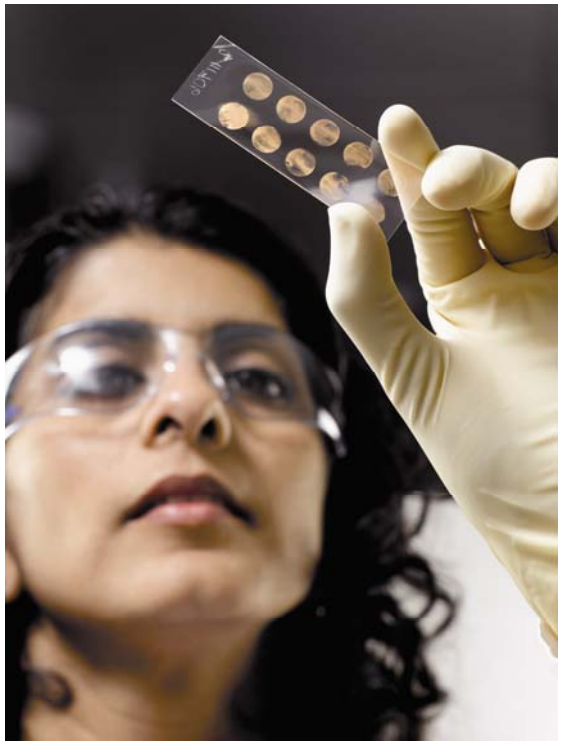
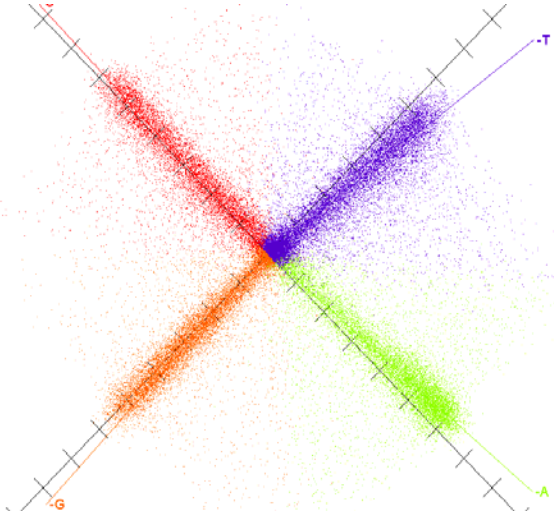


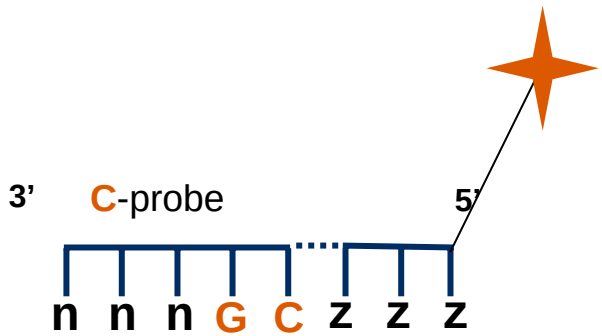


SOLiD™ Data – 2 Base Encoding

Applied Biosystems

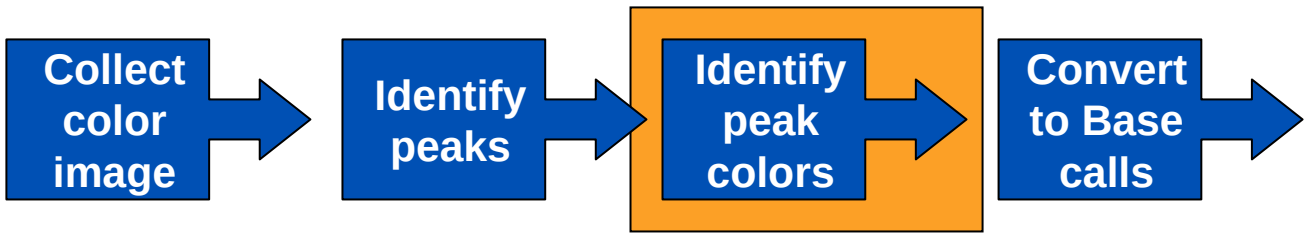
What is two base encoding?

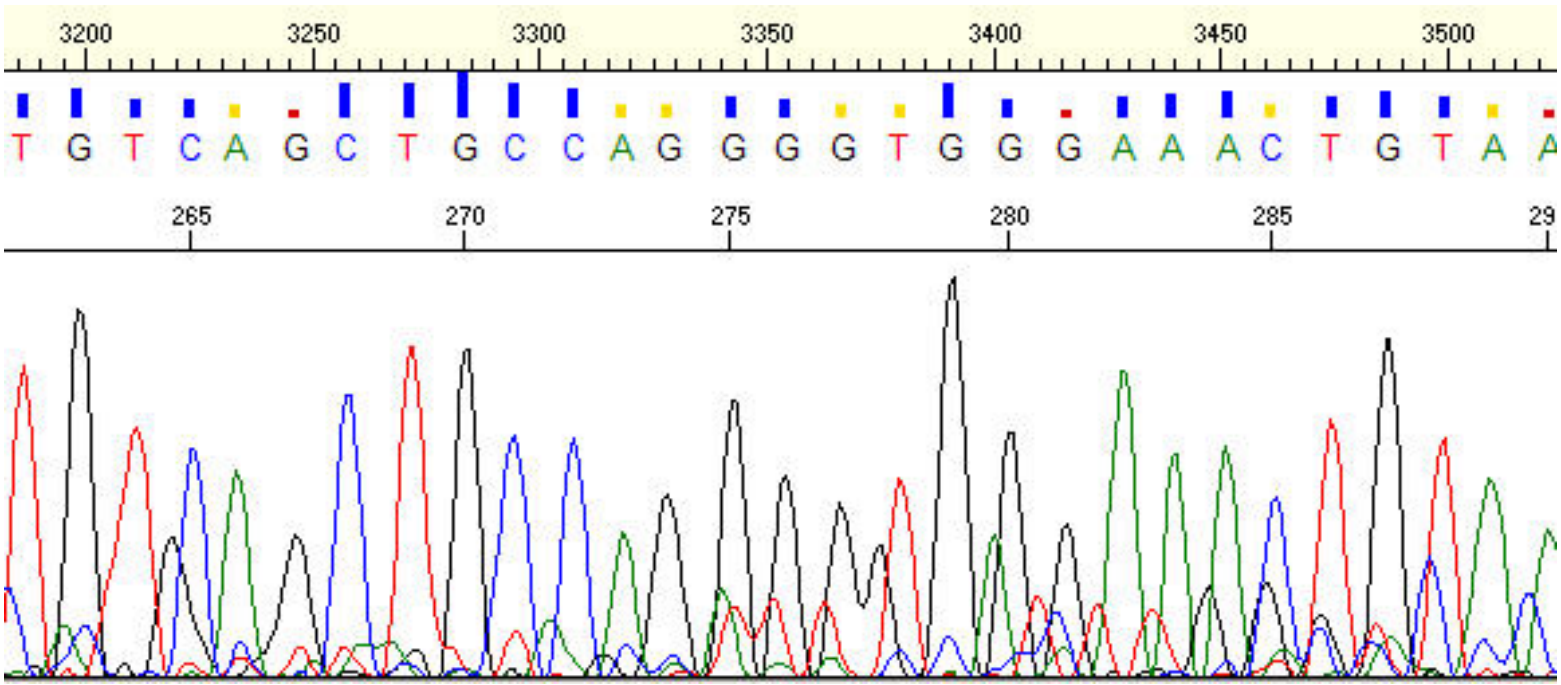
- Rather than a probe , reading out the single base present at the 5th position, a two base encoded probe tells us information about the 4th and 5th bases which needs further information to resolve the base call



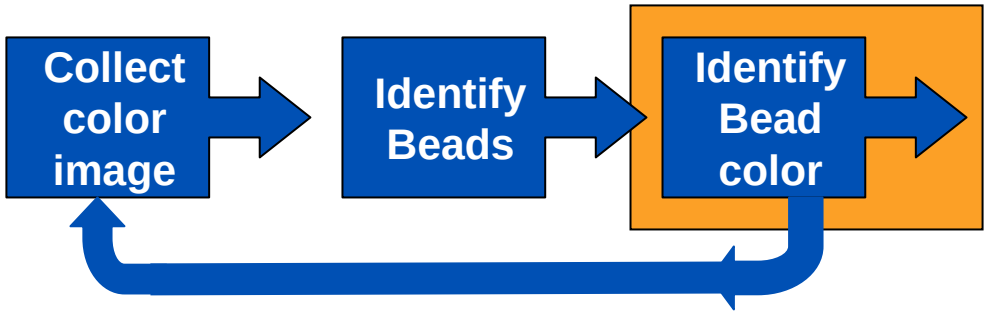
- In order to do this we use the concept of color space

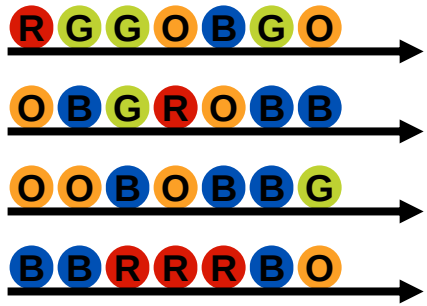
Color Space - Capillary electrophoresis

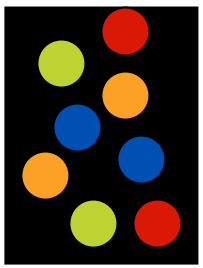


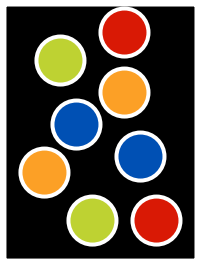


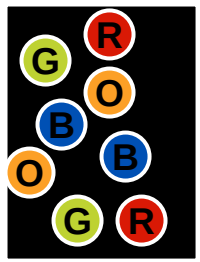
Color Space – SOLiD (Dual Base encoding)





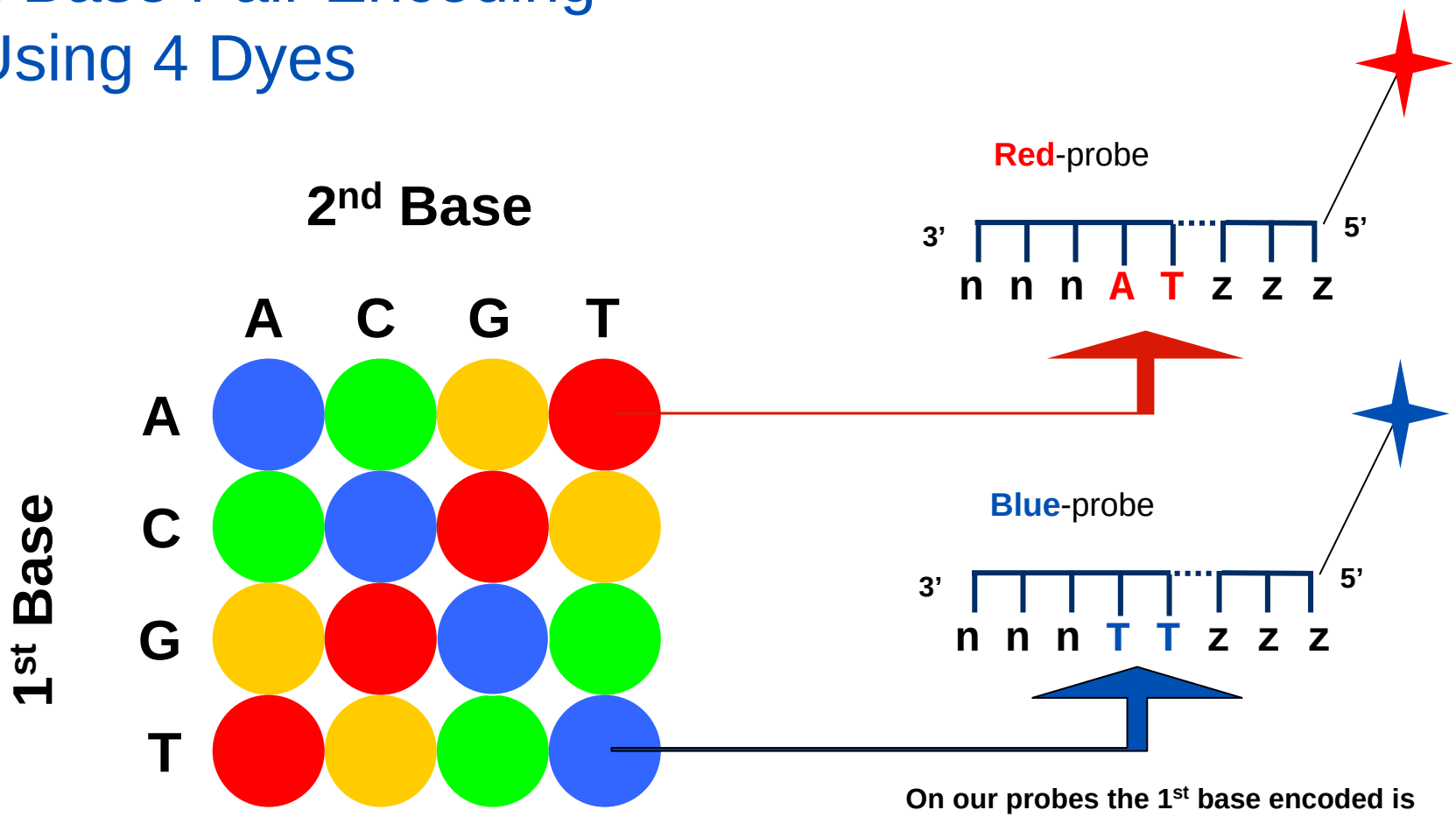




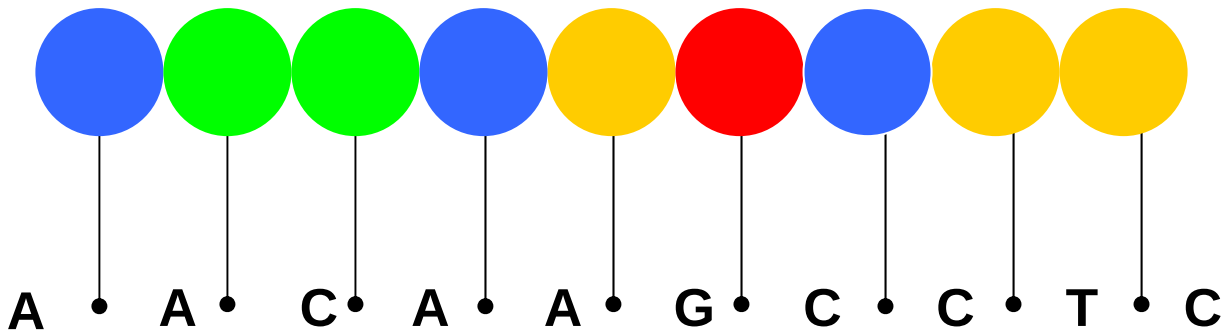


record colors for each bead over consecutive cycles

2 Base Pair Encoding Using 4 Dyes

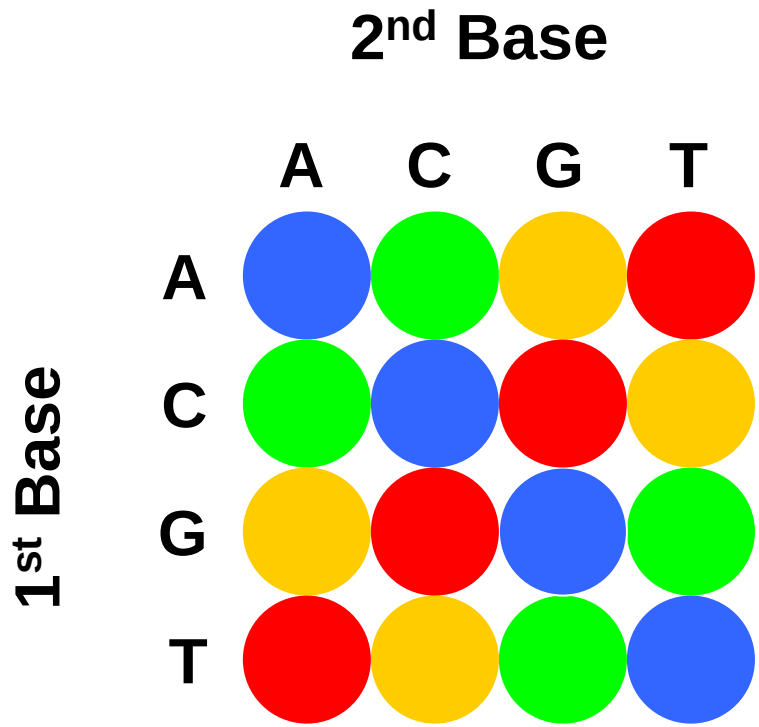


Ball and Stick Model

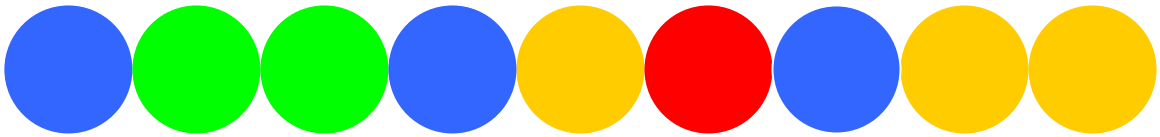


Consequences of 2 Base Pair Encoding

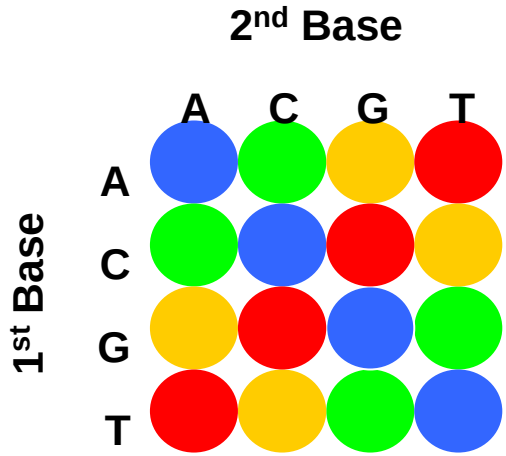
- Detecting a single color does **not** indicate a **base**
- Each reading contains information from two bases
- To decode the bases you **must** know **one** of the bases in the sequence



Example of decoding (i)

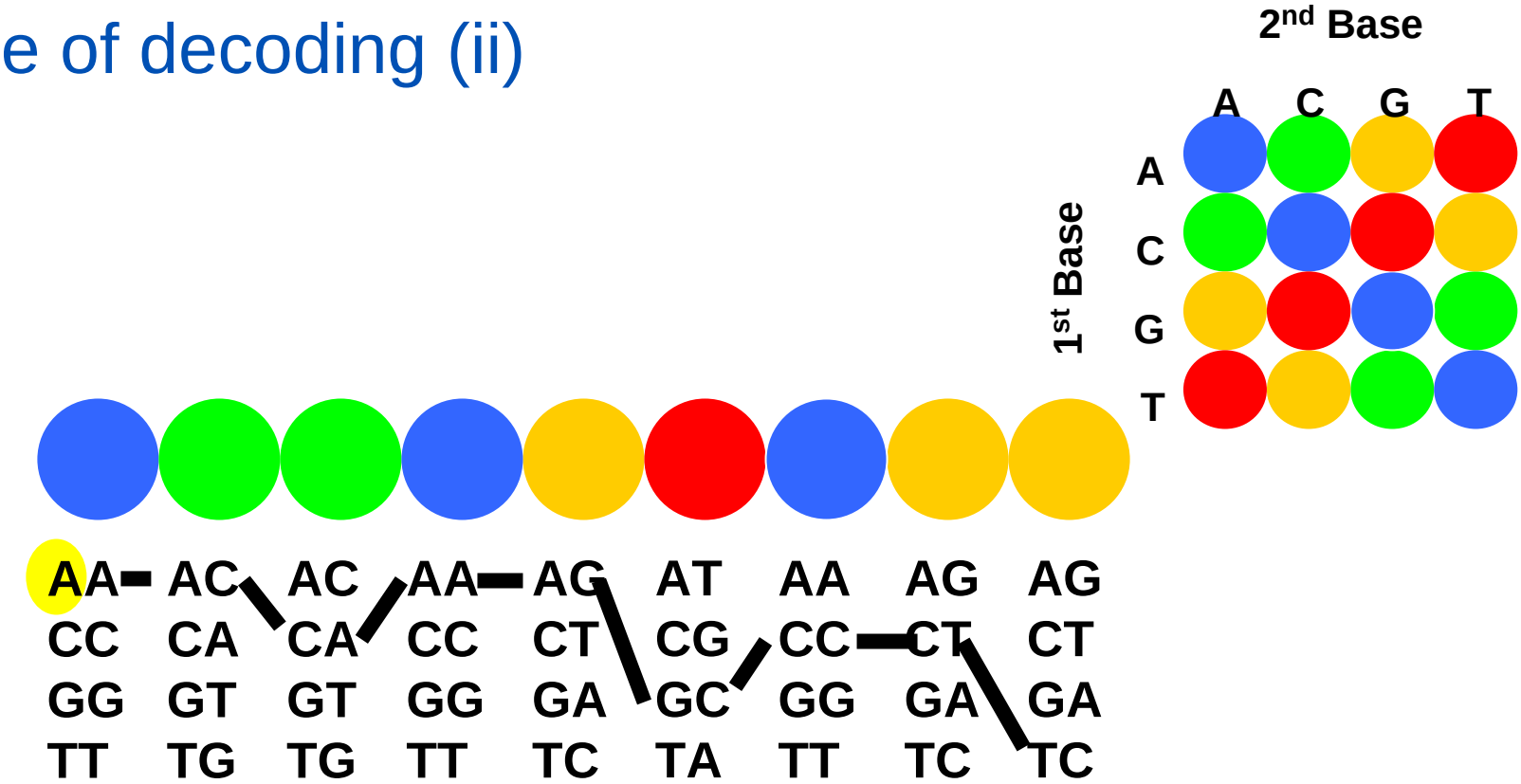


AA	AC	AC	AA	AG	AT	AA	AG	AG
CC	CA	CA	CC	CT	CG	CC	CT	CT
GG	GT	GT	GG	GA	GC	GG	GA	GA
TT	TG	TG	TT	TC	TA	TT	TC	TC



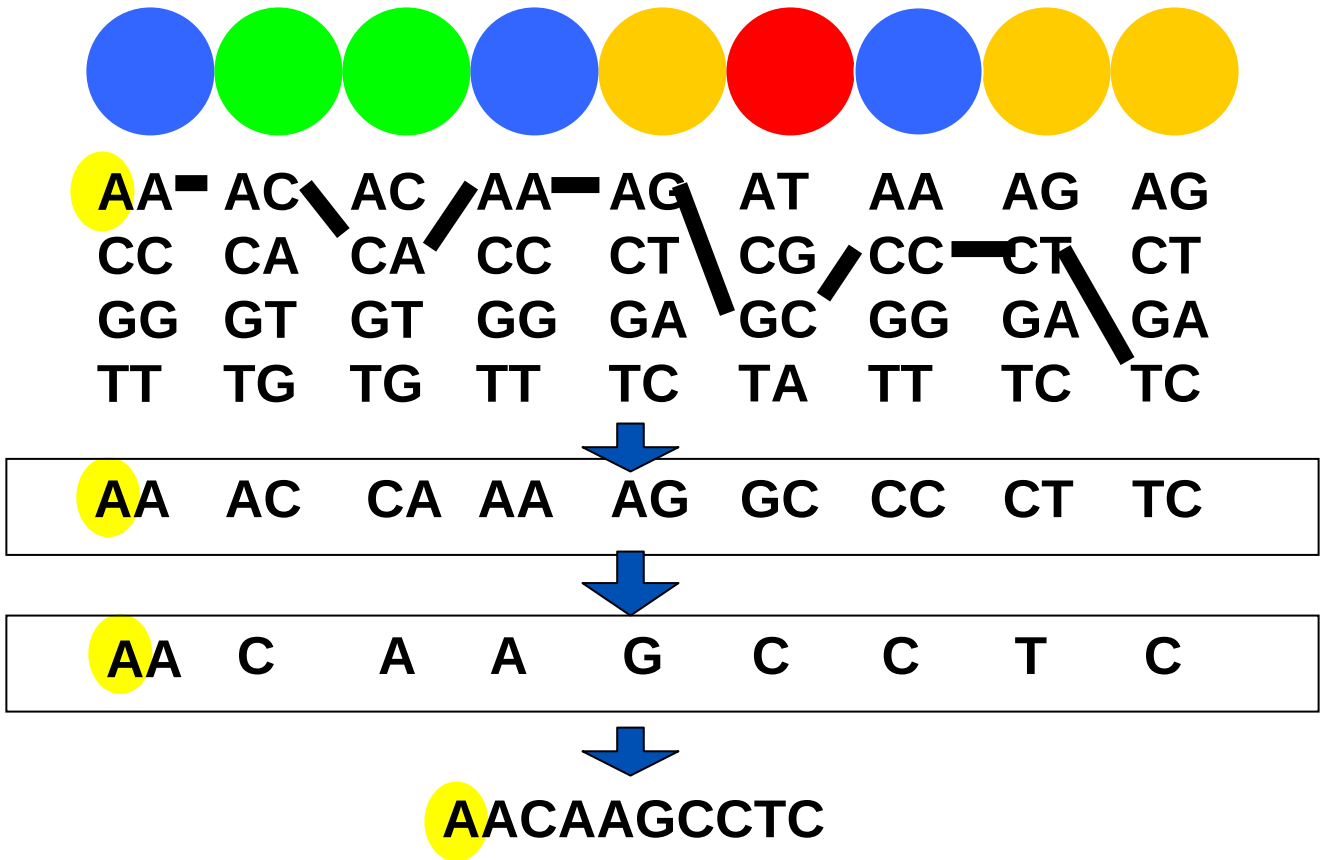
Cannot determine any of the bases

Example of decoding (ii)



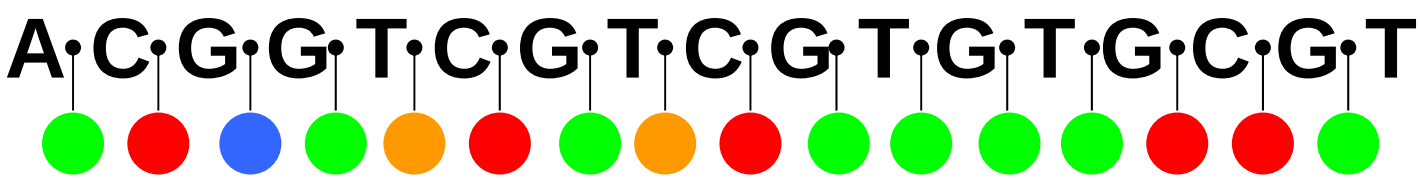
If know first base is an A then immediately it decodes 2nd base. This must be an A as Blue translates 2nd base A if first base A

Summary of decoding

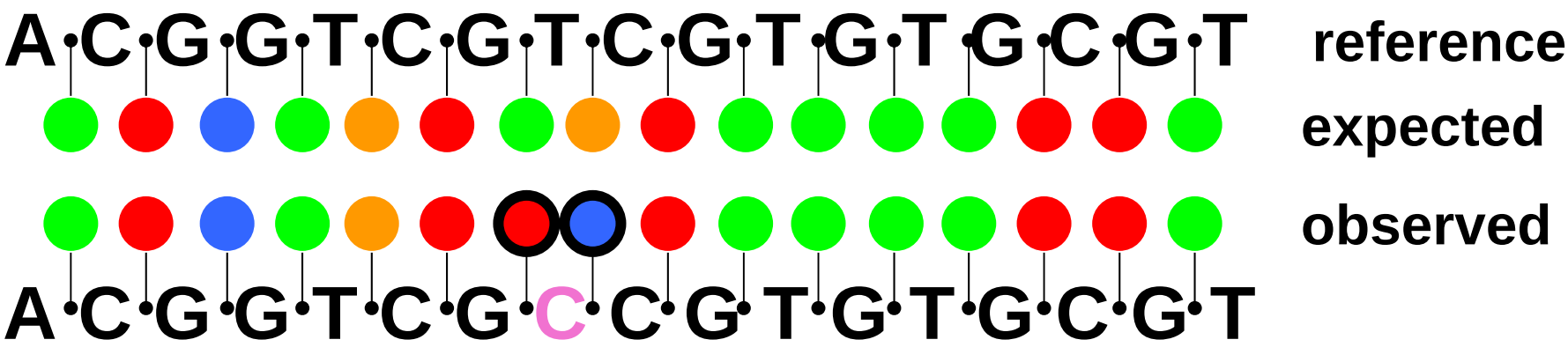


Advantages of 2 base pair encoding

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

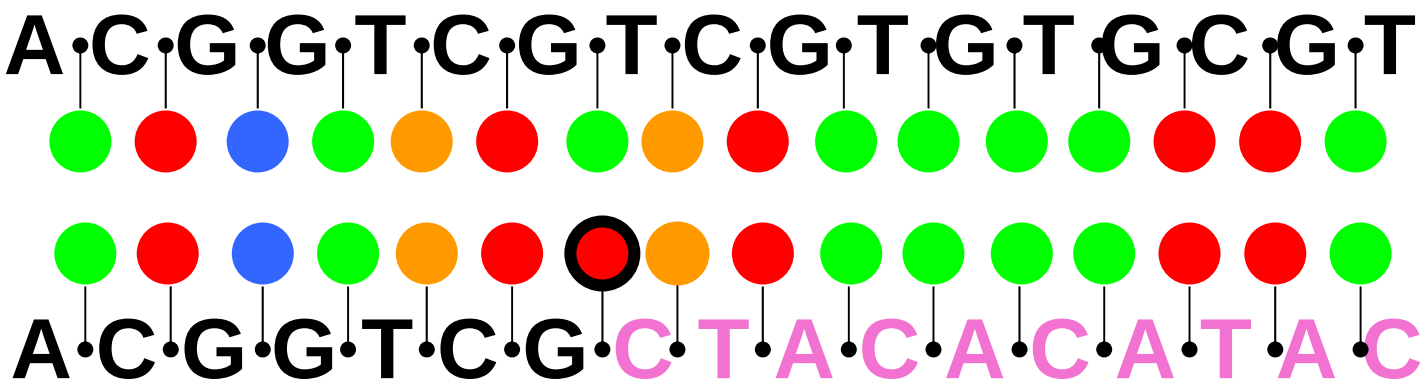


Advantages of 2 base pair encoding
Real SNP

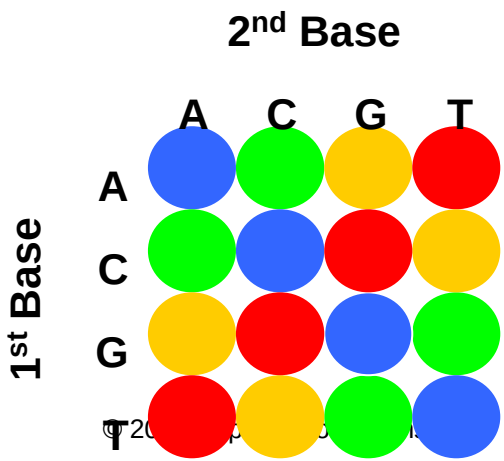


Two color changes represent only a single mismatch to reference sequence (SNP)

Advantages of 2 base pair encoding
Miscall

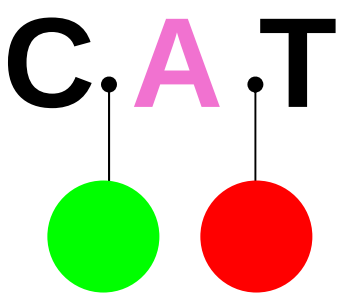


Single color change, represents sequencing error.



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.

(There are only 9 possibilities where *both* colors have changed)

The only allowed color changes

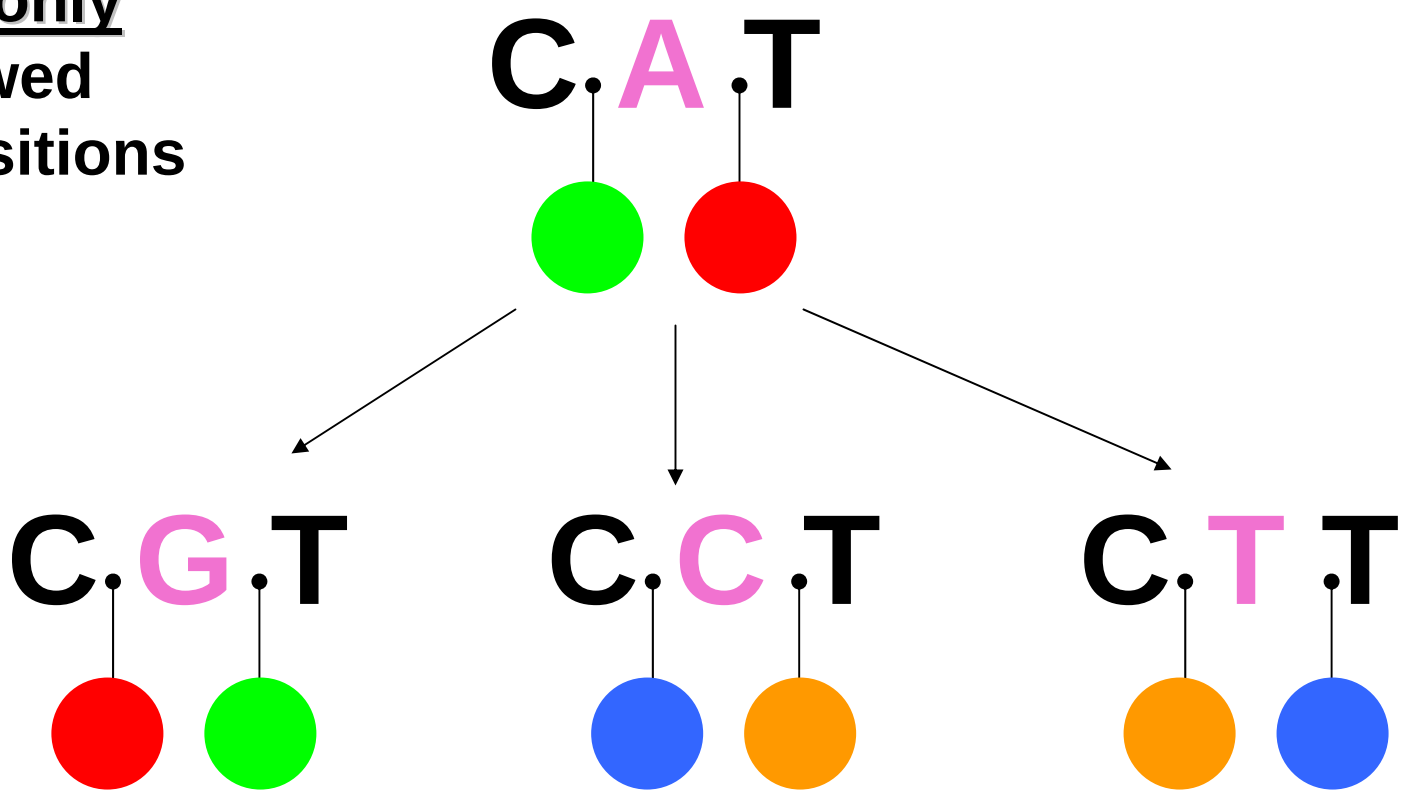
If two colors present (eg B,R)

- **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)**

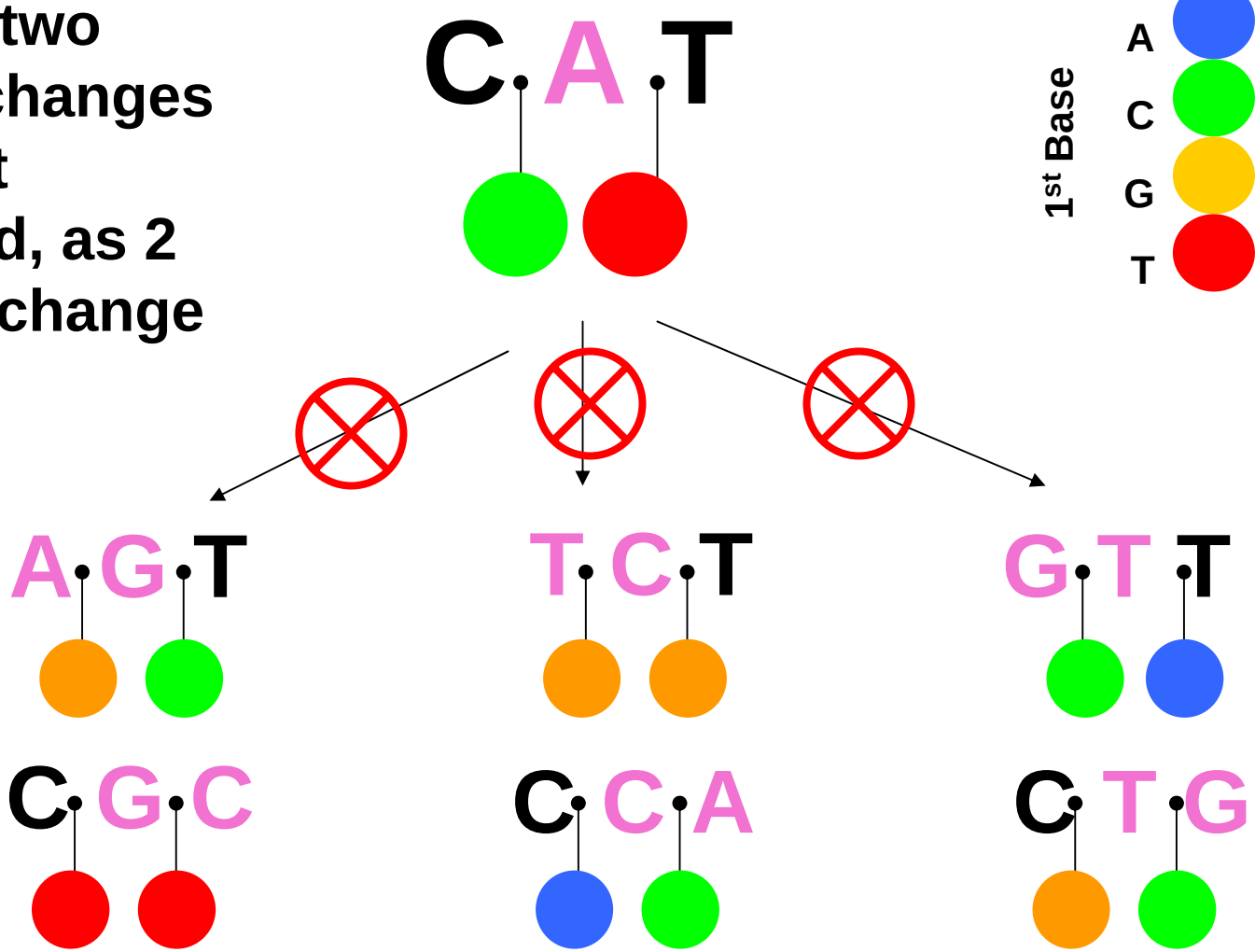
The only allowed transitions

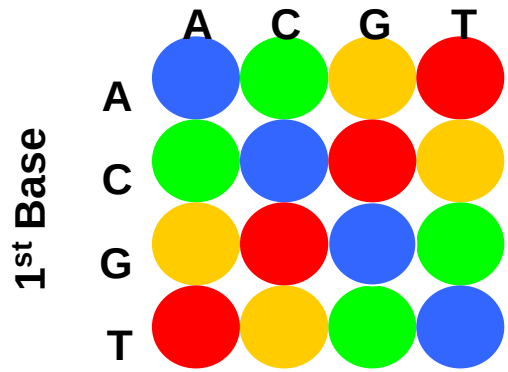


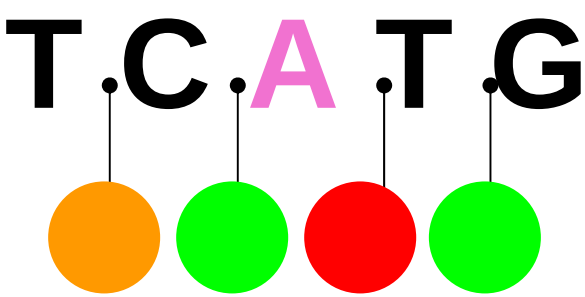
Reverse Colors **Other two colors (both orientations)**

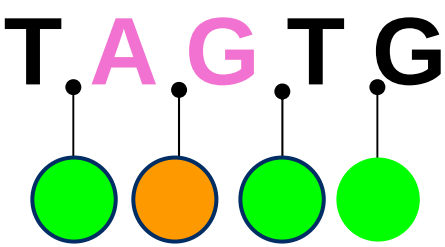
Any other transitions would require the outer two bases to change

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

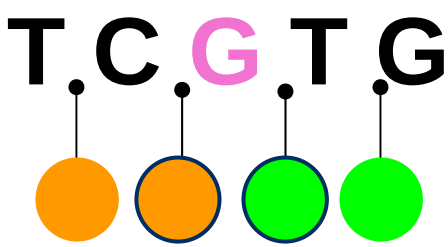




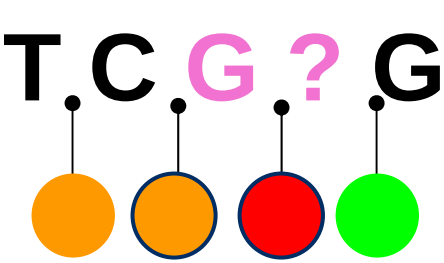




Allowed 3 colors changed



Allowed 2 colors changed acceptable “transition”

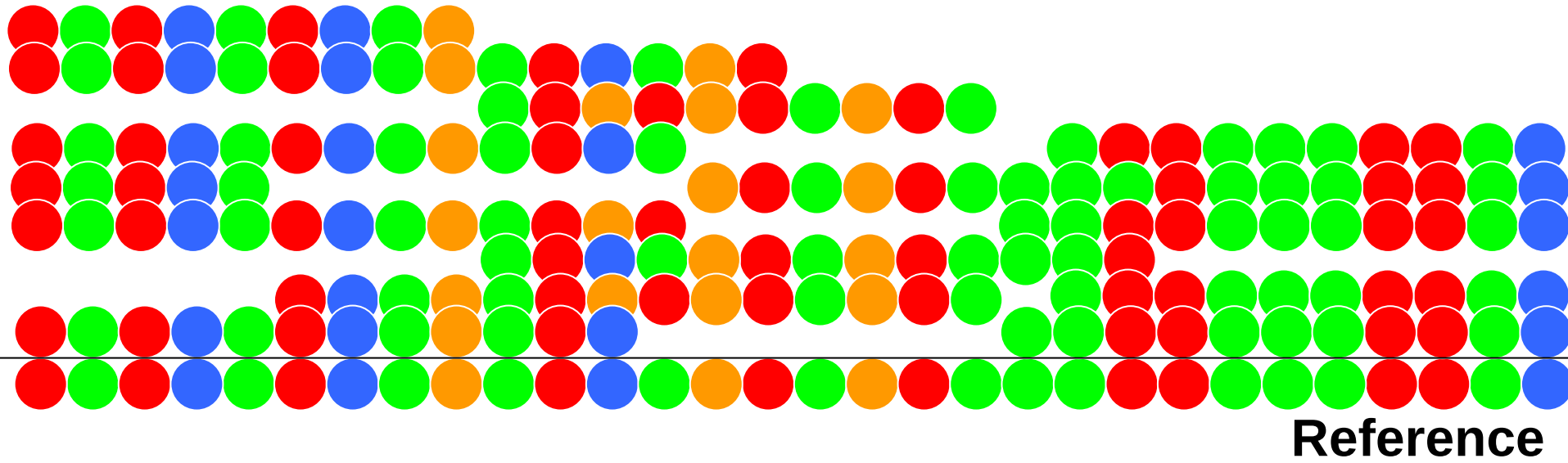


Not allowed 2 colors changed but a forbiden “transition”

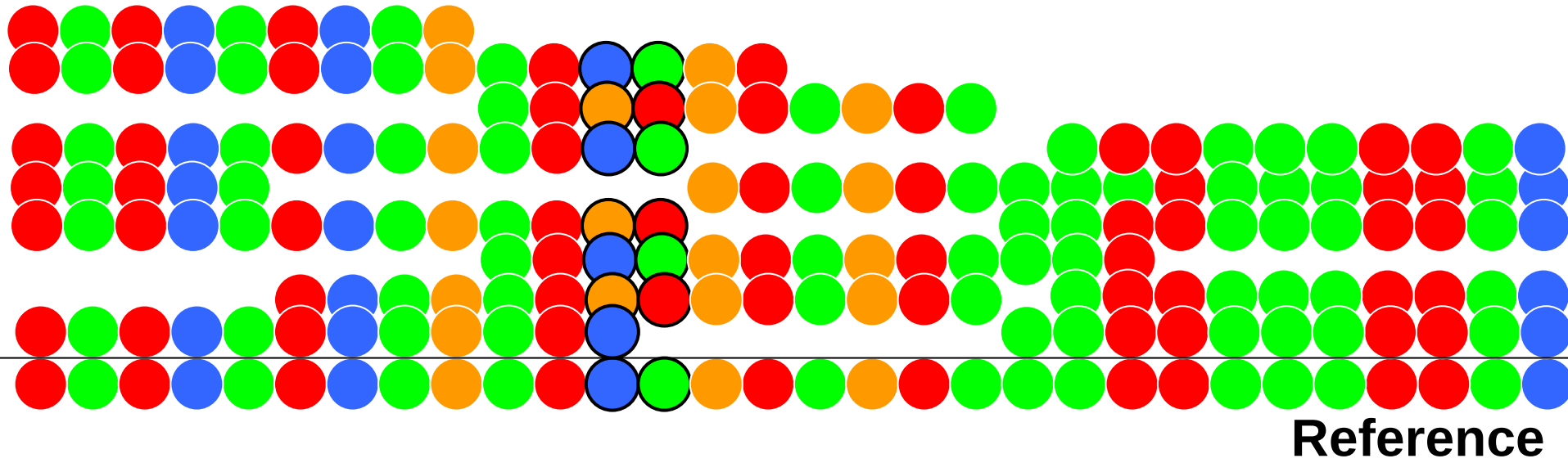
Why leave color space?

color space

Why leave color space?
Align color space reads against color space reference

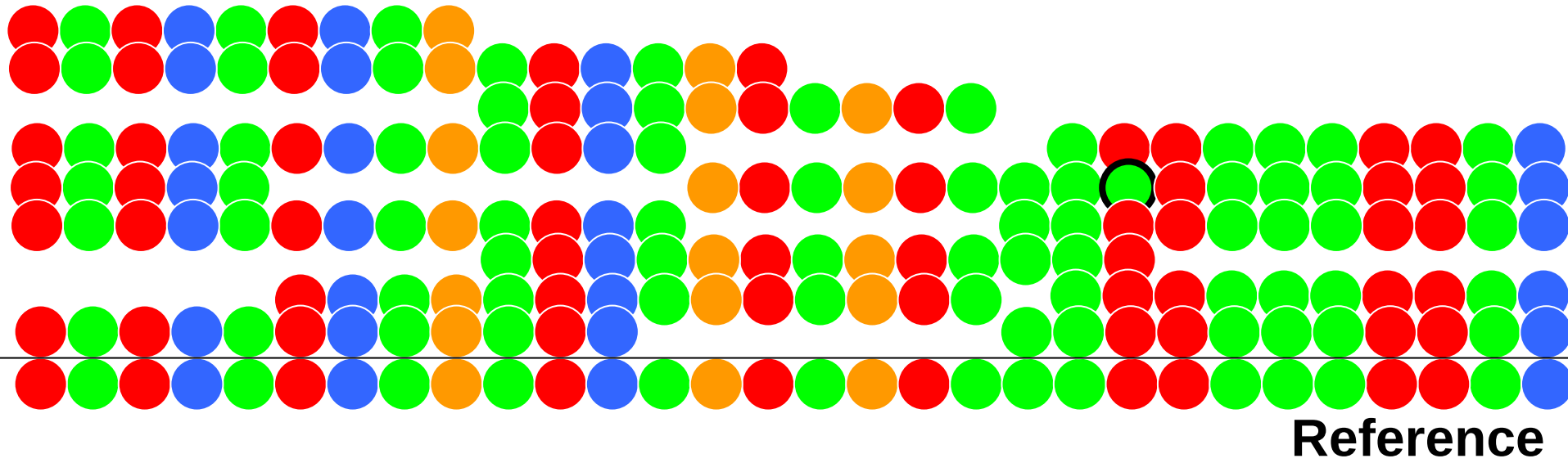


Why leave color space?
Align color space reads against color space reference



SNP 2 colors change

Why leave color space?
Align color space reads against color space reference



Incorrect call , single change in color space

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