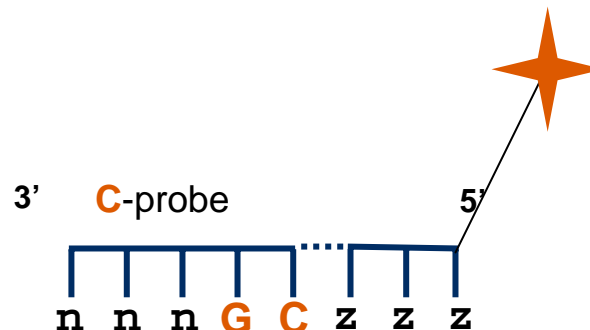


SOLiD™ Data – 2 Base Encoding
Sequencing by **O**ligonucleotide **L**igation and **D**etection

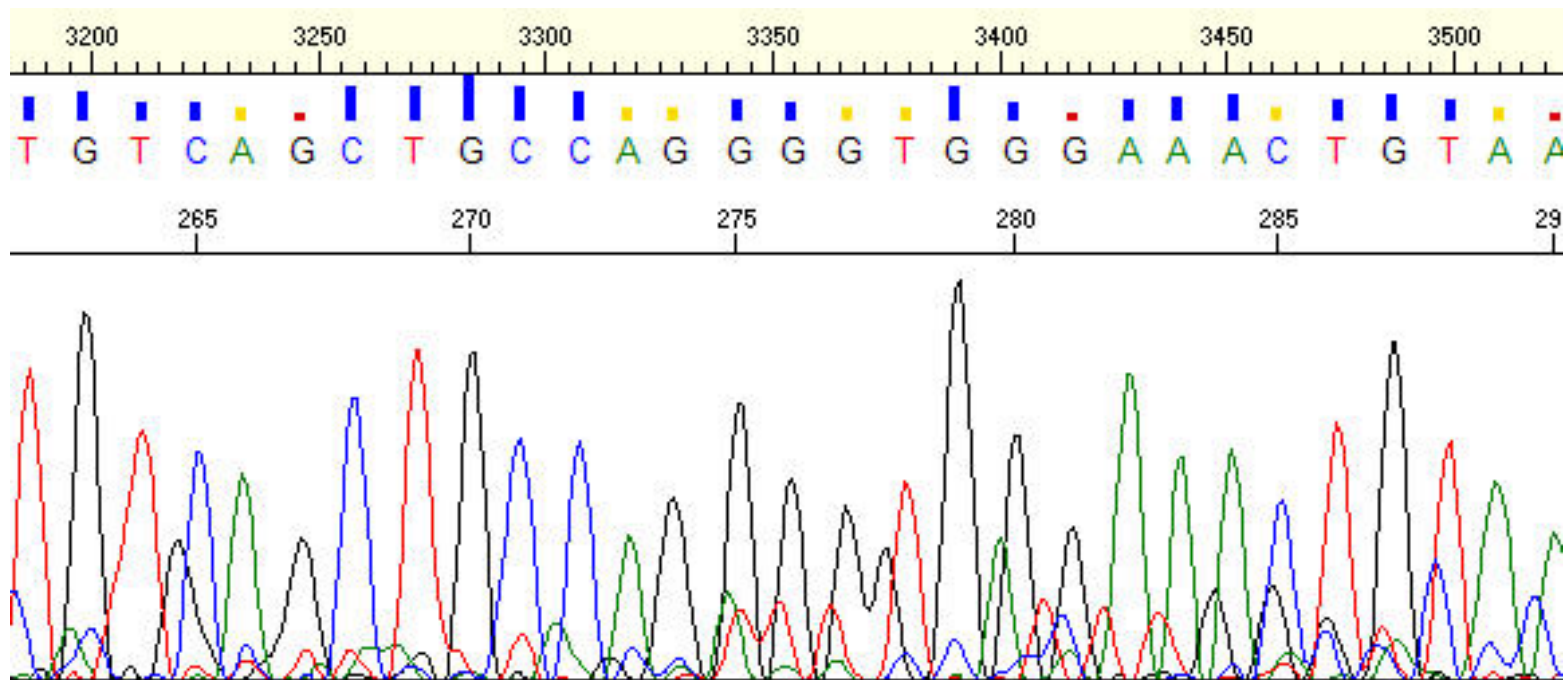
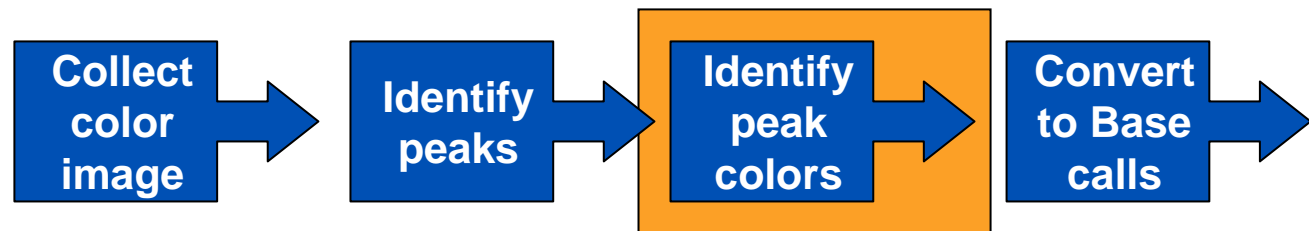
What is two base encoding?

- Rather than a probe, reading out the single base present at the 5th position, a two base encoded probe tells us information about the 4th and 5th bases which needs further information to resolve the base call

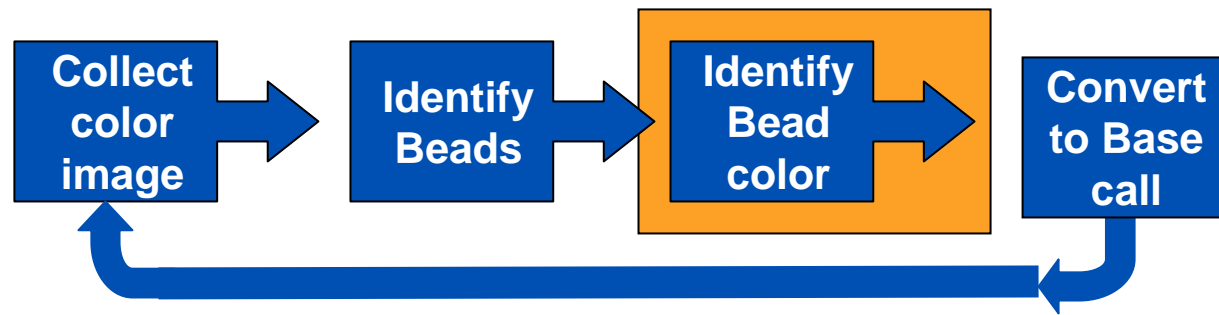


- In order to do this we use the concept of color space

Color Space - Capillary electrophoresis

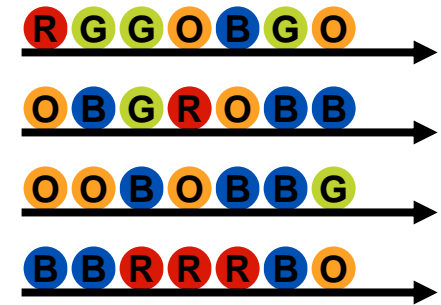
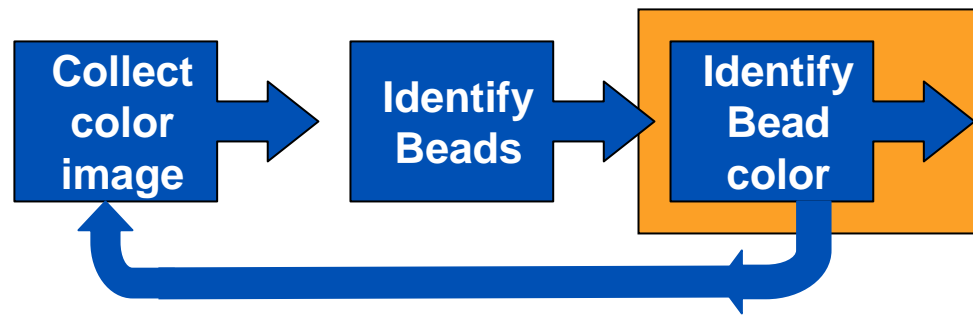


Color Space – SOLiD (single Base encoding)



(One Base encoding)

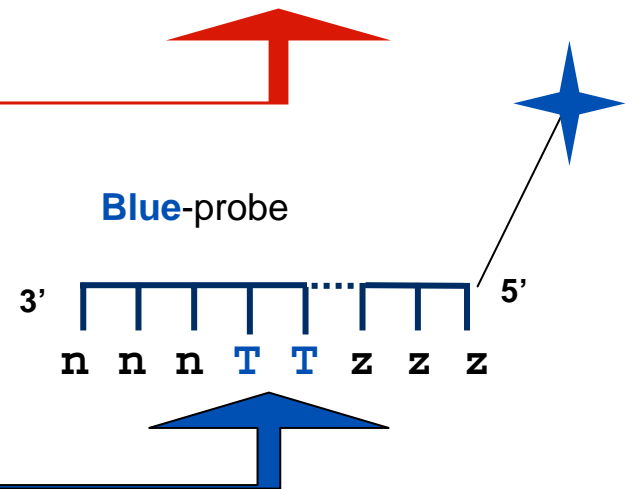
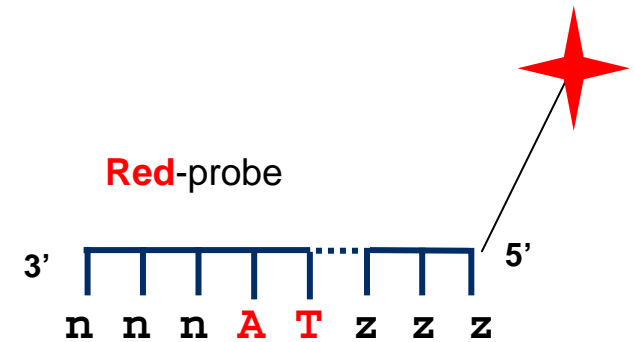
Color Space – SOLiD (Dual Base encoding)



**record colors
for each
bead over
consecutive
cycles**

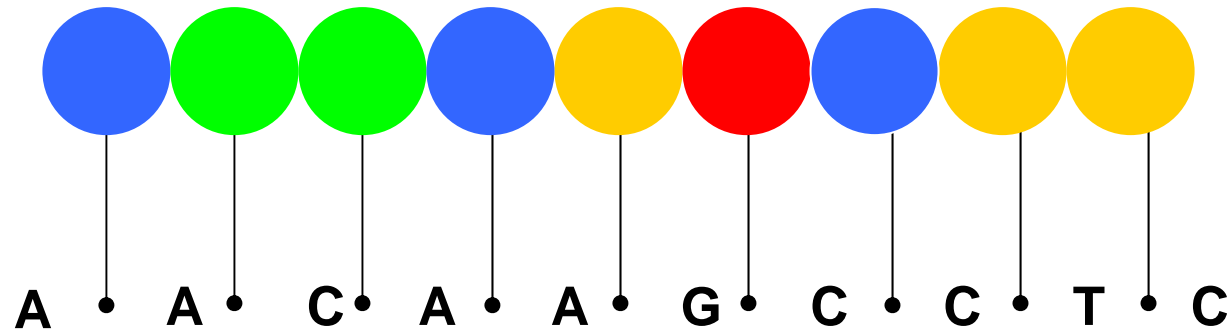
2 Base Pair Encoding Using 4 Dyes

	2nd Base			
	A	C	G	T
1st Base				
A	Blue	Green	Yellow	Red
C	Green	Blue	Red	Yellow
G	Yellow	Red	Blue	Green
T	Red	Yellow	Green	Blue




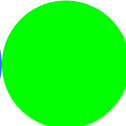
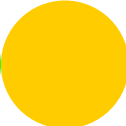
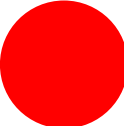
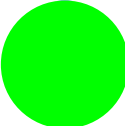

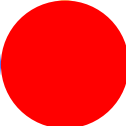

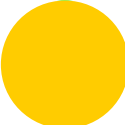
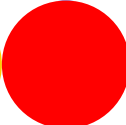

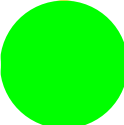

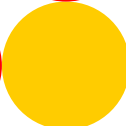
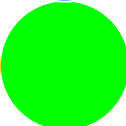

On our probes the 1st base encoded is position 4
the 2nd base encoded is position 5

Ball and Stick Model

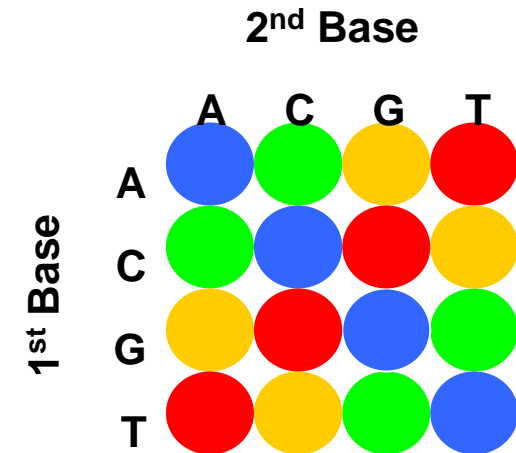
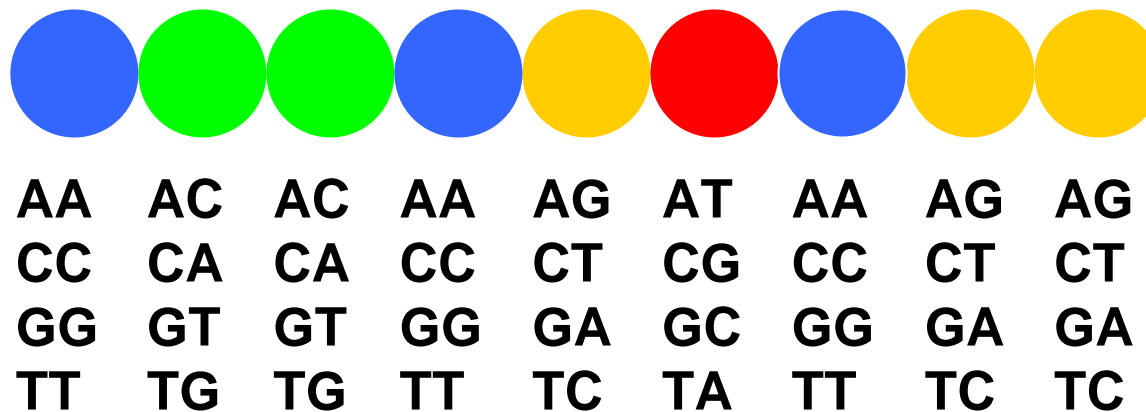


Consequences of 2 Base Pair Encoding

- Detecting a single color does **not** indicate a **base**
- Each reading contains information from two bases
- To decode the bases you **must** know **one** of the bases in the sequence

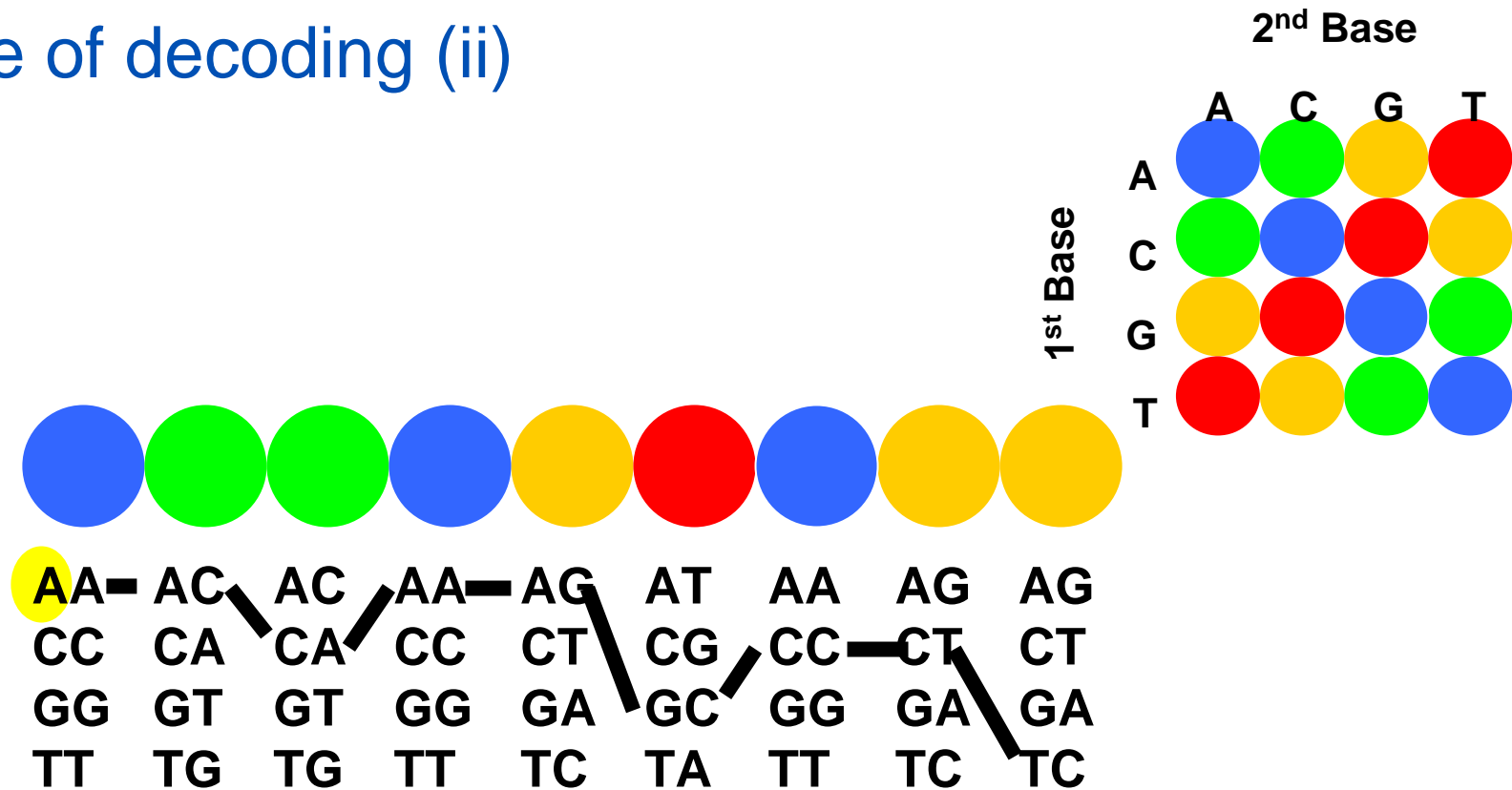
		2 nd Base			
		A	C	G	T
1 st Base	A				
	C				
	G				
	T				

Example of decoding (i)



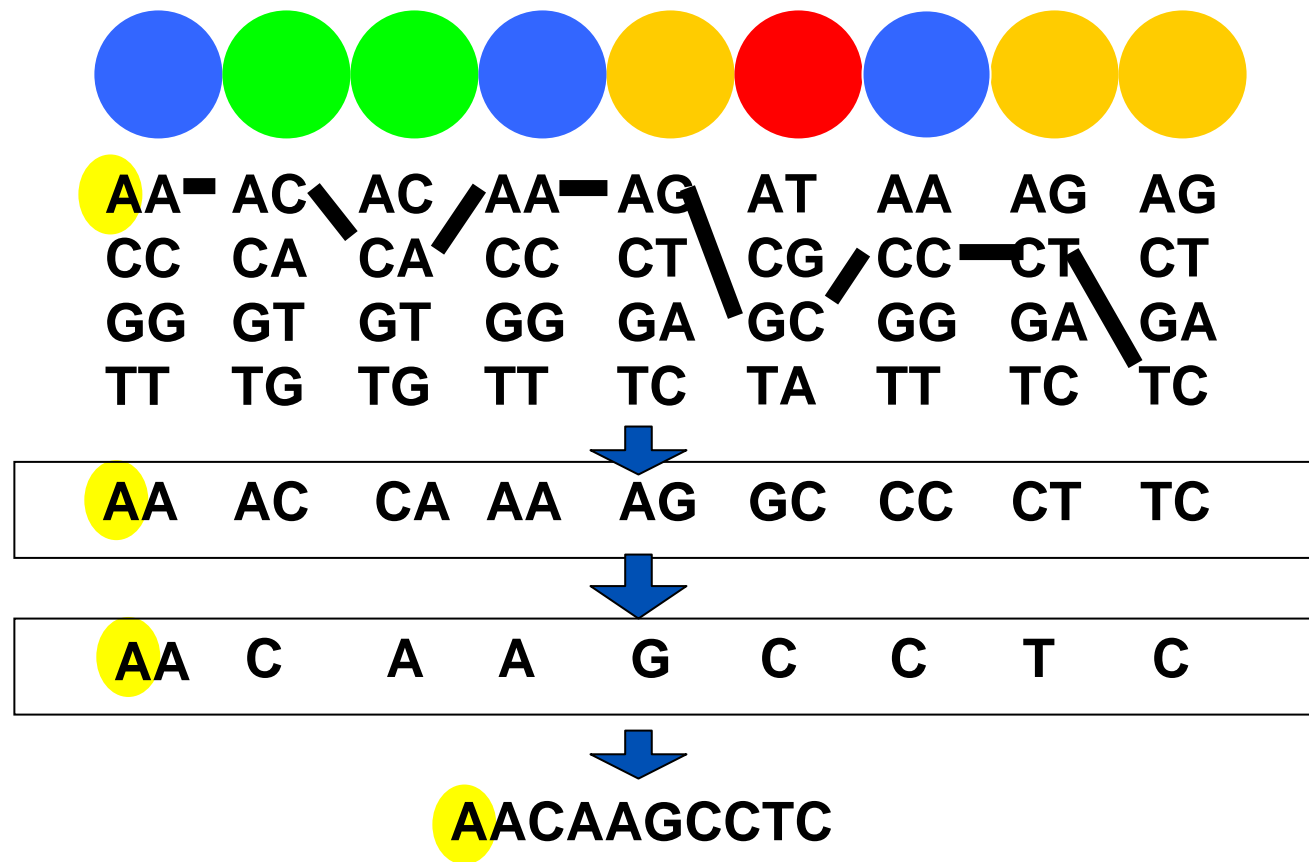
Cannot determine any of the bases

Example of decoding (ii)



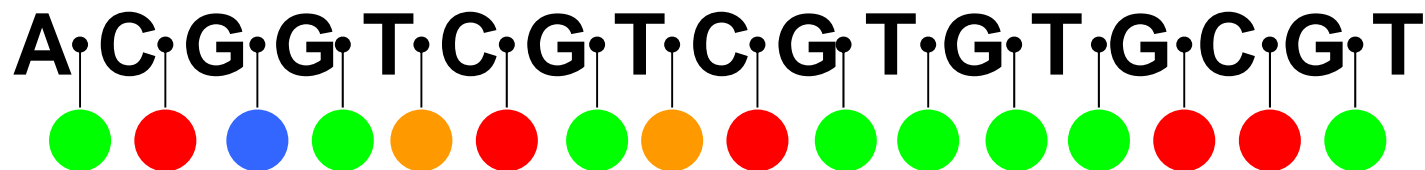
If know first base is an A then immediately it decodes 2nd base. This must be an A as Blue translates 2nd base A if first base A

Summary of decoding

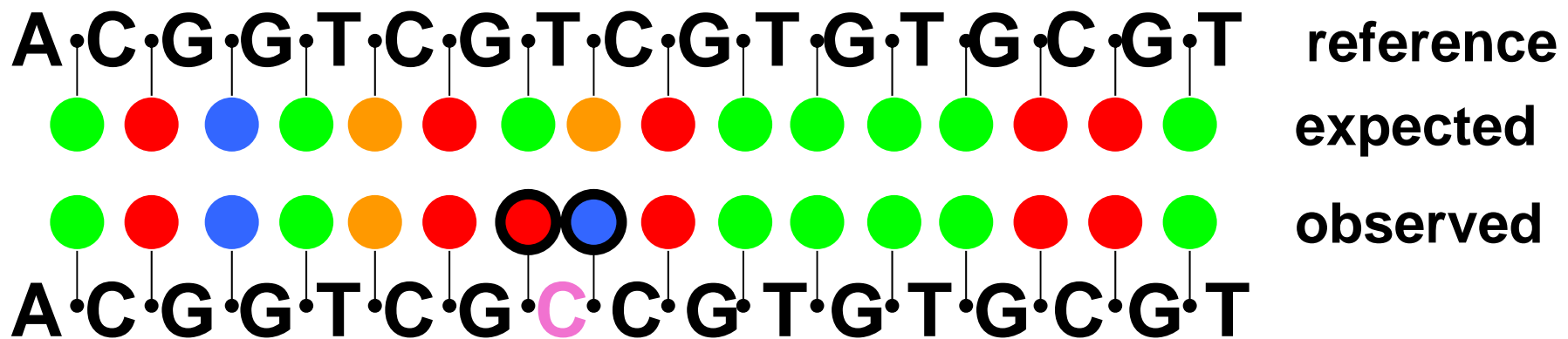


Advantages of 2 base pair encoding

- Double base interrogation eases the discrimination between system **errors** and **true** polymorphism



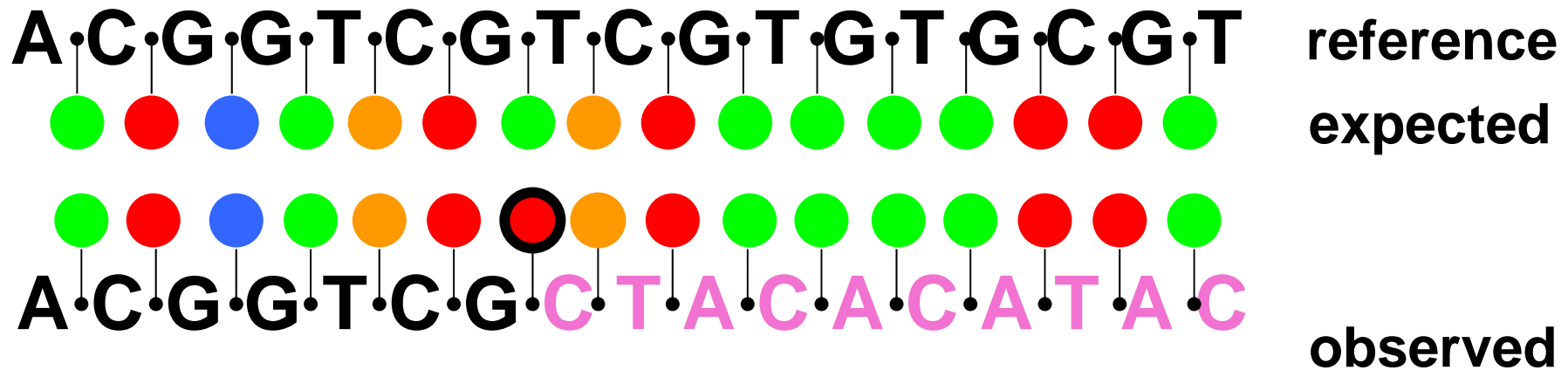
Advantages of 2 base pair encoding Real SNP



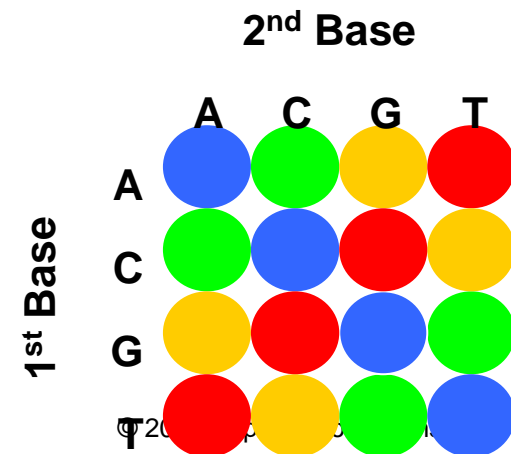
**Two color changes represent only a
single mismatch to reference
sequence (SNP)**

Advantages of 2 base pair encoding

Miscall

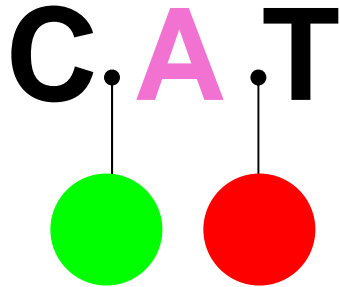


Single color change, represents sequencing error.



But theres more...

only certain transitions are allowed for a real SNP



Consider a triplet of bases, they define 2 colors.

There are only 3 possibilities for a change in the middle base, hence only 3 possibilities for the 2 colors to change to.

Any of the other 6 possibilities for a 2-color change are not allowed and most probably represent measurement errors.

The only allowed color changes

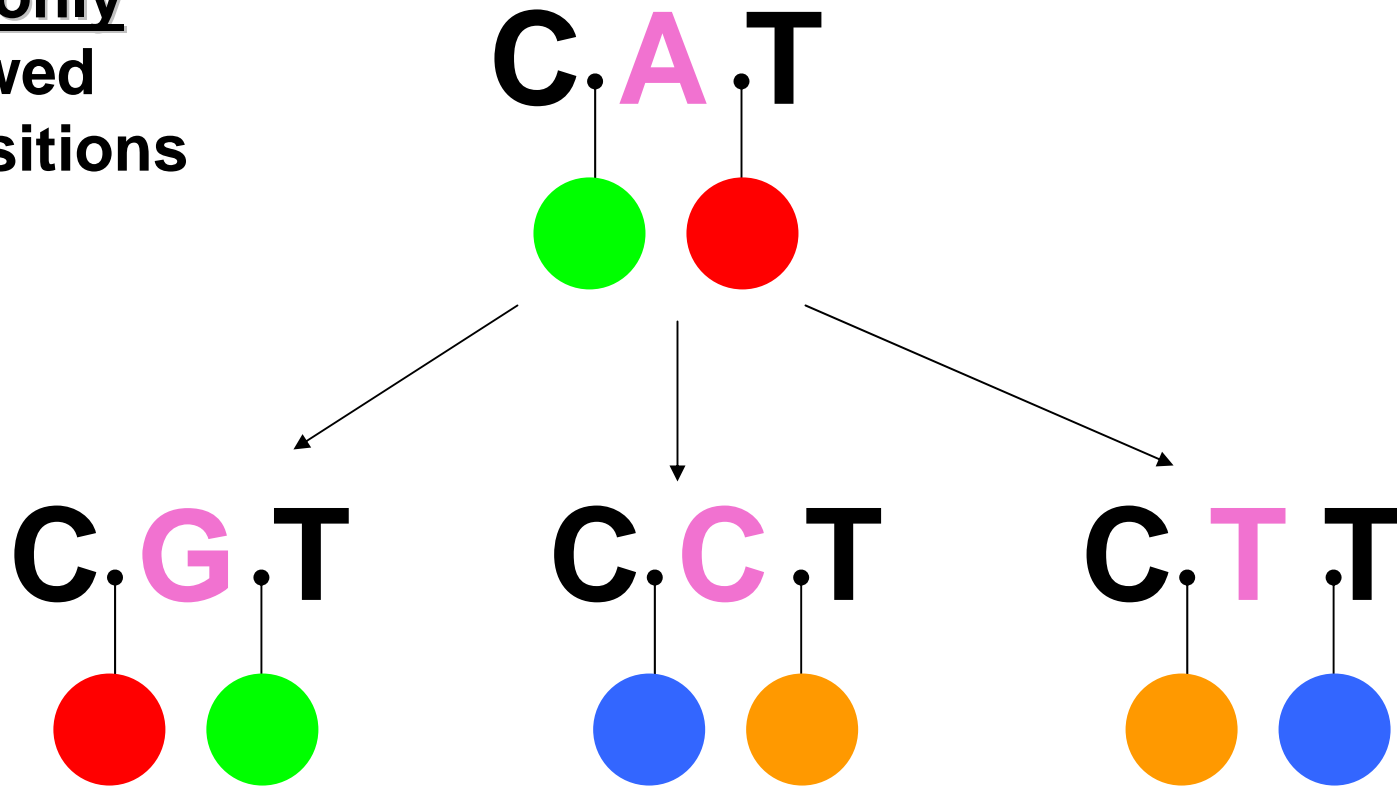
If two colors present (eg **B,R**)

- Reverse the colors (eg **R, B**)
- Use the other two colors , both combinations eg **O,G** and **G,O**

If only one color is present (eg **B,B**)

- The three other color pairs (eg **G,G** or **R,R**, or **O,O**)

The only
allowed
transitions

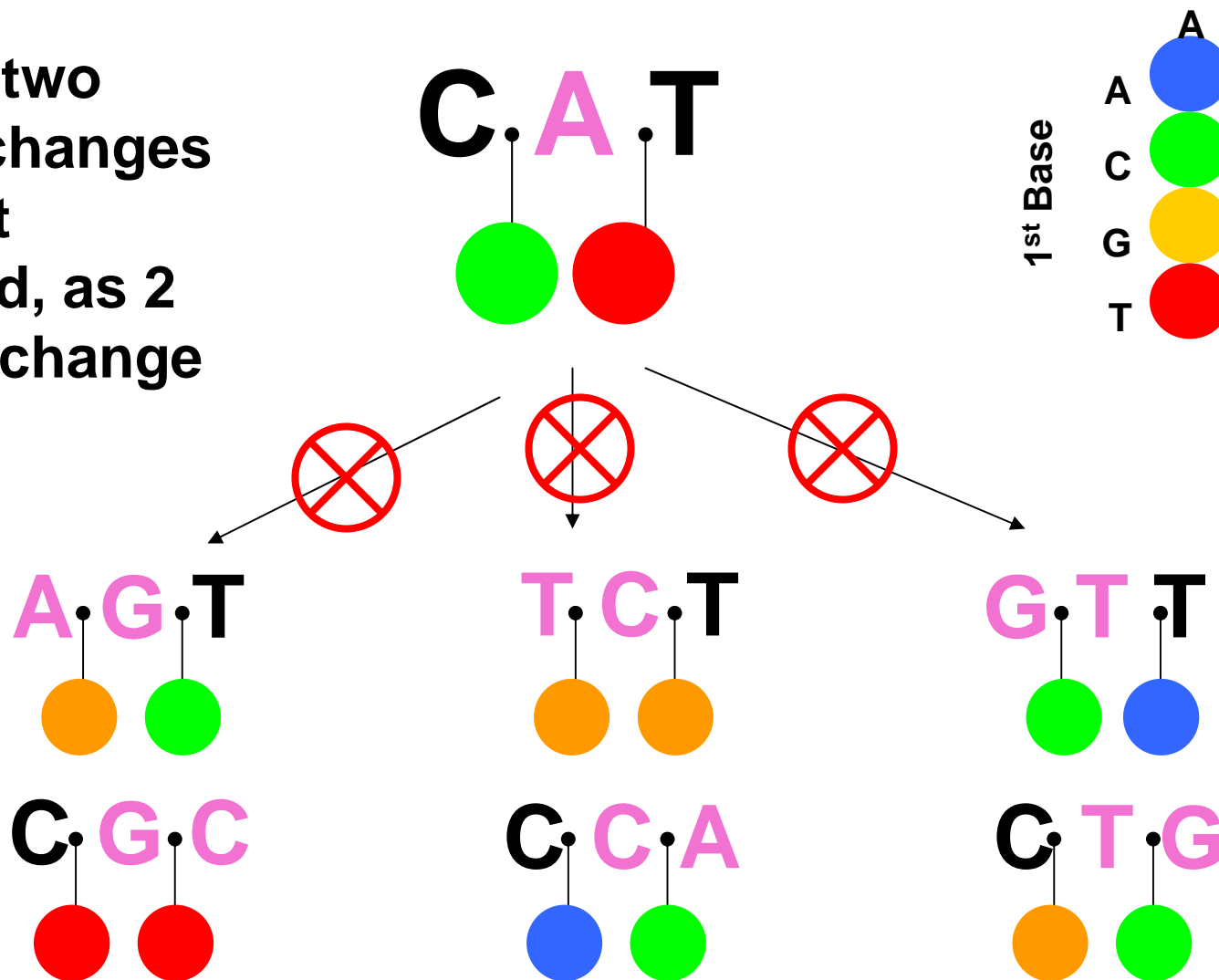


Reverse Colors

Other two colors (both orientations)

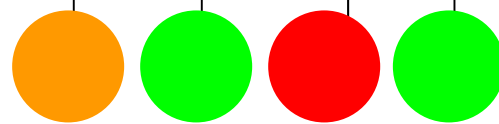
Any other transitions would require the outer two
bases to change

These two color changes are not allowed, as 2 bases change



	A	C	G	T
A				
C				
G				
T				

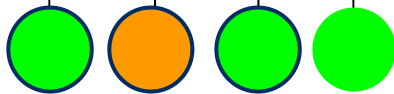
T.C.A.T.G



1st Base

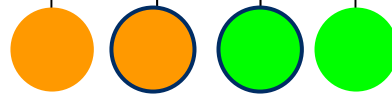
	A	C	G	T
A				
C				
G				
T				

T.A.G.T.G



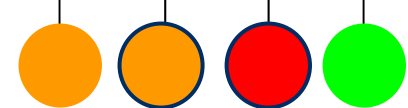
Allowed 3 colors
changed

T.C.G.T.G



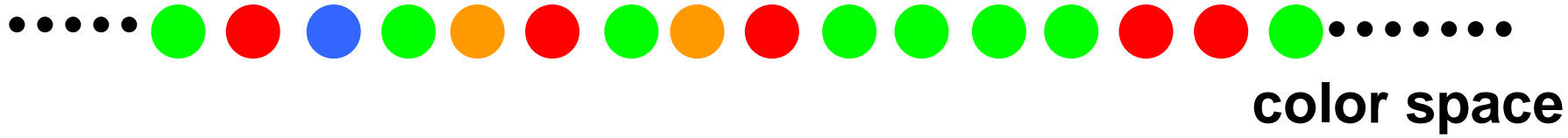
Allowed 2
colors changed
acceptable
“transition”

T.C.G.?G



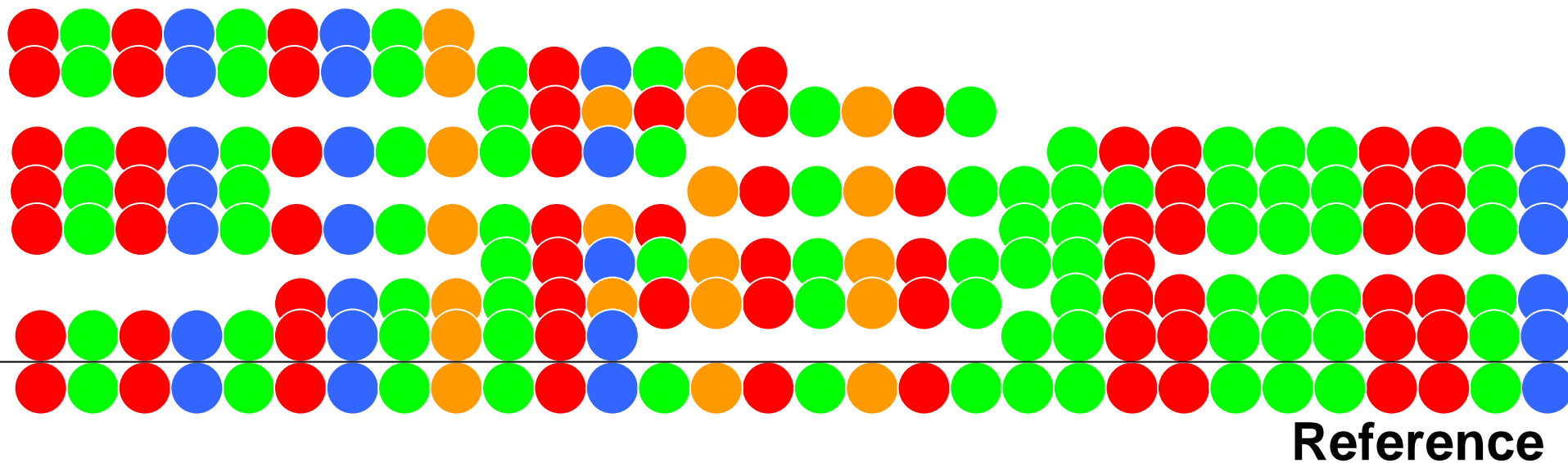
Not allowed 2
colors changed
but a forbidden
“transition”

Why leave color space?



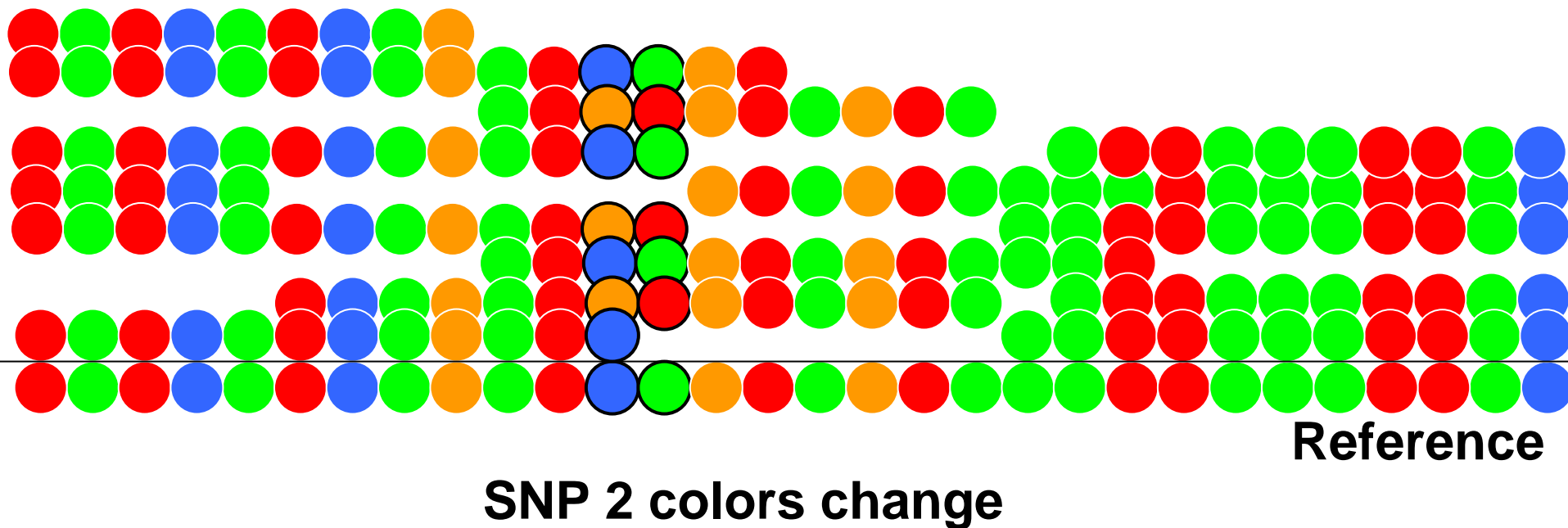
Why leave color space?

Align color space reads against color space reference



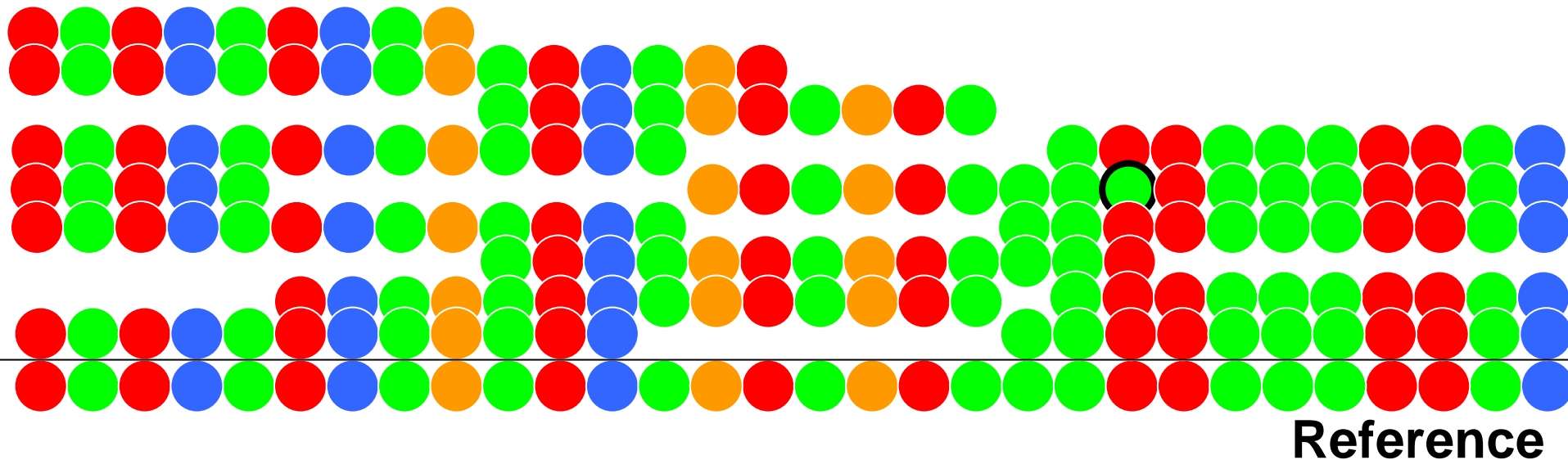
Why leave color space?

Align color space reads against color space reference



Why leave color space?

Align color space reads against color space reference



Incorrect call , single change in color space

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