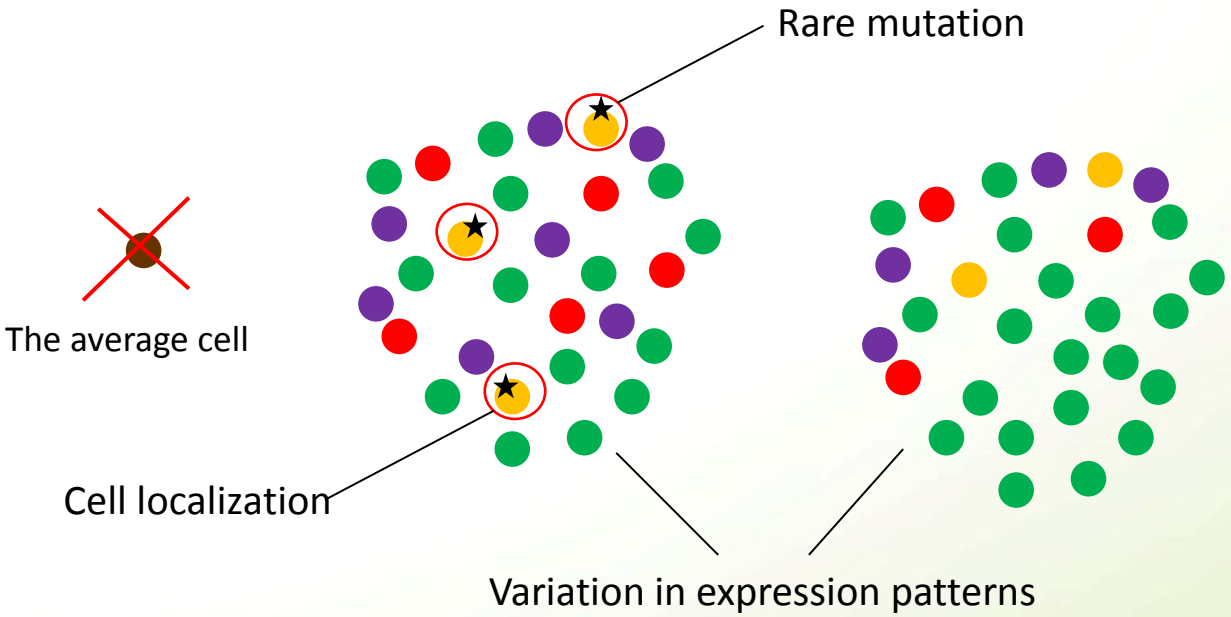


Molecular analysis



What is our contribution?

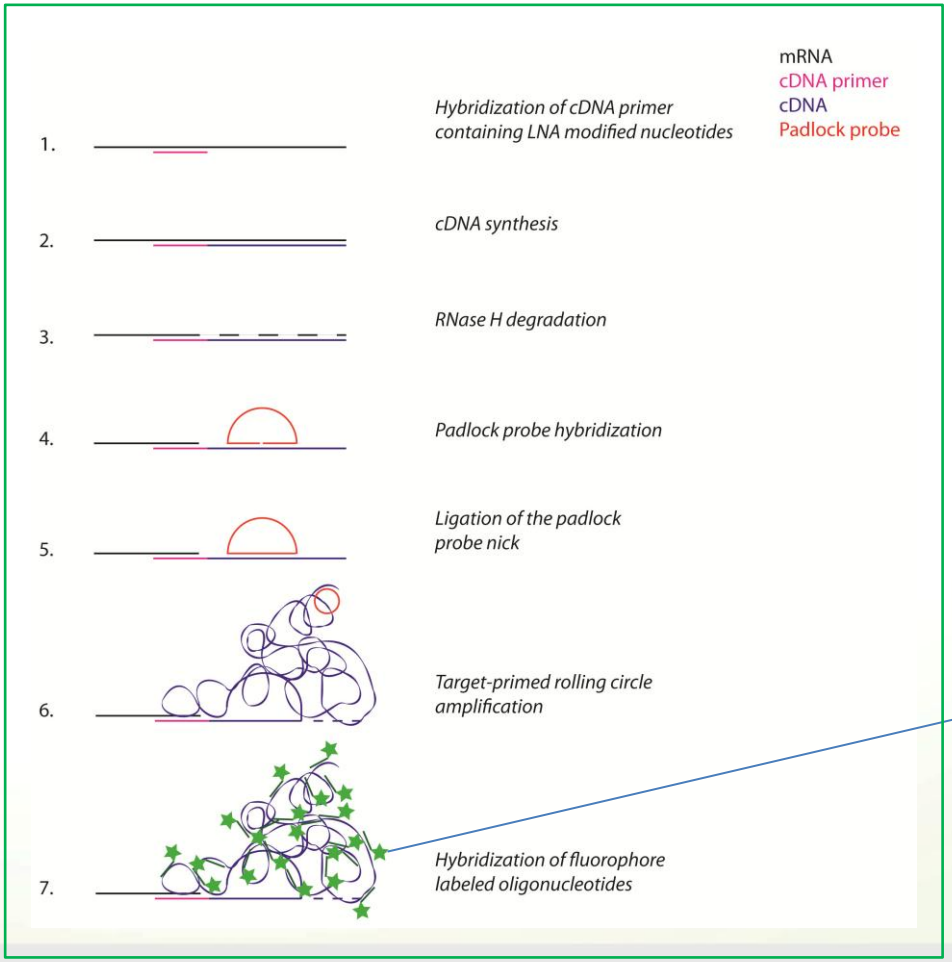
Single molecule detection of single nucleotide differences down to single cell level *in situ*

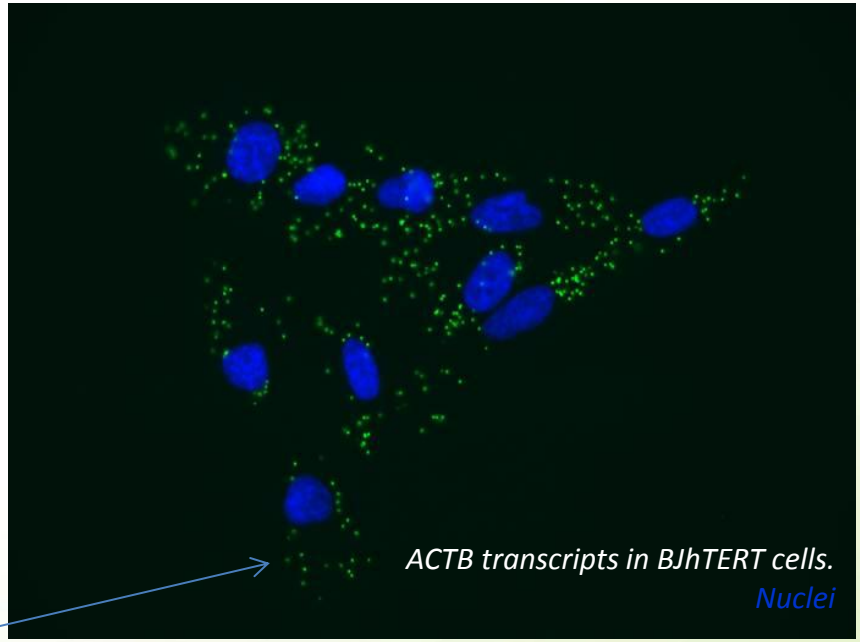


Works as a complement to the currently used methods in cancer diagnostics by adding important information regarding mutation profile and expression patterns of a tumor down to single-cell level.

The method

Detection of single transcripts *in situ*





Larsson, C. *et al.* (2010) *Nature Methods,***7**, 395–397.

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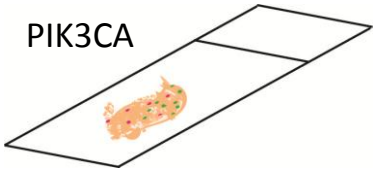
Application examples

Targeted mutation detection

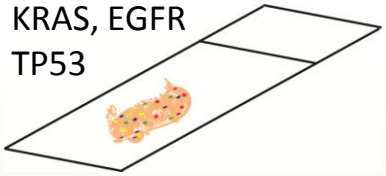
Multiplexed mutation detection KRAS

Expression profiling

PIK3CA



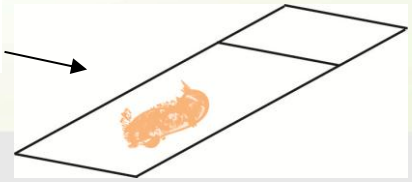
KRAS, EGFR
TP53

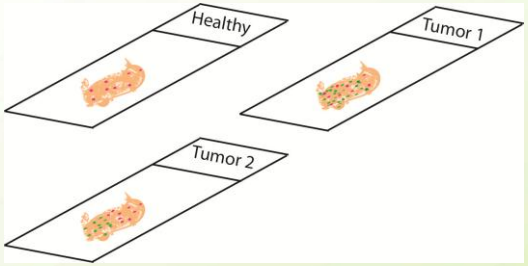


Name	KRAS
plp_KRAS_wt_c12_pos1_GGT	GTGGCGTAGGCAAGATCCTAGTAATCAGTAGCCGTGACTATCGACTGGTTCAAAGTGGTAGTTGGAGCTG
plp_KRAS_wt_c12_pos2_GGT	TGGCGTAGGCAAGAGTCCTAGTAATCAGTAGCCGTGACTATCGACTGGTTCAAAGGGTAGTTGGAGCTGG
plp_KRAS_wt_c13_pos2_GGC	CGTAGGCAAGAGTGCTCCTAGTAATCAGTAGCCGTGACTATCGACTGGTTCAAAGAGTTGGAGCTGGTGG
plp_KRAS_Q61Hwt_82_DO	*GAGGAGTACAGTGCA TCCTAGTAATC AGTAGCCGTGACTATCGACT GGTTCAAAG GACACAGCAGGTCAA*
plp_KRAS_mut_c12_pos2_GAT	TGGCGTAGGCAAGAGTTCTAGATCCCTCAATGCACATGTTTGGCTCCGGTTCAAGGGTAGTTGGAGCTGA
plp_KRAS_ mut_c12_ pos2_GTT	TGGCGTAGGCAAGAGTTCTAGATCCCTCAATGCACATGTTTGGCTCCGGTTCAAGGGTAGTTGGAGCTGT
plp_KRAS_ mut_c12_ pos2_GCT	TGGCGTAGGCAAGAGTTCTAGATCCCTCAATGCACATGTTTGGCTCCGGTTCAAGGGTAGTTGGAGCTGC
plp_KRAS_ mut_c12_ pos1_AGT	GTGGCGTAGGCAAGATTCTAGATCCCTCAATGCACATGTTTGGCTCCGGTTCAAGTGGTAGTTGGAGCTA
plp_KRAS_ mut_c12_ pos1_CGT	GTGGCGTAGGCAAGATTCTAGATCCCTCAATGCACATGTTTGGCTCCGGTTCAAGTGGTAGTTGGAGCTC
plp_KRAS_ mut_c12_ pos1_TGT	GTGGCGTAGGCAAGATTCTAGATCCCTCAATGCACATGTTTGGCTCCGGTTCAAGTGGTAGTTGGAGCTT
plp_KRAS_ mut_c13_ pos2_GAC	CGTAGGCAAGAGTGCTTCTAGATCCCTCAATGCACATGTTTGGCTCCGGTTCAAGAGTTGGAGCTGGTGA
plp_KRAS_Q61Hmut_Lin33	*GAGGAGTACAGTGCA CGCTAGATC CCTCAATGCACATGTTTGGCTCC GGTTCAAG GACACAGCAGGTCA T*

KRAS padlock probe cocktail

Lung tissue





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Images of unpublished results have been removed.

In situ genotyping

RESULTS

To conclude...

- Detect rare mutations in a complex matrix
- Interrogate multiple mutations in one assay and get spatial information about them
- Expression pattern visualization

→ To be applied in clinical diagnostics and for cancer research

What's next?

In situ sequencing of tags to enable detection of several transcripts in situ in parallel.

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