Tips for evaluating new (q)PCR reagents

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1. PROTOCOL: New reagent has its own personal protocol, read it and follow
2. CONTROLS: Set up the right controls & you'll never have to repeat the experiment
3. MIXING: Do not underestimate it. Always mix all reagents very well before each use
4. CYCLING: Run an annealing temperature gradient for a new PCR system, keep cycling short
5. SAFETY: Follow all measures of typical PCR contamination prevention

Fast & simple SOP for PCR comparison (information check-up)

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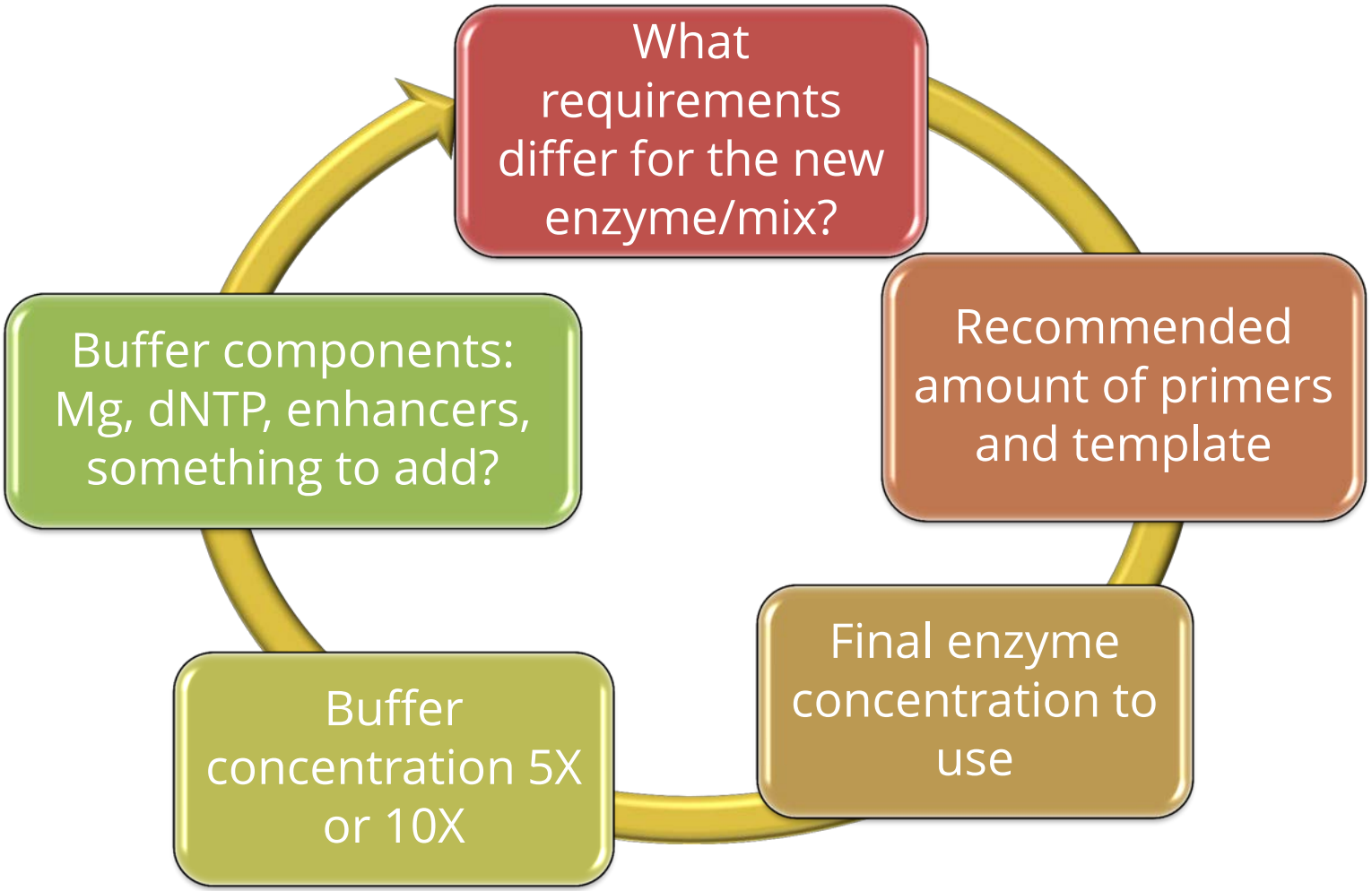
Compare protocols & product descriptions of both new & old enzyme or mix. Identify reaction mix differences:



Fast & simple SOP for PCR comparison (reaction set-up)

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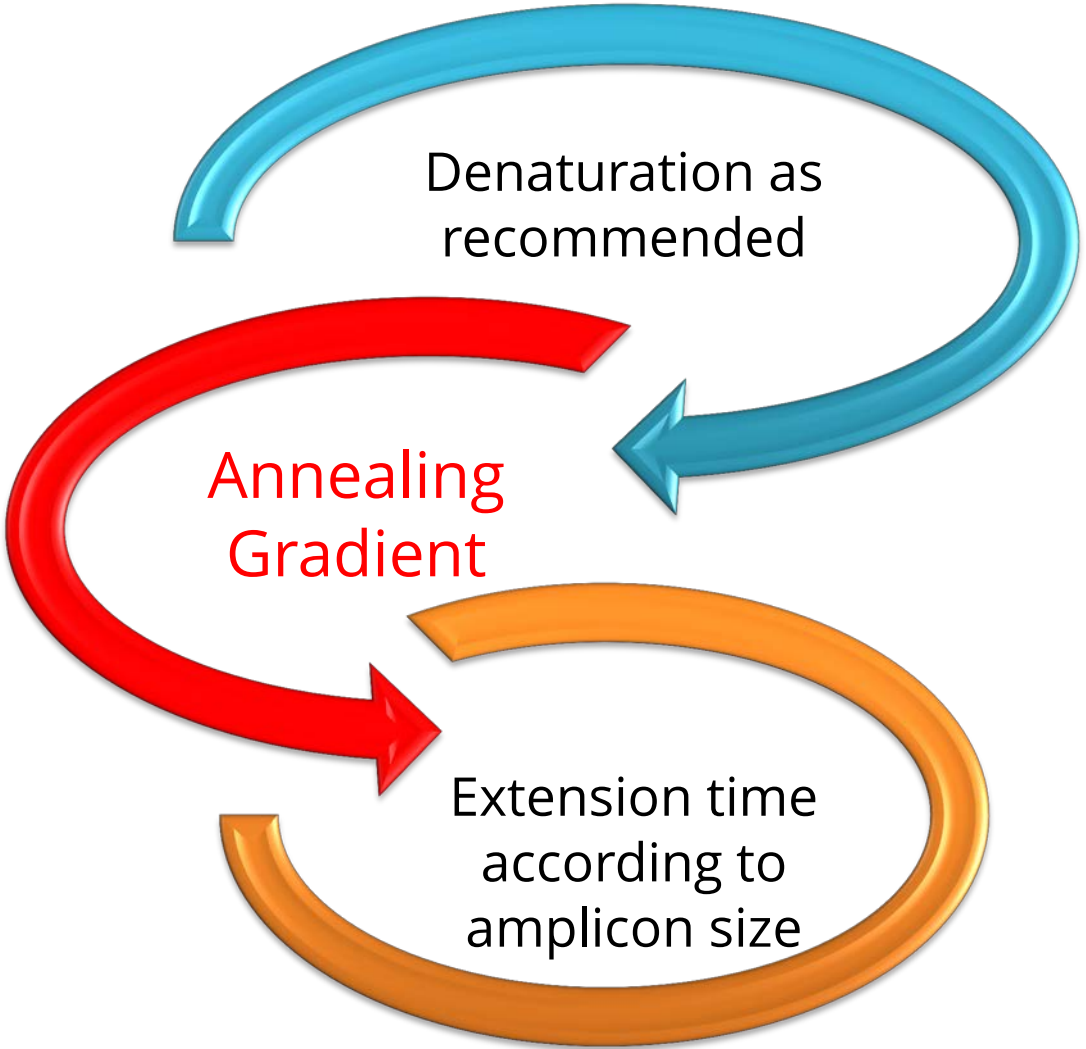
Prepare a set of reactions in at least 3 repeats with controls to run the annealing t° gradient & to compare both enzymes in parallel

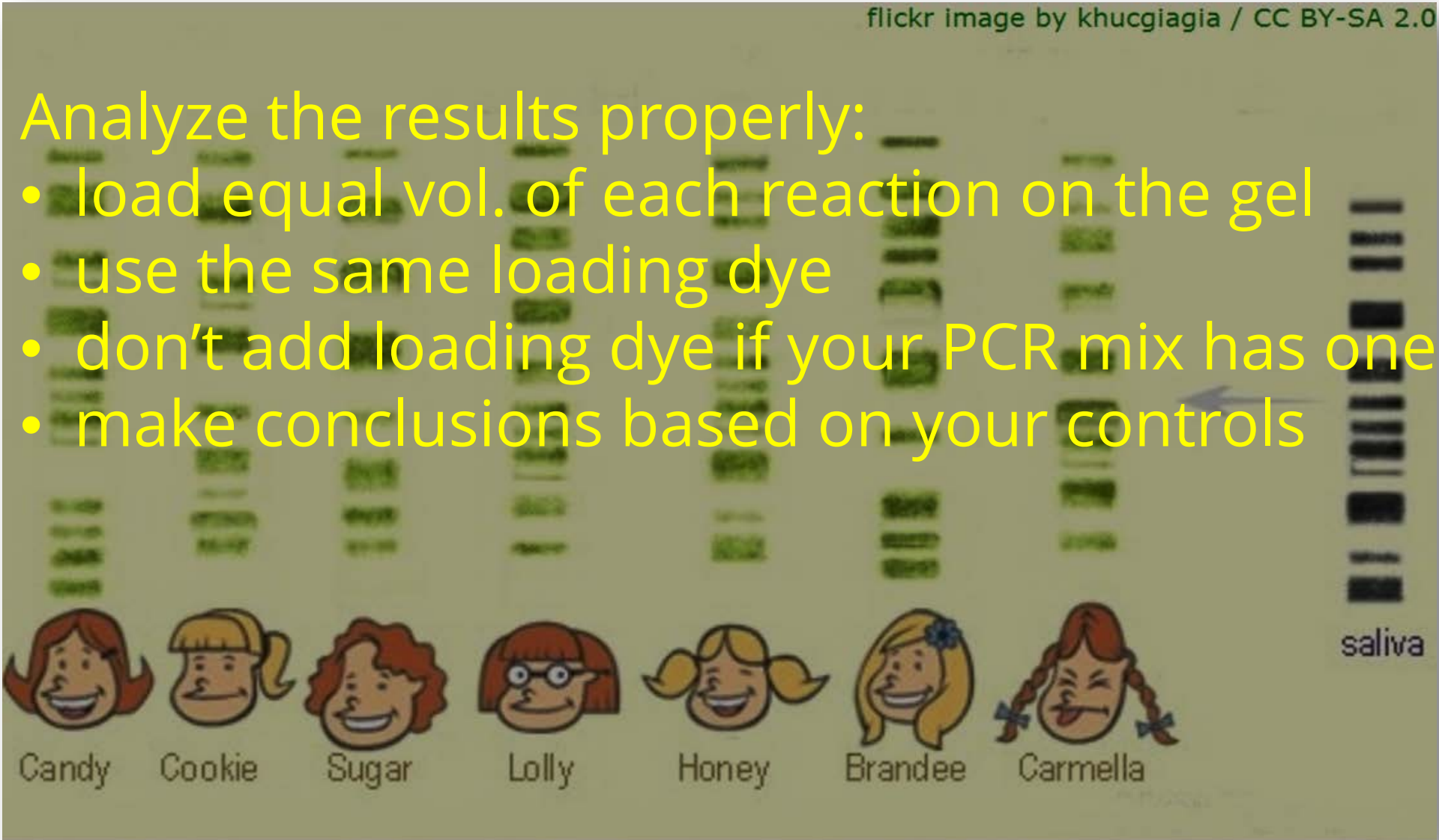
Reaction/ Components	1 Old enz.	2 New enz.	3 Old enz.	4 New enz.	5 Old enz.	6 New enz.	7 Old enz.	8 New enz.	9 Old enz.	10 New enz.
Water	Use the same water for all reactions									
Buffer	Use the buffer supplied with each enzyme									
dNTPs Salts (Mg) Additives	Only if needed/recommend by supplier, in recommend concentrations. All components might be included in the buffer!									
Template										
Primers										
Control Templ.										
Control Primers										
Enzyme										

Fast & simple SOP for PCR comparison (cycling)

Follow cycling recommendations of each supplier for cycle number, denaturation, annealing and extension. Less is better than more.



Fast & simple SOP for PCR comparison (analysis)



Analyze the results properly:

- load equal vol. of each reaction on the gel
- use the same loading dye
- don’t add loading dye if your PCR mix has one
- make conclusions based on your controls